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THÈSE présentée par :

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Développement de la cavité buccale :

du gène à l'expression clinique chez l'Homme

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« La science consiste à passer d'un étonnement à un autre. »

Aristote

INTRODUCTION

1 INTRODUCTION

1.1 Introduction générale et objectifs

Les anomalies dentaires, qu'elles soient de nombre, forme, taille, structure ou d'éruption/résorption sont une des manifestations phénotypiques des maladies rares ou syndromes, fixant ainsi dans le temps, grâce à la minéralisation des tissus dentaires, les troubles issus du développement et sont en lien direct avec les notions de mise en place de patrons, de centres de signalisation, d'histomorphogenèse….

Les maladies rares sont des affections touchant un nombre restreint de personnes, à savoir moins d'une personne sur 2000 selon le seuil admis en Europe. On dénombre environ 7000 maladies rares dont 80% sont d'origine génétique, mais chaque semaine (2012),de nouvelles maladies rares sont définies. Au total, elles concernent 3 à 4 millions de personnes en France, et près de 25 millions en Europe. Parmi ces maladies rares, 919 présentent des manifestations cliniques phénotypiques bucco-dentaires et 750 des fentes labio/palatines.

Ce travail de thèse se place au cœur d'un processus de recherche translationnelle qui met le patient au centre du processus de recherche et considère la cavité buccale et les dents comme des marqueurs et des points d'entrée en développement et en pathologie.

Des approches complémentaires et convergentes de recherche en biologie du développement chez la souris, en particulier par l'étude de modèles animaux, de bioinformatique, de génétique et leur confrontation aux données cliniques observées chez l'homme sont ainsi combinées pour améliorer les connaissances et la compréhension des phénomènes étiopathogéniques à l'origine du développement de la bouche, du palais et de la dentition - et de leurs anomalies.

Le développement crânio-facial et l'odontogenèse en particulier se mettent en place grâce à la migration des cellules des crêtes neurales céphaliques vers le premier arc pharyngien et leurs interactions avec l'ectoderme buccal. L'odontogenèse, classiquement divisée en 5 stades ; la lame, le bourgeon, le capuchon, la cloche et l'édification radiculaire avec l'éruption dentaire, est contrôlée par des interactions épithelio-mésenchymateuses entre les compartiments ectomésenchymateux et épithélial, et est régulée par des voies de signalisation conservées dans l'évolution et bien connues (Fgf, Bmp, Shh, Wnt, Tgfβ, Notch), identifiées en particulier grâce aux travaux de recherche sur la souris. Les différenciations terminales de cellules hautement spécialisées dans les deux compartiments et les synthèses

de matrices protéiques spécifiques suivies de leur minéralisation modèlent la formation coronaire et se poursuivent par la mise en place de la racine et les phénomènes d'éruption.

De nombreuses souris génétiquement modifiées, modèles de maladies rares, reproduisent dans leurs phénotypes les anomalies crânio-faciales rencontrées chez l'homme et autorisent ainsi une étude plus fine des conséquences des dysfonctionnements moléculaires associés. Le développement dentaire représente donc un modèle très intéressant pour l'étude de mécanismes génétiques et moléculaires de l'organogenèse et de ses anomalies.

Le premier axe de ce projet de thèse a consisté à étudier des gènes impliqués dans des syndromes chez l'Homme affectant le développement palatin et/ou dentaire, mais dont l'expression et le mode d'action ne sont pas (ou encore mal) connus, et d'autre part à identifier de nouveaux gènes candidats impliqués dans le développement dentaire en exploitant les données produites par l'analyse à grande échelle des profils d'expression génique (hybridation *in situ* de coupes sériées d'embryons/foetus de souris au stade E14.5: capuchon dentaire) dans le cadre du programme européen EURExpress (www.eurexpress.org) et répertoriées dans la base de données « Odontogenèse » créée par notre équipe (http://lbgi.igbmc.fr/ImAnno collaboration avec R. Ripp, IGBMC). Cette approche nous a permis de sélectionner plusieurs gènes ayant une expression tissu-spécifique au niveau des bourgeons dentaires et des tissus faciaux **(Publication 1 à 3).**

Très rapidement, il est apparu nécessaire de compléter les données d'expression obtenues par hybridation *in situ* par une analyse globale du transcriptome (puces à ADN Affymetrix) du capuchon dentaire au stade E14.5, afin de comparer les profils d'expression génique pour les molaires mandibulaires et maxillaires ainsi que pour les incisives. Ces données de transcriptome complètent les données d'expression (hybridation *in situ*), permettent une approche plus quantitative et éclairent à la lumière de possibles différences d'expression entre molaires et incisives les phénotypes différentiels observés en pathologie chez l'Homme (anomalies dentaires rencontrées plutôt dans le champ incisif ou molaire) **(Publication 4 et 5).**

Le second axe de mon projet est une étude approfondie de modèles animaux présentant des anomalies du développement de la cavité buccale. Des approches par

microtomodensitométrie (microCT) (Collaboration avec le Pr A. Constantinesco et le Dr P. Choquet) et par analyse de puces à ADN sont utilisées. L'étude porte plus particulièrement sur la lignée de souris mutées pour le gène *Rsk2* (collaboration avec le Dr A. Hanauer, IGBMC).

La famille des RSK est une famille de sérine-thréonine kinases qui compte 4 membres (RSK1, 2, 3 et 4) chez l'Homme comme chez la souris : le gène *RSK1* est situé sur le chromosome 3, *RSK3* se trouve sur le chromosome 6 et *RSK2* et *4* sur le chromosome X (en Xp22.2-p22.1, Xq21 respectivement). Les 4 protéines RSK présentent au moins 75% de similitude de séquence, suggérant une possible redondance fonctionnelle, et sont impliquées dans des évènements cellulaires divers et importants comme la prolifération, la différenciation, l'apoptose et la réponse cellulaire au stress.

Des mutations dans le gène *RSK2* causent chez l'Homme le syndrome de Coffin-Lowry (CLS), lié à l'X et caractérisé par un retard mental sévère et un dysmorphisme touchant notamment la face, les mains et le squelette. On observe chez ces patients des anomalies bucco-dentaires telles qu'un palais étroit, un sillon lingual central, une malocclusion, une hypodontie, des incisives riziformes et une perte précoce de certaines dents.

Mon travail se décompose en plusieurs volets. Une analyse macroscopique de la souris *Rsk2-/Y* (un modèle de perte de fonction créé par l'équipe du Dr Hanauer) a mis en évidence une taille réduite chez ces souris, une déviation au niveau nasal chez certains individus et la présence de prémolaires surnuméraires ainsi que des modifications de gradients de taille dans le champ molaire.

Une analyse par microtomodensitométrie (microCT) a été réalisée afin de préciser ces anomalies osseuses, mais également de mieux comprendre l'origine des dents supplémentaires en s'intéressant globalement au champ molaire, à la taille du diastème, mais aussi au nombre total de racines. La participation des incisives au phénotype a aussi été explorée **(Publication 5)**

Des analyses de transcriptome (puces à ADN Affymetrix) ont été réalisées afin d'étudier les gènes dérégulés au stade E14.5 (capuchon) dans les incisives inférieures, les molaires inférieures et les molaires supérieures de souris *Rsk2-/Y*. Cette étude révélant des modifications très hétérogènes du transcriptome parmi les mutants, nous avons décidé d'inactiver *in vitro,* en culture organotypique, le gène *Rsk2* dans des explants dentaires au

stade E14.5 afin de minimiser l'hétérogénéité créée sans doute par des phénomènes de compensation liés aux tissus environnants et par une expression variable du gène. De plus, l'inactivation se faisant uniquement sur 24h, les phénomènes de compensation n'ont pas le temps de se mettre en route. Nous avons donc testé l'inactivation du gène *Rsk2* à l'aide d'un ARN interférent (shRNA). Afin d'optimiser la pénétration de l'ARN, des approches par microinjection puis électroporation des explants dentaires ont été testées **(Publication 5).**

L'analyse de patrons d'expression de gènes et leur mise en relation avec les anomalies dentaires rencontrées en cas d'inactivation de ces mêmes gènes offrent des éléments de compréhension des mécanismes reliant l'odontogenèse et ses anomalies. L'analyse du transcriptome permet d'appréhender les différences moléculaires liées à la différence de forme et de morphologie soit l'identité molaire ou incisive, identité parfois malmenée en contexte pathologique (molarisation des incisives par exemple).

L'approche innovante d'électroporation *ex vivo* d'un shRNA dans des explants de dents suivie d'une culture organotypique durant 24h développée durant ce travail de thèse permet d'ouvrir de nouvelles perspectives d'études fonctionnelles par inactivation de gènes impliqués dans le développement dentaire.

1.2 L'odontogenèse

1.2.1 Le développement dentaire

Le développement dentaire s'inscrit dans le développement crânio-facial en général. Les cellules pluripotentes issues des crêtes neurales céphaliques vont migrer le long du premier arc pharyngien et vont ainsi permettre, en association avec des cellules mésodermiques, le développement de nombreuses structures du massif crânio-facial (Cobourne and Mitsiadis, 2006; Knight and Schilling, 2006; Noden and Schneider, 2006).

L'odontogenèse chez l'Homme se traduit par la morphogenèse de couronnes et de racines spécifiques à chaque type de dents, par l'histogenèse de l'organe de l'émail et les cytodifférenciations des odontoblastes, des améloblastes et des cémentoblastes. L'étude de

l'évolution des mammifères s'intéresse souvent à une analyse détaillée de la morphologie dentaire. Pour que les patrons moléculaires puissent jouer un rôle sur l'évolution dentaire, des différences d'expression de gènes doivent pouvoir être reliées à des variations morphologiques (Jernvall, 2000; Plikus et al., 2005; Prochazka et al.; Salazar-Ciudad and Jernvall) .

La dent embryonnaire chez les mammifères et en particulier chez les souris est un modèle très intéressant en Biologie du Développement. Chez l'Homme, la formule dentaire s'exprime au sein de deux dentitions (temporaire et permanente) et est composée de dents de morphologies différentes avec en avant les incisives suivies des canines puis des prémolaires et des molaires. La souris quant à elle, ne possède qu'un jeu de dents (monophyodontie) et sa formule dentaire, contrairement à celle de l'Homme, n'est composée que de deux types de dents (3 molaires et 1 incisive par hémi-arcade) séparées par un espace appelé diastème. De plus, la souris possède des incisives à croissance continue ayant un développement asymétrique avec dépôt de l'émail uniquement sur la partie labiale et une partie linguale analogue de racine. La forme générale des dents est différente entre les deux espèces **(Figure 1)**. Malgré ces différences, les processus de formation et de développement dentaire chez l'Homme et la souris sont similaires dans la globalité mais dans le détail. En effet, le même langage de communication cellulaire a été conservé au cours de l'évolution, ce qui permet de considérer la souris comme un modèle pertinent de l'odontogenèse humaine et d'aborder les différences propres à chaque type dentaire (molaires mandibulaires, molaires maxillaires, incisives inférieures et incisives supérieures), d'étudier les mécanismes impliqués dans la détermination du nombre et/ou du type de dents, mais aussi d'étudier la problématique des cellules souches (de par la présence d'une niche de cellules souches située dans la lèvre cervicale épithéliale du côté labial de l'incisive) (Yoshiba et al., 1998).

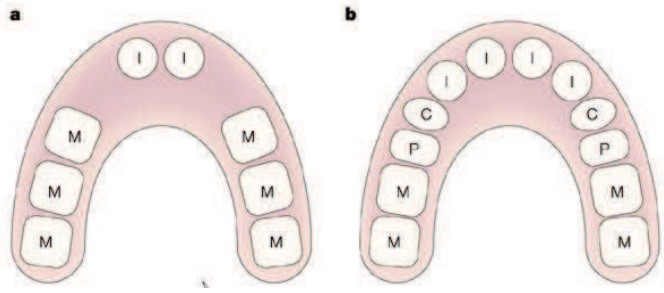
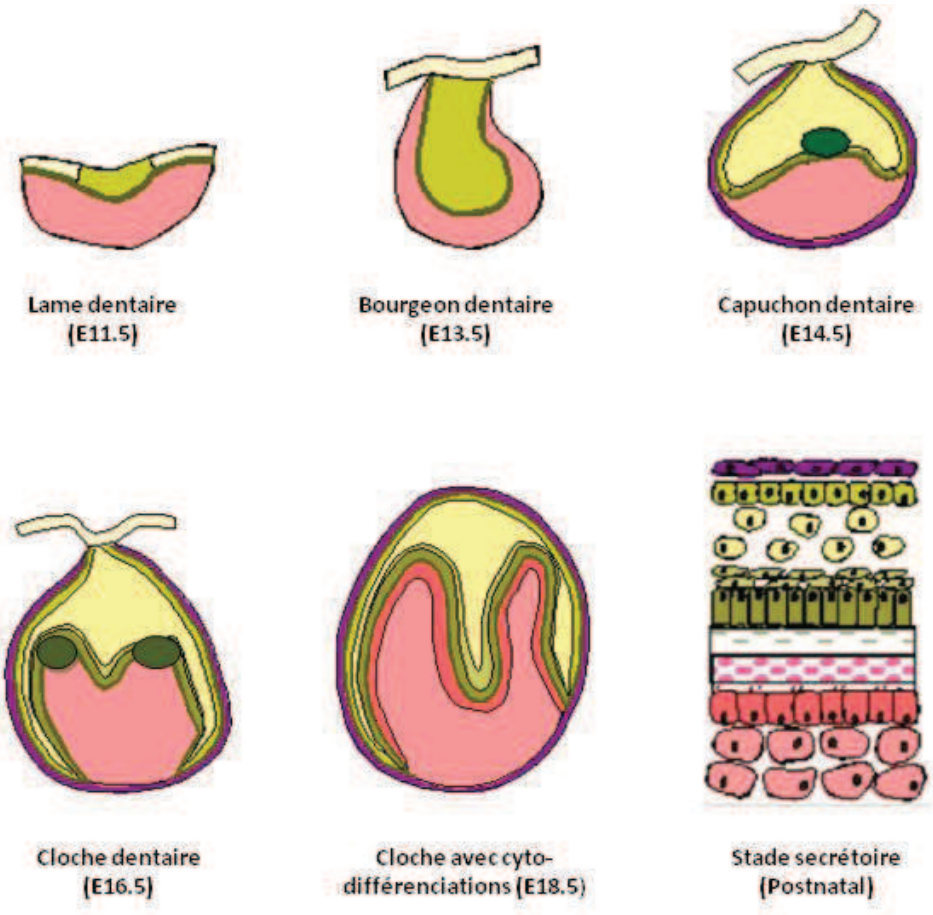


Figure 1 : Comparaison des dentitions humaine et murine. Les souris ont trois molaires et une incisive sur chaque hémi-arcade, qui sont séparées par un espace appelé diastème **(a)**. La dentition humaine est plus complexe car elle est composée de quatre types de dents (deux incisives, une canine, deux prémolaires et trois molaires par hémi-arcade) **(b)**. La denture lactéale est composée de cinq dents sur chaque hémi-arcade : deux incisives, une canine et deux molaires (d'après (Tucker and Sharpe, 2004).

Les étapes continues et progressives du développement dentaire ont été classiquement divisées en stades de lame dentaire, bourgeon, capuchon, cloche, formation radiculaire et éruption. L'odontogenèse est un processus dynamique, contrôlé par des interactions épithélio-mésenchymateuses à médiation matricielle, entre l'ectoderme du premier arc branchial et des cellules ectomésenchymateuses originaires des crêtes neurales céphaliques (Peters and Balling, 1999; Thesleff, 2003b; Thesleff and Aberg, 1999; Tucker and Sharpe, 2004). Ces cellules contribuent à la formation du mésenchyme dentaire, de la pulpe dentaire , des odontoblastes, de la matrice de la dentine, du cément, du parodonte (Chai et al., 2000; Miletich and Sharpe, 2004) **(Figure 2)**.



EO : épithélium oral ; RS : réticulum stellaire ; SI : stratum intermedium ; NE : nœud de l'émail ; MB : membrane basale ; MD : mésenchyme dentaire ; SD : sac dentaire ; LP : ligament périodontal

Figure 2 : Les étapes progressives du développement dentaire : L'odontogenèse est classiquement divisée en stades. Vue schématique d'une coupe frontale d'un germe dentaire représentant : la lame dentaire (E11.5 chez la souris), le bourgeon (E13.5), le capuchon (E14.5), la cloche dentaire (E16.5), la cloche avec cytodifférenciations des améloblastes et des odontoblastes, le stade postanatal (PN0) avec formation de la dentine et de l'émail. (D'après le site http://bite-it.helsinki.fi)

La morphogenèse dentaire comme le développement embryonnaire en général est sous contrôle génétique strict. Les gènes qui régulent l'odontogenèse sont identifiés de plus en

plus rapidement et plus de 300 d'entre eux sont répertoriés dans une base de données, créée par Pekka Nieminen de l'Université d'Helsinki, et illustrant leurs patrons d'expression aux différents stades du développement dentaire (http://bite-it.helsinki.fi). De plus, dans le cadre du projet EURExpress (www.eurexpress.org), des données d'expression ont été générées par hybridation *in situ* sur coupes sériées d'embryons de souris au stade E14.5 (capuchon dentaire) (Diez-Roux et al., 2011). Notre équipe a analysé ces profils d'expression de manière approfondie dans les structures dentaires, et a répertorié les données dans la base "Odontogenèse" (http://lbgi.igbmc.fr/ImAnno; collaboration avec R. Ripp, IGBMC), ceci afin de sélectionner des gènes "intéressants", c'est à dire montrant une expression spécifique au niveau des bourgeons dentaires et/ou des tissus faciaux. A ce jour, plus de 900 gènes ont ainsi été répertoriés **(Figure 3)**.

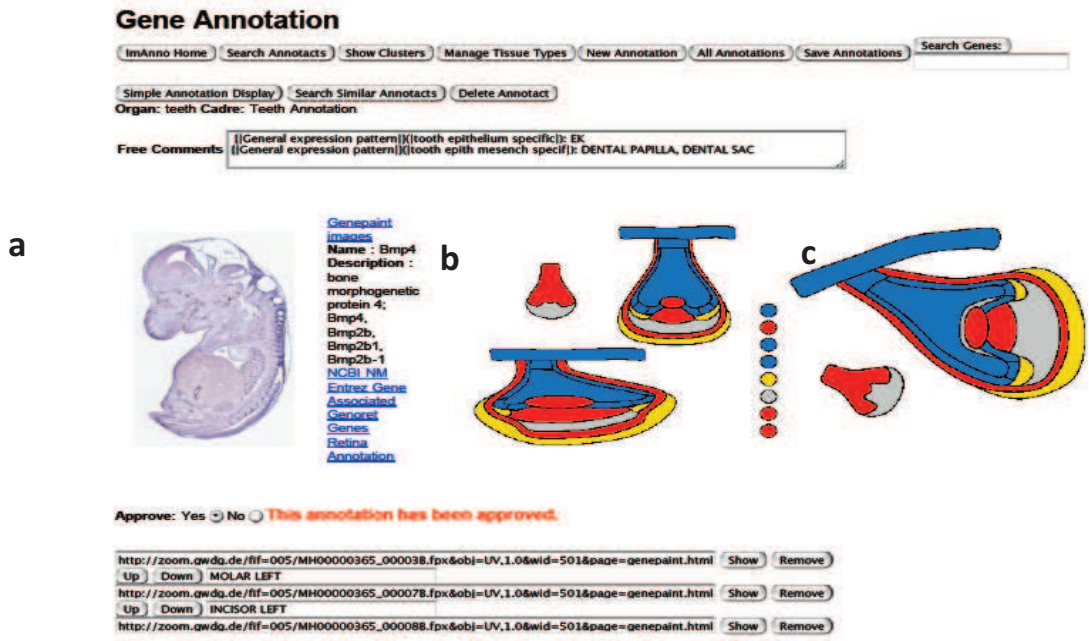


Figure 3 : Exemple d'annotation d'un gène (*Bmp4)* dans la base de données « Odontogenèse ».

Photo d'une coupe de l'hybridation *in situ* du gène *Bmp4* sur un embryon E14.5 provenant du site EURExpress (www.eurexpress.org) **(a)**. Le schéma d'une molaire est représentée en coupe frontale et en coupe transversale **(b)** et le schéma d'une incisive est représenté en coupe transversale **(c)**. Les différentes zones des deux types de dents sont représentées en différentes couleurs. La couleur bleu signifie une absence d'expression , la couleur jaune une expression faible et la couleur rouge une expression forte dans le tissu.

1.2.2 Les interactions épithélio-mésenchymateuses

Un langage de communication cellulaire conservé durant l'évolution est utilisé aussi bien au cours du développement embryonnaire que de l'odontogenèse. Ce langage implique des molécules de signalisation et des facteurs de croissance définissant plusieurs grandes familles : la famille TGF-beta (Transforming growth factor beta) incluant les BMP (Bone morphogenetic proteins, les activines et la follistatine ; la famille des FGF (Fibroblast growth factors) ; Hedgehog (seul Sonic hedgehog est connu pour son rôle dans l'odontogenèse) ; ou la famille des Wnts (Cobourne and Sharpe, 2005; Dassule et al., 2000; Hardcastle et al., 1999; Nie et al., 2006a; Nie et al., 2006b). Ces molécules transmettent leur message via des voies de signalisation et des récepteurs de la surface cellulaire jusqu'au noyau. Des facteurs de transcription vont ensuite moduler l'expression de gènes cibles et induire des modifications de la réponse et du comportement cellulaire **(Figure 4)**. Les rétinoïdes participent également au développement dentaire et crânio-facial (Mark et al., 1992)

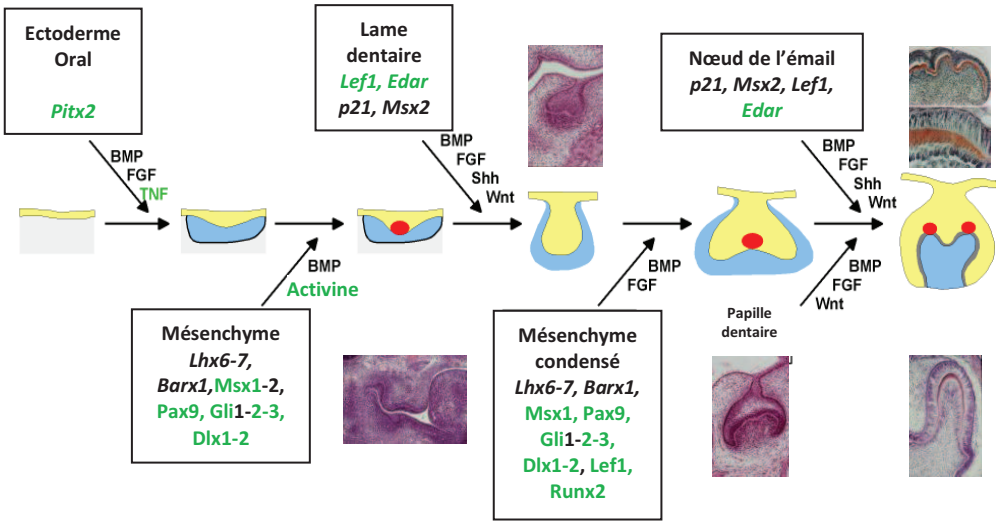


Figure 4 : Les voie de signalisation régulant le développement dentaire (D'après Bloch-Zupan, 2012, adapté de (Thesleff and Mikkola, 2002)

Les gènes en vert sont responsables, lorsqu'ils sont inactivés, d'un arrêt du développement dentaire.

Les cheveux, les plumes, les écailles, le bec, les ongles, les cornes et certaines glandes exocrines (mammaires, sudoripares, salivaires, lacrymales…) tout comme les dents sont

dérivés de l'ectoderme. Ces organes sont très différents de par leurs formes et leurs fonctions, mais ils partagent certaines caractéristiques développementales. En effet, ils proviennent tous de deux couches cellulaires adjacentes composées pour l'une de tissu épithélial d'origine ectodermique et pour l'autre de tissu mésenchymateux provenant du mésoderme ou dérivé des cellules des crêtes neurales. Généralement le mésenchyme envoie le premier signal, puis des interactions entre l'épithélium et le mésenchyme contrôlent les premières étapes du développement de tous ces organes d'origine ectodermique. Le premier signe de développement est un épaississement de l'épithélium, aboutissant à la formation d'une placode, puis les cellules du mésenchyme se condensent et vont former une papille sous la placode qui va donner naissance à un bourgeon. Chaque organe va ensuite suivre sa propre morphogenèse qui impliquera une croissance des tissus épithéliaux et mésenchymateux en association avec des replis et, parfois, des bifurcations dichotomiques ("branching morphogenesis") de l'épithélium, pour aboutir à la taille et à la forme finale de l'organe (Pispa and Thesleff, 2003) **(Figure 5).**

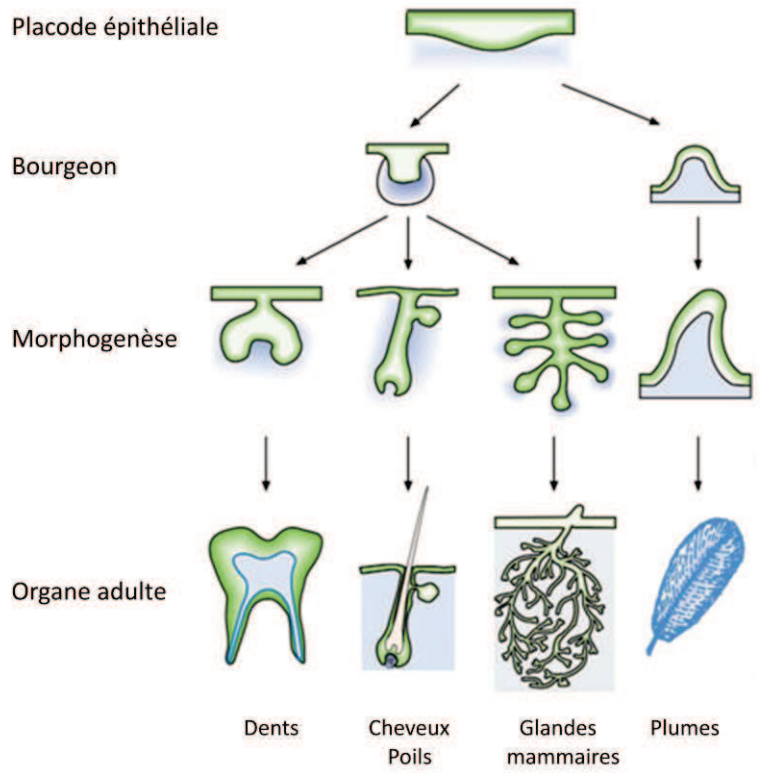


Figure 5 : Développement des organes d'origine ectodermique. Des organes très différents morphologiquement, comme les dents, les cheveux, les poils, les glandes mammaires ou encore les plumes se développent tous à partir de deux tissus adjacents : l'épithélium (vert) et le mésenchyme (bleu). Une placode épithéliale se forme et va ensuite bourgeonner à l'intérieur ou à l'extérieur du mésenchyme. Au cours de la morphogenèse, les repliements et les embranchements de l'épithélium détermineront ensuite la forme propre à chaque organe. (D'après (Pispa and Thesleff, 2003))

1.2.3 Les étapes du développement dentaire

1.2.3.1 *Initiation*

La localisation, l'identité, la forme et la taille de la dent sont déterminées pendant les stades précoces de son développement. Le contrôle moléculaire du patron ("patterning") de

la dentition avec les molaires multicuspidées à l'arrière (dorsal, distal) et les incisives monocuspidées à l'avant (ventral, mésial, proximal) est mis en place avant tout signe de développement dentaire à E10.5.

L'information permettant le développement de chaque type de dents est contenue dans le mésenchyme dentaire. En effet, de nombreux gènes à homéoboîte sont exprimés dans des régions spécifiques du mésenchyme du maxillaire et de la mandibule. La mandibule est divisée entre la région orale (proche de la bouche, rostrale) exprimant *Lhx6* et *7*, aborale (caudale) exprimant *Gsc*, distale territoire présomptif des incisives exprimant *Msx1* et *2* et proximale territoire présomptif des molaires exprimant *Dlx1* et *2, Barx1* et *Pitx1* (Thomas et al., 1997; Tucker et al., 1998; Tucker and Sharpe, 1999) **(Figure 6b)**. Les premiers signaux proviennent cependant de l'ectoderme oral où la présence de BMP4 dans la région incisive va induire l'expression de *Msx1* et *Msx2* dans le mésenchyme sous-jacent, et inhiber l'expression de *Barx1* dans le mésenchyme présomptif de la région incisive (Tucker et al., 1998). *Fgf8* et *9* sont exprimés dans la région molaire de l'ectoderme oral et vont induire l'expression de *Barx1* dans le mésenchyme présomptif de la région molaire. *Bmp4* et *Fgf8* s'inhibent mutuellement (Wilson and Tucker, 2004). L'expression de *Fgf8* est contrôlée positivement par *Pitx2* (paired-related homeobox gene) (Mucchielli et al., 1997).En effet, chez les souris mutantes pour *Pitx2*, l'expression de *Fgf8* est réduite tandis que celle de *Bmp4* est augmentée (Lu, 1999). De plus, *Bmp4* et *Islet1* s'auto-induisent dans une boucle de rétrocontrôle positif (Mitsiadis et al., 2003)**(Figure 6a)**. *Pitx1* est exprimé dans le mésenchyme des molaires mandibulaires, mais est absent au niveau des molaires maxillaires (Mitsiadis and Drouin, 2008).

Beaucoup de ces facteurs de transcription appartiennent à la même famille (c'est à dire codent pour des protéines à homéodomaine) et sont partiellement coexprimés, participant ainsi à une certaine redondance permettant des phénomènes de compensation et de sauvetage phénotypique en cas de dysfonctionnement. L'activation stade- et tissu-spécifique de ces gènes est nécessaire au bon développement dentaire, comme le démontre le phénotype des souris mutées. Le développement dentaire est en effet arrêté au stade de l'initiation lorsque *Msx1* et *Msx2,* ou *Dlx1* et *Dlx2,* sont inactivés (Satokata et al., 2000; Thomas et al., 1997).

La mise en place des territoires au sein de la mandibule ne se restreint pas à l'action de gènes à homéoboîte. En effet, dans sa partie distale, les facteurs de transcription à domaine basique-hélice-boucle-hélice (bHLH) *Hand1* et *Hand2* définissent la zone médiane de la face. Lorsqu'ils sont mutés, la région médiane disparaît et les incisives sont soudées (Barbosa et al., 2007).

Satb2, un membre d'une nouvelle famille de facteurs de trancription qui se lient à la matrice nucléaire et régulent l'organisation tissu-spécifique de la chromatine, joue un rôle dans la mise en place de la région intermédiaire entre la région distale et la région proximale (Britanova et al., 2006; Depew and Compagnucci, 2008) (**Figure 6b)**.

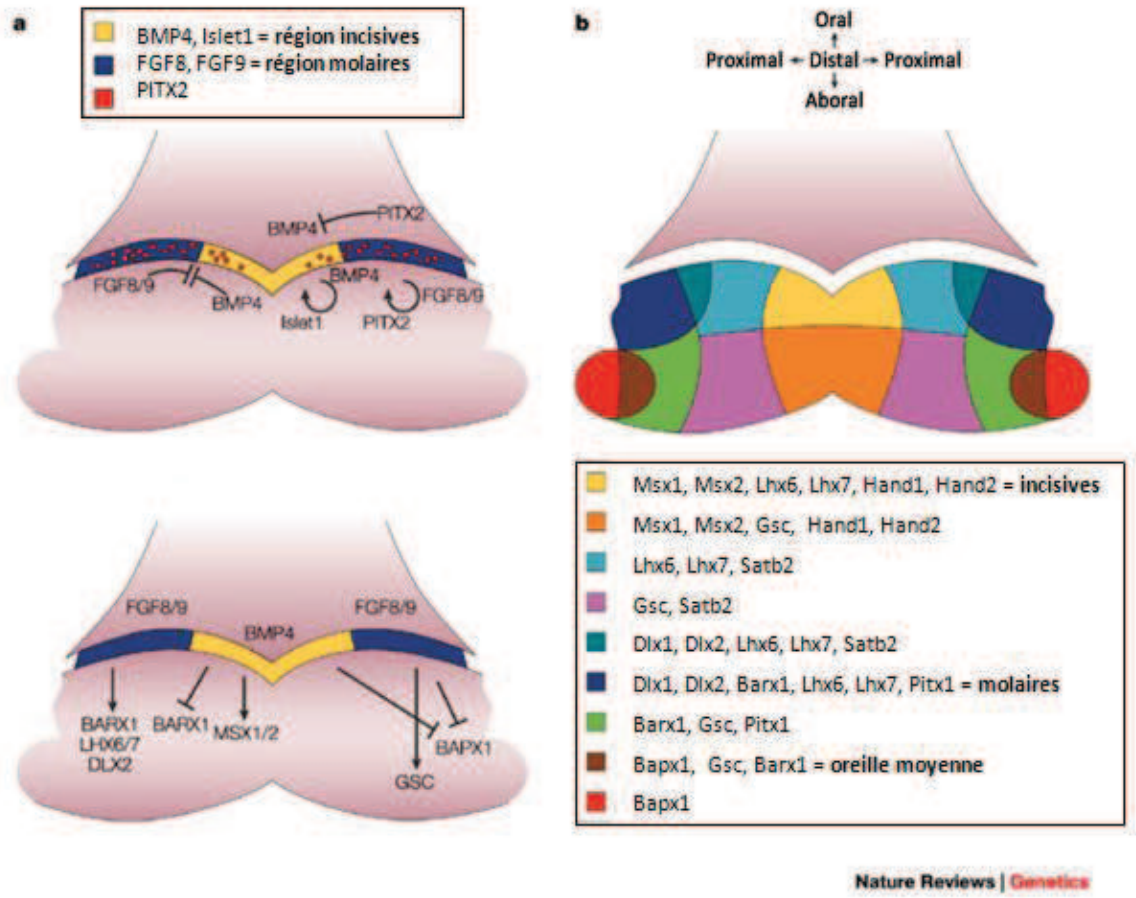


Figure 6 : Le patron d'expression des gènes au cours de l'initiation du développement dentaire

Signalisation et interactions au sein de l'épithélium, et entre l'épithélium et le mésenchyme, au jour embryonnaire E10.5. Le schéma montre la mandibule. Les boucles d'auto-régulation positive ou de répression mutuelle dans l'épithélium mènent à la formation de frontières dans l'expression de gènes qui mettent en place les champs molaire et incisive. Les membres des familles de protéines BMP (bone morphogenetic proteins) et FGF (fibroblast growth factors) exprimés dans l'épithélium induisent l'expression de plusieurs gènes à homéoboîte dans le mésenchyme sous-jacent. Ceci engendre un patron d'expression complexe de gènes dans le mésenchyme le long de l'axe proximo-distal, mais aussi de l'axe oral/aboral **(a)** Schéma des patrons d'expression de gènes codant pour des facteurs de transcription ou d'organisation de la chromatine. L'expression chevauchante des gènes dans la mandibule crée un code qui définit l'emplacement de chaque type de dents. *Bapx1*, bagpipe-like homeobox gene 1; *Barx1,* BarH-like homeobox 1; *Dlx,* distal-less homeobox; Gsc*,* goosecoid; *Lhx,* LIM-homeodomain genes; *Msx*, msh-like homeobox; *Pitx*, paired-related homeobox gene; *Satb2*, Special AT-rich binding protein 2 **(b)**. (Figure tirée de (Tucker and Sharpe, 2004).

1.2.3.2 *Formation de la placode*

Un des aspects clé du développement dentaire est la formation de placodes ectodermiques par épaississement de l'épithélium à l'emplacement de chaque famille de dents. Des placodes similaires initient le développement de tous les organes se développant comme des appendices ectodermiques (Pispa and Thesleff, 2003), comme les cheveux, les ongles, les glandes salivaires, mammaires et sudoripares. Des molécules de signalisation appartenant aux 4 grandes familles mentionnées précédemment participent au développement de la placode. Les FGF et les Wnts agissent comme des activateurs alors que les BMP jouent un rôle de répresseurs pour le développement des poils et des cheveux (Jung et al., 1998; Millar, 2002), et il semble qu'il en soit de même au cours de l'odontogenèse. Des gènes codant pour des molécules impliquées dans la formation de la placode et appartenant à la voie de signalisation TNF/NF-kappaB, ainsi que le proto-oncogène p63, sont responsables de dysplasies ectodermiques lorsqu'ils sont mutés, menant à des maladies impliquant tous les appendices épithéliaux et se manifestant par des hypodonties (moins de 6 dents manquantes), des oligodonties (6 dents manquantes ou plus) et même des anodonties (Laurikkala et al., 2006; Mikkola and Thesleff, 2003; Rinne et al., 2007; Smahi et al., 2002).

1.2.3.3 *Transition du stade bourgeon au stade capuchon*

Le mésenchyme sous-jacent contrôle la croissance et le repliement de l'épithélium dentaire. Les signaux mésenchymateux induisent dans l'organe de l'émail la formation de centres de signalisation appelés nœuds de l'émail ("Enamel knots"). Ce sont des structures transitoires où sont synthétisées plusieurs molécules de signalisation au stade capuchon (Thesleff and Jernvall, 1997).

Sonic hedgehog (Shh) est un des signaux essentiels pour la prolifération épithéliale, mais son action semble cibler le mésenchyme où il induit des boucles de rétro-contrôle vers l'épihélium (Gritli-Linde et al., 2002). La signalisation Wnt et BMP régule la formation des nœuds de l'émail. BMP4 induit l'arrêt du cycle cellulaire dans les cellules du nœud de l'émail

via l'expression de l'inhibiteur de kinase cycline-dépendant p21. Les Wnts sont nécessaires à l'expression de *Fgf4* dans les nœuds de l'émail (Bei et al., 2000; Bloch-Zupan et al., 1998; Jernvall et al., 1998; Kratochwil et al., 1996). Les FGF et leurs récepteurs sont exprimés à la fois dans le mésenchyme et l'épithélium, et ils régulent de manière réciproque la prolifération cellulaire dans les tissus adjacents (Kettunen and Thesleff, 1998; Wang and Thesleff, 2006). Trois facteurs de transcription sont présents à ces stades du développement dans le mésenchyme qui se condense, Msx1, Pax9 (paired homeobox 9) et Runx2 (runt-related homeobox 2). Leur expression est régulée par des signaux épithéliaux. *Msx1* est induit par BMP4, *Pax9* et *Runx2* par FGF8 (Aberg et al., 2004b; Bei and Maas, 1998; Peters et al., 1998a; Vainio et al., 1993). Les souris déficientes pour le gène *Runx2* montrent un développement dentaire arrêté au stade bourgeon (Aberg et al., 2004a; D'Souza et al., 1999). Les molécules de signalisation de la famille TNF (Eda/Edar) participent à la formation des cuspides via le nœud de l'émail (Charles et al., 2009b; Courtney et al., 2005; Tucker et al., 2000).

La voie de signalisation Wnt est impliquée dans le cycle de remplacement des dents lactéale en dents permanentes(Cho et al., 2007a; Cho et al., 2007b; Jarvinen et al., 2006).

1.2.3.4 *Le stade cloche*

Le développement des cuspides des molaires est initié par les nœuds de l'émail secondaires qui apparaissent dans l'épithélium dentaire interne aux sites de formations des pointes des futures cuspides. Ils expriment de nombreuses molécules de signalisation dont *Fgf4* et stimulent la croissance des cuspides. Le lien entre le nœud de l'émail primaire et les noeuds secondaires n'est pas encore totalement élucidé (Coin et al., 2000; Matalova et al., 2005).

La prolifération cellulaire au voisinage des nœuds de l'émail et la non prolifération dans les nœuds coordonnent les repliements de l'épithélium dentaire interne autour du mésenchyme qui se condense (Jernvall et al., 1994; Vaahtokari et al., 1996). L'apoptose dans les nœuds va induire la disparition progressive et la fin de l'activité de signalisation de ces structures à la fin du stade capuchon et au début du stade cloche (Matalova et al., 2004; Tucker and Sharpe, 1999).

1.2.3.5 *Les différenciations cellulaires*

Après la disparition des nœuds de l'émail secondaires, les cellules constituant la dent commencent leur différenciation terminale. Les cellules de l'épithélium dentaire interne se différencient en améloblastes tandis que les cellules mésenchymateuses de la pulpe dentaire se différencient en odontoblastes. Cette différenciation est régulée tout comme les stades précédents par des interactions épithélio-mésenchymateuses.

Les odontoblastes sont des cellules ciliées spécialisées (Magloire et al., 2004). Des signaux provenant de l'épithélium dentaire interne (EDI) induisent la formation des odontoblastes dans le mésenchyme dentaire (Begue-Kirn et al., 1994; Ruch et al., 1982). Des études *in vitro* ont montré que les odontoblastes peuvent être induits par TGFβ1, les FGF, et BMP2, tandis que IGF1 (insulin-like growth factor 1) induit la polarisation de ces cellules (Begue-Kirn et al., 1994; Martin et al., 1998).

Les odontoblastes se différencient suivant un gradient temporo-spatial défini par les cellules de l'épithélium dentaire interne et allant de la pointe des cuspides jusqu'à la zone cervicale de la dent (Thesleff et al., 2001). Cette différenciation temporo-spatiale a été récemment liée au fait que l'expression de *Wnt10a* se déplace des nœuds de l'émail secondaires vers les préodontoblastes sous-jacents (Yamashiro et al., 2007). La surexpression en culture de *Wnt10a* mène à l'induction de *Dspp*, un des marqueurs clés des odontoblastes, indiquant un rôle de la signalisation Wnt dans les stades précoces de la formation des odontoblastes (Yamashiro et al., 2007).

Les odontoblastes synthétisent les protéines de la dentine (Butler et al., 2003) : Les collagènes (I, III, V, VI) et les protéines non collagèniques, et les protéines de la famille SIBLING (Small Integrin-Binding LIgand, N-linked Glycoprotein) telles que l'ostéopontine, MEPE (une phosphoprotéine de la matrice extra-cellulaire), BSP (bone sialoprotein), DMP1 (dentin matrix protein 1), les sialophosphoprotéines de la dentine (DSPP ou DSP et DPP) (Fisher and Fedarko, 2003; MacDougall et al., 2006). Les autres molécules qui contribuent à la formation de la dentine incluent les protéoglycanes ; les protéines du sérum telle que l'albumine ; les protéines de l'émail telles que les amélogénines ; les métalloprotéinases (MMPs), les TIMPs (tissue inhibitors of metalloproteinases), les cathepsines et les phospholipides (Embery et al., 2001; Goldberg et al., 2003; Goldberg et al., 1995; Goldberg

et al., 2002). Les améloblastes se différencient suivant le même gradient spatio-temporel, mais plus tardivement. L'amélogenèse a lieu en présence de prédentine/dentine et après la disparition de la membrane basale. Cette différenciation nécessite la présence d'odontoblastes fonctionnels (Karcher-Djuricic et al., 1985; Zeichner-David et al., 1995). Les améloblastes participent à la minéralisation et résorbent les protéines de l'émail durant les stades de maturation via les protéinases enamelysine (MMP20) puis kallikréine 4 (KLK4) (Simmer and Hu, 2002).

Les protéines structurales présentes dans l'émail incluent les amélogénines, l'améloblastine, l'énaméline, la tuftéline et l'amélotine (Bartlett et al., 2006a; Iwasaki et al., 2005). D'autres protéines telles que les glycoprotéines sulfatées, les protéines se liant au calcium, DPP (dentine phosphoprotein), ainsi que les lipides et phospholipides participent également à la formation de l'émail (Goldberg and Septier, 2002).

Les différenciations terminales des odontoblastes et des améloblastes, ainsi que la synthèse de la dentine et de l'émail, sont régulées par des molécules de la famille TGFβ, en particulier les BMP, et par les FGF (Begue-Kirn et al., 1992; Fincham et al., 1999; Lesot et al., 2001; Ruch et al., 1996). Ces molécules participant aux interactions épithélio-mésenchymateuses sont synthétisées par les préodontoblastes et les préaméloblastes et sont émises par l'intermédiaire de la membrane basale et de la dentine/prédentine. L'inhibition du signal BMP4 (signal envoyé par le mésenchyme puis par les préodontoblastes et les odontoblastes vers l'épithélium dentaire interne puis vers les préaméloblastes) par la follistatine porte atteinte à la différenciation terminale des améloblastes de la lèvre épithéliale labiale chez la souris, comme c'est le cas dans le processus normal au niveau de la lèvre épithéliale linguale dépourvue d'émail (Wang et al., 2004b). Les cellules du stratum intermedium dont le développement est relayé par Msx2, seraient impliquées dans la différenciation des améloblastes via la signalisation Shh (Koyama et al., 2001).

La formation de la dentine et de l'émail est interdépendante. Les odontoblastes sécrètent les amélogénines (Ye et al., 2006a) et les améloblastes sécrètent transitoirement DSP et DPP pendant la formation de la jonction émail/dentine (White et al., 2007).

1.2.3.6 *L'éruption*

L'éruption est un phénomène régulé par les interactions tissulaires et nécessite plusieurs signaux (Yokohama-Tamaki et al., 2006). PtHrP (parathyroid hormone-related peptide) semble être impliqué dans la régulation de la résorption de l'os entourant les dents lors de l'éruption dentaire (Philbrick et al., 1998; Wise et al., 2000). Runx2 pourrait participer à la cémentogenèse, à la formation du ligament parodontal et à l'éruption (Camilleri and McDonald, 2006).

1.2.4 Le développement de l'incisive

Bien que suivant le même processus développemental que chez l'Homme, les incisives chez la souris montrent des différences telles que leur croissance continue due à la présence d'une niche de cellules souches actives dans les lèvres cervicales apicales (Harada et al., 1999; Ohshima et al., 2005; Smith, 1980), et leur développement asymétrique. La différenciation des améloblastes et le dépôt de l'émail ont lieu seulement sur la partie labiale de l'incisive. La partie linguale est beaucoup plus petite et fonctionne comme un analogue de racine avec seulement la différenciation des odontoblastes (Tummers and Thesleff, 2008) **(Figure7)**.

La façon dont se met en place cette asymétrie est encore relativement mal connue. Des études de recombinaison tissulaire ont montré que le mésenchyme dentaire, aussi bien dans la partie labiale que linguale, peut induire la différenciation des améloblastes dans l'épithélium dentaire labial, mais ne peut pas induire cette différenciation dans l'épithélium dentaire lingual, indiquant un défaut de réponse de cet épithélium (Amar et al., 1986; Amar et al., 1989).

La follistatine est exprimée dans l'épithélium dentaire de la lèvre cervicale linguale **(Figure7)**, et sa surexpression dans l'épithélium dentaire labial inhibe la différenciation des améloblastes *in vivo*, suggérant que cette molécule joue un rôle dans l'absence d'émail du côté lingual de l'incisive de souris (Wang et al., 2004b). La follistatine est un inhibiteur de protéines de la superfamille TGFβ incluant les BMP et l'activine, suggérant que la

différenciation des améloblastes du côté lingual nécessiterait la dérépression d'un membre de cette famille. Des expériences de culture d'explants d'incisives ont montré que BMP4 pouvait induire la différenciation des améloblastes, faisant de cette molécule le meilleur candidat pour être la molécule de signalisation provenant des odontoblastes (Wang et al., 2004a).

La follistatine pourrait aussi être impliquée dans la régulation des FGF produits par le mésenchyme, qui sont normalement exprimés dans la papille dentaire adjacente aux lèvres cervicales. *Fgf3* est uniquement exprimé du côté labial **(Figure7)** et joue un rôle important en interagissant avec *Fgf10* dans le maintien des cellules précurseurs des améloblastes (Harada et al., 1999; Harada et al., 2002). La surexpression de la follistatine dans des souris transgéniques empêche l'expression de *Fgf3* dans la papille dentaire près de la lèvre cervicale labiale. Au contraire, chez les souris porteuses d'une inactivation du gène *follistatine*, *Fgf3* est exprimé de manière ectopique au niveau de la lèvre cervicale linguale (Wang et al., 2007). BMP4 réprime *Fgf3,* tandis que l'activine qui est préférentiellement exprimée dans le mésenchyme labial, inhibe l'effet de BMP4 et stimule l'expression de *Fgf3* dans le mésenchyme labial. Des études récentes sur les gènes *Sprouty* montrent l'effet de ces antagonistes des récepteurs tyrosine kinase sur le maintien des précurseurs des améloblastes dans les incisives. L'inactivation de *Sprouty 4* couplée à l'inactivation d'un allèle de *Sprouty 2* génère des incisives produisant de l'émail des deux côtés. Ces souris ont alors des incisives extrêmement longues car elle ne peuvent pas s'éroder (Klein et al., 2006). Ce phénotype est complété par l'interruption de l'effet inhibiteur de Sprouty sur la boucle de signalisation FGF du côté lingual. *Fgf9* est exprimé dans l'épithélium dentaire interne du côté labial, son expression se superpose à celle de *Fgf3* et *Fgf10* dans le mésenchyme. Dans le mutant *Sprouty 2/4, Fgf9, 3* et *10* se retrouvent surexprimés du côté lingual (Klein et al., 2006). Ceci met en évidence l'importance d'une expression asymétrique de *Fgf3*, produisant une « dose » supérieure de signalisation FGF du côté labial et permettant la différenciation des améloblastes.

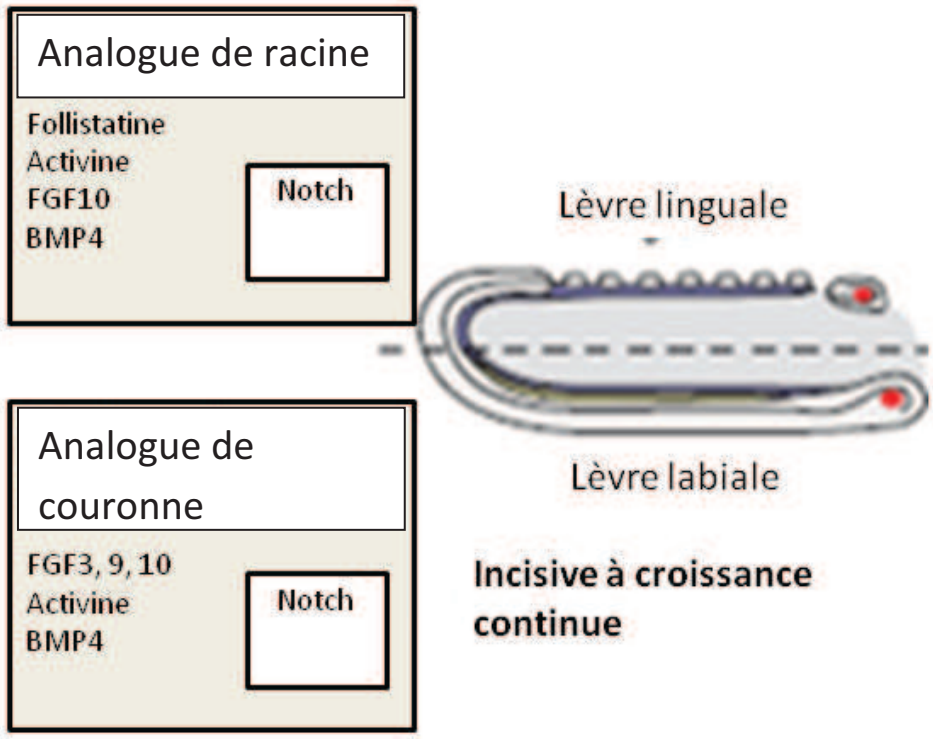


Figure 7: Expression génique variable entre les deux lèvres de l'incisive. Le côté lingual est un analogue de racine et exprime la follistatine, l'activine, FGF10 et BMP4 tandis que le côté labial analogue de couronne exprime FGF3, 9, 10, l'activine et BMP4. La follistatine est essentielle au développement de la lèvre linguale tandis que FGF3 est essentiel au développement de la lèvre labiale.

1.2.5 La formation des molaires et le contrôle du nombre de dents

Chez la souris, la lame dentaire se développe aussi au niveau du diastème. Des ébauches dentaires transitoires se forment dans cette région, elles involuent normalement et cette perte évolutive des bourgeons dentaires aux stades précoces de leur développement aboutit à la formule dentaire de la souris avec une incisive et 3 molaires par quadrant séparées par ce diastème. Ces ébauches dentaires arrêtent leur développement avant le stade de capuchon et régressent par apoptose (Peterkova et al., 2000, 2003).

Les ancêtres des rongeurs ont perdu ces dents dans le diastème il y a plus de 100 millions d'années lors de l'évolution des mammifères placentaires à partir d'un ancêtre commun avec 3 incisives, une canine, 4 prémolaires et 3 molaires (Butler, 1939; Peyer, 1968; Meng et al., 1994; Ji et al., 2002). La quatrième prémolaire aurait progressivement réduit de taille au cours de l'évolution pour finir par être incorporée à la première molaire, augmentant ainsi la taille de sa partie mésiale (Figure 6). Cette incorporation récente à l'échelle de l'évolution, explique l'instabilité de cette région mésiale de la première molaire durant le développement embryonnaire.

Au niveau maxillaire, des ébauches rudimentaires (R2, la plus distale, R1, D1-D5) sont visibles dans le diastème en avant de la première molaire M1 au jour 14.5 du développement embryonnaire (E14.5), elles disparaîtront ensuite totalement. Au niveau de la mandibule, des ébauches rudimentaires (R2, la plus distale, MS) sont visibles dans le diastème en avant de la première molaire M1 à E14.5. Si MS disparaît, R2 sera incorporée dans la première molaire mandibulaire et contribuera à la formation de la partie antérieure ou mésiale de cette dent (Lesot et al., 1996; Viriot et al., 2000) **(Figures 8 et 9)**.

L'incorporation des vestiges de la dernière prémolaire à la première molaire pourrait échouer chez certaines souris mutantes, donnant naissance à un élément dentaire autonome constituant une dent surnuméraire en avant de la première molaire. C'est ce qui semble se passer notamment chez les souris *Tabby* mutées pour le gène *Eda* (Ectodysplasin A) (Boran et al., 2005). En effet chez ces souris, une dent surnuméraire se développe en avant de la première molaire, dont la partie antérieure se retrouve en conséquence réduite. L'origine de cette dent surnuméraire proviendrait d'un défaut de l'incorporation du bourgeon prémolaire R2 à la M1, ce bourgeon participant alors au développement d'une dent surnuméraire autonome (Peterkova et al., 2002). Des dents surnuméraires dans le diastème sont également visibles dans des souris mutantes pour les gènes *Lrp4*, *Sprouty2/4*, *Wise*, *Polaris*, et *Gas1* (Zhang et al., 2003; Kassai et al., 2005; Klein et al., 2006; Murashima-Suginami et al., 2008; Ohazama et al., 2008, 2009, Charles 2011). Ces données montrent que les voies de signalisation FGF et Shh sont régulées négativement (inhibées) au niveau du diastème, pour justement prévenir l'apparition de germes dentaires surnuméraires. En cas de dysfonctionnement des voies FGF, Shh, mais aussi Wnt et NF-kappaB dans les modèles murins génétiquement modifiés, l'apparition de dents surnuméraires au niveau du diastème

(Hacohen et al., 1998; Kim and Bar-Sagi, 2004; Klein et al., 2006, Ohazama et al., 2009 ; Porntaveetus, 2011) prouve que la souris conserve des potentialités génétiques pouvant s'exprimer par le développement d'éléments dentaires perdus au cours de l'évolution.

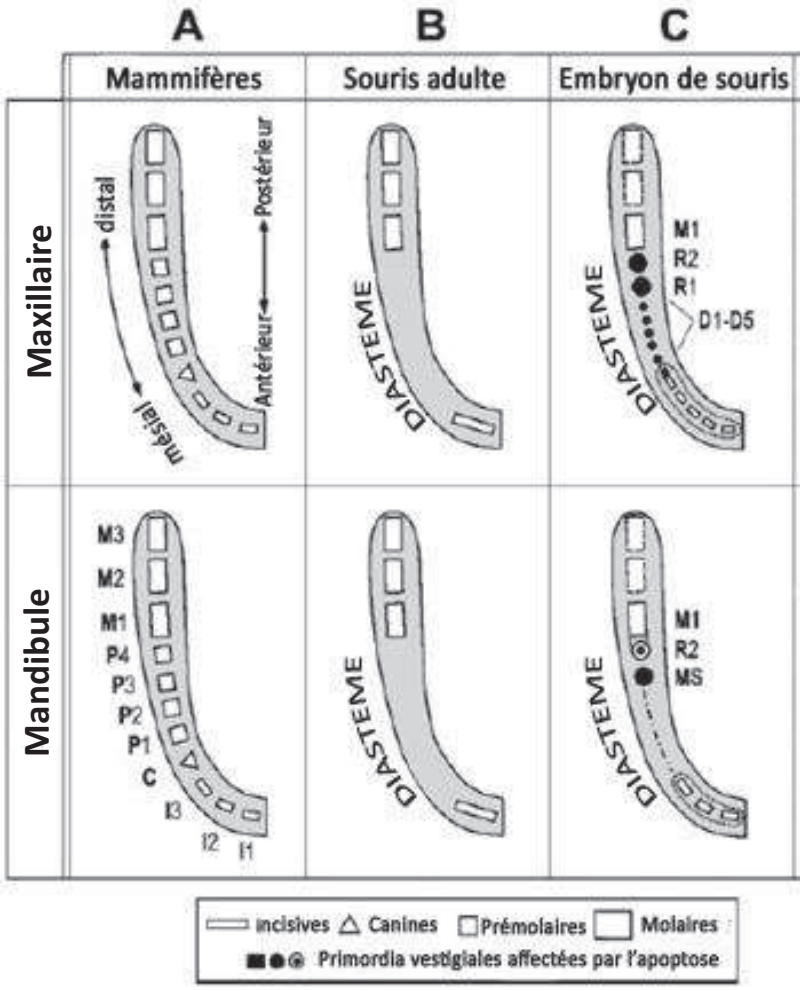


Figure 8 : Comparaison de la distribution dentaire dans la mandibule et dans le maxillaire. (A) La dentition de base chez les mammifères comprend 3 incisives (I1-I3), une canine (C), 4 prémolaires (P1-P4) et 3 molaires (M1-M3). **(C)** Dentition fonctionnelle de souris. **(B)** Embryon de souris : les incisives maxillaires se développent à partir de six placodes et les incisives mandibulaires à partir de 3 placodes. Des placodes transitoires (D1-D5) apparaissent dans la partie mésiale du diastème maxillaire. Deux bourgeons se développent dans la partie distale du diastème maxillaire (R1 et R2) et deux dans la partie distale du diastème mandibulaire (MS et R2). D'après (Peterkova et al., 2006)

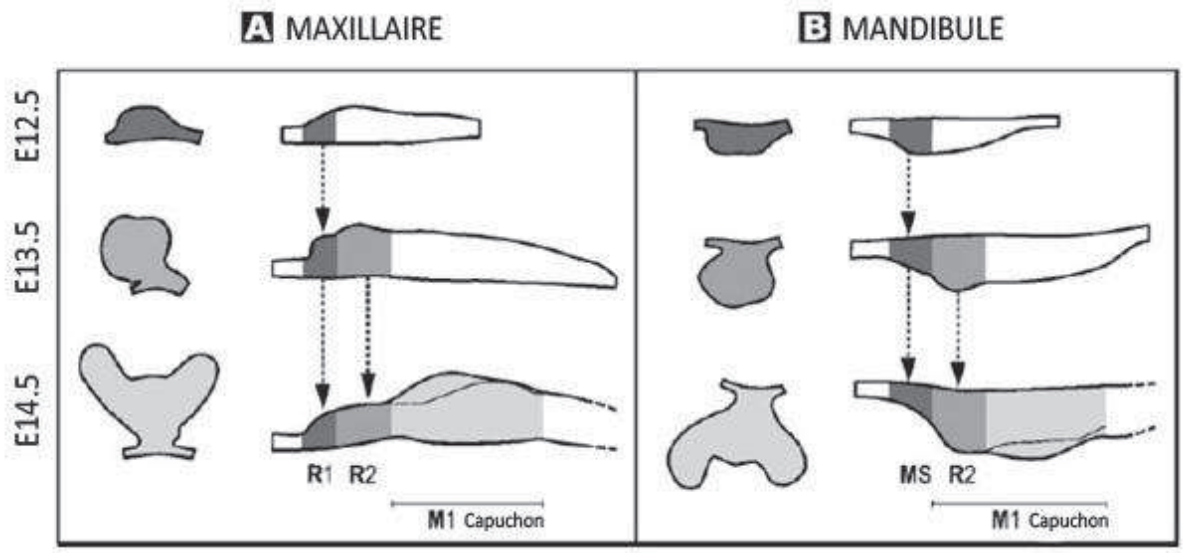


Figure 9 : Schéma des origines consécutives des dents chez la souris. Dans le maxillaire **(A)** et la mandibule **(B)** les ébauches des dents sont présentées en sections frontales (E12.5 lame, E13.5 bourgeon, E14.5 capuchon). Trois structures distinctes se développent consécutivement le long de l'axe mésio-distal. Au jour embryonnaire E12.5, R1 et MS, ébauches transitoires, émergent dans le diastème respectivement dans le maxillaire et la mandibule. A E13.5, deux autres structures, sont visibles dans le diastème (R2) en avant de la première molaire. Les bourgeons R1 et MS régressent. A E14.5, au stade capuchon de la première molaire (M1), R2 dégénère au niveau maxillaire, tandis que cet élément est incorporé et forme la partie mésiale de la première molaire à la mandibule. D'après (Peterkova et al., 2006).

1.3 Syndromes et anomalies dentaires

1.3.1 Introduction

Les anomalies dentaires peuvent exister de manière isolée ou être associées à des signes cliniques extra-oraux dans les syndromes. Elles peuvent être d'origine génétique ou dues à l'action de tératogènes (Alaluusua, 2006; Alaluusua and Lukinmaa, 2006; Alaluusua et al., 1999; Berdal, 2003; Koch, 2003; Weerheijm, 2003). Chaque anomalie dentaire peut être classée dans des catégories variées, anomalies de nombre, de forme, de taille, de formation des structures des tissus durs, de formation radiculaire et d'éruption et de résorption. Elles trouvent leur origine dans des anomalies développementales (Bloch-Zupan, 2004), pouvant

concerner la mise en place du patron de la dentition, la localisation et la définition de l'identité dentaire, la morphogenèse, l'histogenèse, les différenciations terminales des améloblastes et des odontoblastes, la synthèse de la dentine et de l'émail, la minéralisation, la formation des racines et l'éruption dentaire (Salazar-Ciudad and Jernvall, 2002; Thesleff, 2003a; Thesleff, 2003b; Thesleff, 2006). Des interférences dans ces processus développementaux peuvent mener à des anomalies cliniques (Aldred et al., 2003; MacDougall, 2003; Thesleff, 2000; Thesleff, 2006), certaines interférences pouvant même mener à des tumeurs provenant des cellules dentaires épithéliales (tumeurs odontogéniques) (Papagerakis et al., 1999).

Les anomalies dentaires d'origine génétique sont un des aspects phénotypiques de nombreuses maladies rares ou syndromes. Les maladies rares, par définition, affectent moins d'un individu sur 2000, mais il en existe plus de 7000 différentes dont 80% sont d'origine génétique. Ainsi, elles sont estimées affecter environ 3 à 4 millions de personnes en France et 25 milions de personnes en Europe. Parmi ces maladies rares, plus de 900 présentent des manifestations cliniques phénotypiques bucco-dentaires, et 750 des fentes labio-palatines, selon la base de données LDDB (London Dysmorphology DataBase) (Fryns and de Ravel, 2002; Guest et al., 1999; Winter and Baraitser, 1987) .

Le Dr R.J. Gorlin a été un pionnier dans les domaines de la génétique et de la dentisterie (Bloch-Zupan, 2007; Gorlin et al., 2001; Pirinen, 1998). De nombreux syndromes associés à des malformations dento-oro-crâniofaciales ou à des fentes labiales et/ou palatines sont décrits dans ses publications (Gorlin et al., 2001) pour revue). Les anomalies dentaires sont souvent décrites dans le cadre de syndromes (Kurisu and Tabata, 1998; Miletich and Sharpe, 2003; Townsend et al., 1998), en association avec des malformations d'autres organes ou systèmes (Pirinen, 1998). Ceci s'explique par le fait que les mêmes gènes et voies de signalisation régulent le développement dentaire et le développement d'autres organes comme les yeux par exemple. Les anomalies dentaires sont particulièrement présentes dans de nombreux syndromes affectant les dérivés éctodermiques telle que les dysplasies ectodermiques telles que les dysplasies ectodermiques (Pispa and Thesleff, 2003; Rinne et al., 2007; Smahi et al., 2002). De plus, dans le cadre d'un même syndrome, différentes anomalies dentaires peuvent être associées, telles que l'hypodontie, les défauts de l'émail, et/ou un retard dans l'éruption : ceci est sans

doute lié au fait que les gènes responsables ont des fonctions séquentielles à différents stade du développement dentaire.

1.3.2 Dents manquantes : hypodontie, oligodontie

Les dents manquantes (anomalies de nombre), incluant l'agénésie dentaire, l'hypodontie, l'oligodontie, l'anodontie (Arte and Pirinen, 2003), sont associées à des mécanismes régulant le patron de la dentition et la transition du bourgeon au capuchon. L'hypodontie est caractérisée par moins de 6 dents manquantes, en excluant les troisièmes molaires. En effet, chez l'Homme, l'absence des troisièmes molaires est considérée comme une variabilité naturelle et n'est pas considérée comme de l'hypodontie. Elle est très rare dans la dentition lactéale et plus commune chez les femmes que chez les hommes avec un ratio 3:2. En plus des dents manquantes, les personnes ayant une hypodontie peuvent avoir des dents très petites ou très coniques (McKeown et al., 2002).

L'oligodontie est définie comme une absence de six dents ou plus, et l'anodontie comme l'absence de toutes les dents. Statistiquement, environ 5% de la population a au moins une dent manquante, mais seulement 0,3% de la population est affectée par l'oligodontie.

Les hypo-, oligo- ou anodonties peuvent apparaître de manière isolée ou être associées à des syndromes. Des mutations des gènes à homéoboîte, tels *MSX1* ou *PAX9,* peuvent engendrer des dents manquantes de manière isolée **(Table I)**. Les dents manquantes sont l'anomalie pour laquelle le plus de gènes ont été identifiés comme étant impliqués. En effet, des facteurs de transcription, des gènes des voies Wnt, Hedgehog, TGFβ, TNF/Nfκb, FGF, mais aussi d'autres voies de signalisation, ont été identifiés comme étant impliqués dans des syndromes incluant des dents manquantes. Divers exemples sont détaillés ci-dessous.

1.3.2.1 *Les gènes à homéoboîtes*

1.3.2.1.1 MSX1

Les gènes à homéboîte de type Msx ont été identifiés comme homologues du gène Msh (muscle segment homeobox) de drosophile. Une mutation dans le gène MSX1 (substitution d'une arginine par une proline dans l'homéodomaine) a été associée à une absence des deuxièmes prémolaires et troisièmes molaires (Vastardis et al., 1996). Une mutation dans ce gène a aussi été associée à des fentes oro-faciales et à une agenèse dentaire (van den Boogaard et al., 2000) **(Table 1).**

Une mutation non sens dans le gène *MSX1 est* responsable du syndrome de Witkop ou TNS (Tooth and Nail Syndrome) (Jumlongras et al., 2001). Ce syndrome à transmission autosomique dominante est une dysplasie ectodermique affectant les dents, les ongles et les cheveux. Les principales caractéristiques sont des cheveux clairsemés fins et très friables, des ongles des mains et des pieds absents à la naissance, puis poussant très lentement. Au niveau dentaire, les principales caractéristiques sont des dents manquantes dans les deux dentitions, le plus fréquemment les incisives mandibulaires et les canines maxillaires, ainsi que la présence de dents coniques (Devadas et al., 2005; Hodges and Harley, 1999; Hudson and Witkop, 1975; Jumlongras et al., 2001; Redpath and Winter, 1969; Wicomb et al., 2004) **(Table 1).**

MSX1 est également délété chez certains patients atteints du syndrome de Wolf-Hirschhorn avec oligodontie tandis que chez d'autres patients c'est le facteur de transcription WHSC1 qui code pour une protéine contenant un domaine SET qui est muté. Ce syndrome qui atteint 2 personnes sur 100 000 apparaît dans la majorité des cas de manière isolée dans les familles. Il est caractérisé par un handicap d'apprentissage, des convulsions, un retard de croissance, une apparence faciale typique avec un hypertélorisme, une fente palatine et une hypodontie (Babich et al., 2004; Battaglia et al., 2001; Breen, 1998; Iwanowski et al., 2005; Johnston and Franklin, 2006; Kant et al., 1997; Lo et al., 1994; Nieminen et al., 2003; Sase et al., 2005; Zollino et al., 2000; Zollino et al., 2003) **(Table 1)**.

1.3.2.1.2 PAX9

Des mutations dans le gène *PAX9* sont, tout comme certaines mutations de *MSX1*, associées à des hypodonties ou oligodonties isolées. Des mutations dans ce gène sont transmises dans les familles de manière autosomique dominante. Chez certaines familles, elles entraînent une oligodontie caractéristique des molaires permanentes (Mostowska et al., 2006; Nieminen et al., 2001), mais les molaires primaires peuvent aussi être touchées (Das et al., 2002). Certains individus ont également des deuxièmes prémolaires maxillaires et des incisives centrales mandibulaires absentes (Frazier-Bowers et al., 2002; Stockton et al., 2000). La sévérité de l'agénésie dentaire semble corrélée à la capacité de la protéine *PAX9* mutée à se lier à l'ADN (Wang et al., 2009b) **(Table I).**

1.3.2.1.3 Le syndrome d'Axenfeld-Rieger

Le syndrome d'Axenfeld-Rieger est un syndrome à transmission autosomique dominante dont il existe 3 types différents. Le premier est lié à des mutations dans le gène *PITX2* (Pituitary homeobox transcription factor). Pour le deuxième type, le gène n'est pas encore identifié, mais il correspond à des mutations sur le bras long du chromosome 13 (13q14). Le troisième type est lié à des mutations dans *FOXC1* (Kelberman et al., 2011). En général, les patients avec des défauts dentaires ou ombilicaux ont des mutations dans le gène *PITX2*, alors que les patients avec une anomalie de la chambre antérieure de l'oeil isolée ont des mutations dans *FOXC1*. Cependant, des patients avec des anomalies dentaires et des mutations dans *FOXC1* ont été décrites (Tumer and Bach-Holm, 2009). Les caractéristiques principales sont un cryptorchidisme, une lobulation foetale du rein, des défauts cardiaques congénitaux, une sténose anale, un défaut d'involution de la peau périombilicale, un déficit en hormone de croissance et des défauts de la chambre antérieure de l'oeil. Les principales caractéristiques dentaires sont une hypodontie des incisives maxillaires, des dents coniques, des microdonties, une hypoplasie de l'émail et des mauvais positionnements de dents (Amendt et al., 2000; Amendt et al., 1998; Brooks et al., 1989; Dressler et al., 2010; Hjalt and Semina, 2005; Idrees et al., 2006; Jena and Kharbanda, 2005;

Kelberman et al., 2011; O'Dwyer and Jones, 2005; Singh et al., 2003; Tumer and Bach-Holm, 2009; Wang et al., 2003b; Weisschuh et al., 2011) **(Table 1)**.

SYNDROME	Caractéristiques	Gène	Type de molécule	Transmission	Locus
Oligodontie #106600	Fente palatine	*MSX1*	Facteur de transcription	Autosomique dominante	4p16.1
Syndrome de Witkop #189500	Dysplasie des ongles	*MSX1*	Facteur de transcription	Autosomique dominante	4p16.1
Syndrome de Wolf-Hirschhorn #194190	Retard de croissance, microcéphalie, dysmorphie de la face, handicap intellectuel	*MSX1*	Facteur de transcription	Chromosomique	4p16
Oligodontie #604625 #106600		*PAX9*	Facteur de transcription	Autosomique dominante	14q12-q1
Syndrome de Rieger #180500 #602482 #601499	Anomalies de la chambre antérieure des yeux, défauts de l'ombilic	*PITX2* *FOXC1* *?*	Facteur de transcription Facteur de transcription	Autosomique dominante Autosomique dominante	4q25-q26 6p25 13q14

Table 1 : Gènes à homéoboîte impliqués dans des syndromes associés à des dents manquantes (D'après Bloch-Zupan et al., 2012)

1.3.2.2 *La voie Wnt*

1.3.2.2.1 AXIN2

Des mutations dans le gène *AXIN2*, un suppresseur de tumeur qui contrôle négativement la voie de signalisation Wnt, entraînent une susceptibilité au cancer colorectal associée à une hypodontie avec moins de 6 dents permanentes manquantes (Lammi et al.,

2004; Liu et al., 2000). *AXIN2* semble également impliqué dans des formes sporadiques d'agénésie des incisives ou des oligodonties isolées (Bergendal et al., 2011) **(Table 2)**.

1.3.2.2.2 Dysplasie odonto-onycho-dermale (OODD)

Des mutations dans le gène *WNT10A* peuvent conduire au syndrome de dysplasie odonto-onycho-dermale (OODD). Ce syndrome se caractérise par une hypodontie sévère, une dystrophie ongulaire allant de l'onychodysplasie à l'absence totale d'ongles, une langue lisse, une peau sèche, une kératodermie et une hyperhydrose des paumes et des plantes (Nawaz et al., 2009); (Adaimy et al., 2007). Des mutations dans ce gène sont aussi responsables d'oligodontie sévère isolée (Kantaputra and Sripathomsawat, 2011) ou du syndrome de Schopf-Schulz-Passarge qui associe de nombreux kystes le long du bord des paupières, un risque accru de développer des tumeurs bénignes ou malignes de la peau, une kératose palmo-plantaire et une hypodontie (Bohring et al., 2009) **(Table 2).**

1.3.2.2.3 LEF1

Des mutations du gène *LEF1* sont associées à une dysplasie ectodermique et une oligodontie sevère associée à des défauts de l'os alvéolaire (Bailleul-Forestier et al non publié 2011) **(Table 2).**

SYNDROME	Caractéristiques	Gène	Type de molécule	Transmission	Locus
Oligodontie avec néoplasie #608615	Néoplasie coloréctale	*AXIN2*	Régulateur négatif de la voie Wnt canonique	Autosomique dominante	17q24
Dysplasie Odonto-onycho-dermique (OODD; MIM #257980)	Hypodontie sevère, dystrophie ongulaire, langue lisse, peau sèche, kératoderme et hyperhydrose des paumes et des plantes	*WNT10A*	Molécule signal	Autosomique récessive	2q35
Syndrome de Schopf-Schulz-Passarge #224750	Nombreux kystes sur le bord des paupières, tumeurs bénignes et malignes de la peau, kératoses palmo-plantaires et hypodontie	*WNT10A*	Molécule signal	Autosomique récessive	2q35
Hypodontie isolée Agénésie sélective #150400	Hypodontie variable touchant les incisives latérales et les prémolaires	*WNT10A*	Molécule signal	Autosomique dominante	2q35
Dysplasie ectodermique et oligodontie	Oligodontie sévère, défauts sévères de l'os alvéolaire	*LEF1*	Facteur de transcription impliqué dans la voie LEF1/beta-caténine (boucle de régulation négative sur EDA/EDAR)	?	4q25

Table 2 : Gènes de la voie Wnt impliqués dans des syndromes associés à des dents manquantes

1.3.2.3 *La voie TNF/NF-κB*

Des dysfonctionnements de cette voie de signalisation altèrent les interactions épithélio-mésenchymateuses et engendrent des dysplasies ectodermiques. Ces dysplasies ectodermiques, forment un groupe hétérogène avec plus de 170 phénotypes décrits. Elles peuvent être classées en différents groupes sur la base de critères cliniques ou moléculaires.

1.3.2.3.1 Dysplasies ectodermiques hypohydrotiques

Les patients atteints de dysplasie ectodermique hypohydrotique (HED) présentent des degrés variables d'hypodontie ou d'oligodontie, une hypohydrose et des cheveux clairsemés. La prévalence est de 1 pour 17 000. Il en existe trois sortes qui sont cliniquement similaires mais ont des modes de transmission différents. La transmission récessive liée à l'X est due à des mutations du gène *EDA* qui code pour une protéine transmembranaire, l'Ectodysplasine A. D'autres gènes sont responsables de transmissions autosomique récessive ou dominante (Pinheiro and Freire-Maia, 1979a; Pinheiro and Freire-Maia, 1979b; Pinheiro and Freire-Maia, 1979c). La forme la plus commune est la forme liée à l'X et concerne 80% des cas. Les formes à transmission autosomique sont liées à des mutations dans les récepteurs de l'ectodysplasine 1 (EDAR) et le "death domain" associé à EDAR (EDARADD). EDAR est impliqué dans environ 25% des cas de transmission autosomique (Chassaing et al., 2006). Quatre-vingt dix pour cent des cas sont liés à seulement quatre gènes différents (*EDA, EDAR, EDARADD et WNT10A*) (Cluzeau et al., 2011). Les signes caractéristiques sont une absence de cheveux à la naissance, une peau sèche due à la quasi absence de glandes sudoripares, une absence de poils et des ongles sous-développés. Les caractéristiques crânio-oro-dentaires sont un front proéminent, des cils et des sourcils peu formés ou absents, une sécrétion salivaire diminuée, une dépression du pont nasal, une hyperpigmentation périorbitale, des lèvres protubérentes, un maxillaire hypodéveloppé et une absence presque totale de dents dans les deux dentitions (oligodontie ou anodontie). Les dents présentes peuvent être coniques, avec taurodontisme, émail peu calcifié, et/ou absence d'os alvéolaire **(Table 3).**

1.3.2.3.2 NEMO

La dysplasie ectodermique avec déficit immunitaire (HED-ID) est caractérisée par des cheveux fins et des glandes d'origine ectodermique sous-développées, des dents coniques, une hypodontie ou une oligodontie, ainsi qu'une hypersensibilité aux infections (Puel et al., 2005). Des mutations dans le gène *NEMO* sont responsables de la maladie. *NEMO* code pour une protéine (IKK gamma) qui joue un rôle crucial dans la voie de signalisation NF-kB en régulant les composants du complexe IKK (Ikappa B kinase). Ceci mène à une altération de l'activation de NF-kB en réponse, par exemple, au récepteur Toll-like (TLR), résultant en diverses infections **(Table 3).**

Des mutations dans ce gène sont aussi responsables de l'*Incontinentia Pigmenti* aussi connue sous le nom de Syndrome de Bloch-Sulzberger, qui est caractérisé par des vésicules qui vont se transformer en verrues puis en marques pigmentées sur la peau, des anomalies des yeux, du système squelettique, du système nerveux central et des dents. Les anomalies dentaires sont présentes chez 80% des patients avec un retard de l'éruption, une hypodontie ou une oligodontie, des dents surnuméraires, des dents coniques, des dents malformées avec élongation de la couronne des dents antérieures, des incisives centrales en forme de tulipe, des cuspides surnuméraires et des racines courtes (Minic et al., 2006; Van den Steen et al., 2004; Wu et al., 2005). Quatre-vingt quinze pour cent des personnes affectées sont des femmes et la prévalence est de 0,2/100 000 (Orphanet, 2009). C'est une maladie liée à l'X à transmission dominante qui est généralement mortelle périnatalement chez l'Homme **(Table 3).**

1.3.2.3.3 L'ectrodactylie avec dysplasie ectodermique et fentes (EEC)

L'ectrodactylie avec dysplasie ectodermiques et fentes est caractérisée par des anomalies des mains et des pieds, de nombreuses manifestations de dysplasies ectodermiques et des fentes de la lèvre et du palais. La prévalence est de 1/50 000 et la transmission est autosomique dominante. Les principales caractéristiques sont : l'absence d'au moins un doigt, une dysplasie de la peau, des cheveux, des ongles, des anomallies

dentaires, une perte d'audition, et une déficience en hormone de croissance. Les anomalies orodentales sont une fente labio-palatine bilatérale dans 60 à 75% des cas, des fentes isolées dans 10% des cas, une absence des dents permanentes, des dents coniques, une hypoplasie de l'émail (Brunner et al., 2002; Buss et al., 1995; Fete et al., 2009; Lacombe et al., 1993; Maas et al., 1996; O'Quinn et al., 1998; Rinne et al., 2007; Rinne et al., 2006; Rodini and Richieri-Costa, 1990b; Roelfsema and Cobben, 1996; Scherer et al., 1994; South et al., 2002; Vanbokhoven et al., 2011; Yang et al., 1999) **(Table 3).**

1.3.2.3.4 Le syndrome CLPED1

Le syndrome CLPED1 (fente labio/palatine et dysplasie ectodermique récessive) est caractérisé par une fente labiale et palatine, une dysplasie ectodermique, un handicap d'apprentissage et des syndactylies. Les caractéristiques dentaires sont une hypodontie ou une anodontie et une microdontie (Avila et al., 2006; Barron et al., 2008; Bowen and Armstrong, 1976; Bustos et al., 1991; Ogur and Yuksel, 1988; Rodini and Richieri-Costa, 1990a; Sozen et al., 2001; Suzuki et al., 2000; Yoshida et al., 2010; Zlotogora, 1994; Zlotogora and Ogur, 1988; Zlotogora et al., 1987). Le gène impliqué est *PVRL1* (poliovirus receptor-like 1). Il code pour Nectin-1, une immunoglobuline transmembranaire qui compose les jonctions adhérentes. Des mutations de ce gène peuvent aussi conduire à une fente labiale et palatine de façon non syndromique (Avila et al., 2006) **(Table 3).**

1.3.2.3.5 Le syndrome d'Ellis-van Creveld

Le syndrome d'Ellis-van Creveld (EVC) est un syndrome rare (0,9/100000) à transmission autosomique récessive. Il est caractérisé par un nanisme acromésomélique (avants-bras et jambes courtes, raccourcissement moins marqué de l'humérus et du fémur), une polydactylie (6 doigts à chaque main), une dysplasie ectodermique hydrotique avec des ongles malformés, des défauts des cheveux et des anomalies orodentales typiques incluant des dents à la naissance, une hypodontie, des dents surnuméraires, des dents coniques et microdontes, des incisives en forme de pelle, un taurodontisme, une hypoplasie de l'émail, un retard dans l'éruption dentaire, des transpositions dentaires. On note également une

absence de repliement du maxillaire mucobuccal due à la fusion de la muqueuse labiale de la gencive, et une crête alvéolaire mandibulaire antérieure qui est traversée par des freins lui donnant un aspect dentelé.

Le syndrome est dû à des mutations dans les gènes *EVC1* ou *EVC2*. Le phénotype est identique selon que l'un ou l'autre de ces gènes est muté (Aminabadi et al., 2010; Atasu and Biren, 2000; Baujat and Le Merrer, 2007; Blair et al., 2011; Cahuana et al., 2004; Galdzicka et al., 2002; Hattab et al., 1998; Hunter and Roberts, 1998; Katsouras et al., 2003; Kurian et al., 2007; McKusick, 2000; McKusick et al., 1964; Mintz et al., 2005; Mostafa et al., 2005; Orphanet, 2009; Ruiz-Perez et al., 2007; Ruiz-Perez and Goodship, 2009; Ruiz-Perez et al., 2000; Ruiz-Perez et al., 2003; Sajeev et al., 2002; Shen et al., 2011; Tompson et al., 2007; Tompson et al., 2001; Valencia et al., 2009; van Hagen et al., 2005) **(Table 3)**. Le gène *EVC2* code pour une protéine prédite pour avoir un segment transmembranaire, trois regions "coiled-coil" et un domaine RhoGEF. Des mutations des genes *EVC1* et *EVC2* sont aussi responsables de la dysostose acrodentale de Weyers (Curry and Hall, 1979; Gorlin et al., 2001; Howard et al., 1997; Roubicek and Spranger, 1984; Ruiz-Perez et al., 2007; Ruiz-Perez and Goodship, 2009; Ruiz-Perez et al., 2000; Shapiro et al., 1984; Spranger and Tariverdian, 1995; Valencia et al., 2009; Weyers, 1952; Weyers, 1953; Wittig et al., 1998; Ye et al., 2006b).

1.3.2.3.6 Le syndrome de Van der Woude (VWS)

Le syndrome de Van der Woude (VWS) est caractérisé par une fente labio-palatine, des sinus paramédians, des dépressions de la lèvre inférieure, une syndactylie des doigts 3 et 4, des puits labiaux, une fente palatine et/ou labiale, une fusion du maxillaire et de la mandibule, un palais très arqué, une ankyloglossie et une hypodontie affectant principalement les incisives et les prémolaires. Dans certaines familles touchées par le syndrome, l'hypodontie peut être le seul signe visible chez certains individus (Burdick et al., 1985; Desmyter et al., 2010; Ingraham et al., 2006; Item et al., 2005; Koillinen et al., 2001; Kondo et al., 2002; Lees et al., 1999; Marazita et al., 2009; Mostowska et al., 2005; Peyrard-Janvid et al., 2005; Rizos and Spyropoulos, 2004; Rorick et al., 2011; Rutledge et al., 2010;

Sertie et al., 1999; Stottmann et al., 2010; Vieira et al., 2007; Wang et al., 2003a; Wong and Gustafsson, 2000; Yeetong et al., 2009) **(Table 3).**

Il en existe deux types. Le type 1 est le plus fréquent et il est lié au locus 1q32-q4 ; le type 2 est lié au locus 1p34. Sa fréquence est estimée entre 1/35 000 à 1/100 000. il concernerait donc 2% des patients présentant une fente labio-palatine. Sa transmission est autosomique dominante. Des mutations du gène *IRF6 (*interferon regulatory factor-6) sont responsables du type 1. IRF6 est un facteur de transcription à domaine hélice-boucle-hélice.

IRF6 est également impliqué dans des fentes labio-palatines non syndromiques et des cas d'agénésie dentaire (Burdick et al., 1985; Desmyter et al., 2010; Ingraham et al., 2006; Item et al., 2005; Koillinen et al., 2001; Kondo et al., 2002; Lees et al., 1999; Marazita et al., 2009; Mostowska et al., 2005; Peyrard-Janvid et al., 2005; Rizos and Spyropoulos, 2004; Rorick et al., 2011; Rutledge et al., 2010; Sertie et al., 1999; Stottmann et al., 2010; Vieira et al., 2007; Wang et al., 2003a; Wong and Gustafsson, 2000; Yeetong et al., 2009). *WDR65* pourrait être un nouveau gène impliqué dans le syndrome de Van der Woude et les fentes orales (Rorick et al., 2011) **(Table 3)**.

1.3.2.3.7 Le syndrome oro-facial-digital de type I (OFD I)

Le syndrome oro-facial digital de type I (OFD I) est une ciliopathie caractérisée par une pseudo-fente de la lèvre supérieure, de multiples freins oraux et de nombreuses anomalies digitales. Les patients présentent une petite taille, une brachydactylie des doigts 2 à 5 et des degrés variables de syndactylie, un front bossu, un affinement du nez avec hypoplasie des cartilages ailaires, un déplacement latéral du canthus interne des yeux, une pseudo-fente sur la ligne médiane de la lèvre supérieure, une petite mandibule, une hypodontie (incisive supérieure latérale manquante), des dents surnuméraires, et une hypoplasie de l'émail (Diz et al., 2011; Feather et al., 1997a; Feather et al., 1997b; Fenton and Watt-Smith, 1985; Ferrante et al., 2001; Gorlin et al., 1961; Macca and Franco, 2009; Romero et al., 2007; Romio et al., 2004; Thauvin-Robinet et al., 2006; Whelan et al., 1975). Sa prévalence est estimée à 1/ 50 000 et sa transmission est dominante liée à l'X. Il est létal

chez l'Homme. Il est dû à des mutations dans le gène *OFD1* (*CXORF5*) qui code pour une protéine contenant des domaines coiled-coil et à hélices alpha **(Table3).**

OFD1 est une protéine centrosomale localisée au niveau des corps basaux à l'origine des cils primaires, et dans le noyau. Cette protéine est capable de s'auto-associer et son interaction est guidée par sa région riche en domaines coiled-coil. Elle pourrait faire partie d'un complexe multi-protéique jouant un rôle dans différentes fonctions biologiques. Elle joue un role crucial dans la formation des cils primaires (Macca and Franco, 2009; Zullo et al.) **(Table 3).**

1.3.2.3.8 Le syndrome de Williams

Le syndrome de Williams est caractérisé par des anomalies crâniofaciales, cardiovasculaires et comportementales. Les anomalies dentaires incluent une agénésie dentaire, une hypodontie, une microdontie, des dents coniques, et une hypoplasie de l'émail (Amenta et al., 2005; Axelsson, 2005; Axelsson et al., 2003; Hertzberg et al., 1994; Joseph et al., 2008; Lacroix et al., 2009; Makeyev and Bayarsaihan, 2011; Mass and Belostoky, 1993; Moskovitz et al., 2005; Oncag et al., 1995; Pober, 2010; Schubert, 2009; Schubert and Laccone, 2006; Tarjan et al., 2003; Tassabehji, 2003; Tassabehji and Donnai, 2006). Sa prévalence est de 1: 20 000 et sa transmission est autosomique dominante.

Ce syndrome résulte d'une délétion de la région 7q11.23 du chromosome 7 contenant les gènes élastine (*ELN*) et des génes voisins dont *RFC2* (replication factor C, subunit 2), *LIMK1* (LIM kinase-1), *GTF2IRD1/GTF2I* (un membre de la famille de facteurs de transciption généraux TFII-I), FKBP6 (FK506-binding protein) **(Table 3).**

SYNDROME	Caractéristiques	*Gène*	Type de molécu	Transmission	Locus
Dysplasie ectodermique hypohidrotique #305100	Cheveux clairsemés, pas de production de sueur	*EDA*	Signal TNF	Liée à l'X	Xq12-q13.1
Oligodontie non syndromique Agénésie dentaire sélective #313500	Touche tous les types dentaires Absence des dents antérieures, des incisives inférieures et supérieures	*EDA*	Signal TNF	Liée à l'X	Xq12-q13.1
Dysplasie ectodermique hypohidrotique # 129490		*EDAR*	Récepteur TNF	Autosomique dominante et récessive	2q11-q13
Dysplasie ectodermique hypohidrotique #129490		*EDARADD*	Protéine adaptatrice des "death domain"	Autosomique dominante	1q42.2-43
Oligodontie non syndromique		*EDARADD*	Protéine adaptatrice des "death domain"	Autosomique dominante	1q42.2-43
HED-ID #300291	Dysplasie ectodermique anhidrotique, immunodéficience	*NEMO (protéine IKKgamma)*	Activateur de facteurs de transcription	Liée à l'X	Xq28
HED-ID #300291	Dysplasie ectodermique anhidrotique, immunodéficience	*NFKBIA, IKBA 164008*	Le complexe NFKB est inhibé par les protéines I-kappa-B (NFKBI A et B)	Autosomique dominante	14q13

Incontinentia Pigmenti #308300	Pigmentation plus marquée de la peau et des yeux, problèmes neurologiques	*IKKgamma, NEMO*		Liée à l'X	Xq28
Autres Dysplasies ectodermiques					
EEC3 #604292	Dysplasie ectodermique ectrodactylie, fente palatine	*P63*	Facteur de transcription	Autosomique dominante	3q27
CLPED1 #225060	Fente labiale et/ ou palatine, dysplasie ectodermique	*PVRL1* (nectin-1)	Molécule d'adhésion cellulaire	Autosomique récessive	11q23-q24
Syndrome d' Ellis-van Creveld #225500	Dents néonatales, hypodontie, éruption prématurée Membres courts, côtes courtes, polydactylie, ongles et dents dysplasiques	*EVC1, EVC2*	Protéines transmembranaires des cils, régulateurs positifs de la voie shh	Autosomique récessive	4p16
Dystose acrofaciale de Weyers #193530	Incisive centrale unique, dents coniques, incisives permantes irrégulières, petites ou absentes, polydactylie, membres courts	*EVC1*		Autosomique dominante	4p16

Syndrome de Van der Woude #119300	Puits et/ou sinus de la lèvre inférieure, fente labiale et/ou palatine	*IRF6*	Interferon regulatory factor-6	Autosomique Dominante	1q32-q41
OFD1 #311200	Pseudo fente de la lèvre supérieure, multiples freins oraux, nombreuses anomalies digitales, petite mandibule, hypodontie (incisives supérieures latérales manquantes), dents surnuméraires, hypoplasie de l'émail, reins polykystiques	*OFD1* *(Cxorf5/71-7a)*	Protéine contenant des domaines "coiled coil" et hélices alpha	Liée à l'X	Xp22
Syndrome de Williams(-Beuren) ###194050	Handicap intellectuel, faciès non habituel, sténoses aortiques, comportement inhabituel, hypodontie, microdontie, hypoplasie de l'émail	*ELN* *RFC2* *LIMK1* *GTF2IRD1/GTF2I* *FKBP6*	Elastine Kinase LIM	Autosomique dominante	7q11.23 Délétion continue

Table 3 : Gènes de la voie TNF/NF-KB impliqués dans des syndromes associés à des dents manquantes

1.3.2.4 *La voie TGFβ*

1.3.2.4.1 La dysplasie ASPED (angel-shaped phalangoepiphyseal dysplasia)

La dysplasie ASPED (Angel-Shaped PhalangoEpiphyseal Dysplasia) est une dysplasie spécifique des os caracterisée par une brachydactylie, une hypodontie, un émail anormal, un retard de l'éruption dentaire, la persistance de dents primaires, une perte prematurée des dents, une malocclusion, une ossification fémorale retardée, et une arthrose dégénérative précoce de la hanche. Sa transmission est autosomique dominante et elle est causée par des mutations du gène *GDF5* (growth/differentiation factor 5) aussi connu sous le nom de *CDMP1* (cartilage derived morphogenetic protein 1), une molécule signal qui participe à la morphogenèse squelettique (Bachman, 1967; Faiyaz-Ul-Haque et al., 2002; Giedion et al., 1993; Holder-Espinasse et al., 2004; Polinkovsky et al., 1997; Storm et al., 1994; Takahara et al., 2004; Thomas et al., 1996).

1.3.2.5 *La voie Sonic Hedgehog*

1.3.2.5.1 Solitary median maxillary central incisor (SMMCI)

Des mutations du gène *SHH* sont responsables d'un défaut de la ligne médiane conduisant à la présence d'une incisive centrale unique (SMMCI). Les mutations de *SHH* peuvent également entraîner des holoprosencéphalies. La prévalence du syndrome SMMCI est de 1 pour 50 000. Ses caractéristiques principales sont une petite taille, une glande pituitaire anormale, un handicap d'apprentissage, une microcéphalie, un hypertélorisme, un palais en V, et une incisive maxillaire centrale unique (Artman and Boyden, 1990; Balci et al., 2011; Becktor et al., 2001; Berry et al., 1984; Cohen, 2006a; Dubourg et al., 2007; El-Jaick et al., 2007; Fryns and Van den Berghe, 1988; Garavelli et al., 2004; Hall, 2006; Hall et al., 1997; Hehr et al., 2004; Lana-Elola et al., 2011; Lertsirivorakul and Hall, 2008; Liberfarb et al., 1987; Mass and Sarnat, 1991; Miertus et al., 2006; Nanni et al., 2001; Oberoi and Vargervik, 2005; Roessler and Muenke, 1998; Seppala et al., 2007; Tabatabaie et al., 2008; Tavin et al., 1994; Verloes and Lesenfants, 2001; Vissers et al., 2004; Wallis and Muenke, 2000).

1.3.2.6 *La voie FGF*

1.3.2.6.1 Le syndrome Lacrimo-auriculo-dento-digital (LADD)

Le syndrome Lacrimo-auriculo-dento-digital (LADD ou syndrome de Levy-Hollister) est caractérisé par des anomalies dentaires et des canaux lacrimaux, d'une perte d'audition et de malformations digitales. Les anomalies dentaires incluent une hypodontie, des incisives coniques, des molaires malformées, une microdontie, une hypoplasie de l'émail, et une éruption retardée. Il résulte de mutations des gènes codant pour les récepteurs *FGFR2* et *FGFR3*, ou de*FGF10* (Azar et al., 2000; Bamforth and Kaurah, 1992; Calabro et al., 1987; Charles et al., 2011; Cortes et al., 2005; Ensink et al., 1997; Entesarian et al., 2007; Francannet et al., 1994; Guven et al., 2008; Haktanir et al., 2005; Harada et al., 2002; Hennekam, 1987; Hollister et al., 1974; Horn and Witkowski, 1993; Inan et al., 2006; Jaskoll et al., 2005; Klein et al., 2006; Lacombe et al., 1993; Lehotay et al., 2004; Lemmerling et al., 1999; Meuschel-Wehner et al., 2002; Milunsky et al., 2006; Onrat et al., 2003; Orphanet, 2009; Ostuni et al., 1995; Ramirez and Lammer, 2004; Rohmann et al., 2006; Roodhooft et al., 1990; Thompson et al., 1985; Wiedemann and Drescher, 1986) **(Table 4).**

1.3.2.6.2 Le syndrome de Kallmann

Le syndrome de Kallmann (KS) est caractérisé par un hypogonadisme central avec des troubles de l'odorat, des anomalies rénales, une syndactylie, une surdité, une fente labio-palatine, une agénésie dentaire, une hypodontie ou oligodontie avec microdontie (le plus fréquemment absence des incisives mandibulaires inférieures, des deuxièmes prémolaires et des incisives latérales maxillaires). Sa transmission est autosomique dominante et sa prévalence est de 7,7 pour 100 000 naissances. Cinq gènes sont connus pour être impliqués dans ce syndrome : *KAL1*, *FGFR1, PROK2, CHD7* et *FGF8* (Bailleul-Forestier et al., 2010; de Zegher et al., 1995; Kim and Layman, 2011; Molsted et al., 1997; Pallais et al., 1993; Pitteloud et al., 2006a; Pitteloud et al., 2006b; Takamori et al., 2008; Trokovic et al., 2003) **(Table 4).**

Des mutations du gène FGFR1 peuvent aussi causer une agénésie dentaire isolée *(Vieira et al., 2007)* **(Table 4).**

SYNDROME	Caractéristiques	Gène	Type de molécule	Transmission	Locus
Syndrome Lacrimoauriculo-dentodigital #149730	Hypodontie, glande parotide absente, petites glandes lacrymales , agénésie rénale	*FGFR2* *FGFR3* *FGF10*	Fibroblast growth factor receptor-2/3 Fibroblast growth factor 10	Autosomique dominante	10q26 4p16.3 5p13-p12
Syndrome de Kallman 2 KAL2 #147950	Hypogonadisme central, perte de l'odorat, dans certains cas aplasie rénale, surdité, syndactylie, fente lèvre/palais, agénésie dentaire	*FGFR1* KAL1, localisé sur X, KAL3 PROKR2, KAL 4 PROK2, KAL5 CHD7, KAL 6 FGF8	Fibroblast growth factor receptor-1	Autosomique dominante	8p11.2-p11.1 Xp22.31 (KAL1)
Agénésie dentaire Isolée	Agénésie préférentielle des molaires	*FGFR1*	Fibroblast growth factor receptor-1	Autosomique dominante	8p11.2-p11.1

Table 4 : Gène de la voie FGF impliqués dans des syndromes associés à des dents manquantes

1.3.2.7 *Autres voies de signalisation*

Des gènes d'autres voies de signalisation sont également impliqués dans des syndromes avec anomalies dentaires. Les principales caractérisitiques de ces syndromes sont résumés dans la **Table 5.**

1.3.2.7.1 Le syndrome de Coffin-Lowry

La famille des sérine/thréonine kinases ribosomales S6 comporte 4 membres chez l'Homme : RSK1 (chromosome 3), RSK2 (Xp22.2-p22.1), RSK3 (chromosome 6) et RSK4 (Xq21) qui présentent 75% d'identité de séquences et sont impliquées dans différents

évènements cellulaires (prolifération, différenciation, réponse au stress cellulaire, apoptose). Des mutations dans le gene *RSK2* chez l'Homme causent le syndrome de Coffin-Lowry caractérisé par un déficit d'apprentissage sévère, et un dysmorphisme affectant la face, les mains et le squelette (Hanauer and Young, 2002; Temtamy et al., 1975a; Temtamy et al., 1975b). Les anomalies orodentaires typiques sont un palais haut et étroit, un sillon lingual, une malocclusion, une hypodontie, des incisives coniques et une perte prématurée de la dentition primaire (Day et al., 2000; Hanauer and Young, 2002; Igari et al., 2006). Des dents espacées et de larges incisives médianes ont aussi été rapportées **(Table 5).**

Un des substrats connus des RSK est IkappaB alpha. Cette protéine est un inhibiteur de la voie NF-κB. Lorsqu'elle est phosphorylée par les protéines RSK elle est dégradée, et la voie de signalisation NF-κB est active. Le gène codant pour IkappaB alpha (*NFKBIA*) a été associé à des dysplasies ectodermiques anhidrotiques autosomiques avec immunodéficience des cellules T (Courtois et al., 2003).

1.3.2.7.2 Le syndrome de Bloom (BS, BLS)

Le syndrome de Bloom est un syndrome à transmission autosomique récessive caracterisée par une déficience de la croissance, des lésions de la peau, une peau hypo- ou hyperpigmentée, une déficience immunitaire, un hypogonadisme, une prédisposition aux malignités, une dolichocéphalie, des oreilles proéminentes, un nez petit et court, une petite mandibule, une hypodontie ou oligodontie, une absence des incisives latérales supérieures (Chester et al., 2006; Chester et al., 1998; Ellis and German, 1996; Ellis et al., 1995; German et al., 2007; Hanada and Hickson, 2007; Oddoux et al., 1999; Roa et al., 1999; Wang and Heddle, 2004; Weinstein, 2007) **(Table 5).**

1.3.2.7.3 Le syndrome de Rothmund-Thomson

Le syndrome de Rothmund-Thomson est caractérisé par des anomalies cutanées, des cataractes juvéniles, une sensibilité à la lumière du jour, des anomalies squelettiques, une petite taille, des défauts des ongles, des cheveux, un handicap intellectuel, un

hypogonadisme et une apparence faciale typique. Les anomalies dentaires sont des dents manquantes, des dents surnuméraires, une malformation des couronnes, des racines courtes, une microdontie, une éruption retardée (Anbari et al., 2000; Bottomley and Box, 1976; Hall et al., 1980; Haytac et al., 2002; Kerr et al., 1996; Kraus et al., 1970; Kumar et al., 2007; Leusink et al., 1991; Mizuk et al., 2005; Nanda et al., 1989; Orphanet, 2009; Piquero-Casals et al., 2002; Roinioti and Stefanopoulos, 2007; Simon et al., 2010; Snels et al., 1998; Yin et al., 2004; Zinke et al., 1985) **(Table 5).**

1.3.2.7.4 Dysplasie Diastrophique

La dysplasie diastrophique est caractérisée par une chondrodysplasie avec un nanisme et de nombreuses anomalies squelettiques sévères de la colonne vertébrale aux pieds, une fente palatine, un palais très arqué, des cartilages du larynx anormalement mous, et une hypodontie dans 31% des cas (Dawson, 2011; Dawson and Markovich, 2005; Forlino et al., 2006; Forlino et al., 2005; Galante and Schwarzbauer, 2007; Karlstedt et al., 1998; Karlstedt et al., 1996; Kere, 2006; Rintala et al., 1986; Rossi and Superti-Furga, 2001; Yokoyama, 2001) **(Table5).**

1.3.2.7.5 Le syndrome de Johanson-Blizzard

Le syndrome de Johanson-Blizzard (JBS) est un syndrome autosomique récessif qui est caractérisé par une insuffisance pancréatique, une petite taille, des ailes du nez sous-développées, un handicap intellectuel, une perte d'audition, une oligodontie, une absence denombreuses dents permanentes à l'exception des premières molaires et des incisives supérieures centrales, des dents primaires hypoplasiques (Auslander et al., 1999; Baraitser and Hodgson, 1982; Braun et al., 1991; Bresson et al., 1980; Daentl et al., 1979; Dumic et al., 1998; Durie, 1996; Durie, 1997; Fichter et al., 2003; Fox et al., 1976; Gershoni-Baruch et al., 1990; Gould et al., 1989; Guzman and Carranza, 1997; Hurst and Baraitser, 1989; Iwasaki, 2001; Jones et al., 1994; Kaeriyama et al., 1986; Kobayashi et al., 1995; Koizumi, 1993; Koizumi, 2000; Kristjansson et al., 1988; Kulkarni et al., 2004; Lerch et al., 2006; Lerner and

Iancu, 1988; Mardini et al., 1978; Maunoury et al., 1999; McHeik et al., 2002; Moeschler and Lubinsky, 1985; Moeschler et al., 1987; Motohashi et al., 1981; Nagashima et al., 1993; Ono et al., 1987; Orphanet, 2009; Prater and D'Addio, 2002; Reichart et al., 1979; Rezaei et al., 2011; Rosanowski et al., 1998; Rudnik-Schoneborn et al., 1991; Sandhu and Brueton, 1989; Sarles, 2003; Steinbach and Hintz, 2000; Swanenburg de Veye et al., 1991; Szilagyi et al., 1987; Takahashi et al., 2004; Trellis and Clouse, 1991; Vanlieferinghen et al., 2003; Vanlieferinghen et al., 2001; Vaughn et al., 1998; Vieira et al., 2002; Zenker et al., 2006; Zenker et al., 2005; Zerres and Holtgrave, 1986) **(Table5).**

1.3.2.7.6 Le syndrome de Kabuki

Le syndrome de Kabuki est caractérisé par une apparence faciale typique, une petite taille, une hypotonie, un retard de développement, une fente labiale/palatine, un palais très arqué, une langue bifide, des dents manquantes (incisives latérales supérieures et centrales inférieures le plus fréquemment), des dents surnuméraires, des dents coniques, des molaires taurodontiques (Abdel-Salam et al., 2011; Cogulu et al., 2008; Courtens et al., 2000; dos Santos et al., 2006; Glaser et al., 2006; Hannibal et al., 2011; Li et al., 2011; Maas et al., 2007; Matsumoto and Niikawa, 2003; Matsune et al., 2001; Mhanni and Chudley, 1999; Mhanni et al., 1999; Micale et al., 2011; Ng et al., 2010; Paulussen et al., 2011; Petzold et al., 2003; Rocha et al., 2008; Spano et al., 2008; Teixeira et al., 2009) **(Table 5).**

SYNDROME	Caractéristiques	Gène	Type de molécule	Transmission	Locus
Syndrome de Coffin-Lowry *#303600*	palais haut et étroit, sillon lingual, malocclusion, hypodontie, incisives coniques, perte prématurée de la dentition lactéale	*RSK2*	Sérine thréonine-kinase	Liée à l'X	Xp22.2-p22.1
Syndrome de Bloom #210900	Croissance anormale, sensibilité au soleil, télangiectasie, peau hypo ou hyper pigmentée predisposition to malignancy; and chromosomal instability	*RECQL3*	Hélicase	Autosomique Récessive	15q26.1
Syndrome de Rothmund-Thomson #268400	Génodermatose poikiloderma,petite taille , défaut du squelette, anomalies dentaires, cataracte, risque élevé de cancer Microdontie, éruption retardée, dents surnuméraires, dents manquantes, racines multiples, anomalies de forme	*RECQL4*	Hélicase	Autosomique Récessive	8q24
Dysplasie diastrophique #222600	Osteochondrodysplasie	*DTDST*	Transporteur du sulfate	Autosomique Récessive	5q32-q33.1

Syndrome de Johanson-Blizzard #243800	Insuffisance pancréatique, microcéphalie, handicap intellectual, petite taille, hyp/oligodontie de la dentition permanente	*UBR1*	Ubiquitine ligase	Autosomique Récessive	15q15.2
Syndrome de Kabuki #147920	Handicap intellectual, déficience de croissance, absence des incisive latérales et des incisive centrales inférieures	*MLL2*	Histone methyltransférase	Autosomique Dominante	12q12-q14
Syndrome de Karvajal/Naxos	Cheveux laineux, keratoderme palo-plantaire, cardiomiopathie dilatée biventriculaire, hypo/oligodontie	*DSP*	Protéine desmosomique	Autosomique Récessive	6p24

Table 5 : Gène impliqués dans des syndromes associés à des dents manquantes

1.3.3 Dents surnuméraires

1.3.3.1 *Dysplasie cleido-crâniale (CCD)*

La dysplasie cleido-crâniale (CCD) est caractérisée par des anomalies crâniales, dentaires et claviculaires. Sa transmission est autosomique dominante et liée à la mutation du gène *RUNX2* (Runt-related transcription factor 2), un régulateur de la différenciation des ostéoblastes. Les caractéristiques principales en sont une absence bilatérale totale ou partielle des clavicules, un cou court, des anomalies spinales et pelviennes, une scoliose. Les principales caractéristiques crânio-oro-dentales sont un crâne brachycéphalique, des fontanelles et des sutures qui restent ouvertes, des sinus paranasaux sous-développés, un

développement pauvre du prémaxillaire, une dépression du pont nasal, une perte d'audition, une fente palatine, des dents surnuméraires multiples avec pseudo-anodontie due à l'éruption retardée, une hypoplasie de l'émail, une malocclusion (Angle and Rebellato, 2005; Chung et al., 2004; Cohen, 2006b; Cohen, 2009; Cooper et al., 2001; Counts et al., 2001; Feldman et al., 1995; Golan et al., 2004; Komori, 2008; Lee et al., 1997; Mundlos, 1999; Mundlos et al., 1996; Mundlos et al., 1997; Otto et al., 1997; Pal et al., 2007; Petropoulos et al., 2004; Sillence et al., 1987; Suba et al., 2005; Suda et al., 2010; Verstrynge et al., 2006; Zou et al., 2003) **(Table 6).**

1.3.3.2 *Polypose adénomateuse familiale (FAP)*

La polypose adénomateuse familiale (FAP, Familial adenomatous polyposis) est une maladie génétique caractérisée par de multiples polypes intestinaux adénomateux, des ostéomes, des fibromes, des lésions de la peau et des fibroses mammaires. Les caractéristiques dentaires principales incluent des dents manquantes et/ou surnuméraires, des racines anormales, et des odontomes. Sa prévalence est de 5,25/ 100 000 et sa transmission est autosomique dominante. Elle résulte de mutations du gène suppresseur de tumeur *APC* (adenomatous polyposis coli), un fort répresseur de la voie Wnt-beta caténine. Les tumeurs se développent du fait de l'activation de la voie de signalisation Wnt. Un autre gène, *MUTYH* (*MYH*) (1p34.3-p32.1), a aussi été associé à des formes atténuées de ce syndrome (Burt and Jasperson, 1993; Carl and Sullivan, 1989; Clarke, 2007; Fodde et al., 1994; Gardner, 1962; Half et al., 2009; Hasegawa et al., 2002; Hes et al., 2008; Karazivan et al., 2000; Nielsen et al., 2007a; Nielsen et al., 2007b; Sondergaard et al., 1987; Su et al., 1992; Wallis et al., 1999; Wang and Fan, 2011; Wang et al., 2009a; Wijn et al., 2007; Woods et al., 1989) **(Table 6).**

1.3.3.3 *Le syndrome de Nance-Horan (NHS)*

Le syndrome de Nance-Horan est caractérisé par l'association chez les patients masculins de cataractes congénitales avec une microcornée, une microphtalmie, des anomalies dentaires et un dysmorphisme facial. Sa transmission est liée à l'X et est semi-dominante. Un dysmorphisme facial est observé avec une face longue et rectangulaire, un prognathisme dans certains cas, un nez large, de grandes oreilles, et un retard mental est observé dans 30% des cas. Les anomalies oro-dentaires sont trouvées dans 100% des cas et constituent donc un bon signe diagnostic. On trouve un diastème entre les incisives maxillaires, des incisives maxillaires centrales coniques, des cuspides caractéristiques sur les incisives, les canines sont élargies et globulaires, les prémolaires et les molaires sont globulaires avec parfois des cuspides surnuméraires, des dents surnuméraires (incisives ou dents postérieures), des dents manquantes, une éruption retardée, des anomalies de la pulpe (taurodontisme, large chambre pulpaire, pulpe anormalement calcifiée), et un mauvais positionnement dentaire. La fonction du gène *NHS* et de la protéine correspondante est importante pour coordonner le remodelage de l'actine et maintenir la morphologie cellulaire. La régulation de ce gène est complexe car il produit différentes isoformes, par exemple NHS-A et NHS-1A. L'isoforme NHS-A est localisée au niveau de la membrane cellulaire et colocalise avec la protéine des jonctions serrées ZO-1 dans les cellules épithéliales tandis que NHS-1A est une protéine cytosolique. (Brooks et al., 2004a; Brooks et al., 2010; Brooks et al., 2004b; Coccia et al., 2009; Florijn et al., 2006; Huang et al., 2007; Huang et al., 2006; Lewis et al., 1990; Nance et al., 1974; Ramprasad et al., 2005; Reches et al., 2007; Sharma et al., 2006; Sharma et al., 2009; Stambolian et al., 1994; Stambolian et al., 1990; Toutain et al., 1997a; Toutain et al., 2002; Toutain et al., 1997b) **(Table 6).**

1.3.3.4 *Les syndromes tricho-rhino-phalangéaux*

Les syndromes tricho-rhino-phalangéaux sont un groupe de désordres développementaux (types I, II et III) caractérisés par des anomalies des cheveux, de la face, et de certains os. Leur transmission est autosomique dominante. Les types I et III sont dus à

une haploinsuffisance du gène codant pour une protéine à doigts de zinc, TRPS1, qui est un potentiel facteur de transcription pouvant agir comme répresseur transcriptionnel de type GATA. Le type II est un syndrome de microdélétion à la fois de *TRPS1* et du gène *EXT1*. L'expression de TRPS1 module la formation de matrice osseuse minéralisée dans les cellules ostéoblastiques en différenciation par la régulation de la transcription de l'ostéocalcine. TRPS1 peut aussi se lier au promoteur de *RUNX2* et inhibe son activité *in vitro*.

Les principales caractéristiques de ces syndromes sont une petite taille, une scoliose, une dysplasie de la hanche, des épiphyses des os des phalanges coniques, des métacarpes et métatarses courts, des ongles fins. Les caractéristiques crânio-oro-dentales sont des cheveux fins et clairsemés, de grandes ou petites dents, une éruption retardée et une malocclusion (Bennett et al., 1981; Braga et al., 1994; Dumic et al., 1993; Ferrando et al., 1981; Gai et al., 2011; Gellis and Feingold, 1972; Giedion, 1966; Goodman et al., 1981; Gorlin et al., 1969; Hussels, 1971; Kantaputra et al., 2008; Machuca et al., 1997; Malik et al., 2002; Napierala et al., 2005; Ohta et al., 1987; Parkhurst et al., 1972; Paterson and Thomas, 2000; Piscopo et al., 2009; Scheffer et al., 1981; Sommermater et al., 1978) **(Table 6).**

SYNDROME	Caractéristiques	Gène	Type de molécule	Transmission	Locus
Dysplasie cléido-crâniale #119600	Dysplasie des os	*RUNX2 (CBFA1)*	Facteur de Transcription	Autosomique Dominant	6p21
FAP (Familial adenomatous polyposis) #175100	Polypes du colon, odontomes, dents surnuméraires et dents n'ayant pas fait éruption	*APC*	Régulateur négatif de Wnt	Autosomique Dominant	5q21-q22
Syndrome de Nance-Horan #302350	Cataractes, faciès inhabituel, incisive conique	*NHS*	Protéine nucléaire	Liée à l'X	Xp22.13
Syndromes Tricho-Rhino-Phalangéaux (TRPS1) #190350 TRPS2 #150230 TRPS3 #190351	Cheveux fins et clairsemés, lèvre supérieure fine, oreilles protubérantes, épiphyses des os, des phalanges de forme coniques, malformations de la hanche	*TRPS1* *TRPS1 et EXT1* *TRPS1*	Protéine nucléaire avec 9 domaines en doigts de zinc, incluant un domaine de type GATA et un domaine carboxy-terminal de type Ikaros	Autosomique Dominant	8q23.3 8q24.11-q24. 8q23.3
Syndrome d'Ellis-van Creveld #225500	Dents néonatales, hypodontie, éruption prématurée , membres courts, côtes courtes, polydactylie, ongles et dents dysplasiques, défauts cardiaques congénitaux	*EVC1, EVC2*	Protéines transmembranaires des cils Régulateurs positifs de la voie Shh	Autosomique Récessive	4p16

Table 6 : Gène impliqués dans des syndromes associés à des dents surnuméraires

1.3.4 Les anomalies de forme et de taille

La forme et la taille des dents est dépendante du nombre de cellules postmitotiques (odontoblastes et améloblastes), de leur localistaion spatio-temporelle et de leur activité fonctionnelle (secrétion de la matrice et minéralisation) (Ruch, 1990; Ruch, 1995; Ruch, 1998; Ruch et al., 1995; Ruch et al., 1996).

1.3.4.1 *Le syndrome de Rubinstein-Taybi*

Le syndrome de Rubinstein-Taybi (RTS) est caractérisé par un retard de croissance et mental, des pouces larges et de gros orteils, un faciès inhabituel avec un front proéminent, une petite taille, un hirsutisme, des anomalies cardiaques, une microphtalmie, une perte d'audition, des anomalies squelettiques, une microcéphalie et un retard dans la fermeture des fontanelles. Les caractéristiques dentaires sont une hypodontie, des dents surnuméraires, une élongation des incisives supérieures centrales, des cuspides supplémentaires sur les molaires primaires, une opacité et une hypoplasie de l'émail, , des dents à la naissance (Baker, 1987; Bartsch et al., 2010; Bartsch et al., 2005; Bloch-Zupan et al., 2007; Coupry et al., 2002; Gardner and Girgis, 1979; Hennekam et al., 1990; Hennekam and Van Doorne, 1990; Ikuno et al., 1987; Josselyn, 2005; Kinirons, 1983; Oike et al., 1999b; Roelfsema et al., 2005; Rohlfing et al., 1971; Rubinstein, 1990; Rubinstein and Taybi, 1963; Tanaka et al., 1997; Taybi and Rubinstein, 1965; Wood et al., 2005; Zhang et al., 2004) **(Table 7).**

Sa prévalence est de 1 pour 100 000 et sa transmission est autosomique dominante. Il est causé soit par une microdélétion en 16p13.3, soit par des mutations du gène de la protéine de liaison à CREB (*CREBBP* ou *CBP*) ou dans le gene *EP300* (22q13). Ces deux gènes ont une forte homologie et codent pour des histones acétyltransférases (HAT), qui sont des co-activateurs transcriptionnels impliqués dans de nombreuses voies de signalisation.

1.3.4.2 *Le syndrome otodental*

Ce syndrome est caractérisé par des dents larges et bulbeuses (globodontie des canines, prémolaires et molaires) affectant la dentition primaire et secondaire et une perte de l'audition. Sa transmission est autosomique dominante et elle est liée à des mutations dans le gène *FGF3* (Gregory-Evans et al., 2007). Les principales caractéristiques orodentales sont des canines, des molaires et des prémolaires à larges racines bulbeuses, une fusion des molaires et des prémolaires donnant des dents macrodontes, des dents coniques surnuméraires, des fissures verticales de l'émail, des racines courtes, et des odontomes. De manière intéressante, dans ce syndrome toutes les incisives sont intactes (Alvarez et al., 2003; Bloch-Zupan and Goodman, 2006; Chen et al., 1988; Colter and Sedano, 2005; Cook et al., 1981; Gregory-Evans et al., 2007; Hatch et al., 2007; Levin and Jorgenson, 1974; Levin et al., 1975; Mansour et al., 1993; Mesaros and Basden, 1996; Santos-Pinto et al., 1998; Sedano et al., 2001; Witkop et al., 1976) **(Table 7).**

1.3.4.3 *Le syndrome oculo-facio-cardio-dentaire (OFCD)*

Le syndrome oculo-facio-cardio-dentaire (OFCD) est très rare et sa transmission est dominante liée à l'X. Il se caractérise par des radiculomégalies dentaires, des cataractes congénitales, un dysmorphisme facial et des anomalies cardiaques congénitales. Les anomalies dentaires caractéristiques incluent une radiculomégalie des canines et de certaines prémolaires, une oligodontie, des dents fusionnées, des dents surnuméraires, un retard d'éruption, et une persistance des dents primaires **(Table 7).** Le gène co-répresseur de BCL6 (*BCOR*) est responsable du syndrome. La protéine BCOR, en association avec BCL6, a la capacité de réduire l'activation de la transcription par AF9, un partenaire de fusion de MLL (mixed-lineage leukaemia) (Barthelemy et al., 2001; Blake et al., 2009; Cai et al., 2010; Cogulu and Ertugrul, 2008; Fan et al., 2009; Francis et al., 2002; Gorlin et al., 1996; Hedera and Gorski, 2003; Horn et al., 2005; Kawamoto et al., 2004; McGovern et al., 2006; Ng et al., 2004; Numabe and Numabe, 2001; Oberoi et al., 2005; Obwegeser and Gorlin, 1997; Opitz et al., 1998; Schulze et al., 1999; Tsukawaki et al., 2005; Turkkahraman and Sarioglu, 2006).

1.3.4.4 *Le syndrome KBG*

Le syndrome KBG consiste en un dysmorphisme typique de la face associé à une petite taille, un handicap d'apprentissage, des anomalies squelettiques et dentaires. Sa transmission est autosomique dominante. Des mutations du gène *ANKRD11* sont à l'origine de ce syndrome. Les dents sont macrodontes, avec une agénésie des incisives latérales maxillaires, une fusion des incisives centrales et latérales, des puits de l'émail, et une perte prématurée des dents. Les molaires ne sont pas affectées dans ce syndrome (Brancati et al., 2004; Brancati et al., 2006; Davanzo et al., 2005; Devriendt et al., 1998; Dowling et al., 2001; Fryns and Haspeslagh, 1984; Herrmann et al., 1975; Kumar et al., 2009; Maegawa et al., 2004; Mathieu et al., 2000; Rivera-Vega et al., 1996; Sirmaci et al., 2011; Skjei et al., 2007; Smithson et al., 2000; Soekarman et al., 1994; Tekin et al., 2004; Tollaro et al., 1984; Zollino et al., 1994) **(Table 7).**

1.3.4.5 *SMOC2*

SMOC2 appartient à la famille des protéines de la matrice cellulaire qui régulent les interactions entre les cellules et cette matrice. Des mutations dans ce gène sont responsables de microdontie marquée, d'oligodontie, d'anomalies de forme avec des cuspides supplémentaires, de la persistance d'une double dentition permanente des incisives, d'un taurodontisme des molaires, d'un émail fin, de racines courtes avec un os alvéolaire fin (Bloch-Zupan et al.) **(Table 7).**

SYNDROME	Caractéristiques	Gène	Type de molécule	Transmission	Locus
MOPD II (microcephalic osteodysplastic primordial dwarfism, type II) #210720	Extrême petite taille, microcéphalie, Extrême microdontie Dents opalescentes et de forme anormale, molaires sans racines, os alvéolaire hypoplastique	*PCNT2*	Protéine centrosomale	Autosomique Récessive	21q22.3
Microdontie/ Oligodontie	Microdontie/ Oligodontie	*SMOC2*	Protéine se liant au calcium	Autosomique Récessive	6q27
Surdité avec LAMM #610706	Surdité, aplasie du labyrinthe, microdontie, dents coniques	*FGF3*	Facteur de croissance	Autosomique Récessive	11q13.3
Syndrome de Rubinstein-Taybi #180849	Anomalies des cuspides, microcephalie, handicap d'apprentissage, face dysmorphique, pouces et orteils larges	*CREBBP* *EP300*	Protéine se liant à CREB Coactivateur transcriptionnel Voie de signalisation shh	Autosomique Récessive	16p13.3 22q13
Syndrome Otodental #166750	Globodontie (larges molaires bulbaires), perte d'audition	*FGF3*	Facteur de croissance	Autosomique Dominante	11q13.3
Syndrome OFCD #300166	Cataracte, anomalies cardiaques, radiculomégalie, oligodontie, dents fusionnées, dentition lactéale persistante	*BCL-6 co-repressor (BCOR)*	Co-répresseur transcriptionnel	Liée à l'X	Xp11.4
Syndrome KBG 148050	Petite taille, brachycéphalie, macrodontie des incisives supérieures, handicap d'apprentissage, anomalies squelettiques	*ANKRD11*		Autosomique Dominante	

Table 7 : Gènes impliqués dans des syndromes associés à des dents à anomalie de forme ou de taille

1.3.5 Les anomalies de la dentine

Les anomalies héréditaires de la dentine sont dues à des mutations de gènes codant pour les protéines majeures qui constituent la dentine, en particulier les collagènes ou les phosphoprotéines. Ces dernières appartiennent à la famille SIBLINGs (small integrin-binding ligand, N-linked glycoproteins), comportant l'ostéopontine, les sialoprotéines, DMP1 (dentine matrix protein), DSPP (dentine sialophosphoprotein), et MEPE (matrix extracellular phosphoglycoprotein). Les dentinogenèses sont classées en plusieurs types et les principales carctéristiques des dentinogenèses de type II et III sont résumées dans la **Table 8.**

1.3.5.1 *Dentinogenèses imparfaites*

Les dysplasies de la dentine sont caractérisées par des dents à courtes racines, coniques, avec croissance aberrante de la dentine dans la chambre pulpaire. Il en existe plusieurs types et leur prévalence est de 1 pour 100 000. Le type I affecte plutôt les racines alors que le type II affecte plutôt les couronnes. La seule mutation connue responsable du type II est localisée dans le gène *DSPP* (dentine sialophosphoprotein) (Beattie et al., 2006; Lee et al., 2008b; McKnight et al., 2008).

1.3.5.2 *Ostéogeneses Imparfaites (OI)*

Les ostéogenèses imparfaites sont caractérisées par des anomalies de la formation des os, avec une masse osseuse faible et des tendances aux fractures. Parfois il y a des anomalies associées de formation de la dentine. Elles sont divisées en plusieurs types, chaque type étant divisé en sous-types dont les caractéristiques sont résumées dans la **Table 8.**

Dans approximativement 90% des cas, des mutations dans les chaînes pro-alpha1 et pro-alpha3 du collagène de type 1 peuvent être identifiées [*COL1A1* (17q21.31-q22.05) ou *COL1A2* (7q 22.1)] (Aubin et al., 2005; Basel and Steiner, 2009; Baujat et al., 2008; Binger et

al., 2006; Cheung and Glorieux, 2008; Christiansen et al., 2010; Glorieux, 2008; Goldberg et al., 2008; Hall et al., 2002; Homan et al., 2011; Kamoun-Goldrat et al., 2008; Kamoun-Goldrat and Le Merrer, 2007; Kindelan et al., 2003; Koreeda-Miura et al., 2003; Lapunzina et al., 2010; Lee and Ertel, 2003; Lopez Franco et al., 2005; Madenci et al., 2006; Malmgren and Lindskog, 2003; Malmgren and Norgren, 2002; Marini et al., 2007; Pallos et al., 2001; Rauch et al., 2010; Rios et al., 2005; Roughley et al., 2003; Sanches et al., 2005; Shapiro et al., 1995; Van Dijk et al., 2009a; van Dijk et al., 2009b; Waltimo-Siren et al., 2005).

Parmi les 10% restant on trouve des mutations de *LEPRE1* (leprecan) et *CRTAP* (cartilage-associated protein), des protéines impliquées dans le complexe responsable de l'hydroxylation post-traductionnelle de la proline en position 3 de COL1A1. Des formes récessives résultent de mutations dans des enzymes modifiant le collagène telles que *CRTAP, LEPRE1, PPIB, FKBP10*, et *SERPINH1* (Christiansen et al.) (**Table 8).**

1.3.5.3 *Autres syndromes*

Une dentine anormale est aussi présente dans d'autres syndromes tel que : Ehlers Danlos (De Coster et al., 2007), Goldblatt (Bonaventure et al., 1992), dysplasie immune osseuse de Schim (da Fonseca, 2000), syndrome brachio-squeletto-génital (Kantaputra, 2001; Wedgwood et al., 1983) **(Table 8).**

SYNDROME	Caractéristiques	Gène	Type de molécule	Transmission	Locus
Dentinogenèses imparfaites I et II DGI-II DGI-I #125490	Défauts de la dentine, perte d'audition progressive	*DSPP*	Protéine de la dentine	Autosomique Dominant	4q21.3
Dentinogenèses imparfaites III	Défauts de la dentine	*DSPP*	Protéine de la dentine	Autosomique Dominant	4q21.3
Dysplasie de la dentine type II #125420	Dysplasie coronale de la dentine, dents opalescentes, racines courtes	*DSPP*	Protéine de la dentine	Autosomique Dominant	4q21.3
Ostéogenèse imparfaite avec ou sans dentinogenèse imparfaite type I DGI-I	Défauts de la dentine et des os	*COL1A1, COL1A2* *CRTAP* *LEPRE1* *Ou P3H1* *SERPINF1* *SERPINH1* *SP7, OSX*	Protéine de la dentine Protéine associée au cartilage Leprecan	Autosomique Dominant et Récessive Autosomique Récessive Autosomique Récessive Autosomique Récessive	17q21.31-q22 7q22.1 3p22 1p34 17p13.3 11q13.5 12q13.13
Syndrome d'Ehlers-Danlos type I #130000	Hyperextensibilité de la peau , hypermobilité articulaire, fragilité des tissus, apparence anormale de la dentine	*COL1A1* *COL5A1* *COL5A2*	Collagène type I Collagène alpha-1(V) Collagène alpha-2(V)	Autosomique Récessive	17q21.33 9q34.3 2q31
Syndrome d'Ehlers-Danlos type VIIc #225410	Hyperextensibilité des ligaments, extensibilité modérée de la peau, Hypodontie, anomalies de forme de la dentition lactéale, défauts de la dentine et des racines, usure de l'émail, hyperplasie gingivale, micrognathie, subluxations mandibulaire	*ADAMTS2*	Protéase du procollagène	Autosomique Récessive	5q35.3

Table 8 : Gènes impliqués dans des syndromes associés à des dents avec anomalie de la dentine

1.3.6 Les anomalies de l'émail

1.3.6.1 *Les amélogenèses imparfaites*

Les amélogenèses imparfaites (AI) représentent un groupe d'altération de la composition de l'émail associées à des désordres métaboliques ou des syndromes. Certaines sont associées à des anomalies comme le taurodontisme et des défauts de la dentine (Winter et al., 1969), des anomalies d'éruption/résorption, ou même des anomalies squelettiques (Poulsen et al., 2008; Witkop, 1988). La prévalence est de 1 : 4000 à 1: 15 000. Elles ont été classées selon leurs manifestations cliniques et le stade du développement dentaire au cours duquel elles interviennent. Si l'altération survient pendant la formation de l'émail, cela donne lieu à une hypoplasie ; si elle a lieu durant les premières étapes de la calcification et de la minéralisation, cela se traduit par une hypocalcification; enfin, si elle a lieu durant la seconde étape de calcification et de minéralisation (maturation), cela se traduit par une hypomaturation de l'émail.

La majorité des AI (63%) sont transmises de manière autosomique dominante ; 12% sont autosomiques récessives, 6% liées à l'X, et dans 19% des cas, le mode de transmission n'est pas élucidé (Aldred et al., 2003; Crawford et al., 2007; Hart et al., 2002a; Hart et al., 2004; Hart et al., 2002b; Hart et al., 2003a; Hart et al., 2000a; Hart et al., 2003b; Wright, 2006; Wright et al., 2006). Certains gènes ont été identifiés comme responsables d'amélogenèses imparfaites. Parmi les gènes identifiés : *AMELX* (Hart et al., 2000a), *ENAM* (Kim et al., 2005a; Rajpar et al., 2001), *ENAMELYSIN* ou *MMP20* (Kim et al., 2005b), *KALLIKREIN 4* (Hart et al., 2004), *DLX3* (Dong et al., 2005), *FAM83H* (Kim et al., 2008; Lee et al., 2008a; Mendoza et al., 2007), *WDR72* (El-Sayed et al., 2009).

Les principales caractéristiques cliniques en fonction du gène muté figurent dans la **Table 9.**

1.3.6.2 *Les anomalies de l'émail associées à des syndromes*

Les anamolies peuvent aussi être associées à des syndromes tels que le syndrome tricho-dento-osseux (TDO), les épidermolyses bulleuses, les scléroses tubéreuses, la dysplasie oculodentogénitale, la piknodysostose et le rachitisme. Les principales caractéristiques de ces syndromes sont répertoriées dans la **Table 9.**

SYNDROME	Caractéristiques	Gène	Type de molécule	Transmission	Locus
Amelogenese imparfaite AIH1 #301200	Hypoplasie, hypominéralisation, hypomaturation	*AMELX*	Protéine de l'émail	Liée à l'X	Xp22.3-p22.1
Amelogenese imparfaite TYPE IB; AI1B = AIH2 #104500 TYPE IC; AI1C #204650	Forme hypoplasique, dents lisses, puits, béance antérieure	*ENAM*	Protéine de l'émail	Autosomique dominante	4q21
Amelogenèse imparfaite , type III, AI3 #130900	Hypominéralisation	*FAM83H*		Autosomique dominante	8q24.3
Amelogenese imparfaite et hyperplasie gingivale 204690? (O'Sullivan et al., 2011)	Hyperplasie gingivale	*FAM20A*	Glycoprotéine secrétée	Autosomique récessive	17q24.2
Amelogenese imparfaite #204700 IIA1; AI2A1	Hypomaturation Pigmentée	*KLK4*	Sérine protéase	Autosomique récessive	19q13.3-q13.4
Amelogenese imparfaite #612529 IIA2, AI2A2	Hypomaturation	*MMP20*	Métalloprotéase	Autosomique récessive	11q22.2
Amelogenese imparfaite #613211 IIA3, AI2A3	Hypomaturation	*WDR72*	Hélice beta	Autosomique récessive	15q21.3
Amelogenese imparfaite #104510 TYPE IV; AI4	Hypoplasie Hypomaturation Taurodontisme	*DLX3*	Facteur de transcription	Autosomique dominante	17q21.3-q22
Amelogenese imparfaite Et dystrophie des cônes et des bâtonnets #217080		*CNNM4*	Transporteur de métaux	Autosomique récessive	2q11
Syndromes avec défauts de l'émail					
Syndrome tricho-dento-osseux #190320	Hypoplasie de l'émail, taurodontisme, anomlies des cheveux et des os	*DLX3*	Facteur de transcription	Autosomique dominante	17q21.3

Epidermolyse bulleuse simplex #131760 (EBS Dowlin-Meara type) #131800 (EBS Weber-Cockayne type) #131900 (EBS Koebner type)	Cloques orales	keratin 5 (*KRT5*) keratin 14 (*KRT14*)		Autosomique dominante	12q13 17q12-q21
Epidermolyse bulleuse jonctionelle EBJ #226700 Et type non Herlitz (#226650)	Puits de l'émail, cloques sur la peau et dans la bouche	*LAMA3* *LAMB3* *LAMC2* *COL17A1*	Protéines de l'émail	Autosomique récessive	18q11.2 1q32 1q25-q31 10q24.3
Epidermolyse bulleuse dystrophique EBD #226600		*COL7A1* *MMP1,* gène modificateur		Autosomique récessive	3p21.3 11q22-q23
Sclérose tubéreuse # 191100	Puits de l'émail	*TSC1* *TSC2*	Suppresseur de tumeurs	Autosomique dominante	9q34 16p13.3
Dysplasie oculodentodigitale ODDD #164200	Hypoplasie de l'émail, microdontie	*GJA1*	Protéine des jonctions communicantes	Autosomique dominante	6q21-q23.2
Pyknodysostose # 265800	Hypoplasie de l'émail, oligodontie, éruption des dents retardée, malocclusion	*CTSK*	Cathépsine K	Autosomique récessive	1q21
Rachitisme vitamine D dépendant #264700 (type I), #277440 (type II)	Retard de croissance, hypotonie, convulsions, tétanie, fractures pathologiques, émail hypoplastique	*CYP27B1*	Hydroxylase rénale	Autosomique récessive	12q13.3

Table 9 : Gènes impliqués dans des syndromes associés à des dents avec anomalie de l'émail

1.3.7 Les anomalies d'éruption/résorption

L'éruption dentaire dépend de la présence des follicules dentaires, de la création d'un chemin à travers l'os alvéolaire par les ostéoclastes, et la capacité des ostéoclastes à former un nouvel os (Wise, 2009). Dans les maladies où la formation ou la fonction des ostéoclastes est réduite comme dans les ostéopétroses **(Table 10)**, l'éruption dentaire est affectée voire absente.

Les maladies dans lesquelles la formation des ostéoclastes ou leur fonction est augmentée (comme dans les ostéolyses expansiles et les maladies osseuses de Paget) sont associées à des anomalies dentaires comme l'hypercémentose, une résorption des racines et une perte des dents prématurée (Helfrich, 2005). La perte prematurée des dents peut aussi être à l'origine de périodontes anormaux et de formation du cément anormale, comme c'est le cas dans le Syndrome de Papillon-Lefèvre (Beertsen et al., 1999) **(Table 10).**

1.3.7.1 *Le syndrome de Sotos*

Le syndrome de Sotos est caractérisé par une croissance excessive, une dolichocéphalie, un âge osseux avancé et un handicap d'apprentissage. Ce syndrome serait également associé à un risque accru de tumeurs. Les anomalies dentaires incluent une éruption prematurée des dents, une hypodontie et une hypoplasie de l'émail. Sa prévalence est de 7 : 100 000. Dans 75% des cas, il est dû à des mutations et des microdélétions dans le gène *NSD1* ("nuclear receptor-binding Su-var, enhancer of zeste, and trithorax domain protein 1") codant pour une histone méthyltransférase impliquée dans la régulation transcriptionnelle (Baujat and Cormier-Daire, 2007; Berio et al., 1992; Callanan et al., 2006; Faravelli, 2005; Gomes-Silva et al., 2006; Inokuchi et al., 2001; Lapunzina, 2005; Leventopoulos et al., 2009; Niikawa, 2004; Park et al., 2006; Scaglioni et al., 1982; Staffolani et al., 1994; Takei et al., 2007; Tatton-Brown et al., 2005a; Tatton-Brown et al., 2005b; Villaverde and Da Silva, 1971; Ward et al., 2000; Welbury and Fletcher, 1988) **(Table 10).**

1.3.7.2 *Rachitisme vitamine D résistant*

Il est caractérisé par une hypophosphatémie associée à une diminution de l'absorption de phosphate inorganique par les reins, une normocalcémie et une excrétion accrue de phosphate par les tubules rénaux. Les anomalies dentaires comportent une éruption retardée, une hypominéralisation de l'émail, une chambre pulpaire anormalement grande, une dentine globulaire et un cément anormal (Baroncelli et al., 2006; Batra et al., 2006; Boukpessi et al., 2006; Chaussain-Miller et al., 2007; Chaussain-Miller et al., 2003;

Douyere et al., 2009; Gaucher et al., 2009; Holm et al., 2001; Pereira et al., 2004; Yamamoto, 1997; Yuan et al., 2008).

Des mutations dans le gène PHEX (phosphate regulating endopeptidase homologue, X-linked) sont impliquées dans ce désordre. Ce gène code pour une endoprotéase liée à la membrane qui est préférentiellement exprimée dans les ostéoclastes et qui régule le métabolisme des phosphates. PHEX régule la fonction de *FGF23*, le facteur le plus important dans la physiopathologie des perturbations hyperphosphaturiques. *FGF23* (12p13.3) est responsable d'une forme autosomique dominante de la maladie, *DMP1* d'une forme autosomique récessive et *ClCN5* d'une forme récessive liée à l'X.

1.3.7.3 *Hypophosphatasie*

L'hypophosphatasie est caractérisée par un large spectre de défauts des tissus minéralisés (os, dents) et est causée par des déficiences dans le gène *ALPL* codant par la phosphatase alkaline non tissu-spécifique (TNSALP) (Brun-Heath et al., 2005; Cole, 2008; Fauvert et al., 2009; Greenberg et al., 1990; Hartsfield, 1994; Herasse et al., 2003; McKee et al., 2011; Mornet, 2007; Mornet et al., 2011a; Mornet and Simon-Bouy, 2004; Mornet et al., 2011b; Moulin et al., 2009; Reibel et al., 2009; Stoll et al., 2002; Taillandier et al., 2005; van den Bos et al., 2005; Whyte, 2010).

1.3.7.4 *Le syndrome de Haim-Munk*

Ce syndrome se caractérisé par des doigts longs, des callosités des paumes et des plantes, et des périodontes. Il est lié à une mutation de la protéase lysosomale cathepsine C (*CTSC* ; 11q14.1-q14.3). (Berdowska, 2004; Cury et al., 2005; Hart et al., 2000b; Janjua et al., 2008; Lidar et al., 2004; Pahwa et al., 2010; Van Steensel et al., 2002).

1.3.7.5 *Le syndrome d'ostéolyse expansile familiale*

Il est caractérisé par des ostéolyses expansiles, des douleurs osseuses, des fractures, une perte d'audition, une résorption des racines, des dents qui bougent, et une perte prématurée des dents. Il est dû à des mutations dans le gène *TNFRSF11A* (tumour necrosis factor receptor superfamily, member 11a) (18q22.1) qui code pour RANK (receptor activator of NF-kappa B). Ce récepteur peut interagir avec de nombreuses protéines de la famille TRAF à travers lesquelles il induit l'activation des voies de signalisation NF-kappa B et MAPK8/JNK. (Crone and Wallace, 1990; Daneshi et al., 2005; Dickson et al., 1991; Esselman et al., 1996; Helfrich, 2005; Hughes et al., 2000; Hughes et al., 1994; Johnson-Pais et al., 2003; Marik et al., 2006; Mitchell et al., 1990a; Mitchell et al., 1990b; Olsen et al., 1999; Osterberg et al., 1988; Palenzuela et al., 2002; Ralston, 2008; Wallace et al., 1989; Whyte and Hughes, 2002; Whyte et al., 2002).

SYNDROME	Caractéristiques	Gène	Type de molécule	Transmission	Locus
Syndrome de Sotos *#117550*	Eruption prematurée des dents, croissance rapide, acromégalie, faciès typique et handicap d'apprentissage	*NSD1*	Domaine se liant au réceptaire nucléaire	Cas isolés	*5q35*
Hypophosphatémie et rachitisme vitamine D résistant *#307800*	Très petite taille, fragilité des extrémités, éruption dentaire retardée, défauts de la dentine, cément anormal	*PHEX*	Endoprotéase	Liée à l'X	*Xp22.2-1*
Pycnodysostoses *#265800*	Petite taille, déformations du crâne, du maxillaire et des phalanges, ostéosclérose et fragilité des os, micrognathie, éruption retardée et persistance des dents lactélaes, éruption retardée des dents permantes, hypodontie, hypoplasie de l'émail	*CTSK*	Cathépsine K	Autosomique récessive	*1q21*
Echec de l'éruption des dents lactéales *#125350*	Hypodontie Béance postérieure Ankylose des dents lactéales	*PTHR1*	Récepteur de l'hormone parathyroïdienne	Autosomique dominante	*3p22-p21.1*
Ostéopétroses *#259710* *#607649*	Ostéosclérose Absence ou retard d'éruption des dents	*TNFSF11* *TCIRG1 sous-unité*	Facteur de nécrose tumorale Régulateur de la réponse immunitaire	Autosomique récessive	*13q14* *11q13.4-q13.5*
Syndrome d' hypomyélinisation et leukoencéphalopathie 4H /ADDH *#607694*	Syndrome neurodégénératif, retard de l'éruption de la dentition lactéale, rétention complète des incisives maxillaires centrales lactéales et anormalies de forme des incisives maxillaires	*POLR3A*	Sous-unité de l'ARN polymérase III C	Autosomique récessive	*10q22.3*

	centrales permanentes, oligodontie				
Hypophosphatasie, infantile *#241500, 241510, 146300*	Anomalies squelettiques, absences, perte prématurée des dents	*ALPL Alkaline phosphatase*	Enzyme	AR, AD	*1p36.1-p34*
Syndrome de Papillon Lefevre *#245000*	Hyperkératose des paumes et des plantes, perte prématurée des dents, parodontite sévère	*CTSC Cathepsin C gene*	Enzyme Lysosomale	Autosomique récessive	*11q14.1-q14.*
Syndrome de Haim-Munk *#245010*	Kératose palmoplantaires, perte prématurée des dents, parodontite sévère, onychogrypose, arachnodactylie,acrostéolyse	*CTSC Cathepsin C gene*	Enzyme Lysosomale	Autosomique récessive	*11q14.1-q14.*
Parodontite sévère *#170650*		*CTSC Cathepsin C*	Enzyme lysosomale	Autosomique récessive	*11q14.1-q14.3*
Ostéolyse familiale expansible #174810 *Hyperphosphatasie squelettique expansible*	Résorption des racines	*TNFRSF11A*	RANK	Autosomique dominante	*18q21.1-22*

Table 10 : Gènes impliqués dans des syndromes associés à des dents avec anomalie de l'éruption ou de la résorption

1.4 Les modèles murins

La souris est un modèle très intéressant pour étudier les anomalies dentaires. En effet de nombreux modèles de souris mimant les anomalies rencontrées chez l'Homme sont

aujourd'hui disponibles (les mécanismes moléculaires qui sous-tendent le développement dentaire chez l'Homme et chez la souris étant très conservés).

Le tableau ci-dessous récapitule les différents modèles murins disponibles, et précise les anomalies dentaires trouvées chez la souris **(Table 11).**

MALADIE CHEZ L'HOMME	GENE IMPLIQUE	MODELE MURIN	ANOMALIES DU MODELE MURIN	BIBLIOGRAPHIE
# OMIM	Localisation chromosomique			
Anomalies de nombre par défaut				
Hypodontie/Oligodontie #106600	*MSX1* (4p16.1)	Haploinsuffisance	Fente palatine, développement des molaires arrêté au stade bourgeon, absence des incisives, sous développement du maxillaire et de la mandibule, anomalies des os du crâne, et de l'oreille moyenne	(Satokata and Maas, 1994)
Hypodontie #106600 #604625	*PAX9* (14q12-q13)	*Pax9*-/-	Mort peu après la naissance, fente palatine, absence de toutes les dents, du thymus et des glandes parthyroïdes, anomalies squelettiques	(Peters et al., 1998b)
S. Axenfeld-Rieger #180500 % 601499 #602482	*PITX2* (4q24-26)	*Pitx2*-/-	Arrêt du développement dentaire au stade bourgeon	(Lu et al., 1999; Sclafani et al., 2006)
S. Wolf-Hirschhorn #194190	*WHSC1* (4p16.3)	*Whsc1* +/-	Retard de croissance, fente palatine	(Naf et al., 2001)
S. du cancer colorectal et oligodontie #608615	*AXIN2* (17q24)	*Axin2*-/-	anomalies des structures du crâne ressemblant à la craniosynostose humaine	(Yu et al., 2005)
Oligodontie et dysplasie ectodermique #153245	*LEF1* (4q25)	*Lef1*-/-	Développement dentaire arrêté au stade bourgeon	(van Genderen et al., 1994)

Dysplasie ectodermique hypohydrotique #305100	*EDA* (Xq13.1)	*Tabby*	Prémolaires surnuméraires, hypercémentose des racines, petit nœud de l'émail, anomalies des cuspides	(Charles et al., 2009a; Peterkova et al., 2005; Pispa et al., 1999; Tucker et al., 2000)
Dysplasie ectodermique hypohydrotique #129490	*EDAR* (2q12.3)	*Downless*	Absence du nœud de l'émail, dents plus petites, nombre de cuspides réduit	(Charles et al., 2009b; Tucker et al., 2000)
Dysplasie ectodermique hypohydrotique #129490	*EDARADD* (1q42-43)	*Crinkled*	Dents plus petites, nombre de cuspides réduit	(Tucker et al., 2004)
Ectrodactylie, dysplasie ectodermique et fentes #1299000 #602077 #604292	*p63 (7q11.2-q21.3)*	*p63-/-*	Anomalie des membres et des dérivés ectodermiques, pas de formation des placodes dentaires et ongulaires	(Brunner et al., 2002; Mills et al., 1999)
Incontinentia Pigmenti #308300	*NEMO = IKKgamma* (Xq28)	*IKKgamma+/-*	Femelles : lésions de la peau , Mâles : létalité et tous les signes de la maladie humaine	(Makris et al., 2000; Nenci et al., 2006; Rudolph et al., 2000)
S. CLPED1 #225000	*PVRL1* (11q23-24)	Absence de la protéine (nectin-1)	Défaut de formation de l'émail	(Barron et al., 2008)
S. d'Ellis Van Creveld #225500	*EVC1/EVC2* (4p16)	*Evc-/-*	Développement d'anomalies similaires au syndrome humain avec membres courts et côtes courtes et anomalies dentaires similaires	(Ruiz-Perez et al., 2007)
S. de Van der Woude #119300	*IRF6* (1q32-41)	*Irf6-/-*	Anomalies de la peau, des membres et du développement crâniofacial, fente palatine	(Ingraham et al., 2006)
S. oro-facio-diagital #311200	*OFD1* (Xp22.3-p22.2)	*Ofd1-/-*	Anomalies similaires au syndrome humain avec une sévérité plus élevée	(Ferrante et al., 2006)
ASPED #105835	*CDMP1 (GDF5)* (20q11.2)	*Gdf5-/-*	Défauts développementaux des membres, de la queue et du squelettes	(Storm et al., 1994; Takahara et al., 2004)

Incisive centrale médiane #147250	*SHH(7q36)*	*Noggin-/-*	Incisive centrale maxillaire médiane	(Lana-Elola et al.)
Incisive centrale médiane #147250	*SHH* (7q36)	*Gas1-/-*	Fusion des incisives	(Seppala et al., 2007)
S. de Kallmann #147950	*FGFR1* (8p11.23-p11.22)	*Fgfr1 -/-* inactivation tissu spécifique	Défauts amélaires importants	(Takamori et al., 2008)
S. lacrimoauriculodentodigital #149730	*FGFR2 (10q26.13)*	*Fgfr2b -/-*	Développement dentaire arrêté au stade bourgeon	(De Moerlooze et al., 2000)
S. lacrimoauriculodentodigital #149730	*FGF*	*Sprouty2, 4 -/-*	Dent surnuméraire en avant de la première molaire, incisives surnuméraires	(Klein et al., 2006)
S. de Bloom #210900	*RECQL3 (BLM)* (15q26.1)	*Recql3-/-*	Mort embryonnaire au stade E13.5 ou viable avec de nombreux cancers et anomalies chromosomiques	(Chester et al., 2006)
S. de Rothmund-Thomson #268400	*RECQL4* (8q24.3)	*Recql4-/-*	Anomalies de la peau et du squelette, instabilité génomique entraînant une susceptibilité aux cancers	(Mann et al., 2005)
Dysplasie diastrophique #222600	*DTDST* (5q32-q33.1)	*Dtdst-/-*	Retard de croissance et anomalies squelettiques	(Forlino et al., 2006; Forlino et al., 2005)
S. de Johanson-Blizzard #243800	*UBR1* (15q15.2)	*Ubr1-/-*	Insuffisance pancréatique exocrine	(Zenker et al., 2005)
S. de Kabuki #147920	*MLL2*(12q13.12)	*Mll2-/-*	Arrêt du développement embryonnaire au stade E11.5	(Glaser et al., 2006)
S. de Coffin-Lowry #303600	*RSK2* (Xp22.2-p22.1)	*Rsk2-/Y*	Taille réduire, crâne moins long, déviation nasale, dent surnuméraire	(Publication 5)
Anomalies de nombre par excès				
Dysplasie cléido-crâniale #119600	*RUNX2* (6p21.1)	*Runx2-/-*	Arrêt du développement dentaire au stade bourgeon, mort à la naissance	(Aberg et al., 2004a)

Polype adénomateux familial #175100	*APC* (5q21-22)	*Apc-/-*	Dents surnuméraires, néoplasies de l'épithélium intestinal	(Wang et al., 2009a)
S. de Nance-Horan #302350	*NHS* (Xp22.13)	Mutant *Xcat (Nhs1)*	Anomalie de la lentille	(Huang et al., 2006)
Anomalies de forme				
S. de Rubinstein-Taybi #180849	*CREBBP* (16p13.3) ou EP300 (22q13)	*Crebbp-/- ou EP300-/-*	Mort embryonnaire entre E9.5 et E10.5	(Oike et al., 1999a)
S. de Rubinstein-Taybi #180849	*CREBBP* (16p13.3) ou EP300 (22q13)	*Crebbp+/- ou EP300+/-*	Défaut squeletiques, cardiaques et hématopoïétiques, croissance retardée	(Oike et al., 1999a)
S. oculo-facio-cardio-dentaire #300166	*BCL6 = BCOR* (Xp11.4)	*Bcor -/-*	Modèle pas encore décrit	
Dentinogenèse imparfaite				
Dentinogenèse imparfaite #125490	*DSPP* (4q21.3)	*Dspp-/-*	Défaut de minéralisation de la dentine	(Sreenath et al., 2003)
Ostéogenèse imparfaite #166210	*COL1A2* (7q21.3)	*Col1a2 -/- ou Col1a2 -/+*	Dentinogenèse imparfaite	(Lopez Franco et al., 2005)
Amélogenèse imparfaite				
Amélogenèse imparfaite #301200	*AMELX* (Xp22.3-p22.1)	*Amelx-/-*	Défaut de l'émail : couleur blanc crayeux, les molaires montrent une usure excessive, hypoplasie de l'émail	(Wright et al., 2009)
Amélogenèse imparfaite #104510	*DLX3* (17q21.33)	*Dlx3 -/-*	Mort embryonnaire à E9,5/10	(Morasso et al., 1999)
Amélogenèse imparfaite #204650, #104500	*ENAM* (4q13.3)	*Enam-/-*	Hypoplasie de l'émail, incisives de couleur blanc crayeux	(Seedorf et al., 2007)
Amélogenèse imparfaite #612529	*MMP20* (11q22.2)	*Mmp20 -/-*	Hypoplasie de l'émail, hypominéralisation de l'émail	(Bartlett et al., 2006b; Wright et al., 2009)
S. d'épidermolyse bulleuse jonctionnelle #226650	*COL17*(10q24.3-q25.1)	*Col17-/-*	Incisives jaunes, retard de calcification, prismes d'émail irréguliers, les molaires montrent des signe d'usure avancée	(Asaka et al., 2009)

Anomalies d'éruption et de resorbtion				
S. de Sotos #117550	*NSD1* (5q35)	Nsd1-/-	Arrêt du développement embryonnaire au cours de la gastrulation	(Rayasam et al., 2003)
Rachitisme vitamine D résistant #307800	*PHEX* (Xp22.2-1)	*hyp-mouse* (déletion dans le gène)	Hypophosphatémie	(Ogawa et al., 2006)
S. d'ostéolyse expansive familiale #174810	*TNFRS11A* (18q21.33)	Surexpression de la protéine *RANK*	Eruption dentaire prématurée, élongation radiculaire accélérée	(Castaneda et al.; Dougall et al., 1999)
Hypophosphatasie #241500	*ALPL* (1p36.12)	*Alpl-/-*	Défaut similaire d'un point de vu métabolique et squelettique à l'hypophastasie infantile, hypoméniéralisation de la dentine, du cément et del'os alvéolaire	(McKee et al., 2011)

Table 11 : Modèles murins disponibles mimant des syndromes associés à des anomalies dentaires

RESULTATS

2 Résultats

Nous avons voulu, en utilisant une approche de médecine translationnelle, examiner et comprendre la mise en place des anomalies dentaires rencontrées dans les maladies rares grâce à des approches combinées de biologie du développement, de bioinformatique, de transcriptomique, l'étude de modèles murins couplées à une analyse des phénotypes cliniques chez les patients concernés.

L'étude transcriptomique réalisée au cours de ce travail de thèse servira de fil rouge pour naviguer entre les différents axes d'étude.

Afin d'identifier et de comprendre le rôle de nouveaux gènes impliqués dans les anomalies dentaires, nous avons entrepris une étude du transcriptome dentaire murin au stade capuchon E14.5 en comparant le transcriptome de molaires mandibulaires et des incisives inférieures mais aussi des molaires mandibulaires et maxillaires. Nous avons fait le choix de ne pas inclure les incisives maxillaires pour travailler sur des organes provenant d'une population de cellules des crêtes neurales homogène **(Publication 6).**

Afin de valider statistiquement les données obtenues lors de cette étude nous avons réalisés une analyse en composante principale. L'analyse des molaires mandibulaires et des incisives inférieures a démontré que les échantillons se regroupent en deux groupes distincts reflétant des différences d'expression génique entre ces deux groupes. Lorsqu'on nous considérons les gènes avec un facteur de changement de 1,2 nous obtenons 3071 gènes différentiellement exprimés entre les deux types dentaires tandis que lorsque nous considérons un facteur de changement plus stringent de 2 nous obtenons 231 gènes. L'expression de gènes jouant un rôle majeur dans le développement dentaire sur le transcriptome, nous permet de valider biologiquement ces données cependant leur expression ne semble pas être différentielle. De même lors de la comparaison en composante principale des molaires mandibulaires et maxillaires, les échantillons se séparent en deux groupes reflétant des différences d'expression géniques.

La validation statistique de ces données a mis en évidence 17995 gènes exprimés dans la dent au stade capuchon en considérant 80% des intensité les plus fortes sur les puces à ADN. **(Publication 6).**

Nous avons voulu connaître le pourcentage de ces 17995 gènes impliqués dans les maladies rares. En croisant nos données avec celles de la base de données OMIM (Online Mendelian Inheritance in Man) qui regroupe les gènes impliqués dans des maladies génétiques. Nous avons mis en évidence 2513 gènes impliqués dans des maladies génétiques dont 466 ont dans leur phénotype clinique des anomalies dentaires décrites.

Parmi ces 466 gènes nous retrouvons des gènes (*Irf6 , Nfkbia, Map2k1, Ercc3 et Evc2*) que nous avions sélectionnés au début de ce projet sur la base de 2 critères : - gènes

impliqués dans des anomalies du développement dentaire rencontrés dans les maladies rares ici à titre d'exemple les dysplasies ectodermiques et pour le second critère une expression spécifique dans le germe dentaire visualisée et analysée grâce à l'atlas de transcriptome issu du projet européen EURExpress (projet dirigé à Strasbourg par le Dr. Pascal Dollé) et la base de données dérivée créée dans le laboratoire suite à une collaboration avec Raymond Ripp. Cet atlas Eurexpress regroupe les données d'expression de plus de 20 000 gènes par hybridation in situ au jour E14.5 du développement embryonnaire de la souris, Odontogenèse détaille à ce jour les patrons pour 900 gènes au stade de capuchon.

Nous nous sommes en particulier demandé comment les patrons d'expression de ces gènes pouvaient expliquer les phénotypes cliniques rencontrés chez l'Homme (**Publications 1, 2 et 3).**

Le gène *Irf6* est responsable lorsqu'il est muté chez l'Homme du syndrome de van der Woude caractérisé au niveau bucco-dentaire par une hypodontie une fente palatine et ou labiale et des puits de la lèvre inférieure. L'expression de ce gène est épithéliale aussi bien au niveau molaire qu'incisif tout au long du développement dentaire. Son expression aux stades précoces du développement dentaire pourrait être rapprochée des anomalies de nombre par défaut rencontrées chez l'Homme **(Publication 1).**

Le gène *Nfkbia* est impliqué chez l'homme dans une dysplasie ectodermique associée à un déficit immunitaire. Son expression chez la souris est épithéliale tout au long du développement et devient également mésenchymateuse à partir du stade E16.5. Son expression au stade E12.5 lame dentaire permet d'expliquer les anomalies de nombre par défaut présentes chez les patients tandis que son expression au stade capuchon correspondant au stade d'histomorphogenèse permet d'expliquer les anomalies de forme (dents coniques) et de taille (microdontie) **(Publication 1).**

Le gène *Ercc3* est impliqué dans 3 maladies rares affectant les mécanismes de réparation de l'ADN, *Xeroderma pigmentosum*, la trichothidystrophie et le syndrome de Cokayne. Le gène *Evc2* est responsable lorsqu'il est muté chez l'Homme du syndrome d'Ellis van Creveld caractérisé au niveau buccp-dentaire par Dents neo-natales ou à la naissance. Les profils d'expression correspondent, sur le plan temporel et en ce qui concerne les tissus marqués, aux phénomènes qui lorsqu'ils sont perturbés peuvent générer les anomalies dentaires observées chez les patients (hypodontie/oligodontie, petite dents coniques, hypoplasie de l'émail) atteints du syndrome d'Ellis Van Creveld (Publications 1 et 3).

Parmi les 466 gènes exprimés à E14.5 et impliqués dans des maladies avec anomalies dentaires nous retrouvons également *Smoc2* un gène qui a été identifié grâce à une famille informative du centre de référence des manifestations buccodentaires des maladies rares présentant un phénotype clinique particulier (famille étudiée par cartographie par homozygotie classique et étude de gène candidat en Collaboration avec le Pr. Hélène

DOLLFUS). L'expression de ce gène à différents stades du développement permet d'expliquer le spectre des anomalies dentaires rencontrées **(Publication 4).** Il est important de noter qu'il existe une expression différentielle de *Smoc 2* entre les molaires et les incisives.

Ce qui nous permet d'aborder un autre aspect de l'étude transcriptomique correspondant à l'identification de gènes impliqués dans l'identité de chaque type de dents. Pour revenir à notre approche maladies rares il existe en effet des syndrome ou seul un type particulier de dents est affecté comme c'est le cas dans le syndrome KBG ou otodental.

Dans un premier temps nous avons voulu mettre en évidence les gènes différentiellement exprimés entre molaires mandibulaires et incisives inférieures. Parmi les gènes les plus fortement changeant on retrouve *Barx1* et *Six2* qui sont connus pour définir le champ molaire et *Isl1* et *Hand2* qui sont connus pour définir le champ incisif. Ces gènes permettent de valider biologiquement ces données transcriptomiques. Nous nous sommes alors intéressés aux gènes impliqués dans les grandes voies de signalisation impliquées dans le développement dentaire. Nous en avons identifié 107 et parmi eux 88 n'avaient jamais été identifiés dans la dent et 99 ne sont pas connus pour définir l'identité dentaire **(Publication 6).**

Dans un deuxième temps nous avons voulut mettre en évidence les gènes différentiellement exprimés entre molaires mandibulaires et maxillaires. Parmi les gènes les plus fortement changeant on retrouve *Nkx2-3* ainsi que des gènes connus pour être exprimés au niveau dentaire. Ces gènes permettent de valider biologiquement ces données transcriptomiques. La distance génétique séparant les molaires mandibulaires des molaires maxillaires semble être plus petite que celle séparant les molaires mandibulaires des incisives inférieures. Nous nous sommes alors intéressé aux gènes impliqués dans les grandes voies de signalisation du développement dentaire. Nous en avons identifié 62 et parmi eux 53 n'avaient jamais été identifiés dans la dent et 58 ne sont pas connus pour définir ces morphogenèse et morphologies spécifiques **(Publication 6).**

Nous abordons maintenant grâce à l'atlas de transcriptome la dernière partie de ce travail consacrée à l'étude d'un modèle murin de maladie rare. Nous nous sommes également intéressé au gène *RSK2* (the ribosomal S6 family of serine/threonine kinase) responsable lorsqu'il est muté chez l'Homme du syndrome de Coffin-Lowry (OMIM #303600).

Comme nous disposions du modèle murin nous avons tout d'abord voulu savoir quelles sont les anomalies crânio-faciales du modèle murin *Rsk2-/Y* . Nous avons analysé ces souris par micro-tomographie. Cette technique consiste à sonder un objet par plans de coupes successifs sous différents angles de vue, de façon à reconstruire numériquement une représentation tridimensionnelle de l'objet. Certains crânes de souris mutantes présentent une déviation nasale mais le phénotype va de la normalité au mutant très affecté. Pour

préciser ces anomalies nous avons voulu calculer des distances euclidiennes entre différents points qui correspondent à des repères anatomiques facilement identifiables. Les analyses statistiques ont montré que ces mutants avaient un crâne moins long mais pas moins large. L'analyse macroscopique du phénotype bucco-dentaire a également mis en évidence la présence d'une prémolaire surnuméraire en avant de la M1. Cette dent surnuméraire est très intéressante d'un point de vu évolutif car on sait qu'au court de l'évolution la formule dentaire de la souris a été réduite.

Une première analyse par microCT à une résolution de voxel de 25µm a été mené en collaboration avec le Pr Constantinesco et le Dr Choquet. Celle-ci nous a permis de mettre en évidence que cette prémolaire surnuméraire peut être de taille variable et qu'il semble exister un phénotype intermédiaire de fusion entre les cuspides t2 et t5. De plus le phénotype est très variable allant de la normalité à la présence de 4 dents sur chaque hémi arcade.

Nous avons ensuite souhaité préciser l'origine de cette prémolaire supplémentaire en analysant le champ molaire quant à sa forme, taille, répartition des éléments dents et des racines. Cette analyse a permis de mettre en évidence au niveau de la mandibule que les souris mutantes qui ne présentent que 3 molaires ont quand même un champ molaire réduit avec une M2 et une M3 plus petites. Les souris mutantes à 4 dents quant-à elle ont un champ molaire de taille équivalente avec réduction de la M1, la M2 et la M3. La prémolaire supplémentaire entraîne la présence d'une racine supplémentaire propre à cette dent **(Publication 5).** Au niveau du maxillaire, les molaires M1 et M2 sont toujours plus petites. Le champ molaire est plus petit lorsque le mutant a 3 molaires et plus grand lorsque le mutant a 4 dents. La première molaire a deux racines au lieu de 3 en présence d'une prémolaire surnuméraire **(Publication 5).**

Une collaboration initiée récemment avec le Pr Viriot et Pauline Marangoni nous a permis de scanner les souris à plus haute résolution avec un voxel de seulement 3µm. Cette meilleure résolution permettra de mieux préciser les anomalies au niveau des cuspides.

Très vite il est apparu intéressant d'utiliser l'outil transcriptomique également dans le cadre de cette analyse. C'est pourquoi nous avons comparé les molaires mandibulaires de souris mutées et sauvages.

La comparaison de 4 échantillons ne nous a pas permis de séparer les deux groupes d'échantillons par analyse en composante principale tandis que l'analyse de 8 échantillons permettait une bonne séparation des deux groupes et mettant en évidence 494 gènes différentiellement exprimés entre souris mutantes et sauvages. Cette nécessité d'avoir un nombre élevé d'échantillon démontre que le phénotype n'est pas seul à être hétérogène chez ces mutants mais que le transcriptome est lui aussi hétérogène.

La validation biologique de ces données par qPCR sur 3 échantillons en duplicata a montré que seul un gène reproduisait les résultats obtenus par analyse transcriptomique.

Ceci s'explique par l'hétérogénéité du transcriptome à laquelle il faudrait pallier. Nous avons donc voulu savoir s'il était possible de pallier à cette hétérogénéïté. Nous avons donc mis au point tous les paramètres nécessaire à la microinjection puis à l'électroporation d'un shRNA dans le tissu dentaire. Le shRNA a été microinjecté directement dans la molaire qui sera ensuite électroporée puis mise en culture organotypique durant 24h avec extraction de l'ARN. De manière intéressante cette méthode a permis de diminuer l'expression de Rsk2 de près de 4 fois et de confirmer l'expression différentielle des 2 gènes testés. L'inactivation uniquement au niveau molaire par isolation du tissu et la culture durant 24h seulement permettrait de s'affranchir de l'hétérogénéïté qui existe chez le mutant.

Publication 1 : De la transcription des gènes impliqués dans les dysplasies ectodermiques à la compréhension des anomalies dentaires associées

From the transcription of genes involved in ectodermal dysplasias to the understanding of associated dental anomalies

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Running title: Expression of ectodermal dysplasia-causing genes

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Abstract

Orodental anomalies are one aspect of rare diseases and become increasingly identified as diagnostic and predictive traits. To understand the rationale behind gene expression during tooth or other ectodermal derivatives development and the disruption of odontogenesis or hair and salivary glands formation in human syndromes we analyzed, in mouse, by *in situ* hybridization, the expression patterns of a set a genes (*Irf6, Nfkbia, Ercc3, Evc2, Map2k1)* involved in human ectodermal dysplasias. The expression patterns of *Nfkbia*, *Ercc3 and Evc2* during odontogenesis had never been reported previously. All genes were indeed transcribed in different tissues/organs of ectodermal origin, however for *Nfkbia, Ercc3, Evc2, Map2k1* signals were also present in the ectomesenchymal components of the tooth germs. These expression patterns were consistent in timing and localization with the known dental anomalies (teeth agenesis, microdontia, conical shape, enamel hypoplasia) encountered in syndromes resulting from mutations in those genes. They could also explain the similar orodental anomalies encountered in some of the corresponding mutant mouse models. Translational approaches in development and medicine are relevant to gain understanding of molecular events underlying clinical manifestations.

Introduction

Oral cavity and dental developmental anomalies are one aspect of rare diseases or syndromes. These diseases, that encompass about 8000 different entities, affect 4 million people in France and almost 25 millions in Europe. Per se and definition they affect less than a person among 2000 and are for 80% of them genetically driven. Among more than 7000 known syndromes, at least 900 have a dento/oro/facial phenotype and 750 display in their clinical synopsis a cleft lip/palate. This is understandable as the same genes and signalling pathways regulate palate, tooth development, and other systems organogenesis. Considering the dual origin of teeth (the oral ectoderm for the enamel organ and the derived ameloblasts synthesising enamel matrix, and ectomesenchyme originating from the cephalic neural crest cells for the mesenchymal part of the tooth including pulp tissues, odontoblasts and the periodontium), orodental anomalies are very often present in syndromes involving ectodermal derivatives like ectodermal dysplasias where abnormalities of tooth number (missing teeth), shape (conical crown, taurodontic molars) or hard tissues structure (enamel hypoplasia) are part of the phenotype. These anomalies, associated to the clinical synopsis of these syndromes become increasingly identified as diagnostic and predictive traits.

The aim of this study is to increase knowledge about genes involved in tooth development and anomalies in a syndromic context within the group of ectodermal dysplasias through the analysis of their expression patterns during mouse odontogenesis, as well as in other ectodermal organs like salivary glands and vibrissae. Using a bioinformatic approach, we selected known genes involved in ectodermal dysplasias within online databases (OMIM, Online Mendelian Inheritance in Man; Orphanet [Ayme, 2003; Ayme et al., 1998] and the literature [Gorlin et al., 2001; Hennekam et al., 2010]; PubMed (http://www.ncbi.nlm.nih.gov/pubmed/) with limited information about their expression pattern or role during odontogenesis. The online EURExpress in situ hybridization atlas (http://www.eurexpress.org; http://www.genepaint.org/ [Diez-Roux et al., 2011] was then used to identify genes showing detectable expression in tooth buds of embryonic day (E)14.5 mice. Five

genes were thus selected, for which we performed a detailed analysis of their expression patterns by in situ hybridization at various stages of mouse development.

Materials and Methods

Sample preparation

Mouse embryos/fetuses were collected at E12.5, E14.5, E16.5, and on the day of birth (hereafter referred to as E19.5), after natural matings between C57BL6 mice. For E14.5 and older samples, the whole head was embedded in OCT 4583 medium (Tissue-TEK, Sakura) and frozen on the surface of dry ice. E12.5 embryos were fixed overnight in 4% paraformaldehyde (pH 7.5, w/v) in phosphate-buffered saline (PBS), cryoprotected by overnight incupation in 20% sucrose (w/v) in PBS, and cryoembedded as described above. Cryosections (Leica CM3050S cryostat) at 10 µm were collected on Superfrost plus slides and stored at -80°C until hybridization. E12.5 and E14.5 samples were sectioned in a frontal plane, whereas other stages were sectioned sagittally.

Probe synthesis

All probes were synthesized from PCR-generated DNA templates kindly provided by the EURExpress consortium (http://www.eurexpress.org). The template sequences are given as online supplementary information (table S1).

Digoxigenin (DIG)-labeled antisense riboprobes were transcribed in vitro by incubation for 2 h at 37°C using 1 µg of the PCR product, 20 U RNA polymerase, 5x transcription buffer (Promega), 10x DIG RNA labeling Mix (Roche), 0.5 M DTT, 20 U RNAse inhibitor (Roche) in a 20 µl volume. The following RNA polymerases (Sigma) were used: T7 polymerase (*Ercc3* and *Evc2* probes), T3 polymerase (*Irf6* and *Map2k1* probes), and SP6 polymerase (*Nfkbia* probe). The reaction was stopped with 2 µl EDTA (0.2 M, pH 8), and RNA was precipitated with 1 µl yeast tRNA (10 mg/ml), 2.5 µl Li Cl (4 M) and 75 µl absolute ethanol, followed by an incubation for 30 min at -80°C and centrifugation at 12000 rpm (30 min at 4°C). The pellet was washed with 0.5 ml ethanol 70% and re-centrifuged. The supernatant was discarded and the pellet was allowed to dry. The probe was then diluted in 20 µl sterile H_2O. The quality of the probe was verified by electrophoresis in a 1% agarose gel. If no smear was observed

and the size was as expected, the probe was considered to be ready for use. The quantity of RNA was evaluated by a Nanodrop (ND-1000 Spectrophotometer, Labtech) and adjusted to 150 ng/µl in hybridization buffer, and stored at -20°C until use.

In situ hybridization

Slides were allowed to thaw to room temperature (RT) for 2 h. Then they were post-fixed on ice in 4% paraformaldehyde (diluted in PBS) for 10 min and rinsed in PBS. The hybridization buffer was composed of 50% deionized formamide, 10% dextran sulfate, 1 mg/ml yeast tRNA, 1x Denhardt's solution, 1x Salts (0.195 M NaCl, 5 mM Tris pH 7.2, 1.13 M NAH_2PO_4, $1H_2O$, 0.4 M Na_2PO_4, $12H_2O$, 5 mM EDTA pH 8). The probe was diluted in hybridization buffer at a concentration of 1 µg/ml. The probe mix was denaturated by a 10 min incubation at 70°C and placed on ice. One hundred µl was applied on each slide, which were covered by coverslips and allowed to hybridize overnight at 65°C in a humidified chambers. The slides were then washed two times for 30 min at 65°C in 1x standard saline citrate (SSC), 50% formamide, 0.1% tween-20, and two times for 30 min at RT in MABT buffer (1x MAB [Maleic acid buffer : Maleic Acid (Roche) 0.5M, NaCl 0.75M, NaOH to ph 7.5], 0.1% Tween-20).

Probe detection was performed using antibodies and reagents from Roche. Slides were incubated for 1 h at RT with a blocking solution (20% goat serum, 2% blocking reagent in MABT). The anti-DIG antibody was diluted 1:2500 in blocking solution, and 200 µl was added to each slide, which were covered by Parafilm and incubated overnight at 4°C. Slides were washed five times in MABT for 20 min and then two times 10 min in NTMT buffer (100 mM NaCl, 100 mM Tris pH 9.5, 50 mM $MgCl_2$, $6H_2O$, 0.1% Tween-20). Two hundred µl of freshly prepared staining solution [3.5 µl nitro-blue tetrazolium chloride (NBT) (Roche), 3.5 µl 5-bromo-4-chloro-3'-indolyphosphate p-toluidine salt (BCIP) (Roche) in NTMT buffer] was placed on each slide, covered by a Parafilm and incubated overnight in the dark at RT. The staining solution was changed every day and when signal was optimal the slides were rinsed two times during 5 min in NTMT Buffer. The slides were further rinsed by PBS and water, allowed to dry overnight, and mounted in Coverquick 2000 mounting medium (Labonord).

Results

Selection of candidate genes

As described in the Introduction, five genes were selected on the basis of their involvement in human rare diseases and orodental anomalies [Allanson et al., 2011; Courtois et al., 2003; Kondo et al., 2002; McDonald et al., 2007; Ruiz-Perez et al., 2000; Rutledge et al., 2010; Vermeulen et al., 1994; Vieira et al., 2007; Weeda et al., 1997] (for an example, see fig. 1), and preliminary observation of *in situ* hybridization signals in the developing tooth buds and/or oral ectoderm of E14.5 mouse fetuses in the EURExpress/GenePaint databases (http://www.eurexpress.org; http://www.genepaint.org/ [Diez-Roux et al., 2011]. A brief description of each gene is given hereafter, with table 1 providing additional information on the proteins encoded, mode of inheritance, and symptomatology of the corresponding syndromes or rare diseases.

Irf6 encodes an interferon regulatory transcription factor. It was recently reported that p63 and Irf6 interact epistatically in palatogenesis, and that Irf6 is a target of p63 in the midface [Thomason et al., 2010]. Members of the Irf family are known to activate the canonical NF-κB pathway [Hiscott, 2007].

Nfkbia (formerly known as *Ikba*: NF-κB inhibitor alpha) is a member of the NF-κB inhibitor family. Mutations in this gene were discovered in autosomal dominant anhidrotic ectodermal dysplasia with T-cell immunodeficiency [Courtois et al., 2003; Lopez-Granados et al., 2008].

Ercc3, the excision repair cross-complementing 3/xeroderma pigmentosum B (ERCC3/XPB) DNA helicase, a subunit of the transcription factor TFIIH complex, is also involved in the DNA nucleotide excision repair (NER) mechanism.

Evc2 is a positive regulator of the Hedgehog signalling pathway, and encodes a cilia transmembrane protein located at the basal body of primary cilia. It is also found in the nucleus, where its function remains to be clarified [Blair et al., 2011].

Map2k1 (formerly known as *Mek1* or *Mapkk1*), is a member of the dual specificity protein kinase family which acts as a mitogen-activated protein (MAP) kinase kinase and is involved in many cellular processes such as proliferation, differentiation, transcriptional regulation and development. Developmental syndromes involving dysregulation of the RAS/MAPK pathway are referred to as RASopathies [Rauen et al., 2010].

In situ hybridization expression analysis

Tooth and oral cavity development

Table 2 provides a summary of the various transcript distributions observed in developing tooth tissues, which are described below.

Irf6 transcripts were detected in the epithelial compartment of the teeth: at E12.5 in the oral ectoderm and dental lamina (fig. 2A,B); at E14.5 throughout the whole enamel organ, especially in the future epithelial loop area (fig. 2C,D); at E16.5 in the outer dental epithelium, the stellate reticulum, and more strongly in the stratum intermedium and the epithelial loops (fig. 2E,F); and at E19.5 in the outer dental epithelium, the stratum intermedium, the preameloblasts, the inner dental epithelium and both epithelial loops (fig. 2G,H). Areas containing highly proliferative cells (such as the epithelial loops or stem cell niche) were strongly labelled.

The *Nfkbia* probe labelled the oral ectoderm and dental lamina at E12.5 (fig. 3A,B). At E14.5 the transcripts were scattered in the enamel organ of all teeth, with a particularly strong labeling of the enamel knot (fig. 3C,D). The epithelium lining the palatal shelves was labelled prior to contact (data not shown). At E16.5 at the bell stage, the epithelial loops, inner dental epithelium and outer dental epithelium were most prominently labelled (fig. 3E,F). At E19.5 the transcripts were localized in the inner and outer dental epithelium, in the preameloblasts area and in the epithelial loops (for the second molars), as well as in odontoblasts (fig. 3G,H).

Ercc3 expression was detected in the oral ectoderm at E12.5 and E14.5 (as seen on cap stage lower incisors fig. 4A and data not shown). *Ercc3* was expressed in the enamel organ and more weakly in the mesenchyme both in molar and incisor at E14.5 (fig. 4A) and E16.5 (fig. 4B and data not shown). The in situ hybridization signal was quite faint and dotty. At E19.5 the labeling was clearly asymmetric and, for the incisors, was higher in the labial area within the epithelial loop and facing mesenchyme. The preameloblasts were also labelled (fig. 4C). The most differentiated epithelial cells were devoid of signal. At E19.5, the signal was visible both in the first and second molars in the inner dental epithelium, the preameloblasts, and the epithelial loops (data not shown). In the second molars it was widespread in the mesenchyme, whereas it was more focused to the base of the cusps and the area facing the epithelial loops for the first molars. In the inner dental epithelium the

signal was clearly visible in the basal region of cells in contact with the basement membrane.

Evc2 was expressed in the oral ectoderm and the dental lamina at E12.5 (data not shown). At E14.5 the signal was observed in the molar cap both in the enamel organ and within the ectomesenchyme (fig. 4D). The molar gubernaculum was also labelled. At E16.5 cartilaginous tissues including Meckel cartilage were strongly expressing this gene (data not shown). A faint signal was detected in the incisor and molar mesenchyme. At E19.5 the transcripts were detected in the mesenchyme and in the preodontoblasts/odontoblasts layers (data not shown).

Map2k1 was expressed in the oral ectoderm and the epithelial dental lamina at E12.5 (data not shown). The signal was then apparent both in the epithelial and mesenchymal compartments of the cap stage teeth at E14.5 (fig. 4E and data not shown). At E16.5, in addition to cartilaginous areas, the transcripts were detected in the mesenchyme and the epithelial inner, outer and loop areas for both the incisors and molars (fig. 4F and data not shown). At E19.5 the transcripts were mainly mesenchymal (fig. 4G).

Salivary glands and vibrissae

The expression patterns of *Irf6*, *Nfkbia*, *Ercc3*, *Evc2* and *Map2k1* are further illustrated in two organs in which specialized ectodermal-derived tissues are developing, the salivary (submandibular) glands and the vibrissae follicles (fig. 5). With the exception of *Evc2*, all genes displayed distinct expression in the epithelial cell component of the developing salivary glands (fig. 5A-E). *Irf6* and *Map2k1* were particularly strongly expressed in the epithelial layer of the vibrissae follicle shafts, whereas *Nfkbia* displayed weaker discontinuous labeling, and *Ercc3* and *Evc2* no obvious expression, in this epithelial compartment (fig. 5F-J).

Discussion

Tooth development is embedded within craniofacial development. It originates from pluripotent cephalic neural crest cells which migrate towards the first pharyngeal arch, triggering in combination with mesodermal cells the development of many craniofacial structures [Cobourne and Mitsiadis, 2006; Knight and Schilling, 2006;

Noden and Schneider, 2006]. Odontogenesis leads to specific crown and root morphogenesis for each type of tooth (incisors and molar for the mouse), to enamel organ histomorphogenesis and to terminal cytodifferentiation of odontoblasts, ameloblasts and cementoblasts. Evolutionary study of mammals is often focused on detailed analysis of teeth shapes. Molecular patterning may influence dental evolution via differences in gene expressions correlated with morphological variations [Jernvall et al., 2000; Plikus et al., 2005; Salazar-Ciudad and Jernvall, 2002]. The continuous and progressive stages of odontogenesis have classically been divided into the dental lamina, placode, bud, cap and bell stages, root formation and tooth eruption. Tooth development is a dynamic process mediated by epithelio-mesenchymal interactions between ectomesenchymal cells originating from cephalic neural crest cells and the first pharyngeal arch ectoderm [Peters and Balling, 1999; Thesleff, 2003b; Thesleff and Aberg, 1999; Tucker and Sharpe, 2004; Tucker and Sharpe, 1999]. These cells contribute to the formation of the dental mesenchyme, the dental pulp, odontoblasts, dentine matrix, cement and periodontium [Chai et al., 2000; Miletich and Sharpe, 2004]. Extracellular matrix (i.e. basement membrane, predentine, dentine) participates in odontogenesis either as a substrate for interaction with receptors of the plasma membrane, or as a putative reservoir of endocrine or paracrine factors like peptide growth factors. Tooth mophogenesis is under strict genetic control and the participating genes are being discovered at an increasing speed. By 2008, more than 300 of these genes had been listed in the database created by P. Nieminen (Helsinki University, Finland) gathering expression patterns at various stages of odontogenesis from worldwide laboratories [Nieminen et al., 1998] (http://bite-it.helsinki.fi).

Developmental dental anomalies may exist in isolation or may be associated with extraoral clinical manifestations in syndromes, and can be of genetic origin or due to the action of teratogens [Alaluusua, 2006; Alaluusua and Lukinmaa, 2006; Alaluusua et al., 1999; Berdal, 2003; Koch, 2003; Weerheijm, 2003]. They are correlated to specific genetic and developmental biology problematics [Bloch-Zupan, 2004; Bloch-Zupan et al., 2012] such as the embryonic origins of dental cells, the patterning of the dentition, the defined location of tooth development, tooth identity, specific morphogenesis, histogenesis, terminal differentiation of odontoblasts and ameloblasts, dentine and enamel matrix synthesis followed by mineralization, root

and periodontium formation and eruption of teeth [Salazar-Ciudad and Jernvall, 2002; Thesleff, 2003a; Thesleff, 2003b; Thesleff, 2006]. Any interference with these developmental processes can lead to clinical anomalies and defects [Aldred et al., 2003; MacDougall, 2003; Thesleff, 2000; Thesleff, 2006] and some may even lead to tumours from dental epithelial cells [Papagerakis et al., 1999]. Ectodermal dysplasias (ED) are disorders characterized by alterations in two or more ectodermal structures affected in the following decreasing order of frequency: hair, teeth, nails, sweat glands, salivary glands and any other ectodermal appendage [Visinoni et al., 2009]. So far, identification of a wide variety of genes has led to the molecular characterization of around 30% of ED and to a reconsideration of their clinical classification. The genes selected in the present study are involved in various rare diseases belonging to the ED spectrum: *IRF6* in Van der Woude syndrome [Kondo et al., 2002], non syndromic cleft lip/palate [Desmyter et al., 2010; Rutledge et al., 2010; Vieira et al., 2007], hypodontia [Vieira et al., 2007]; *NFKBIA* in ED with immune deficit [Courtois et al., 2003; Lopez-Granados et al., 2008]; *ERCC3* in Xeroderma pigmentosum [Bootsma et al., 1995] and trichothiodystrophy [Weeda et al., 1997]; *EVC2* in Ellis Van Creveld syndrome [Galdzicka et al., 2002]; *MAP2K1* [Rodriguez-Viciana et al., 2006] in cardiofaciocutaneous syndrome.

While the same genes are indeed involved in different diseases with overlapping phenotypes, it is also interesting to note that mutations in different genes may account for similar diseases. Cardiofaciocutaneous syndrome, for example, is caused by gain of function mutations in *BRAF* (7q34), *KRAS2* (12p12.1), *MEK2* (7q32) and *MAP2K1* (15q21) [Allanson et al., 2011]; Ellis-van Creved syndrome is caused by mutations in *EVC* and *EVC2* (4p16.2) [Ruiz-Perez et al., 2003].

Our expression *in situ* hybridization data confirmed the transcription of the selected murine genes in different tissues/organs of ectodermal origin (teeth, salivary glands, vibrissae). However *Nfkbia, Ercc3, Evc2 and Map2k1* signals were also present in the ectomesenchymal compartments of the tooth germs. Indeed ED are groups of conditions presenting similar ectodermal signs, but the associated defects may primarily concern mesenchymal structures, for example bone in Ellis-van Creveld syndrome. Expression of *Irf6* was previously described [Blackburn et al., 2012; Knight et al., 2005; Kondo et al., 2002] as occurring in the ectoderm covering the facial processes during the formation of the lip, the primary palate, and the

secondary palate from day 14.5-E15 mouse embryos. Recently, information was provided about the localization of *Irf6* transcripts from E10.5 to E18.5 during odontogenesis [Blackburn et al., 2012]. No precise description at the tissular level (outer dental epithelium, stratum intermedium, inner dental epithelium, preameloblasts) was given, however. For example the strong signal and localization within the future epithelial loops area of the cap, then bell stage teeth (i.e. the proliferating area) were not described.

MAP2K1 immunolocalization was described in human third molar tooth germs enucleated for othodontic reasons at the early crown mineralization stage. Strong reactivity was observed in inner dental epithelium, and weak to moderate staining was visible in outer dental epithelium, stratum intermedium and stellate reticulum [Kumamoto et al., 2004]. These results are in agreement with the expression pattern described for *Map2k1* in the present paper, except for the localization within the stellate reticulum and stratum intermedium sometimes difficult to assess.

The expression patterns of *Nfkbia*, *Ercc3 and Evc2* during odontogenesis had never been reported previously. The expression patterns described herein are consistent in timing and localization with the known dental anomalies (hypodontia/oligodontia, smaller and conical teeth, enamel hypoplasia) occurring in patients [Clauss et al., 2008; Gros et al., 2010]. Disruption of molecular/biological events at early stages of odontogenesis (dental lamina, transition bud to cap stage) are linked to missing teeth, at the cap stage to anomalies of tooth shape and size, at the bell stage and terminal cytodifferentiation with anomalies of tooth structure (dentine and enamel). It is also interesting to notice that cleft lip and palate are symptoms present in the clinical synopsis of Ellis Van Creveld and Van der Woude syndromes and that the corresponding genes (*Evc2* and *Irf6*) are expressed in the palatal medial epithelial edge and seam (data not shown).

Mouse models generated by targeted gene mutations often mimick the phenotypes encountered in corresponding human rare disases [Fleischmannova et al., 2008]. The *Ercc3* knock-in mice [Andressoo et al., 2009] recapitulate the UV sensitivity typical for Xeroderma Pigmentosum, but fail to show overt Cockayne syndrome features, i.e. no observation or mention of an orodental phenotype was described for the mutant mice. *Evc* null-mutant mice develop an EVC-like syndrome, including short ribs, short limbs and dental abnormalities [Ruiz-Perez et al., 2007].

Mutants showed small dysplastic incisors, and conical lower molars. The size of the first molar was reduced, and enamel defects were visible. Null-mutant mice for *Irf6* have abnormal skin, limb and craniofacial development [Ingraham et al., 2006]. Histological and gene expression analyses indicate that the primary defect is in keratinocyte differentiation and proliferation. The cleft palate 1 (*clft1*) mutant mouse also displays a mutation in *Irf6* (Van der Woude syndrome mutation) [Ingraham et al., 2006; Stottmann et al., 2010]. The mandible in the *Irf6* mutant was smaller with a narrower angle than in the wild-type, and the snout was also shorter with cleft palate [Ingraham et al., 2006]. Protruding incisors were described in *Irf6* mutant mice, pointing toward an important role of Irf6 in tooth epithelial invagination [Blackburn et al., 2012]. The disruption of the murine *Map2k1* gene leads to an embryonic lethal phenotype at mid-gestation from an abnormal placenta development and vascularization [Bissonauth et al., 2006]. *Ikbia*-deficient mice show skin defects and die at 9 day post-natally with severe widespread dermatitis and increased levels of TNF-alpha mRNA in the skin [Klement et al., 1996]. These mice develop a severe hematological disorder [Rupec et al., 2005]; however, no features reminiscent of the ectodermal dysplasia and dental abnormalities observed in human patients with *NFKBIA* mutations were described in mice.

Conclusions

Orodental anomalies of transgenic mouse model are often unsufficiently described, thus making it difficult to fully compare mouse and human disease phenotypes. However the mouse orodental anomalies quite frequently recapitulate the counterpart human malformations belonging to the clinical synopsis of syndromes, confirming the informative role of these models to study tooth development and anomalies. This paper described the expression pattern of murine homologues of human genes involved in tooth development and disease, focusing on the ectodermal dysplasia spectrum. It reinforces the utility of translational approaches in development and medicine to gain understanding of the molecular events underlying the clinical manifestations, especially the orodental anomalies accompanying these rare diseases.

Acknowledgments

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Figure Legends

Fig. 1. Orodental phenotype observed in an ectodermal dysplasia, here hypohidrotic ectodermal dysplasia shown as an example, includes missing teeth (hypodontia, oligodontia), microdontia and abnormalities of tooth shape (conical teeth). The anomalies of tooth number, shape and size are associated and represent a continuum of anomalies.

Fig. 2. *In situ* hybridization analysis of *Irf6* gene expression in the developing teeth and oral cavity. Selected sections are shown at E12.5 (A: mandible and incisor dental lamina; B: palate, with vertical palatal shelves on each side of the tongue, and molar dental lamina), E14.5 (C: lower and upper cap stage incisors; D: palate and lower and upper cap stage first molars), E16.5 (E: bell stage lower incisor; F: mandibular first molar) and E19.5 (G: lower incisor; H: first and second molars). All section planes are coronal (frontal), except for E,F,G,H (sagittal). Abbreviations: DL dental lamina, DP dental papilla, EL epithelial loop, EO enamel organ, Gu gubernaculum, IDE inner dental epithelium, In incisor, M1 first molar, M2 second molar, Md mandible, ODE outer dental epithelium, PreAm preameloblasts, SI stratum intermedium, SR stellate reticulum, To tongue.

Fig. 3. *In situ* hybridization analysis of *Nfkbia* expression in the developing teeth and oral cavity. The selected sections show the mandibular incisor dental lamina (A), posterior tongue/pharyngeal region and molar dental lamina (B) at E12.5, the lower incisors (C) and the first molar caps (D) at E14.5, the lower incisor (E) and the first mandibular molar (F) at E16.5, and the lower incisor (G) and first and second molars (H) at E19.5. All section planes are coronal, except for E,F,G,H, which are sagittal. Abbreviations: DL dental lamina, EK enamel knot, EL epithelial loop, IDE inner dental epithelium, La labial, Lin lingual, M1 first molar, M2 second molar, Md mandible, ODE outer dental epithelium, PreAm preameloblasts, To tongue.

Fig. 4. *In situ* hybridization expression patterns observed for *Ercc3* (A-C), *Evc2* (D), and *Map2k1* (E-G). The selected sections show the lower cap stage incisors (A,E) and lower first molar (D) at E14.5, the mandibular incisor (B,F) at E16.5, the lower incisor (C) and first and second molars (G) at E19.5. All section planes are sagittal, except for A,B,E which are frontal. Abbreviations: DP dental papilla, EL epithelial loop, EO enamel organ, Gu gubernaculum, IDE inner dental epithelium, PreAm preameloblasts, La labial, Lin lingual, M1 first molar, M2 second molar.

Fig. 5. Expression of ectodermal dysplasia-related genes in the E16.5 developing submandibular salivary gland (A-E), and vibrissae follicles (F-J). In all cases where expression is detected (A-C,E-G,J), it is selectively seen in the epithelial compartment.

Table captions

Table 1. Selected genes and corresponding rare diseases within the spectrum of ectodermal dysplasias.

Table 2. Summary of the *in situ* hybridization data during odontogenesis.
Abbreviations: In, incisor ; M1, first molar ; M2, second molar ; M3, third molar;
+ indicates presence of signal.

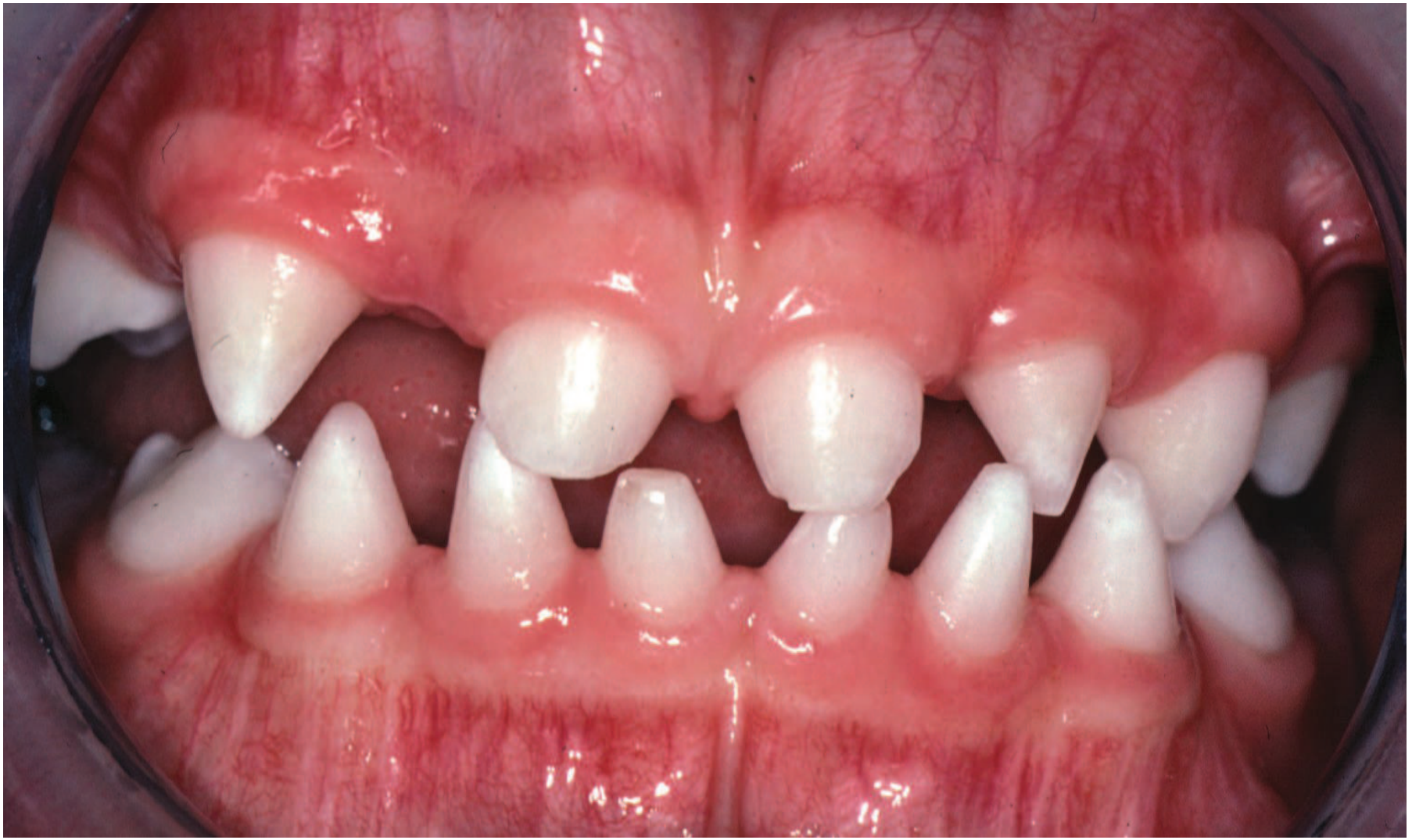

Figure 1

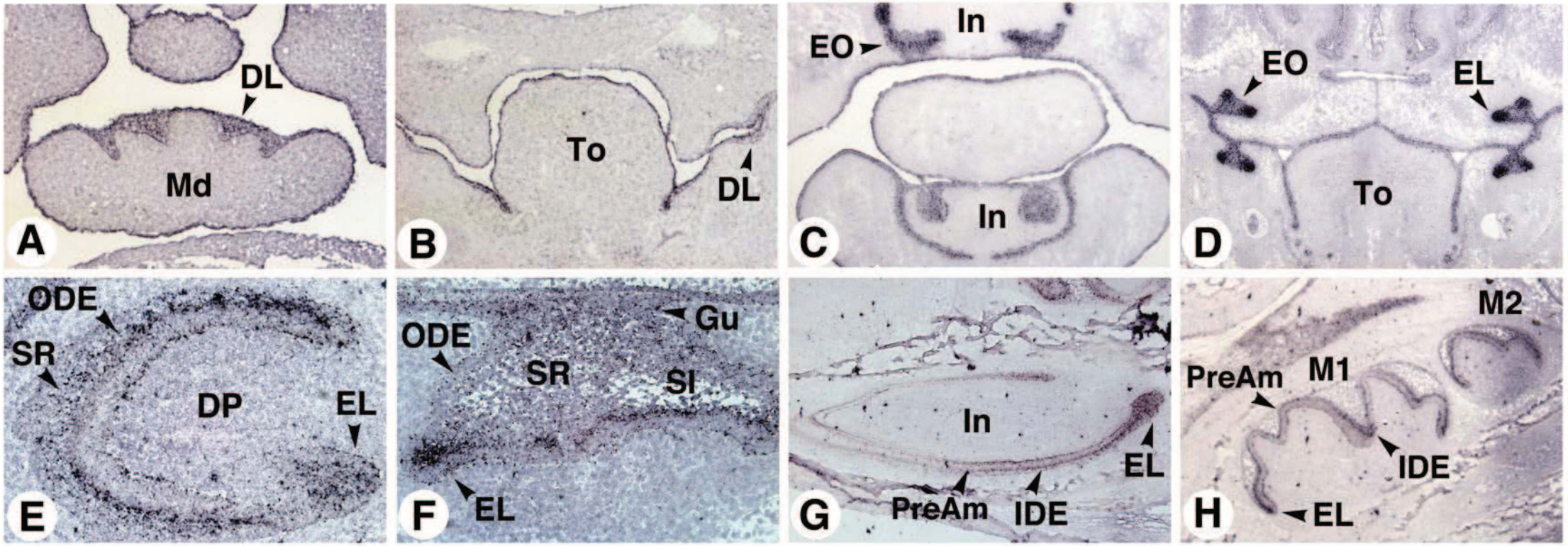
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Figure 2

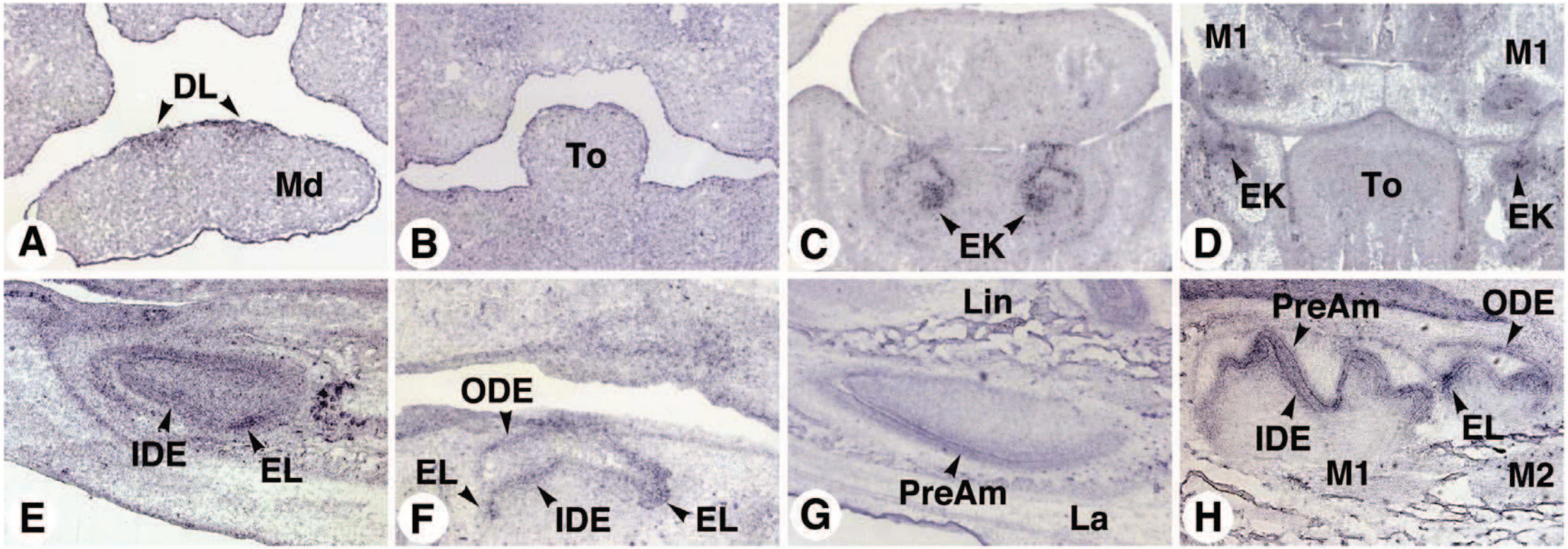
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Figure 3

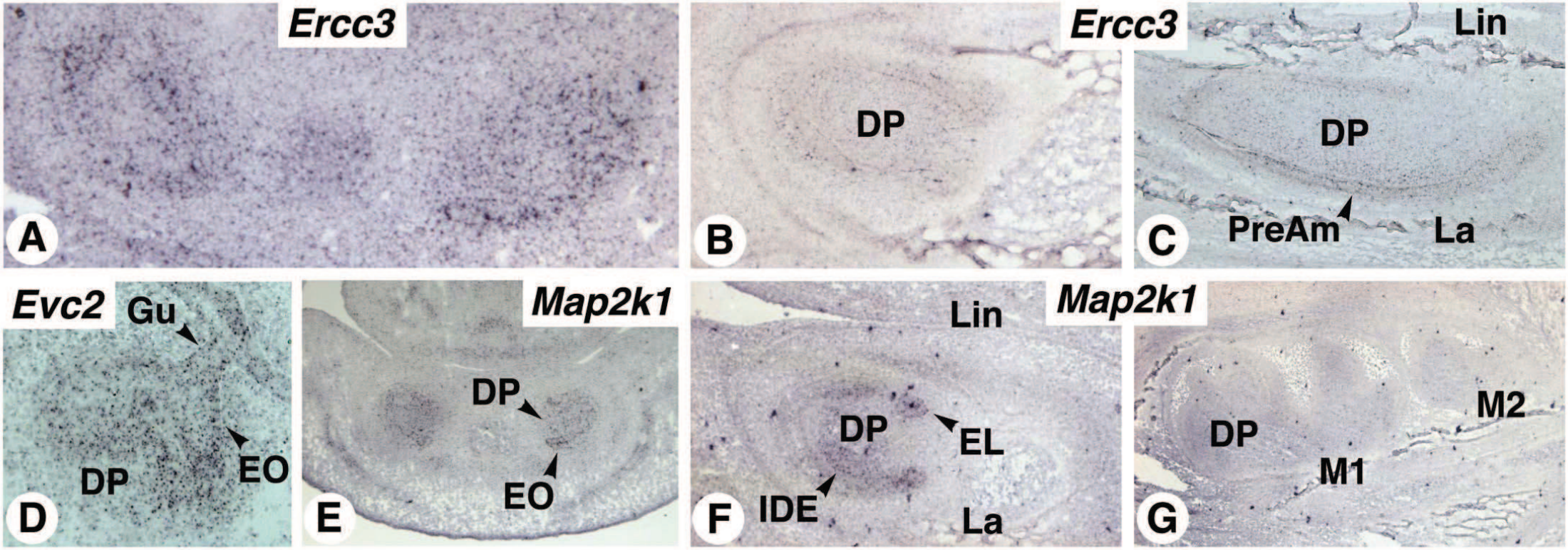
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Figure 4

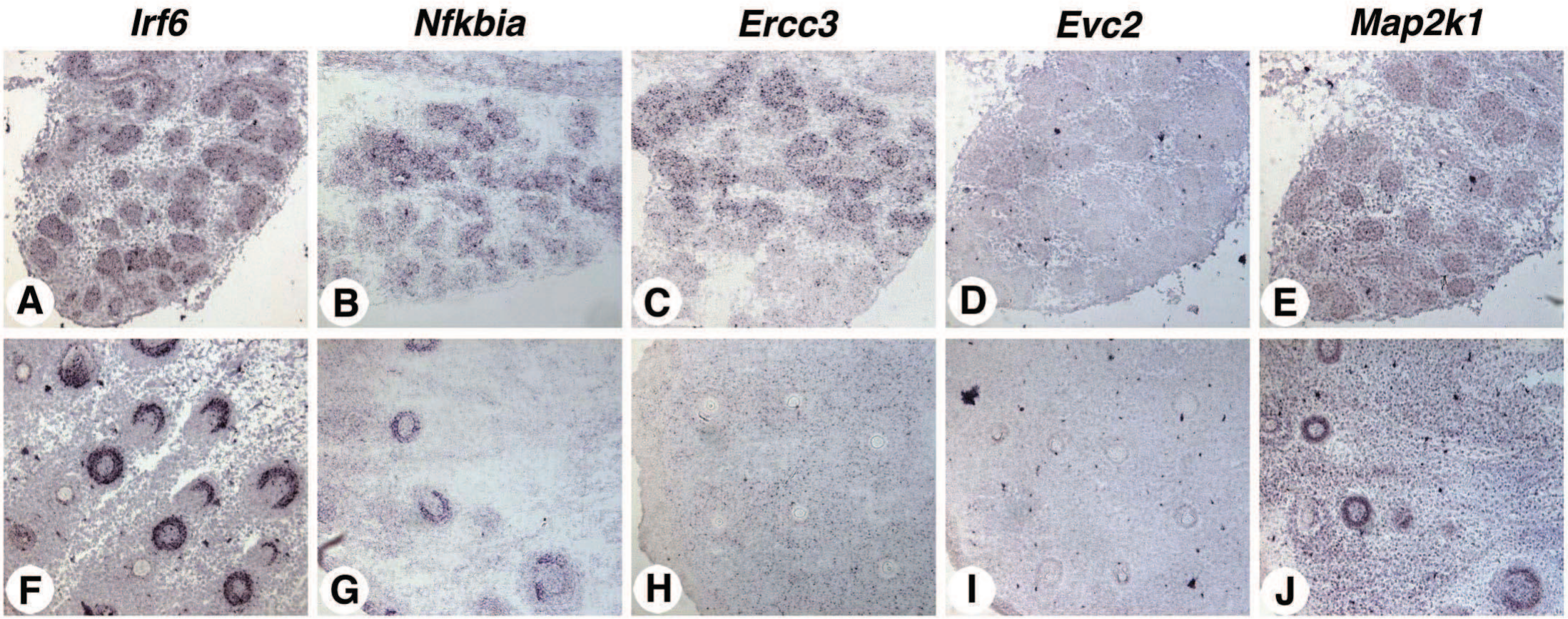

Figure 5

Table 1. Selected genes and corresponding rare diseases within the spectrum of ectodermal dysplasias

Gene symbol (Human) Full name	Rare disease or syndrome *Main clinical features*	OMIM ref. #	Gene product	Mode of inheritance Chromosomal location	Orodental phenotype	Selected references
IRF6 Interferon regulatory factor 6	Van der Woude syndrome (Popliteal pterygium syndrome) Non syndromic cleft palate Hypodontia *Lower lip pits and/or sinuses, cleft lip and/or cleft palate*	#119300 #119500	Transcription factor whose function is related to epidermal development	Autosomal dominant 1q32-q41	- hypodontia with preferential tooth agenesis of incisors and premolars - lip pits - lower lip bulges might represent a discrete sign - lip pits and cleft lip/palate (~ 30%) - lip pits and cleft palate or submucous CP (~ 30%) - lip pits without cleft (~ 65%) - cleft lip and palate - cleft or bifid uvula - syngnathia - narrow high arch palate - ankyloglossia	[Kondo et al., 2002] [Desmyter et al., 2010; Rutledge et al., 2010] [Vieira et al., 2007]
NFKBIA NF-kappa-B (Nuclear factor of kappa light chain *gene* enhancer in B cells) inhibitor alpha	Anhidrotic ectodermal dysplasia with immune deficit *Recurrent infections, T cell deficiency* *Dry, rough skin (lack of sweat glands), sparse hair*	#612132	Member of the NF-κB inhibitor complex	Autosomal dominant 14q13	- hypodontia, oligodontia - cone-shaped teeth	[Courtois et al., 2003; Lopez-Granados et al., 2008]
ERCC3 Excision repair cross-complementing rodent repair deficiency, complementation group 3	Xeroderma Pigmentosum complementation group B (XPB) *Sun sensitivity, pigmentation abnormalities* *Risk of malignancy* *Sometimes associated*	#610651 #601675	Helicase subunit of transcription/DNA repair factor TFIIH	Autosomal recessive 2q13.3	- dysplastic teeth, - enamel hypoplasia	Xeroderma Pigmentosum : [Bootsma et al., 1995] Trichothiodystrophy : [Weeda et al., 1997]

	with a Cockayne-like phenotype Trichothiodystrophy (TTD) *Brittle hair and nails, ichthyotic skin, growth retardation learning deficit, photosensitivity*					
EVC2 Ellis-van Creveld syndrome 2	Ellis-van Creveld syndrome *Congenital cardiac defects* *Short limbs, short ribs, Postaxial polydactyly, and dysplastic nails*	#225500	Cilia transmembrane protein - positive regulators of shh signaling	Autosomal recessive 4p16	- natal or neo-natal teeth - hypodontia - supernumerary teeth - microdontic and conoid teeth - shovel shaped incisor - talon cusp - taurodontism - hypoplastic enamel - delayed teeth eruption - premature exfoliation - teeth transposition - absent maxillary mucobuccal fold - accessory labiogingival frenula - gingival hypertrophy - partial cleft lip - malocclusion	Ellis Van Creveld syndrome : [Galdzicka et al., 2002]
MAP2K1 Mitogen activated protein kinase kinase1	Cardio-facio-cutaneous syndrome *Distinctive face* *Heart defects* *Intellectual deficit* *Ectodermal abnormalities: sparse hair, skin lesions, generalized ichthyosis*	#115150	Mitogen-activated protein (MAP) kinases, also known as extracellular signal-regulated kinases (ERKs), interact in a common RAS/ERK – MAPK pathway that regulates cell differentiation, proliferation, and apoptosis	Autosomal dominant 15q22.31	- dysplastic teeth	[Rodriguez-Viciana et al., 2006]

Table 2. Summary of the *in situ* hybridization data during odontogenesis

Developmental stage Tooth tissues	*Irf6*				*Nfkbia*				*Ercc3*				*Evc2*				*Map2k1*			
	In	M1	M2	M3	In	M1	M2	M3	In	M1	M2	M3	In	M1	M2	M3	In	M1	M2	M3
E12.5																				
Oral epithelium	+	+			+				+				+				+	+		
Dental lamina	+	+			+	+			+	+			+				+	+		
Mesenchyme									+	+										
E14.5																				
Gubernaculum	+	+			+	+								+			+	+	+	
Enamel organ	+	+			+	+			+	+			+	+			+	+	+	
Inner dental epithelium	+	+			+	+			+	+			+	+			+	+	+	
Enamel knot	+	+			+	+								+			+	+	+	
Dental papilla									+	+			+	+			+	+	+	
Dental sac																				
E16.5																				
Gubernaculum	+	+				+												+		
Outer dental epithelium	+	+			+	+											+	+		
Stellate reticulum	+	+																		
Stratum intermedium	+	+																		
Inner dental epithelium	+	+			+	+			+	+	+						+	+		
Epithelial loop	+	+			+	+											+	+		
Dental papilla									+	+	+		+	+			+	+		
E19.5 (newborn)																				
Outer dental epithelium	+	+	+			+	+													
Stellate reticulum			+																	
Stratum intermedium	+	+	+																	
Inner dental epithelium	+	+	+			+	+			+	+									
Preameloblasts	+	+			+	+			+	+										
Ameloblasts																				
Epithelial loop	+	+	+				+		+	+	+									
Preodontoblasts														+						
Odontoblasts					+	+								+						
Dental papilla									+	+	+		+	+	+		+	+	+	
Dental sac																				

Table S1 : Template DNA sequences used to generate riboprobes

Ercc3
Template size : 1027 bp
Amplicon size : 494 bp (partial)
Sequence :
GCGGTGTGCCGGCCTGCCTGCATCTAGAGCTGTTATGGGCAAAAGAGATCGAGTGGACCGCGA
CAAAAAGAAATCCAAGAAGAGGCAGTATGAAGAGGAAGAGGAAGACGAAGACGACATTCCTGGG
AACGAGTCTCAGGAGGCGGTGCCCTCCGCCGCTGGGAAACAGGTGGACGAGTCCAGCACCAAA
GTGGATGAGTATGGAGCAAAGGACTACAGACAGCAGATGCCACTAAAGGGTGACCATACCTCCA
GGCCCCTCTGGGTGGCTCCTGATGGCCACATTTTCTTGGAAGCCTTCTCTCCAGTTTACAAATAT
GCCCAAGACTTCCTGGTGGCAATTGCAGAGCCGGTGTGCCGGCCTACTCATGTACATGAATACA
AGCTAACCGCCTACTCCCTCTATGCAGCGGTCAGTGTTGGGCTGCAAACCAGTGACATTACTGAG
TACCTCAGAAAGCTCAGTAAGACTGGAGTTCCTGATGGAATTATCC

Evc2
Template size : 4500 bp
Amplicon size : 4107 bp (full length)
Sequence :
CCACGCGTCCGCGCGACTCTGCGGGGCCTGTGTGGGGTCTGTAGCCACCAGATGGGGGCGAC
GGGCCCCACGGGCGCTGGGGGACGAGCCACGTGGGTGCTGGCTGGGAATATCCTGGCCGCTG
CCCTGGTACTGGGCTCGGGTCCGCGTGCACTTCCACCATCCTTCCCTGCCCTGGGCCCAGGGT
CACCATCTCGGCCCGGCCCAGCAGGACCCTGGGCAAGCTCGCAGTACAGCGATATTTCAAGAGA
AGCCCGAGGTCCCTTTGAGAATGGAGTGATATTTCAAAAATGCTCACTGGTGTCTGGGCAGAGC
GAATCGCAGACCATGCACGTGCAGCTGTCCGTGAACAACACCAGGACGCCCACATCAGTCAACC
TTTCGAACCTGCTCGTGCTAGATGAGATCACTGGCCTTGCTGTGAAGGAGTCGCCTGGAAATAAT
ACTCAGGATGGAATCCAGACTTTCAGAAAGAGCTTTCTGCAAGTGGGAGAATGCTACTCTGTCAG
CTACACAGCCTCGCTGGACCCCACAGCCCTTGGGACTGGGGAGAGCCTGGATCTTCCTGCCCG
GCTCATCTTTCAGAGCCCCTCACAGAACAGAACTCAGCTAAAAGCCCCCTTTACCATCACAGTGG
AAGAAAAGATAATGGTTCTTCCTAACCATGGTCTTCACGCAGCAGGCTTCATCGCAGCCTTCCTC
ATCTCCCTCCTCCTGACCGTGGCAGCCCTGTTCTTCTTGGCGAGGGGTCGATGTCTGCAGGGAG
GCATGCTGTCCAGATGCCGGATTCAGCATCCTGAGAACAAGCTGGAGCCCTCGCCCTTCACCTC
TGCTAACGGTGTGAGCCAGGACCTTTCCCTCAATGACCAAGTGGTTGCCATCCTGACTTCTGAGG
AGCCTGGGAGCATGCTTCAAGCCCTAGAAGAGTTGGAGATCGCAACCCTGAATCAGGCAGATGC
GGATCTGGAGGCTTGTCGAAACCAGATTAGTAAGGACATCATTGCCCTTTTGATGAAGAATCTGG
TCAGCGGTGGTCACCTCTCTCCGCAAACAGAAAGGAAGATGGCCGCTGCTTTCAAAAAGCAGTTT
CTGCTGTTGGAAAATGAAATACAGGAGGAGTATGAGCGGAAGATGTTGGCACTGACGGCAGAGT
GTGACCTAGAGATGAGGAAGAAGACAGAAAACCAGTACCAGAGGGAGATGGTGGCCATGGAGG
AAGCAGAAGAGGTGTTGAAGAGAGTTAGCGAGAGGTCTGCTGCCGAGTGCAGCAGCCTTTTGCG
GACCCTCCATGGGCTGGAGCAGGAGGACATGCAGAGGTCCCTTACTCTGGACCAAGCCGAGGA
CTTTGCTCAAGCACACAGGCAGCTGGCTGTTTTCCAGAGGAATGAATTACACAGCATCGTCTATA
CCCAGATACAGAGTGCTGTCTCCAAAGGGGAACTGAGGCCTGAGGTGGCTAAAATGATGCTGCA
AGACTACTCTAAAACACAGGAGAGTGTAGAAGAGCTGATGGACTTCTTCCAGGCCACCAAGCGAT
ACCATCTCAGCAAGAGGTTTGGCCACAGAGAGTACCTGGTCCAGAGACTGCAGGCCATGGAGAC
CCGGGTGCAAGGGCTGCTGAACACCGCGGCTACCCAGCTAACAAGCCTCATCCATAAACATGAG
AGGGCTGGCTACTTGGATGAAGATCAGATGGAAACGCTATTGGAGCGTGCACAGACAGAAACCT
TTTCTATAAAACAGAAGCTGGACAATGACTTAAAGCAGGAAAAGAAAAGGCTCCACCAGAGATTA
ATAACCAGGAGGCGGCGAGAGCTTCTGCAGAAGCACAAGGAGCAGCAGAAGGAGCAGGTGTCC
CTTGGCGAGGCCTCCAGCACCGCGGAGGACGCAGTCCAGTACCTGCACCAGTGGAGGAGCGTG
ATGGCCGAGCACACCGCTGCCCTGGAGGAGCTGCAGGAACGTCTGGACCAGGCTGCCCTGGAT
GACCTCCGGGTCCTGACTGTGTCGCTGTCGGAGAAAGCCACGGAAGAGCTGAGGCGCCTGCAG
AGCACGGCCATGACACAGGAGCTGCTGAAGCGCAGTGCTCCCTGGCTCTTCCTGCAGCAGATCT
TAGAGGAGCACAGCCGGGAGTCTGCGGCTCGCACCACGCAACTGGAGGCAGAGGAGAGGGAA
CGTGGCCAGGAGCTGGTGCAGGGCGTGAGGCAGAGGCTACAGCAGGATGCACTGGAGGCCTA
CACGGAAGAGCAGGCAGAGCTGAGGCACTGGGAGCACTTGGTGTTCATGAAACTCTGCTGTGCG
GCCATCTCATTGTCTGAAGAAGACCTGCTCCGGGTAAGGCAGGAGGCACAGGGCTGCTTCAGCC
AGCTGGACCGGAGCTTGGCCCTCCCCAGAGTCCGTGCACGAGTGTTGCAGCAGCAGGCGCAGA
TGGCCTGGCGGGAAGCAGAGTTTCGGAAACTGGACCAGGCCCTGGCTGCGCCTGAGCTGCAGT

CCAAGGCAAGAAAGCTGCGTTCCAAGGGCAGAGGCAAGGCCGATCTTCTGAAGAAGAACCTGGA
GGATAAGATCCGACTATTCGAGGAGCGGGCCCCTGTAGAACTGGCTGACCAGGTGCGAGGGGA
ATTGCTCCAAGAGAGGGTCCAGAGGCTGGAGGCCCAGGAGGCGCACTTTGCTGAGTCACTTGTG
GCTCTGCAGTTCCAGAAGGTGGCCCGGGCTGCTGAGACCCTCTCAGTCTACACCGCCCTGCTCA
GCATTCAAGACCTGCTGCTGGGGGAGCTGAGTGAGTCGGAGACCCTGACCAAGTCAGCCTGTGT
GCAGATCCTGGAGTCCCACAGGCCGGAGCTGCAGGAGCTGCAGGAGCTGGAGAGGAAGCTGGA
GGACCAGCTGGTGCAGCAGGAAGAGGCCGAGCAGCAGCGGGTCCTGGAGAGCTGGCAGCGGT
GGGCGGCCGATGGACCTGGGCTGAGCGAACCTGAGGAGATGGATCCTGAAAGGCAGGTCTCTG
CCATCCTGCGGCAAGCCCTGAACAAGGGCCAGAAGCTGCTGGAGCAGCATCAGCAGAGGGTGA
GAGAGGAGTGGCAGAACGGTGCGGTGCTGGAAGATTCTCTGGAAAGCATTGAAGCTGACACCAT
GGCCAGCCTTTGCAGCCAGGGACTGAGGCTGGTGTCATACCTCTCAAGGATGACAATGGTACCA
GGCAGCACACTACTGCGACTCCTGAGTGTGGTGCTTCCAGCGGCTTCACAGCCTCAGCTACTGG
CCCTGCTGGACGCAGTCAGTGAGAAGCACTCCGACCACACAGCTGAGAATGAGAGCAGTGGTGA
GCAGGCTCAGGCAGAGCAGAGCAAGAGGAGGAAACACCAAGTTTGGTGGAAGGTTTTGGACAG
CAGATTCAGAGCAGATCTGGTGAGTCAAGGACTGGAGAGGATGCTCTGGGCCCGCCAGAAGAA
GGAGCGCATATTGAAGAAGATATATGTTCCCGTCCAGGAGAGGGTGATGTTTCCTGGGAAAGGA
AGCTGGCCACACCTGTCCCTAGAACCCATTGGAGAACTGGCTCCCATCCCCATCACTGGGGCAG
ACGCTATGGACATATTAAACACGGGAGAAAAGATCTTTGTATTCAGAAGCCCAAGGGAGCCCGAG
ATCTCTTTGCGTGTTCCTCCCAGGAGGAAAAAGAATTTCCTGAATGCCAAGAAGGCCAACAGGGC
CTTGGGCTTGGACTGACCCAGGGGGAAGCACCAGGGGCTGTCTCAAGACAGGTGCATCTTTCCT
CCAGCCCACGTGGGTTGGTCTGTCCGTCTATATCATACAGCTTGCAGTATACATATGCTTGCAGC
CTTGACACAGTGCCACGCAAGTTGGCCCTCCGGTGTTCAGGTCCAGTCTCTTTTGTGCCACAGG
ACACAAACATACCTTCCTTCTAAGTCCGTATTACCATGATCTAGAGGGAACCCACCTTACCCCACC
TTCCTCACACAGACCACTGGCTGTGGCTGGGATGGGTCCCCACCCCACCCAGTTTATGTGAGGA
AGGTGCAGCTCTCTATTCTTACAATGTGTGTTACATGCTCTGTTTTTCCATAAAGGGAACTTTAAAA
ATGTAAAAAAAAAAAAAA

Irf6
Template size : 1027 bp
Amplicon size : 510 bp (partial)
Amplified sequence :
TGTTCNNAAAAGTNAAAAGTAACCCATTACATANNNAGTGGGAGGGTAGCTGTGGAATAGGGGAC
ATTTCCTTTCCCTTCTGTGACAAGTCAGCAGGCTATGAGGAATGTCCAGACAACAGGATCTGGTA
AAGAATGTTGAAAAATATGTTCTGAGGGCTATGGATTGAGTTATTAGTGTGTGACCATAATGAAAA
ATTGACAAATATGTTGGGACAATGCATGATTTTGTGTGGTCCTATCTAGGCAGTCCAGTTTCTTGT
CTTGCATCTTTTTCGGAATGCTGGGAGCACACACTACATGCAGGATCTTCTCTTGTCATATTCAGT
AAGCTTTCTCTGGTTTTAGTAGATACCAAGTCCCCAGGAGCTCTAAGTTTCCCAATGCTCCCATTC
ACTCTCCAGCTGTCTTCTTCCTGCCTTCATTTGAAGACTCAGAAGGGAGGAGGATTTCCCCCAGT
TCCTCCTTTTCCTTACTGTGCACTCCANATTAACTCCAATGTTAAAAGAAN

Map2k1
Template size : 905 bp
Amplicon size : 562 bp (partial)
Sequence :
TGGCCTCGAGGCCAGATTCGGCACGAGGTATGGTTACTCCCCTAAGTGGATTGGCTTTGTGCTT
GGGGCTATTTGTCTGTTCATCAAACACATGCCAGGCTGAACTACAGTGAAACCCTAGTGACCTGG
GTGGTCGTTCTTACTGATGTTTGCACTGCTGTTCATCGTGACTCACTAGCTGGCTGCCTGTATTGT
CAGGATTCTCGGACCTTGGTACTTCACTCTTGCTGGTGACCTCTCAGTCTGAGAGGGAGCCTTGT
GAGACCCTTCACAGGCAGTGCATGCATGGAAAGCATGCTTTGCTGCTACTGAAATGAGCATCAGA
ACGTGTACGTCATGGTATTTTTATTTTTTGCTTTTGGTATAGAACTCAGCAATTCCCATCAAAAAAA
CCTAAGCAGAGCCCATCACTGCCATGATAGCTGGGCTTCAGTCTGTCTACTGTGGTGATTTTTAG
ACTTCTGGTTGTATTTCTATATTTATTTTTAAATATACAGTGTGGGATACTTAGTGGTGTGTGTCTC
TAAGTTTGGATTAGTGTTTCTAAATTGGTGGTTATTTT

Nfkbia
Template size : 750bp
Amplicon size : 815 bp (full length)
Sequence :
ACTCCATGAAGGACGAGGAGTACGAGCAAATGGTGAAGGAGCTGCGGGAGATCCGCCTGCAGC
CGCAGGAGGCGCCGCTGGCCGCCGAGCCCTGGAAGCAGCAGCTCACGGAGGACGGAGACTCG
TTCCTGCACTTGGCAATCATCCACGAAGAGAAGCCGCTGACCATGGAAGTCATTGGTCAGGTGAA
GGGAGACCTGGCCTTCCTCAACTTCCAGAACAACCTGCAGCAGACTCCACTCCACTTGGCTGTG
ATCACCAACCAGCCAGGAATTGCTGAGGCACTTCTGAAAGCTGGCTGTGATCCTGAGCTCCGAG
ACTTTCGAGGAAATACCCCTCTACATCTTGCCTGTGAGCAGGGCTGCCTGGCCAGTGTAGCAGT
CTTGACGCAGACCTGCACACCCCAGCATCTCCACTCCGTCCTGCAGGCCACCAACTACAATGGC
CACACGTGTCTGCACCTAGCCTCTACTCACGGCTACCTGGCCATCGTGGAGCACTTGGTGACTTT
GGGTGCTGATGTCAACGCTCAGGAGCCCTGCAATGGCCGGACAGCCCTCCACCTTGCGGTGGA
CCTGCAGAATCCTGACCTGGTTTCGCTCTTGTTGAAATGTGGGGCTGATGTCAACAGGGTAACCT
ACCAAGGCTACTCCCCCTACCAGCTTACCTGGGGCCGCCCAAGTACCCGGATACAGCAGCAGCT
GGGCCAGCTGACCCTGGAAAATCTCCAGATGCTACCCGAGAGCGAGGATGAGGAGAGCTATGA
CACGGAGTCAGAATTCACAGAGGATGAGCTGCCCTATGATGACTGTGTG

Publication 2 : Etude du profil d'expression au cours de l'odontogenèse du gène Ube3a responsable lorsqu'il est muté chez l'Homme du syndrome d'Angelman.

DÉVELOPPEMENT DE LA CAVITÉ BUCCALE : DU GÈNE À L'EXPRESSION CLINIQUE CHEZ L'HOMME

DEVELOPMENT OF THE ORAL CAVITY: FROM THE GENE TO THE CLINICAL EXPRESSION IN HUMANS

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Origine des crédits : IFRO 2009, API HUS 2009-2012, UdS

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Les pathologies ou anomalies du développement de la cavité buccale sont un des aspects cliniques voire diagnostiques, souvent peu considéré en particulier dans leur prise en charge, des maladies génétiques ou syndromes.
Sur plus de 7000 maladies rares connues, 850 ont une composante dento/oro/faciale et plus de 300 présentent dans leur tableau clinique une fente labio/palatine.
Ce projet de recherche original propose de combiner des approches complémentaires et convergentes de recherche en bioinformatique et en biologie du développement, en particulier par l'étude de modèles animaux murins, afin d'améliorer les connaissances et la compréhension de la formation de la cavité buccale et en particulier du palais et de la dentition. Dans cette première étape, il précise, par hybridation in situ, chez la souris, au cours de l'odontogenèse, le patron d'expression du gène *Ube3a*.
Ce gène est responsable chez l'homme du syndrome d'Angelman.

Pathologies or developmental anomalies of the oral cavity are one – often underestimated, especially in their management – clinical and diagnostic aspect of genetic diseases or syndromes.
Among more than 7000 known rare diseases, 850 have dental/oral/facial manifestations and more than 300 include in their clinical synopsis a cleft lip/palate.
This original research project proposes to combine complementary and convergent approaches in bioinformatics and developmental biology, more particularly through the study of genetically engineered mice, to improve the knowledge and understanding of the formation of the oral cavity and specifically of the palate and dentition. In this first step, it details by in situ hybridization, the expression pattern of the Ubea3 *gene during mouse odontogenesis. This gene is involved in humans in the Angelman syndrome.*

CAVITÉ BUCCALE
PALAIS
DENTS
DÉVELOPPEMENT
ANOMALIES
MALADIES GÉNÉTIQUES
MALADIES RARES
SYNDROMES
SOURIS
HOMME

ORAL CAVITY
PALATE
TOOTH
DEVELOPMENT
ANOMALIES
GENETICS
RARE DISEASES
SYNDROMES
MOUSE
HUMAN

INTRODUCTION

Les pathologies ou anomalies de la cavité buccale et des dents (anomalies dentaires de nombre (Nieminen, 2009), forme, taille, structure (Barron et al., 2008; Crawford et al., 2007), éruption (Wise & King, 2008), position, résorption) génétiquement conditionnées sont un des aspects des maladies rares ou syndromes (Bailleul-Forestier et al., 2008a; Bailleul-Forestier et al., 2008b; Hart & Hart, 2009). Ces maladies rares qui regroupent quelques 7000 pathologies différentes, concernent 4 millions de personnes en France et près de 25 millions en Europe. Par définition, elles touchent moins d'une personne sur 2000 et sont à 80% d'origine génétique. Sur plus de 7000 syndromes génétiques connus, 850 ont une composante dento/oro/faciale et plus de 300 présentent dans leur tableau clinique une fente palatine (Gorlin et al., 2001; Gritli-Linde, 2008). Ces anomalies bucco-dentaires du développement retiennent de plus en plus l'attention du fait de leur caractère diagnostique voire prédictif dans certaines maladies génétiques (Bloch-Zupan, 2007). C'est tout à fait compréhensible car les mêmes gènes et les mêmes voies de signalisation régulent le développement du palais et de la dent et celui d'autres organes (Gritli-Linde, 2007; Tummers & Thesleff, 2009). La cavité buccale, dans cette approche, est considérée comme un marqueur et une porte d'entrée en développement et pathologie.

Les objectifs de ce programme de recherche sont de combiner des approches complémentaires et convergentes, de recherche en bioinformatique et en biologie du développement en particulier par l'étude de modèles animaux, pour améliorer les connaissances et la compréhension des phénomènes étiopathogéniques à l'origine du développement de la cavité buccale et en particulier du palais et de la dentition et de leurs anomalies. Les souris génétiquement modifiées reproduisent souvent les phénotypes rencontrés chez l'homme en particulier bucco-dentaires et constituent de ce fait d'excellents modèles d'étude et de compréhension des maladies génétiques (Fleischmannova et al., 2008).

Les développements palatin et dentaire chez la souris sont des modèles très pertinents pour l'étude des mécanismes génétiques et moléculaires de l'organogenèse (participation des crêtes neurales céphaliques, interactions épithélio-mésenchymateuses, cytodifférenciations, transition épithélio-mésenchymateuse, interventions des grandes voies de signalisation…). Ils permettent également d'aborder les problématiques des cellules souches (les incisives de souris ayant une croissance continue) et les phénomènes de minéralisation (émail, dentine, os) et de métabolisme osseux (os alvéolaire, mandibulaire, maxillaire) (Caton & Tucker, 2009; Salazar-Ciudad & Jernvall, 2002; Thesleff, 2003a; Thesleff, 2003b; Thesleff, 2006).

Sont ainsi étudiés des gènes spécifiquement impliqués dans des syndromes humains affectant les développements dentaire et/ou palatin, et associant en particulier des anomalies cranio-faciales et/ou oculaires en s'appuyant sur des approches intégrées :

1) Sélection de gènes connus mais dont l'expression et/ou le mode d'action n'est pas (ou mal) caractérisé ;

2) Identification de nouveaux gènes candidats, en particulier par l'analyse des profils d'expression dans les bourgeons dentaires et les tissus faciaux - ceci en exploitant les données produites pour l'ensemble du génome murin dans le cadre du projet EURExpress, 6e PCRD ;

3) Caractérisation de manière approfondie des profils d'expression, chez la souris, des gènes les plus intéressants aux stades suivants de l'odontogenèse (E12.5, E14.5, E16.5. P0, PN6) ;

4) Etude des modèles animaux par des approches d'analyse phénotypique notamment par imagerie (micro-CT), et d'analyse fonctionnelle en privilégiant les modèles murins disponibles et/ou produits lors de projets de mutagenèse à grande échelle (programmes européens EUMORPHIA, EMPReSS, EUCOMM, EUMODIC en partenariat avec l'Institut Clinique de la Souris).

5) Analyse du rôle des gènes d'intérêt par des approches classiques de biologie du développement (immunohistochimie pour la localisation protéique ; culture organotypique de germes dentaires et de bougeons palatins et manipulation in vitro des gènes et voie de signalisation impliqués).

Ce programme fédère des scientifiques et des cliniciens autour de la compréhension des anomalies bucco-dentaires et vise à proposer des hypothèses diagnostiques autour de ces anomalies basées sur la preuve scientifique dans le but d'améliorer la santé et la prise en charge générale et bucco-dentaire de ces patients et d'adapter les traitements utilisés ou de proposer de nouvelles thérapeutiques (comme par exemple le traitement des agénésies dentaires par stimulation de l'odontogenèse in situ, ingénierie tissulaire…).

Dans le cadre de cet appel à projet de l'IFRO, nous avons sélectionné des gènes connus, responsables de maladies rares mais dont l'expression et/ou le mode d'action n'est pas (ou mal) caractérisé et avons précisé par hybridation in situ leurs profils d'expression, chez la souris au cours de l'odontogenèse.

LE DÉVELOPPEMENT DES DENTS ET DU PALAIS

Le développement dentaire s'inscrit dans le développement cranio-facial en général qui trouve son origine dans la formation des cellules des crêtes neurales céphaliques et dans la migration consécutive de ces cellules pluripotentes vers les arcs pharyngiens pour former en combinaison avec des cellules mésodermiques de nombreux éléments du massif cranio-facial (Cobourne & Mitsiadis, 2006; Knight & Schilling, 2006; Noden & Schneider, 2006).

La dent embryonnaire des mammifères et en particulier de souris est un modèle intéressant en biologie du développement. L'odontogenèse se traduit par des morphogenèses coronaires et radiculaires spécifiques pour chaque type de dents (incisive, canine, prémolaire, molaire) par l'histogenèse de l'organe de l'émail et les cytodifférenciations des

odontoblastes, des améloblastes et des cémentoblastes. L'étude de l'évolution des mammifères s'intéresse souvent à une analyse détaillée de la morphologie des dents (Renaud et al., 2009). Pour que les patrons moléculaires puissent jouer un rôle sur l'évolution dentaire, des différences d'expression de gènes doivent pouvoir être reliées à des variations morphologiques (Charles et al., 2009; Jernvall et al., 2000; Plikus et al., 2005; Salazar-Ciudad & Jernvall, 2002).

Les étapes continues et progressives du développement dentaire ont été classiquement divisées en stades de lame dentaire, bourgeon, capuchon, cloche, formation radiculaire et éruption. L'odontogenèse est un processus cinétique cellulaire dépendant, contrôlé par des interactions épithélio-mésenchymateuses à médiation matricielle, entre l'ectoderme du premier arc branchial et des cellules ectomésenchymateuses originaires des crêtes neurales céphaliques (Peters & Balling, 1999 ; Thesleff, 2003b ; Thesleff & Aberg, 1999 ; Tucker & Sharpe, 2004 ; Tucker & Sharpe, 1999). Ces cellules contribuent à la formation du mésenchyme dentaire, de la pulpe dentaire, des odontoblastes, de la matrice de la dentine, du cément, du parodonte (Chai et al., 2000; Miletich & Sharpe, 2004). La matrice extracellulaire (membrane basale, prédentine, dentine) intervient dans l'odontogenèse soit comme substrat à spécificité temporospatiale en liaison avec des récepteurs de la membrane plasmique, soit comme réservoir potentiel de facteurs endocrines, paracrines ou autocrines comme des facteurs de croissance peptidiques.

La morphogenèse dentaire comme le développement embryonnaire en général est sous contrôle génétique strict. Les gènes qui régulent l'odontogenèse sont identifiés de plus en plus rapidement et plus de 300 d'entre eux sont répertoriés dans une base de données, créée par Pekka Nieminen de l'Université d'Helsinki, illustrant leurs patrons d'expression aux différents stades du développement dentaire (http://bite-it.helsinki.fi) (Nieminen et al., 1998).

Le même langage de communication cellulaire est utilisé au cours du développement embryonnaire en général et dentaire en particulier et a été largement conservé au cours de l'évolution. Il comprend les molécules « signal » et facteurs de croissance (famille des Tgfß comprenant les Bmps, les activines, la follistatine ; les Fgfs ; hedgehog (seul sonic hedgehog shh joue un rôle au cours de l'odontogenèse) ; et les Wnts) (Cobourne & Sharpe, 2005; Dassule et al., 2000; Hardcastle et al., 1999; Nie et al., 2006a; Nie et al., 2006b; Pispa & Thesleff, 2003). Ces molécules transmettent leur message par l'intermédiaire de récepteurs membranaires et de voies effectrices relayant le signal vers le noyau. Des facteurs de transcription modulent alors l'expression de gènes cibles, provoquant une réponse et un changement d'attitude cellulaire. Il est intéressant de noter que la plupart des gènes de la base de données citée précédemment (Nieminen et al., 1998) interviennent dans la communication cellulaire et que des mutations retrouvées parmi certains de ces gènes sont à l'origine d'anomalies dentaires (Thesleff, 2003a).

La formation du palais représente un événement majeur du développement cranio-facial (Bloch-Zupan, 2002; Gritli-Linde, 2007; Pungchanchaikul et al., 2005). Elle résulte de la confluence dans une suture en forme de Y de trois bourgeons: le bourgeon prémaxillaire ou palais primaire dérivant du bourgeon frontonasal et les deux processus palatins, émanations des bourgeons maxillaires. Ce processus morphogénétique aboutit au cloisonnement du stomodeum, ou cavité buccale primitive, en cavité buccale définitive et fosses nasales. La fusion de ces bourgeons implique une jonction (suture) locale de leurs épithélia de recouvrement suivie de la dispersion de cette barrière épithéliale aboutissant à la continuité du mésenchyme. Des perturbations de ces événements morphogénétiques complexes sont à la base d'une des anomalies congénitales les plus fréquentes: les fentes palatines (Gritli-Linde, 2008).

MATÉRIEL ET MÉTHODES

SÉLECTION DES GÈNES CANDIDATS

Une approche bioinformatique et bibliographique est utilisée croisant les données d'ouvrage de référence « Syndromes of the Head and Neck » (Gorlin et al., 2001), d'Orphanet (Ayme, 2003; Ayme et al., 1998), de Online Mendelian Inheritance in Man (OMIM), de l'atlas de transcriptome du programme européen EURExpress (http://www.eurexpress.org; http://www.genepaint.org/ (Visel et al., 2004)) et PubMed (http:// www.ncbi.nlm.nih.gov/pubmed/).

HYBRIDATION IN SITU

Matériel

Nous utilisons des souris de la souche C57Bl/6. Le jour de détection du « bouchon vaginal » (sperme coagulé) est considéré comme le jour E0.5 de la gestation.

Préparation des tissus

Après prélèvement, les têtes des foetus E14.5, E16.5, E19.5, et de nouveaux nés à PN6 sont inclus directement dans l'OCT 4583 (Tissue-TEK, Sakura) et stockées à -80°C jusqu'à leur utilisation. Avant inclusion, les embryons E12.5 sont fixés toute la nuit dans le paraformaldéhyde (4% (w/v) pH 7,5) dans du PBS 1X puis dans le sucrose 20% (w/v) et du PBS 1X toute une nuit également. Les têtes des embryons sont coupées au cryostat à -20°C (Modèle Leica® CM3050S) à une épaisseur de 10µm. Les têtes E12.5 et E14.5 sont coupées en frontal sur 5 séries (5 sondes), celles de E16.5, E19.5 et PN6 sur 7 séries en sagittal. Les coupes sont récoltées sur des lames Superfrost plus. Les lames sont ensuite stockées à -80°C jusqu'à leur utilisation.

Préparation des sondes

• ***Amplification du gène*** **Ube3a**

L'hybridation in situ est réalisée à partir d'un produit PCR qui provient du programme EURExpress (www.eurexpress.org). La séquence de ce produit correspond à celle du gène *Ube3a* flanquée des séquences de promoteurs T3 et T7 (**Figure 1**).

Référence (base de données EURExpress.org)

NM_173010
T2322

Séquence amplifiée

GGCCTCNAGCCAGATTCGCGACATCCATAGTCCTGGGTCTGGCTATTTACAATAATTGTATACTGGATGTCCATTTTCCCATGGTTGTATACAG-
GAAGCTAATGGGGAAAAAAGGAACCTTTCGTGACTTGGGAGACTCTCACCCAGTTTTATATCAGAGTTTAAAGGATTTATTGGAATATGAAGG-
GAGTGTGGAAGATGATATGATGATCACTTTCCAGATATCACAGACAGATCTTTTTGGTAACCCAATGATGTATGATCTAAAAGAAAATGGTGA-
TAAAATTCCAATTACAAATGAAAACAGGAAGGAATTTGTCAATCTCTATTCAGACTACATTCTCAATAAATCTGTAGAAAAACAATTCAAGGCAT
TTCGCAGAGGTTTTCATATGGTGACTAATGAATCGCCCTTAAAATACTTATTCAGACCAGAAGAAATTGAATTGCTTATATGTGGAAGCCG-
GAATCTAGATTTCCAGGCACTAGAAGAAACTACAGAGTATGACGGTGGCTATACGAGGGAATCTGTTGTGATTAGGGAGTTCTGG-
GAAATTGTTCATTCGTTTACA

Taille : 573 paires de bases

Figure 1. Séquence amplifiée du gène Ube3a.

• Transcription in vitro de la sonde antisens

Pour synthétiser la sonde antisens, 1 µg de produit PCR du gène *Ube3a* et 20U de polymérase T7 sont incubés 2h à 37°C dans du tampon de transcription (Transcription Buffer 5X (Promega), DIG RNA Labelling Mix 10X (Roche), DTT 0,5M, RNAsin 20U). La réaction a lieu dans un volume final de 20µl. Le DIG RNA Labelling contient les quatre nucléotides ainsi que des UTP marqués à la digoxygénine afin de permettre la synthèse d'une ribosonde marquée.

• Purification de la sonde

La réaction de synthèse est arrêtée par ajout de 2µl d'EDTA 0,2M à pH 8. L'ARN est précipité par ajout d'un µl de tRNA à 10mg/ml, 2,5µl de Chlorure de Lithium 4M, 75µl d'éthanol 100%. Le mélange est ensuite incubé à -80°C pendant 15min et centrifugé à 12000 rpm à 4°C pendant 30 minutes.

Le culot est repris dans 22µl d'eau, puis, l'ARN est à nouveau précipité par ajout de 2,5µl de Chlorure de Lithium 4M et 75µl d'éthanol 100%, suivi d'une incubation de 30 minutes à -80°C et d'une centrifugation à 12000 rpm à 4°C pendant 30 minutes.

Le culot est lavé dans 0,5ml d'éthanol 70% par une centrifugation de 15 minutes à 12000 rpm à 4°C. Le surnageant est ensuite retiré puis le culot est laissé à sécher à l'air libre.

La sonde est reprise dans 20µl d'eau stérile et la concentration est vérifiée.

• Contrôle qualitatif

Le contrôle s'effectue par électrophorèse sur un gel d'agarose 1%. 1µl de la sonde est dilué dans 4µl d'H2O-DEPC et est laissé sur glace 3min. L'échantillon est déposé sur le gel après ajout d'1µl de tampon de charge. L'électrophorèse est lancée à 120V pendant 45 min. La sonde est jugée de bonne qualité si on n'observe aucun « smear » et que la bande a la masse moléculaire escomptée.

• Contrôle quantitatif

La quantité d'ARN récupérée est évaluée par spectrophotométrie. Le volume est alors ajusté à 150 ng/µl. La sonde est ensuite diluée dans 100 à 200µl de tampon d'hybridation en fonction de la quantité de transcrits et stockée à -20°C jusqu'à utilisation.

Hybridation in situ

• Post-fixation et perméabilisation

Les lames sont sorties de leur stockage à -80°C et laissées à température ambiante pendant 2 heures. Ensuite, elles sont post-fixées sur glace en formaldéhyde à 4% (PBS 1x) pendant 10 minutes. La fixation est arrêtée dans du PBS puis les lames sont perméabilisées dans une solution de protéinase K à 0,6µg/ml pendant 2 fois 10 minutes puis rincées dans du PBS. Les lames sont ensuite acétylées pendant 2 minutes (Triethanolamine 1,3%, HCl 0,06%, Anhydride acétique 0,18%), puis rincées dans du PBS.

• Hybridation et lavages post-hybridation

Le tampon d'hybridation est composé de 50% de formamide desionisée, 10% de sulfate dextran, tRNA 1mg/ml, solution de Denhardt 1X et Salt 1X (NaCl 0,195M, Tris 0,005M pH 7,2, NaH2PO4,1H20 1,13M, Na2PO4, 12H2O 0,4M, EDTA 0,005M pH8)

La sonde est mélangée au tampon d'hybridation à une concentration comprise entre 0,1 et 1µg/ml. Ce mélange est ensuite mis à dénaturer 10 minutes à 70°C. 100 µl sont déposés sur chaque lame qui est ensuite recouverte d'une lamelle puis mises à 65°C dans un bain-marie toute la nuit pour permettre l'hybridation. Les lames sont ensuite lavées deux fois pendant 30 min à 65°C dans du 1X SSC, 50% formamide, 0,1% tween-20. Ces lavages sont suivis de deux bains de 30 min à température ambiante dans du tampon MABT (MAB 1X, 0,1% Tween-20).

• Détection de la sonde

La détection de la sonde hybridée est précédée par un blocage des sites aspécifiques pendant 1h à température ambiante dans une solution de blocage (20% de sérum de chèvre normal et 2% de Blocking Reagent (Roche) dans du MABT). 200 µl d'anticorps primaire anti-DIG (Roche) dilués au 1 : 2500e dans la solution de blocage sont déposés sur chaque lame. Les lames sont ensuite recouvertes de parafilm et mises pendant une nuit à 4°C. Le lendemain, les lames

sont lavées cinq fois 20 min dans du tampon MABT puis rincées deux fois 10 min dans du tampon NTMT (100mM NaCl, Tris pH9,5 100mM, MgCl2,6H2O 50mM, Tween-20 0,1%). 200 µl de solution de coloration (3,5µl/ml NBT (Roche), 3,5µl/ml BCIP (Roche) dans du NTMT 1X) fraîchement préparée sont déposés sur chaque lame, elles sont ensuite recouvertes d'un parafilm puis révélées toute une nuit à température ambiante à l'abri de la lumière.

Le milieu de coloration est renouvelé chaque jour, si besoin. Après obtention d'une coloration optimale, les lames sont rincées deux fois 10 minutes dans du NTMT 1X. La coloration est arrêtée par un court rinçage dans du PBS puis dans de l'eau. Les lames sont ensuite laissées à sécher toute une nuit, puis montées avec du DPX.

RÉSULTATS

SÉLECTION DES GÈNES CANDIDATS

Une analyse précise des données de la littérature et des ressources de l'Internet, menée entre janvier et décembre 2009 et croisant les données de (Gorlin et al., 2001), d'Orphanet (Ayme, 2003; Ayme et al., 1998) et de Online Mendelian Inheritance in Man, (OMIM) a permis d'identifier 51 gènes peu étudiés dans le contexte de la cavité buccale et responsables de maladies rares comportant dans leur tableau clinique des anomalies dentaires/et ou palatines. Ces informations sont confrontées aux données de l'atlas de transcriptome du programme européen EURExpress (http://www.eurexpress.org; http://www.genepaint.org/ (Visel et al., 2004) (**Figure 2**) qui vise à cartographier par hybridation in situ robotisée, au jour 14,5 du développement embryonnaire, l'ensemble du génome de la souris et comprend plus de 18 000 gènes analysés. Au jour E14,5, l'organe dentaire se trouve au stade de capuchon ce qui correspond à la formation des centres de signalisation (nœud de l'émail) et à la mise en place de l'épithélium dentaire interne (histomorphogenèse). Le dysfonctionnement de ces structures peut entraîner des anomalies de nombre, de forme, de taille des dents et éventuellement plus tard de formation de l'émail voire de la dentine. Après analyse des profils d'expression de la base de données EURExpress, 21 gènes laissent entrevoir la présence de transcrits au stade de capuchon dentaire. Un ultime processus de sélection via PubMed élimine les gènes déjà étudiés ou en cours d'étude. 12 gènes feront ainsi l'objet d'une analyse détaillée dont le gène *Ube3a* présenté ici.

HYBRIDATION IN SITU

Les transcrits de *Ube3a* sont retrouvés au cours de l'odontogenèse depuis le stade de lame dentaire (faiblement) jusqu'au stade de cloche dentaire avancée à la fois dans le compartiment épithélial (en particulier au niveau de l'épithélium dentaire interne) et dans le compartiment mésenchymateux ; ceci aussi bien au niveau des incisives que des molaires. Les cellules en voie de différenciation (préaméloblastes, préodontoblastes) et différenciées (améloblastes et odontoblastes) expriment aussi ce gène (**Figure 3 et Tableau 1**).

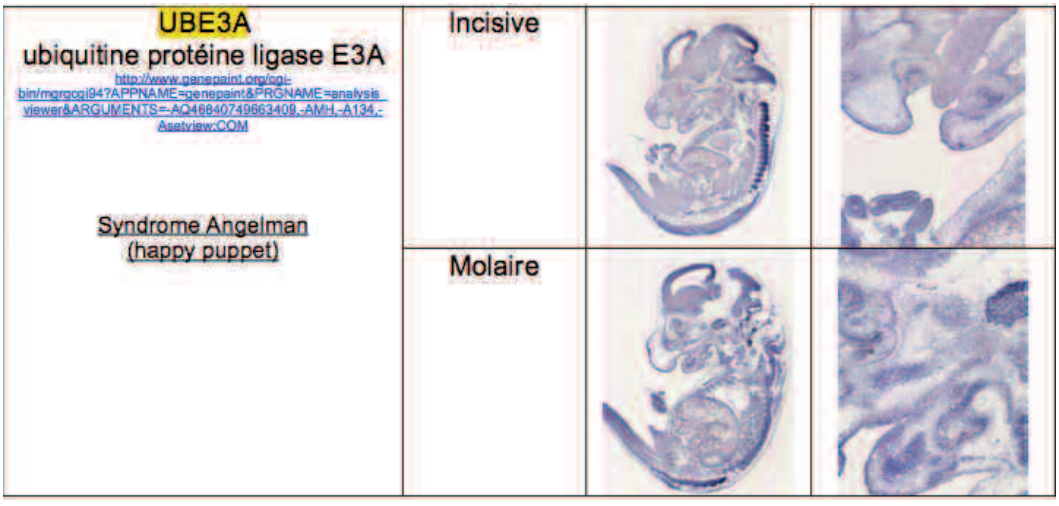

Figure 2. Données de l'atlas de transcriptome EURExpress consultées via www.genepaint.org

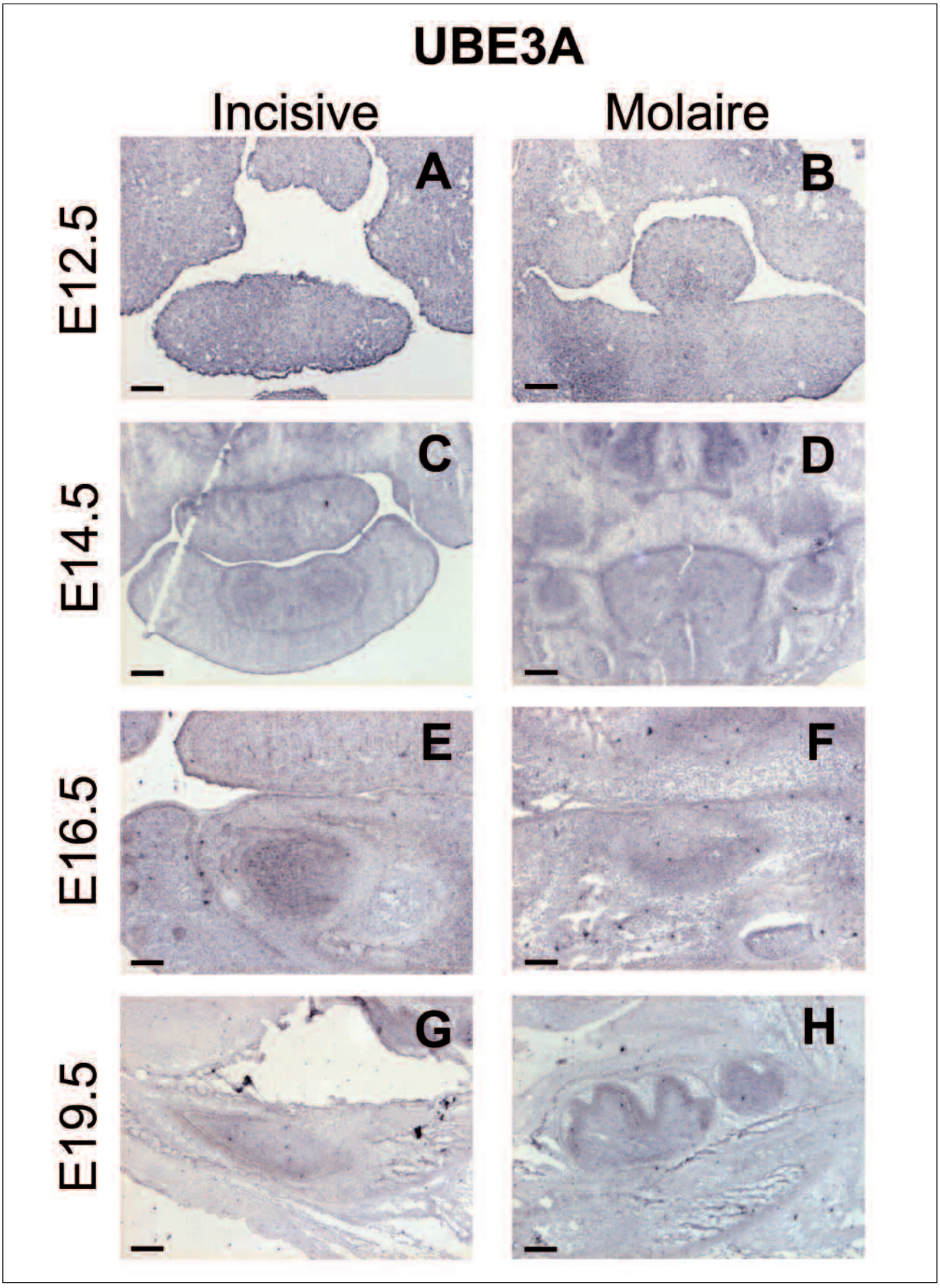

Figure 3. Expression du gène Ube3a au cours de l'odontogenèse
Barre d'échelle: A, B, E, F = 140µm; C, G = 200µm; D, H, J = 250µm.
A, B : lame dentaire ; C, D : capuchon ; E, F : cloche ; G, H : cytodifférenciations ; A,C,E,G incisive ; B,D,F,H molaire

Gène Ube3a	Inc	M1	M2	M3
E12.5				
Epithelium buccal				
Lame dentaire	+	+		
Mésenchyme	+	+		
E14.5 capuchon				
GUB				
Organe de l'émail	+	+		
EDI	+	+		
Nœud de l'émail				
Papille dentaire	+	+		
Sac dentaire				
E16.5 cloche				
GUB				
EDE				
RS				
SI				
EDI	+	+		
Lèvres épithéliales	+	+		
Papille dentaire	+	+		
E19.5 PO				
EDE				
RS				
SI				
EDI	+	+	+	
PreAm	+	+		
Am	+	+		
Lèvres épithéliales		+	+	
Od	+	+		
PreOd	+	+		
Papille dentaire	+	+	+	
Sac Dentaire				

Tableau 1. Localisation des transcrits du gène Ube3a en fonction des compartiments cellulaires et structures histologiques présents aux différents stades de l'odontogenèse (incisive et molaire) chez la souris.
(GUB : gubernaculum ; EDI, épithélium dentaire interne ; EDE, épithélium dentaire externe ; RS, réticulum stellaire ; SI, stratum intermédium ; PreAm, préaméloblastes ; Am, améloblastes ; PreOd, préodontoblastes ; Od, odontoblastes, Inc, incisive ; M1 première molaire ; M2, deuxième molaire ; M3, troisième molaire).

DISCUSSION

La méthode de discrimination utilisée pour identifier des gènes impliqués dans le développement de la dent et du palais et plus généralement de la cavité buccale est adaptée est a permis la sélection de plusieurs gènes candidats intéressants, dont le gène *Ube3a*.

Des mutations ou délétions, perte de fonction, de *Ube3a* (15q11-q13), affectant l'allèle maternel, sont impliquées chez l'homme, dans le syndrome d'Angelman (Lalande & Calciano, 2007; Van Buggenhout & Fryns, 2009) (10% des cas ; OMIM # 105830). Le gène code pour une protéine ubiquitine-ligase E3A encore appelée E6-AP (papillomavirus humain E6-protéine associée) de la famille HECT E3. Cette protéine a été initialement découverte comme indispensable à l'association du papillomavirus humain et du proto-oncogène, suppresseur de tumeur p53 ; elle participe d'ailleurs à la dégradation de p53 (Bernassola et al., 2008).

La protéolyse médiée par l'ubiquitine, par l'intermédiaire des actions du protéasome, est la voie majeure pour la dégradation sélective contrôlée de protéines intracellulaires dans les cellules eucaryotes. La modification par l'ubiquitine de diverses cibles protéiques à l'intérieur de la cellule s'avère être importante dans un certain nombre de fonctions cellulaires fondamentales telles que la régulation de l'expression génique, la régulation du cycle cellulaire, la modification de récepteurs cellulaires de surface, la biogenèse des ribosomes, et la réparation de l'ADN. Une fonction majeure du système à médiation par l'ubiquitine consiste à contrôler les demi-vies de protéines cellulaires. Les substrats sont reconnus soit directement par des enzymes conjuguées à l'ubiquitine soit par des protéines associées de reconnaissance de substrat, les protéines E3, également connues sous l'appellation d'ubiquitine ligases. E6-AP a également des fonctions distinctes de coactivateur des récepteurs des hormones stéroïdes (Ramamoorthy & Nawaz, 2008). Ces protéines sont donc impliquées en pathologie dans le cancer (mutations gain de fonction de E6-AP et cancer du col de l'utérus) et les maladies génétiques (Jiang & Beaudet, 2004; Matentzoglu & Scheffner, 2008).

Le syndrome d'Angelman, cette maladie rare neurogénétique (prévalence estimée à 1 sur 12 000) touche le cerveau et se traduit par un ensemble de signes cliniques incluant des troubles du développement moteur (acquisition de la marche, ataxie), un déficit intellectuel avec un langage minimal ou absent, des crises d'épilepsie, des troubles du sommeil, un visage aux traits caractéristiques et un comportement gai avec des rires très faciles.

Plusieurs autres mécanismes génétiques sont impliqués et la majorité des cas (60-75%) est due à une délétion d'une région critique du chromosome 15.

Au niveau du phénotype cranio-facial sont décrits : une microcéphalie, une brachycéphalie, un prognatisme, une macrostomie avec une langue en protrusion, un bavage excessif, des dents espacées et donc de nombreux diastèmes. Des troubles de la succion/déglutition et des comportements excessifs de mastication sont présents.

L'expression de *Ube3a*, en particulier son empreinte maternelle, a surtout été étudiée chez la souris, dans les tissus neuronaux (cerveau, hippocampe, cervelet, cellule de Purkinje, bulbe olfactif) (Albrecht et al., 1997; Yamasaki et al., 2003). L'analyse, dans le cadre de notre projet, de son patron d'expression montre clairement son implication au cours du développement dentaire sans toutefois préciser définitivement son rôle.

p53, une cible possible de *Ube3a* est présent au cours de l'odontogenèse et signale en particulier les compartiments de prolifération comme les portions de l'épithélium dentaire interne en base de cuspides, les lèvres épithéliales ou certaines zones du mésenchyme en regard (Leveillard et al., 1998). Les patrons d'expression des deux gènes trouvent donc des zones de coïncidence.

Nawaz et collaborateurs (Ramamoorthy & Nawaz, 2008) ont montré que E6-AP pouvait aussi agir en tant que coactivateur des récepteurs des hormones stéroïdes comme le récepteur de la progestérone (PR), le récepteur des oestrogènes (ER), le récepteur des androgènes (AR), le récepteur des glucocorticoïdes (GR), le récepteur α de l'acide rétinoïque (RAR-α) et le récepteur des hormones thyroïdiennes (TR). Certains de ces récepteurs sont aussi exprimés au cours de l'odontogenèse (Bloch-Zupan et al., 1994; Ferrer et al., 2005; Thompson et al., 2004).

Les anomalies bucco-dentaires rencontrées chez les patients Angelman, qui seraient reliées, comme les autres défauts, essentiellement à la perte de l'activité ligase, ne sont pas explicables par l'analyse de ces données de transcriptome.

Des analyses complémentaires (immunohistochimie, analyse des modèles murins (Jiang et al., 1998)), portant en particulier sur l'allèle d'origine maternelle et son dérivé protéique, seront nécessaires.

CONCLUSION

Ce travail détaille, au cours de l'odontogenèse le patron d'expression du gène *Ube3a* impliqué chez l'Homme dans le syndrome d'Angelman. Il précise ainsi les connaissances autour des gènes impliqués dans les maladies rares ayant des répercussions à la cavité buccale. La stratégie utilisée pour identifier et ensuite étudier ce gène a été reprise avec succès pour d'autres gènes qui feront l'objet de publications ultérieures.

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http://www.orpha.net/ Portail des maladies rares et des médicaments orphelins (Ayme, 2003; Ayme et al., 1998)

PubMed ressource développée et maintenue par le « National Center for Biotechnology Information (NCBI), au U.S. National Library of Medicine (NLM), localisé au National Institutes of Health (NIH) http://www.ncbi.nlm.nih.gov/pubmed/

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Cranio-oro-dental Features in Cockayne Syndrome

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Running title: Cockayne syndrome orodental phenotype

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Cockayne Syndrome CS (type A CSA; OMIM #216400; type B CSB OMIM #133540) is a rare autosomal recessive neurological disease caused by defects in DNA repair characterized by progressive cachectic dwarfism, intellectual disability with cerebral leukodystrophy, microcephaly, progressive pigmentary retinopathy, sensorineural deafness and photosensitivity. We studied orodental findings in a group of 17 CS patients participating in the National Hospital Program for Clinical Research (PHRC) 2005 « Clinical and molecular study of Cockayne syndrome » All were examined by two investigators, using the Diagnosing Dental Defects Database record form.

Various orodental features were found: small mouth; retrognathia; micrognathia; highly arched and narrow palate; expressed crowding; hypodontia (missing permanent lateral incisor, second premolars or molars) screwdriver incisors, microdontia, radiculomegaly, and enamel hypoplasia. Eruption was usually normal.

Cephalometric analysis revealed hypo-development of the face and skull.

Caries, a minor disease criterion, was associated with enamel defects, soft food diet, poor oral hygiene, dry mouth, gastro-oesophageal reflux. Mouse tooth transcriptome analysis confirmed expression of genes involved in CS syndromes and their possible involvement in odontogenesis and related anomalies.

Specific attention for these anomalies should facilitate diagnosis and help adequate management.

Keywords: Cockayne Syndrome, Phenotype, Tooth development, Tooth

abnormalities, Cephalometry, ERCC6, ERCC8

INTRODUCTION

Cockayne Syndrome CS (type A CSA; OMIM #216400; type B CSB OMIM #133540) is a rare autosomal recessive neurological disease caused by defects in DNA repair via nucleotide excision repair (NER), a molecular mechanism of disease shared also by Xeroderma Pigmentosum (XP) and Trichothiodystrophy (TTD) [de Boer and Hoeijmakers, 2000; Lehmann, 2003; Subba Rao, 2007]. The incidence in Western Europe has been recently evaluated as 2.7 per million [Kleijer et al., 2008]. The main clinical features are a progressive cachectic dwarfism, intellectual disability with cerebral leukodystrophy, microcephaly, progressive pigmentary retinopathy, sensorineural deafness and photosensitivity [Nance and Berry, 1992]. Type I is defined as the classical milder form of the syndrome whereas type II is the early onset severe form which can lead to an early death. Cerebro-oculo-facio-skeletal syndrome (COFS) is a more severe prenatal form of CS with similar clinical expression to type II. Type III has mild symptoms and onset in late childhood. Different severity groups have however been described and renamed recently : severe, moderate and mild CS. Mean age of death is 5, 16 and 30 years in these groups, respectively. Very severe cases with prenatal onset and very mild cases with adult-onset have also been identified at both ends of the clinical spectrum [Natale, 2011; Spivak, 2004]. CS is caused by mutations in the excision-repair, cross-complementing group 8 gene (*ERCC8*) (at 5q12) for CSA and in the excision-repair, cross-complementing group 6 gene (*ERCC6*) (at 10q11) for CSB with no genotype/phenotype correlations described [Henning et al., 1995; Laugel et al., 2008; Mallery et al., 1998]. Other genes like *XPB (ERCC3)*, *XPD (ERCC2)*, *XPG (ERCC5), XPF (ERCC4)* involved in XP are

causative in patients presenting with a combination of XP and CS type II [Kraemer et al., 2007; Nouspikel, 2008; Rapin et al., 2000].

Craniofacial dysmorphism associated with CS is partially described in the literature. Microcephaly with retrognathia, prominence of the facial bones, micrognathia [Tan et al., 2005], mandible prognathism [Macdonald et al., 1960] have been reported.

High arched palate, atrophy of the alveolar process, condylar dysplasia, absence of permanent teeth and short roots were also described [Arenas-Sordo Mde et al., 2006; Civantos, 1961; Cotton et al., 1970; Dumic et al., 1995; Hamamy et al., 2005; Macdonald et al., 1960; Nance and Berry, 1992; Schneider, 1983; Scott-Emuakpor et al., 1977; Sorin, 1994; Yuen et al., 2001]. Small mouth opening with reduced mandibular movement was reported by [Boraz, 1991].

Caries is considered to be a minor diagnostic criterion by Nance and Berry [Nance and Berry, 1992] with photosensitivity, progressive retinitis pigmentosa, perception deafness, dysmorphic traits. Other orodental features like delayed deciduous tooth eruption, malocclusion, absent/hypoplastic teeth were also described in this milestone paper [Nance and Berry, 1992].

The aim of the present study was to investigate the craniofacial and orodental findings in a series of 17 patients, to ascertain these specific aspects of the phenotype and their variability, to provide quantitative data as cephalometric analysis and to assess their usefulness for the clinical diagnosis through possible genotype/phenotype correlations. Guidelines for clinical management are also proposed.

PATIENTS AND METHODS

A total of 17 CS patients from France, the Netherlands, Switzerland, Morocco participated in this sub-study of the PHRC 2005 “Clinical and molecular study of Cockayne syndrome”.

Families gave informed consent. All clinical and molecular studies were approved by the Local Ethics Committee of the Strasbourg University Hospital. For each patient, the diagnosis of CS was confirmed using cellular (defect in TCR pathway) and molecular (identified mutations in *CSA* or *CSB*) analyses. Mutations have been previously reported in [Laugel et al., 2010]).

Patients were examined clinically by 2 different dentists in the Reference Centre for Orodental Manifestations of Rare Diseases, Pôle de Médecine et Chirurgie Bucco-Dentaires, Hôpitaux Universitaires, Strasbourg, France. Inter-investigator agreement was assessed through comparison of cases and discussion. The orodental findings were documented using the D[4]/phenodent registry: a **D**iagnosing **D**ental **D**efects **D**atabase (see www.phenodent.org, to access assessment form). This registry allows standardisation of data collection and, therefore, assists in orodental phenotyping. It facilitates providing clinical care to patients, a basis for genotype/orodental phenotype correlations, and sharing of data and clinical material between clinicians.

Computed tomography (CT) examination of the whole body was acquired for each patient within the Department of Radiology, University Hospital, Strasbourg, by a high resolution spiral equipment (SOMATOM Sensation 16® scanner, Siemens® Medical Solutions, Erlangen, Germany) [Koob et al., 2010]. Axial images of 1mm thickness

were made with 0.7 intervals and a field of view of 220 mm.

All the axial CT data were reformated to generate images parallel to the Frankfurt reference plane. Lateral and frontal cranial cephalometric projections were obtained from the 3D MIP reconstruction of the skull. Panoramic and cross-sectional images of the maxilla and mandible were generated for examination of the teeth and periodontium.

Cephalometric analyses were performed in *norma lateralis* and *norma frontalis* from the CT-cranial projections [Lux et al., 2004; Muller et al., 1983; Ricketts, 1960; Ricketts, 1961; Tweed, 1954; Tweed, 1962; Tweed, 1966]. The method, landmarks, reference lines, and measurements are described in TABLES I and II (Supplementary material) and illustrated in Figures 1 and 2. Dental radiographic examination comprised the panoramic and cross-sectional reconstructions. Dental abnormalities of number, shape, size, structure, eruption… such as agenesis, impacted teeth, were then analyzed for each patient.

Wild type mouse tooth germ transcriptome analysis

Total RNA was extracted from E14.5 tooth germs (14.5 embryonic day post coitum) with the RNAeasy micro Kit from Qiagen. RNA quality from four lower incisors and four lower and upper molars was verified by analysis on the 2100 Bioanalyzer (Agilent). All samples displayed an RNA Integrity Number greater than 9.8. Biotinylated single strand cDNA targets were prepared, starting from 300 ng of total RNA, using the Ambion WT Expression Kit (Cat # 4411974) and the Affymetrix GeneChip® WT Terminal Labeling Kit (Cat # 900671), according to the manufacturer's instructions. Following fragmentation and end-labeling, 1.9 µg of

cDNAs were hybridized for 16 hours at 45°C on GeneChip® Mouse Gene 1.0 ST arrays (Affymetrix) interrogating 28,853 genes represented by approximately 27 probes spread across the full length of the gene. The chips were washed and stained in the GeneChip® Fluidics Station 450 (Affymetrix) and scanned with the GeneChip® Scanner 3000 7G (Affymetrix). Finally, raw data (.CEL Intensity files) were extracted from the scanned images using the Affymetrix GeneChip® Command Console (AGCC) version 3.1. CEL files were further processed with the Partek® software. Using the principal component analysis (PCA) from Partek® software, the samples could be grouped according to their identity (molars or incisors). After Partek® analysis, we decided to exclude one incisor sample because it did not regroup with the others. Analyses were therefore based on three incisors and four molars samples. Expression intensity values table (.CEL Intensity files) was then transferred to Excel® software for further statistical analysis.

RESULTS

Seventeen patients aged between 1 to 28 years and diagnosed clinically with CS were examined between September 2006 and October 2009 during the PHRC « Clinical and molecular study of Cockayne syndrome ». The clinical diagnosis was confirmed with the discovery of mutations in *CSA* gene for 5 patients and *CSB* gene for 10 patients. For 2 brothers (patients 12 and 14) no mutations were found also there was total absence of *CSA* mRNA (Table III and [Laugel et al., 2010]).

Seven patients were in the primary dentition stage, 5 patients had mixed dentition and 5 patients had a permanent dentition.

For all patients except one it was not possible to use standard panoramic radiograph. Patients were sedated for the whole body CT examination. We used the data related to the craniofacial area.

Cephalometric analyses unraveling skeletal dysmorphology were performed only on 9 patients aged between 6.7 and 28.5 years to allow comparison with known standards (Table IV). Seven patients were below 4 years of age. One patient had no radiographic data. The *norma lateralis* analysis showed a typical profile characterized by skeletal Class II, vertical growth with posterior rotation of the mandible, increase of the lower facial stage and chin in retreat (Table IV Fig. 1). Measurements in *norma frontalis* suggested a general tendency to an hypo-development of the face and the skull (Table IV, Fig. 2).

A summary of all major findings related to orodental anomalies is provided in Table

III. The orodental phenotype displayed extreme heterogeneity and variability concerning both the type of anomalies and their severity. Dental findings can be classified into:

Anomalies of tooth number: hypodontia (fewer than 6 missing permanent teeth excluding third molars) was discovered on radiographs for 2 individuals only. The missing teeth were the upper right lateral incisor (12), the upper second premolars (15,25,35,45) as well as the second molars (17,37,47) (Fig. 3a).

Anomalies of tooth size and shape: Shovel or screw-driver shaped upper permanent central incisors (11,21) were the most striking features (Fig. 3b) affecting 4 individuals. Other anomalies included abnormal shape of the upper lateral incisors (patient 10), hyper developed cingula on the lateral incisors (Fig. 3c) and globular premolars. Five children had microdont primary teeth (Fig. 3e). A microdont upper permanent lateral incisor (22) facing a missing contra-lateral tooth (12) was visible in patient 8 (Fig. 3d). Taurodontic first permanent molars (patients 10,17) (Fig. 3a) and radiculomegaly on canines, premolars and molars (patient 16) (Fig. 3f,g) were also observed on X-Rays.

Anomalies of tooth structure: generalized demarcated enamel opacities and hypoplasia and pits have been noticed in 16 out of 17 patients both in the primary (Fig. 3e,h,i) and permanent dentitions (Fig. 3b,j,k). Intrapulpal calcifications were discovered in one patient (17). Hypoplasia in the primary dentition was clearly visible in very young patients and affected surfaces of teeth rarely exposed to decay confirming that the enamel defect occurred prior to secondary carious lesions (4(Fig. 3e), 2 (Fig. 3h), 6 (Fig. 3i), 1, 3, 7, 5). For example, the patient (6) illustrated in Fig 3i was never mouth fed.

Anomalies of tooth eruption/exfoliation: Eruption was usually normal. Two patients however showed early eruption of the primary and permanent teeth. A child of 20 months (1) had delayed eruption with only 4 erupted primary teeth.

Caries was present in 9 patients, with some individuals being severely affected (dmft decayed, missing, filled teeth score ranking between 16 and 20 for the primary teeth (out of 20) and between 13 (Fig. 3k) and 28 for the permanent teeth (DMFT, out of 32). 47% of patients had no decay.

In preschool children and in the primary dentition the 2 patients showing the higher dmft index were, both affected by a severe form of the disease, presented enamel hypoplasia, had deficient or absent oral hygiene and gastro-oesophageal reflux.

It is interesting to notice that most of the patients with high caries rate were suffering from gastro-oesophageal reflux.

Most patients needed assistance to maintain oral hygiene. For patient 5 the cleaning of the oral cavity was performed solely using gauze compress. Assisted brushing became more difficult as patients grew older and subsequently patients had gingivitis associated with dental plaque and poor or deficient oral hygiene habits (Fig. 3b,j). The marginal gingiva had a thin biotype (Fig. 3e).

Occlusion: Crowding and tooth malposition were prevalent in the mixed and the permanent dentitions (Fig. 3k).

Functional defects included mixed breathing (mouth and nose) leading to dry mouth, atypical or immature deglutition (normal under 8-10 years of age) and parafunctions

like bruxism (3/17 patients). Gastro-oesophageal reflux and vomiting were anamnestically present in 35% of patients (6 out of 17 patients). Feeding was always difficult, leading to failure to thrive, even with soft diet and gastrostomy.

Access to dental treatment (see Table III)

For 3 patients below 3 years of age, the participation to the research program was their first opportunity to have an examination of the oral cavity and to discuss with the dentist. Two patients presenting with all decayed primary teeth had not yet received dental treatment. Four patients (moderate to mild type) had treatment in dental practices (1 had fissure sealants under conscious sedation, 2 had local anesthesia). 5 patients had previous GA for dental treatment (2 of them numerous time from 2 to 4; patient 12 had 12 primary and 4 permanent teeth extracted and patient 14 had 12 primary and 22 permanent teeth extracted). 1 patient (mild) had orthodontic treatment.

To attempt to correlate orodental anomalies with disruption of the molecular and developmental sequences underlying odontogenesis we investigated if genes mutated in CS were possibly active or expressed during wild type mouse tooth development through transcriptome analysis. Transcriptome analysis is a more sensitive technique than *in situ* hybridization and allows the detection of low-abundance transcripts. This studied tooth developmental cap stage is of great interest as it allows to explore molecular events related to tooth patterning, signaling and histomorphogenesis.

Results analysis within the DNA microarray demonstrated that hybridization signals vary from 1 to 14 (values in log base 2). 30% of lowest signals were considered as not detected (threshold at 5.72).

Ercc 2,3,4,5,6,8 expressions were indeed detected in each of our molar and incisor wild type mouse samples (hybridization signal mean values between 6.65 and 10.36). A fold change around 1 means that the genes were expressed equally in molars and incisors at this embryonic cap stage (Table V) therefore not accounting for any difference in the genesis of developmental anomalies related to tooth type.

DISCUSSION

Our patients' cohort displayed all the orodental anomalies described in the literature in Table VI ([Arenas-Sordo Mde et al., 2006; Bertola et al., 2006; Boraz, 1991; Civantos, 1961; Cockayne, 1936; Colella et al., 1999; Cook, 1982; Cotton et al., 1970; Dumic et al., 1995; Falik-Zaccai et al., 2008; Fujimoto et al., 1969; Guzzetta, 1967; Hamamy et al., 2005; Lieberman et al., 1961; Macdonald et al., 1960; Mallery et al., 1998; Marie et al., 1958; Meira et al., 2000; Nance and Berry, 1992; Natale, 2011; Rowlatt, 1969; Schmickel et al., 1977; Schneider, 1983; Scott-Emuakpor et al., 1977; Sorin, 1994; Tan et al., 2005; Wang et al., 2011].

Various anomalies of the number, shape, size, structure and eruption of teeth demonstrated disruption of tooth development. If agenesis of upper lateral incisors (12,22) and second premolars (15,25,35,45) are relatively common in the general population and the most frequently absent teeth [De Coster et al., 2009; Nieminen, 2009], of special interest were the agenesis of second permanent molars and the radiculomegaly. Molar agenesis has been associated with *PAX9* mutations [Stockton et al., 2000] and recently with desmoplakin gene (*DSP*) mutations in Carvajal/Naxos syndrome [Chalabreysse et al., 2011].

Radiculomegaly has been described in Oculofaciocardiodental (OFCD) syndrome due to mutations in *BCOR* gene [Ng et al., 2004] a transcriptional corepressor through the proto-oncoprotein, BCL6.

Dental anomalies are fixed in time thanks to hard tissue mineralization and therefore are not subject to changes related to age or aging.

The dental developmental anomalies described in CS might further substantiate the hypothesis of a transcriptional defect in the pathogenesis of developmental symptoms in CS [Proietti-De-Santis et al., 2006].

Clearly caries, an acquired, multifactorial, infectious disease, stated as a CS minor disease criterion, was developing on preexisting enamel developmental defects (opacities, hypoplasia) and its formation was accelerated by soft diet, poor oral hygiene, dry mouth, gastro-oesophageal reflux and vomiting**.** Caries was secondary to the developmental enamel defects. The preexisting enamel lesions could explain the speed and the extent of caries progression. This is the first analysis of the multifactorial origin of this diagnostic criterion. [Natale, 2011] stated that dental caries occurred widely in CS but that no correlation with severity groups was found.

Severely affected patients (6) had neither seen a dentist nor received previous oral health care advises or treatment. 2 of them were suffering from extensive decay.

Patients with moderate to mild form of CS benefited from a wide range of dental treatments from simple preventive and control visits to conventional filling in the dentist office sometimes under local anesthesia or with the help of conscious sedation to more extensive restorative or surgical (tooth extraction) treatments under general anesthesia. The treatment modalities were chosen taking into account the possible cooperation of the patient (age group, severity of the disease) and the extent of the existing oral pathology. There are reports of difficulties with general anesthesia procedures in CS such as difficult airway and intubation management, increased risks of gastric aspiration, cachexia and accelerated aging issues [Raghavendran et al., 2008; Wooldridge et al., 1996]. A benefice risk decision shared by all the heath professionals caring for the patients is necessary to program dental treatment under

general anesthesia. Emphasis should be definitely made on early preventive oral health measures alleviating the added burden of general anesthesia requirement to treat infectious consequences of decay.

No cephalometric data explaining the craniofacial dysmorphism in CS were found in the literature. The use of lateral cephalograms in the differential diagnosis of jaw and craniofacial anomalies and treatment planning, has become generally accepted as the standard in orthodontics since cephalograms were first simultaneously and independently described in 1931 by [Broadbent, 1931; Hofrath, 1931]. As a result of further innovation in X-ray technology, digital radiology has become increasingly important, and is now used regularly in dental and orthodontic diagnostics [Jackson et al., 1985]. With complex anomalies, such as CS, it may also become necessary to use modern imaging methods such as cone-beam computed tomography (CBCT) or conventional computed tomography (CT) to obtain sufficient detailed information for diagnosis, treatment planning and assessing therapeutic prognoses. To avoid multiple imaging and the ensuing increased exposure to radiation, it seems reasonable to use existing CT datasets to obtain virtual frontal and lateral skull images and evaluate them with the aid of computers. In programs for processing of Dicom datasets, it is possible to calculate virtual summation images from the three-dimensional volume datasets that closely resemble conventional X-ray images. Recent studies demonstrated conventional frontal and lateral cephalograms were not necessary, as they could be created from the CT dataset with comparable evaluative accuracy [Greiner et al., 2007; Kumar et al., 2007]. Thus we performed cephalometric analyses in *norma lateralis* and *norma frontalis* from the CT-cranial projections. Measurements in *norma frontalis* suggested a general tendency to an hypo-

development of the face and the skull. The *norma lateralis* showed a typical profile characterized by skeletal class II, vertical growth with posterior rotation of the mandible, increase of the lower facial stage and chin in retreat. Facial morphology and therefore dysmorphology changes markedly with age; however it was not possible to observe evolutive aspects of the skeletal dysmorphology pattern on the samples of 9 patients aged between 6.7 and 28.5 years on which cephalometric analyses were performed.

No genotype/phenotype correlation related to craniofacial and orodental anomalies was detected in this patients' sample. Most of the studies published so far describe also variation in phenotype but no specific genotype/phenotype correlations [Laugel et al., 2010; Nance and Berry, 1992; Natale, 2011].

Tooth development is embedded within craniofacial development. It originates from pluripotential cephalic neural crest cells which subsequently migrate towards the first pharyngeal arch to trigger (in combination with mesodermal cells) the development of many elements of the craniofacial structures [Cobourne and Mitsiadis, 2006; Knight and Schilling, 2006; Noden and Schneider, 2006]. The continuous and progressive stages of odontogenesis have classically been divided into the dental lamina, bud, cap and bell stages, root formation and tooth eruption. Tooth development is a kinetic dependent process mediated through epithelio-mesenchymal interactions between ectomesenchymal cells originating from cephalic neural crest cells and the first pharyngeal arch ectoderm [Peters and Balling, 1999; Thesleff, 2003b; Thesleff and Aberg, 1999; Tucker and Sharpe, 2004; Tucker and Sharpe, 1999; Tummers and

Thesleff, 2009]. Each dental anomaly (of tooth number, shape, size (a continuum of anomalies), of structure for hard tissues formation, of root formation and eruption and of resorption) are correlated to specific genetic and developmental biological processes [Bloch-Zupan, 2004] involving the embryonic origins of dental cells, the patterning of the dentition, the defined location of tooth development, tooth identity, specific morphogenesis, histogenesis, terminal differentiation of odontoblasts and ameloblasts, dentine and enamel matrix synthesis followed by mineralization, root and periodontium formation and eruption of teeth [Salazar-Ciudad and Jernvall, 2002; Thesleff, 2003a; Thesleff, 2003b; Thesleff, 2006].

Mouse molar and incisor E14.5 transcriptome analysis demonstrated that the genes involved in CS were indeed expressed at this developmental stage and could possibly, when mutated, be involved in the genesis of the described orodental anomalies.

Mouse models recapitulate features of rare diseases and are valuable to explore dental anomalies in humans [Fleischmannova et al., 2008]. Craniofacial and orodental aspects should be further explored using transgenic mouse mimicking CS.

CONCLUSIONS

CS patients have specific orodental features, some of them being described for the first time in this paper (agenesis of second molars and radiculomegaly). The expression of the genes involved in CS in a mouse tooth transcriptome analysis demonstrated that the incriminated genes and associated proteins might be able to play a role during tooth development and anomalies.

Recognized as a minor criterion of diagnosis, the high susceptibility to develop rampant caries lesions is related to a conjunction of factors (for example feeding, vomiting, gastro-oesophageal reflux, oral biofilm) on the ground of hypoplastic enamel. Dental health education for parents and children including oral hygiene with assisted brushing, the appropriate use of topical fluoride and regular visits to the dentist should be scheduled within the overall management of children suffering from CS. Prevention at an early age, meaning as soon as primary teeth erupt, is the key. Oral hygiene should be maintained even when gastrostomy feeding is in place.

Due to the discrepancy in the size of the jaws and the teeth volume, malocclusion and crowding are frequent. Some patients may benefit from orthodontic treatment with extraction of permanent teeth mainly premolars.

Reference centers for rare diseases play an instrumental role in the knowledge and management of orodental manifestations encountered in CS patients.

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LEGEND

Figure 1 3D MIP reconstruction of the skull (a) and cephalometric analysis in *norma lateralis* (b) of patient 8 (6.7 years) (See Table IV).

The names and definitions of the landmarks and measured euclidien distances and angles are given in TABLES I and II. Observe the vertical growth direction of lower jaw (angle 9 FMA) and retrognathia (diminished angle 2 Facial depth) or skeletal class II (angle 11 ANB).

Figure 2 Cephalometric analysis in *norma frontalis* of patient 16 (16.5 years). Correspondence of landmarks and measurements are detailed in TABLES I and II respectively (supplementary material). Reported to age related standards, transversal craniofacial hypodevelopment is patent.

Figure 3 Orodental phenotype encountered in patients presenting with Cockayne syndrome (see also TABLE III.)

Anomalies of tooth number (a) like missing second molars 17, 37, 47 and the lower inferior left premolar (35) in patient 10 - shape (b) shovel/and or screwdriver shape incisors (11, 21 patient 15); hyper developed cingulum on the permanent upper lateral incisors (c) (patient 12) – size with microdontia in the primary dentition (e) (patient 4, notice the diastemata separating the smaller primary teeth or in the permanent dentition with a microdont upper left lateral incisor (d) (here 22 in patient 8, the 12 is missing); taurodontic permanent first molars (a) in patient 10; radiculomegaly of canines, premolars or even molars (f,g, patient 16) – structure with enamel

hypoplasia in the primary (h patient 2, i patient 6) or the permanent dentition. Dental plaque and biofilm subsequent to poor oral hygiene as well as gingivitis were seen in patient 11 (j). Dental crowding was visible in (k) for patient 17.

(3a patient 10; 3b patient 15; 3c patient 12; 3d patient 8; 3e patient 4; 3f;g patient 16; 3h patient 2; 3i patient 6; 3j patient 11; 3k patient 17).

TABLE I. Definition of selected landmarks used in the cephalometric analysis in *norma lateralis and frontalis.*

(Supplementary material)

TABLE II. Measurement definitions and correspondence with Fig. 1 and 2.

(Supplementary material)

TABLE III. Genotype and orodental phenotype encountered in our cohort of 17 CS patients

pd: primary dentition ; PD: permanent dentition; screw d : screw driver ; dmft/DMFT : Decayed Missing Filled Teeth for primary (dmft) or permanent (DMFT) teeth ; Perio: periodontium; The level of oral hygiene was correlated to the abundance of dental plaque and confronted to the brushing habits (from assisted AB to absent NB); Dental treatment was either: this consultation was the first visit to the dentist First visit, regular visit to the dentist VI, dental treatment in the chair under local anesthesia LA, dental procedures under conscious (inhalation) MEOPA/nitrous oxide sedation (CSE),

dental treatment under general anesthesia GA, previous orthodontic assessment or treatment or Ortho ass or ttt; FD: functional defects, MB: mixed breathing (mouth and nose), D: deglutition or swallowing, R: reflux, Para: parafunctions, brux: bruxism ; Ø none ; + present.

TABLE IV. Results of the cephalometric analysis in *norma lateralis and frontalis*

For each measurement, the age-corresponding cephalometric standards are given (Mean; S.D.) **N** : Normal values. ↑ : Increased values. ↓ : Reduced values.

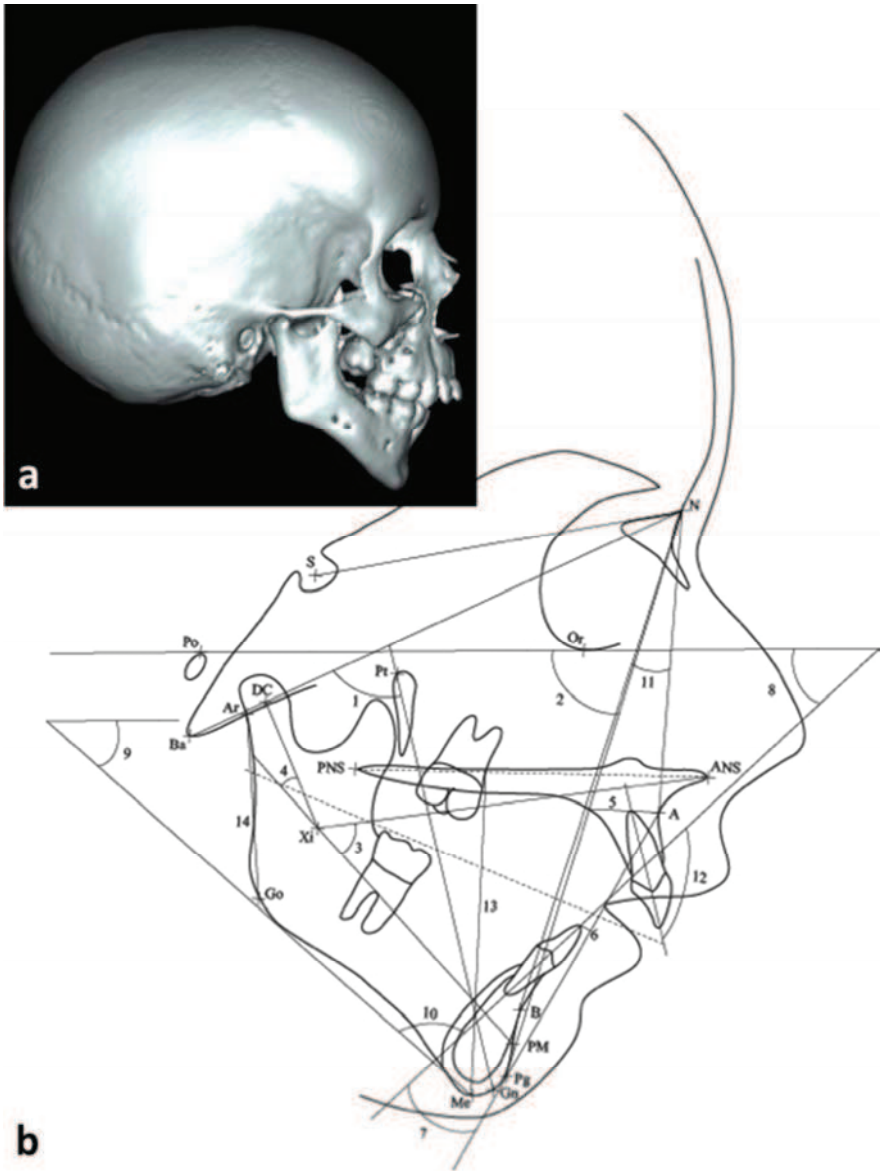
In patient 8 angle ANB is 15 ° compared to standard angle measure of 4.7° and confirms the skeletal class II. The standard deviation is 2.2.

TABLE V Transcriptome analysis of *Ercc* genes expression during E14.5 mouse tooth development.

Genes with an expression signal higher than 5 (20th percentile) were considered to be expressed. Genes with a fold change higher than 1,2 or lower to -1,2 were considered to be differentially expressed between tooth types. All analyzed *Ercc* genes were expressed in each tooth types (signal means ranging between 6,65 and 10,36 ; SD : standard deviation). *Ercc8* was the less expressed gene whereas *Ercc3* was the more strongly expressed. Fold changes (FC) were around 1 meaning that genes were not differentially expressed between the lower incisors, lower and upper molars.

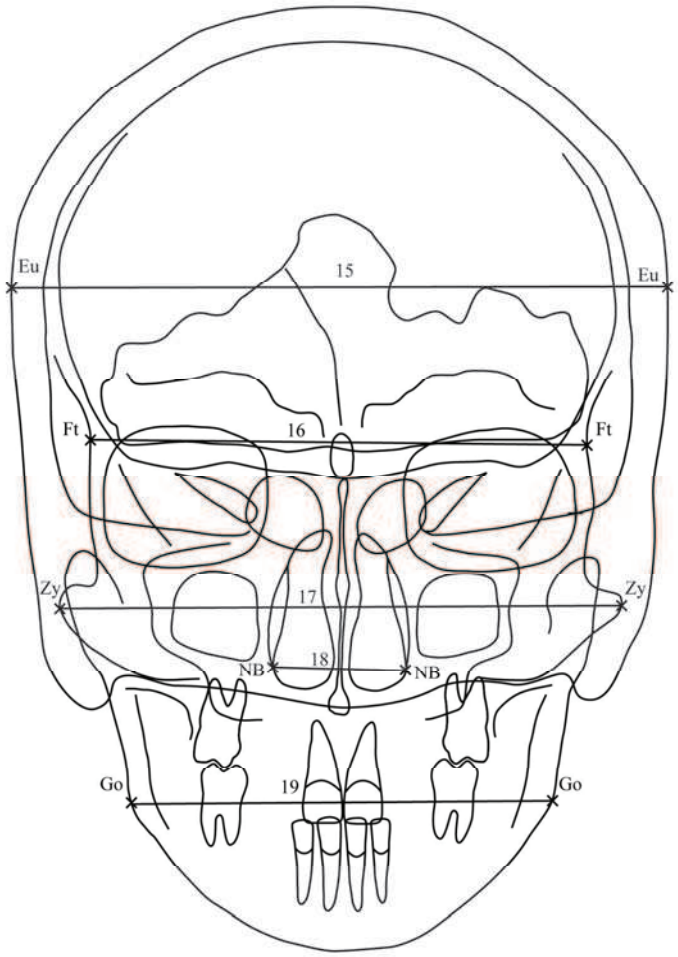
TABLE VI. Literature review of craniofacial and orodental findings in CS**.**

The description of the anomalies appears as stated in the reviewed papers using the following wording:
Mandibular micrognathia : Mandibular hypoplasia [Arenas-Sordo Mde et al., 2006], Underdeveloped mandible [Cook, 1982], Retruded chin [Schneider, 1983], Retruded small mandible [Sorin, 1994]
Micrognathia : Small oral cavity [Boraz, 1991]
Agenesis : Congenitally absent of 14, 23, 24 [Arenas-Sordo Mde et al., 2006], Congenitally absent mandibular second premolars [Schneider, 1983], Absent/hypoplastic teeth [Nance and Berry, 1992]
Macrodontia : Inappropriately large teeth [Cook, 1982]
Microdontia : Very small teeth [Sorin, 1994]
Enamel defects : Opacities/hypoplasia (PHRC), Dark pigmented teeth [Dumic et al., 1995], Discolored teeth [Sorin, 1994], Hypoplasia ([Arenas-Sordo Mde et al., 2006]; PHRC), Absent/hypoplastic teeth [Nance and Berry, 1992]
Ectopic eruption : Ectopically erupted first molars and ectopically placed molars [Schneider, 1983]
Dental caries : Dental extractions [Cook, 1982]
pt primary teeth, PT permanent teeth

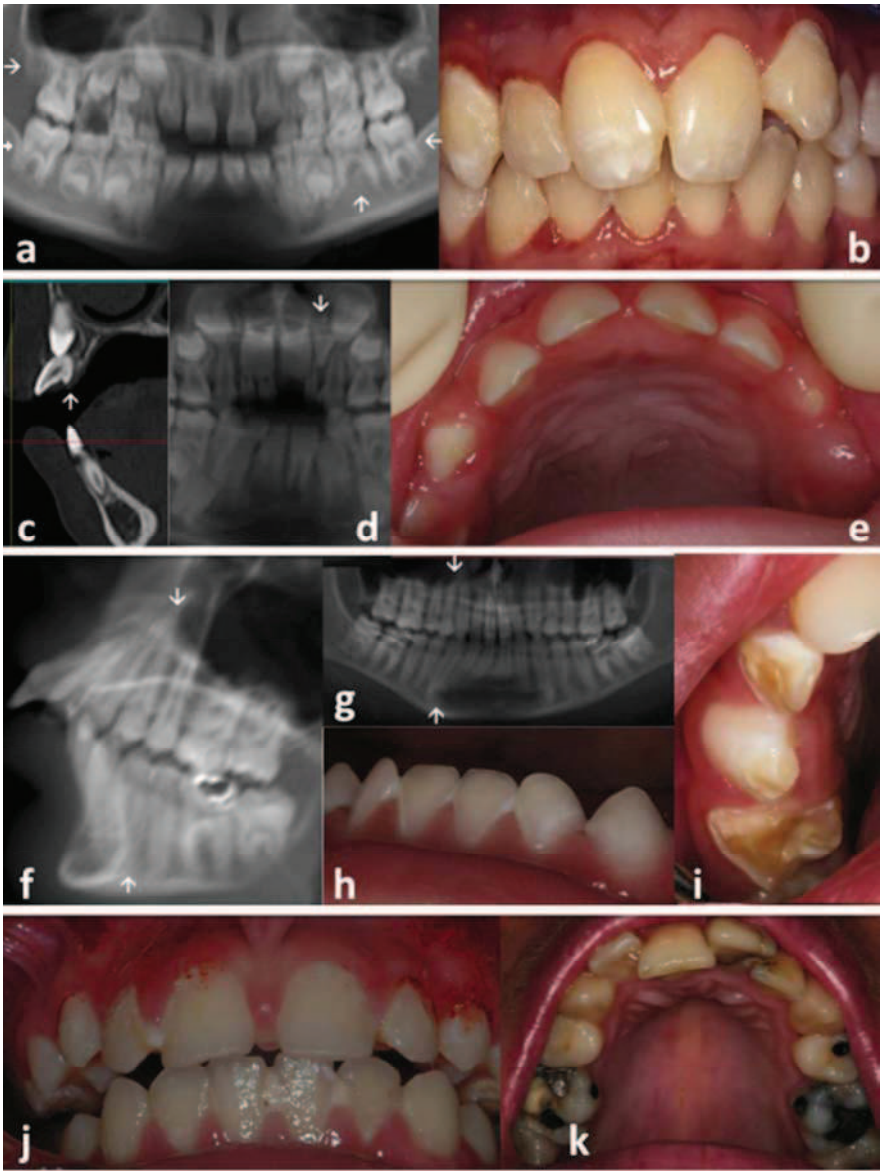


3D MIP reconstruction of the skull (a) and cephalometric analysis in norma lateralis (b) of patient 8 (6.7 years) (See Table IV).
The names and definitions of the landmarks and measured euclidien distances and angles are given in TABLES I and II. Observe the vertical growth direction of lower jaw (angle 9 FMA) and retrognathia (diminished angle 2 Facial depth) or skeletal class II (angle 11 ANB).

190x254mm (72 x 72 DPI)



Cephalometric analysis in norma frontalis of patient 16 (16.5 years). Correspondence of landmarks and measurements are detailed in TABLES I and II respectively (supplementary material). Reported to age related standards, transversal craniofacial hypodevelopment is patent.
752x752mm (72 x 72 DPI)



Orodental phenotype encountered in patients presenting with Cockayne syndrome (see also TABLE III.) Anomalies of tooth number (a) like missing second molars 17, 37, 47 and the lower inferior left premolar (35) in patient 10 - shape (b) shovel/and or screwdriver shape incisors (11, 21 patient 15); hyper developed cingulum on the permanent upper lateral incisors (c) (patient 12) – size with microdontia in the primary dentition (e) (patient 4, notice the diastemata separating the smaller primary teeth or in the permanent dentition with a microdont upper left lateral incisor (d) (here 22 in patient 8, the 12 is missing); taurodontic permanent first molars (a) in patient 10; radiculomegaly of canines, premolars or even molars (f,g, patient 16) – structure with enamel hypoplasia in the primary (h patient 2, i patient 6) or the permanent dentition. Dental plaque and biofilm subsequent to poor oral hygiene as well as gingivitis were seen in patient 11 (j). Dental crowding was visible in (k) for patient 17.

(3a patient 10; 3b patient 15; 3c patient 12; 3d patient 8; 3e patient 4; 3f;g patient 16; 3h patient 2; 3i patient 6; 3j patient 11; 3k patient 17).

190x254mm (72 x 72 DPI)

Patient	Age (y.m)	Type	Gene	Mutation	Orodental anomalies							Crowding	Caries dmft / DMFT	Perio	Oral Hygiene	Dental treatment	FD	Para
					Number of teeth	Shape	Size	Structure		Eruption								
								Enamel	Dentin	pd	PD							
1	1.8	Severe (II)	*CSB*	c.2167C>T c.2578_80delCTG	Ø	Ø	microdontia	opacities	Ø	late	Ø	+	0 / Ø	normal	good AB	First visit	MB	Ø
2	1.9	Very severe COFS	*CSB*	c.2612T>C c.3513dupT	Ø	Ø	microdontia	opacities	Ø	Ø	Ø	Ø	0 / Ø	normal	absent NB	VI	MB	brux
3	2.3	Severe (II)	*CSB*	c.1954C>T c.1954C>T	Ø	Ø	microdontia	hypoplasia	Ø	Ø	Ø	Ø	0 / Ø	normal	good AB	VI	MB	brux
4	2.5	Very severe COFS	*CSB*	c.2960T>C c.2254A>G	Ø	Ø	microdontia	hypoplasia	Ø	Ø	Ø	Ø	0 / Ø	normal	absent NB	First visit	MB R	Ø
5	2.9	Severe (II)	*CSB*		Ø	Ø	Ø	hypoplasia	Ø	Ø	Ø	Ø	20 / Ø	gingivitis	deficient NB	First visit	MB R	Ø
6	3.7	Severe (II)	*CSB*	c.3862C>T	Ø	Ø	Ø	hypoplasia	Ø	Ø	Ø	Ø	20 / Ø	normal	absent NB	VI No treatment	MB R	brux
7	4.11	Moderate (I)	*CSB*	c.2830-2A>G c.3983dupA	Ø	Ø	microdontia	hypoplasia	Ø	Ø	Ø	Ø	0 / Ø	normal	good AB	VI	MB	Ø
8	6.7	Moderate (I)	*CSB*	c.653-2A>G	agenesis 12, 15, 25,35,45	Ø	microdont 22	Ø	Ø	Ø	Ø	+++	2 / Ø	normal	good AB	VI no treatment Ortho ass	MB	Ø
9	7.11	Moderate (I)	*CSA*	c.618–1G>A	Ø	Ø	Ø	hypoplasia	Ø	early	early	++	5 / 0	gingivitis	deficient AB	VI LA	MB D R	Ø
10	8.1	Moderate (I)	*CSA*	c.582G>C c.70dupA	agenesis 35,17,37, 47	11, 21 shovel 12,22 atyp	taurodontism	opacities	Ø	Ø	Ø	Ø	2 / Ø	gingivitis	deficient AB	VI	MB D	Ø
11	9.1	Moderate (I)	*CSA*	c.797A>G c.843+5G>C	Ø	11, 21 screw d.	Ø	opacities	Ø	early	early	Ø	0 / 0	gingivitis	deficient AB	VI Fissure sealants under CSE	MB D	Ø
12	9.5	Moderate (I)	*CSA*	r.0 (no mRNA full length	Ø	12, 22 cingulum	globular 2nd premolar	opacities	Ø	Ø	Ø	+++	16 / 4	gingivitis	deficient NB	2GA	MB R	Ø
13	14.4	Moderate (I)	*CSA*	c.797A>G	Ø	Ø	Ø	hypoplasia	Ø	Ø	Ø	Ø	Ø / 0	gingivitis	deficient AB	1GA Primary dentition	MB D	Ø
14	14.6	Moderate (I)	*CSA*	r.0 (no mRNA full length)	Ø	Ø	Ø	hypoplasia	Ø	Ø	Ø	?	Ø / 28	gingivitis	deficient NB	4GA	MB R	Ø
15	15.10	Mild (III)	*CSB*	c.1913A>G c.2247delT	Ø	11, 21 screw d.	Ø	opacities	Ø	Ø	Ø	+++	Ø / 0	gingivitis	deficient AB	VI, LA Ortho ass	MB	Ø
16	16.5	Moderate (I)	*CSA*	c.797A>G	Ø	Ø	radiculo-megaly	hypoplasia	Ø	Ø	Ø	+	Ø / 2	gingivitis	deficient AB	1GA	MB D	Ø
17	28.5	Mild (III)	*CSB*	c.3778+2T>G c.2203C>T	Ø	11, 21 shovel	taurodontism	pits	Pulp Calcif	Ø	Ø	+++	Ø / 13	gingivitis	deficient AB	1GA Ortho ttt	MB	Ø

TABLE III. Genotype and orodental phenotype encountered in our cohort of 17 CS patients

TABLE IV. Results of the cephalometric analysis in *norma lateralis* and in *norma frontalis*.

Measurement	Patient 8 (6.7)		Patient 9 (7.11)		Patient 10 (8.1)		Patient 11 (9.1)		Patient 12 (9.5)		Patient 14 (14.6)		Patient 15 (15.10)		Patient 16 (16.5)		Patient 17 (28.5)		CS
Norma lateralis																			
Typology																			
Facial axis (°)	81.0	(89.5; 3.8)	79.6	(89.6 ;4.0)	88.0	(89.5; 3.6)	89.0	(89.6; 4.1)	76.0	(89.3; 4.3)	76.0	(89.4; 4.3)	74.0	(89.2; 4.5)	96.0	(88.9; 4.6)	80.0	(89.0; 4.0)	↑
Facial depth (°)	73.0	(83.2; 3.2)	84.1	(83.8 ;2.8)	81.0	(82.1; 3.3)	80.0	(84.3; 3.0)			74.0	(83.3; 3.7)	67.0	(82.9; 4.5)	75.0	(82.5; 3.9)	78.0	(86.0; 2.5)	↓
Lower facial height (°)	56.0	(47.0; 4.0)	53.2	(47.0 ;4.0)	43.0	(47.0; 4.0)	41.0	(47.0; 4.0)	58.0	(47.0; 4.0)	50.0	(47.0; 4.0)	51.0	(47.0; 4.0)	47.0	(47.0; 4.0)	50.0	(47.0; 4.0)	N
Mandibular arch (°)	31.0	(26.0; 3.5)	20.1	(26.0 ;3.5)	26.0	(26.0; 3.5)	52.0	(26.0; 3.5)	13.0	(26.0; 3.5)	18.0	(28.0; 3.5)	22.0	(29.0; 3.5)	16.0	(29.0; 3.5)	22.0	(30.0; 3.5)	↓
FMA (°)	45.0	(29.4; 4.5)	38.8	(28.6 ;3.8)	36.0	(29.4; 4.8)					50.0	(27.7; 5.8)	44.0	(28.5; 6.2)	36.0	(28.7; 5.2)	41.0	(25.8; 3.0)	↑
AFH (mm)	50.0	(57.1; 3.1)	76.4	(53.3;3.3)	46.0	(61.8; 3.6)	46.0	(60.2; 3.6)	50.0	(62.8; 3.9)	52.0	(70.7; 5.5)	51.0	(73.3; 5.8)	49.0	(76.1; 5.6)	58.0	(67.2; 4.3)	↓
PFH (mm)	22.0	(37.9; 3.7)	28.5	(33.6 ;2.7)	25.0	(42.2; 3.4)	23.0	(41.2; 3.5)	25.0	(43.4; 3.3)	19.0	(51.4; 4.6)	22.0	(51.4; 4.6)	26.0	(54.3; 4.1)	30.0	(49.6; 3.9)	↓
FHI (%)	0.4	(0.6)	0.37	(0.6)	0.5	(0.6)	0.5	(0.6)	0.5	(0.6)	0.3	(0.7)	0.4	(0.7)	0.5	(0.7)	0.5	(0.7)	↓
Skelatal analysis																			
Convexity (mm)	10.0	(4.5; 2.2)	11.9	(4.1 ;2.4)	5.0	(4.4; 2.5)	12.0	(3.6; 2.7)	7.0	(3.8; 2.3)	11.5	(2.8; 2.6)	9.0	(2.8; 2.8)	9.0	(2.6; 3.4)	8.5	(1.7; 2.9)	↑
ANB (°)	15.0	(4.7; 2.2)	10.9	(4.6 ;2.4)	8.0	(4.8; 2.2)	14.0	(4.0; 2.6)	10.0	(4.2; 1.9)	13.0	(3.4; 2.0)	11.0	(3.3; 2.1)	11.0	(3.2; 2.3)	10.0	(2.6; 2.4)	↑
Denture analysis																			
i to APg (mm)	4.0	(-0.5; 2.7)	7.2	(0.9 ;2.4)	5.0	(1.1; 2.5)	8.0	(1.6; 2.7)	6.0	(1.8; 2.4)			12.0	(1.9; 2.6)	9.0	(2.8; 2.9)	8.0	(0.8; 2.8)	↑
i to APg (°)	22.0	(15; 7.2)	25.1	(20.7 ;6.3)	44.0	(20.8; 5.2)	32.0	(22.1; 6.2)	24.0	(22.1; 4.8)			35.0	(23.8; 5.4)	37.0	(25.2; 4.9)	33.0	(21.8; 7.3)	↑
FMIA (°)	48.0	(64.8; 7.5)	59.1	(58.0 ;9.1)	45.0	(56.4; 7.7)							36.0	(55.9; 8.1)	42.0	(55.6; 8.2)	43.0	(59.0; 10.7)	↓
IMPA (°)	87.0	(87.9; 7.2)	82.1	(93.1 ;7.0)	99.0	(94.0; 5.7)	105.0	(93.9; 7.2)	81.0	(94.7; 5.7)			100.0	(94.8; 7.2)	102.0	(95.3; 6.6)	96.0	(92.1; 9.0)	N
i/I (°)	128.0	(142.2; 14.2)	110.8	(127.2 ;10.2)	110.0	(128.1; 11.2)	107.0	(125.5; 9.7)	117.0	(126.3; 9.2)			114.0	(129.2; 10.1)	103.0	(126.6; 10.0)	119.0	(133.6; 13.0)	↓
Norma frontalis																			
Cranial width (mm)	103.0	(135.7; 5.4)	180.9	(140.2; 4.3)	137.0	(140.2; 4.3)	132.0	(136.5; 5.6)	103.0	(140.2; 4.3)	116.0	(143.2; 4.7)	117.0	(143.2; 4.6)	120.0	(143.2; 4.6)	132.0	(139.1; 5.5)	↓
Bifrontotemporale width (mm)	69.0	(92.3; 4.9)	129.3	(95.4; 3.0)	85.0	(95.4; 3.0)	92.0	(93.9; 5.5)	72.0	(95.4; 3.0)	81.0	(100.3; 3.6)	79.0	(100.3; 3.6)	92.0	(100.3; 3.6)	93.0	(96.5; 4.5)	↓
Bizygomatic width (mm)	85.0	(109.4; 3.2)	128.4	(114.2; 4.2)	101.0	(114.2; 4.2)	105.0	(112.9; 3.4)	94.0	(114.2; 4.2)	100.0	(128.1; 4.4)	101.0	(128.1; 4.4)	105.0	(128.1; 4.4)	107.0	(122.8; 3.5)	↓
Nasal width (mm)	22.0	(26.2; 1.6)	40.6	(29.5; 2.0)	20.0	(29.5; 2.0)	22.0	(27.5; 1.6)	23.0	(29.5; 2.0)	26.0	(34.3; 2.6)	23.0	(34.3; 2.6)	24.0	(34.3; 2.6)	25.0	(30.5; 1.5)	↓
Bigonial width (mm)	65.0	(80.4; 3.8)	113.9	(83.0; 3.5)	77.0	(83.0; 3.5)	85.0	(83.5; 3.1)	66.0	(83.0; 3.5)	76.0	(94.6; 4.6)	75.0	(94.6; 4.6)	78.0	(94.6; 4.6)	89.0	(91.5; 3.1)	↓

For each measurement, the age-corresponding cephalometric standards are given (Mean; S.D.). **N** : Normal values. ↑ : Increased values. ↓ : Reduced values.

Gene Symbol	Lower Incisor Mean ± SD	Mandibular molars Mean ± SD	Maxillary molars Mean ± SD	FC mandibular molars/lower incisors	FC mandibular molars/ maxillary molars	FC maxillary molars/ lower incisors
Ercc6	8.76 ± 0.10	8.67 ± 0.09	8.60 ± 0.05	1.06	1.04	1.08
Ercc8	7.05 ± 0.15	7.16 ± 0.17	6.65 ± 0.10	-1.08	1.15	1.13
Ercc2	9.04 ± 0.05	9.11 ± 0.08	8.70 ± 0.02	1.05	1.11	1.02
Ercc3	10.17 ± 0.10	10.25 ± 0.14	10.36 ± 0.06	1.06	1.10	1.18
Ercc4	7.56 ± 0.06	7.61 ± .08	7.16 ± 0.07	1.03	1.06	1.02
Ercc5	8.90 ± 0.08	8.79 ± 0.12	8.54 ± 0.08	1.07	1 .04	1.03

Sign or symptom	Author (publication year)																										
	Cockayne (1936)	Marie (1958)	Macdonald (1960)	Civantos (1961)	Lieberman (1961)	Guzzetta (1967)	Fujimoto (1969)	Rowlatt (1969)	Cotton (1970)	Schmickel (1977)	Scott-Emuakpor (1977)	Cook (1982)	Schneider (1983)	Boraz (1991)	Nance & Berry (1992)	Sorin (1994)	Dumić (1995)	Mallery (1998)	Meira (2000)	Hamamy (2005)	Tan (2005)	Arenas Sordo (2006)	Bertola (2006)	Falik-Zaccai (2008)	Natale (2011)	Wang (2011)	PHRC
Craniofacial dysmorphism																											
Facial dysmophism	X	X	X																		X	X		X	X		X
Facial complex underdeveloped horizontally and vertically	X												X	X													X
Transversal hypo-development of the skull and face	X																										X
Straight facial profile													X														
Micrognathia														X							X						X
Sharp mandible																										X	
Mandibular micrognathia												X	X			X						X					X
Small oral aperture												X															
Condyle hypoplasia									X													X					
Deeply arched palate													X			X						X					X
Atrophy of alveolar processes				X							X											X					X
Orodental anomalies																											
Number																											
Agenesis							X	X		X	X		X		X							X					X
Shape																											
Shovel incisors																											X
Screw driver incisors																											X
Size																											
Macrodontia												X										X					X
Microdontia																X											X
Taurodontism																											X
Radiculomegaly																											X
Short roots in PT													X									X					
Structure																											
Enamel defects															X	X	X					X					X
Pulpar calcification																											X
Eruption																											
Delayed eruption PT																											
Delayed eruption pt			X												X				X			X					X
Early eruption in pt and PT																											X
Teeth rotation																						X					
Ectopic eruption													X														X
Crowding												X									X						X
Dental caries	X	X	X		X	X		X				X	X		X	X		X	X	X		X	X	X	X	X	X
Periodontium																											
Marginal gingivitis													X									X					X
Functional defects																											
Restricted mandibular range of motion														X													
Deglutition																											X
Mixed breathing																											X
Parafunction																											
Bruxism																											X
Malocclusion													X		X							X					X
Hypertrophied alveolar ridges																					X						
Others																											
Tooth loss																										X	

TABLE VI. Literature review of craniofacial and orodental findings in CS.

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Table I. Landmark definitions

Landmark	*Definition*
Norma lateralis	
A	A POINT: the most posterior point on the curve of the maxilla between the anterior nasal spine and the supradentale
ANS	ANTERIOR NASAL SPINE: the tip of the median, sharp bony process of the maxilla at the lower margin of the anterior nasal opening
Ar	ARTICULARE : the point of intersection of the inferior cranial base surface and the averaged posterior surfaces of the mandibular condyles
B	B POINT : the point most posterior to a line from infradentale to pogonion on the anterior surface of symphyseal outilne of the mandible
Ba	BASION : the most inferior, posterior point on the anterior margin of foramen magnum
DC	DC POINT : the center of the neck of the condyle on the BaN line
Gn	GNATHION: the most anterior-inferior point on the contour of the bony chin symphysis determined by bisecting the angle formed by the mandibular plane and the line through pogonion and nasion
Go	GONION : the most inferior and posterior point at the angle of the mandible
Me	MENTON : the most inferior point on the symphyseal outline
N	NASION : the junction of the frontonasal suture at the most posterior point on the curve at the bridge of the nose
Or	ORBITALE : the lowest point on the average of the right and left borders of the bony orbit
Pog	POGONION : the most anterior point on the contour of the bony chin. Determined by the tangent through nasion
PM	PROTUBERANCE MENTI : the point on the front border of symphysis between point B and Pog
Po	PORION : the midpoint of the line connecting the most superior point of the radiopacity generated by each of the two ear rods of the cephalostat
PNS	POSTERIOR NASAL SPINE: The most posterior point at the sagittal plane on the bony hard palate
Pt	PTERYGO-MAXILLARY FISSURE : the most posterior and superior point on the contour of the pterygo-maxillary fissure
S	SELLA TURCICA : the center of the pituitary fossa of the sphenoid bone
Xi	Xi POINT : constructed point corresponding to the geometric centre of the mandibular branch (Ricketts, 1961)
Norma frontalis	
Eu	EURION : the point at either end of the greatest diameter of the skull
Ft	FRONTOTEMPORALE : the most medial point on the temporal line of the frontal bone
Zy	ZYGION : the most lateral point of the zygomatic arch
NB	NASAL BREADTH : the greatest distance between the right and left lateral bony walls of the nasal cavity
Go	GONION : the most inferior and lateral point at the angle of the mandible

Table II. Measurement definitions

Measurement	*Definition*
Norma lateralis	
Facial axis (°)	Angle posterior and inferior formed by lines constructed from nasion-basion (N-Ba) and gnathion-pterygomaxillary fissure (Gn-Pt) (1)
Facial depth (°)	Angle posterior and inferior formed by Frankfort plane (porion-orbitale) and line nasion-pogonion (2)
Lower facial height (°)	Angle anterior and superior formed by lines constructed from Xi-anterior nasal spine (ANS) and Xi-protuberance menti (PM) (3)
Mandibular arch (°)	Angle posterior and superior formed by lines constructed from Xi-DC and Xi-protuberance menti (PM) (4)
Convexity (mm)	Distance between A point and line nasion-pogonion (5)
i to APg (mm)	Distance between lower incisor incisal edge perpendicular to line A point-pogonion (6)
i to APg (°)	Angle posterior and inferior formed by lower incisor axis and line A point-pogonion (7)
FMIA (°)	Angle posterior and inferior formed by Frankfort plane and lower incisor axis (8)
FMA (°)	Angle anterior and superior formed by Frankfort plane and Mandibular plane (menton-gonial intersection) (9)
IMPA (°)	Angle posterior and superior formed by lower incisor axis and Mandibular plane (10)
ANB (°)	Angle formed by drawing a line from A point to nasion and from nasion to B point (11)
i/I (°)	Interincisal angle (12)
AFH (mm)	Distance between line anterior nasal spine (ANS)-posterior nasal spine (PNS) and menton (13)
PFH (mm)	Distance between articulare and gonion (14)
FHI (%)	Facial height index corresponding to the ratio posterior facial height (PFH)/anterior facial height (AFH)
Norma frontalis	
Cranial width (mm)	Distance between right and left eurion points (15)
Bifrontotemporale width (mm)	Distance between right and left frontotemporale points (16)
Bizygomatic width (mm)	Distance between right and left zygion points (17)
Nasal width (mm)	The greatest distance between the right and left lateral bony walls of the nasal cavity (18)
Bigonial width (mm)	Distance between right and left gonion points (19)

For each definition, the number in brackets indicates the representation of the measurement in Figure 1.

Publication 4 : Identification par cartographie de l'homozygotie de mutations dans le gène SMOC2 responsable d'anomalies développementales.

Homozygosity Mapping and Candidate Prioritization Identify Mutations, Missed by Whole-Exome Sequencing, in *SMOC2*, Causing Major Dental Developmental Defects

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Inherited dental malformations constitute a clinically and genetically heterogeneous group of disorders. Here, we report on a severe developmental dental defect that results in a dentin dysplasia phenotype with major microdontia, oligodontia, and shape abnormalities in a highly consanguineous family. Homozygosity mapping revealed a unique zone on 6q27-ter. The two affected children were found to carry a homozygous mutation in *SMOC2*. Knockdown of *smoc2* in zebrafish showed pharyngeal teeth that had abnormalities reminiscent of the human phenotype. Moreover, *smoc2* depletion in zebrafish affected the expression of three major odontogenesis genes: *dlx2*, *bmp2*, and *pitx2*.

Dental development is a complex process of reiterative epithelio-mesenchymal interactions between the oral ectoderm and the mesenchymal cells of cephalic neural-crest origin. Tooth development involves numerous genes implied in various signaling pathways such as the Bone Morphogenetic Protein (BMP), Fibroblast Growth Factor (FGF), Sonic hedgehog homolog (SHH), and Wnt pathways.[1,2] Tooth developmental abnormalities can affect numbering, shape, size, hard tissue structures (such as enamel or dentin), roots, and periodontium formation, as well as global developmental processes such as dental eruption and resorption. All of these can be affected alone or together in either inherited disorders limited to the orodental sphere or more complex syndromes.

Here, we report on a unique and complex tooth malformation phenotype suggestive of autosomal-recessive inheritance in two first-degree cousins born from a highly consanguineous family of Turkish origin. Both children were referred to the Reference Center for Rare Orodental Diseases at the Strasbourg University Hospital because, compared to their healthy siblings, they exhibited extreme microdontia and were missing teeth. Both children presented with extreme microdontia, oligodontia, dental shape anomalies, double permanent-tooth formation, thin enamel, and short roots (with a thin associated alveolar bone), as seen in the spectrum of dentin dysplasia type I (Figure 1).[3,4] The eldest child (III.3) was 10 years old on last examination and presented a well-identified, moderate, X-linked, ichthyosis phenotype known to segregate in the family. The youngest child (III.4), III.3's female cousin, was 5 years old at her first visit and received followed-up examinations for the next 5 years. Both children were born after uneventful pregnancies and were normal at birth. Their developmental milestones are normal to date, and their general physical appearance is unremarkable except for obesity in III.4 (not present in III.3) and very mild bone abnormalities in III.4 (not present in III.3). The orodental findings were documented with the D[4]/phenodent Diagnosing Dental Defects Database. Oligodontia was diagnosed because III.4 was missing 13 permanent teeth and III.3 was missing 14. Anomalies of tooth size were observed, and an extreme microdontia affected both primary teeth (all present) and permanent teeth. However, some permanent teeth were macrodont. Anomalies of tooth shape concerned all existing teeth; extra cusps were visible, and crowns were tiny, globular, and malformed, especially in the primary dentition. Double tooth formation (notched and macrodont) was visible on the permanent incisors. Temporary and permanent molars exhibited taurodontism. Moreover, the molars showed tooth-structure anomalies reminiscent of the dentin dysplasia type I spectrum and had very short roots (Figure 1).[3] Compared to dentin in the X-ray, the enamel was very thin and had limited contrast. The alveolar bone associated with the primary teeth was hypodeveloped. The primary teeth were mobile and exfoliated prematurely.

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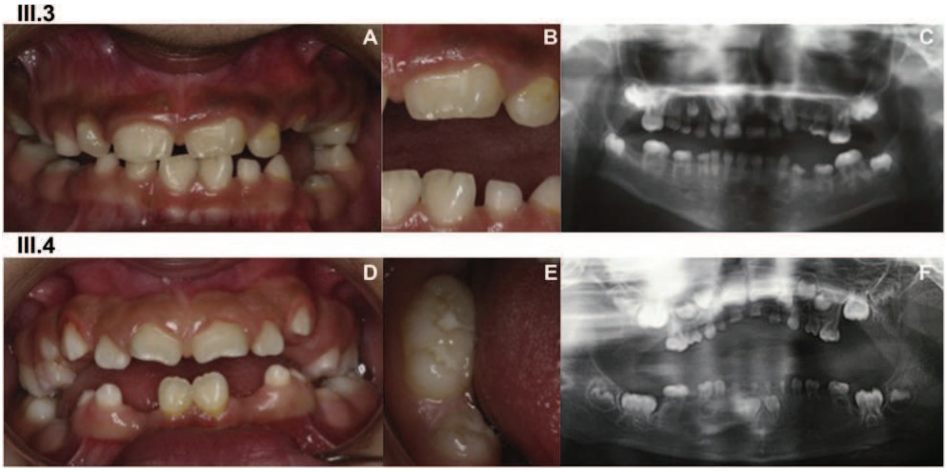

Figure 1. Clinical Description of the Affected Family Members
A clinical description of individuals III.3 (A, B, and C) and III.4 (D, E, and F) shows major dental developmental abnormalities in tooth number, size, shape, structure, eruption, and resorption, as seen in the intraoral photographs (A, B, D, and E) and the panoramic radiographs (C and F).
(A and B) (A) shows an intraoral view of III.3 (10 years old). Beside the microdont primary and permanent teeth, which show spaced dentition, double tooth formation (notched and macrodont) is visible on permanent central incisors 21 and 31; 21 shows a vestibular abnormal relief. These anomalies are clearly seen on (B) in an enlargement of the left incisor region.
(C) A panoramic radiograph shows III.3, who is missing the following permanent teeth: 18, 15, 24, 25, 28, 48, 45, 44, 43, 32, 33, 34, 35, and 38. The primary and permanent molars are taurodont. The roots are extremely short and are slightly more developed in the permanent dentition but are, however, conical with sharp endings. The pulp has a flame-like shape. The enamel is very thin and has limited contrast compared to the dentin in the X-Ray. Teeth 64, 65, 74, and 75 are reincluded.
(D) Intraoral view of III.4 (9.5 years old). Double tooth formation (notched and macrodont) is visible on the permanent central-upper-left incisor (21). The lower arch seems interrupted in the area of missing teeth (45, 43, 42, 32, 33, 34, and 35). Teeth 85 and 75 are reincluded, indicating ankylosis in the alveolar bone.
(E) A close-up on macrodont tooth 46 (lower-right permanent first molar) shows extra cusps and an elongated crown on its mesiodistal axis.
(F) A panoramic radiograph of III.4 at 5 years old shows oligodontia—13 permanent teeth are missing (18, 15, 25, 28, 48, 45, 43, 42, 32, 33, 34, 35, and 38)—and extreme microdontia of all the primary teeth. Note the short and sharp roots and the hypodeveloped alveolar bone.

This study—designed to identify the genetic mutations involved in the dentin dysplasia phenotype—was approved by the ethics committee of the Strasbourg University Hospital. Informed consent was obtained from all individuals who participated in the study. Homozygous mapping via GeneChip Human 250K SNP Affymetrix was performed on affected individuals III.3 and III.4 and nonaffected individuals III.1, III.2, III.5, and III.6. A unique homozygosity region was shared between the two affected individuals and was located between rs2981956 and the end of chromosome 6, defining a 3 Mb region on chromosome 6q27-ter (Figure 2A). According to Ensembl, this interval contained 69 annotated genes. Genes were selected as likely candidates either because of their known implication in inherited dental conditions or because of their potential dental expression, indicated by the following databases: Helsinki University's Gene Expression in Tooth; the UCSC Genome Browser; the 1000 Genomes Browser; the Ensembl Genome Browser; GeneHub-GEPIS; GenePaint; Eurexpress; and the Zebrafish Information Network (see Web Resources).

Two genes were selected with high priority: *DACT2* (Dapper antagonist of beta-catenine 2 [OMIM 608966]) and *SMOC2* (SPARC-related modular calcium-binding protein [OMIM 607223]). Dact2 modulates Wnt signaling by binding to the intracellular protein Dishevelled (Dvl) and might play an important signal-modulating role in tooth development at the level of the epithelial cells that include the enamel-knot signaling centers and the preameloblasts.[5] The sequencing of *DACT2* 4 exons was normal in both affected individuals.

SMOC2 belongs to a family of matricellular proteins that regulate interactions between cells and the extracellular matrix. The GenePaint database indicated a high level of in situ hybridization in the craniofacial region of the mouse at embryonic day 14.5 (E14.5), especially at the level of the tooth mesenchyme. *SMOC2* spans about 226 kb. The coding region of *SMOC2* consists of 13 exons. Each domain of *SMOC2* is encoded by one or more exons, and the domain borders coincide with splice sites. Sequencing of *SMOC2* (ENST00000354536; NM_022138/hg19) revealed a homozygous mutation (c.84+1G>T) in the canonical-splice donor site of intron 1. The parents of both affected children were heterozygous for this mutation, and the children's nonaffected siblings were heterozygous for this mutation (Figure 2B). This mutation was absent in 112 ethnically matched controls. The primer sequences are detailed in Table S1, available online.

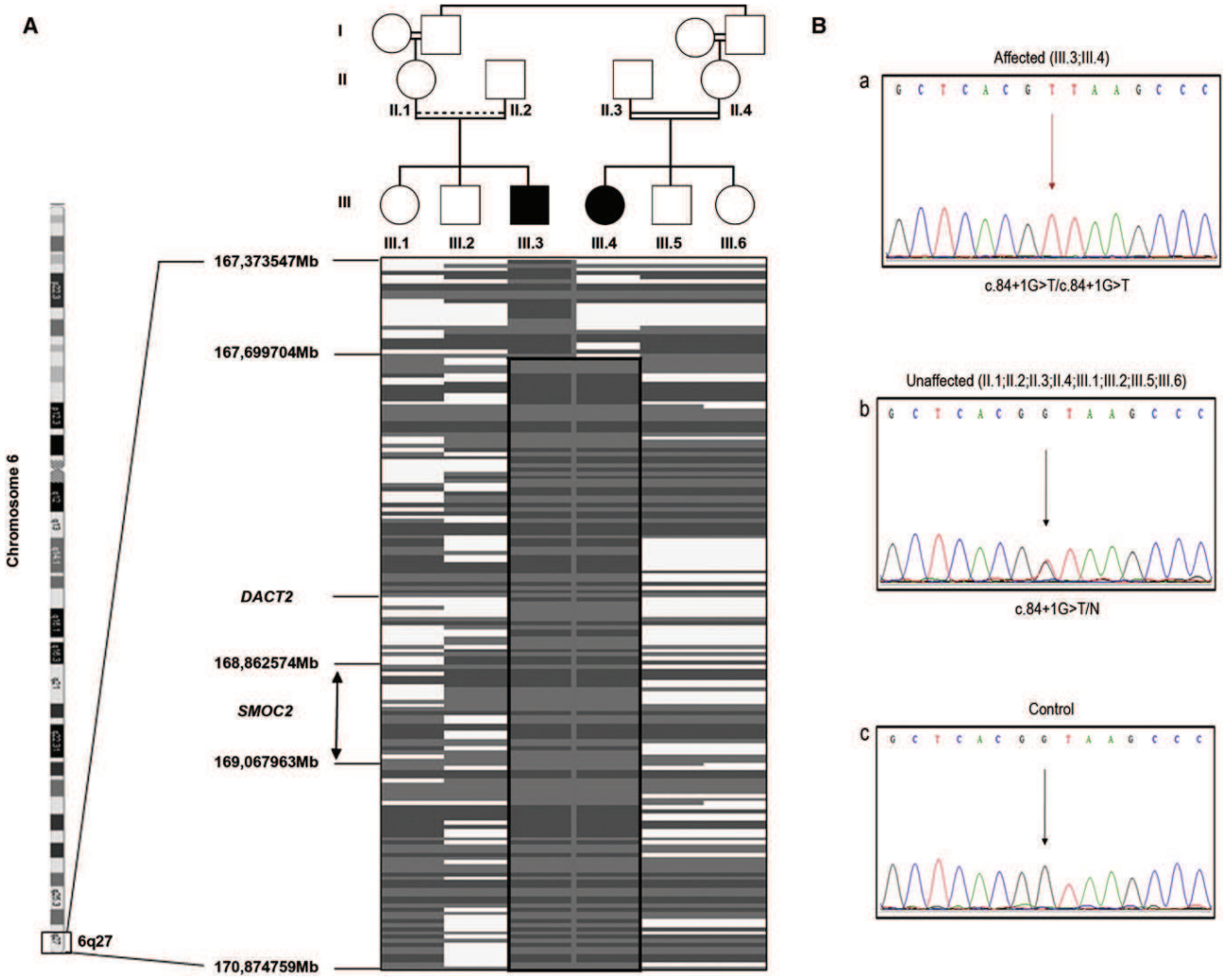

Figure 2. Homozygosity Mapping and Mutation Detection
(A) A simplified pedigree of the family, underlined by corresponding schematic representation of the homozygosity mapping results, shows the chromosome 6 homozygous region that is common in affected individuals: Gray shading indicates homozygous SNPs and white zones indicate heterozygous alleles.
(B) Electropherograms of a part of the *SMOC2* exon1-intron1 boundary show (a) a homozygous c.84+1G>T mutation in an affected child; (b) a heterozygous c.84+1G>T mutation in a nonaffected sibling; and (c) a healthy control individual.

In order to confirm that *SMOC2* was the only gene that carried mutations in the interval, we performed exome sequencing in collaboration with IntegraGen (Evry, France). Exons of patient III.4's DNA were captured via in-solution enrichment methodology (SureSelect Human All Exon Kit v.3, Agilent, Massy, France) with the company's biotinylated oligonucleotide probe library (Agilent Human All Exon 50 Mb Kit v.3). The genomic DNA was then sequenced on a sequencer as paired-end 75 bases (HISEQ, Illumina, San Diego, USA). Image analysis and base calling were performed with Real Time Analysis (RTA) Pipeline version 1.9, set to its default parameters (Illumina). The bioinfomatic analysis of sequencing data was based on the pipeline provided by IntegraGen (Illumina's CASAVA 1.8). CASAVA performs alignment, calls the SNPs on the basis of allele calls and read depth, and detects variants (SNPs and indels). Genetic variation annotation was performed by an in-house pipeline and provided results for the sample in tabulated text files.

Among the sequences that could be analyzed, no obvious truncating or nonsense mutation could be identified in any of the 69 genes. We identified 81 substitutions (47 intronic, or in the untranslated regions; 22 synonymous; and 12 missense, of which all were SNPs), seven deletions (all intronic and four SNPs), and one insertion (all intronic and one SNP).

However, in our 3 Mb region of interest on chromosome 6, 32 out of 279 baits could not be further analyzed because they did not provide enough coverage. A total of 6.6 kb from the 3 Mb region was not covered sufficiently and overlapped with at least one bait (average bait size was 121 bp) in each of 17 genes (from one to four baits per gene). Thus, taking into account that we had already sequenced two of these genes—*SMOC2* and *DACT2*, which

together account for six unread baits—because of their high expected impact on tooth development, 15 genes (out of the 69) that accounted for 5.5 kb were still imperfectly explored and were excluded as interesting candidates in our initial approach.

Interestingly, the region that contains our mutation is poorly covered, and we could not identify by whole-exome sequencing the *SMOC2* mutation located at the end of exon 1. Moreover, this region of *SMOC2* is GC rich, possibly explaining this failure. We compared exome-capture sequencing data from several independent individuals involved in other projects by applying the same setting and confirmed the deficit in the sequence coverage of this specific bait. We would like to point out that although the exome-capture approach is a true revolution in human genetics, it has to be analyzed cautiously; in our case, we would have missed the causative mutation and gene.

Although widespread expression of *SMOC2* in various human tissues (skin, liver, muscle, lung, spleen, colon, pancreas, kidney) is demonstrated by quantitative reverse transcription PCR (QIAGEN Quantitect primer assay, assay name Hs_SMOC2_1_SG Cat N° QT00085687), we did not succeed in comparing the reverse transcription PCR (RT-PCR) of patients to that of controls because the *SMOC2* expression seemed to be very weak in human fibroblasts.

SMOC2 was identified by way of an expressed sequence tag database search for proteins homologous to the BM-40 protein family, also known as secreted protein acidic and rich in cysteines (SPARC).[6] BM-40 matricellular proteins are extracellular proteins that do not contribute structurally to the extracellular milieu but that regulate interactions between cells and the extracellular matrix.[7] The SPARC/osteonectin/BM-40 family is expressed in many cell types and is highly expressed during embryogenesis, wound healing, and other instances where there is extensive tissue remodelling.[8] SMOC2 shares an identical domain structure with SMOC1, another secreted modular calcium-binding protein.[9] In addition to a extracellular calcium-binding (EC) domain homologous to that in BM-40, SMOC1 and SMOC2 share two thyroglobulin-like (TY) domains, an follistatin (FS) domain, and a novel domain. Mutations in *SMOC1* have recently been described in patients with a rare recessive developmental disease—Waardenburg anophthalmia syndrome, which mainly involves severe eye malformations and limb defects.[10,11] *SMOC2* has been reported as a risk locus for generalized vitiligo in an isolated Romanian community, but this finding has been questioned in another study.[12,13] To date, no inherited condition has been clearly related to *SMOC2* mutations. The mutations identified in this family point to a major role of SMOC2 in dental development, and we aimed to gather functional data for such a role.

Mouse *Smoc2* is located on chromosome 17, and its intron-exon structure is highly conserved in comparison to that of the human gene. *Smoc2* is expressed in nearly all adult mouse tissues, and the highest expression is found in the heart, muscles, spleen, and ovaries.[6] We next analyzed the expression of *Smoc2* during mouse orodental development. We used E14.5 tooth germ cDNAs to perform a study with GeneChip Mouse Gene 1.0 ST arrays (Affymetrix). We detected greater *Smoc2* expression in molar than in incisor germs; the opposite pattern was evident for *Smoc1* expression. Moreover, in situ hybridization was performed on mouse embryos at E12.5, E14.5, E16.5, and E18.5, which correspond to dental lamina, cap, bell, and bell with differentiated odontoblast and preameloblast stages, respectively. *Smoc2* expression was found in the oral ectoderm and the outer dental epithelium at E14.5 and in mesenchymal papilla facing the epithelial loops of molars and the only lingual loop of incisors (Figure S1).

To obtain independent functional data on the role of Smoc2 in tooth development, we turned to zebrafish by using the well-established morpholino knockdown technique.[14] The development and structure of zebrafish teeth reflect the evolutionary, ancestral condition of jawed vertebrates. A distinctive feature of zebrafish dentition is the restriction of teeth to a single pair of pharyngeal bones: Teeth are absent from the oral cavity and are restricted to the fifth ceratobranchials.[15] Such dentition is characteristic of the order Cypriniformes.[16] Morphological signs of tooth initiation appear around the time of hatching (2 days after fertilization) in zebrafish, and the first germs become mineralized and attached to the underlying bone within 4 days after fertilization. Tooth development is similar to that of mammals.[17,18] We therefore used the zebrafish as a model to analyze the function of Smoc2 in tooth development. A search of the zebrafish genome sequence (Ensembl, zv9) revealed a 115 amino acid zebrafish Smoc2 protein (ENSDARP00000108925; named Smoc2a) that shared 68% identity with a 123 amino acid human SMOC2 splice variant (GRCh37, ENSP00000440052). We identified the full-length zebrafish *smoc2*, which encodes a 429 amino acid protein (Figure S2). The protein shares 67% overall identity with the longest human SMOC2 splice variant (ENSP00000346537). In particular, the C-terminal calcium-binding domains appear to be evolutionarily conserved: Human *SMOC2* has numerous splice variants, all of which share the C-terminal region, which includes two calcium-binding domains. In situ hybridization of *smoc2a* mRNA revealed expression in the pharyngeal pouches and arches. Expression in the area from which the teeth develop was diffuse at 48 hpf (not shown) but condensed by 56 hpf to two bilateral dots, marking the position of the first pair of teeth (Figures S3C–S3D). Morpholinos are an effective way of transiently knocking down zebrafish gene function.[14] Two morpholinos were created: Mo-*smoc2-1* and Mo-*smoc2-2*. Mo-*smoc2-1* was designed to target the *smoc2a* ATG triplet code to impair the initiation of translation and splicing within larger *smoc2* transcripts. Mo-*smoc2-2* was designed to target the exon2-intron2 boundary of *smoc2a* (Figures S2A–S2F). The efficiency of Mo-*smoc2-2* was controlled by RT-PCR.

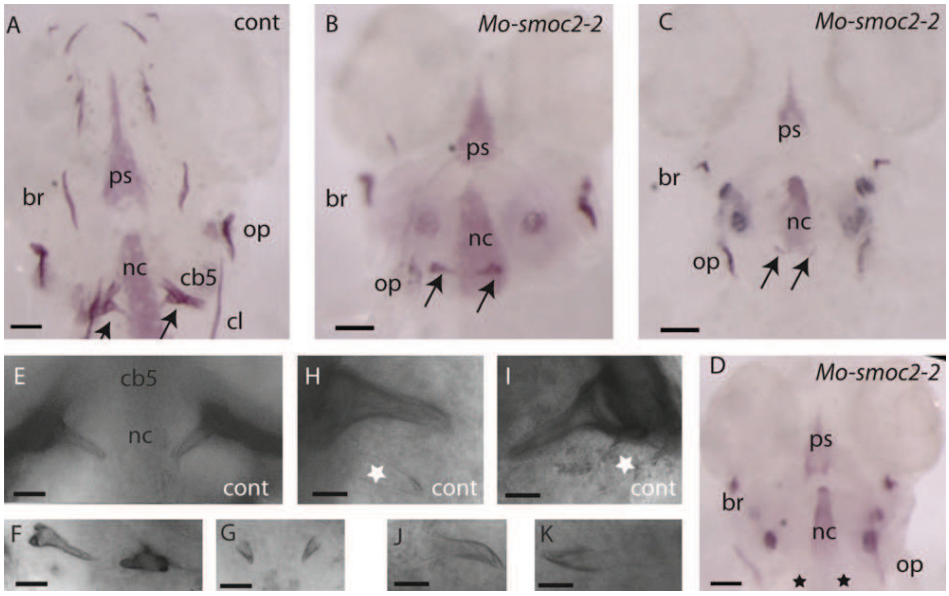

Figure 3. Dentition in *smoc2* Morphants
The heads of the control (A) and the *smoc2-2* morphants (B–D) stained with alizarin red at 5 dpf show different degrees of reduction of pharyngeal tooth size (arrows in B and C) and the complete absence of pharyngeal teeth (black stars in D) in the morphants. Control (E) and morphant (F and G) teeth are shown at higher magnification. Note the misorientation and the difference in the shape of the teeth in (F) compared to the control. Compared to the control (H and J), the morphants (J and K) show no additional emerging teeth. The white stars represent the transparent second tooth. The scale bars represent the following measurements: (A–D): 50 μm; (E–G): 25 μm; and (H–K): 12 μm. The following terms are abbreviated: branchiostegal ray (br); ceratobranchial 5 (cb5); parasphenoid (ps); ceratohyal (cl); notochord (nc); and opercle (op). All embryos are presented in ventral view, anterior up.

The morphant transcript contained both the correctly spliced fragment and a transcript lacking part of the second exon that encodes the calcium-binding domain, leading to a premature stop codon (Figures S2F–S2H). The morpholino appears to activate a cryptic splice site, as previously noted for other morpholinos.[19] We analyzed the development of the teeth in 5-day-old *smoc2-2* morphants by using alizarin red to stain the calcified structures.[20] In both *smoc2* morphants, the first two bilateral teeth were smaller than those of the controls (Figure 3, 40 embryos were analyzed). In about 5% of the morphants, the teeth were even undetectable (Figure 3D). The size and presence of the teeth were probably dependent on the level of *smoc2* depletion. In addition, although the appearance of the second tooth was already visible in the control embryos, it was undetectable in morphants (Figures 3H–3K). A close inspection of tooth shape revealed a very broad tooth base anchored within the fifth ceratobranchial bone in control larvae (Figures 3H and 3I), whereas it appeared very thin in the morphants (Figures 3J and 3K). In addition, compared to the controls, the *smoc2* morphants were missing calcification of some dermal bones and the fifth ceratobranchial bone (Figures 3A–3D), indicating that the skull was affected in morphants. Injections of 0.3 mM of Mo-*smoc2-2* and 0.7 mM of Mo-*smoc2-1* resulted in a slightly reduced head size in 74% and 56% of the embryos, respectively (category 1, Figure S2D). This reduction was independent of the head volume, excluding the possibility that tooth development could have indirectly been affected by overall impairment of head development (Figures 3B and 3C). To further rule out developmental delay, we analyzed the head musculature in wild-type and *smoc2-2* morphants at 5 dpf with a skeletal muscle reporter line (Roostalu, personal communication). All the muscles present in the wild-type were also seen in the morphant, indicating that the development of the head musculature occurred correctly in the morphant (data not shown). In addition, an immunostaining with an antibody against phosphohistone H3 marked proliferating cells. We showed that cell proliferation was not inhibited in the oropharyngeal area of 5 dpf morphants, indicating that the reduced tooth size was not a consequence of an overall reduced proliferation rate (data not shown).

Next, we analyzed the developing zebrafish tooth germs by using probes of genes whose orthologs are expressed during mouse odontogenesis. *dlx2* is an early marker of the dental epithelium in the mouse.[21] The zebrafish possesses two semiorthologs (a duplicate gene pair equally related to a single ortholog in another species)[22] of human *DLX2*: *dlx2a* and *dlx2b*.[23,24] Both duplicates are expressed in tooth germs from 48 hr onward.[25] *dlx2b* is expressed initially in the thickened dental epithelium, but not in the underlying mesenchyme.[26] The expression of this gene marks the location of the tooth germ undergoing morphogenesis before mineralization. *dlx2b* expression in 56 hpf and 72 hpf *smoc2* morphants was undetectable in the pharyngeal region where teeth would normally form (Figures 4A–4D). This absence of expression was observed

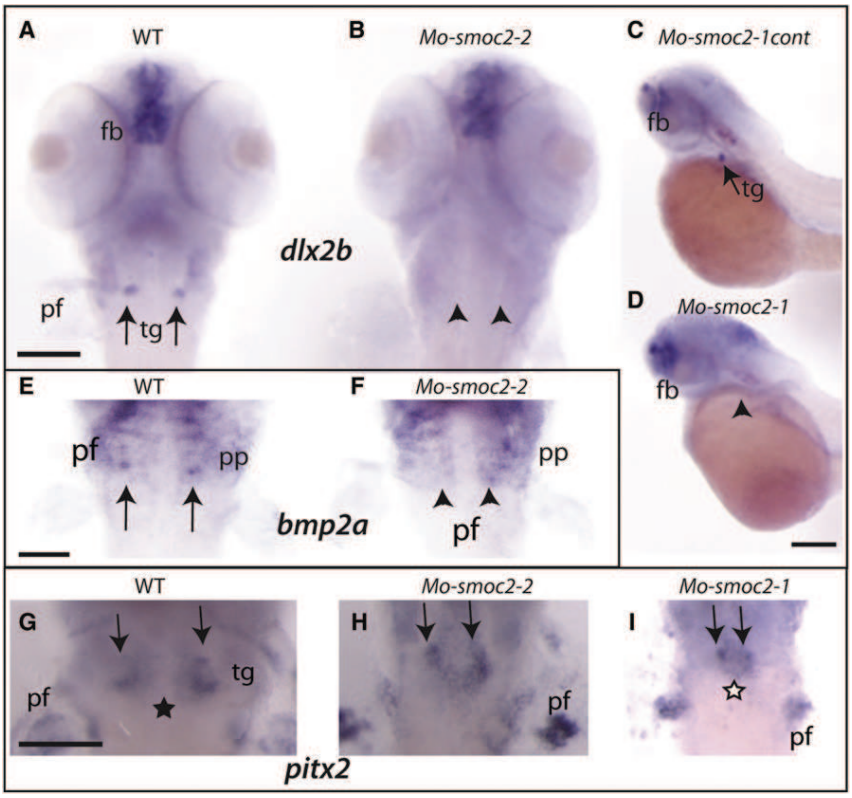

Figure 4. *smoc2* Morphants Exhibit Tooth-Germ Defects

(A–I) In situ hybridization of both *dlx2b* (A–D; probe obtained from D.W. Stock, Colorado, USA), *bmp2a* (E and F; probe obtained from M. Hammerschmidt, Cologne, Germany) and *pitx2* (G–I, *pitx2* full-length cDNA was cloned into the Flc3 plasmid [Riken]) on wild-type (A, E, and G), *smoc2-2* (B, F, and H), *smoc2-1* (D and I), and *smoc2-1cont* morphants (C) shows a loss of *dlx2b* and *bmp2* expression in *smoc2* morphants (B, F, and D) and reduced and fused expression of *pitx2* (H and I). The *smoc2-1cont* morphants do not show any defects.

The arrows represent teeth germs; the arrow heads represent missing *dlx2b* or *bmp2a* expression. The black star represents a gap within *pitx2* expression, the white star represents fused *pitx2* expression. Abbreviations are as follows: pf, pectoral fin; tg, teeth germs; fb, forebrain; and pp, pharyngeal pouches. Lateral (C and D) and ventral (A, B, E–I) views of embryos, 56 hpf. The scale bar represents 100 μm.

in 90% of *smoc2-1* and *smoc2-2* morphants (20 embryos were analyzed for each morpholino, Figures 4B–4D). In contrast, 100% of the control larvae ($n > 30$) showed normal expression (Figure 4C). Other *dlx2* domains, including the forebrain, did not seem to be impaired in the morphants. Because morpholinos can cause cell death in an unspecific manner, we coinjected a morpholino targeting p53 and analyzed *dlx2b* expression at 72 hpf. Even when apoptosis was blocked, *dlx2b* expression was gone from the tooth germ area in the morphant (Figures S3I–S3M). In conclusion, apoptosis was not the cause of a lack of *dlx2b* expression.

In contrast to the lack of *dlx2b* expression, no obvious reduction of expression of the semiortholog *dlx2a* in *smoc2a* morphants was revealed through in situ hybridization (Figures S3C and S3D), suggesting that the two orthologs were regulated differently.

Bone morphogenetic proteins (BMPs) are known to play multiple roles in tetrapod tooth development and evolution.[27,28] *bmp2a* was shown to be expressed in the pharyngeal tooth germ in zebrafish.[28] In situ hybridization of this transcript revealed a loss of pharyngeal tooth expression of *bmp2a* in *smoc2* morphants (100% for both morpholinos, 20 embryos were analyzed for each) compared to the control embryos (Figures 4E and 4F).

In mice, the pituitary homeobox transcription factor PITX2, a DNA- and RNA-binding protein, is expressed in the stomodeal ectoderm from which teeth are eventually derived.[29] Zebrafish *pitx2* is strongly expressed in bilateral patches in the pharyngeal epithelium.[26] Epithelial *pitx2* expression in 56 hpf morphants was not as drastically affected as that of *dlx2b* (Figures 4G–4I). *pitx2,* which is normally expressed in bilateral patches, showed a reduced expression domain (90% of the *smoc2-2* and *smoc2-1* morphants, $n = 20$). In addition, the bilateral patches were often fused (80% of the *smoc2-1* morphants, 20 embryos were analyzed for each). This was never observed in the control embryos (20 embryos were analyzed). Other regions of *pitx2* expression were unaffected in the morphants.

Reduction in the expression of genes considered as tooth germ markers is likely to affect the tooth development itself. *Dlx2* has been shown to be involved in the patterning of murine dentition, given that the loss of function of Dlx1 and Dlx2 results in early failure of upper-molar development. Mice null for *pitx2* have, among other defects, impaired determination and proliferation of tooth organogenesis.[30] The effect of *smoc2* knockdown on *dlx2b*, *bmp2a*, and *pitx2* expression suggests that *smoc2* plays a crucial role in zebrafish dental development upstream of these factors. The fact that, unlike *dlx2* expression, *smoc2* expression is not initially restricted to the tooth germ suggests that it defines a broader domain from which the tooth germ can develop. Similarly, it was shown that knockout of *prdm1a*, which is required for posterior arch development, leads to tooth depletion in zebrafish.[31] Overall, the zebrafish *smoc2* analysis suggests that *smoc2* has an important function in oropharyngeal development. In light of the highly similar human phenotype—characterized by a very rare dental developmental abnormality—we conclude that Smoc2 plays an evolutionarily conserved role in tooth development. However, the exact role of Smoc2 during development warrants further investigation.

Smoc1 and *Smoc2* have been shown to be widely expressed in both embryonic and adult mice—*Smoc1* mainly in basement membranes of organs and *Smoc2* mainly in the extracellular matrix.[9,6, 32] Similarly, the phenotype that we observed in the heads of zebrafish morphants was not limited to teeth. Hence, in addition to tooth development, other morphogenetic events appear to require Smoc2 function.

The molecular function of Smoc2 (and Smoc1, which is often studied in conjunction) has been partially uncovered. Smoc2 has been shown to interact with αvβ1 and αvβ6 integrins and contributes to cell-cycle progression by maintaining integrin-linked kinase (ILK) activity during the G1 phase of the cell cycle.[33] This suggests a role in linking the extracellular matrix with the intracellular effector ILK.

Another finding is that Smoc2 can regulate the mitogenic and angiogenic effects of vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), and FGF acting in the related pathways.[34] Developmental studies in mice have shown that Smoc2 (and Smoc1) might mediate intercellular signaling and cell-type-specific differentiation during gonadal and reproductive duct development.[35] The data collected here from mouse in situ hybridization shows that the ectomesenchymal *Smoc2* expression is indeed localized within the proliferative compartment facing the epithelial loop at E18.5. The asymmetric mesenchymal labeling observed in the continuously growing incisor on its lingual side might be linked to the short-root anomaly.

Using a knockout mouse to further characterize Smoc2 would improve our knowledge of the exact role of this protein during dental development. Moreover, a possible interaction with other factors such as Pitx2, Dlx2, or other extracellular proteins warrants further investigation. Interestingly, in Axenfeld-Rieger syndrome,[36] dental abnormalities due to *PITX2* (OMIM 601542) mutations share common features with the phenotype reported herein, suggesting that *PITX2* and SMOC2 may have concurrent developmental functions. The dental phenotype disclosed by the patients has been seldom reported in the literature and resembles that of dentin dysplasia type I, yet it has major differences. Teeth affected by dentin dysplasia generally appear clinically unremarkable and have normal shape and consistency. Radiographically, the roots are sharp with conical and apical constrictions. Pre-eruptive pulpal obliteration leads to a crescent-shaped pulpal remnant parallel to the cementoenamel junction in the permanent dentition and to the total pulpal obliteration in the deciduous teeth.[3] When combined with certain features of dentin dysplasia type I, the phenotype described in patients III.3 and III.4 very closely matches the root phenotype but is, however, distinct because the patients' teeth were extremely microdont and presented various shape anomalies. This phenotype is very similar to the phenotype described by Ozer and already qualifies as an atypical case.[4]

Although the reports on the biological activities of SMOC2 suggest a widespread effect, other proteins might compensate for the absence of SMOC2. Indeed, the patients reported herein mainly presented with an orodental phenotype with very minor, if any, developmental traits.

Interestingly, a transcriptome study on human periodontal ligaments has highlighted the expression of 13 extracellular matrix genes, among which is *SMOC2*.[37] In contrast to SMOC1, human SMOC2 appears to be particularly important for dental development but does not play a major role in eye and limb development.

In conclusion, although exome capture is a powerful approach to identifying genes, classical homozygosity mapping followed by candidate-gene selection remains an efficient process, especially for regions of the genome that are poorly covered. This is the first report showing that *SMOC2* is an early dental developmental gene in human beings and highlighting this protein as potentially useful in regenerative dentistry.

Supplemental Data

Supplemental Data include three figures and one table and can be found with this article online at http://www.cell.com/AJHG/.

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Web Resources

The URLs for data presented herein are as follows:

1000 Genomes Browser, http://browser.1000genomes.org
BDGP, http://www.fruitfly.org
D[4]/phenodent Diagnosing Dental Defects Database, http://www.phenodent.org
dbSNP, http://www.ncbi.nlm.nih.gov/projects/SNP
Ensembl Genome Browser, http://www.ensembl.org
Eurexpress, http://www.eurexpress.org/ee/
GenBank, http://www.ncbi.nlm.nih.gov/Genbank
Gene Expression in Tooth, http://bite-it.helsinki.fi
GeneHub-GEPIS, http://www.cgl.ucsf.edu/Research/genentech/genehub-gepis/genehubgepis-search.html

GenePaint, http://www.genepaint.org
HSF2.4.1, http://www.umd.be/HSF
NCBI, http://www.ncbi.nlm.nih.gov/
Online Mendelian Inheritance in Man (OMIM), http://www.omim.org
UCSC Genome Browser, http://genome.ucsc.edu/cgi-bin/hgGateway
UniGene, http://www.ncbi.nlm.nih.gov/unigene
The Zebrafish Model Organism Database (ZFIN), http://zfin.org/cgi-bin/webdriver?MIval=aa-ZDB_home.apg

Accession Numbers

The GenBank accession number for the zebrafish *smoc2* sequence reported in this paper is JQ085591.

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Supplemental Data

Homozygosity Mapping and Candidate Prioritization Identify Mutations, Missed by Whole-Exome Sequencing, in *SMOC2*, Causing Major Dental Developmental Defects

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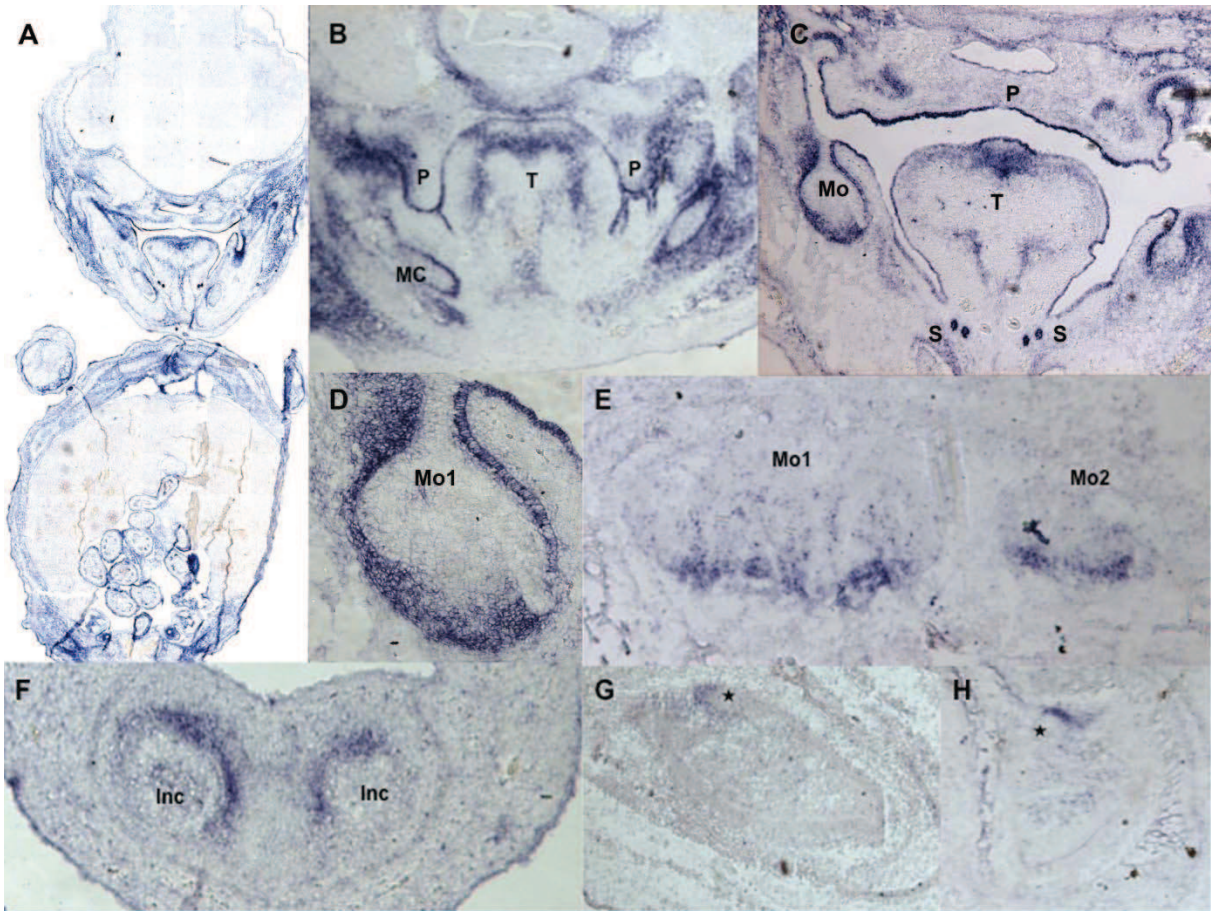
Figure S1. In situ hybridization of *Smoc2* during mouse development



(A) General view of an E14.5 dpc mouse embryo, *Smoc* 2 was expressed in various organs and systems like the digestive, urinary, respiratory (lung), nervous systems, vibrissae, olfactory nasal epithelium, axial skeleton.

(B) On this frontal view of the oral cavity, at E12.5 mouse embryo, *Smoc 2* transcripts were detected in the oral ectoderm especially in the maxilla and lining the palatal shelves (P) or the tongue (T), in the gingivolingual sulcus and in various mesenchymal area within the craniofacial region - preenchondral

condensation especially the skull basis - preossification area - around Meckel's cartilage – and within specific region of tongue mesenchyme.

(C) On this view frontal of the oral cavity (E14.5), *Smoc 2* labeling was fainting but still visible in the oral ectoderm, the salivary glands excreting canals (S), the perichondrium of Meckel's cartilage (MC), tongue muscle bundles (T) and in the condensing mesenchymal cells of the maxillary/palatal bone rudiments.

The expression of *Smoc2* was present from day E14.5 onwards both during molar (Mo) and incisor (Inc) development.

(D) At E14.5 the signal was visible both in ectodermal (asymmetric labeling of the outer dental epithelium) and mesenchymal structures (cervical region of the dental papilla, follicular sac) of the cap stage first molar (Mo1).

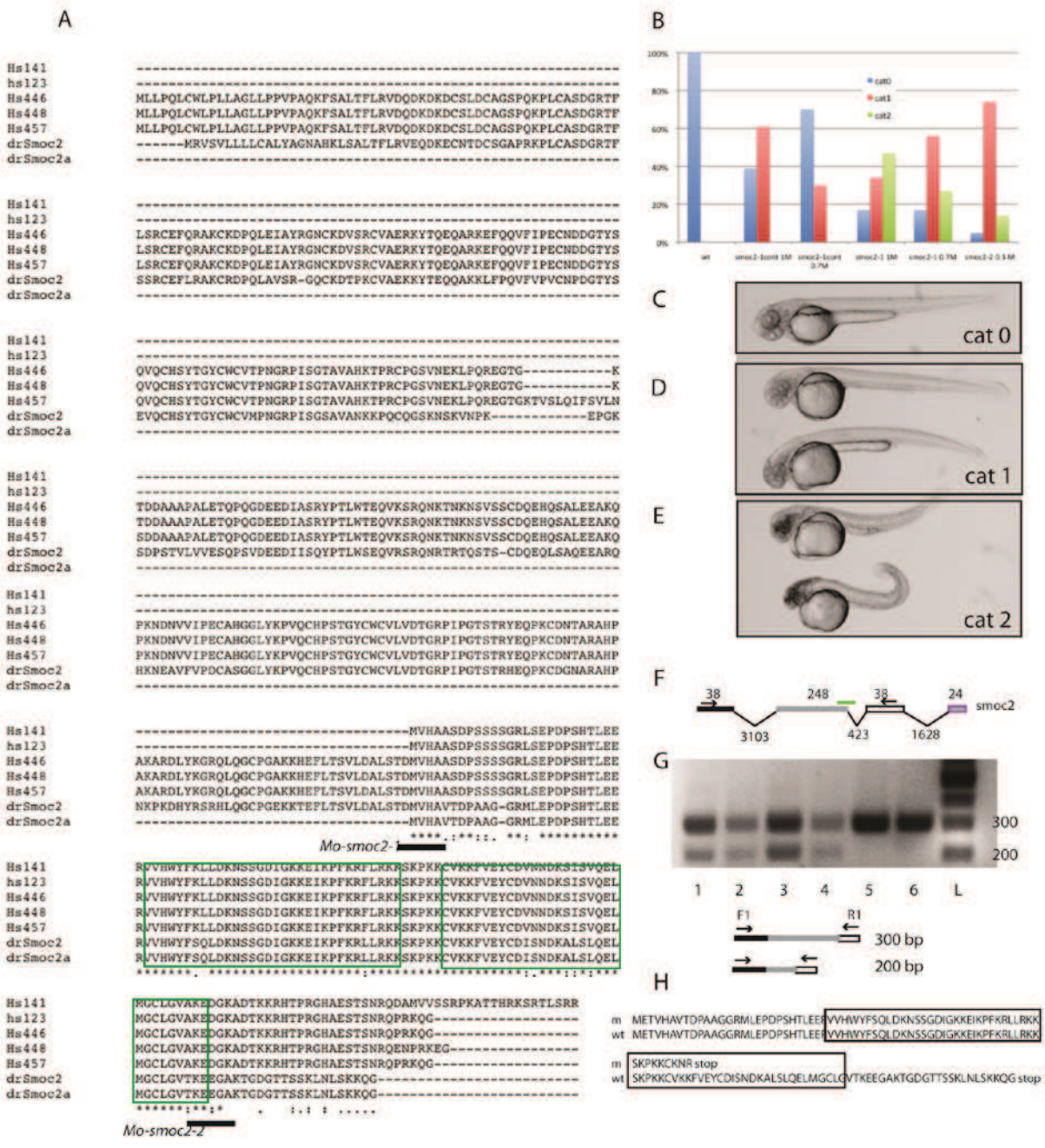
(E) On this sagittal view of the lower first (Mo1, bell stage with differentiated odontoblast) and second molar (Mo2) (early bell stage) at day E18.5 the labeling was restricted to the mesenchymal dental papilla and specifically to the area facing the epithelial loop (proliferative compartment) from which the root will further develop.

(F) Frontal view of lower incisors (Inc) at E14.5, the *Smoc 2* transcripts were located in the dental papilla especially on the lingual side.

(G) At E16.5 the labeling was restricted to the posterior part of the lower incisor (sagittal section) and stronger in the area facing the lingual epithelial loop (★).

(H) The asymmetrical lingual mesenchymal signal (★) was still visible on day E18.5 upper incisor (sagittal plane). Note that the lingual side of the continuously growing incisor is considered to be the analogue root side. Ameloblasts differentiation and enamel deposition occur only on the opposite labial side.

Figure S2. Zebrafish *smoc2* morpholino efficiency



(A) Alignment of human (Hs) and zebrafish (Dr) SMOC2 proteins. Human proteins correspond to 5 different transcripts (hs141, hs446, hs448, hs457, and hs123, amino acid numbers are indicated). The isolated full length zebrafish sequence (DrSmoc2, amplified by PCR with primers forward: gagcacagtctgcgacagca and reverse: caggttcagtttactagatg) and the short splice transcript Smoc2a (ENSDARP00000108925). Green blocks: calcium binding domain.

Black bars show the *smoc2-2* (CAGGAAACAGTGAGACTCATT) and *smoc2-1* (CTGGGTCAGTTACTGCATGGACCAT) morpholino positions. Morpholino control is:

CTGGcTCAcTTAgTGgATcGACCAT

(B) Chart showing the percentage of the different phenotypic categories obtained after morpholino injection.

(C–E) three different categories of phenotype after *smoc2-1* or *smoc2-2* morpholino injection. C: Cat 0 (wild-type); D: cat 1 (small head, slightly curved); E: cat 2 (necrotic head, strongly curved, reduced body size).

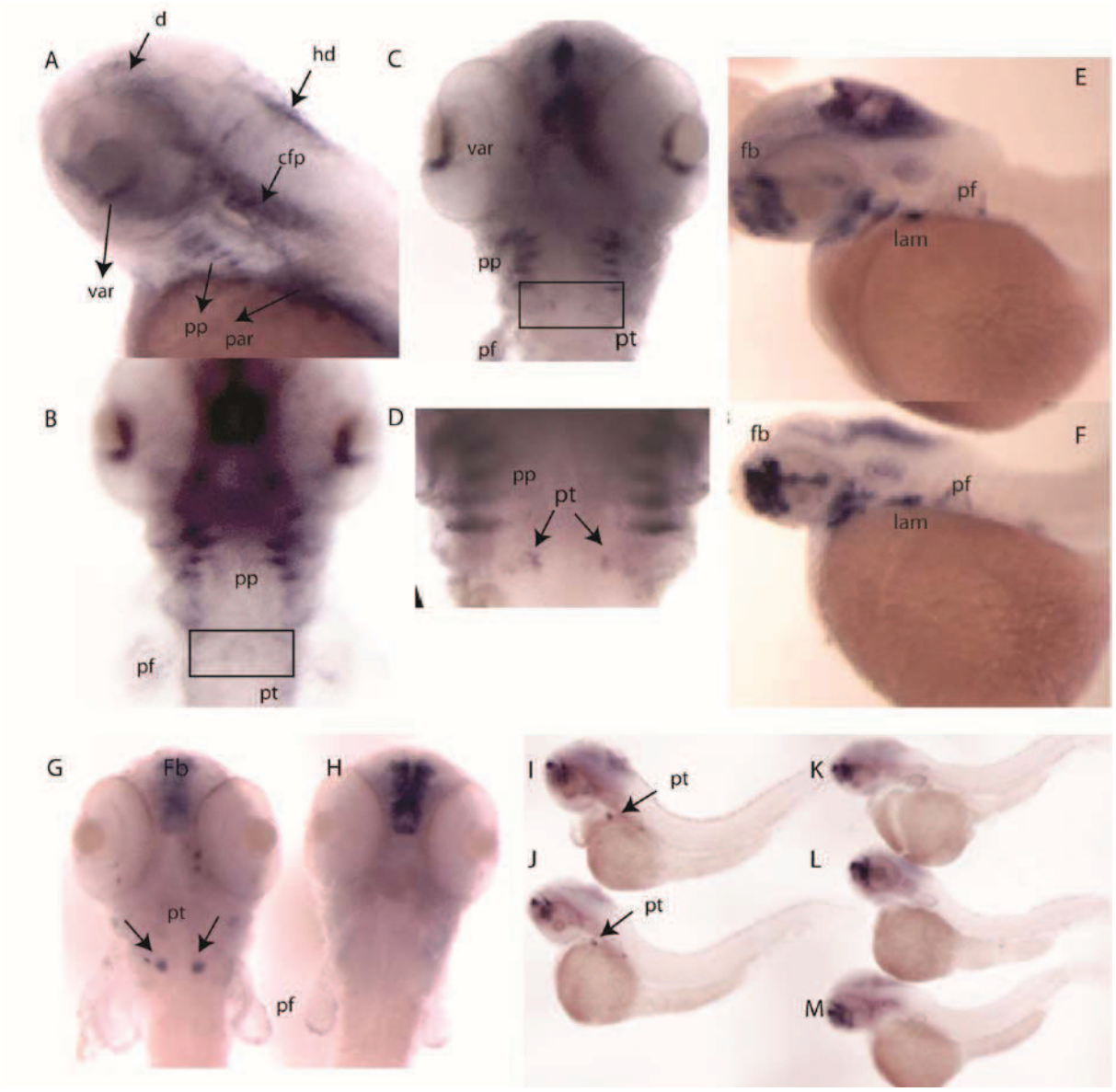
(F–H) Determination of *Mo-smoc2-2* efficiency

(F) *smoc2a* pre-mRNA. Bars represent the 4 exons flanking the 3 introns. Arrows show location of the primers (F1: 5'-GCAGTAACTGACCCAGCAGC-3' ; R2 : 5'- CTAGATGTTGTGCCATCACCTG-3'). Green line represents the morpholino target site. Numbers above indicate the size of exons, and below the size of introns (in bp).

(G) PCR amplification of *smoc2* cDNA prepared from single *smoc2-2* morphant (lane 1-4) shows 2 products: the 300 bp fragment indicates the correctly spliced transcript, and the 200 bp fragment represents the transcript with partial loss of exon 2. Wild-type siblings (lane 5-6) show only the correctly spliced variant (300 bp). All the products were sequenced. Scheme shows the corresponding amplified product. Lane L: DNA ladder.

(H) Tentative protein sequence of truncated Smoc2 in *smoc2-2* morphants. Sequencing of the shortest fragment (200 bp) revealed the formation of a premature stop codon removing the second calcium binding domain.

Figure S3. Zebrafish *smoc2* is expressed in future dental germ and inhibits specifically *dlx2b*



(A–D) *In situ* hybridization on wild type embryos with *smoc2* probe (900 bp). A: lateral view, C-D: ventral view of embryos without yolk. A, B: 56 hpf; C, D: 72 hpf. D: magnification of C. Black box: pharyngeal tooth area.

(E–F) *In situ* hybridization with *dlx2a* probe. Control (E) and *smoc2-2* morphant (F) embryos at 56 hpf did not reveal any obvious difference. Lateral view.

(G–M) *dlx2b* expression in 72 hpf embryos. G, I: control, H, K: 0.3 M *smoc2-2* morphant; J: 0.3 mM *p53* morphant; L: 0.2m M morphant; M: 0.3 mM *smoc2-2*/ 0.3 mM *p53* morphant. G-H: ventral view, I-M: lateral view.

var: ventral and anterior retina ; cfp: cephalic floor plate ; par : pharyngeal area ; pp: pharyngeal pouches ; d: diencephlon. pf: pectoral fin. pt: pharyngeal teeth area. Fb: forebrain; lam: lateral arch mesenchyme: pf: pectoral fin.

Table S1. Primer sequences of *SMOC2* gene used in this study

Primer name	Sequence (5' -> 3')
SMOC2-ex1F	GAAGCTCCGGGGTATTTGAC
SMOC2-ex1R	GCTGCGTCCTACCTGCTC
SMOC2-ex2F	TGAGAACCGGGTGGTGTTAT
SMOC2-ex2R	GAAATTACTCTTCTTCCTTACATGCTG
SMOC2-ex3F	GCTCCCTTTTCTGAGAGCTG
SMOC2-ex3R	GCTGATTTCCCCAGAGAACA
SMOC2-ex4F	GGGTCAAGACCTGCACACTT
SMOC2-ex4R	GCTTCACCCTTCCCCTCTTA
SMOC2-ex5F	AGCGCCAGAAGCAGAAACTA
SMOC2-ex5R	GAGCTGGTGATCGTGAGTGA
SMOC2-ex6F	TTCCTTCCTCATCTGCCCTA
SMOC2-ex6R	CGTGGTCTTTTCTGTCTGTGTC
SMOC2-ex7F	TGGATAGAGAGAAGAATGCCAAA
SMOC2-ex7R	GGCTGCCAGTCTACAAGAGG
SMOC2-ex8F	GAGGTTCTGCCTCTCCTGCT
SMOC2-ex8R	CCAAAGCCTCCCATGGTC
SMOC2-ex9F	CCACAAAGGTGACAGAAAGGA
SMOC2-ex9R	CCTTCCATACACAGCAGCAG
SMOC2-ex10F	AATGCCTGTGAATTTTAGTCTCA
SMOC2-ex10R	GCAACTTCCTGTGTTTGCAG
SMOC2-ex11F	TTGTTATATCTGATGACCAGTGCT
SMOC2-ex11R	CGCCAATTATCATTATTATTTCTGC
SMOC2-ex12F	GGAACCAATCCCTTCTGGAT
SMOC2-ex12R	GTAAGCTTGCCTCCCTTGAA
SMOC2-ex13F	CCAGCCCTCTTTGGTTCA
SMOC2-ex13R	TCTCCATTTTCACAGATTAAAACA

Publication 5 : Rsk2 un modulateur du développement dentaire.

Rsk2 a modulator of tooth development

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Running title: Rsk2 and cranio-oro-dental development

Key words: RSK2 • Coffin-Lowry syndrome • Craniofacial development • Dental anomalies •Tooth development • Gene expression • Transgenic Mouse

Introduction

The ribosomal S6 family of serine/threonine kinases is composed of 4 members in Human: RSK1 (chromosome 3), RSK2 (Xp22.2-p22.1), RSK3 (chromosome 6) and RSK4 (Xq21) which present 75% of homology and are implicated in several important cellular events (proliferation, differentiation, cellular stress response, apoptosis). Mutations in *RSK2* cause X-linked Coffin-Lowry syndrome (OMIM #303600) with severe learning deficit, characteristic dysmorphism, most notably affecting the face and hands and skeletal changes [Hanauer and Young, 2002; Temtamy et al., 1975a; Temtamy et al., 1975b]. Typical orodental findings include a high narrow palate, a midline lingual furrow, malocclusion, hypodontia and peg shaped incisors. Premature loss of the primary dentition was also observed [Day et al., 2000; Hanauer and Young, 2002; Igari et al., 2006]. Wide spaced teeth and large medial incisors were reported.

These RSK kinases are also cytosolic substrates of extracellular signal-regulated kinases (ERK) in the Ras/MAPK signaling pathway [Romeo et al., 2012]. *RSK2* is highly expressed in the hippocampus, and *Rsk2* knockout mice display spatial learning and memory impairment [Poirier et al., 2007]. Murine *Rsk2* through its high expression during somitogenesis seems also to play a specific role during skeletal and muscle development, may be in relation with the skeletal abnormalities (delayed bone age, kyphoscoliosis…) encountered in the syndrome [Kohn et al., 2003]. It is interesting to notice that *Rsk2* shows a specific pattern of expression in the maxillary and the mandibular components of the first branchial arch [Kohn et al., 2003] and that craniofacial and dental anomalies are indeed present in the clinical synopsis of Coffin-Lowry syndrome.

The mouse dentition is a powerful and useful model to study the genesis of Human dental anomalies despite its intrinseque differences (monophyodontia, continuous growth of incisors, dental formula consisting of incisors and molars separated by a diastema) [Peterkova et al., 1995]. Analysis of the literature shows that the mutant mouse models generated so far and displaying dental defects mimic the pathology encountered in Human in syndromic and non-syndromic situations [Fleischmannova et al., 2008]. Some differences may however exist [Aberg et al., 2004]. When mouse models are generated, they are usually created to study a specific organ or system; rarely is the craniofacial or even oral phenotype considered. Therefore we investigated in this study the craniofacial and orodental phenotype of *Rsk2-/Y* and *Rsk1,2,3 -/-* mutant mice.

Materials and methods

Mutant mice

Rsk2 gene targeting was previously described in [Yang et al., 2004]. Triple *Rsk1,2,3* KO mutant mice were obtained by breeding of single knockout mice, themselves obtained by excision of the first or the second or the two exons leading to frameshift mutations [Yang et al., 2004].

MicroCT analysis

A cohort of 6 mutant *Rsk2-/Y* male mice (150, 155, 700, 702, 728, 731) and 6 wild type littermate (149, 154, 699, 727, 729, 730) were analyzed through X-ray micro-computed tomography Micro-CT (X-ray µCT; GE eXplore Vision CT120). For craniofacial exploration the µCT was performed using 220 projections with an angle increment of 0.877 degrees (Parker mode) and one average frame per projection (70.0 kV, 32 mA, and exposition time of 16 milliseconds). Reconstructions were done using a Feldkamp algorithm giving voxels of 100 x 100 x 100 μm^3. For teeth a second µCT acquisition was performed with a smaller voxel size (50 x 50 x 50 μm^3).

Anatomical landmarks were identified as remarkable points (for instance cranial sutures) that could be easily recognized on each specimen. Figure S1 (supplementary material) illustrates this step, showing on a 3D isosurface rendering of a Tc1 mouse the landmarks (red points) and their anatomical definitions. A similar approach was used for molar analysis (Figure S2)

Calculation involved euclidean distances between selected pairs of points that could be compared to identify statistical significant differences between WT and transgenic mice. The methodology is based upon the work of [Richtsmeier et al., 2000; Richtsmeier et al., 1995]. To avoid any bias in landmarks position, landmarks were placed two times by one investigator (intra-investigator reproducibility) and one time by another (inter-investigator reproducibility). As no statistical differences were observed between distances measured within or in between investigators, we decided to base our study on the average of the three measured distances.

Statistical analysis comparing WT and mutant mice was performed using Mann-Whitney non parametric test. P-values lower than 0.05 were considered as significant.

Analysis of incisor morphology

All image processing tasks were done using MicroView (GE Healthcare, Waukesha, USA). Right and left mandibular incisors were isolated by manual segmentation from mandible µCT acquisitions of the two mice groups (6 mutants *Rsk2-/Y* (150, 155, 700, 702, 728, 731) and 6 wild type mice (littermate 149, 154, 699, 727, 729, 730). Several measurements were achieved for morphology description, on the lateral and dorsal views of a 3D isosurface rendering of incisors (threshold value = 700 Hounsfield units): the length of the longest and external bow (Li), the height of median part of the incisor (hi), the total height (Hi), the horizontal length joining the tip of the incisor and the distal extremity of the root (li), projected area in lateral view of the incisor (lpa), thickness of the median part of the incisor (ti) (Figure 3S). A Wilcoxon-Mann Whitney test was used to compare both groups.

In situ hybridization on cryosections

The following partial cDNA (Table SI supplementary information) and plasmids were used and in situ hybridization was performed according to an automated procedure described in [Diez-Roux et al., 2011]. Mouse embryos/fetuses (C57BL6) were collected at E12.5, E14.5, E16.5, and on the day of birth (hereafter referred to as E19.5). For E14.5 and older samples, the whole head was embedded in OCT 4583 medium (Tissue-TEK, Sakura) and frozen on the surface of dry ice. E12.5 embryos were fixed overnight in 4% paraformaldehyde (pH 7.5, w/v) in phosphate-buffered saline (PBS), cryo-protected by overnight incubation in 20% sucrose (w/v) in PBS, and cryo-embedded as described above. Cryosections (Leica CM3050S cryostat) at 10 µm were collected on Superfrost plus slides and stored at -80°C until hybridization. E12.5 and E14.5 samples were sectioned in a frontal plane, whereas other stages were sectioned sagitally.

Whole mount *in situ* hybridization

Whole mount *in situ* hybridization of mandible collected at E14.5 was performed with a digoxigenin-labeled *Shh* riboprobe as described [Chotteau-Lelievre et al., 2006], using an Intavis InSituPro robot (for details, see http://www.empress.har.mrc.ac.uk/browser/, Gene Expression section). The *Shh* template plasmid was kindly provided by A. McMahon (Harvard University).

Microarray

Tissue preparation

Pregnant female were killed by cervical dislocation. Embryos at E14.5 were dissected and mandibular molars were isolated. The tail was put aside for embryo genotyping. Individualized tissue samples were frozen in liquid nitrogen and keep at -80C until genotyping and further use.

Microarray hybridization

To obtain enough RNA for the hybridization on DNA chip, total RNA was extracted from molar tooth germs (pooling 4 teeth per sample from mice either WT or *Rsk2-/Y* as confirmed by genotyping). Total RNA was extracted with the RNAeasy micro Kit from Qiagen. RNA quality was verified by analysis on the 2100 Bioanalyzer (Agilent). All samples displayed a RNA Integrity Number greater than 9.8. 8 *Rsk2-/Y* and 8 WT samples where hybridized and compared.

Biotinylated single strand cDNA targets were prepared, starting from 300ng of total RNA, using the Ambion WT Expression Kit (Cat # 4411974) and the Affymetrix GeneChip® WT Terminal Labeling Kit (Cat # 900671), according to Affymetrix recommendations.

Following fragmentation and end-labeling, 1.9 µg of cDNAs were hybridized for 16 hours at 45oC on GeneChip® Mouse Gene 1.0 ST arrays (Affymetrix) interrogating 28,853 genes represented by approximately 27 probes spread across the full length of the gene. The chips were washed and stained in the GeneChip® Fluidics Station 450 (Affymetrix) and scanned with the GeneChip® Scanner 3000 7G (Affymetrix). Finally, raw data (.CEL Intensity files) were extracted from the scanned images using the Affymetrix GeneChip® Command Console (AGCC) version 3.1.

Data are available in the GEO database http://www.ncbi.nlm.nih.gov/geo under the accession number ---.

Microarray analysis

CEL files were further processed with the Partek software to obtain principal componant analysis (PCA) and to select and considered only genes with a signal value above 5 (20th percentile of all expression values) in at least one sample.

Genes were considered as differentially expressed if the false discovery rate from Benjamini and Hochberg test was under 0,1 and if the fold change was greater than 1.2 or lower than -1.2.

Microinjection and inactivation

A DNA solution containing 1 µg of Rsk2 shRNA (SABiosciences – linearized pGeneclip Neomycn vector), Sucrose (0.5%) and Fast Green (Sigma; 1/10,000) was injected into the developing molars with Femtojet Eppendorf (t=0.2 s, P=61hPa). Two platinium needle electrodes (0,1 mm tip -Sonidel) were inserted into the molar lying in a cover round platinium plat electrode (Sonidel). DNA was then transferred into the cells using an ECM 830 Electroporation System (BTX Harvard Apparatus), applying 1 set of 5 pulses. Electroporation settings were set to 5V for 50ms, spaced of 50ms intervals. On control conditions, explants were electroporated in the absence of DNA or with a random shRNA control sequence.

In vitro culture

The chemically-defined culture medium was previously described in [Mark et al., 1992].

After electroporation, the explant is embedded into a 14 µl rat collagen drop (BD Biosciences), the collagen drop containing 0.2 ng of laminin (BD Biosciences), 50 µl of DMEM 10x concentrated, 5 µl of HEPES (1mM), 60 µl of 7.5% NaHCO3. The collagen drop was kept on ice to prevent it from polymerizing. The collagen was then polymerized at 37 degrees for 10-15 min and then the culture medium was added.
All explants were then cultured for 24 hours in a DMEM-HAM F12 medium supplemented with Vitamin C (18mg/ml); L-Glutamine (200mM); Streptomycine (1000U/ml) and 20% foetal calf serum for optimal tooth growth. Explants were then processed to RT-qPCR analysis.

Real-time quantitative RT-PCR

RT-PCR assays were performed in duplicate comparing three RNA samples electroporated with the *Rsk2* shRNA and three non-electroporated samples. RNA extractions were performed as previously described. Oligo-dT primed cDNAs were generated using the Superscript II kit (Invitrogen) according to the manufacturer's protocol with. Quantitative real-time PCR was achieved using SybrGreen and LightCycler 480 (Roche). The sequences of primers used for the various tested genes are given in Supplementary Table II. A probe set for detection of mouse Gapdh (a housekeeping gene) was used for normalisation. For each sample the ratio between signals for the gene of interest and Gapdh was calculated to normalize concentration values. To verify if genes were differentially expressed between electroporated and non-electroporated samples, the average of ratios were then compared.

Results

Rsk2 -/Y craniofacial phenotype

The craniofacial phenotype was assessed by microCT imaging and analysed through euclidean distances measurement and comparison. This phenotype was variable ranging from normality (mutant mice 702, 731) to obvious craniofacial dysmorphology (mutant 150 Figure 1B). In between these extremities of the phenotypical spectrum other mutants showed intermediate phenotypes. When affected mutant skulls were smaller in length but not in width.

The length reduction was essentially caudal (distances 1-4, 1-5, 2-3, 2-4, 2-5 being more reduced than 1-2 or 1-3: measurements were performed according to anatomical landmarks detailed in Fig. S1, supplementary information). A nasal deviation was observed in some mutant mice (Figure 1B,C,E; Table I). Craniofacial microCT analysis of *Rsk1,2,3-/-* mice was not performed.

Rsk2-/Y and *Rsk1,2,3-/-* dental phenotype

Molar phenotype

We analyzed 15 *Rsk2-/Y* mice and 9 R*sk1,2,3 -/- mice* (see Table II and Figure 2).

A supernumerary molar S1 (mesial to the first molar M1) was visible in all except 2 mice (702, 731). This anomaly was highly penetrant in the upper jaw compared to the lower jaw. The supernumerary molar shape and size was variable from a single cusp to a well-shaped molar. Its size was always smaller than the adjacent first molar. The sole inactivation of *Rsk2* was sufficient to generate the phenotype. In the presence of S1 the crown morphology of the first molar was altered.

Even when the phenotype looked rather normal with the presence of 3 molars (M1, M2, M3), a significant reduction of M1 (mesiodistal dimension) (only in the maxilla), and of M2, M3 (only in the mandible) was observed (Table III).

In the presence of a supernumerary molar S1, the mean value of the mesiodistal dimension was always reduced for M1 and also M2 (Table III). S1 was always longer than M3.

The vestibulo-lingual dimensions of the molars were not changed for M1, however they were reduced for M2 (except when only 3 molars were present in the mandible) and for M3 (Table IV).

Abnormalities of the molar shape were

- for lower M1: the L1 cusp was flattened or non existent in the presence of an adjacent S1 (Figure 3).
- for the upper molar, T2 cusp was dysmorphic and sometimes was linked to T5 by an additional crest (Figure 3).

The total molar field length was significantly reduced both in the lower and upper jaw when only 3 molars were present. When a supernumerary molar existed, the molar field size seemed rather normal when compared to the wild type in the mandible, but was however significantly increased in the maxilla (Table III).

In *Rsk1,2,3-/-* mice the phenotype was slightly different, in some instance only 3 molars were visible with however the second (probably M1) bigger than the first considered as the S1 (supernumerary molar); in this case, M3 was missing.

The tooth phenotype was independent of the craniofacial malformations. Mutant mouse 155, for example, did dot display any craniofacial dysmorphology, and however presented an abnormal tooth phenotype with 4 molars in each quadrant.

Incisor phenotype

No statistically significant differences were revealed between right and left incisors in both groups ($p>0.05$). As a consequence, results from right and left incisors were merged (Table V). No statistical difference was observed between WT and *Rsk2-/Y* ($p>0.05$).

Abnormal molar root development in Rsk2-/Y mice

The root numbers of the mandibular molars in the wild type mouse are M1 (2), M2 (2), M3 (1), the total number of roots in one mandibular quadrant is 5; and of the maxillary molars are M1 (3), M2 (3), M3 (1 but more often 2 to 3), the total number of roots in one maxillary quadrant is then 8 to 9. The roots numbers and shape also reflect the tooth identity.

In *Rsk2-/Y* mice, in the case of the presence of a supernumerary molar in the lower jaw the total number of roots was 6 meaning that S1 had a unique root and that the number of roots of the other molars was identical to the wild type situation.

In the upper jaw, the total number of roots ranked 9 to 8 in the presence of a supernumerary molar; S1 could have 1 or 2 roots and surprisingly the root pattern of M1 was altered with the presence of only 2 roots. The root pattern of the second molar was unchanged (Table VI and Figure supplemental).

Rsk2-/Y and the diastema

The maxilla diastema was significantly reduced (compared to wild type mice) in the presence of 4 molars (one supernumerary). This was not the case for the mandibular diastema whatever the number of molars (Table VII).

Expression patterns of Rsk genes during mouse odontogenesis

To investigate whether the dental anomalies observed in the *Rsk* mutants may correlate with distinct patterns of expression during odontogenesis, we performed an in situ hybridization analysis of *Rsk1*, *Rsk2*, *Rsk3* and *Rsk4* gene transcripts at various stages of tooth development. All four genes were found to be expressed in specific areas of the tooth anlagen, as illustrated in Figure 4 for the developing first molars, and Figure 5 for the mandibular incisors.

Rsk2 transcripts were mainly localized in the mesenchymal compartment or dental papilla from E12.5 (dental lamina stage) to E19.5 (late bell stage). At E16.5 (bell stage), the transcripts were scattered in the area facing the epithelial loops or at the base of the cusps; the second molar dental mesenchyme was uniformly labelled. In the incisor the transcripts were also located in the mesenchyme, and the signal was more intense in the labial area and posterior area.

The expression of *Rsk1* and *Rsk4* was mainly epithelial, marking the inner dental epithelium and especially the epithelial loop areas. A faint signal was also observed for *Rsk4* in the posterior mesenchymal area facing the epithelial loops especially the labial loop for incisors (E16.5 and 19.5).

Rsk3 transcripts were mainly visible in the dental papilla at E14.5 (cap stage), E16.5 bell stage and E19.5 (Figure 4 and Table VIII). At E19.5 the transcripts were concentrated in the cervical part of the papilla for the first molar and had a wider distribution in the second molar mesenchyme.

Rsk2-/Y supernumerary molar is associated with an extra mesial placode

An extra molar placode, and subsequent enamel knot labelled with *Shh* as a marker, was clearly visible at E14.5 in the mandible mesial to M1 in *Rsk2-/Y* mice when compared to WT littermates (Figure 5).

Rsk2-/Y transcriptome analysis

The transcriptome data did not reveal any statistically significant differential expression pattern for incisors (not shown).

For molars however it was possible to discriminate between the molar samples according to their genotype (WT versus *Rsk2-/Y*) (see Principal composant analysis Figure 6).

504 genes or clones were retrieved as being differentially expressed with a fold change higher than 1.2 and false discovery rate under 0.1. The fold change were however rather low ranking between 2.044 and -1.979 suggesting that rather minimal molecular changes were taking place. These changes could be also minimized by a rather heterogeneous sample for *Rsk2-/Y* molars ranking as previously described between normality and a 4 molars phenotype; the phenotype was not known precisely at this E14.5 cap stage. The top ten genes and a selection of genes being up (+) or down (-) regulated were presented in Table IX A,B.

Eaf2 is an oncogene involved in cancer and embryogenesis acting with Wnt4 in an autoregulatory feedback loop [Wan et al., 2010]. Inactivation of this gene causes numerous tumours in mice [Xiao et al., 2008]. *Eaf2* was down regulated in *Rsk2-/Y* mutants.

Rdh1 retinol dehydrogenase 1 (all trans), in addition to retinol dehydrogenase activity, has strong 3alpha-hydroxy and weak 17beta-hydroxy steroid dehydrogenase activities. Rdh1 has widespread and intense mRNA expression in tissues of embryonic (for example within the neural tube, gut, and neural crest at embryo day 10.5) and adult mice. [Zhang et al., 2001]. *Rdh1* was found to be the most up regulated gene in *Rsk2-/Y* mutant mice.

We tried unsuccessfully to confirm those results on a selection of genes by qRT-PCR comparing three RNA Rsk2-/Y molar samples with three RNA WT molar samples (not shown). This negative result reflected the heterogeneous nature of the *Rsk2-/Y* E14.5 samples and did not invalidate, to our belief, the microarray data.

Rsk2* inactivation *in vitro

In order to overcome this problem of a heterogeneous molar sample dissected from *Rsk2-/Y* mice we decided to go back to an *in vitro* system inactivating directly *Rsk2* via the microinjection and electroporation of an *Rsk2* shRNA. In vivo electroporation in the mandible, as previously described by [Wise et al., 2011] was not suitable for our study, because of the lack of precision on the targeted zone and our aim to inactivate the gene directly within the tooth anlagen.

We optimized the experimental settings *ex vivo* to inject and electroporate *Rsk2* shRNA in cap stage molars. Control explants were electroporated with a random shRNA construct.

After electroporation the explants were put into collagen drop culture to maintain their morphology [Wright et al., 2004] and kept in a define culture medium for 24 hours, before processing to qRT-PCR analysis. Tooth morphology was preserved and no apoptosis nor cell death were induced, as seen by activated caspase3 and TUNEL assays (data not shown).

Rsk2 shRNA electroporation efficiently diminished Rsk2 expression by 75% (Figure 7). We then analyzed *Eaf2* and *Rdh1* expressions and found that *Eaf2* expression was indeed down regulated (50% decrease), while *Rdh1* expression was up-regulated (Figure 7). These results were in accordance with the microarray data.

The new combined technique allowed us not only to optimize a protocol for studying the molecular mechanisms during early tooth development, but also to set up a new original and reliable way for microarray validation.

Discussion

A peculiar dental phenotype

Mice are commonly studied mammals for investigating the mechanisms of tooth development and the orodental phenotype of transgenic mice indeed mimick dental anomalies encountered in rare diseases [Fleischmannova et al., 2008].

Placental mammals have evolved from a common ancestor with three incisors, one canine, four premolars, and three molars [Ji et al., 2002]. Actual mouse dentition comprises 1 incisor and 3 molars per quadrant separated by a toothless space called diastema. Rudimentary tooth primordia develop however within the mouse diastema through the initial stages of odontogenesis as remnants of the evolutionary lost teeth but cease before the cap stage and regress by apoptosis [Peterka et al., 2000; Peterkova et al., 2003; Viriot et al., 2000].

The tooth phenotype encountered in the *Rsk2-/Y* mutant mouse was remarkable with the presence of a supernumerary tooth in the place and position of the ancestral, lost through evolution fourth premolar and with the associated cusp pattern anomaly like in the upper molar the presence of an additional crest linking T2 and T5. This crown shape anomaly is very unique and has never observed in any other published mouse mutant. These supernumerary teeth, mesial to the first molar, were associated to the compressions of the anteroconides in the lower first molars and anterocones in the

upper first molar (the mesial portion of the first molar adjacent to the diastema that is indeed formed through the incorporation of transitory tooth germs developing within the distal part of the diastema) [Lesot et al., 1996; Peterkova et al., 1996].

Mouse models with supernumerary molars

Supernumerary molars within the diastema were also described in other mouse models mutants for ectodin, Lrp4, Sprouty2/4, Wise, Polaris, and Gas1, [Charles et al., 2011; Kassai et al., 2005; Klein et al., 2006; Murashima-Suginami et al., 2008; Ohazama et al., 2010; Ohazama et al., 2009; Ohazama et al., 2008; Porntaveetus et al., 2011].

The dysfunctions of Fgf and Shh, Wnt, NF-KappaB signalling pathway in transgenic mice, inducing formation of these supernumerary teeth within the diastema, confirm that mouse maintain genetic potentialities that could be stimulated and induce the formation of these supernumerary teeth [Hacohen et al., 1998; Kim and Bar-Sagi, 2004].

A similar phenotype as the tabby mouse

Ectopic teeth in the diastema were also observed in mice misexpressing ectodysplasin A1 (EdaA1) and EdaA1 receptor (Edar) [Mustonen et al., 2003; Peterkova et al., 2005; Pispa et al., 2003; Tucker et al., 2004]. The dental phenotype of the *Rsk2-/Y* mouse resembles the dental defects encountered in the tabby mouse (a mouse model for X-linked hypohidrotic ectodermal dysplasia (OMIM #305100, gene map locus Xq12-q13.1, ectodysplasin-A gene mutations, ED1) [Charles et al., 2009; Kristenova et al., 2002; Lesot et al., 2003; Peterkova et al., 2002]. Ontogenetic and phylogenetic data support that the supernumerary tooth in tabby mice arises due to the segregation of the distal premolar vestige from the molar dentition and thus represents an evolutionary throwback (atavism). This supernumerary cheek tooth in tabby/EDA mice was considered to be a reminiscence of the premolar in mouse ancestors [Peterkova et al., 2005].

Known cytosolic Rsk substrates include IkappaBalpha [Ghoda et al., 1997; Romeo et al., 2012; Schouten et al., 1997] a member of the NF-kappaB pathway. This pathway is involved in development and diseases with the associated ectodermal dysplasia affecting ectodermal derivatives like the tooth, hair, salivary, mammary glands, skin… IkappaBalpha has been associated with autosomal dominant anhidrotic ectodermal dysplasia and T cell immunodeficiency [Courtois et al., 2003].

The fact that IkappaBalpha might be phosphorylated leading to an increase of NF-kappaB activity by Rsk may explain some of the dental anomalies encountered in the *Rsk2-/Y* transgenic mice and in Coffin Lowry patients as it has however not yet been shown which signalling pathways were involved in EdaA1/Edar for tooth formation in the diastema.

Recently is has demonstrated that tooth number was regulated by fine-tuning levels of receptor-tyrosine kinase signalling [Charles et al., 2011].
IkappaBalpha was however not found in the microarray data.

A variable phenotype

The craniofacial and dental phenotypes were independent and highly variable ranging from normality to a rather dysmorphic and asymmetric skull and the presence of 4 supernumerary molars.

The phenotype in the light of Coffin-Lowry syndrome

A mouse model for Coffin-Lowry syndrome, obtained by inactivation of the *Rsk2* gene, was described by [Yang et al., 2004]. Mutant mice weigh 10% less and were 14% shorter than their wild-type littermates. This was in agreement with a role of Rsk2 in growth. These mice exhibited impaired learning and poor co-ordination, providing evidence that Rsk2 has similar roles in mental functioning both in mice and humans. No previous description of the craniofacial or dental phenotype was previously reported.

The craniofacial features described in Coffin-Lowry syndrome are a thick calvarium, hyperplastic supra-orbital ridges, hypertelorism, flat malar region, prominent mandible/prognathism. It was difficult in the light of our observations to relate the *Rsk2-/Y* craniofacial phenotype to the anomalies encountered in patients. The small size of the skull could be associated to the general growth defect.

If the *Rsk2-/Y* dental phenotype demonstrated the presence of supernumerary teeth, it is interesting to notice that in Coffin-Lowry patients teeth agenesis or hypodontia are described. This apparent opposite phenotype has however already been described in another rare disease the cleido-cranial dysplasia syndrome characterised by skeletal defects, numerous supernumerary teeth, and delayed eruption and the *Cbfa1*-/- corresponding mouse model presenting with arrested tooth development [Aberg et al., 2004].

Cell cycle progression and cell proliferation

Tooth number, size and shape are linked to cell proliferation / apoptosis events [Boran et al., 2005; Coin et al., 1999; Lesot et al., 1998; Viriot et al., 1997].

It is interesting to notice that *Rsks* were expressed throughout tooth development especially in proliferating area (Figures 4 A and B) either in the mesenchyme (Rsk2, Rsk3) or in the epithelial loops (Rsk1, Rsk4) or both (Rsk3, Rsk4). This was very

similar to the general expression pattern described for the Rsk [Guimiot et al., 2004; Kohn et al., 2003; Zeniou et al., 2002].

Rsk1 was highly expressed in regions harbouring highly proliferating cells like liver, lung, thymus and olfactory and respiratory epithelia. Particularly intense *Rsk1* expression was observed in the gut epithelium. From the expression patterns observed, Rsk1 seemed to be more strictly linked to cellular proliferation.

Rsk2 was expressed in somites and transcripts were more abundant in skeletal muscle, heart and pancreas.

Rsk3 was mainly expressed in the nervous system but also in the thyroid gland and testis cords. Rsk3 may regulate proliferation and differentiation of neuroepithelial cells during development [Zeniou et al., 2002].

Rsk4 ubiquitous expression at lower level was observed throughout development.

In teeth *Rsk4* expression was rather specific marking the epithelial loops.

RSKs control cell proliferation through the regulation of mediators of the cell cycle [Romeo et al., 2012]. RSK2 for example promotes cell-cycle progression by phosphorylating c-Fos, a transcription factor regulating the expression of cyclin D1 during G1/S transition. RSK2 activates and phosphorylates p53 (Ser15) *in vitro* and *in vivo* and colocalizes with p53 in the nucleus. The RSK2-p53-histone H3 complex may likely contribute to chromatin remodelling and cell cycle regulation [Cho et al., 2005].

A list of substrates of the RSK isoforms was reported by [Romeo et al., 2012]. Rsk2, c-Fos, NHE-1, Erα, p27^{kip1}, Sos1, RanBP13, YB-1, Erp1, eIF4B, rpS6, Bad, DAPK, Nur77, NFAT3, TIF-1A, ATF4, ATF1, MEF2c, Filamin A, Raptor, CCTβ, CRHSP24, Shank were listed as phosphorylation substrates, none of these genes was however present in our differential *Rsk2-/Y*/WT microarray data.

However other genes related to the cell cycle (*ccna1, hmgb1, mdm2, Tpd52*), to modulation of transcription and DNA binding (*Sp6, L3mbtl4, Zkscan17, Neurog3, Zfp35, Zfp78*), to apoptosis (*Ndufaf4, Trim69, Npy5r, hmgb1, Naip2, Dffa, Cflar*), to growth (*Ngf, bradykinin receptor, beta 1, Bdkrb1, Amhr2*), to cancer (*eaf2*), retinoid pathway (*Rdh1*), were detected in the microarray.

Rsk and the Ras/MAPK pathway

Recently altered ERK/MAPK signalling (abnormally increased phosphorylation of ERK1/2) in the hippocampus of the *Rsk2* knockout mouse model of Coffin-Lowry syndrome ERK/MAPK was reported [Schneider et al., 2011]. RSK2 was proven also to exert a feedback inhibitory effect on the ERK1/2 pathway. We could not show a

similar result with our microarray data. *Ngf* was the only gene referring to this pathway and tooth development [Mitsiadis and Luukko, 1995] detected on the array.

RSK2 can also be phosphorylated in response to other pathways via FGFR ([Kang et al., 2007] and Src activation (EGF stimulation) [Kang et al., 2008].

Unravelling target genes and involved pathways

The heterogeneity of the molar samples and the variability of the molar phenotype impaired the analysis of the microarray data and the subsequent confirmation of target genes expression by qRT-PCR.

However genes involved in transforming growth factor beta receptor signalling pathway (Amhr2), Fgf (Fgfbp3), Wnt (Sfrp5) were shown to be affected by the inactivation of *Rsk2*. These pathways are well known pathway involved in tooth development [Tummers and Thesleff, 2009] and anomalies both in mouse models and men [Bloch-Zupan et al., 2012].

The analysis of a transcriptome profile in *Rsk2-/Y* mouse was however successful for the hippocampus of the R*sk2*-knockout mice revealing AMPA receptor dysfunction [Mehmood et al., 2011].

The *in-vitro* essay through micro-injection and electoporation of a *Rsk2* sh-RNA in molar tooth germs proved to be more valuable to assess quantitative changes in target genes expression and revealed an interesting interference with the retinoid pathway.

Conclusion

Analysis of *Rsk2-/Y* mutant mouse revealed an unexpected role of Rsk2 in tooth development and patterning allowing the reappearance of a supernumerary molar considered as a remnant of evolutionary lost teeth. *Rsk2* and *Rsk1,3,4* expression patterns were clearly correlated to tooth proliferating area confirming a biological function of Rsk2 in cell-cycle, cell growth and cell survival. Transcriptome profile analysis was difficult because of the heterogeneity and variability of the molar phenotype but however pointed towards interesting target genes and pathways. *In vitro inactivation* of *Rsk2* using *Rsk2* sh-RNA was more efficient to address target genes and showed an interference with the retinoid pathway.

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Legends

Figure 1 The craniofacial phenotype of *Rsk2-/Y* mice assessed by microCT imaging.

Figure 2 Macroscopic view of the dental phenotype encountered in Rsk2-/Y (mutant #184 A,B,C) or Rsk1,2,3-/- mice (mutants #793 E, #111 D,G, 1283 F). Supernumerary molars of various size and shape were visible in from of the first molar.

Figure 3 Variation of molar shape size and number in Rsk2-/Y mice assessed by microCT imaging. WT wild type, UR upper right quadrant, LL lower left quadrant.

Figure 4 Expression pattern of Rsks during mouse molar and incisor odontogenesis at E12.5 (dental lamina), E14.5 (cap), E16.5 (bell), E18.5 (late bell) stages by *in situ* hybridization.

Figure 5 Whole mount in situ hybridization with Shh riboprobe showing the molar and incisor placodes within the mandible at E14.5 in the wild type mice (A,B) and in *Rsk2-/Y* mouse (C). Two molar placodes are clearly visible in C.

Figure 6 Principal composant analysis (PCA) on Wild Type (WT) mandibular molars samples versus *Rsk2-/Y* mutant mandibular molars samples (KO). WT samples are represented in red whereas KO samples are represented in blue. Samples segregate in two distinct groups showing relevant transcriptional differences.

Figure 7 Graphical representation of *In vitro* inactivation of *Rsk2* and subsequent consequences on target genes following electroporation and microinjection in the first mandibular molar of a *Rsk2* shRNA assessed by qRT-PCR 24 hours after injection.

A: *Rsk2* is down regulated 3.84 times after electroporation

In the microarray the value for Rsk2 was 1.05 which was in accordance with the expected presence of *Rsk2* transcripts. *Rsk2*-deficient mice were generated using a targeting vector containing a neomycin resistant (NeoR) gene and 3 stop codons in three different frames at the end of the coding sequence inserted into exon 2. No protein was however produced [Yang et al., 2004].

B: *Eaf2* was significantly down regulated and *Rdh1* was upregulated after electroporation.

C: The expression values retrieved from the microarray data for the 3 selected genes

The numerical corresponding values are listed in the following Table :

Gene	*Rsk2*	*Eaf2*	*Rdh1*
microarray	1.05	-1.23	2.04
qRT-PCR *Rsk2-/Y*		-1.49	1.09
qRT-PCR shRNA *Rsk2*	-3.84	-2.48	1.17

Table I Distances between skull landmarks on wild type and Rsk2 mutant mice. In bold are distances significantly different between wild type (WT) and mutants (all these distances were shorter in mutants). Anatomical landmarks are explained in Figure S2 (supplementary information). Diagonal distances were smaller in mutants probably due to the nasal deviation. STD : standard deviation.

Fig. 1

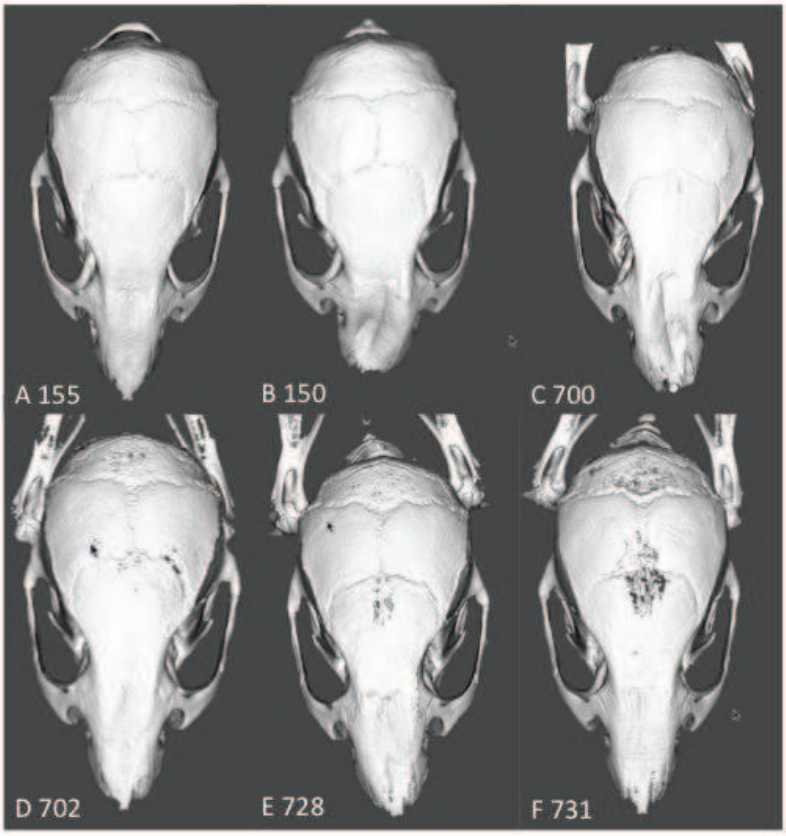

A 155
B 150
C 700
D 702
E 728
F 731

Fig. 2

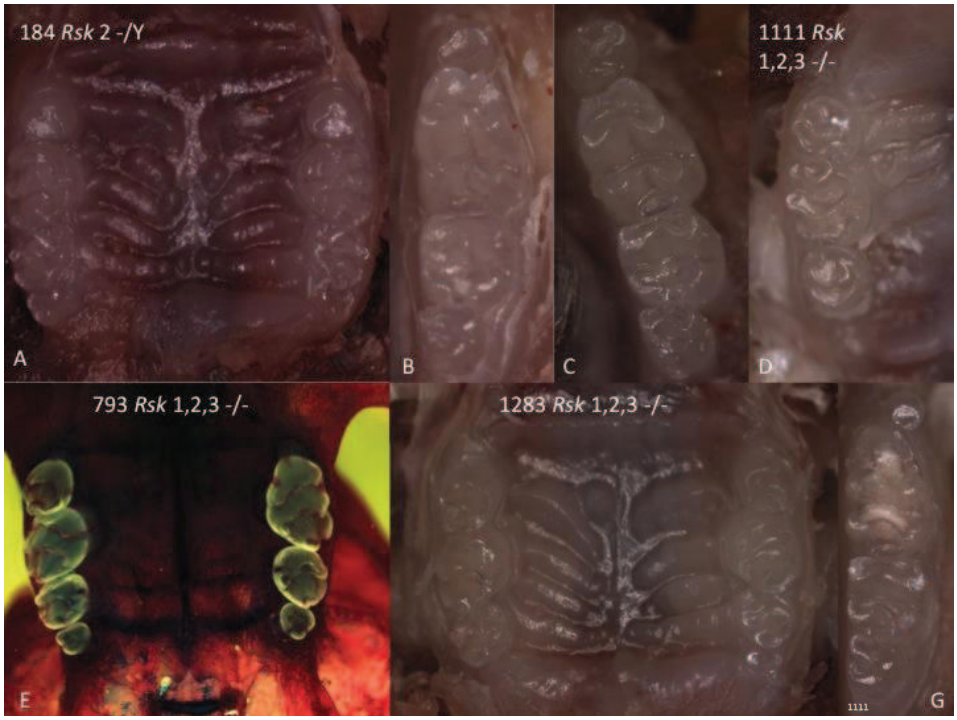

184 Rsk 2 -/Y
A
B
C
1111 Rsk
1,2,3 -/-
D
793 Rsk 1,2,3 -/-
E
1283 Rsk 1,2,3 -/-
G

Fig. 3

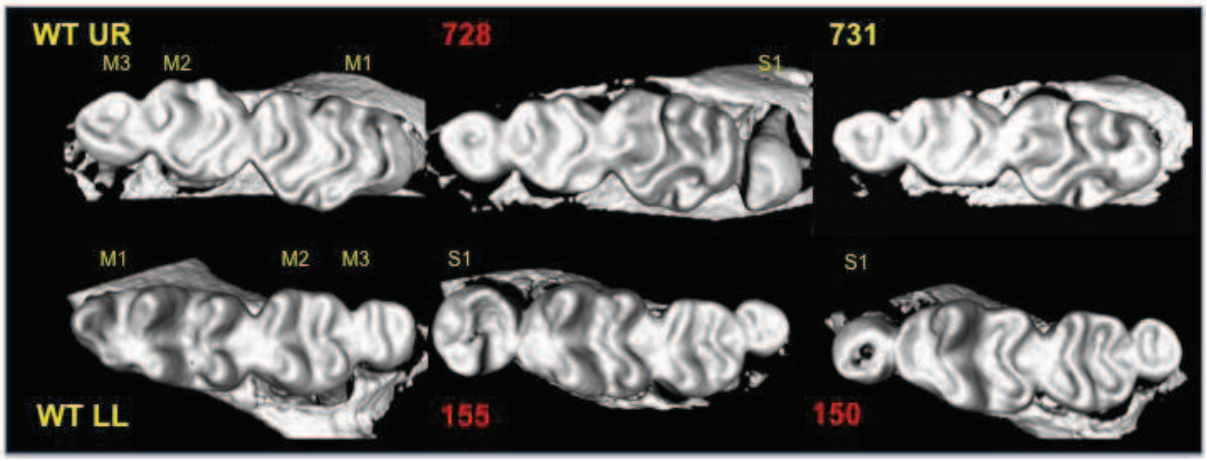

WT UR
728
731
M3
M2
M1
S1
M1
M2
M3
S1
S1
WT LL
155
150

Fig . 4A

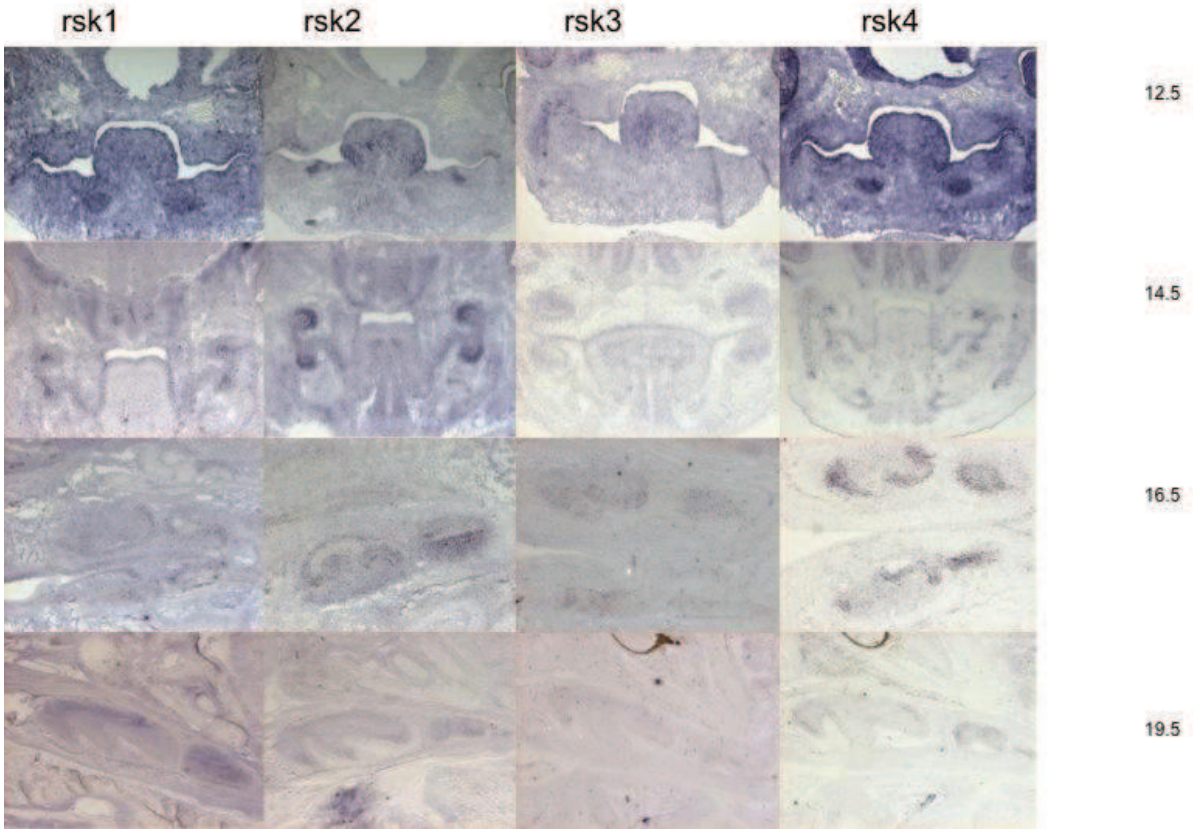

rsk1
rsk2
rsk3
rsk4
12.5
14.5
16.5
19.5

Fig. 4B

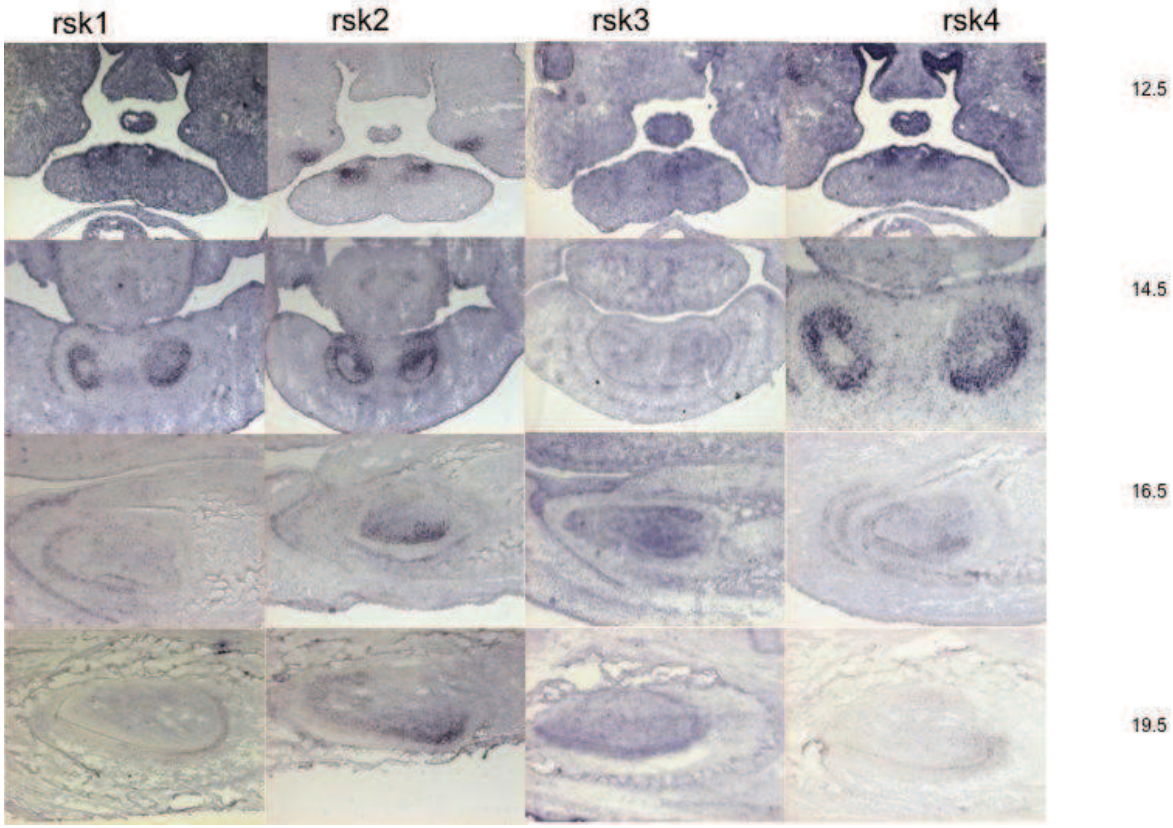


Fig. 5

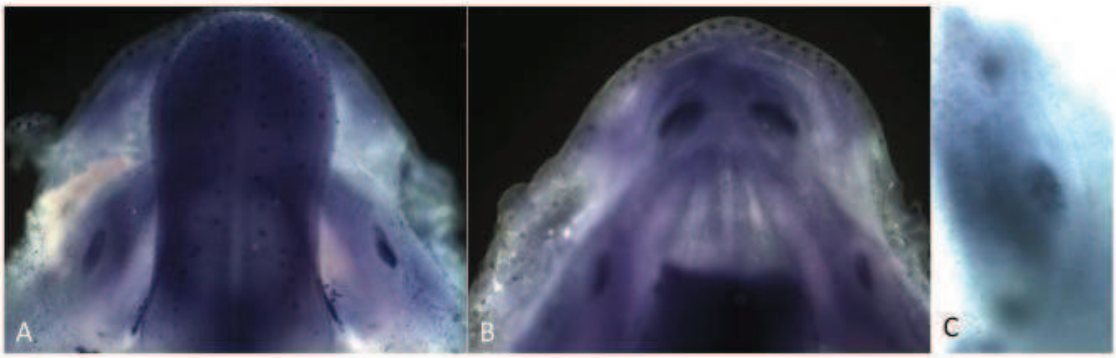


Fig. 6

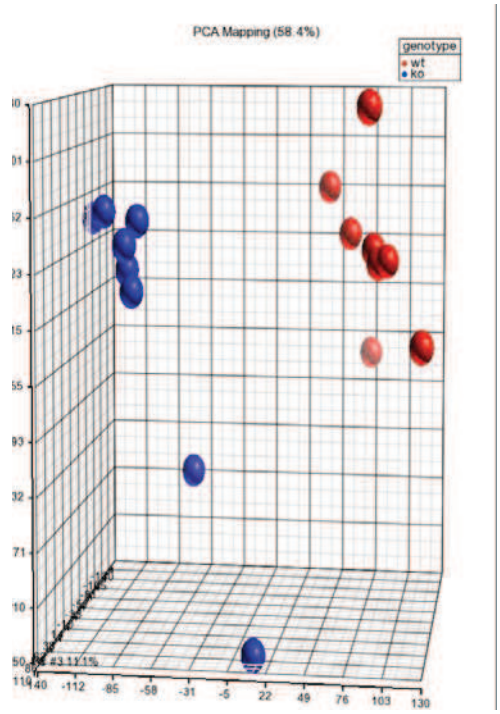

PCA Mapping (58.4%)
genotype
wt
ko
-140
-112
-85
-58
-31
-5
22
49
76
103
130

Fig . 7

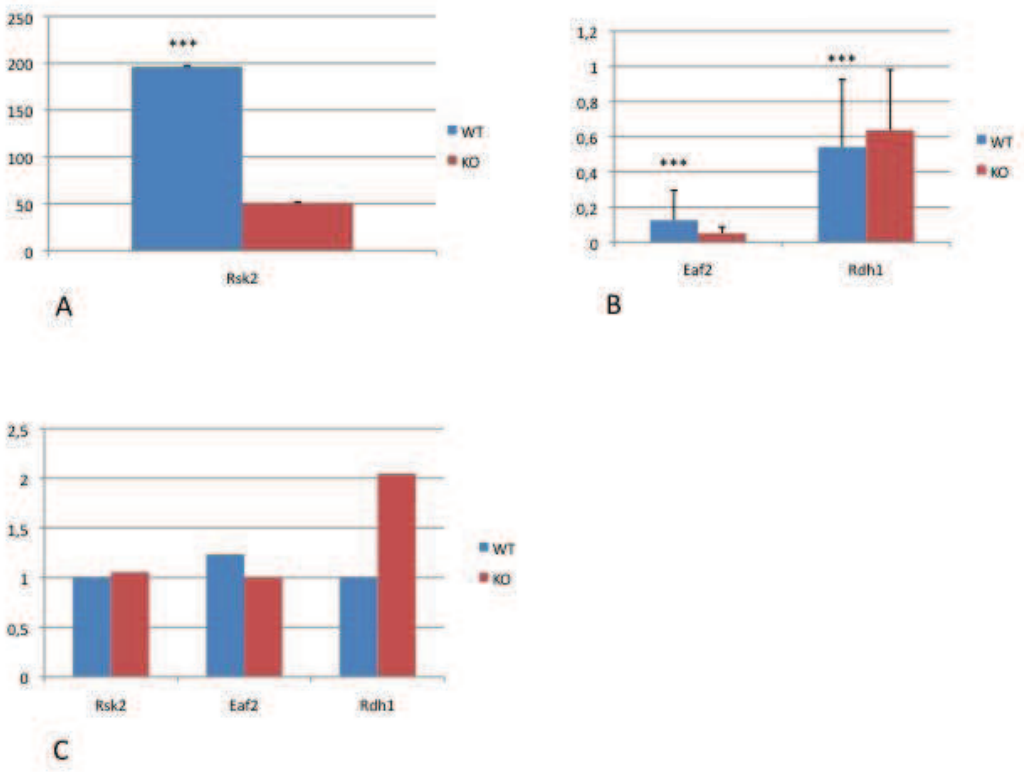
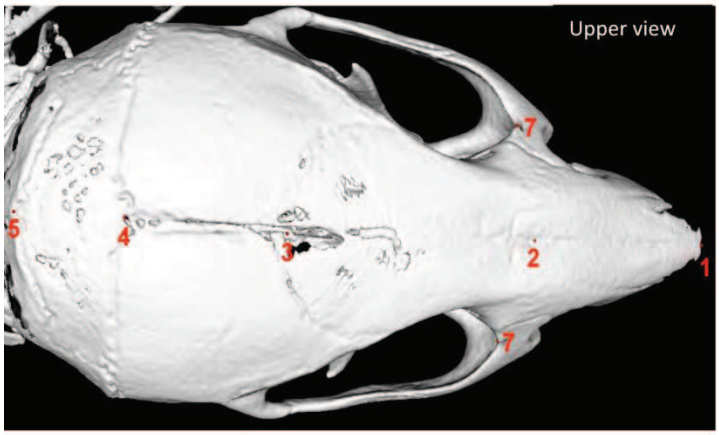
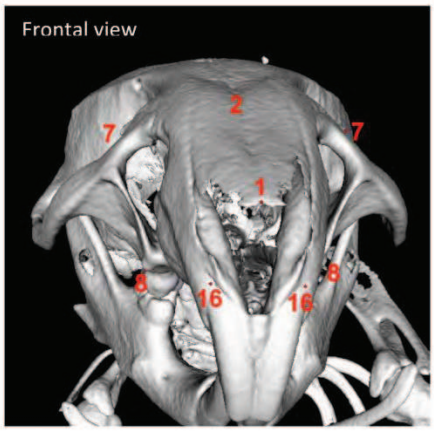

250
200
150
100
50
0
***
WT
KO
Rsk2
A
1,2
1
0,8
0,6
0,4
0,2
0
***
***
WT
KO
Eaf2
Rdh1
B
2,5
2
1,5
1
0,5
0
WT
KO
Rsk2
Eaf2
Rdh1
C

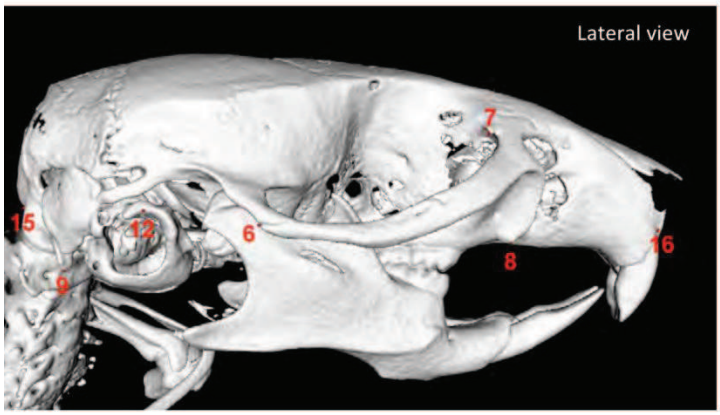
Table I

Distances	Mean WT	STD WT	Mean *Rsk2* mutants	STD *Rsk2* mutants	p-value
Between 1 and 2	**7,10**	**0,59**	**6,84**	**0,35**	**0,03**
Between 1 and 3	**15,05**	**0,46**	**13,92**	**0,59**	**0,01**
Between 1 and 4	**18,59**	**0,41**	**17,24**	**0,57**	**0,005**
Between 1 and 5	**21,87**	**0,49**	**20,48**	**0,48**	**0,005**
Between 2 and 3	**8,07**	**0,45**	**7,25**	**0,47**	**0,005**
Between 2 and 4	**11,72**	**0,38**	**10,69**	**0,45**	**0,005**
Between 2 and 5	**15,21**	**0,35**	**14,13**	**0,31**	**0,005**
Between G and D 6	12,08	0,17	12,16	0,13	0,47
Between G and D 7	6,42	0,21	6,45	0,27	0,68
Between G and D 8	4,77	0,11	4,79	0,73	0,22
Between G and D 9	6,27	0,25	6,13	0,12	0,06
Between G and D 10	0,74	0,06	0,71	0,08	0,47
Between G and D 11	1,05	0,05	1,13	0,11	0,29
Between G and D 12	10,08	0,37	10,22	0,28	0,29
Between G and D 15	4,53	0,19	4,72	0,51	0,37
Between G and D 16	2,29	0,22	2,23	0,23	0,57
Between 1 and 6D	**15,24**	**0,34**	**14,39**	**0,52**	**0,008**
Between 1 and 6G	**15,20**	**0,32**	**14,47**	**0,31**	**0,008**
Between 1 and 7D	**7,92**	**0,28**	**7,18**	**0,58**	**0,008**
Between 1 and 7G	**7,89**	**0,22**	**7,24**	**0,34**	**0,005**
Between 1 and 8D	6,90	0,24	6,29	0,46	0,88
Between 1 and 8G	6,90	0,25	6,35	0,29	0,68
Between 1 and 9D	**20,65**	**0,47**	**19,55**	**0,46**	**0,008**
Between 1 and 9G	**20,62**	**0,48**	**19,56**	**0,32**	**0,008**

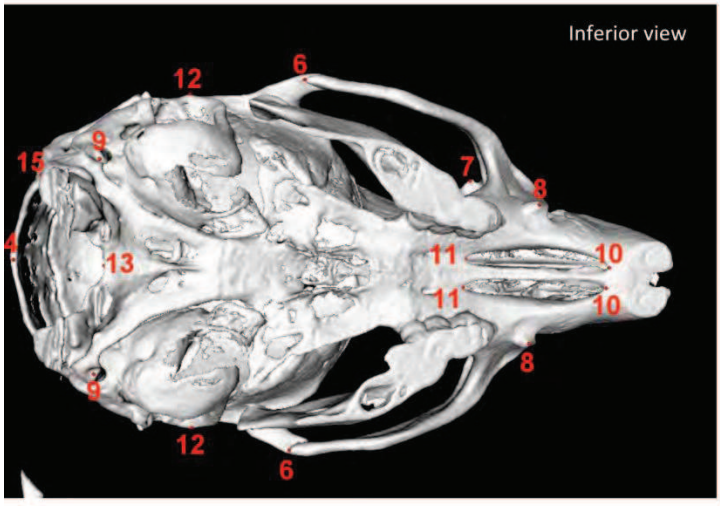
Fig. 1S Craniofacial Landmarks







1: Nasion, intersection of nasal bones, rostral point
2: Nasion, intersection of nasal bones, caudal point
3: Bregma
4: Intersection of parietal bones with anterior aspect of interparietal bone at midline
5: Intersection of interparietal bone with occipital bone at midline
6: Most posterior point of the intersection of the maxillary bone process with the zygoma bone
7: Lacrymal protuberance
8: Maxillary bone protuberance
9: Para occipital process
10: Most anterior point of the anterior palatine foramen
11: Most posterior point of the anterior palatine foramen
12:Most posteroinferior point on the superior portion of the tympanic ring
13: Basion
14: Opisthion
15: Intersection of occipital condyle with foramen magnum, taking the most lateral point of the curve
16: Centre of the alveolar ridge over maxillary incisor



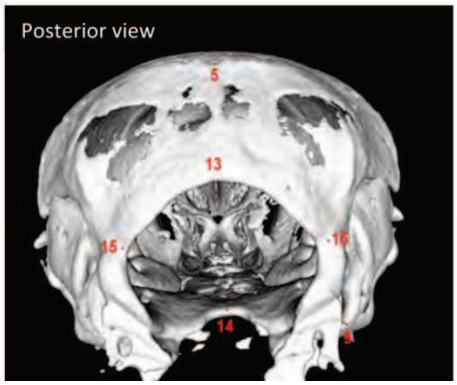


Fig .2S Molar Landmark

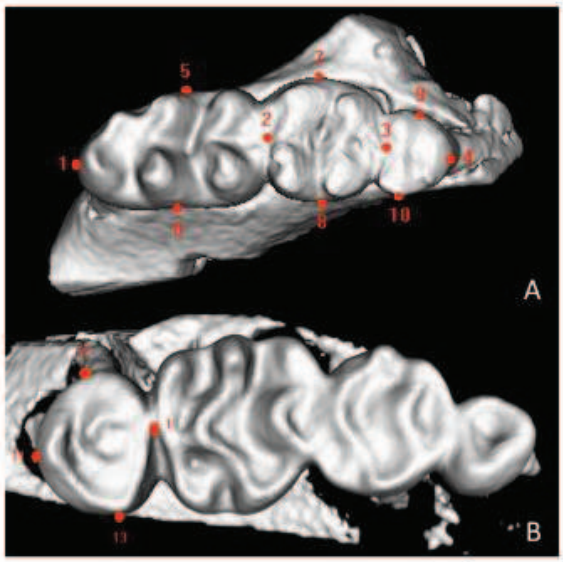


Fig. 3S Incisor Landmarks

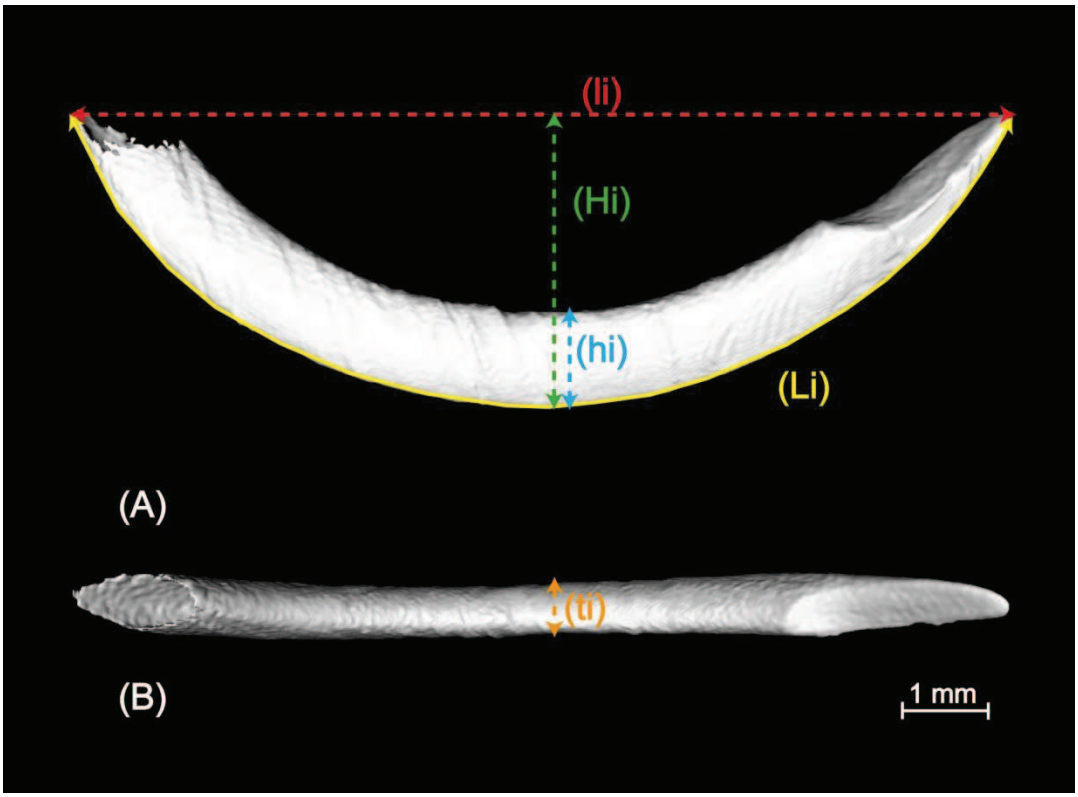


Table IS Templates for in situ hybridization

GENE	Genbank NM_	Sequence	VECTOR	RNA POLYMERASE
Rsk1 Rps6ka1	NM_009097	*1313-2098*	pCMV.SPORT6	*SP6 sens*
Rsk1		*3097-2282*	pCMV.SPORT6	*T7 antisens*
Rsk2 Rps6ka3	NM_148945	*3025-2117*	pT7T3D-PacI	*T3 antisens*
Rsk2		*2224-3208*	pT7T3D-PacI	*T7 sens*
Rsk3 Rps6ka2	NM_011299	*3234-4245*	CMV.SPORT6	*SP6 sens*
Rsk3		*5374-4403*	CMV.SPORT6	*T7 antisens*
Rsk4 Rps6ka6	NM_025949	*20-759*	CMV.SPORT6	*SP6 sens*
Rsk4		*4330-3405*	CMV.SPORT6	*T7 antisens*

Publication 6 : Développement de la molaire et de l'incisive : identification de différences transcriptionnelles au stade capuchon par puces à ADN.

Molar and incisor development: show your microarray IDs

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Running title: Tooth-specific transcriptomic analysis

Key words: Tooth development • Molar • Incisor • Gene expression • Mouse • Microarray•

Abstract

Background: One of the key questions in developmental biology is how from universally shared molecular mechanisms and pathways, is it possible to generate organs displaying similar or complementary functions, with a wide range of different shapes or tissue organization? The dentition represents a valuable system to address the issues of differential molecular signatures generating specific tooth types. We performed a comparative transcriptomic analysis of developing murine lower incisors, mandibular molars and maxillary molars at the developmental cap stage (E14.5) prior to recognizable tooth shape and cusp pattern.

Results: 231 genes were identified as being differentially expressed between mandibular incisors and molars, with a fold change higher than 2 and a false discovery rate lower than 0.1, whereas only 96 genes were discovered as being differentially expressed between mandibular and maxillary molars. Numerous genes belonging to specific signaling pathways (the Hedgehog, Notch, Wnt, FGF, TGFβ/BMP, and retinoic acid pathways), and/or to the homeobox gene superfamily, were also uncovered when a less stringent fold change threshold was used. Differential expressions for 10 out of 12 (mandibular incisors versus molars) and 9 out of 10 selected genes were confirmed by quantitative reverse transcription-PCR (qRT-PCR). A bioinformatics tool (Ingenuity Pathway Analysis) used to analyze biological functions and pathways on the group of incisor versus molar differentially expressed genes revealed that 143 genes belonged to 9 networks with intermolecular connections. Networks with the highest significance scores were centered on the TNF/NFκB complex and the ERK1/2 kinases. Two networks ERK1/2 kinases and tretonin were involved in differential molar morphogenesis.

Conclusion: These data allowed us to build several regulatory networks that may distinguish incisor versus molar development, and may be useful for further investigations of these tooth-specific ontogenetic programs, some of which may be dysregulated in transgenic animal models and in human diseases.

Background

One of the key questions in developmental biology is how from universally shared molecular mechanisms and pathways, is it possible to generate organs displaying similar or complementary functions, with a wide range of different shapes or tissue organization? The dentition represents a valuable system to address the issues of differential molecular signatures generating specific tooth types. The mouse dentition is composed of one incisor and three molars on each hemiquadrant, separated by a toothless gap called diastema. Although molars and incisors develop according to the same basic developmental sequences, they display several important differences besides their recognizable tooth shape. Rodent incisors have, for instance, a continuously growing ability through life, linked to the presence of an active stem cell niche located within the apical cervical loops [1]. They also exhibit asymmetrical development: ameloblasts differentiate and deposit enamel matrix solely on the labial side, whereas the lingual side is much smaller and functions as a root analogue with solely odontoblast differentiation [2].

From a common developmental layout with pluripotent cephalic neural crest cells migrating towards the first pharyngeal arch and mesodermal cells participating to the development of many elements of the craniofacial region, including teeth [3-5], odontogenesis proceeds through several stages. Odontogenesis initiates at the dental lamina stage by the appearance of a localized thickened area of the oral ectoderm, and proceeds to bud, cap and bell stages, odontoblasts and ameloblasts terminal differentiations, dentin and enamel matrix deposition and mineralization, root formation and finally tooth eruption. Odontogenesis is controlled by epithelio-mesenchymal interactions between the ectomesenchymal cells and the oral ectoderm [6-9], and is regulated by well known conserved signaling pathways (FGF, BMP, Shh, Wnt, TGFβ, Notch, TNF/NFκB) [10-16]. Transcription factors including several homeobox gene products [17-19] and genes from the retinoic acid pathway [20] also play a role in tooth development.

The differential location, identity, shape and size of teeth are already determined during early stages of development [21]. At embryonic day (E)10.5 the first molecular signals (BMP4, FGF8) initiating differential tooth morphogenesis are found in the oral ectoderm in mutually exclusive and complementary territories, allowing tooth development to be restricted to the oral cavity [9, 22] and triggering subsequent mesenchymal signaling. Tooth

development was postulated not to involve *Antennapedia* class homeobox (Hox) genes [23], although recent expression studies showed specific expression of some of these genes in distinct tooth bud tissues [24]. Developing teeth express a number of other homeobox genes that are expressed in nested patterns across the jaw. The mandible is divided into oral (expressing *Lhx6* and *7*), aboral (expressing *Gsc*), distal (presumptive incisor, expressing *Msx1* and *2*) and proximal (presumptive molar, expressing *Dlx1* and *2*, *Barx1*, *Pitx1*) domains [17-19]. These expression patterns are defined by positive and negative signals from the oral epithelium. *Bmp4*, for example, is initially expressed in the distal epithelium and induces expression of *Msx1* in the underlying (presumptive incisor) mesenchyme, while at the same time it negatively regulates expression of *Barx1*, so as to restrict its expression to the presumptive molar region [18]. *Fgf8*, meanwhile, is expressed adjacent to *Bmp4* in the proximal oral epithelium and positively induces *Barx1* expression in the underlying presumptive molar epithelium [18]. The expression of *Fgf8* is positively controlled by the paired-related homeobox gene *Pitx2* (*Otlx2*) [25, 26]. Patterning of the mandible also involves other transcription factors. The basic helix-loop-helix (bHLH) factors *Hand1* and *2* are expressed at the distal end of the developing mandible, where they define the midline area [27]. *Islet1* (*Isl1*) expression in distal (presumptive incisor) epithelium requires signals from the mesenchyme. Isl1 plays an important role in regulating distal gene expression during jaw and tooth development [28].

Tooth shape specification from the dental lamina stage is then exclusively contained within the ectomesenchyme. At the cap stage (E14.5 in the mouse) the condensing dental mesenchymal papilla controls the growth and folding of the inner dental epithelium. Mesenchymal signals induce within the enamel organ the formation of a signaling center called the primary enamel knot. It is a transitory structure of non proliferative cells, which produces several signaling molecules [29] and is essential to crown and cusps development and shape. The patterning role of the mesenchyme and dental papilla has also been adressed by heterologous recombination experiments from E13 to E16 between molar and incisor dental papilla and enamel organs, allowing the development of teeth of shape and type corresponding to the mesenchymal identity [30, 31].

Alterations of these precisely regulated molecular and cellular sequences of development lead to dental anomalies, i.e. anomalies of teeth number, shape and size, of

hard structures (enamel and dentin), of root formation and eruption. These malformations are observed in transgenic mouse models [32, 33] mimicking human diseases and within the clinical phenotypes of syndromes or rare genetic diseases [34, 35]. Indeed, at least 900 of the ~ 7000 known rare diseases or syndromes include oro-dental anomalies. In some syndromes only molars and canines are affected, like in oto-dental syndrome caused by deletions of the *FGF3* gene and characterized by grossly enlarged molar teeth (globodontia) [36], or in *PAX9* related molar oligodontia [37]. In other syndromes, only incisors are affected like in KBG syndrome caused by mutation in *ANRD11* and characterized by intellectual disability associated with short stature, facial dysmorphism and macrodontia of the upper central incisors, often with an agenesis of maxillary lateral incisors [38]. *SATB2* was involved in dental anomalies like incisor agenesis both in human in the 2q33.1 microdeletion syndrome [39] and in the corresponding mouse model [40]. We also recently identified *SMOC2*, a gene causing when mutated severe developmental dental defects with a dentin dysplasia phenotype associated to major microdontia, oligodontia, and shape abnormalities [41]. Furthermore, we showed a differential expression of this gene between molars and incisors.

In order to discover new candidate genes implicated in the molecular events responsible for differential histomorphogenesis of the molars and incisors, we performed a transcriptomic analysis of developing murine lower incisors, mandibular molars and maxillary molars at the cap stage of development (E14.5). Here we report a global analysis of the identified differentially expressed genes. These data allowed us to build several regulatory networks that may distinguish incisor versus molar development, and may be useful for further investigations of these tooth-specific ontogenetic programs, some of which may be dysregulated in human diseases.

Results and Discussion

Analysis of tooth specific transcriptional profiles

We decided to compare gene expression profiles in developing murine lower incisor and molars, as well as between the lower and upper (mandibular and maxillary) first molars. The

developing tooth buds were collected by microdissection from E14.5 wild-type C57Bl6 mice, and total RNA was extracted with the RNAeasy micro Kit (Qiagen, see Materials and Methods), after pooling 4 tooth germs per sample in order to obtain enough RNA for DNA microarray hybridization. Altogether, 4 lower incisors samples, 4 maxillary molars samples, and 8 mandibular molars samples were hybridized on Affymetrix mouse exon 1.0 ST microarrays. Principal component analysis (PCA) was performed using the Partek Software to assess the consistency of the results. According to this analysis, the transcriptional profiles of three incisors samples (one sample of dubious quality was discarded) and eight mandibular molars samples showed that samples segregated in two distinct groups, showing relevant transcriptional differences between mandibular molars and lower incisors (Additional file 1). PCA performed on transcriptional profiles of eight mandibular molars samples and four maxillary molars samples also showed a clear segregation of samples between the two groups. This analysis indicated that transcriptional differences existed both between lower incisors and molars, as well as between mandibular and maxillary molars.

Our microarray data analysis allowed identification of several genes already known to be involved in tooth development (see Introduction), which did not show statistically different expression levels between distinct tooth samples. For instance, there was no significant difference in *Bmp4, Fgf8, Msx1*, *Pitx1, Pitx2, Gsc, Dlx2, Runx2, Msx2, Lhx6, Hand1* or *Satb2* expression between lower incisors and mandibular molars, and in *Bmp4, Fgf8, Msx1, Dlx2, Runx2, Msx2* and *Satb2* expression between mandibular and maxillary molars. Altogether, these data validated the sensitivity of the microarray analysis, and confirmed that several important regulators of tooth development were expressed at comparable levels in distinct tooth types at the stage analyzed.

Many genes exhibited statistically significant differential expression levels between specific tooth types. The distribution of differentially expressed genes is illustrated in Fig. 1A (mandibular incisor versus first molar) and 1B (mandibular versus maxillary first molar). In these diagrams—as well as in all subsequent Tables—negative “fold changes” reflect an enriched expression in incisor (Fig. 1A) or in maxillary molar (Fig. 1B), whereas positive values indicate an enriched expression in mandibular molar. Genes plotted in red are those exhibiting a fold change superior to 2 (> 2 fold change) between the two types of samples (with statistical significance). We focused our analysis on such genes exhibiting at least a 2

fold change in expression in a given tooth type. However, in order not to overlook genes that may be relevant even if their differential expression is not as pronounced, we also considered all genes belonging to specific signaling pathways (the Hedgehog, Notch, Wnt, FGF, TGFβ/BMP, and retinoic acid pathways) and/or to the homeobox gene superfamily, as many important regulators of tooth development belong to these families. For analysis of these selected pathways and gene families, we applied a less stringent threshold (+ or - 1.2 fold change). For all identified genes we performed a detailed analysis of the literature, and hereby distinguish genes previously reported to be expressed (and sometimes functionally involved) in tooth development—including rare cases of reported differential expression between tooth types—from new candidate genes potentially involved in establishing tooth-specific programs.

Expression profiling of mandibular molars versus incisors

Genes with a fold change higher than 2

Among the 35,556 probe sets represented in the microarrays, about 10% of the genes were excluded for any further analysis because of their low expression level, and 231 genes were differentially expressed between mandibular incisor and molar, with a fold change higher than 2 and a false discovery rate lower than 0.1 (corresponding to a p-value lower than 6.89E-04) (Fig. 1A). The top ten genes exhibiting the highest expression in mandibular molars were *Barx1*, *C1qtnf3*, *Adcy8*, *Cntn6, Six2*, *Tcfap2b, Odz1, Vstm2a, Nptx1* and *Has2.* The top ten genes showing highest expression in incisors were *Hpse2*, *Alx1*, *Hand2*, *Sfrp4*, *Pax3*, *Alx3, Isl1, Mcpt2, Cacna2d3* and *Irx4* (Table 1A). Interestingly, among these genes, four were already known to be differentially expressed, with *Barx1* and *Six2* preferentially expressed in molars [42, 43] and *Hand2* and *Isl1* in incisors [28, 44].

Additional literature searches revealed that, among the remaining 231 genes, only 22 were previously described as being expressed during tooth development, including 9 genes known to be differentially expressed between molar and incisors. Thus, our analysis revealed nearly 200 “new”, potentially interesting genes not previously described as differentially expressed between developing incisors and molars (a complete list is given in Additional file 2). Table 1B provides data for selected genes with high fold change and/or belonging to families for which other member(s) are involved in odontogenesis. *Sfrp2* and *Sfrp4*, for

example, belong to the family of secreted frizzled-related proteins, for which *Sfpr1* was already known to be expressed in teeth [45, 46]. *Tlx2* and *Bmp5* also have two paralogues, *Tlx1* and *Bmp4,* that were previously described as being differentially expressed between tooth types [47, 48]. Two *Alx* genes, *Alx1* and *Alx3* were differentially expressed in our microarray experiments, with very high fold changes. The corresponding human genes are mutated in frontonsal dysplasia affecting the midline facial structures [49]. We also found two members of the *Iroquois* homeobox gene family, *Irx4* and *Irx6*, suggesting a role of these genes in defining incisor identity.

Genes from selected pathways or families

From the 3078 genes exhibiting a fold change higher than 1.2 and a false discovery rate lower than 0.1, 107 belonged to pathways or families selected as being important for tooth development (the FGF, TGFβ/BMP, Wnt, Hedgehog, retinoic acid, and Notch pathways, and the homeobox gene superfamily: see Introduction). Among these, 88 had not been reported to be expressed in teeth and were considered as new potential genes involved in tooth development (Table 2). Twenty genes were already known to be expressed in teeth (Table 2, gene names in bold), and among them 11 were known to be differentially expressed between the two tooth types (Table 2, underlined). Considering genes not previously known to be expressed in teeth, and genes not yet described to be differentially expressed between tooth types, we found in total 99 genes not yet involved in differential tooth morphogenesis.

Quantitative RT-PCR analysis

Twelve of these 99 genes were selected for validation of the microarray data by quantitative RT-PCR (qRT-PCR). These were: *Ihh* (from the hedgehog pathway), *Dll1* (from the Notch pathway), *Sfrp1* and *Sfrp2* (from the Wnt pathway), *Fgf12* (from the FGF pathway), *Bmp5* (from the TGFβ pathway/superfamily), *Cyp26c1* and *Cyp1b1* (encoding two retinoic acid-metabolizing enzymes), *Alx1* and *Shox2* (members of the homeobox gene superfamily). We also decided to verify two genes known to be expressed in teeth and exhibiting a fold change higher than 2: *Smoc2* because we recently detected by *in situ* hybridization a differential expression between molars and incisors [41] and *Prkcq,* which belongs to the NFκB pathway (Table 1B). qRT-PCR was performed on the same RNA samples as used for microarray hybridization. From these 12 genes, 10 were found to be statistically differentially expressed

between molar and incisors by qRT-PCR, in agreement with the microarray data (Fig. 2). The two exceptions were *Ihh*, which did not exhibit differential expression, and *Dll1*, which displayed an opposite expression (molar > incisor, not statistically significant) when compared to the microarray data .

Gene network analysis

Relevant networks when considering all genes with a fold change higher than 2

To gain insight into interactions that may occur between the differentially expressed genes and/or proteins, we constructed biologically relevant networks using the Ingenuity pathway analysis software. From the 231 differentially expressed genes with a fold change higher than 2, 143 genes were mapped in nine networks. The most relevant network (score=48) was centered on the NFκB complex and contained 24 differentially expressed genes (Fig. 3A). *Barx1, Dlx1, Sox2, Cited1, Nr2f1, Nr2f2, Vsnl1, Cxcl6, Dusp6, Has2, Lpl, Tfap2b, Rgs5, Sfrp1* and *Sfrp2* were more strongly expressed in mandibular molars than in incisors. *Otx1, Isl1, Cyp2c19, Foxa3, Pappa, Rgs7, Rgs20, Cyp17a1* and *Sfrp4* were more expressed in incisors. This network highlighted two genes from the nuclear receptor superfamily (*Nr2f1* and *Nr2f2*, also known as COUP-TFI and II), both expressed at higher levels in molars. On the other hand, several genes from the *Sfrp* family were differentially expressed, with *Sfrp1* and *Sfrp2* being more expressed in mandibular molars and *Sfrp4* more expressed in incisors (Fig. 3A).

The second network (score=42) was centered on ERK1/2 and contained 22 differentially expressed genes (Fig. 3B). *Hdac9, Entpd1, Ampa Receptor, Grp and Gria2* were expressed at higher levels in mandibular molars, whereas *Hand1, Hand2, Myocd, Cacna1d, Ppargc1a, C1qtnf2, Ptprr, Ace2, Nts, SSt, Alx3, Ins1, Glis3, Nlrp5, Dsc1, Tlx1* and *Reln* were preferentially expressed in lower incisors. *Hand1* and *Hand2* were also preferentially expressed in the incisor area, as previously reported at E10.5 [44].

Relevant networks when considering genes from selected pathways or families

From the 107 differentially expressed genes in the selected pathways, 50 genes were mapped in only 4 different networks involved in embryonic or tissue development (Additional file 3). The first network (score=24) contained 16 differentially expressed genes and was focused on *Six1,* a gene highly expressed in molars. This network contained two additional *Six* genes, *Six2* and *Six4,* also more expressed in molars, as well as *Six5,* which was

not differentially expressed. It further contained *Irx4* and *6,* preferentially expressed in incisors, whereas *Irx2* was not differentially expressed. In this network we also found *Dlx1,* a gene preferentially expressed in molars, whereas *Dlx2* and *Dlx5* were not differentially expressed. *Hmx2, Arx, Gsx2, Crx* and *Wnt11* were more expressed in incisors. Some Hox genes appeared in this network. Hox genes were classically considered as being not expressed in the maxillo-mandibular region, which derives from the first embryonic branchial arch [23], but recent expression studies have revealed expression of some Hox genes in specific developing tooth compartments [24]. This network also contained genes known to act during tooth development, like *Bmp4* or *Fgf10,* but these were not differentially expressed.

The second network (score=20) contained 14 differentially expressed genes and was centered on *Shox2,* a homeobox gene known to be expressed in teeth at E14.5 (Additional file 3). Only this gene and *Sfrp2* were more expressed in molars in this network. All others genes like *Tlx2, Brtc, Cer1, Hhex*, or *Lmx1b,* were preferentially expressed in incisors. This nework also contained genes known to be involved in odontogenesis, like *Runx2* and *Pitx2*, and which were not differentially expressed. The third network was centered on *Pparg* (encoding PPARγ, a member of the nuclear receptor superfamily) and all molecules from this network were more highly expressed in incisors except one nuclear receptor gene, *Nr2f2* (encoding COUP-TFII). The fourth network was centered on *fos* and contained several genes from the retinoic acid pathway preferentially expressed in incisors (Additional file 3).

Expression profiling of lower versus upper molars

Genes with a fold change higher than 2

We found 96 genes differentially expressed between mandibular and maxillary molars with a fold change higher than 2 (Table 3; Additional file 4 for a full list). The gene with highest expression in maxillary molars was *Cyp26c1*, a gene previously shown to be expressed in teeth [50] and found to be also differentially expressed between molars and incisors in our microarray analysis. The *Nefl* gene, responsible for Charcot Marie Tooth disease, was the most highly enriched in mandibular molars (Table 3A). *Nkx2-3* had already been reported in the literature as differentially expressed between the two tooth types [51] (Table 3A). Examination of *Nkx2-3* null mice revealed defects in maturation and cellular organization of

the sublingual glands. Furthermore, cusps were absent from mandibular molars and the third molar was occasionally missing [51].

Other genes previously described as acting during odontogenesis were identified as being differentially expressed in our microarray analysis. Seven of them were expressed with a fold change higher than 2 (Table 3B). Among these, *Pitx1* had already been described as being differentially expressed between upper and lower molars [19]. Inactivation of the *Pitx1* gene in mice affected mandibular tooth morphogenesis [19].

Among the "new" genes unravelled by our microarray analysis, several belong to gene families with other members known to act during odontogenesis. *Lhx1* and *Lhx9* were identified as displaying enriched expression in mandibular molars (Table 3B). Their paralogues *Lhx6* and *Lhx7* are implicated in tooth patterning at E10.5 [52]. *Lhx6/7* double mutant embryos lacked molar teeth. Despite molar agenesis, *Lhx6/7*-deficient animals had normal incisors which, in the maxilla, were flanked by a supernumerary pair of incisor-like teeth [53]. *Nkx6-1* and *Nkx2-1* appeared interesting as their paralogue *Nkx2-3* is already known to be differentially expressed between mandibular and maxillary molars [51]. *Msx3* was identified as being enriched in mandibular molars; its homologues *Msx1* and *Msx2* are known to play a role in mouse dentition patterning at E10.5 [54]. *Alx1*, which was differentially expressed in mandibular molars vs. incisors in our microarray analysis, was also found to be expressed at higher levels in maxillary molars (Table 3A).

Genes from selected pathways or families

Among the 2070 genes with a fold change higher than 1.2 and a p-value lower than 0.1 in mandibular vs. maxillary molars, 61 belonged to the pathways or families selected for further analysis (Table 4). Only nine genes were known to be expressed in teeth (Table 4, in bold), four of these being reported to be differentially expressed betwen the two tooth types (Table 4, underlined). Fifty-three genes had not yet been described as being expressed or acting during odontogenesis. In total we found 58 new genes not known to be differentially expressed between the two tooth types.

Quantitative RT-PCR analysis

To further validate our microarray experiments, a subset of 10 genes were selected for quantitative RT-PCR analysis. We focused our analysis on genes encoding known signaling

molecules or their effectors: *Wnt11*, the FGF receptor gene *Fgfr4*, *Gli1* (an effector of the Hedgehog pathway), and *Dll1* (Delta-like 1) acting in the Notch pathway. We also chose the *Rorb* and *Cyp26c1* genes from the retinoic acid signaling pathway, and *Alx1* as a homeobox gene. We further decided to analyze one of the integrin genes (*Itga8*) identified as being differentially expressed, *Prkcq* (which was also found as differentially expressed between incisor and molar; see above), and *Adamtsl3*. Among the ten genes analyzed by qRT-PCR, eight were confirmed to be differentially expressed as detected by microarray analysis (Fig. 4), whereas two (*Gli1* and *Wnt11*) were not found to be statistically differentially expressed.

Gene network analysis

Relevant networks when considering all genes with a fold change higher than 2

Among the 96 genes with a fold change higher than 2, 36 genes were mapped in two networks. The first network (score=27) was centered on ERK1/2 and included 13 genes identified as being differentially expressed between the two molar types (Fig. 5A). *Chnrq, Chnra1, Acp1, Angtpl1, Plac8, Fgfr4, Il1r1, Grap2, Cftr, Prkcq, Ankrd1* and *Mypn* were more highly expressed in mandibular molars, whereas *Pla2g7* was enriched in maxillary molars. The second network (score=25) was centered on tretinoin (a retinoic acid derivative, used as a medication for skin diseases) and contained 12 differentially expressed genes (Fig. 5B). *Rorb* and *Pla2g7* were the only two genes more expressed in maxillary molars, whereas *Pitx1*, *Tbx4*, *Gsc*, *Nkx2-3, Corin, Barx2, Otx1, Dlx6, Gjb2* and *Pgfr4* were more expressed in mandibular molars.

Relevant networks when considering genes from selected pathways or families

From the 62 differentially expressed genes belonging to the pathways selected for analysis, 23 genes were mapped in only 2 different networks involved in embryonic or tissue development (Additional file 5). The first network contained 11 differentially expressed molecules and was centered on *Dlx1* (a gene known to be expressed in the presumptive molar region at E10.5 [17]. *Dlx1* and *Itga8* were the only two genes identified as being more highly expressed in maxillary molars, whereas *Nkx6-1, Phox2b, Gsx1, Rhox4b* were found to be enriched in mandibular molars. The second network contained 12 differentially expressed genes and was focused on *Gli3*. Only *Aldh1a1* was more expressed in maxillary molars,

whereas *Barx2, Nkx3-2, Nkx2-1, Cer1, Lhx1, Gsc, Camk2b, Camk2a, Hmx2* and *Dll1* were preferentially expressed in mandibular molars.

Conclusions

This study provides the first comprehensive analysis of differential gene expression between developing murine tooth types, leading to new insights into the regulatory mechanisms involved in the ontogenesis of mammalian teeth. The issues of differential tooth morphogenesis were explored looking at the molecular identity of E14.5 mouse lower incisors and mandibular and maxillary molars by microarray analysis. The chosen stage (cap stage) is also called the dental histomorphogenesis stage and is occurring prior to visual tooth shape identification. Patterning instructions are already set up earlier in the developing jaws by the expression of the so-called divergent homeobox genes, and it is of no surprise to discover these genes still being differentially expressed at the histomorphogenesis stage. Molecules belonging to well-known pathways involved in various aspects of development (such as the Wnt, TGFβ/BMP, or FGF pathways) were also discovered as potentially carrying information for differential tooth morphogenesis. Of interest is the involvement of the retinoic acid pathway [55], as retinoids have marked effects on molar and incisor morphogenesis [20, 56]. Treatment with retinoic acid specifically inhibited cusp formation when E14.5 molars were cultured for 6 days on serum-supplemented medium, thus displaying a monocuspal, incisor-like shape [20].

Tooth morphology and its evolution in various mammalian species were proven to be related to dosage effect of signaling molecules, like for instance FGF3 being able to modify the cusps pattern [16, 57]. Our microarray analysis highlighted the molecules more or less expressed in a given tooth type, reinforcing the model of dosage modulating mechanisms. That specific tooth types are related to response to dosage modulation is further supported by transgenic mouse models displaying dysmorphism of specific tooth types [19, 53]. Gene dosage abnormalities are likely to occur in human rare diseases presenting with a tooth family specific dental phenotype [36, 38, 58]. Some of the corresponding genes were not retrieved in our analysis of differential gene expression in lower incisors versus lower or upper molars, suggesting that other levels of regulation, post-transcriptionally via effectors

of a given pathway or via fine tuning of kinase signalling (e.g. ref. [59]), will undoubtedly also participate in the molecular identity leading to specific tooth morphology.

Methods

Tissue collection

Pregnant female mice (C57Bl6 genetic background) were euthanized at 14.5 days of gestation (E14.5), embryos were collected and tooth samples (lower incisors, mandibular and maxillary first molars) were microdissected. Tissue samples were frozen in liquid nitrogen and kept at -80°C until use.The CERBM-GIE/ICS/IGBMC complies with the French national and European laws and regulations relating to the transport, housing and use of animals in research.

Microarray hybridization

Total RNA was extracted with the RNAeasy micro Kit (Qiagen) from pools of 4 tooth germs to obtain enough RNA for subsequent microarray hybridization. RNA quality was verified by analysis on a 2100 Bioanalyzer (Agilent). All samples displayed a RNA Integrity Number (RIN) greater than 9.8. Biotinylated single strand cDNA targets were prepared, starting from 300 ng of total RNA, using the Ambion WT Expression Kit (Cat #4411974) and the Affymetrix GeneChip WT Terminal Labeling Kit (Cat #900671), according to Affymetrix recommendations. Four lower incisors samples, 4 maxillary molars samples and 8 mandibular molars samples were hybridized on Affymetrix GeneChip Mouse Exon 1.0 ST arrays. Briefly, following fragmentation and end-labeling, 1.9 µg cDNA was hybridized for 16 h at 45^{o}C on the arrays interrogating 28,853 genes represented by approximately 27 probes spread across the full length of the gene. The chips were washed and stained in the GeneChip Fluidics Station 450 (Affymetrix), and scanned with the GeneChip Scanner 3000 7G (Affymetrix). Finally, raw data (.CEL Intensity files) were extracted from the scanned images using the Affymetrix GeneChip Command Console (AGCC) version 3.1. One incisor sample was excluded from the analysis because a technical problem occured during hybridization washing.

Microarray analysis

CEL files were further processed with the Partek software to obtain principal component analysis (PCA) and to select only genes with a signal value above 5 (20th percentile of all expression values) in at least one sample. The analysis was done only on three lower incisors samples as a technical problem during hybridization occurred for one of the 4 samples (high background). Genes were considered as differentially expressed if the false discovery rate from Benjamini and Hochberg test was under 0.1.

Ingenuity pathways analysis

Biologically relevant networks were created using the Ingenuity Pathway Analysis software (http://www.Ingenuity.com). Based on the algorithmically generated connectivity between gene–gene, gene–protein, and protein–protein interactions, the program develops functional molecular networks that overlay genes in the dataset. This program calculated p-values for each network by comparing the number of genes that were mapped in a given network, relative to the total number of occurrences of those genes in all networks. The score for each network is given as the negative log of the p-value, which indicates the likelihood of finding a set of genes in the network by random chance. For instance, a score of 20 indicates that there is a 10^{-20} chance that the genes in focus would be in a network because of random chance. Networks taking in account direct and indirect interactions have been generated for genes with a fold change higher than 2, whereas networks involving only direct interactions have been created for genes that were selected as members of pathways or families of interest with a fold change higher than 1.2.

Real-time quantitative RT-PCR

RT-PCR assays were performed in duplicate on three RNA samples for each tooth type analyzed, and included samples obtained from E14.5 WT mice. RNA extractions were performed as previously described. Oligo-dT primed cDNAs were generated using the Superscript II kit (Invitrogen) according to the manufacturer's protocol with. Quantitative

real-time PCR was achieved using SybrGreen and LightCycler 480 (Roche). The sequences of primers used for the various tested genes are given in Additional file 6. A probe set for detection of mouse *Gapdh* (a housekeeping gene) was used for normalisation. For each sample the ratio between signals for the gene of interest and *Gapdh* was calculated to normalize concentration values. To verify if genes were differentially expressed in different tooth types, the average of ratios calculated for lower incisors, mandibular molars and maxillary molars were then compared.

Authors' contributions

All authors contributed to the overall experimental design. VLH and MP performed the tissue collection, RNA extraction, and real-time RT-PCR experiments. CTC did the microarray hybridization. VLH and DD performed all statistical analyses. VLH, PD and ABZ wrote the manuscript. All authors read, contributed to, and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

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Table I

A

	Genes	Fold change	P-value	Known in teeth	Known as differentially expressed	References
Enriched in molars	*Barx1*	8,60	3,47E-09	Yes	Yes	[42]
	C1qtnf3	7,30	4,37E-08	No	No	
	Adcy8	5,75	2,76E-08	No	No	
	Cntn6	4,91	2,94E-07	No	No	
	Six2	4,82	4,84E-08	Yes	Yes	[43]
	Tcfap2b	4,31	1,88E-05	No	No	
	Odz1	4,24	5,02E-09	No	No	
	Vstm2a	4,09	7,28E-08	No	No	
	Nptx1	4,06	1,21E-05	No	No	
	Has2	3,94	1,66E-09	No	No	
Enriched in incisors	*Irx4*	-5,31	1,92E-07	No	No	
	Cacna2d3	-5,66	2,55E-08	No	No	
	Mcpt2	-6,39	2,90E-08	No	No	
	Isl1	-6,44	3,53E-08	Yes	Yes	[28]
	Alx3	-8,36	6,59E-12	No	No	
	Pax3	-11,72	1,68E-13	Yes	No	[60]
	Sfrp4	-12,38	9,88E-10	No	No	
	Hand2	-13,20	6,89E-14	Yes	Yes	[44]
	Alx1	-15,95	1,35E-10	No	No	
	Hpse2	-27,41	2,23E-11	No	No	

B

	Genes	Fold change	P-value	Known in teeth	Known as differentially expressed	References
Enriched in molars	*Lhx6*	3,69	8,95E-08	Yes	Yes	[52]
	Sfrp1	3,56	7,94E-06	Yes	No	[61]
	Smoc2	3,28	3,28E-08	Yes	Yes	[41]
	Shox2	2,85	1,08E-08	Yes	No	[62]
	Dlx1	2,81	4,49E-11	Yes	Yes	[17]
	Fgf12	2,22	3,32E-06	Yes	No	[63]
	Sfrp2	2,16	4,40E-11	No	No	
	Dbx2	1,97	8,51E-06	Yes	No	[64]
	Six4	1,95	8,05E-07	Yes	Yes	[43]
	Six1	1,83	8,69E-06	Yes	Yes	[43]
	Lhx8	1,64	2,87E-06	Yes	No	[65]
	Bmpr1a	1,36	7,12E-07	Yes	No	[66]
	Mapk1	1,30	3,20E-06	Yes	No	[67]
Enriched in incisors	*Gas1*	-1,25	6,93E-06	Yes	No	[68]
	Hoxa2	-1,41	9,43E-07	Yes	No	[23]
	Tlx2	-1,47	7 ,35E-07	No	No	
	Irx6	-1,58	3,09E-08	No	No	
	Gdf6	-2,00	4,01E-06	Yes	No	[69]
	Aqp1	-2,16	9,51E-08	Yes	No	[70]
	Amtn	-2,28	1,65E-08	Yes	Yes	[71]
	Prtg	-2,3	9,93E-07	Yes	No	[72]
	Slitrk6	-2,37	2,60E-07	Yes	No	[73]
	Wnt5a	-2,38	1,38E-05	Yes	No	[74]
	Hand1	-2,55	4,01E-06	Yes	Yes	[27]
	Prkcq	-2,75	7,17E-07	Yes	No	[75]
	Tlx1	-2,84	6,05E-08	Yes	No	[47]
	Bmp5	-3,20	3,00E-08	No	No	
	Cyp26c1	-4,05	3,10E-09	Yes	No	[50]
	Nts	-4,43	9,56E-10	Yes	No	[76]
	Irx4	-5,31	1,92E-07	No	No	

Table 2

Pathway/Family	Gene names (fold change)	Number of genes	Differentially expressed in teeth	Not known to be differentially expressed in teeth	New genes in teeth	Total new genes and new differentially expressed genes
FGF: molars	***Fgf12 (2,24)***	1	0	1	0	1
FGF: incisors	*Fgf22 (-1,41)*	1	0	0	1	1
TGFβ: molars	*Thbs2 (1,77); Gdf7 (1,73);* ***Bmpr1a (1,36);*** *Ppp2r1b (1,35);* ***Mapk1 (1,3);*** *Smurf2 (1,25)*	6	0	2	4	6
TGFβ: incisors	***Bmp5 (-3,20)****; Gdf6 (-2,00); Acvr1c (-1,59); Inhbe (-1,52); Nodal (-1,45)*	5	0	1	4	5
Wnt: molars	***Sfrp1 (3,56);*** *Sfrp2 (2,16); Plcb4 (1,66); Camk2d (1,48); Ccnd2 (1,44); Ppp2r1b (1,35); Cul1 (1,30); Ppp3cb*	8	0	1	7	8
Wnt: incisors	*Sfrp4 (-12,38);* ***Wnt 5a (-2,38);*** *Cer1 (-1,64); Wnt9b (-1,51); Wnt1 (-1,45); Camk2a(-1,34); Ppp3r2 (-1,32)*	7	0	1	6	7
hedgehog incisors	*Ihh (-1,30); Btrc (1,26);* ***Gas1 -(1,25)***	3	0	1	2	3
Retinoic acid: molars	***Cyp1b1****; Nr2f1 (3,59); Nr2f2 (2,69), Aldh7a1 (1,50)*	4	0	0	4	4
Retinoic acid: incisors	***Cyp26c1 (-4,05);*** *Cyp2c54 (-2,26); Rdh1 (-1,94); Cyp2c66 (-1,81); Cyp2a12 (-1,76); Rarres1 (-1,57); Rdh9 (-1,57); Ugt1a9 (-1,57); Aldh1b1 (-1,51); Rbp3 (-1,49); Pram1 (-1,43); Rarres2 (-1,35); Adh7 (-1,33); Cyp2b19 (-1,31); Rdh8 (-1,29)*	15	0	1	14	15
Notch: incisors	*Rbpjkl (-2,12); predicted gene 5109 (-1,71); Dll1 (-1,37)*	3	0	0	3	3
Homeobox genes: molars	***Barx1 (8,59); Six2 (4,81); Lhx6 (3,69);*** *Shox2 (2,85);* ***Dlx1 (2,81); Dbx2 (1,97); Six4 (1,95); Six1 (1,83); Lhx8 (1,64)***	9	6	2	1	3
Homeobox genes: incisors	*Alx1 (-15,94* ***)*** *Alx3 (-8,36);* ***Isl1 (-6,44) ;*** *Irx4 (-5,31);* ***Tlx1 (-2,83);*** *Otx1 (-2,48) ; Hoxa11 (-1,92); Hoxd8 (-1,86); Hoxd3 (-1,84); Hoxd4 (-1,81); Obox5 (-1,76); Lbx2 (-1,74); Rhox6 (-1,72); Hoxd10 (-1,66); Rhox1 (-1,62); Lmx1b (-1,61); Hoxd11 (-1,60); Hoxd1 (-1,60); Nkx2-1 (-1,60);Hnf1b (-1,59); Hoxc6 (-1,58); Irx6 (-1,58); Sebox (-1,57); Hhex (-1,55); Lhx4 (-1,54); Rhox12 (-1,54); Rhox2a (-1,53); Hoxc4 (-1,52); Tlx2 (-1,47); Rhox7 (-1,46); Hoxa9 (-1,44);* ***Hoxa2 (-1,41);*** *Pdx1 (-1,40); Hoxb9 (-1,40); Esx1 (-1,39); Crx (-1,36) ; Hoxb7 (-1,36); Hoxa6 (-1,35); Arx (-1,33); Dux (-1,33), Lbxcor1 (-1,32); Gsx2 (-1,29); Hmx2 (-1,27); Hoxb2 (-1,27); Gbx1 (-1,26)*	45	2	1	42	43
Total		**107**	**8**	**11**	88	**99**

Table 3

A

	Genes	Fold change	P-value	Known in teeth	Known as differentially expressed	References
Enriched in lower molars	*Nefl*	6,28	1,68E-08	No	No	
	Ostn	4,12	6,22E-06	No	No	
	Nkx2-3	3,97	5,32E-10	Yes	Yes	[51]
	Tnnt1	3,19	9,08E-08	No	No	
	Chrna1	3,04	4,47E-06	No	No	
	Nefm	3,01	1,54E-08	No	No	
	Myf5	2,94	7,14E-09	No	No	
	Klhl31	2,92	4,58E-08	No	No	
	Plac8	2,91	4,68E-09	No	No	
	Synpo2l	2,87	3,71E-08	No	No	
Enriched in upper molars	*Naalad2*	-2,21	6,48E-09	No	No	
	Kcnb2	-2,29	1,42E-06	No	No	
	Itga8	-2,34	4,57E-08	No	No	
	Atp6	-2,84	1,26E-07	No	No	
	Alx1	-2,85	2,50E-08	No	No	
	Gabrb2	-3,06	7,32E-09	No	No	
	Ndst4	-3,48	4,21E-06	No	No	
	Pla2g7	-3,99	1,34E-08	No	No	
	Nmbr	-4,25	5,52E-10	No	No	
	Cyp26c1	-5,04	1,74E-10	Yes	No	[50]

B

	Genes	Fold change	P-value	Known in teeth	Known as differentially expressed	References
Enriched in lower molars	*Dlx6*	2,76	1,01E-07	Yes	No	[77]
	Gsc	2,21	1,31E-06	Yes	No	[78]
	Pitx1	2,19	6,09E-08	Yes	Yes	[19]
	Prkcq	2,15	6,71E-07	Yes	No	[75]
	Barx2	2,00	3,47E-07	Yes	No	[79]
	Lhx9	1,86	1,12E-06	No	No	
	Nkx6-1	1,62	4,43E-08	No	No	
	Lhx1	1,48	2,87E-06	No	No	
	Msx3	1,43	8,33E-07	No	No	
	Nkx2-1	1,40	1,84E-06	No	No	
Enriched in upper molars	*Gli1*	-1,34	6,21E-07	Yes	No	[80]
	Dlx1	-1,36	2,18E-06	Yes	No	[78]
	Lhx8	-1,48	2,57E-06	Yes	No	[65]

Table 4

Pathway/Family	Gene names (fold change)	Number of genes	Differentially expressed in teeth	Not known to be differentially expressed in teeth	New genes in teeth	Total new genes and new differentially expressed genes
FGF: lower molars	Fgfr4 (2,58); *Fgf16 (1,86)*	2	0	0	2	2
TGFβ: lower molars	*Amhr2 (1,68); Acvr2b (1,26)*	2	0	1	1	2
TGFβ: upper molars	*Smad9 (-1,53)*	1	0	0	1	1
Wnt: lower molars	*Camk2a (1,83); Fzd8 (1,67); Wnt9b (1,55); Camk2b (1,50); Wnt11 (1,39); Plcb2(1,39); Fzd5 (1,29); Cer1 (1,26)*	8	0	0	8	8
Wnt: upper molars	*Vangl1 (-1,22)*	1	0	0	1	1
Hedgehog: upper molars	***Gli1 (-1,34)***	1	1	0	0	0
Retinoic acid: lower molars	*Rorb (2,09); Rbp2 (1,59); Crabp2 (1,58); Polr2l (1,37);Rdh8 (1,27)*	5	0	0	5	5
Retinoic acid: upper molars	***Cyp26c1 (-5,04);*** *Aldh1a1 (-1,98); Dhrs3 (-1,51)*	3	0	1	2	3
Notch: lower molars	*Dtx4 (1,81); Dll1 (1,41); Rfng (1,31); Dll3 (1,36)*	4	0	0	4	4
Homeobox genes: lower molars	***Nkx2-3 (3,97), Dlx6 (2,76); Pitx1 (2,18) ; Gsc (2,21); Barx2 (2,01);*** *Hoxa7 (1,91); Lhx9 (1,86); Rhox11 (1,82); Hoxb7 (1,78); Vsx1 (1,69); Phox2b (1,68); Hoxa6 (1,67); Rhox4f (1,65); Nkx6-1 (1,62); Hoxb9 (1,57); Hoxa10 (1,55); Tgif2 (1,53); Lhx1 (1,48); Hmx1 (1,47); Hoxa3 (1,47); Hoxb2 (1,46); Hoxc12 (1,43); Prox2 (1,43); Msx3 (1,43); Gsx1 (1,42); Mixl1 (1,40); Nkx2-1 (1,40); Hoxd8 (1,39); Gbx1 (1,38); Hmx2 (1,36); Lmx1a (1,34); Rhoxa2 (1,23)*	32	3	2	27	29
Homeobox genes: upper molars	*Alx1 (-2,85);* ***Lhx8 (-1,48); Dlx1 (-1,36)***	3	1	1	1	2
Total		**62**	**4**	**5**	53	**58**

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Figure Legends

Figure 1. Volcano plots representing corrected p-values (ordinates) from Student t-test of the mRNA levels compared in mandibular molars vs. incisors (A), and mandibular vs. maxillary molars (B). Genes with a fold change (abscissae) higher than 2 and a false discovery rate lower then 0.1 are shown in red. In both plots, positive values correspond to genes more highly expressed in mandibular molars, and negative values to genes enriched in expression in incisors (A) or maxillary molars (B).

Figure 2. Real-time quantitative RT-PCR analysis of genes selected for their differential expression between mandibular molars vs. lower incisors as detected by Affymetrix microarrays. Histograms show expression levels in molars (blue) and incisors (red) as values normalized with respect to *Gapdh* expression. Data (mean ±SEM) were analyzed with Student t-test; ***p<0.001; **p<0.01; *p<0.05.

Figure 3. Ingenuity pathway gene network analysis of incisor vs. molar expressed genes. The two most significant gene networks identified in the Ingenuity pathway analysis of our microarray data are shown. These networks (see Results for details) are centered on the NFκB complex (A) and the ERK1/2 kinases (B). Many of the key genes highlighted in these networks are members of the ontology groups that include receptors, ligands and interacting proteins, and two families of transcription factors: homeodomain (homeobox encoded) proteins and nuclear receptors. The networks are displayed graphically as nodes (genes/gene products) and edges (biological relationships between the nodes). Differentially expressed genes are shown in two colors, the intensity of the colors reflecting the degree of enrichment in molar (red) versus incisor (green) tooth buds. Nodes are displayed using various shapes representing the functional class of the gene product (flat oval: transcription factor; tall oval: transmembrane receptor or interacting protein; losange: enzyme; triangle: kinase; rectangle: G protein-coupled receptor; circle: other). Interactions are depicted by arrows ("acts on", with dashed arrows indicating "indirect" interactions) or straight lines (binding only).

Figure 4. Real-time quantitative RT-PCR analysis of genes selected for their differential expression between mandibular vs. maxillary molars as detected by Affymetrix microarrays. Histograms show expression levels in mandibular molars (gray) and maxillary molars (black), with values normalized with respect to *Gapdh* expression. Data (mean ±SEM) were analyzed with Student t-test; ***p<0.001; **p<0.01; *p<0.05.

Figure 5. Ingenuity Pathway gene network analysis of maxillary (upper) vs. mandibular (lower) molar expressed genes. The three most relevant networks identified by Ingenuity Pathway analysis are centered on ERK1/2 (A), and tretinoin (13-*cis*-retinoic acid, an active retinoid used in therapy) (B). All differentially expressed genes are shown in color, the intensity reflecting the degree of enrichment in mandibular (red) versus maxillary (green) molars. See Legend to Figure 3 for explanations on symbols and types of interactions.

Table Captions

Table 1. Overview of genes differentially expressed between lower (mandibular) incisor and molar. The table is subdivided in two sections highlighting: (A) The "top ten" genes showing the highest degree of enrichment in incisor (negative values) or molar (positive values); (B) additional examples of differentially expressed genes, some already known from the literature as being expressed in developing teeth, others not predicted from the literature. Separate columns indicate those genes already known as being expressed in developing teeth ("Known in teeth"), and sometimes as being differentially expressed in both tooth types ("Known as differentially expressed"). In all cases, one relevant reference has been selected. For a complete list of differentially expressed genes (with fold changes > 2 or < -2), see Additional file 2.

Table 2. Overview of genes belonging to selected signaling pathways (FGF, TGFβ/BMP, Wnt, Hedgehog, Retinoic acid, Notch) or to the homeobox-containing superfamily, showing differential expression in mandibular molar or incisor. Genes are listed as being enriched in expression in molar or incisor, with fold changes in expression in parentheses. Genes already known from the literature to be expressed in teeth appear in bold, and those for which a differential expression was reported for the two tooth types are underlined. Additional columns summarize the literature survey, scoring genes previously described as expressed in developing teeth ("Known in teeth"), as differentially expressed in both tooth types ("Known as differentially expressed"), or "new" (i.e. not described in the literature: right-most column).

Table 3. Overview of genes differentially expressed between lower (mandibular) and upper (maxillary) molars. As for Table 1, this table is organized in two sections showing: (A) The top ten genes showing highest expression in mandibular (lower) molars (negative values) or maxillary (upper) molars (positive values); (B) examples of genes known from the literature as being expressed in developing teeth, only a minority being described as differentially

expressed in upper vs. lower molars ("Known as differentially expressed"), or not described in the literature. Among the top ten genes, only two were known to be expressed in developing teeth. For a complete list of differentially expressed genes (with fold changes > 2 or < -2), see Additional file 4.

Table 4. Overview of genes belonging to selected signaling pathways (FGF, TGFβ/BMP, Wnt, Hedgehog, Retinoic acid, Notch) or to the homeobox gene superfamily, showing differential expression in mandibular (inferior) versus maxillary (superior) molars. Fold changes in expression are incicated in parentheses. As in Table 2, genes known from the literature to be expressed in teeth appear in bold, and those for which a differential expression was reported are underlined. Additional columns scoring the genes previously described as being expressed in developing teeth ("Known in teeth"), as being differentially expressed in both molar types ("Known as differentially expressed"), or "new" (not previously described).

Additional material

Additional file 1 (Word, docx). Principal component analysis (PCA) of mandibular molar vs. lower incisor samples (A), and mandibular vs. maxillary molar samples (B). Mandibular molar samples are represented in red, and incisor or maxillary molar samples in blue. For both analyses, samples segregate in two distinct groups, showing relevant transcriptional differences between the two tooth types.

Additional file 2 (Word, docx). This table presents an overview of genes showing differential expression in developing mandibular incisors versus molars. Only the genes exhibiting at least a two fold change in expression according to Affymetrix microarray analysis are listed. Genes with the highest expression in incisors (positive values) or molars (negative values) appear on top and bottom of the list, respectively.

Additional file 3 (Word, docx). Ingenuity pathway gene network analysis of genes belonging to selected pathways and/or superfamily (homeobox genes), showing differential expression in incisor or molar tooth buds. Four relevant networks were constructed by Ingenuity pathway analysis. The networks are displayed graphically as nodes (genes/gene products) and edges (biological relationships between the nodes). Differentially expressed genes are shown in two colors, the intensity of the colors reflecting the degree of enrichment in molar (red) versus incisor (green) tooth buds. Nodes are displayed using various shapes representing the functional class of the gene product (flat oval: transcription factor; tall oval: transmembrane receptor or interacting protein; losange: enzyme; triangle: kinase; rectangle: G protein-coupled receptor; circle: other). Interactions are depicted by arrows ("acts on", with dashed arrows indicating "indirect" interactions) or straight lines (binding only).

Additional file 4 (Word, docx). Overview of genes showing differential expression in developing mandibular (lower) versus maxillary (upper) molars. Only the genes exhibiting at least a two fold change in expression according to Affymetrix microarray analysis are listed. Genes with the highest expression in upper molars (positive values) or lower molars (negative values) appear on top and bottom of the list, respectively.

Additional file 5 (Word, docx). Ingenuity pathway gene network analysis of genes belonging to selected pathways and/or superfamily (homeobox genes), showing differential expression in upper versus lower molars. Two relevant networks are centered on *Dlx1* (network 1) and *Gli3* (network 2). See Legend to Additional file 3 for key and explanations.

Additional file 6 (Word, docx). Sequences of primers used for real-time qRT-PCR assays.

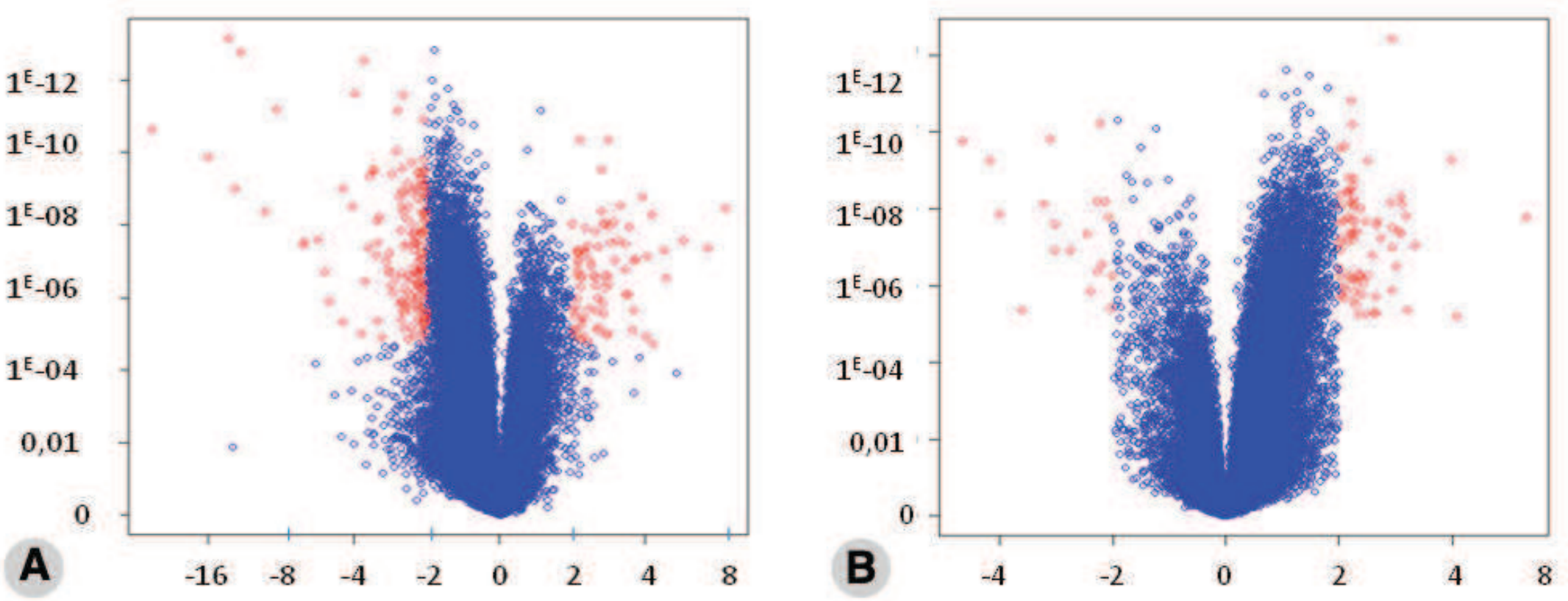


Figure 1

Figure 1

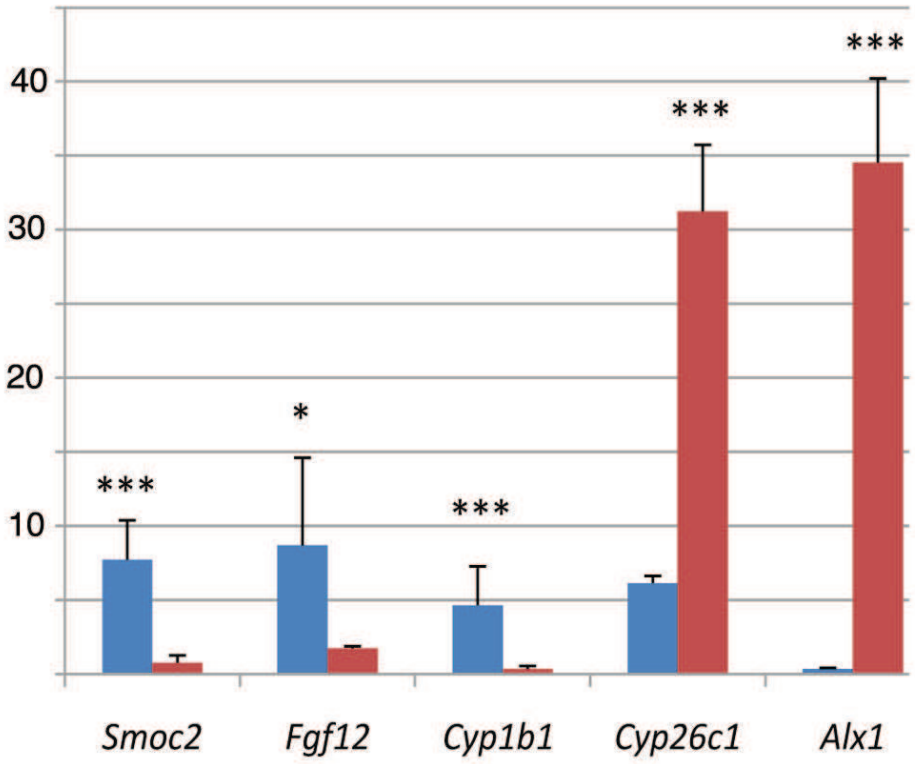


Figure 2

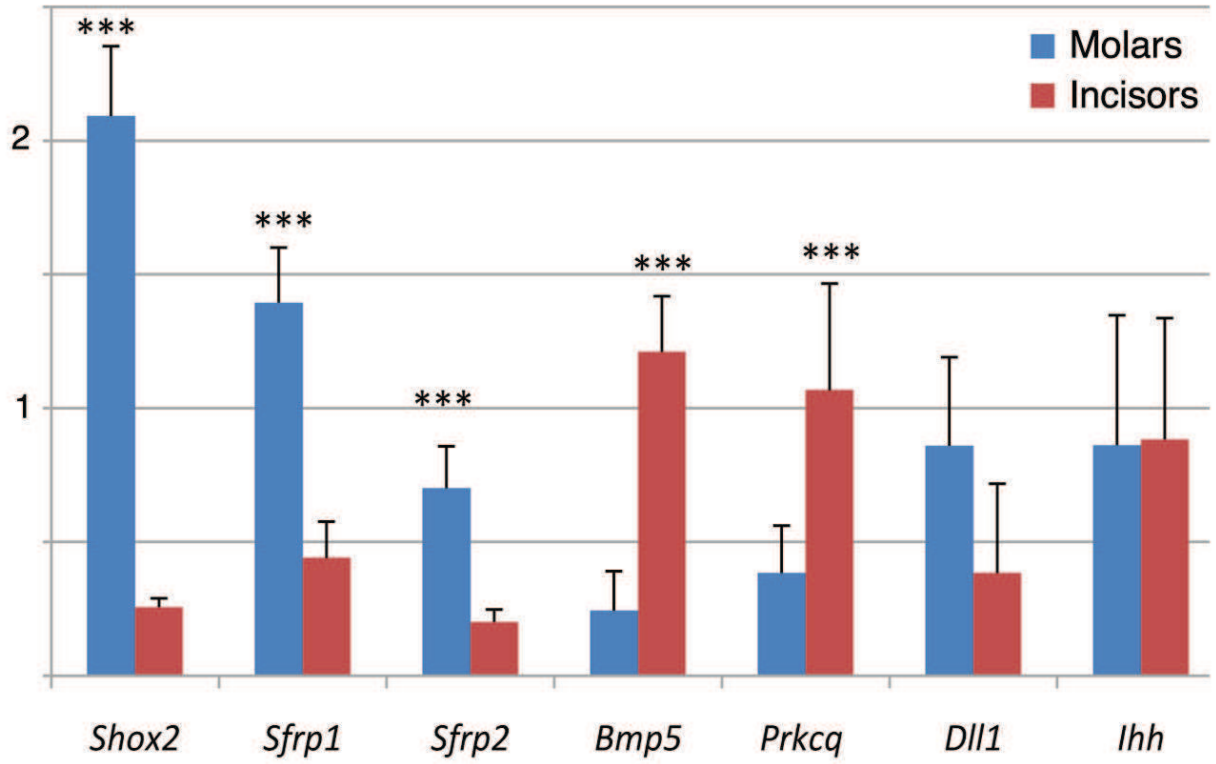


Figure 2

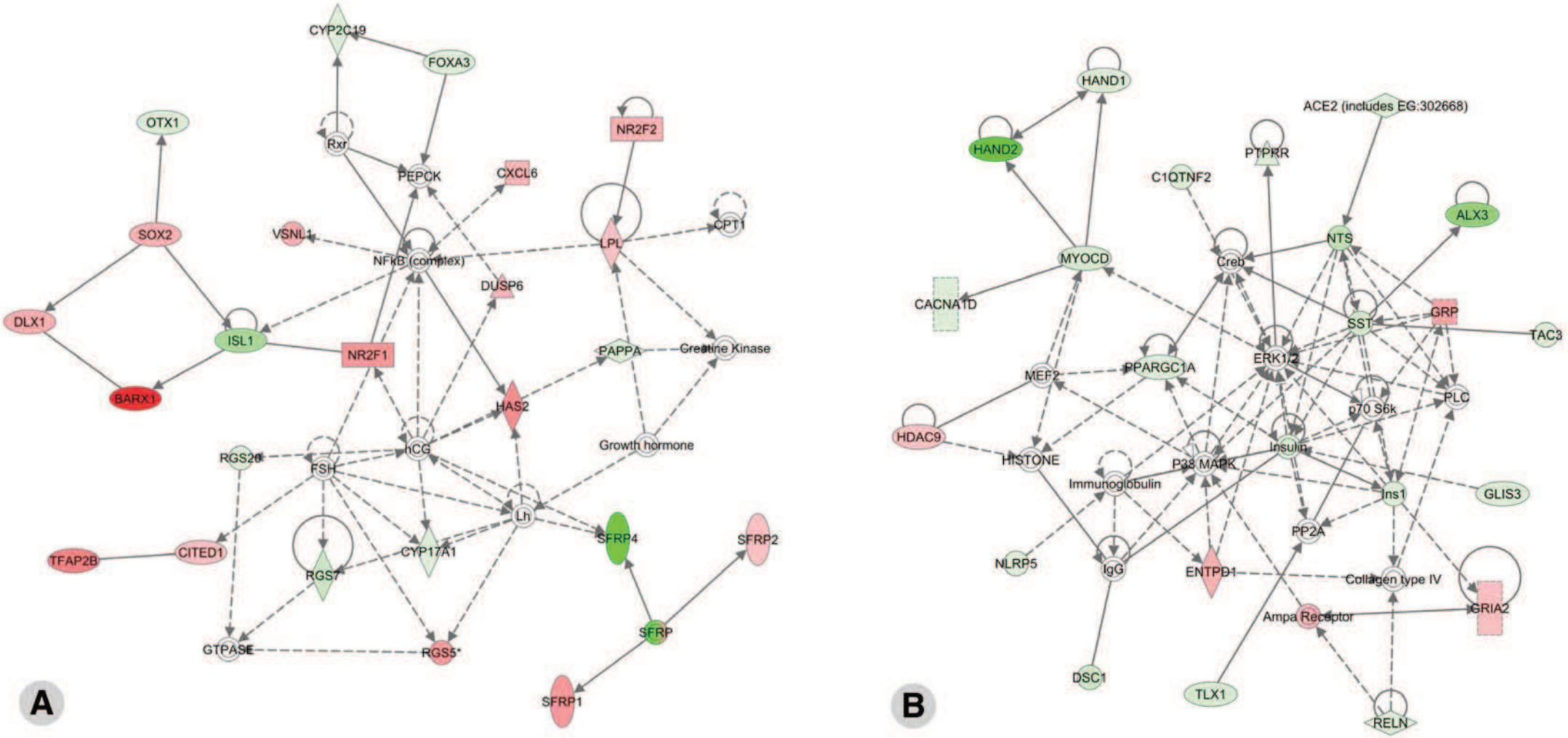


Figure 3

Figure 3

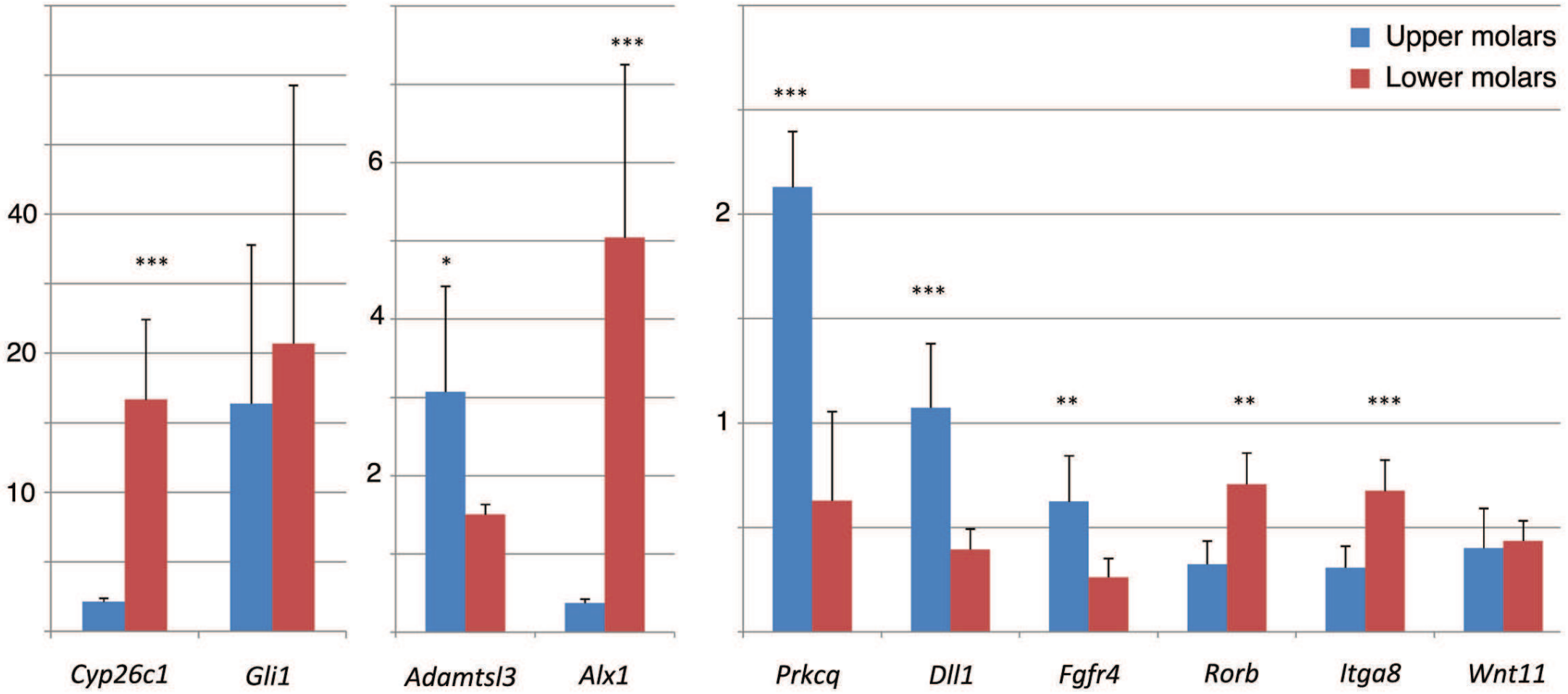


Figure 4

Figure 4

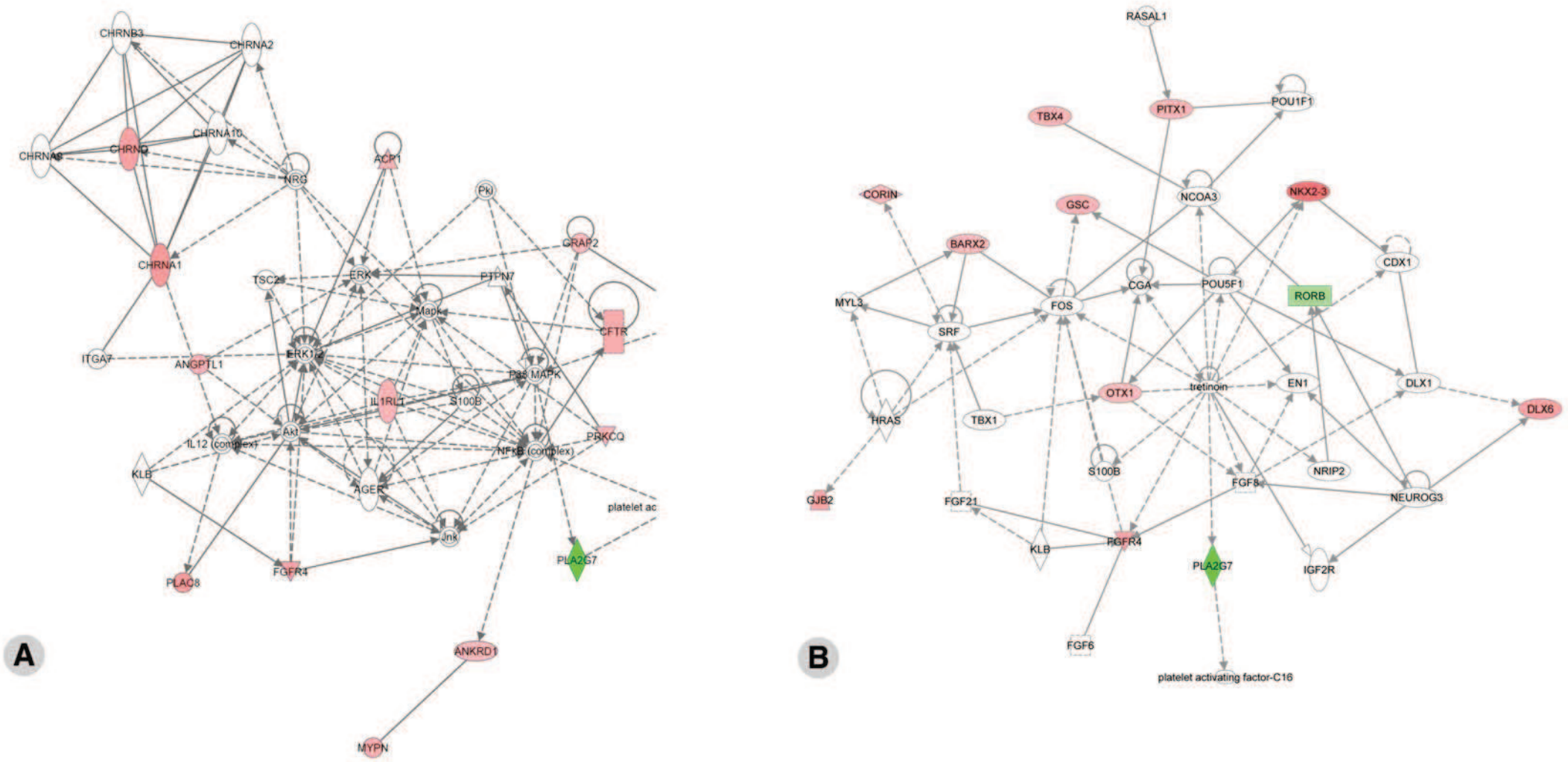
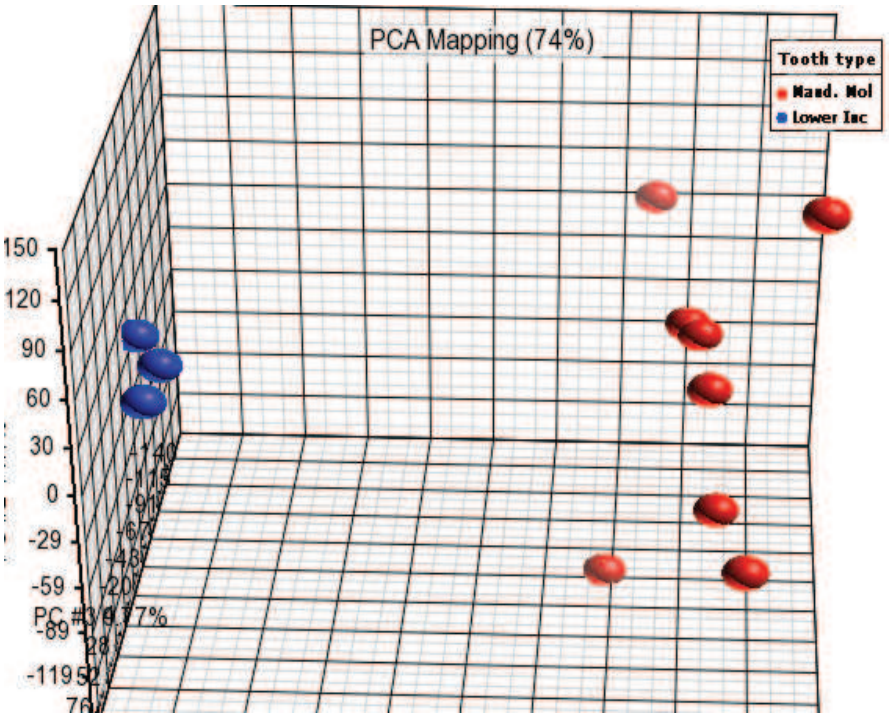


Figure 5

Figure 5

Additional File 1

A



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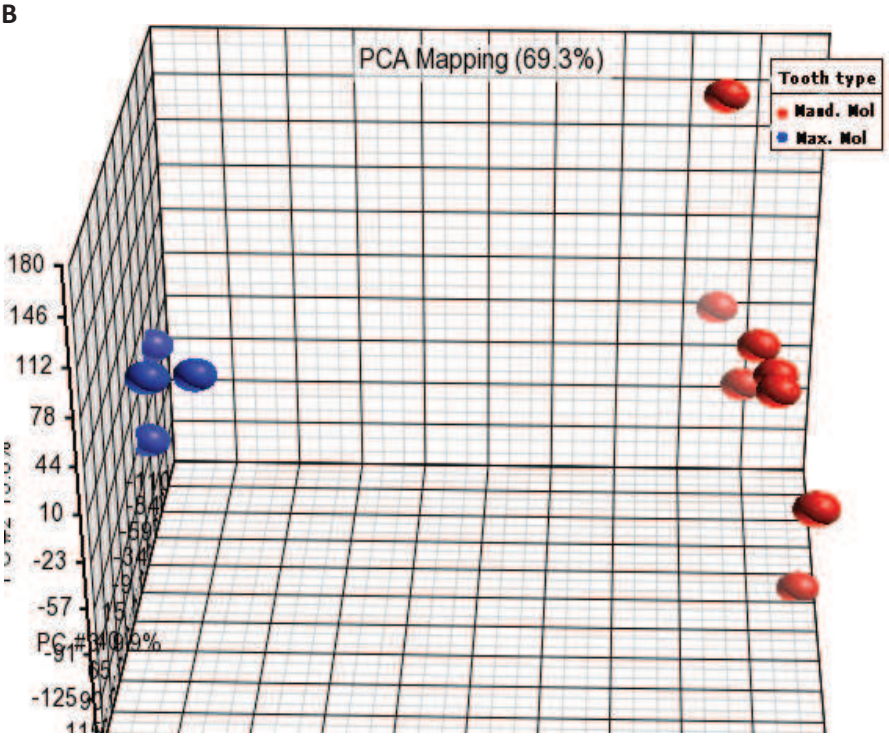

Additional File 2

Gene Symbol	Gene Description	GO Cellular Component Term	GO Molecular Function Term	p-value	Fold change
Barx1	BarH-like homeobox 1	nucleus	transcription factor activity	3,47E-09	8,60
C1qtnf3	C1q and tumor necrosis factor related protein 3	extracellular region	not determined	4,37E-08	7,30
Adcy8	adenylate cyclase 8	membrane fraction	adenylate cyclase activity	2,76E-08	5,75
Cntn6	contactin 6	plasma membrane	protein binding	2,94E-07	4,91
Six2	sine oculis-related homeobox 2 homolog (Drosophila)	nucleus	transcription factor activity	4,84E-08	4,82
Tcfap2b	transcription factor AP-2 beta	nucleus	transcription factor activity	1,88E-05	4,31
Odz1	odd Oz/ten-m homolog 1 (Drosophila)	integral to plasma membrane	---	5,02E-09	4,24
Vstm2a	V-set and transmembrane domain containing 2A	not determined	not determined	7,28E-08	4,09
Nptx1	neuronal pentraxin 1	cytoplasmic vesicle	metal ion binding	1,21E-05	4,06
Has2	hyaluronan synthase 2	membrane	transferase activity, transferring glycosyl groups	1,66E-09	3,94
Lhx6	LIM homeobox protein 6	nucleus	DNA binding	8,95E-08	3,69
Cyp1b1	cytochrome P450, family 1, subfamily b, polypeptide 1	endoplasmic reticulum	iron ion binding	9,56E-08	3,60
Nr2f1	nuclear receptor subfamily 2, group F, member 1	nucleus	transcription factor activity	2,24E-06	3,59
Sfrp1	secreted frizzled-related protein 1	extracellular region	protein binding	7,94E-06	3,56
Rgs5	regulator of G-protein signaling 5	---	signal transducer activity	1,15E-08	3,45

Itgbl1	integrin, beta-like 1	not determined	receptor activity //	8,44E-07	3,42
Slc25a21	solute carrier family 25 (mitochondrial oxodicarboxylate carrier), member 21	mitochondrion	binding	8,03E-07	3,32
Ncam2	neural cell adhesion molecule 2	plasma membrane	protein binding	1,72E-07	3,31
Smoc2	SPARC related modular calcium binding 2	extracellular region	glycosaminoglycan binding	3,28E-08	3,29
Nxph1	neurexophilin 1	extracellular region	receptor binding	2,78E-09	3,18
Grp	gastrin releasing peptide	extracellular region	neuropeptide hormone activity	2,71E-08	3,11
Gria1	glutamate receptor, ionotropic, AMPA1 (alpha 1)	membrane fraction	ionotropic glutamate receptor activity	9,28E-08	3,01
Ppfia2	protein tyrosine phosphatase, receptor type, f polypeptide (PTPRF), interacting protein (liprin), alpha 2	cytoplasm	protein binding	8,06E-09	2,96
Tmem30b	transmembrane protein 30B	not determined	not determined	3,07E-08	2,90
Shox2	short stature homeobox 2	nucleus	transcription factor activity	1,08E-08	2,85
Dgkb	diacylglycerol kinase, beta	membrane fraction	diacylglycerol kinase activity	3,70E-07	2,82
Dlx1	distal-less homeobox 1	nucleus	chromatin binding	4,49E-11	2,81
Slc5a7	solute carrier family 5 (choline transporter), member 7	plasma membrane	choline:sodium symporter activity	2,51E-07	2,81
Kera	keratocan	extracellular region	protein binding	9,92E-06	2,80
Vsnl1	visinin-like 1	---	calcium ion binding	1,02E-05	2,76
Gabra1	gamma-aminobutyric acid (GABA) A receptor, subunit alpha 1	plasma membrane	GABA-A receptor activity	8,46E-09	2,75
2900055J20Rik	RIKEN cDNA 2900055J20 gene	not determined	not determined	5,19E-07	2,74

Gabrb2	gamma-aminobutyric acid (GABA) A receptor, subunit beta 2	membrane fraction	ion channel activity	2,37E-07	2,72
Sox2	SRY-box containing gene 2	nucleus	DNA binding	2,80E-06	2,69
Nr2f2	nuclear receptor subfamily 2, group F, member 2	nucleus	transcription factor activity	9,19E-09	2,69
Entpd1	ectonucleoside triphosphate diphosphohydrolase 1	basal lamina	protein binding	1,47E-06	2,66
Nup62cl	nucleoporin 62 C-terminal like	not determined	not determined	4,14E-09	2,64
Clstn2	calsyntenin 2	endoplasmic reticulum	calcium ion binding // protein binding	2,95E-10	2,64
Galnt13	UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 13	Golgi apparatus	sugar binding	6,92E-06	2,57
Cysltr1	cysteinyl leukotriene receptor 1	plasma membrane	receptor activity	4,02E-08	2,56
Bmper	BMP-binding endothelial regulator	extracellular region	protein binding	1,06E-06	2,56
Dusp6	dual specificity phosphatase 6	cytoplasm	protein tyrosine phosphatase activity	7,62E-06	2,52
Cxcl5	chemokine (C-X-C motif) ligand 5	extracellular region	chemokine activity	4,59E-07	2,51
Nell1	NEL-like 1 (chicken)	extracellular region	structural molecule activity	2,27E-06	2,51
AI427809	expressed sequence AI427809	not determined	not determined	1,44E-08	2,50
Pax9	paired box gene 9	nucleus	transcription factor activity	2,66E-07	2,49
Gabra2	gamma-aminobutyric acid (GABA) A receptor, subunit alpha 2	plasma membrane	GABA-A receptor activity	2,41E-07	2,46
Vps33b	vacuolar protein sorting 33B (yeast)	not determined	syntaxin-2 binding	1,32E-07	2,41
Leprel1	leprecan-like 1	endoplasmic reticulum	oxidoreductase activity	9,24E-08	2,38

Calml4	calmodulin-like 4	---	calcium ion binding	1,94E-06	2,35
Msr1	macrophage scavenger receptor 1	cytosol	scavenger receptor activity	3,83E-08	2,33
Cdh12	cadherin 12	not determined	calcium ion binding	1,69E-05	2,32
Ptchd1	patched domain containing 1	not determined	hedgehog receptor activity	1,22E-08	2,27
Fgf12	fibroblast growth factor 12	nucleus	protein binding	6,11E-08	2,24
Slitrk5	SLIT and NTRK-like family, member 5	membrane	protein binding	4,65E-08	2,22
Rgs5	regulator of G-protein signaling 5	---	GTPase activator activity	3,32E-06	2,22
Cldn10a	claudin 10A	plasma membrane	structural molecule activity	1,11E-05	2,21
Ucma	upper zone of growth plate and cartilage matrix associated	extracellular region	---	4,13E-06	2,20
Dach1	dachshund 1 (Drosophila)	nucleus	DNA binding	2,48E-07	2,20
Gria2	glutamate receptor, ionotropic, AMPA2 (alpha 2)	membrane fraction	ionotropic glutamate receptor activity	1,46E-05	2,18
Urb2	URB2 ribosome biogenesis 2 homolog (S. cerevisiae)	not determined	not determined	5,89E-06	2,18
Pid1	phosphotyrosine interaction domain containing 1	not determined	not determined	5,00E-07	2,16
Sfrp2	secreted frizzled-related protein 2	extracellular region	Wnt-protein binding	4,40E-11	2,16
Cited1	Cbp/p300-interacting transactivator with Glu/Asp-rich carboxy-terminal domain 1	nucleus	protein binding	2,69E-07	2,16
Ccdc53	coiled-coil domain containing 53	not determined	not determined	5,38E-08	2,15
Lpl	lipoprotein lipase	extracellular space	lipoprotein lipase activity	8,09E-08	2,13

Nlgn1	neuroligin 1	plasma membrane	protein binding	1,04E-07	2,11
Scd3	stearoyl-coenzyme A desaturase 3	integral to membrane	oxidoreductase activity	5,58E-08	2,11
Myo9a	myosin IXa	intracellular	motor activity	1,64E-07	2,11
Nmbr	neuromedin B receptor	plasma membrane	receptor activity	2,01E-06	2,09
Prr15	proline rich 15	not determined	not determined	2,22E-07	2,09
Cdc40	cell division cycle 40 homolog (yeast)	not determined	not determined	5,22E-08	2,09
Lrrtm1	leucine rich repeat transmembrane neuronal 1	endoplasmic reticulum	protein binding	1,12E-06	2,09
Bard1	BRCA1 associated RING domain 1	intracellular	protein binding	1,13E-05	2,07
Hdac9	histone deacetylase 9	histone deacetylase complex	histone deacetylase activity	7,24E-06	2,05
Man1a	mannosidase 1, alpha	Golgi membrane	calcium ion binding	3,15E-06	2,03
Npas3	neuronal PAS domain protein 3	nucleus	signal transducer activity	8,60E-06	2,02
Ror1	receptor tyrosine kinase-like orphan receptor 1	integral to plasma membrane	protein kinase activity	9,37E-09	2,01
Mgat4a	mannoside acetylglucosaminyltransferase 4, isoenzyme A	extracellular region	transferase activity	2,01E-06	2,01
Sdcbp2	syndecan binding protein (syntenin) 2	not determined	protein binding	5,30E-09	-2,00
Fam69c	family with sequence similarity 69, member C	not determined	not determined	3,03E-06	-2,00
Hmcn1	hemicentin 1	extracellular region	not determined	2,59E-06	-2,00
Mrgprd	MAS-related GPR, member D	plasma membrane	receptor activity	1,36E-09	-2,00

Foxa3	forkhead box A3	nucleus	DNA binding	3,73E-09	-2,00
Mir200c	microRNA 200c	---	---	4,70E-06	-2,00
Gm16432	predicted gene 16432	not determined	not determined	4,61E-08	-2,00
Gm16498	predicted gene 16498	not determined	not determined	4,12E-06	-2,01
Cma2	chymase 2, mast cell	not determined	peptidase activity	7,24E-10	-2,01
A430089I19Rik	RIKEN cDNA A430089I19 gene	not determined	not determined	2,25E-08	-2,02
A430089I19Rik	RIKEN cDNA A430089I19 gene	not determined	not determined	2,25E-08	-2,02
Epyc	epiphycan	extracellular region	protein binding	3,88E-07	-2,03
Ccdc81	coiled-coil domain containing 81	not determined	not determined	7,88E-09	-2,03
Rgs20	regulator of G-protein signaling 20	nucleus	GTPase activator activity	3,41E-10	-2,04
4921508D12Rik	RIKEN cDNA 4921508D12 gene	not determined	not determined	2,81E-10	-2,04
Fam163a	family with sequence similarity 163, member A	not determined	not determined	6,72E-07	-2,04
Gm10385	predicted gene 10385	not determined	not determined	7,22E-09	-2,04
Gm10210	predicted gene 10210	---	---	8,92E-06	-2,04
Cyp17a1	cytochrome P450, family 17, subfamily a, polypeptide 1	mitochondrion	steroid 17-alpha-monooxygenase activity	8,36E-10	-2,05
Gm10304	predicted gene 10304	not determined	not determined	1,72E-06	-2,05
Olfr46	olfactory receptor 46	integral to membrane	receptor activity	7,09E-09	-2,05

Gm9276	eukaryotic translation elongation factor 1 gamma pseudogene	---	---	7,67E-07	-2,05
Tac2	tachykinin 2	extracellular region	---	1,02E-06	-2,05
Fam155a	family with sequence similarity 155, member A	not determined	not determined	1,59E-10	-2,06
Vmn2r51	vomeronasal 2, receptor 51	not determined	not determined	5,04E-06	-2,06
Il16	interleukin 16	extracellular region	protein binding	3,84E-06	-2,06
Olfr810	olfactory receptor 810	integral to membrane	receptor activity	4,38E-09	-2,06
Gm7849	predicted gene 7849	not determined	not determined	2,55E-06	-2,06
5930412G12Rik	RIKEN cDNA 5930412G12 gene	not determined	not determined	2,18E-09	-2,06
D830046C22Rik	RIKEN cDNA D830046C22 gene	not determined	not determined	1,36E-09	-2,07
Cma1	chymase 1, mast cell	extracellular region	serine-type endopeptidase activity	1,56E-07	-2,07
Olfr698	olfactory receptor 698	integral to membrane	olfactory receptor activity	1,24E-11	-2,07
Smok2b	sperm motility kinase 2B	not determined	not determined	1,41E-08	-2,07
Wscd2	WSC domain containing 2	not determined	not determined	4,62E-10	-2,07
4930467E23Rik	RIKEN cDNA 4930467E23 gene	not determined	not determined	8,03E-06	-2,07
Gm2736	predicted gene 2736	---	---	8,56E-08	-2,08
F830212C03Rik	RIKEN cDNA F830212C03 gene	not determined	not determined	5,08E-07	-2,08
A430089I19Rik	RIKEN cDNA A430089I19 gene	not determined	not determined	7,87E-10	-2,08

A430089I19Rik	RIKEN cDNA A430089I19 gene	not determined	not determined	7,87E-10	-2,08
Reln	reelin	extracellular region	protein binding	5,84E-06	-2,08
Pou2f3	POU domain, class 2, transcription factor 3	nucleus	transcription regulator activity	1,45E-05	-2,09
Wdr64	WD repeat domain 64	not determined	not determined	1,51E-08	-2,10
A430089I19Rik	RIKEN cDNA A430089I19 gene	not determined	not determined	4,57E-09	-2,10
1700026D08Rik	RIKEN cDNA 1700026D08 gene	not determined	not determined	1,60E-08	-2,11
Gm7735	predicted gene 7735	not determined	not determined	1,45E-08	-2,11
4930467E23Rik	RIKEN cDNA 4930467E23 gene	not determined	not determined	3,28E-07	-2,11
Lrrn3	leucine rich repeat protein 3, neuronal	membrane	protein binding	7,55E-08	-2,12
C1qtnf2	C1q and tumor necrosis factor related protein 2	extracellular region	receptor binding	5,88E-08	-2,12
Rbpjl	recombination signal binding protein for immunoglobulin kappa J region-like	nucleus	transcription factor activity	1,37E-07	-2,12
A430089I19Rik	RIKEN cDNA A430089I19 gene	not determined	not determined	5,43E-10	-2,13
Clec2h	C-type lectin domain family 2, member h	plasma membrane	transmembrane receptor activity	1,89E-07	-2,13
Gm7174	predicted gene 7174	not determined	not determined	4,87E-07	-2,14
Epgn	epithelial mitogen	membrane	epidermal growth factor receptor binding	3,30E-08	-2,15
4930467E23Rik	RIKEN cDNA 4930467E23 gene	not determined	not determined	1,75E-06	-2,15
Ins1	insulin I	extracellular region	insulin receptor binding	3,70E-10	-2,15

5330417C22Rik	RIKEN cDNA 5330417C22 gene	not determined	protein binding	2,07E-06	-2,16
Olfr1200	olfactory receptor 1200	integral to membrane	receptor activity	2,29E-07	-2,16
Aqp1	aquaporin 1	integral to membrane of membrane fraction	water transmembrane transporter activity	9,51E-08	-2,16
E030019B06Rik	RIKEN cDNA E030019B06 gene	not determined	not determined	3,49E-10	-2,16
A430089I19Rik	RIKEN cDNA A430089I19 gene	not determined	not determined	1,84E-09	-2,17
A430089I19Rik	RIKEN cDNA A430089I19 gene	not determined	not determined	1,84E-09	-2,17
A430089I19Rik	RIKEN cDNA A430089I19 gene	not determined	not determined	1,84E-09	-2,17
Olfr878	olfactory receptor 878	integral to membrane	receptor activity	9,41E-10	-2,17
Ly6g6c	lymphocyte antigen 6 complex, locus G6C	not determined	not determined	3,95E-06	-2,18
Gzme	granzyme E	---	serine-type endopeptidase activity	4,39E-07	-2,19
Masp1	mannan-binding lectin serine peptidase 1	extracellular region	serine-type endopeptidase activity	3,74E-07	-2,19
4930467E23Rik	RIKEN cDNA 4930467E23 gene	not determined	not determined	1,45E-06	-2,19
Dnahc3	dynein, axonemal, heavy chain 3	not determined	not determined	1,95E-07	-2,19
Tgs1	trimethylguanosine synthase homolog (S. cerevisiae)	nucleus	methyltransferase activity	1,03E-06	-2,19
Scara5	scavenger receptor class A, member 5 (putative)	plasma membrane	scavenger receptor activity	3,96E-07	-2,21
1700029M20Rik	RIKEN cDNA 1700029M20 gene	not determined	not determined	2,04E-07	-2,22
Kcnh7	potassium voltage-gated channel, subfamily H (eag-related), member 7	not determined	inward rectifier potassium channel activity	3,28E-08	-2,23

Dio2	deiodinase, iodothyronine, type II	membrane	thyroxine 5'-deiodinase activity	2,90E-08	-2,23
Kcna1	potassium voltage-gated channel, shaker-related subfamily, member 1	voltage-gated potassium channel complex	voltage-gated ion channel activity	6,84E-07	-2,24
Nrn1	neuritin 1	plasma membrane	---	1,04E-05	-2,25
9630013D21Rik	RIKEN cDNA 9630013D21 gene	not determined	not determined	9,60E-10	-2,26
Cyp2c54	cytochrome P450, family 2, subfamily c, polypeptide 54	endoplasmic reticulum	iron ion binding	4,98E-08	-2,26
Serpinb9e	serine (or cysteine) peptidase inhibitor, clade B, member 9e	not determined	not determined	1,87E-08	-2,27
Amtn	amelotin	extracellular region	protein binding	1,65E-08	-2,28
6330403A02Rik	RIKEN cDNA 6330403A02 gene	not determined	not determined	9,87E-09	-2,28
Bpil2	bactericidal/permeability-increasing protein-like 2	not determined	lipid binding	1,48E-07	-2,29
Mug1	murinoglobulin 1	extracellular region	serine-type endopeptidase inhibitor activity	1,91E-10	-2,29
Prtg	protogenin homolog (Gallus gallus)	membrane	---	9,93E-07	-2,30
Lman1l	lectin, mannose-binding 1 like	not determined	sugar binding	1,23E-09	-2,32
Gm10664	predicted gene 10664	not determined	not determined	6,70E-06	-2,33
Lrrc4	leucine rich repeat containing 4	plasma membrane	protein binding	2,16E-06	-2,34
2810055G20Rik	RIKEN cDNA 2810055G20 gene	not determined	not determined	3,08E-09	-2,36
Pappa	pregnancy-associated plasma protein A	extracellular region	peptidase activity	3,32E-07	-2,37
Slitrk6	SLIT and NTRK-like family, member 6	membrane	protein binding	2,60E-07	-2,37

Qrfpr	pyroglutamylated RFamide peptide receptor	plasma membrane	receptor activity	1,38E-06	-2,37
Wnt5a	wingless-related MMTV integration site 5A	extracellular region	receptor binding	1,38E-05	-2,38
Tceal6	transcription elongation factor A (SII)-like 6	not determined	translation elongation factor activity	3,51E-08	-2,40
Glis3	GLIS family zinc finger 3	intracellular	DNA binding	7,34E-07	-2,40
Nlrp5	NLR family, pyrin domain containing 5	nucleus	protein binding	6,94E-10	-2,40
4930467E23Rik	RIKEN cDNA 4930467E23 gene	not determined	not determined	6,58E-09	-2,44
Glis1	GLIS family zinc finger 1	intracellular	DNA binding	6,78E-06	-2,47
Cacna1d	calcium channel, voltage-dependent, L type, alpha 1D subunit	plasma membrane	voltage-gated ion channel activity	1,25E-09	-2,47
Ppargc1a	peroxisome proliferative activated receptor, gamma, coactivator 1 alpha	nucleus	nucleic acid binding	2,22E-06	-2,48
Ptprr	protein tyrosine phosphatase, receptor type, R	cytoplasm	protein tyrosine phosphatase activity	1,45E-06	-2,48
Ctnna2	catenin (cadherin associated protein), alpha 2	cytoplasm	protein binding	3,76E-08	-2,48
Otx1	orthodenticle homolog 1 (Drosophila)	nucleus	transcription factor activity	5,56E-07	-2,48
Olfr1126	olfactory receptor 1126	integral to membrane	receptor activity	2,46E-12	-2,49
AI593442	expressed sequence AI593442	not determined	not determined	2,49E-06	-2,50
Klhl14	kelch-like 14 (Drosophila)	not determined	not determined	4,34E-09	-2,51
Ace2	angiotensin I converting enzyme (peptidyl-dipeptidase A) 2	extracellular region	carboxypeptidase activity	3,02E-09	-2,52
Ramp3	receptor (calcitonin) activity modifying protein 3	membrane	protein transporter activity	1,28E-06	-2,53

Pla2g2f	phospholipase A2, group IIF	extracellular region	calcium ion binding	2,78E-10	-2,54
Hormad1	HORMA domain containing 1	not determined	not determined	1,15E-09	-2,54
Adamts16	a disintegrin-like and metallopeptidase (reprolysin type) with thrombospondin type 1 motif, 16	not determined	metalloendopeptidase activity	2,12E-07	-2,54
Hand1	heart and neural crest derivatives expressed transcript 1	nucleus	transcription factor activity	4,01E-06	-2,55
Olfr166	olfactory receptor 166	integral to membrane	receptor activity	1,42E-08	-2,57
Cyp4f39	cytochrome P450, family 4, subfamily f, polypeptide 39	not determined	monooxygenase activity	1,40E-06	-2,58
Sprr1b	small proline-rich protein 1B	cornified envelope	structural constituent of cytoskeleton	1,69E-09	-2,60
Olfr1043	olfactory receptor 1043	integral to membrane	receptor activity	7,07E-12	-2,64
Syt17	synaptotagmin XVII	trans-Golgi network	transporter activity	9,08E-11	-2,67
Unc5c	unc-5 homolog C (C. elegans)	plasma membrane	netrin receptor activity	2,98E-07	-2,68
Prkcq	protein kinase C, theta	immunological synapse	protein kinase activity	7,17E-07	-2,75
Stfa3	stefin A3	intracellular	cysteine-type endopeptidase inhibitor activity	3,87E-10	-2,79
Gm10396	predicted gene 10396	not determined	not determined	4,71E-07	-2,80
Myocd	myocardin	nucleus	transcription coactivator activity	2,06E-07	-2,83
Tlx1	T-cell leukemia, homeobox 1	nucleus	transcription factor activity	6,05E-08	-2,84
Sel1l3	sel-1 suppressor of lin-12-like 3 (C. elegans)	not determined	not determined // binding	1,57E-07	-2,87
Krt6b	keratin 6B	intermediate filament	structural molecule activity	9,61E-08	-2,94

Krt10	keratin 10	intermediate filament	protein binding	1,31E-05	-3,07
Sst	somatostatin	extracellular region	hormone activity	6,01E-09	-3,13
Cnnm1	cyclin M1	plasma membrane	not determined	4,40E-06	-3,19
Serpinb12	serine (or cysteine) peptidase inhibitor, clade B (ovalbumin), member 12	not determined	peptidase inhibitor activity	7,46E-09	-3,20
Bmp5	bone morphogenetic protein 5	extracellular region	protein binding	3,00E-08	-3,20
Mcpt4	mast cell protease 4	intracellular	serine-type endopeptidase activity	6,99E-08	-3,24
Rxfp1	relaxin/insulin-like family peptide receptor 1	plasma membrane	receptor activity	2,96E-10	-3,29
Dsc1	desmocollin 1	plasma membrane	protein binding	3,23E-10	-3,31
Gm10001	predicted gene 10001	not determined	not determined	4,35E-10	-3,46
Prg4	proteoglycan 4 (megakaryocyte stimulating factor, articular superficial zone protein)	extracellular region	polysaccharide binding	4,20E-08	-3,50
Serpinb3a	serine (or cysteine) peptidase inhibitor, clade B (ovalbumin), member 3A	not determined	not determined	3,49E-07	-3,62
Rgs7	regulator of G protein signaling 7	nucleus	GTPase activator activity	2,90E-13	-3,64
Dpep1	dipeptidase 1 (renal)	plasma membrane	metalloexopeptidase activity	9,65E-06	-3,73
Fcrl6	Fc receptor-like 6	membrane	---	2,38E-12	-3,96
Cyp26c1	cytochrome P450, family 26, subfamily c, polypeptide 1	---	oxidoreductase activity	3,10E-09	-4,05
Nts	neurotensin	extracellular region	neuropeptide hormone activity	9,56E-10	-4,43
Sprr3	small proline-rich protein 3	cytoplasm	protein binding	4,79E-06	-4,44

4930578G10Rik	RIKEN cDNA 4930578G10 gene	not determined	not determined	1,26E-06	-5,05
Irx4	Iroquois related homeobox 4 (Drosophila)	nucleus	transcription factor activity	1,92E-07	-5,31
Cacna2d3	calcium channel, voltage-dependent, alpha2/delta subunit 3	membrane	voltage-gated ion channel activity	2,55E-08	-5,66
Mcpt2	mast cell protease 2	intracellular	serine-type endopeptidase activity	2,90E-08	-6,39
Isl1	ISL1 transcription factor, LIM/homeodomain	intracellular	chromatin binding	3,53E-08	-6,44
Alx3	aristaless-like homeobox 3	nucleus	transcription factor activity	6,59E-12	-8,36
C130021O09Rik	RIKEN cDNA C130021O09 gene	not determined	not determined	4,27E-09	-9,31
Pax3	paired box gene 3	nucleus	chromatin binding	1,68E-13	-11,72
Sfrp4	secreted frizzled-related protein 4	extracellular region	Wnt-protein binding	9,88E-10	-12,38
Hand2	heart and neural crest derivatives expressed transcript 2	nucleus	transcription factor activity	6,89E-14	-13,20
Alx1	ALX homeobox 1	nucleus	transcription factor activity	1,35E-10	-15,95
Hpse2	heparanase 2	not determined	not determined	2,23E-11	-27,41

Additional File 3

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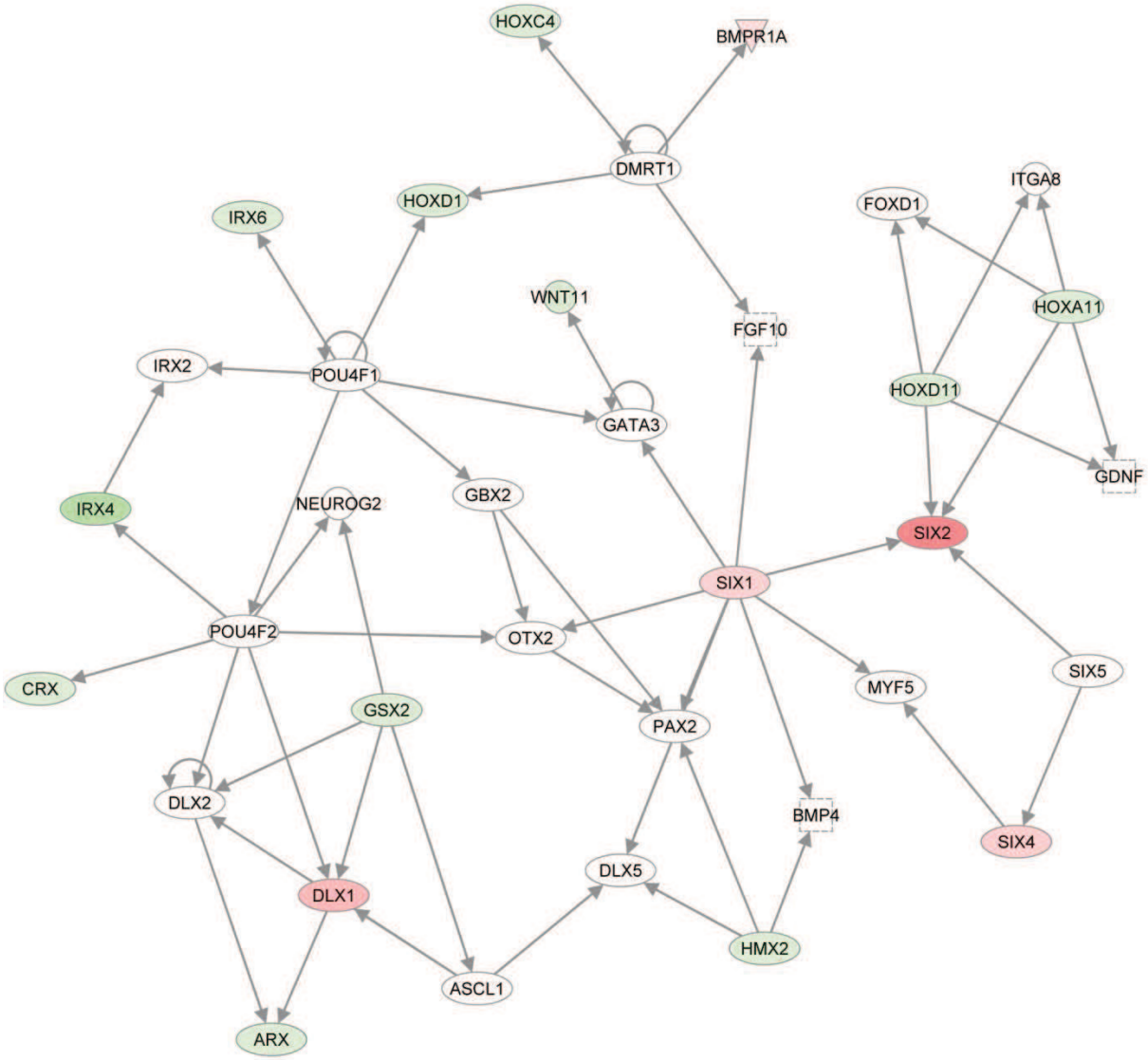

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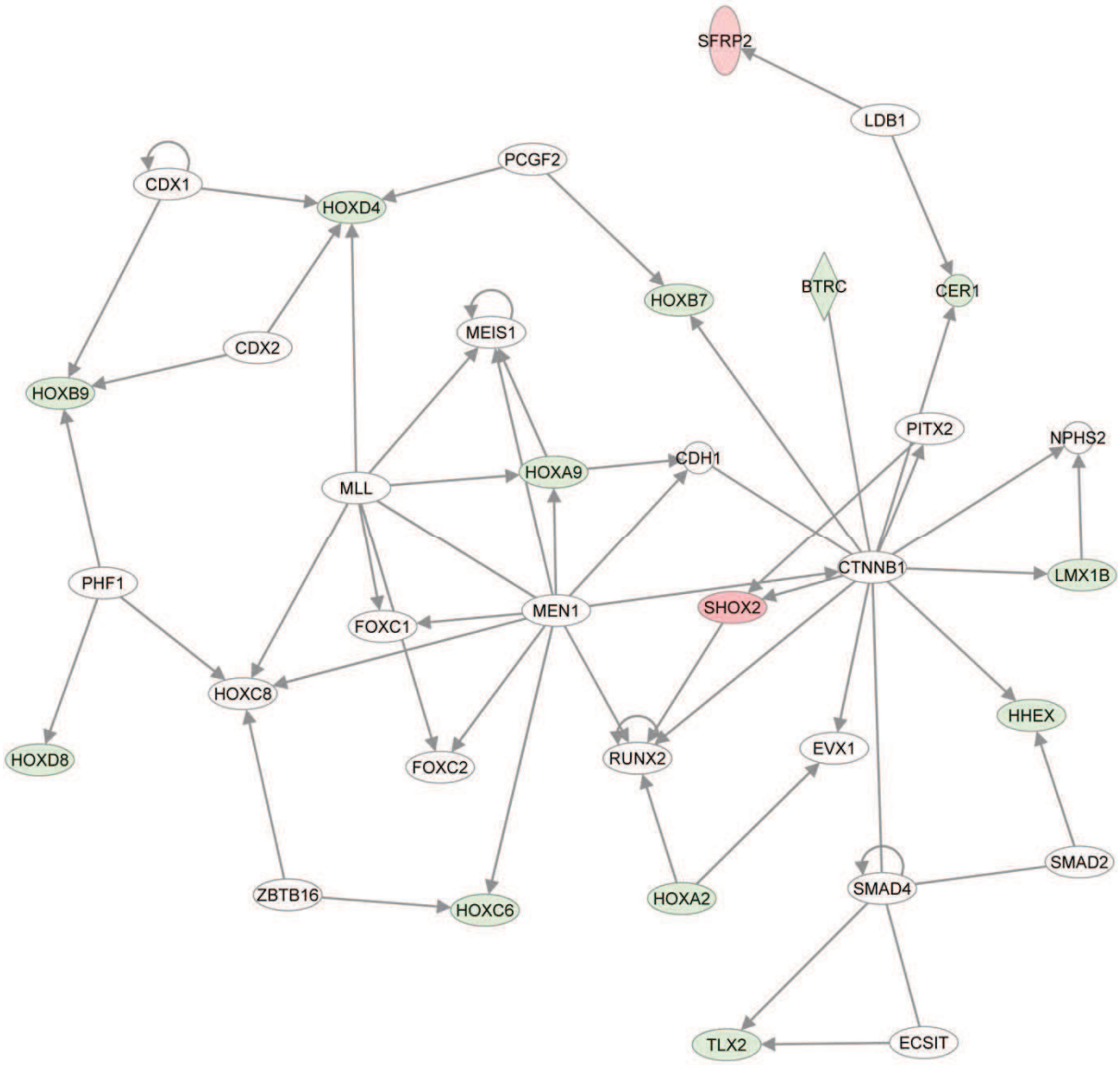
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BTRC
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MEIS1
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NPHS2
CDH1
MLL
HOXA9
CTNNB1
LMX1B
PHF1
MEN1
SHOX2
FOXC1
HOXC8
HHEX
HOXD8
FOXC2
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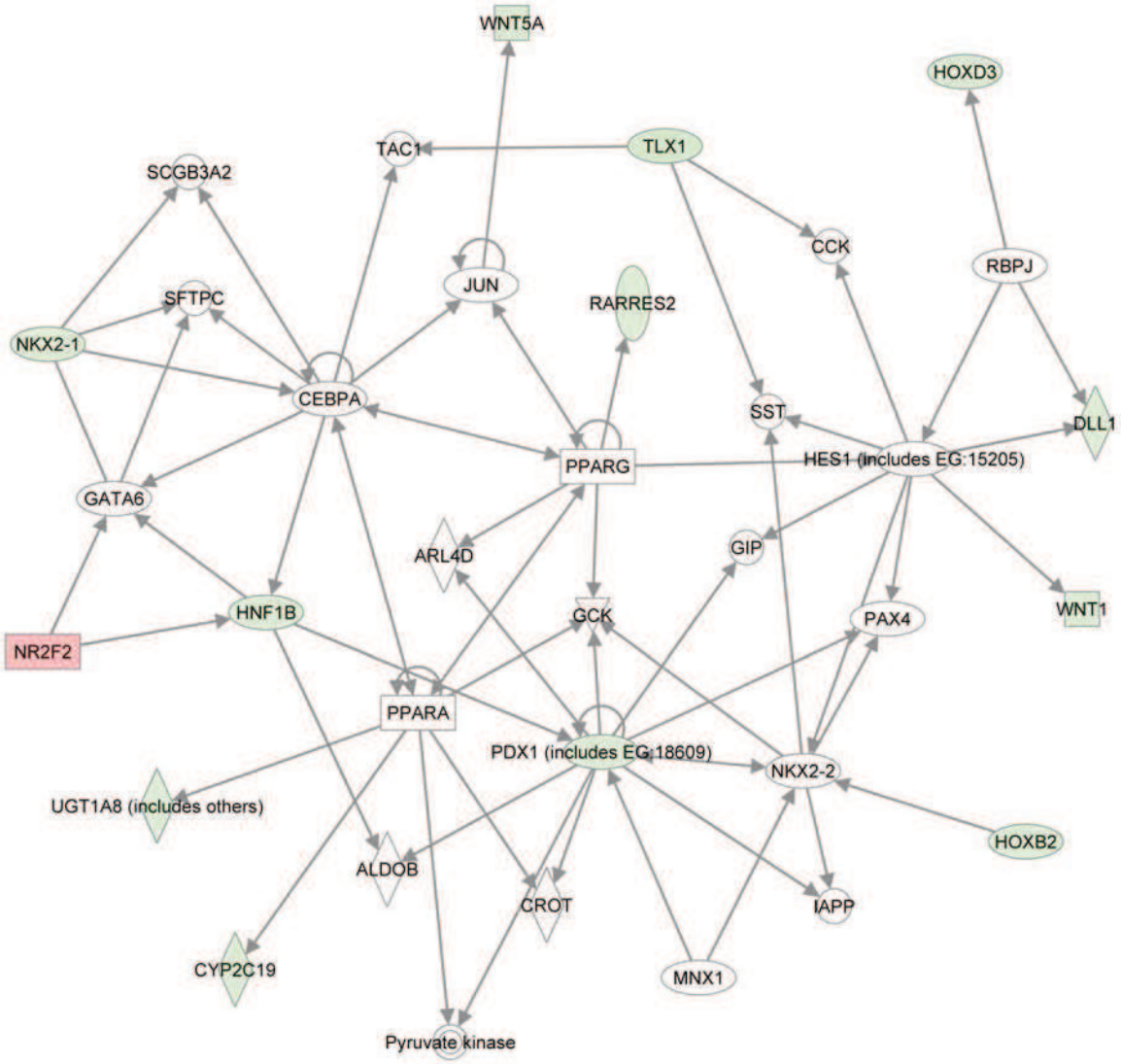
HOXA2
SMAD4
TLX2
ECSIT

Network 3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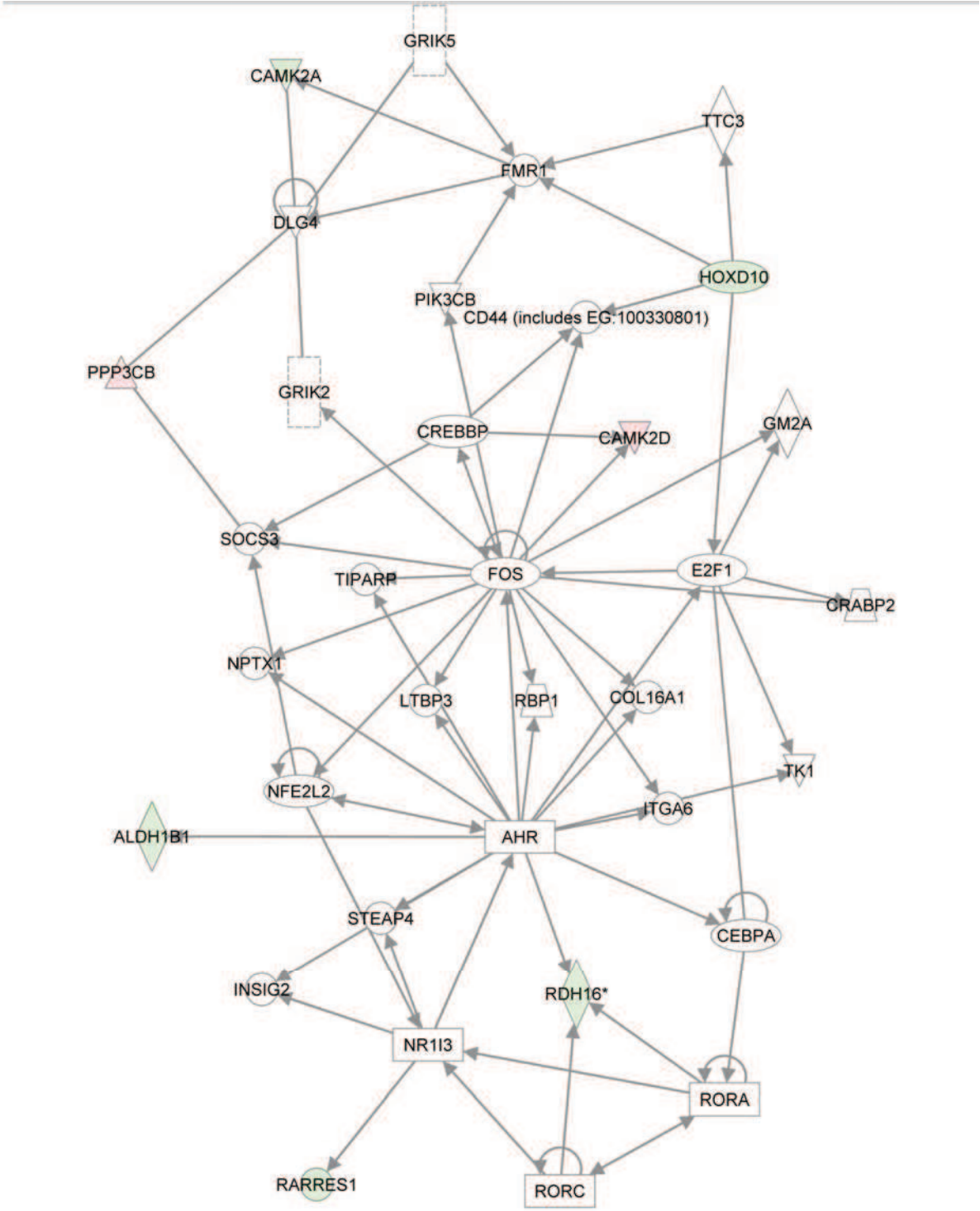
HOXD3
TAC1
TLX1
SCGB3A2
CCK
RBPJ
JUN
RARRES2
SFTPC
NKX2-1
CEBPA
SST
DLL1
PPARG
HES1 (includes EG:15205)
GATA6
GIP
ARL4D
WNT1
HNF1B
GCK
PAX4
NR2F2
PPARA
PDX1 (includes EG:18609)
NKX2-2
UGT1A8 (includes others)
HOXB2
ALDOB
CROT
IAPP
CYP2C19
MNX1
Pyruvate kinase

Network 4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TTC3
FMR1
DLG4
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PIK3CB
CD44 (includes EG:100330801)
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GRIK2
CREBBP
CAMK2D
GM2A
SOCS3
TIPARP
FOS
E2F1
CRABP2
NPTX1
LTBP3
RBP1
COL16A1
TK1
NFE2L2
ITGA6
ALDH1B1
AHR
STEAP4
CEBPA
INSIG2
RDH16*
NR1I3
RORA
RARRES1
RORC

Additional File 4

Gene Symbol	Gene Description	GO Cellular Component Term	GO Molecular Function Term	p-value	Fold change
Nefl	neurofilament, light polypeptide	intermediate filament	structural molecule activity	1,68E-08	6,28
Ostn	osteocrin	extracellular region	hormone activity	6,22E-06	4,12
Nkx2-3	NK2 transcription factor related, locus 3 (Drosophila)	Nucleus	transcription factor activity	5,32E-10	3,97
Tnnt1	troponin T1, skeletal, slow	troponin complex	protein binding	9,08E-08	3,19
Chrna1	cholinergic receptor, nicotinic, alpha polypeptide 1 (muscle)	plasma membrane	nicotinic acetylcholine-activated cation-selective channel activity	4,47E-06	3,04
Nefm	neurofilament, medium polypeptide	cytoskeleton	structural molecule activity	1,54E-08	3,01
Myf5	myogenic factor 5	Nucleus	transcription regulator activity	7,14E-09	2,94
Klhl31	kelch-like 31 (Drosophila)	not determined	protein binding	4,58E-08	2,92
Plac8	placenta-specific 8	not determined	not determined	4,68E-09	2,91
Synpo2l	synaptopodin 2-like	cellular_componen	actin binding	3,71E-08	2,87
Slc25a21	solute carrier family 25 (mitochondrial oxodicarboxylate carrier), member 21	mitochondrion	not determined	3,15E-07	2,83
Trim55	tripartite motif-containing 55		metal ion binding	1,29E-06	2,77
Chrnd	cholinergic receptor, nicotinic, delta polypeptide	plasma membrane	receptor activity	3,16E-08	2,77
2510003E04Rik	RIKEN cDNA 2510003E04 gene	not determined	not determined	3,60E-13	2,76
Dlx6	distal-less homeobox 6	Nucleus	transcription factor activity	1,01E-07	2,76

Aass	aminoadipate-semialdehyde synthase	mitochondrion	catalytic activity	6,86E-09	2,74
Fgfr4	fibroblast growth factor receptor 4	membrane	nucleotide binding	5,80E-08	2,58
Gjb2	gap junction protein, beta 2	plasma membrane	protein binding	2,08E-08	2,53
Clrn1	clarin 1	membrane		2,58E-08	2,51
Atp1b4	ATPase, (Na+)/K+ transporting, beta 4 polypeptide	chromatin	sodium:potassium-exchanging ATPase activity	5,19E-06	2,50
Cdh15	cadherin 15	plasma membrane	calcium ion binding	1,88E-06	2,49
Myom2	myomesin 2	cytoskeleton	structural constituent of cytoskeleton	4,88E-06	2,48
Cftr	cystic fibrosis transmembrane conductance regulator homolog	cytoplasm	ion channel activity	1,59E-07	2,46
Gm10000	predicted gene 10000	not determined	not determined	5,61E-10	2,39
Adamtsl3	ADAMTS-like 3	not determined	not determined	5,43E-07	2,38
Gm9558	predicted gene 9558			2,15E-08	2,35
Mypn	myopalladin	Nucleus	actin binding	5,55E-06	2,35
1700055N04Rik	RIKEN cDNA 1700055N04 gene	not determined	not determined	8,33E-07	2,32
Aldh3b2	aldehyde dehydrogenase 3 family, member B2	not determined	not determined	1,71E-06	2,31
Ppfia2	protein tyrosine phosphatase, receptor type, f polypeptide (PTPRF), interacting protein (liprin), alpha 2	synaptosome	protein binding	9,74E-09	2,28
Abca8a	ATP-binding cassette, sub-family A (ABC1), member 8a	plasma membrane	nucleotide binding	5,45E-07	2,28
Tmem45b	transmembrane protein 45b	integral to membrane	not determined	4,94E-07	2,26

Angptl1	angiopoietin-like 1	extracellular region	receptor binding	7,27E-07	2,26
Arpp21	cyclic AMP-regulated phosphoprotein, 21	cytoplasm	nucleic acid binding	5,55E-06	2,26
Krtap5-4	keratin associated protein 5-4	keratin filament	not determined	4,03E-08	2,23
Fam19a1	family with sequence similarity 19, member A1	extracellular region	not determined	3,11E-06	2,21
Gsc	goosecoid homeobox	transcription factor complex	transcription factor activity	1,31E-06	2,21
Cox8c	cytochrome c oxidase, subunit VIIIc	mitochondrion	cytochrome-c oxidase activity	8,81E-09	2,19
3110018I06Rik	RIKEN cDNA 3110018I06 gene	not determined	not determined	5,59E-09	2,19
Tceal6	transcription elongation factor A (SII)-like 6	not determined	translation elongation factor activity	2,24E-08	2,19
Pitx1	paired-like homeodomain transcription factor 1	Nucleus	transcription regulator activity	6,09E-08	2,19
Fitm1	fat storage-inducing transmembrane protein 1	endoplasmic reticulum		7,07E-07	2,18
Mmd2	monocyte to macrophage differentiation-associated 2	membrane	receptor activity	2,35E-09	2,18
Gm7849	predicted gene 7849	not determined	not determined	3,90E-08	2,18
Arhgap36	Rho GTPase activating protein 36	not determined	not determined	1,53E-09	2,18
Il1rl1	interleukin 1 receptor-like 1	extracellular region	interleukin-33 receptor activity	2,38E-06	2,18
Gm9276	eukaryotic translation elongation factor 1 gamma pseudogene			1,72E-08	2,18
Akap14	A kinase (PRKA) anchor protein 14	not determined	protein domain specific binding	7,37E-09	2,17
01-mars	membrane-associated ring finger (C3HC4) 1	membrane	ligase activity	6,38E-11	2,16

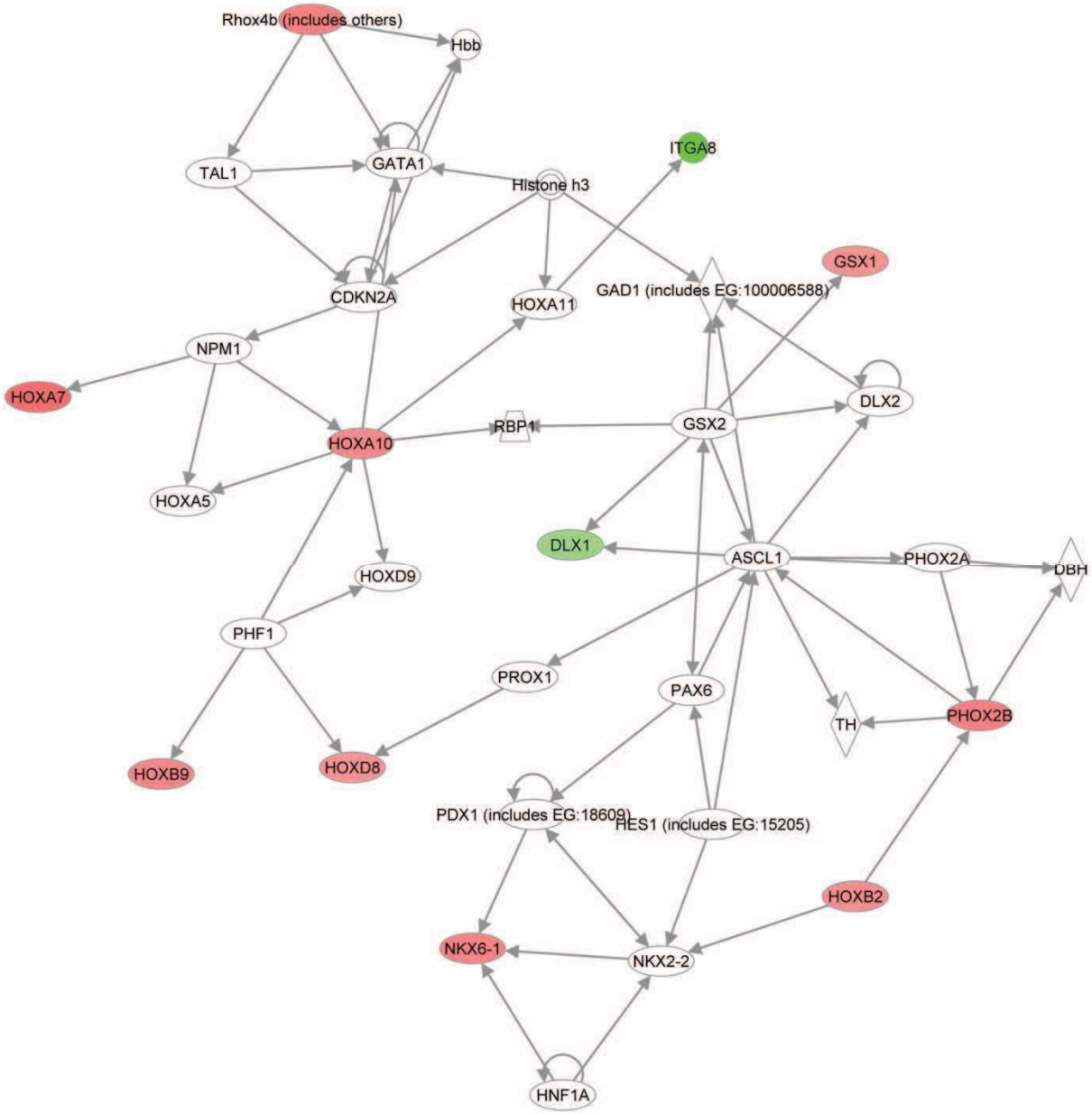
Tbx4	T-box 4	Nucleus	DNA binding	3,20E-09	2,16
Mug1	murinoglobulin 1	extracellular region	endopeptidase inhibitor activity	1,47E-11	2,16
Fam150b	family with sequence similarity 150, member B	not determined	not determined	4,80E-08	2,16
Prkcq	protein kinase C, theta	immunological synapse	protein kinase activity	6,71E-07	2,15
Vwc2	von Willebrand factor C domain containing 2	extracellular region	not determined	4,30E-08	2,15
Speer4d	spermatogenesis associated glutamate (E)-rich protein 4d	Nucleus	not determined	6,33E-09	2,14
Gm9717	predicted gene 9717	not determined	not determined	5,19E-07	2,13
Speer4d	spermatogenesis associated glutamate (E)-rich protein 4d	Nucleus	not determined	3,70E-09	2,12
Olfr29-ps1	olfactory receptor 29, pseudogene 1			1,51E-09	2,12
Fam26d	family with sequence similarity 26, member D	not determined	not determined	3,66E-09	2,12
Dok6	docking protein 6	not determined	insulin receptor binding	7,47E-08	2,11
Gm8985	predicted gene 8985			1,68E-08	2,11
Fibin	fin bud initiation factor homolog (zebrafish)	not determined	not determined	3,53E-09	2,10
Itm2a	integral membrane protein 2A	membrane		1,36E-06	2,08
A430089I19Rik	RIKEN cDNA A430089I19 gene	not determined	not determined	2,41E-10	2,07
1700031M16Rik	RIKEN cDNA 1700031M16 gene	not determined	not determined	2,00E-08	2,06
Olfr444	olfactory receptor 444	integral to membrane	G-protein coupled receptor activity	1,01E-06	2,05

AI593442	expressed sequence AI593442	not determined	not determined	2,08E-06	2,05
Gm5734	predicted gene 5734	not determined	not determined	5,93E-08	2,03
B3galt2	UDP-Gal:betaGlcNAc beta 1,3-galactosyltransferase, polypeptide 2	Golgi apparatus	galactosyltransferase activity	1,91E-06	2,03
Grap2	GRB2-related adaptor protein 2		protein binding	1,12E-07	2,02
Ankrd1	ankyrin repeat domain 1 (cardiac muscle)	transcription factor complex	transcription corepressor activity	5,84E-07	2,01
Chst7	carbohydrate (N-acetylglucosamino) sulfotransferase 7	Golgi membrane	sulfotransferase activity	2,24E-08	2,01
9130404H23Rik	RIKEN cDNA 9130404H23 gene	not determined	not determined	8,26E-09	2,01
Ccl26	chemokine (C-C motif) ligand 26	extracellular space	cytokine activity	2,61E-10	2,01
Corin	corin	plasma membrane	serine-type endopeptidase activity	4,37E-09	2,01
Barx2	BarH-like homeobox 2	Nucleus	chromatin binding	3,47E-07	2,01
Otx1	orthodenticle homolog 1 (Drosophila)	Nucleus	transcription factor activity	8,02E-08	2,00
Llph	LLP homolog, long-term synaptic facilitation (Aplysia)	not determined	not determined	5,36E-07	-2,01
Mdga2	MAM domain containing glycosylphosphatidylinositol anchor 2	plasma membrane		3,76E-06	-2,02
Gabra1	gamma-aminobutyric acid (GABA) A receptor, subunit alpha 1	plasma membrane	GABA-A receptor activity	1,63E-08	-2,05
Rorb	RAR-related orphan receptor beta	Nucleus	transcription factor activity	6,41E-09	-2,09
Zmat4	zinc finger, matrin type 4	not determined	nucleic acid binding	2,84E-07	-2,14
Fcrl6	Fc receptor-like 6	membrane		6,03E-11	-2,17

Rtn4rl1	reticulon 4 receptor-like 1	plasma membrane	receptor activity	4,35E-07	-2,20
Naalad2	N-acetylated alpha-linked acidic dipeptidase 2	not determined	carboxypeptidase activity	6,48E-09	-2,21
Kcnb2	potassium voltage gated channel, Shab-related subfamily, member 2	voltage-gated potassium channel complex	voltage-gated ion channel activity	1,42E-06	-2,30
Itga8	integrin alpha 8	integrin complex	receptor activity	4,57E-08	-2,34
Gabrb2	gamma-aminobutyric acid (GABA) A receptor, subunit beta 2	membrane fraction	ion channel activity	1,21E-07	-2,60
ATP6	ATP synthase F0 subunit 6	mitochondrion	hydrogen ion transmembrane transporter activity	1,26E-07	-2,84
Alx1	ALX homeobox 1	Nucleus	transcription factor activity	2,50E-08	-2,85
2610017I09Rik	RIKEN cDNA 2610017I09 gene	not determined	not determined	1,52E-10	-2,92
Gabrb2	gamma-aminobutyric acid (GABA) A receptor, subunit beta 2	cell junction	ion channel activity	7,32E-09	-3,06
Ndst4	N-deacetylase/N-sulfotransferase (heparin glucosaminyl) 4	Golgi apparatus	catalytic activity	4,21E-06	-3,48
Pla2g7	phospholipase A2, group VII (platelet-activating factor acetylhydrolase, plasma)	extracellular region	hydrolase activity	1,34E-08	-3,99
Nmbr	neuromedin B receptor	plasma membrane	receptor activity	5,52E-10	-4,25
Cyp26c1	cytochrome P450, family 26, subfamily c, polypeptide 1		monooxygenase activity	1,74E-10	-5,04

Additional File 5

Network 1



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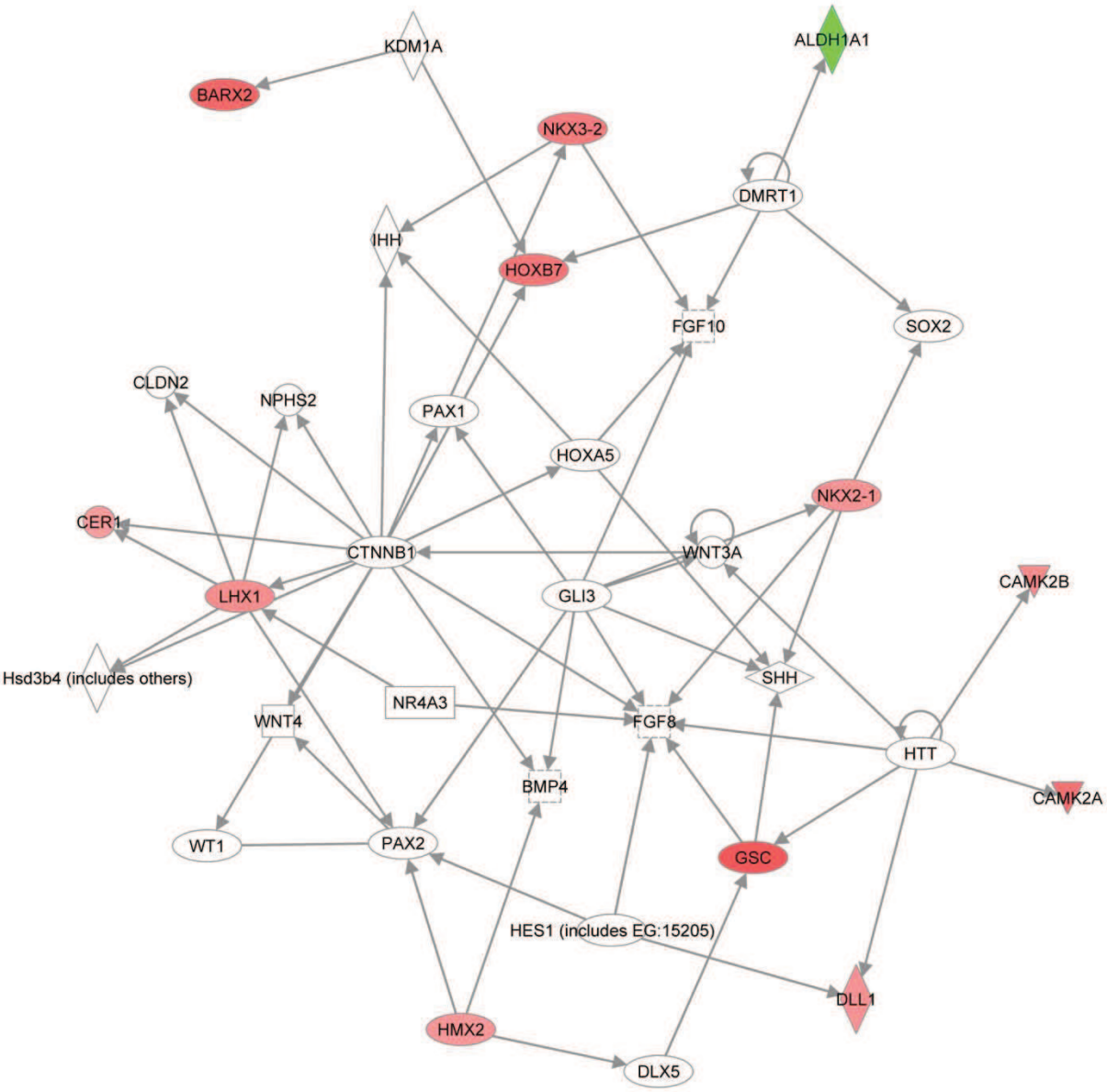
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NKX3-2
DMRT1
IHH
HOXB7
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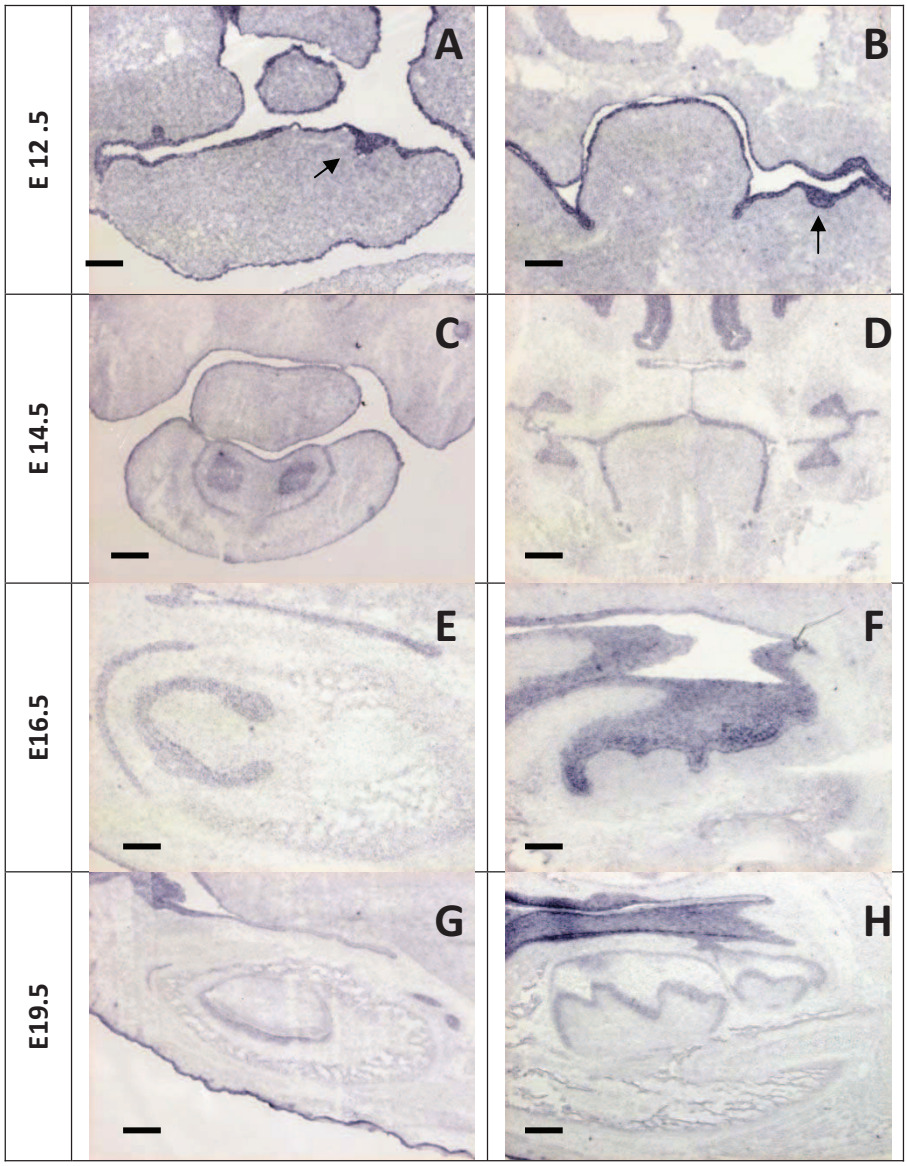
CLDN2
NPHS2
PAX1
HOXA5
NKX2-1
CER1
CTNNB1
WNT3A
LHX1
GLI3
CAMK2B
Hsd3b4 (includes others)
SHH
NR4A3
WNT4
FGF8
HTT
BMP4
CAMK2A
WT1
PAX2
GSC
HES1 (includes EG:15205)
DLL1
HMX2
DLX5

Additional File 6

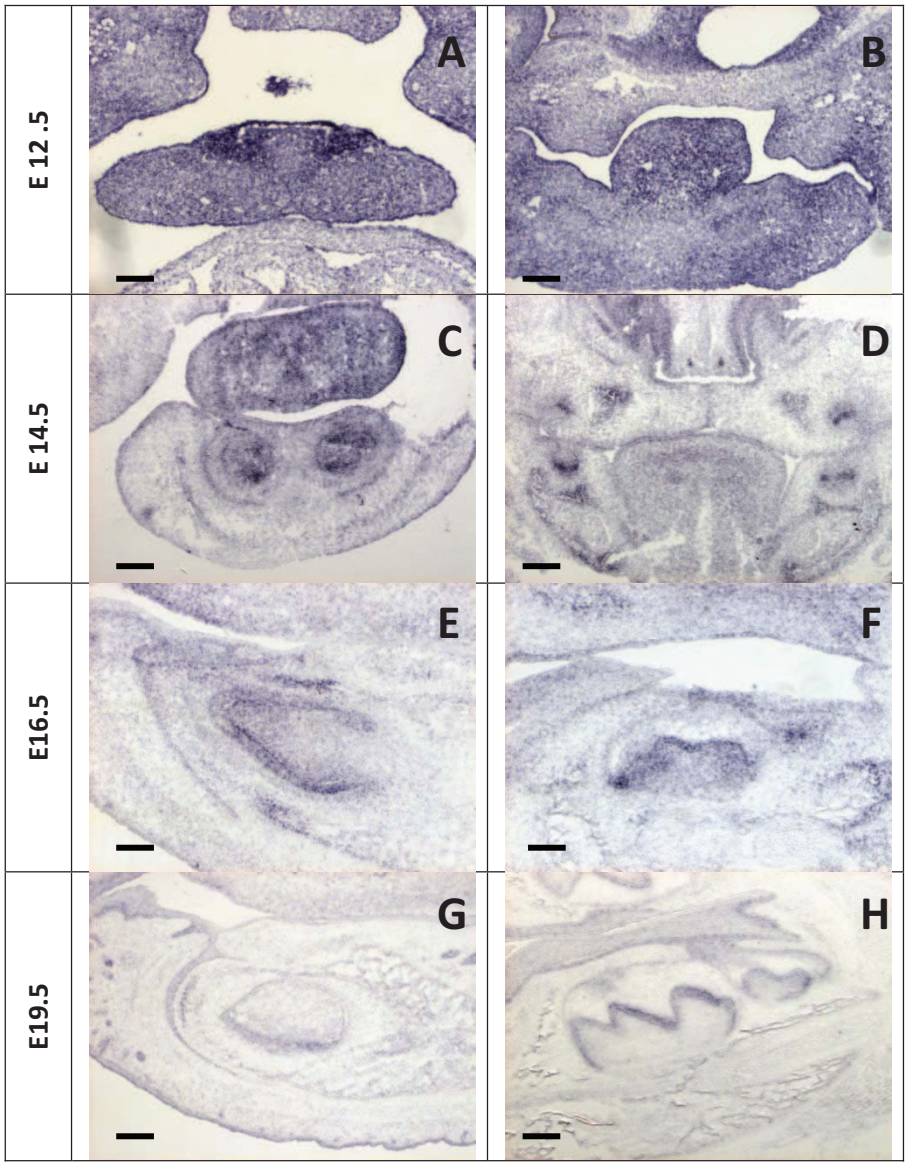
Genes/Primers	Sequences (5' to 3')
Adamtsl3 For.	CCTACAATGACGTGCAGTACC
Adamtsl3 Rev.	GTTCCATCCAACACTTTGGGT
Alx1 For.	CCTTCACACCGAGCTGAATAG
Alx1 Rev.	GACATCCTGAGATTGTTGCAGT
Bmp5 For.	TTACTTAGGGGTATTGTGGGCT
Bmp5 Rev.	TGAACGTGATTGTCTCCCAAG
Cyp1b1 For.	CTTCGCCTCTTTCCGTGTG
Cyp1b1 Rev.	GTGACCGAACGCCAGACTG
Cyp26c1 For.	TGGCCCAACAACTCTGGAC
Cyp26c1 Rev.	CAGCGTTTCACCGAAGAATGG
Dll1 For.	CCCATCCGATTCCCCTTCG
Dll1 Rev.	GGTTTTCTGTTGCGAGGTCATC
Fgf12 For.	CTAATTCCTGTAGGACTGCGTG
Fgf12 Rev.	GGGGTAAAAACATCTGAGCTGT
Fgfr4 For.	TACACATGCCTTGTGGAGAAC
Fgfr4 Rev.	GGAGATAGCTGTAGCGAATGC
Gapdh For.	AGGTCGGTGTGAACGGATTTG
Gapdh Rev.	TGTAGACCATGTAGTTGAGGTCA
Gl1 Rev.	CTTCCGAGTCAGACAGTCCCT
Gli1 For.	TACCATGAGCCCTTCTTTAGGA
Ihh For.	CTCAGACCGTGACCGAAATAAG
Ihh Rev.	CCTTGGACTCGTAATACACCCAG
Itga8 For.	TGTCTGGCGTTCAACTTGGAT
Itga8 Rev.	TCCAGTGAGTAGCCGAAGTAG
Prkcq For.	GAGATGCCGCAAGAACAATGG
Prkcq Rev.	ATTCATTAGCATTCGGCCTTGA
Rorb For.	AGGAACCGTTGCCAACACTG
Rorb Rev.	GACATCCTCCCGAACTTTACAG
Sfrp1 For.	TACTGGCCCGAGATGCTCAA
Sfrp1 Rev.	GAGGCTTCCGTGGTATTGGG
Sfrp2 For.	GGCCACGAGACCATGAAGG
Sfrp2 Rev.	CATGACCAGCGGAATCCAGG
Shox2 For.	CAAAGACGATGCGAAAGGGAT
Shox2 Rev.	AGGGTAAAATTGGTCCGACTTC
Smoc2 For.	GAAGGAGTTCCAGCAAGTGTT
Smoc2 Rev.	AGTATCCTGTGTAGCTGTGACA
Wnt11 For.	AAACTGATGCGTCTACACAA
Wnt11Rev.	CATGGCATTTACACTTCGTTTCC

Profil d'expression des gènes *Ap1m2* et H*you1* au cours de l'odontogenèse

In situ hybridization expression patterns during odontogenesis observed for *Ap1m2*



In situ hybridization expression patterns during odontogenesis observed for *Ap1m2*



Summary of the *in situ* hybridization data during odontogenesis

Developmental stage Tooth tissues	*Ap1m2*				*Hyou1*			
	In	M1	M2	M3	In	M1	M2	M3
E12.5								
Oral epithelium	+	+			+	+		
Dental lamina	+	+			+	+		
Mesenchyme								
E14.5								
Gubernaculum	+	+						
Enamel organ	+	+			+	+		
Inner dental epithelium	+	+			+	+		
Enamel knot	+	+						
Dental papilla								
Dental sac								
E16.5								
Gubernaculum	+	+						
Outer dental epithelium	+	+						
Stellate reticulum								
Stratum intermedium					+	+	+	
Inner dental epithelium	+	+			+	+		
Epithelial loop	+	+			+	+		
Dental papilla	+	+			+	+		
E19.5 (newborn)								
Outer dental epithelium	+	+			+	+		
Stellate reticulum						+		
Stratum intermedium								
Inner dental epithelium	+	+			+	+	+	
Preameloblasts	+	+			+	+		
Ameloblasts					+	+		
Epithelial loop	+	+			+	+		
Preodontoblasts								
Odontoblasts								
Dental papilla			+				+	
Dental sac								

DISCUSSION – PERSPECTIVES

3 DISCUSSION – PERSPECTIVES

Le développement dentaire s'inscrit dans le développement crânio-facial en général. Les cellules pluripotentes issues des crêtes neurales céphaliques vont migrer le long du premier arc pharyngien et vont ainsi permettre, en association avec des cellules mésodermiques, le développement de nombreuses structures du massif crânio-facial (Cobourne and Mitsiadis, 2006; Knight and Schilling, 2006; Noden and Schneider, 2006).

L'odontogenèse chez l'Homme se traduit par la morphogenèse de couronnes et de racines spécifiques à chaque type de dents, par l'histogenèse de l'organe de l'émail et les cytodifférenciations des odontoblastes, des améloblastes et des cémentoblastes. L'étude de l'évolution des mammifères s'intéresse souvent à une analyse détaillée de la morphologie dentaire. Pour que les patrons moléculaires puissent jouer un rôle sur l'évolution dentaire, des différences d'expression de gènes doivent pouvoir être reliées à des variations morphologiques (Jernvall, 2000; Plikus et al., 2005; Salazar-Ciudad and Jernvall).

La souris est un modèle pertinent pour l'étude de l'odontogenèse qui permet d'aborder la mise en place des patrons de la dentition, d'étudier les mécanismes impliqués dans la régulation du nombre de dents, de comprendre les différences reliées aux morphogenèses et morphologies spécifiques des dents (molaires mandibulaires ou maxillaires, incisives inférieures ou supérieures), mais aussi d'étudier la problématique des cellules souches du fait de la présence d'une niche de cellules souches situées dans les lèvres cervicales épithéliales de l'incisive.

Les étapes continues et progressives du développement dentaire ont été classiquement divisées en stades de lame dentaire, bourgeon, capuchon, cloche, formation radiculaire et éruption. L'odontogenèse est un processus cinétique cellulaire dépendant, contrôlé par des interactions épithélio-mésenchymateuses à médiation matricielle, entre l'ectoderme du premier arc pharyngé et des cellules ectomésenchymateuses originaires des crêtes neurales céphaliques (Peters and Balling, 1999; Thesleff, 2003b; Thesleff and Aberg, 1999; Tucker and Sharpe, 2004). Ces cellules de l'ectomesenchyme contribuent à la formation du mésenchyme dentaire, de la pulpe dentaire, des odontoblastes, de la matrice de la dentine, du cément, du parodonte (Chai et al., 2000; Miletich and Sharpe, 2004). L'ectoderme oral va

contribuer à la formation de la partie épithéliale de la dent avec l'organe de l'émail, l'épithélium dentaire interne et les futurs améloblastes qui vont synthétiser les protéines de la matrice de l'émail.

3.1 Gènes impliqués dans des syndromes avec anomalies dentaires

Les anomalies dentaires peuvent exister de manière isolée ou être associées à des signes cliniques extra-oraux dans les syndromes. Elles peuvent être d'origine génétique ou dues à l'action de tératogènes. (Alaluusua, 2006; Alaluusua and Lukinmaa, 2006; Alaluusua et al., 1999; Berdal, 2003; Koch, 2003; Weerheijm, 2003).

Chaque anomalie dentaire peut être classée dans des catégories variées : anomalies de nombre, de forme, de taille, de structure des tissus durs, de formation radiculaire et d'éruption et de résorption. Ces anomalies sont liées à des problématiques développementales et génétiques spécifiques (Bloch-Zupan, 2004) telles que l'origine embryonnaire des cellules dentaires, la mise en place du patron de la dentition, la localisation définie du développement dentaire, l'identité dentaire, la morphogenèse spécifique, l'histogenèse, les différenciations terminales des améloblastes et des odontoblastes, la synthèse de la dentine et de l'émail suivie de la minéralisation, la formation des racines et l'éruption dentaire (Salazar-Ciudad and Jernvall, 2002; Thesleff, 2003a; Thesleff, 2003b; Thesleff, 2006). Des interférences dans ces processus développementaux peuvent mener à des anomalies cliniques (Aldred et al., 2003; MacDougall, 2003; Thesleff, 2000; Thesleff, 2006) et certaines interférences peuvent même mener à des tumeurs provenant des cellules dentaires épithéliales (tumeurs odontogéniques) (Papagerakis et al., 1999).

Les travaux réalisés dans le cadre de cette thèse sont tous inscrits dans le contexte des maladies rares et ont, soit pris comme point de départ les anomalies du développement dentaire rencontrées chez l'Homme et participant au tableau clinique de syndromes, soit se sont attachés à partir d'un modèle murin présentant des anomalies dentaires à contribuer à la compréhension de ces anomalies en les confrontant en particulier aux données cliniques chez l'Homme.

3.1.1 Analyse détaillée de patrons d'expression génique au cours du développement dentaire

Ainsi, le premier axe de ce travail de thèse a consisté à exploiter les données d'*hybridation in situ* produites pour l'ensemble du génome murin au stade E14.5 (capuchon dentaire) dans le cadre du programme européen EURExpress (Diez-Roux et al., 2011) et répertoriées dans la base de données « Odontogenèse » (travail supervisé, pour l'équipe EURExpress IGBMC/ICS, par le Dr P. Dollé) afin de sélectionner les gènes montrant des profils d'expression spécifiques au niveau des bourgeons dentaires et/ou des tissus faciaux, et connus comme étant impliqués dans des syndromes humains affectant l'odontogenèse. Treize gènes ont été ainsi sélectionnés pour une étude approfondie : *Alx3, Alx4, Ercc3, Evc2, Irf6, Mek1, Nhs, Nsd1, Plod1, Sema3e, Tgif, Ube3a* et *Nfkbia.*

3.1.1.1 *Les gènes impliqués dans des dyplasies ectodermiques*

Parmi ces 13 gènes retenus, 5 sont impliqués dans des dysplasies ectodermiques (*Ercc3, Evc2, Irf6, Mek1 et Nfkbia)* (Visinoni et al., 2009). Ces maladies rares sont caractérisées par des altérations des structures ectodermiques telles que les cheveux, les dents (agénésies dentaires, hypodontie, oligodontie voire anodontie, anomalies de forme, de taille et anomalies de l'émail), les ongles, les glandes sudoripares, les glandes salivaires et les appendices ectodermiques (Visinoni et al., 2009). *IRF6* est impliqué dans le syndrome de Van der Woude (Kondo et al., 2002), et dans des cas de fentes labio/palatines non syndromiques (Desmyter et al., 2010; Rutledge et al., 2010; Vieira et al., 2007) ou d'hypodontie (Vieira et al., 2007); *NFKBIA* dans les dysplasies ectdermiques avec déficit immunitaire (Courtois et al., 2003; Lopez-Granados et al., 2008); *ERCC3* dans le Xeroderma pigmentosum (Bootsma et al., 1995) et la trichothiodystrophie (Weeda et al., 1997); *EVC2* dans le syndrome d'Ellis-Van Creveld (Galdzicka et al., 2002); *MAP2K1* (Rodriguez-Viciana et al., 2006) dans le syndrome cardiofaciocutané (cf. Introduction).

Nos données d'expression obtenues par hybridation *in situ* chez la souris confirment la transcription de ces gènes dans différents tissus/organes d'origine ectodermique (dents, glandes, salivaires, vibrisses). *Nfkbia, Ercc3, Evc2 et Map2k1* sont aussi détectés dans les

compartiments ectomésenchymateux des dents. L'expression d'*Irf6* a été précédemment décrite par (Blackburn et al., 2012; Knight et al., 2005; Kondo et al., 2002), mais aucune description précise au niveau tissulaire n'avait été faite à ce jour. Nous avons mis en évidence que ce gène, fortement exprimé dans la zone des futures lèvres épithéliales au stade de capuchon, est également transcrit dans l'épithélium dentaire interne et externe, dans le stratum intermedium et dans les préaméloblastes. L'immunolocalisation de MAP2K1 a été décrite dans la troisième molaire chez l'Homme. Une forte réactivité a été observée dans l'épithélium dentaire interne, et un signal modéré a été montré dans l'épithélium dentaire externe et dans le réticulum stellaire (Kumamoto et al., 2004). Ces données sont en accord avec nos résultats, à l'exception de la localisation dans le réticulum stellaire et le stratum intermedium.

L'expression des gènes *Nfkbia*, *Ercc3 et Evc2* au cours de l'odontogenèse n'avait jamais été décrite. Les profils d'expression correspondent, sur le plan temporel et en ce qui concerne les tissus marqués, aux phénomènes qui, s'ils sont perturbés peuvent générer les anomalies dentaires observées chez les patients (hypodontie/oligodontie, petite dents coniques, hypoplasie de l'émail) (Clauss et al., 2008; Gros et al., 2010). Des perturbations des évènements biologiques et moléculaires aux stades précoces (lame dentaire, transition du stade bourgeon au stade capuchon) sont liées à des dents manquantes, au stade capuchon à des anomalies de taille et de forme, au stade cloche et durant les cytodifférenciations terminales à des anomalies de structures (dentine, émail).

3.1.1.2 *Le syndrome d'Angelmann*

Nous avons également analysé au cours de l'odontogenèse l'expression du gène *Ube3a* qui code pour une protéine ubiquitine-ligase EA3. Des mutations ou délétions, perte de fonction de ce gène affectant l'allèle maternel, sont impliquées chez l'homme dans le syndrome d'Angelmann (Lalande and Calciano, 2007; Van Buggenhout and Fryns, 2009). Ce syndrome est une maladie rare neurogénétique. Les principales caractéristiques sont des troubles du développement moteur, un déficit intellectuel, et des crises d'épilepsie. Elles sont associées à des manifestations crânio-faciales incluant une microcéphalie, une brachycéphalie, un prognathisme, une langue en protrusion et des dents espacées.

La modification par l'ubiquitine de diverses cibles protéiques à l'intérieur de la cellule s'avère être importante pour un certain nombre de fonctions cellulaires fondamentales. Une fonction majeure des ubiquines consiste à contrôler la demi-vie de protéines cellulaires. Les ubiquitines sont donc des protéines impliquées en pathologie dans le cancer et les maladies génétiques (Jiang and Beaudet, 2004; Matentzoglu and Scheffner, 2008). Les anomalies bucco-dentaires rencontrées dans ce syndrome seraient reliées essentiellement à la perte de l'activité ligase, mais l'étude des patrons d'expression ne permet pas de préciser ce rôle. Des analyses complémentaires de modèle murins et des études fonctionnelles seraient nécessaires.

3.1.1.3 *Le syndrome de Cockayne*

Le syndrome de Cockayne est une maladie rare d'origine génétique, conséquence d'une altération des voies de réparation et de transcription de l'ADN. Il se caractérise classiquement par un retard de croissance et une cachexie majeure, une dégradation progressive des fonctions neurologiques et cognitives, une atteinte sensorielle associant une dystrophie rétinienne et une surdité de perception ainsi qu'une photosensibilité inconstante. Dans la forme sévère, le décès survient avant la fin de la première décennie. Une microcéphalie, une proéminence des os faciaux (Tan et al., 2005), un prognathisme de la mandibule (Macdonald et al., 1960), un palais arqué ont été décrits. Du point de vue bucco-dentaire, les anomalies décrites dans la littérature sont : un retard d'éruption des dents temporaires, une absence de dents permanentes, une microdontie, des racines dentaires coniques et courtes, des nombreuses caries dentaires, une atrophie de développement des processus alvéolaires, une dysplasie des condyles, une petite ouverture buccale et des mouvements mandibulaires réduits.

Ce syndrome est dû à des mutations des gènes CSA et dans les gène *ERCC6 (*excision-repair, cross-complementing group 6 gene) et *ERCC8* (excision-repair, cross-complementing group 8). D'autres gènes comme *XPB (ERCC3), XPD (ERCC2), XPG (ERCC5), XPF (ERCC4)* peuvent être incriminés chez des patients présentant une association Xeroderma Pigmentosum et syndrome de Cockayne de type II (Kraemer et al., 2007; Nouspikel, 2008; Rapin et al., 2000).

Des hybridations *in situ* aux différents stades de développement dentaire réalisées pour le gène *Ercc3* ont permis de mettre en évidence une expression au stade capuchon, cloche précoce et tardive à la fois au niveau épithélial et au niveau mésenchymateux. Les analyses du transcriptome de la dent de souris au stade de capuchon dentaire E14.5 ont montré que tous les gènes *Ercc* étaient transcrits et pourraient donc, dans un contexte pathologique, être à l'origine des anomalies dentaires décrites chez l'homme.

3.1.1.4 *Smoc2*

Smoc2 appartient à la famille des protéines matricellulaires Sparc qui régulent les interactions entre les cellules et la matrice extracellulaire. Il a été démontré en 2012, qu'une mutation dans ce gène se caractérise chez l'homme par une oligodontie, une extrême microdontie, des anomalies de forme avec des cuspides supplémentaires, des formations dentaires doubles et un taurodontisme des molaires, un émail fin, des racines courtes (dysplasie dentinaire) avec un os alvéolaire associé très réduit (Bloch-Zupan et al.).

Dans cette étude nous avons mis en évidence que ce gène est exprimé à différents stades du développement dentaire, molaire et incisive, chez l'embryon de souris depuis E12.5 (lame dentaire) jusqu'à E18.5 (cloche avec cytodifférenciations terminales des odontoblastes et améloblastes). A E14.5, stade du capuchon dentaire, le signal est présent à la fois dans l'épithélium (marquage asymétrique au niveau de l'épithélium dentaire externe) et dans des structures mésenchymateuses (la région cervicale de la papille dentaire, le sac folliculaire). Le signal se restreint ensuite aux zones de prolifération du mésenchyme dentaire faisant face aux lèvres épithéliales et plus spécifiquement à la lèvre épithéliale linguale pour l'incisive.

L'expression de ce gène à différents stades du développement permet d'expliquer le spectre des anomalies dentaires rencontrées. L'analyse transcriptionnelle comparative entre molaires et incisives mandibulaires a permis de mettre en évidence que ce gène était plus fortement exprimé au niveau molaire. Les anomalies dentaires étant présentes dans les deux types de dents chez l'homme, il est difficile de corréler cette expression différentielle au phénotype.

Après ces analyses relativement descriptives de l'expression de gènes associés au développement dentaire normal chez la souris et impliqués dans les anomalies dentaires chez l'homme, nous avons voulu caractériser un modèle murin présentant des anomalies du développement dentaire. En effet, les souris génétiquement modifiées sont d 'excellents modèles d'étude des maladies rares et plus spécifiquement de leurs manifestations à la cavité buccale (Fleischmannova et al., 2008).

3.1.2 Analyse détaillée d'un modèle animal du syndrome de Coffin-Lowry

Dans le deuxième axe de ce travail, une étude approfondie de souris mutées présentant des anomalies du développement de la cavité buccale a été mené. Des approches par microtomodensitométrie (microCT) (collaboration avec le Pr A. Constantinesco et le Dr P. Choquet) et par analyse de puces à ADN ont été utilisées. L'étude a porté plus particulièrement sur la souris mutée pour le gène *Rsk2* (collaboration avec le Dr A. Hanauer, IGBMC).

Des mutations dans le gène *RSK2* causent chez l'Homme le syndrome de Coffin-Lowry (CLS) caractérisé par un retard mental sévère lié à l'X, un dysmorphisme touchant notamment la face, les mains, des anomalies osseuses et des anomalies cardiaques; de plus on retrouve chez ces patients des anomalies buccodentaires typiques telles qu'un palais étroit, un sillon lingual central, une malocclusion, une hypodontie, des incisives riziformes et une perte précoce de certaines dents.

Une analyse macroscopique de la souris mutée pour le gène *Rsk2* a mis en évidence une taille réduite chez ces souris, une déviation au niveau nasal chez certains individus ainsi que la présence d'une prémolaire en localisée en avant de la première molaire (M1).

Le phénotype ressemble à celui des souris *Tabby* (mutée pour le gène EDA (Ectodyspalsine A) chez lesquelles une dent surnuméraire se développe en avant de la première molaire, dont la partie antérieure se retrouve en conséquence réduite. L'origine de cette dent surnuméraire proviendrait d'un défaut de l'incorporation du bourgeon prémolaire

R2 à la M1, ce bourgeon participant alors au développement d'une dent surnuméraire autonome (Peterkova et al., 2002).

Une analyse par microtomodensimétrie (microCT) a donc été réalisée afin de préciser les anomalies crânio-faciales, mais également de mieux comprendre l'origine de cette molaire supplémentaire chez les souris *RSk2-/Y.* Pour cela nous nous sommes intéressés au champ molaire, à la taille du diastème mais aussi à l'identité radiculaire spécifiée par le nombre de racines.

Mes analyses ont montré que le phénotype des mutants *Rsk2-/Y* n'est pas homogène, ceci pourrait être dû aux variabilité de l'expression clinique observées dans le syndrome. Ce phénotype crâniofacial oscille entre normalité et forte asymétrie crânienne ; pour les dents la formule dentaire est soit normale, soit enrichie d'une dent surnuméraire.

Le phénotype dentaire ressemble aux défauts retrouvés chez les souris *tabby* (modèle de la dysplasie ectodermique liée à l'X (OMIM #305100, locus Xq12-q13.1, mutations du gène ectodysplasine-A, ED1) (Boran et al., 2005). D'autres modèles murins montrent aussi cette réapparition de dents dans le diastème avec modification de la taille et de la morphologie de la première molaire, qui s'explique par l'expression des potentialités odontogènes du diastème perdues au cours de l'évolution (Peterkova et al., 2002).

L'étude des molaires mandibulaires a montré que le champ molaire ainsi que le diastème sont de taille équivalente chez les mutants *Rsk2-/Y* possédant 4 dents dans le champ molaire et chez les souris sauvages. Ceci laisse suggérer une réorganisation du champ molaire en 4 dents au lieu de 3. Lorsque nous comparons les molaires maxillaires entre le mutant et la souris sauvage, la longueur totale du champ molaire est quant à elle augmentée et le diastème réduit chez le mutant à 4 dents. La molaire surnuméraire maxillaire est plus grande que celle mandibulaire.

Un des substrats cytosoliques des RSK est IkappaBalpha. Des mutations de ce gène ont été associées à des dysplasies ectodermiques hypohidrotiques dominantes avec une immunodéficience des cellules T (Courtois et al., 2003). Cette liaison possible entre RSK et son substrat pourrait expliquer la similitude des phénotypes et des anomalies retrouvées chez les souris transgéniques.

Des analyses de transcriptome (puces à ADN Affymetrix) ont également été réalisées afin d'étudier les gènes dérégulés au stade E14.5 (capuchon) dans les incisives, les molaires inférieures et les molaires supérieures de souris *Rsk2-/Y*. Dans un premier temps nous avions étudié 4 échantillons mutants versus 4 échantillons sauvages pour chacun des types dentaires et ceci ne nous permettait pas de mettre en évidente de différences transcriptionnelles statistiquement significatives entre les deux groupes d'échantillons. Les phénotypes dentaires retrouvés en microCT étant hétérogènes, nous avons décidé de comparer un plus grand nombre d'échantillons pour les molaires inférieures à savoir 8 mutants versus 8 individus sauvages et ceci nous a en effet permis d'identifier des gènes dérégulés dans les molaires inférieures. Ces résultats n'ont pas pu être validés par qPCR quantitative, sans doute à cause de l'hétérogénéité des mutants et parce que ces analyses ont été réalisées uniquement en duplicata sur 3 échantillons mutants versus 3 échantillons contrôle. Cependant, l'augmentation du nombre d'échantillons dans le transcriptome nous a permis de mettre en évidence des gènes qui sont dérégulés au niveau des molaires inférieures chez les mutants et ce quel que soit leur degré d'affectation.

Pour confirmer cette hypothèse de transcriptome hétérogène, nous avons décidé d'inactiver *in* vitro en culture organotypique, le gène *Rsk2* dans des explants dentaires au stade E14.5 afin de minimiser l'hétérogénéité créée sans doute par des phénomènes de compensation liés aux tissus environnants ou à la redondance fonctionnelle possible entre Rsk. De plus, l'inactivation se faisant uniquement sur 24h, ces phénomènes de compensation n'avaient pas le temps de se mettre en route. Nous avons donc testé l'inactivation du gène *Rsk2* à l'aide d'un shRNA. Afin de faciliter la pénétration, une approche par microinjection puis électroporation de shRNA a été réalisée directement dans le germe dentaire disséqué au stade E14.5. L'inactivation de ce gène a été validée par qPCR après un jour de culture des explants dentaires. Suite à l'électroporation du shRNA nous avons vérifié par qPCR, 2 des gènes affectés sur le transcriptome des souris mutées pour le gène *Rsk2* (*Eaf2* et *Rdh1*). Ces gènes ont permis de valider les données obtenues par le transcriptome. Cette approche d'électroporation de shRNA au sein du tissu dentaire permettrait donc de proposer une alternative pour l'étude et le décryptage des données obtenues par analyse sur puces à ADN.

3.2 Nouveaux gènes candidats jouant un rôle au cours de l'odontogenèse

3.2.1 Analyse détaillée de patrons d'expression génique au cours du développement dentaire

L'identification de nouveaux gènes impliqués dans le développement dentaire est essentielle pour la compréhension du développement dentaire et pathologique. L'analyse de la base de données Odontogenèse nous a permis de mettre en évidence quatre gènes exprimés dans les tissus dentaires et/ou crâniofaciaux pour lesquels peu ou pas de données sur leur expression ou leur mode d'action étaient répertoriées dans la littérature (*Hyou1, Galnt1, Ap1m2, Lss*). Nous avons caractérisé par hybridation *in situ* les patrons d'expression de ces gènes aux différents stades de développement dentaire.

AP1M2 (adaptor-related protein complex 1, mu 2 subunit) est la chaine médiane du complexe AP1 qui se trouve dans le réseau trans-Golgi. Il a été mis en évidence que cette protéine à une expression restreinte aux cellules épithéliales (Fölsch & al., 1999) et qu'elle pourrait être impliquée dans les évènements de tri cellulaire spécifiques aux cellules polarisées (Ohno & al., 1999).

HYOU1 (Hypoxia up regulated protein 1) appartient à la famille des protéines "heat shock 70" et joue un rôle important dans le repliement des protéines et dans le reticulum endoplasmique (Bando & al., 2000). Comme son inactivation accélère l'apoptose, elle pourrait également jouer un rôle cytoprotecteur dans l'hypoxie (Ozawa & al., 1999; Tamatanj & al. 2001).

L'expression des gènes *Hyou1* et *Ap1m2* est présente à tous les stades du développement dans les dents mais aussi dans d'autres tissus comme l'épithélium olfactif ou la cochlée. Leur expression est épithéliale cependant de manière intéressante *Hyou1* est également exprimé transitoirement dans le mésenchyme aussi bien dentaire qu'olfactif au stade E16.5. Cette expression mésenchymateuse disparaît à E19.5. L'expression de *Ap1m2* dans les structures de l'organe de l'émail est en accord avec l'expression décrite comme restreinte de ce gène aux cellules épithéliales (Fölsch & al., 1999). Les améloblastes étant décrits comme des cellules sensibles à l'hypoxie, cela pourrait peut être expliquer l'expression de *Hyou1* dans ces cellules.

3.2.2 Les nouveaux gènes impliqués dans l'identité dentaire au stade E14.5

Il est apparu nécessaire de compléter les données d'expression obtenues par hybridation *in situ* par une analyse intégrale du transcriptome (puces à ADN Affymetrix) du capuchon dentaire au jour E14.5 en considérant séparément les molaires mandibulaires et maxillaires et les incisives. L'idée était d'explorer des différences d'expression susceptibles de concourir à la mise en place de phénomènes morphogénétiques aboutissant à une morphologie différencielle, et aussi d'expliquer la régionalisation des anomalies dentaires dans certaines maladies rares (incisives macrodontes dans le syndrome KBG, molaires et canines géantes dans le syndrome Otodental, par exemple).

L'étude comparative des transcriptomes de germes dentaires nous a permis de mettre en évidence des gènes différenciellement exprimés au stade capuchon entre molaires mandibulaires et incisives mais aussi entre molaires mandibulaires et maxillaires. Une grande partie de ces gènes sont de nouveaux gènes non connus comme étant impliqués dans le développement dentaire (88 nouveaux gènes différenciellement exprimés entre molaires mandibulaires et incisives et 53 entre molaires mandibulaires et maxillaires) mais appartenant aux grandes voies de signalisation (FGF, BMP, Shh, Wnt, Tgfβ, Notch) ou à des familles de gènes (gènes à homéoboîtes) impliqués dans le développement dentaire. Dix-sept de ces gènes ont été vérifiés quant à leur expression différencielle par qPCR quantative et 13 d'entres eux valident les résultats obtenus par l'étude des transcriptomes. Les données du transcriptome complètent les données d'expression, permettent une approche plus quantitative et éclairent à la lumière de possibles différences d'expression entre molaires et incisives les phénotypes différenciels observés en pathologie chez l'Homme (anomalies dentaires rencontrées plutôt dans le champ incisif ou molaire).

3.3 Conclusion

Ce travail démontre l'utilité d'une approche translationnelle entre biologie du développement et médecine ou recherche clinique et fondamentale pour élucider les évènements moléculaires à l'origine des manifestations cliniques, et en particulier des anomalies dentaires accompagnant divers syndromes. Mon travail a permis de montrer que des modèles de souris peuvent reproduire les syndromes rencontrés chez l'Homme, confirmant que ce modèle animal est très intéressant pour comprendre le développement dentaire et ses anomalies.

Le projet de recherche a fédéré des scientifiques et des cliniciens autour de la compréhension de ces anomalies. La poursuite des travaux par les membres de l'équipe visera à proposer des hypothèses diagnostiques dans le but d'améliorer la prise en charge de patients, et d'adapter les traitements utilisés ou de proposer de nouvelles thérapeutiques (par exemple le traitement des agénésies dentaires par stimulation de l'odontogenèse *in situ* ; l'ingénierie tissulaire…).

Durant ce travail de thèse une approche innovante de microinjection puis d'électroporation d'un shRNA au stade capuchon a été développée. Cette méthode ouvre des perspectives d'études fonctionnelles par l'inactivation de gène *ex-vivo* à un moment précis dans un tissu donné et de suivre en culture les conséquences morphologiques de cette inactivation. De plus, cette méthode permet de compléter les études de modèles animaux car elle permet de s'affranchir des phénotypes hétérogènes et des phénomènes de compensation qui ont lieu *in vivo* permettant ainsi d'analyser la contribution d'un gène unique au phénotype.

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Développement de la cavité buccale : du gène à l'expression clinique chez l'Homme

Le développement crânio-facial et l'odontogenèse en particulier se mettent en place grâce à la migration des cellules des crêtes neurales céphaliques vers le premier arc pharyngien et leurs interactions avec l'ectoderme buccal. L'odontogenèse, classiquement divisée en 5 stades: la lame, le bourgeon, le capuchon, la cloche, l'édification radiculaire avec l'éruption dentaire, est contrôlée par des interactions épithelio-mésenchymateuses entre les compartiments ectomésenchymateux et épithélial et est régulée par des voies de signalisations conservées et bien connues (Fgf, Bmp, Shh, Wnt, Tgfβ, Notch). Les anomalies bucco-dentaires sont un des aspects du tableau clinique des syndromes. Parmi 7000 syndromes connus à ce jour 900 ont un phénotype oral.
De nombreuses souris génétiquement modifiées, modèles de maladies rares reproduisent dans leurs phénotypes les anomalies crânio-faciales et bucco-dentaires rencontrées chez l'homme et autorisent ainsi une étude plus fine des conséquences des dysfonctionnements moléculaires associés.
Le développement dentaire représente donc un modèle très intéressant pour l'étude de mécanismes génétiques et moléculaires de l'organogenèse et de ses anomalies.
Le but de ce travail est de combiner l'étude de modèles animaux et la bioinformatique pour améliorer la compréhension des mécanismes étiopathogéniques impliqués dans le développement de la cavité buccale et des dents.

Différentes approches ont été utilisées pour mettre en évidence des gènes d'intérêt : (1) sélection de gènes connus comme étant impliqués dans des syndromes mais dont l'expression et/ou le rôle n'étaient pas caractérisés; (2) identification de nouveaux gènes candidats, par l'analyse de leur expression crânio-faciale et dentaire grâce à l'atlas de transcriptome EURExpress; (3) sélection de gènes différentiellement exprimés entre molaires mandibulaires et incisives et entre molaires mandibulaires et maxillaires au stade E14.5 par analyse de transcriptomes. (4) Pour les gènes les plus intéressants, étude des malformations crânio-faciales et bucco-dentaires des modèles animaux correspondant par microtomodensimétrie (microCT).

Les patrons d'expression au cours du développement dentaire ont été réalisés par hybridation *in situ* pour 17 gènes sélectionnés. Treize ont été retenus pour leur implication dans des syndromes (*Alx3, Alx4, Ercc3, Evc2, Irf6, Mek1, Nhs, Nsd1, Plod1, Sema3e, Tgif, Ube3a, Nfkb*ia) (1) et quatre pour leur expression crânio-faciale et/ou dentaire (*Hyou1, Ap1m2, Lss, Galnt1*) (2). L'étude transcriptomique entre les différents types de dents nous a permis de mettre en évidence 88 gènes non connus dans la dent à ce jour et différentiellement exprimés entre molaires mandibulaires et incisives et 53 gènes entre molaires mandibulaires et maxillaires (3). Des mutations dans le gène *RSK2* causent chez l'Homme le syndrome de Coffin-Lowry (CLS) caractérisé par un retard mental sévère lié à l'X, un dysmorphisme touchant notamment la face, les mains et le squelette ; de plus sont retrouvées chez ces patients des anomalies bucco-dentaires telles qu'un palais étroit, un sillon lingual central, une malocclusion, une hypodontie, des incisives riziformes et une perte précoce de certaines dents.
Une analyse de la souris *Rsk2-/Y* a mis en évidence une taille réduite chez ces souris, une déviation au niveau nasal chez certains individus, la présence de molaires surnuméraires ainsi que des modifications de gradients de taille dans le champ molaire. Le phénotype observé ressemble au phénotype des souris Tabby mutées pour le gène EDA (Ectodysplasine A) (4).

Ce projet fédère des scientifiques et des cliniciens autour de la compréhension des anomalies bucco-dentaires afin de stimuler le diagnostic de ces troubles du développement en se basant sur des preuves scientifiques et de proposer, à terme, de nouvelles options thérapeutiques.

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Développement de la cavité buccale : du gène à l'expression clinique chez l'Homme

Résumé : **Développement de la cavité buccale : du gène à l'expression clinique chez l'Homme**

Introduction : L'odontogenèse est sous contrôle génétique strict et elle est contrôlée par des interactions épithelio-mésenchymateuses. Les anomalies bucco-dentaires sont un des signes cliniques des syndromes. Parmi 7000 syndromes connus 900 ont un phénotype oral. Ce travail combine l'étude de modèles animaux et la bioinformatique pour améliorer la compréhension des mécanismes étiopathogéniques impliqués dans le développement dentaire.

Méthodes : (1) Sélection de gènes impliqués dans des syndromes dont l'expression n'est pas caractérisée
(2) Identification de gènes candidats par analyse de leur expression crânio-faciale et dentaire (atlas de transcriptome EURExpress)
(3) Sélection de gènes différenciellement exprimés entre molaires et incisives et entre molaires mandibulaires et maxillaires au stade E14.5 par analyse transcriptomique
(4) Etude par microtomodensitométrie des malformations crânio-faciales et bucco-dentaires des souris*Rsk2-/Y* (modèle du syndrome de Coffin-Lowry)

Résultats : (1) Patrons d'expression au cours du développement dentaire pour 13 gènes
(2) Patrons d'expression pour 4 gènes
(3) 88 gènes différenciellement exprimés entre molaires mandibulaires et incisives et 53 entre molaires mandibulaires et maxillaires
(4) Taille réduite des mutants, déviation nasale et présence de dents surnuméraires

Conclusion :
Ce projet fédère des scientifiques et des cliniciens autour de la compréhension des anomalies bucco-dentaires afin de stimuler le diagnostic de ces troubles du développement en se basant sur des preuves scientifiques et de proposer de nouvelles options thérapeutiques.

Mots clés : Cavité buccale, dents, anomalies, syndromes, souris

Résumé en anglais : **Development of the oral cavity: from gene to clinical phenotype in human**

Introduction: Tooth development is under strict genetic control and is mediated by epithelio-mesenchymal interactions. Oro-dental anomalies are one aspect of the 7000 known syndromes and 900 of these have an oral phenotype. Our goal is to combine the study of animal models and bioinformatics to improve the understanding of etiopathogenic mechanisms involved in oral development.

Methods: (1) Selection of known genes responsible for syndromes but for which the expression and/or roles are not characterised
(2) Identification of new candidate genes, through an analysis of their craniofacial and dental expression patterns using the EURExpress mouse transcriptome-wide atlas
(3) Selection of genes differentially expressed between molars and incisors and between mandibular and maxillary molars at E14.5 by transcriptomic analysis
(4) Study of craniofacial and orodental malformations of Rsk2-/Y mice by microtomodensitometry (model of Coffin-Lowry syndrome)

Results: (1) Expression pattern during odontogenesis for 13 genes
(2) Expression pattern for 4 genes
(3) 88 gènes différentially expressed between molars and incisors and 53 between mandibular and maxillary molars
(4) Smaller mutants, nasal deviation and supplementary teeth

Conclusion: This project federates scientists and clinicians around the understanding of orodental anomalies and should stimulate the implementation of science based evidence diagnosis and new therapeutic options.

Key words: Oral cavity, teeth, anomalies, syndromes, mice