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Ataxies cérébelleuses héréditaires: identification de gènes responsables, description clinique et stratégie diagnostique

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A ma famille…

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1. Introduction

1.1. L’ataxie cérébelleuse

L’ataxie (du grec a, sans; taxis, ordre) est un signe neurologique correspondant à un déséquilibre et à un trouble de la coordination des mouvements. L’ataxie cérébelleuse se caractérise par des troubles de l’équilibre, un tremblement d’intention, un retard d’initiation des mouvements, une adiadococinésie, une asynergie, une dysmétrie ou encore une dysarthrie (Subramony, 2007). Elle révèle un dysfonctionnement au niveau du cervelet, des pédoncules cérébelleux ou des voies spino-cérébelleuses.

Le cervelet contient une portion médiane, le vermis, et deux lobes, ou hémisphères cérébelleux, situés de chaque côté. Il est relié au tronc cérébral par les pédoncules cérébelleux supérieurs, moyens et inférieurs.

Le cortex cérébelleux contient 3 couches laminaires :

- la couche externe ou moléculaire, contenant les arborisations dendritiques des cellules de Purkinje, les axones des cellules olivaires ou fibres grimpantes, les axones des cellules granulaires ou fibres parallèles, ainsi que des interneurones (cellules étoilées et cellules en corbeille).
- la couche moyenne ou couche des cellules de Purkinje, qui contient les corps cellulaires des cellules de Purkinje répartis en une seule assise.
- la couche interne ou granulaire, dont les éléments dominants sont des corps cellulaires très nombreux et de petite taille: les grains ou cellules granulaires. Les axones de ces cellules remontent dans la couche moléculaire et se divisent en "T" en formant de longues branches, les fibres parallèles. La couche granulaire interne contient aussi les cellules de Golgi localisées au voisinage des somas des cellules de Purkinje.

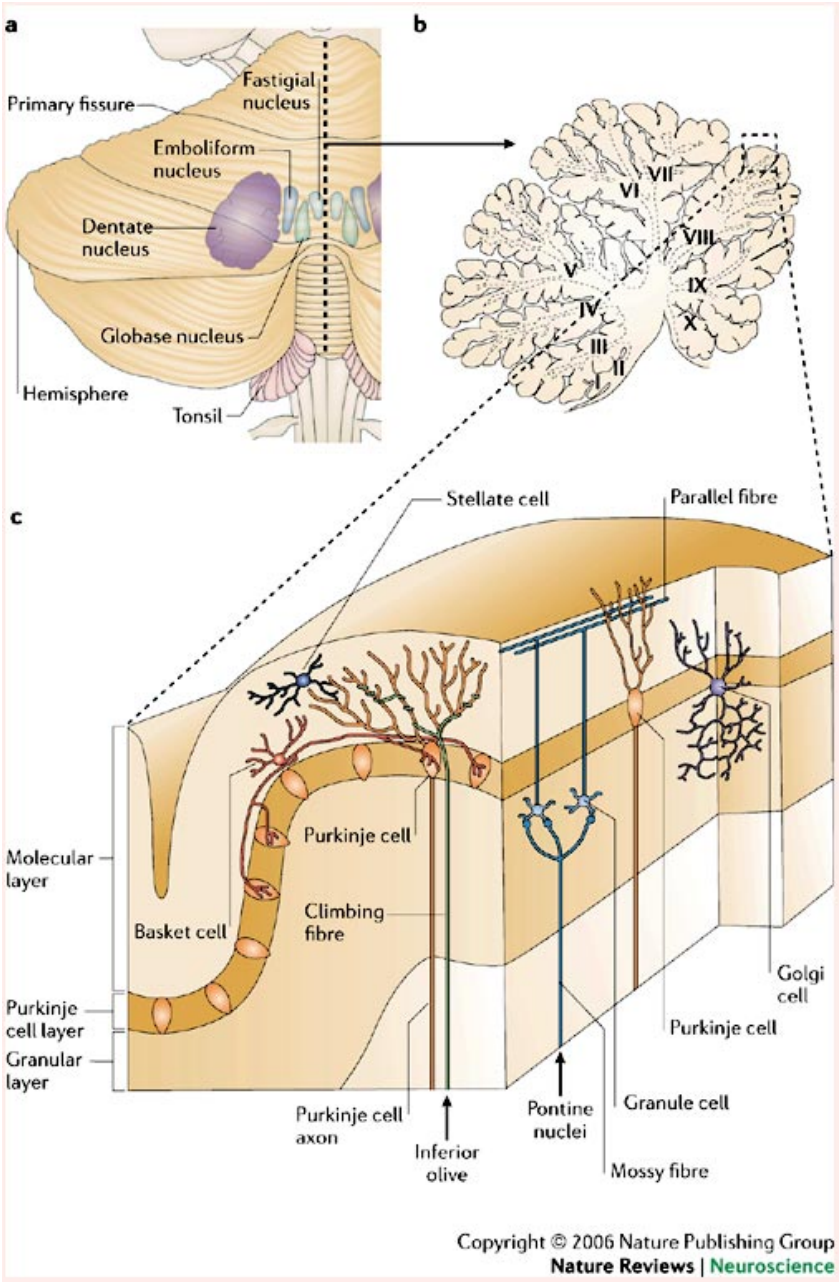

Figure 1: Anatomie du cervelet humain.

a: vue postérieure du cervelet, montrant les noyaux cérébelleux encastrés sous le cortex cérébelleux.

b: coupe transversale du cervelet montrant une organisation lobulaire.

c: organisation microstructurale du cortex cérébelleux indiquant la présence de trois couches. (Ramnani N., 2006)

Le syndrome cérébelleux statique, le plus souvent en lien avec une atteinte du vermis cérébelleux, se caractérise par une instabilité à la marche avec embardées, un élargissement du polygone de sustentation et une hypotonie avec réflexes ostéo-tendineux pendulaires. Le syndrome cérébelleux cinétique, quant à lui, se définit par la présence d'une atteinte segmentaire avec dysmétrie, tremblement d'action et poursuite saccadique. Il est secondaire à une atteinte au niveau des hémisphères cérébelleux ou du pédoncule cérébelleux supérieur.

Les plaintes principales des patients ataxiques sont des difficultés à courir puis une instabilité à la marche avec secondairement la présence de chutes. Il existe également des difficultés à réaliser des gestes fins comme l'écriture. Les patients se plaignent de dysarthrie, pouvant rendre difficile à terme la compréhension pour l'entourage. L'échelle SARA (Scale for the Assessment and Rating of Ataxia) permet de donner un score de sévérité aux ataxies (Schmitz-Hubsch et al., 2006). L'échelle SDFS (Spinocerebellar Degeneration Functional Score) permet de juger du degré d'autonomie d'un patient avec un score allant de 0 à 7. (Anheim et al., 2009)

En dehors des atteintes motrices, on retrouve une atteinte cognitive et émotionnelle du fait de l'atteinte de voies de connexion entre le cervelet et les aires associatives corticales. Elle se caractérise notamment par des troubles dysexécutifs et visuo-spatiaux.

L'ataxie cérébelleuse peut être aigüe ou subaigüe et dans ce cas associée à une cause traumatique (ischémie, hémorragie cérébelleuse), toxique (alcool, iatrogénie, mercure, solvants), infectieuse (abcès cérébelleux, varicelle, rubéole, paludisme) ou encore immunitaire (pathologie démyélinisante (sclérose en plaques), syndrome paranéoplasique). Lorsque l'ataxie apparaît de façon progressive et constante, une cause tumorale ou héréditaire doit être recherchée avant de conclure à une cause neuro-dégénérative de type atrophie multi-systématisée.

Le travail de cette thèse porte exclusivement sur des formes génétiques d'ataxie cérébelleuses. Les ataxies cérébelleuses héréditaires sont des pathologies neuro-dégénératives rares, hétérogènes, complexes affectant le cervelet et parfois la moelle épinière et/ou les nerfs périphériques. Elles se transmettent sur le mode autosomique récessif, dominant ou lié à l'X. Les ataxies cérébelleuses autosomiques récessives ont dans la plupart des cas, un début précoce avant l'âge de 20 ans. Il existe d'autres ataxies héréditaires de découverte récente comme le FXTAS (Fragile X-associated Tremor Ataxia Syndrome), pathologie neuro-dégénérative liée à l'X due à une prémutation du gène *FMR1* et dans laquelle une ataxie cérébelleuse plus tardive est présente.

1.2. Généralités sur les ataxies autosomiques récessives

Les ataxies cérébelleuses autosomiques récessives (ACAR) touchent principalement le cervelet mais aussi les faisceaux spino-cérébelleux et cordonaux postérieurs de la moelle épinière et les nerfs périphériques. Le tableau clinique est habituellement dominé par un syndrome cérébelleux auquel s'associent volontiers d'autres symptômes neurologiques et/ou extra-neurologiques.

La plupart des ACAR se révèlent dès l'enfance ou l'adolescence (en général avant 30 ans), mais des formes de révélation tardive sont possibles comme dans l'ataxie de Friedreich (late onset Friedreich ataxia (LOFA) ou very late Friedreich ataxia (vLOFA) (Lecocq et al., 2015)). La plus fréquente des ACAR est d'ailleurs l'ataxie de Friedreich affectant une personne sur 50 000 en France. Elle doit toujours être cherchée en priorité (répétitions de triplets GAA sur le gène de la frataxine). Ensuite, les ACAR les plus fréquentes sont l'ataxie télangiectasie (AT), l'ataxie avec déficit en vitamine E (AVED),

les ataxies avec apraxie oculomotrice de type 1 et 2 (AOA1 et AOA2) et l'ataxie spastique autosomique récessive de Charlevoix-Saguenay (ARSACS).

La présence de signes neurologiques associés (polyneuropathie, troubles ophtalmologiques ou oculomoteurs, signes pyramidaux) et/ou extra neurologiques (surdité, rétinite pigmentaire, diabète, cardiopathie) permettent d'orienter le diagnostic étiologique. Les examens biologiques d'orientation sont les dosages de la vitamine E, de l'alpha-foetoprotéine (AFP), du cholestérol, de l'albumine, le lipidogramme, la recherche d'acanthocytes, le dosage de l'acide phytanique, des enzymes lysosomales et du cholestanol. On réalisera également un EMG à la recherche d'une neuropathie et une IRM cérébrale à la recherche d'une atrophie cérébelleuse. La prévalence de ces pathologies rares est estimée à 3.5/100 000 habitants. (Ruano et al., 2014)

En fonction du gène, le handicap peut être important et porte essentiellement sur la marche et parfois sur l'atteinte extra-neurologique (cardiopathie dans l'ataxie de Friedreich par exemple). Bien qu'il n'existe pas de traitement dans la plupart des cas pour ralentir l'évolution de la maladie, certains patients peuvent avoir accès à certains traitements spécifiques comme la supplémentation en vitamine E dans l'ataxie par déficit en vitamine E (AVED), l'acide chénodesoxycolique dans la xanthomatose cérébro-tendineuse ou encore le miglustat dans la maladie de Niemann-Pick de type C.

Plusieurs classifications des ACAR ont été proposées, basées soit sur le regroupement des signes cliniques (Harding, 1983, 1993), soit sur la localisation de la dégénérescence au niveau du cervelet, des faisceaux spino-cérébelleux ou de la colonne postérieure de la moelle épinière (Koenig, 2003). De grandes voies physiopathologiques communes à certaines de ces ACAR ont été identifiées, comme les troubles du métabolisme mitochondrial ou les défauts de réparation des cassures de l'ADN. Mais il n'existe pas

pour autant de réelle classification basée sur des données moléculaires tant les gènes impliqués ont des fonctions diverses. Ces classifications sont controversées en raison de la complexité et de l'hétérogénéité phénotypique de ces ataxies.

Un nombre important de gènes d'ACAR est touché par des pertes de fonction partielles hypomorphes comme par exemple dans l'ataxie de Friedreich. Dans cette pathologie, l'expansion du triplet GAA entraîne une perte de fonction partielle de frataxine, l'absence de frataxine n'étant pas viable. Par ailleurs, les données récentes de biologie moléculaire montrent que de nombreuses maladies métaboliques ainsi que les épilepsies myocloniques progressives peuvent se présenter sous une forme pauci-symptomatique avec une ataxie au premier plan. Dans ces cas, les mutations sont également hypomorphes, la protéine résultante étant alors peu active.

Grâce au séquençage à haut débit, le nombre de gènes responsables d'ataxies génétiques ne cesse de croître chaque année et il devient quasi impossible pour un clinicien même expérimenté, d'avoir une connaissance exhaustive de toutes ces pathologies. Comme nous l'avons, il existe des tableaux cliniques complexes pouvant associer à l'ataxie cérébelleuse une atteinte du système périphérique (neuropathie), du système nerveux central (retard mental, épilepsie, paraparésie spastique, mouvements anormaux, etc.) ou d'autres organes (rétinite pigmentaire, diabète, cardiopathie, etc.).

Par ailleurs, la fréquence des présentations sporadiques et la variabilité phénotypique au sein d'une même ataxie génétique rendent encore plus difficile leur diagnostic. L'objectif du clinicien reste d'aboutir à un diagnostic le plus rapidement possible afin d'en informer le patient et sa famille et de débuter un traitement spécifique si il est nécessaire. L'utilisation d'algorithme permet ainsi de guider le diagnostic et éviter des investigations contraignantes, coûteuses et itératives chez le patient. Plusieurs

algorithmes ont été proposés notamment par notre équipe en 2012 (Anheim et al., 2012). Ces algorithmes reposent sur des critères cliniques et paracliniques précis nécessitant une analyse sémiologique neurologique fine et détaillée de la part du clinicien.

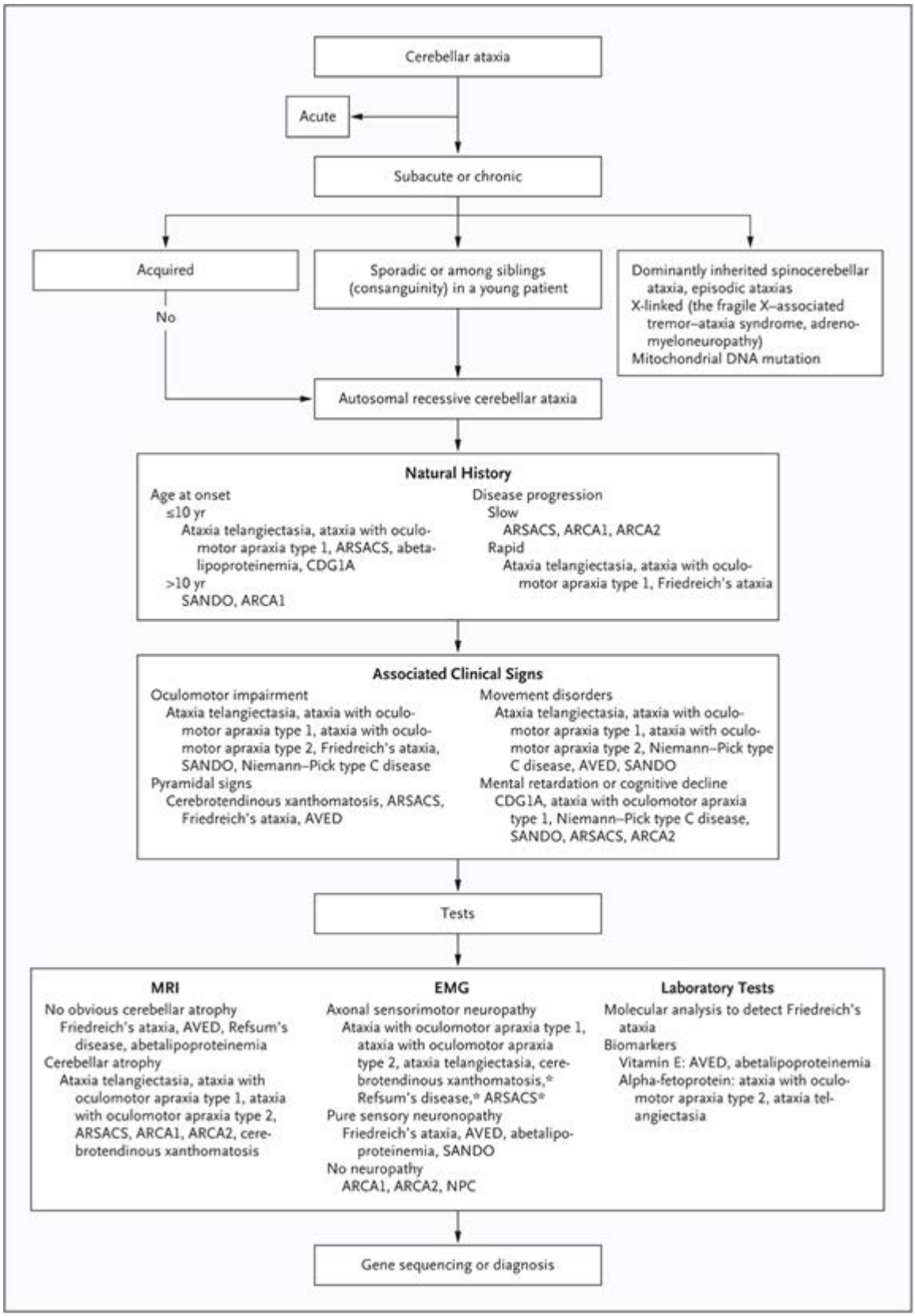

Figure 2 : Algorithme diagnostique devant une ataxie cérébelleuse. (Anheim et al, 2012)

1.3. Le séquençage à haut débit de nouvelle génération

Le séquençage à haut débit de nouvelle génération souvent appelé NGS (next generation sequencing) a l'avantage de séquencer un grand nombre de bases d'ADN en une seule réaction. Il est utilisé pour le diagnostic étiologique de pathologies génétiques selon 2 stratégies à savoir le séquençage ciblé d'un panel de gènes (Targeted-panel Sequencing) et le séquençage de l'exome (Whole Exome Sequencing (WES)) correspondant au séquençage de toutes les régions codantes du génome. Dans le cadre du diagnostic, des techniques de « mini-exome » permettent d'étudier l'ensemble des gènes déjà connus comme étant impliqués dans des pathologies génétiques humaines. Le séquençage à haut débit du génome (Whole Genome Sequencing (WGS)) est parfois utilisé en recherche mais son interprétation est encore plus difficile. Le séquençage Sanger reste utilisé lorsqu'il n'y a qu'un seul gène à séquencer.

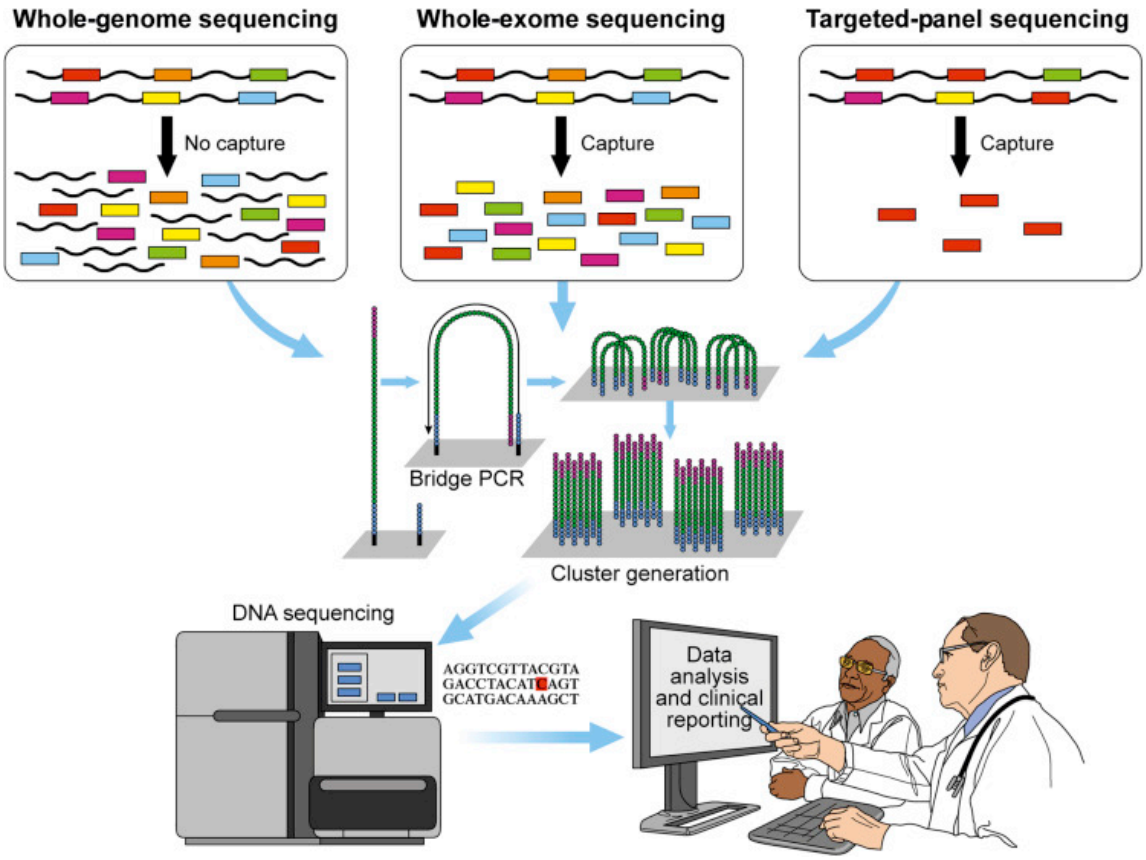

Figure 3: Différentes méthodes utilisées dans le NGS (WGS, WES, targeted-panel NGS). Dans le WGS, aucune étape de capture n'est nécessaire et toutes les régions intergéniques et intragéniques sont séquencées, tandis que le WES étudie uniquement les régions codantes avec l'utilisation de kits de capture d'exome. (Klein et al, 2017)

Pour que le séquençage à haut débit soit fiable, il est nécessaire d'obtenir une bonne couverture des gènes d'intérêt afin de minimiser le risque de manquer une mutation pathogène. La profondeur du séquençage correspond au nombre de séquences obtenues pour une même région. La couverture est le pourcentage de bases séquencées par rapport au nombre de bases totales. Les données du séquençage à haut débit permettent de s'assurer de la qualité du séquençage en donnant les valeurs de profondeur et de couverture. Afin d'obtenir une couverture et une profondeur suffisante, une étape d'enrichissement d'exons est nécessaire. On distingue l'enrichissement par capture d'hybrides: la capture par hybridation des régions cibles est initiée à partir de librairies de séquençage; et l'enrichissement par circularisation de fragments d'ADN: en amont de la préparation de librairies, les fragments d'ADN sont enrichis via une sonde constituée d'une séquence universelle flanquée aux extrémités de séquences spécifiques de la région cible, permettant la circularisation de ces séquences cibles et leur capture à l'aide de la séquence universelle. (Mertes et al. 2011)

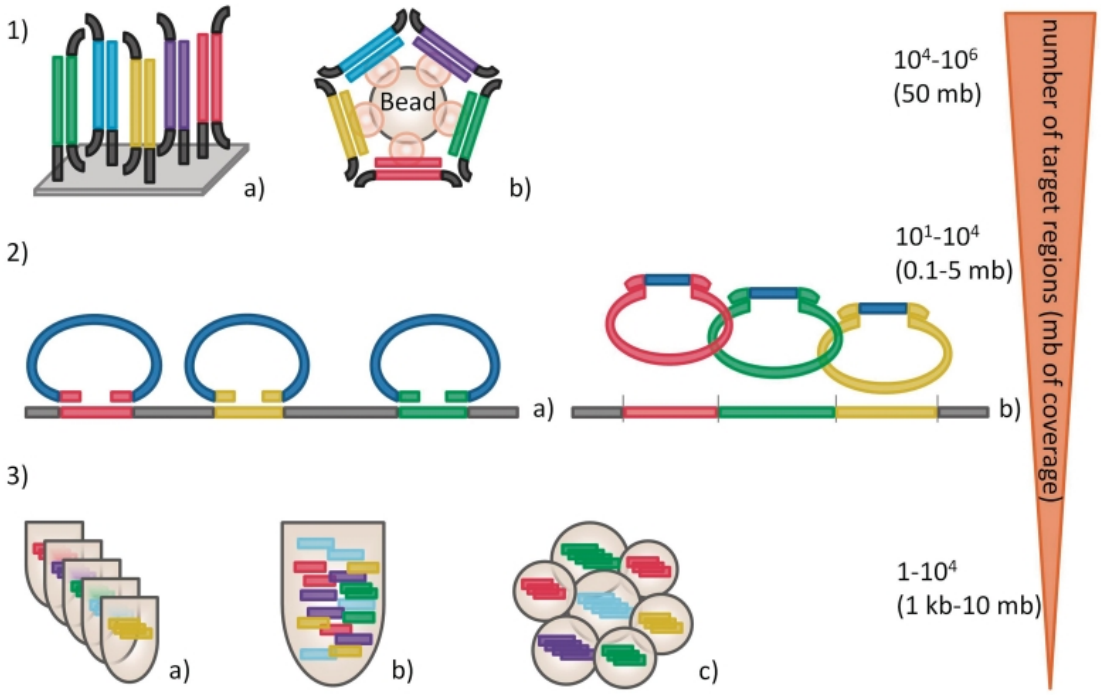

Figure 4: Techniques d'enrichissement ciblées utilisées dans le NGS:

(1) Enrichissement ciblé par capture hybride: Elle peut être effectuée soit (a) sur support solide (microarrays), soit (b) en solution (billes magnétiques). Après hybridation (et couplage des billes), les séquences non ciblées sont éliminées par lavage, l'échantillon enrichi peut alors être élué et séquencé.

(2) Enrichissement par circularisation de fragments d'ADN: les fragments d'ADN (fragmentation enzymatique ou mécanique selon la méthode) sont enrichis via une sonde constituée d'une séquence universelle flanquée aux extrémités de séquences spécifiques de la région cible.

(3) Enrichissement par PCR qui intervient avant la préparation de la librairie et consiste en une PCR multipléxée ciblant les régions d'intérêts. Une étape préliminaire de design des amorces est requise. (Mertes et al, 2011)

Avec le NGS, nous assistons à une explosion des données et des connaissances nécessitant pour chaque pathologie/gène de fonctionner en réseau en s'appuyant d'une part sur l'expertise des cliniciens et d'autre part sur des biologistes référents. Cette coordination est nécessaire pour s'assurer de la qualité de l'interprétation des nombreux résultats générés qui doivent s'appuyer sur des analyses bio-informatiques robustes.

1.4. Objectifs de la thèse

Les objectifs de cette thèse étaient la description phénotypique d'ataxies cérébelleuses héréditaires, la mise en évidence de corrélations du génotype au phénotype et la description de stratégies diagnostiques de ces affections rares.

Notre première étude avait pour objectif de montrer l'importance d'un signe radiologique particulier, l'hypersignal du splénium du corps calleux, dans le FXTAS et de confirmer que ce signe devait être considéré comme un critère radiologique majeur pour le diagnostic de cette pathologie.

Puis, nous avons étudié un exemple de mutations hypomorphes dans les ataxies cérébelleuses récessives à savoir des mutations dans le gène *PEX10* avec un phénotype modéré. Le gène *PEX10* est habituellement impliqué dans des pathologies de la biosynthèse du peroxysome relativement graves menant rapidement au décès.

Nous nous sommes ensuite intéressés à une forme particulière d'ataxie cérébelleuse récessive: l'ataxie avec apraxie oculomotice de type 1 (AOA1). Notre étude visait à étudier les caractéristiques cliniques, paracliniques (IRM cérébrale, EMG, biomarqueurs) et moléculaires d'une population de 80 patients AOA1 pour lesquels un

séquençage d'*APTX* (gène en cause dans AOA1) avait été effectué dans le laboratoire du Pr Michel Koenig depuis 2002.

Nos deux dernières études portent sur la validation et le développement de l'algorithme proposé par Anheim et *al*. en 2012 dans le New England Journal of Medicine pour le diagnostic des ataxies héréditaires, en proposant notamment un algorithme automatisé, informatisé et testé sur une large cohorte internationale de 834 patients souffrant d'ataxies cérébelleuses récessives.

2. Résultats

2.1. Apport de l'hypersignal du splénium du corps calleux dans le diagnostic de FXTAS : étude d'une série multicentrique de 22 cas

2.1.1. Le FXTAS

Le FXTAS (Fragile X-associated Tremor ataxia Syndrome) est une pathologie neurodégénérative complexe, liée à l'X caractérisée par une ataxie cérébelleuse progressive et un tremblement d'action, due à une prémutation du gène *FMR1*.

Les mutations complètes de *FMR1*, correspondant à une expansion du nombre de triplets CGG supérieure à 200 (sur les allèles normaux, la répétition est inférieure à 50 triplets CGG), sont à l'origine du syndrome X-fragile, cause la plus fréquente de retard mental héréditaire, touchant principalement les garçons.

Les prémutations de *FMR1* correspondent à une répétition de 55 à 200 triplets et prédisposent les individus porteurs à développer le FXTAS. La pénétrance du FXTAS augmente avec l'âge et le nombre de triplets CGG. Le FXTAS débute en moyenne vers l'âge de 60 ans, le plus souvent par un tremblement d'action touchant les membres supérieurs, puis survient une ataxie cérébelleuse. (Hagerman et al., 2001, Hagerman et al., 2013, Jacquemont et al., 2003, Berry-Kravis et al., 2004)

Le tremblement est présent chez 80 à 90% des patients, et se présente comme un tremblement essentiel-like, un tremblement cérébelleux ou un tremblement parkinsonien. (Apartis et al., 2012) Un syndrome parkinsonien allant de la simple amimie au syndrome akinéto-rigide avec tremblement de repos est observé dans 30 à 60% des cas. Quelques cas de dopasensibilité ont été rapportés. (Hagerman et al., 2001)

Une neuropathie périphérique est présente chez 50 à 80% des patients. Il peut s'agir d'une neuropathie sensitive axonale non longueur dépendante de type ganglionopathie ou d'une neuropathie à prédominance sensitive axonale longueur dépendante (Soontarapornchai et al., 2001). Une détérioration cognitive avec syndrome sous-cortico-frontal est mise en évidence chez la moitié des patients FXTAS. Les troubles cognitifs peuvent être inauguraux. (Sevin et al., 2009)

L'évolution est marquée par une perte de la marche vers l'âge de 75 ans, puis le décès survenant vers 80 ans, alors que le patient est alité, dysarthrique, parkinsonien et incontinent. (Berry Kravis et al., 2007)

Examination and degree	
Molecular	
Major	55–200 CGG repeats (premutation)
Radiological	
Major	MRI white matter lesions in middle cerebellar peduncle
Minor	MRI white matter lesions in cerebral white matter
Minor	Moderate-to-severe generalised atrophy
Clinical	
Major	Intention tremor
Major	Gait ataxia
Minor	Parkinsonism
Minor	Moderate-to-severe short-term memory deficit
Minor	Executive function deficit
Neuropathological	
Major	FXTAS inclusions
Diagnostic category	
Presence of expanded CGG repeat (molecular), and	
Definite	Presence of one major radiological sign plus (i) one major clinical symptom or (ii) the presence of FXTAS inclusions
Probable	Presence of one major radiological sign and one minor clinical symptom, or two major clinical symptoms
Possible	Presence of one minor radiological sign and one major clinical symptom

Tableau 1: Critères diagnostiques du FXTAS selon Hagerman et Hagerman en 2013.

L’IRM cérébrale constitue un outil majeur pour le diagnostic de FXTAS. L’hypersignal des pédoncules cérébelleux moyens (PCM) est un signe radiologique classique mais non spécifique dans ce contexte. On le retrouve dans d’autres causes d’ataxie comme l’atrophie multi-systématisée, la xanthomatose cérébro-tendineuse ou encore dans des mutations du gène *POLG1*. Il est par ailleurs inconstant puisqu’il est absent chez près de 40% des patients FXTAS. (Leehey et al., 2009) Les hypersignaux de la substance blanche périventriculaire sont fréquents mais non spécifiques, de même que l’atrophie cérébrale globale. D’autres signes radiologiques peuvent être rencontrés comme un hypersignal du tronc cérébral, des noyaux dentelés, une atrophie du corps calleux ou du cervelet (Jacquemont et al., 2003). En 2012, Apartis et al. relève la présence d’un hypersignal du splénium du corps calleux chez des patients FXTAS. Ce signe semble alors au moins aussi fréquent que l’hypersignal des PCM et faciliterait le diagnostic dans certains cas atypiques, notamment chez les femmes ou lorsque le tremblement manque.

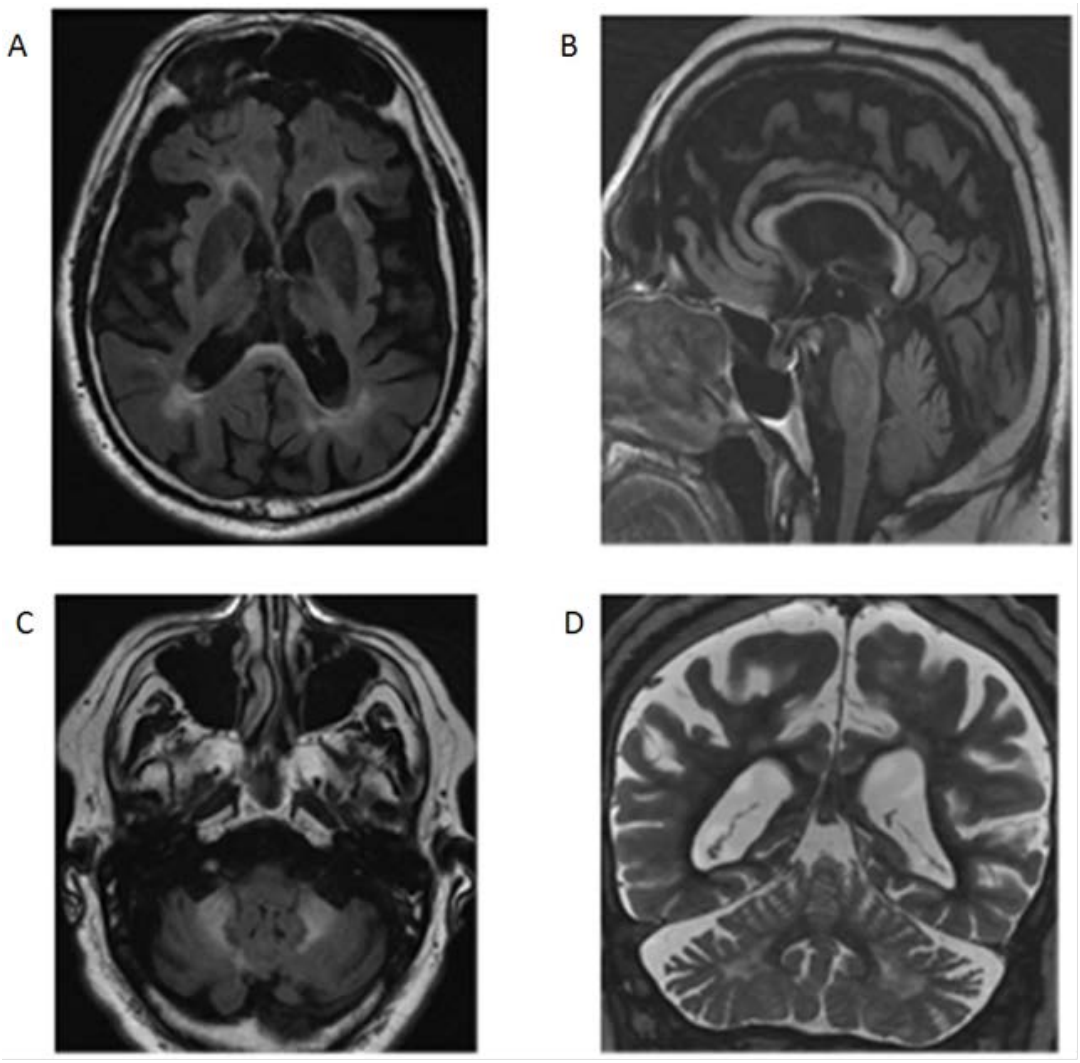

Figure 5: IRM cérébrale avec coupes axiales et sagittale en séquence FLAIR (A, B et C) et coupe coronale en séquence T2 (D) de patients FXTAS: noter l'atrophie cortico-sous-corticale diffuse (A, B et D) avec atrophie du corps calleux (A et B) et cérébelleuse (B et D), l'hypersignal du splénium du corps calleux (A et B), des pédoncules cérébelleux moyens (C) et des noyaux dentelés (D) ainsi que les anomalies sévères de la substance blanche périventriculaire (A).

2.1.2. Manuscrit 1

Relevance of corpus callosum splenium versus middle cerebellar peduncle hyperintensity for FXTAS diagnosis in clinical practice.

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ORIGINAL COMMUNICATION

Relevance of corpus callosum splenium versus middle cerebellar peduncle hyperintensity for FXTAS diagnosis in clinical practice

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Abstract Fragile X-associated tremor ataxia syndrome (FXTAS) is caused by *FMR1* premutation. The features include ataxia, action tremor and middle cerebellar peduncle (MCP) hyperintensity, the latter being the only major radiological criterion in the diagnosis of definite FXTAS until very recently. The importance of corpus callosum splenium (CCS) hyperintensity was recently reported and this sign is now considered as an additional major radiological diagnostic criterion in the diagnosis of FXTAS. However, little is known about its relevance for the diagnosis of FXTAS in clinical practice. We report a practical justification of the relevance of CCS hyperintensity in parallel with MCP hyperintensity for the diagnosis of FXTAS. Clinical and radiological study of 22 *FMR1* premutation carriers with neurological signs that may be encountered in FXTAS compared to series of patients with essential tremor, multiple system atrophy of cerebellar type, Parkinson's disease, Alzheimer's disease and stroke. Among the 22 patients with *FMR1* premutation [17 men, 5 women; mean age, 63 ± 7.5 (46–84)], 14 were diagnosed with definite FXTAS with the initial criteria. Considering CCS hyperintensity as a new major radiological criterion permitted the diagnosis of definite FXTAS in 3 additional patients. Overall CCS proved as frequent as MCP hyperintensity (64 versus 64 %), while 23 % of patients had CCS but not MCP hyperintensity, 14 % of patients had CCS hyperintensity but neither MCP, nor brainstem hyperintensity. In contrast with CCS hyperintensity, MCP hyperintensity proved less frequent in women than in men.

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CCS and MCP hyperintensity were more frequent in FXTAS than in the other neurodegenerative disorders. The combination of CCS and MCP hyperintensity was specific of FXTAS. We confirmed the relevance of CCS hyperintensity in FXTAS and we clarified its interest compared to MCP hyperintensity. Our results support the inclusion of CCS hyperintensity in the diagnostic criteria as a new major radiological criterion.

Keywords FXTAS · Ataxia · Tremor · Corpus callosum · Criteria

Introduction

Fragile X-associated tremor ataxia syndrome (FXTAS) is an X-linked disorder due to *FMR1* premutation, which corresponds to a repeat expansion between 55 and 200 CGG triplets [1–3]. Full mutations (≥200 CGG) of *FMR1* cause fragile X syndrome (FXS). FXTAS is a complex neurodegenerative disease which affects men (more frequently than women) with the premutation occurring between 50 and 80 years of age with an average age at onset of around 60 years. It is characterized by progressive cerebellar ataxia and action tremor with classical but optional middle cerebellar peduncle (MCP) FLAIR hyperintensities [1–7]. Seemingly neglected due to its broad phenotypic spectrum, [8] it is crucial in clinical practice to diagnose FXTAS for an optimum management of the patient and subsequent genetic counselling of the relatives. The diagnosis of FXTAS relies on a combination of major and minor clinical and MRI criteria. In the initial criteria, the only major radiological criterion considered in the diagnosis of definite FXTAS was MCP and/or brainstem hyperintensity, signs that can nevertheless be lacking in up to 30 % of FXTAS patients [2] and that are not specific of FXTAS [9]. We recently reported that a hyperintensity of the corpus callosum splenium (CCS) could assist in identifying more FXTAS patients and if effective should then be considered as a novel major radiological criterion [10].

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More recently, the criteria for the diagnosis of FXTAS have been revised by an expert group, including CCS as an additional major radiological criterion [11]. Our aim here is to report a practical justification of the relevance of CCS hyperintensity in parallel with MCP hyperintensity for the diagnosis of FXTAS in a new cohort of patients, to support the revision of the diagnosis criteria for FXTAS and to better define the value of the CCS sign.

Methods

Between April 2012 and July 2013 we included in the study the patients that were consecutively referred in 7 French neurology centres (Strasbourg, Montpellier, Marseille, Bordeaux, Nantes, Grenoble and Saint Malo) demonstrating the combination of *FMR1* premutation and neurological signs that may be encountered in FXTAS. No preferential recruitment of families expressing FXS was made [2, 8].

Clinical examination

Clinical data were collected via retrospective review of the patients' records. Neurological examination was performed by a neurologist experienced in the field of movement disorders. Examination included a comparative Scale for the Assessment and Rating of Ataxia (SARA) which has been validated for inherited ataxias [12], frontal assessment battery (FAB) [13] and mini-mental state examination (MMSE) [14] scores.

Brain MRI

Brain MRI were recorded for the 22 suspected FXTAS patients as well as for 19 patients consecutively diagnosed with multiple system atrophy of cerebellar type (MSA-C) who were negative for *FMR1* premutation, 24 patients consecutively diagnosed with essential tremor (ET) who were negative for *FMR1* premutation, 30 patients consecutively diagnosed with Parkinson's disease (PD), 22 patients consecutively diagnosed with Alzheimer's disease (AD) and 30 patients consecutively diagnosed with stroke. A comparative, qualitative analysis was performed especially regarding the hyperintensities of periventricular white matter, of the pons, the MCP and the CCS in all these groups. All patients underwent 1.5 T MRI including T1, T2-weighted, and T2 FLAIR images, the latter being considered as the standard for the identification of white matter hyperintensities in FXTAS [10]. Image acquisition was performed in axial, sagittal and coronal planes. The FXTAS patients underwent the MRI in the center they were referred to and the neuroradiologist of each center blindly red the MRI. All the MRI were then read by a

neurologist expert in the field of FXTAS. The MRI of the MSA-C, ET, PD, AD and stroke were performed in Strasbourg and assessed by a neuroradiologist and then by a neurologist.

Electromyography

Electromyography was performed as previously described [10].

FMR1 molecular analysis

PCR reactions were realized as previously reported [15] with primer kit PR155 and PR156; PCR products were further analysed by automated fragment analysis in a 3730*xl* DNA Analyser (Applied Biosystems, Foster City, CA, USA), for assessment of repeat number. Alleles with 55-199 CGG repeats were defined as premutation alleles.

FXTAS diagnostic category

With respect to the clinical, radiological and molecular findings, the diagnosis of FXTAS was established according to the initial FXTAS diagnostic category [2, 8] (with MCP and/or brainstem hyperintensity as the only major radiological criterion) as well as according to the recently proposed new diagnosis criteria taking into account CCS hyperintensity as an additional major radiological criteria [10, 11].

Statistical methods

The statistical analyses included both descriptive and analytical sections. The descriptive section presented the frequency of each value and the cumulative frequency for the qualitative variables along with the mean and standard deviation for the quantitative variables. For the quantitative variables, normality of distribution was verified using the Shapiro–Wilk test. The analytical section was performed under the Bayesian paradigm. Comparisons between qualitative variables were made using logistical regressions while those between quantitative and qualitative variables used linear regressions. Variables that had a bivariate 95 % posterior probability to be different from 0 were considered for multivariate analyses. The analyses were made with R 3.0.0 software and with R2WinBUGS package. Comparisons between different groups (FXTAS, MSA-C, ET, PD, AD and stroke) were performed with χ^2 test.

Standard protocol approvals, registrations and patient consents

Local ethics committees approved the study and all participants being informed gave written consent.

Results

Twenty-two patients (17 men; 5 women) were included.

Demographics and clinical characteristics

The main demographic, clinical, molecular and radiological data are presented in Table 1. While CCS hyperintensity was found in 11/17 men and 3/5 women, MCP hyperintensity was found in 14/17 men and 0/5 women and brainstem hyperintensity found in 7/17 men and 2/5 women (2 patients had brainstem hyperintensity but no MCP hyperintensity and finally 14/17 men and 2/5 women had MCP and/or brainstem hyperintensity). MCP hyperintensity was more frequent in men than in women, while for CCS hyperintensity the gender ratio approached unity. According to the initial criteria of Jacquemont et al. [2] the diagnosis of FXTAS was definite in 14 patients (13 men and one woman), probable in 4 patients (2 men and 2 women) and possible for one woman. Three patients did not meet the criteria of possible FXTAS (2 men and one woman) even if they had both neurological signs that may be encountered in FXTAS (cognitive decline and/or fibromyalgia for instance) [2], abnormal MRI findings (CSS or MCP hyperintensity) and *FMR1* premutation. Considering CCS hyperintensity as a new major clinical sign [11], the diagnosis of FXTAS was definite in 17 patients (15 men and 2 women), probable in 2 patients (2 women), possible for no patients. Two men and one woman still could not meet the criteria for possible FXTAS.

Radiological and neurophysiologic results

The principal radiological and neurophysiological data of premutation carriers are presented in Table 1 and Fig. 1. The neuroradiologists and the neurologists found the same abnormalities in all patients. The results of the comparison between FXTAS and the neurodegenerative diseases (MSA-C, ET, PD and AD) as well as stroke are provided in Table 2. The combination of CCS and MCP hyperintensity was found in 41 % of FXTAS patients and was more frequent in FXTAS than in ET ($p < 0.001$), PD ($p < 0.001$), AD ($p < 0.001$), MSA-C ($p = 0.001$) and stroke ($p < 0.001$). In two FXTAS patients, the CCS hyperintensity affected the lower part of the splenium of the corpus callosum (Fig. 1c), which was never found in the patients with MSA-C, ET, PD, AD and stroke.

Correlation between clinical, morphologic and molecular data

Middle cerebellar peduncle hyperintensity was more frequent in men than in women [OR = 50.55, 95 % CI

Table 1 Main clinical, neurophysiological, morphological and molecular findings of the 22 *FMR1* premutation carriers

	Values
Sex ratio (M/F)	3.4 (17/5)
Age at onset, years, mean ± SD (range)	63 ± 7.5 (46–84)
Current age, years, mean ± SD (range)	68 ± 7.4 (50–87)
Disease duration, years, mean ± SD (range)	5.5 (1–13)
CGG repeat expansion, mean ± SD (range)	95 ± 11.3 (81–127)
No family history of FXS or mental retardation, %	32 ($n = 7$)
FXPOI (fragile X-associated primary ovarian insufficiency), %	4.5 ($n = 1$)
Thyroid dysfunction, %	18 ($n = 4$)
Family history of POI, %	9 ($n = 2$)
Vascular risk factors	
Hypertension, %	23 ($n = 5$)
Diabetes mellitus, %	14 ($n = 3$)
Dyslipidemia, %	27 ($n = 6$)
Smoking, %	18 ($n = 4$)
Stroke, %	0
Migraine, %	9 ($n = 2$)
Coronary heart disease, %	9 ($n = 2$)
Initial sign	
Ataxia, %	45 ($n = 10$)
Isolated ataxia, %	23 ($n = 5$)
Action tremor, %	41 ($n = 9$)
Ataxia and action tremor, %	23 ($n = 5$)
Isolated action tremor, %	18 ($n = 4$)
Isolated parkinsonism, %	4.5 ($n = 1$)
Isolated cognitive impairment, %	18 ($n = 4$)
Isolated dysautonomia, %	4.5 ($n = 1$)
Current clinical signs	
Ataxia, %	86 ($n = 19$)
SARA score, ±SD (range), ($n = 10$)	5/40 ± 4.7 (0–13)
Tremor, %	68 ($n = 15$)
Postural/action/intention tremor, %	63.5 ($n = 14$)
Rest/postural tremor, %	4.5 ($n = 1$)
Cognitive impairment, %	36 ($n = 8$)
MMSE score, mean ± SD (range), ($n = 9$)	27.5/30 ± 3.3 (23–30)
FAB score, mean ± SD (range), ($n = 7$)	16.5/18 ± 1.2 (15–18)
Parkinsonism, %	23 ($n = 5$)
Extensor plantar reflex, %	9 ($n = 2$)
Absent achillean tendon reflex, %	23 ($n = 5$)
Ankle vibration sense loss, %	36 ($n = 8$)
EMG findings ($n = 11$)	
Normal, %	45 ($n = 5$)
Length-dependent sensorimotor neuropathy, %	45 ($n = 5$)
Small fiber sensory neuropathy, %	10 ($n = 1$)
Brain MRI ($n = 22$)	
Normal, %	0
Middle cerebellar peduncle hyperintensities, %	64 ($n = 14$)
Corpus callosum splenium hyperintensity, %	64 ($n = 14$)
Corpus callosum splenium and middle cerebellar peduncle hyperintensities, %	41 ($n = 9$)
Corpus callosum splenium or middle cerebellar peduncle hyperintensities, %	86 ($n = 19$)
Corpus callosum splenium without middle cerebellar peduncle hyperintensities, %	23 ($n = 5$)
Brainstem hyperintensity, %	41 ($n = 9$)
Middle cerebellar peduncle and/or brainstem hyperintensity, %	72 ($n = 16$)
Corpus callosum splenium with neither middle cerebellar nor brainstem hyperintensity, %	14 ($n = 3$)
Dentate nucleus hyperintensities, %	36 ($n = 8$)
Periventricular white matter hyperintensities, %	82 ($n = 18$)
Cerebral atrophy, %	91 ($n = 20$)
Cerebellar atrophy, %	77 ($n = 17$)

FAB frontal assessment battery, *FXS* fragile X syndrome, *FXTAS* fragile X-associated tremor ataxia syndrome, *MMSE* mini-mental state examination, *POI* primary ovarian insufficiency, *SARA* Scale for the Assessment and Rating of Ataxia

(4.300–991.459), PP (OR > 1) = 99.97 %], which proved untrue for CCS hyperintensity [OR = 1.20, 95 % CI (0.153–8.727), PP (OR > 1) = 57.59 %].

MCP hyperintensity was more frequent in patients with initial tremor [OR = 9.65, 95 % credibility interval (CI) (1.226–120.868), posterior probability (PP) (OR > 1) = 98.51 %].

The variables which were negatively associated with the size of the CGG repeat expansion were the age at diagnosis [$\beta = -0.611$, 95 % CI (−1.090 to −0.103), PP ($\beta > 0$) = 1.03 %] and the age at onset [$\beta = -0.332$, 95 % CI (−0.587 to −0.079), PP ($\beta > 0$) = 0.57 %]. The size of the CGG repeat expansion was correlated with initial parkinsonism [$\beta = 17.17$, 95 % CI (1.858–31.200), PP ($\beta > 0$) = 98.51 %].

Discussion

In our series, CCS hyperintensity proved as frequent as MCP hyperintensity. Consideration for the inclusion of CCS as another major radiological sign in the diagnosis of FXTAS is supported by the identification of 5 patients who

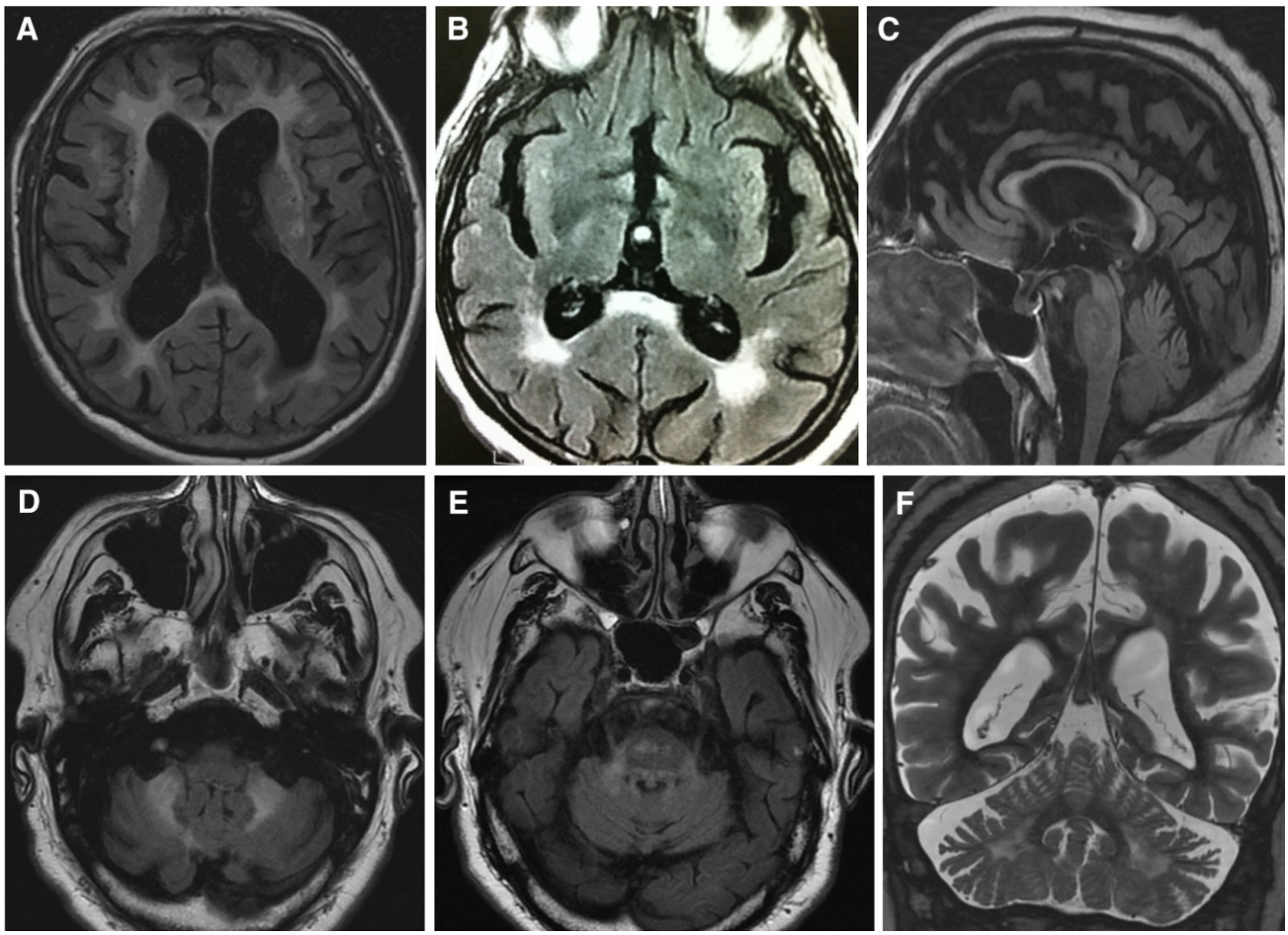

Fig. 1 Main brain MRI abnormalities in FXTAS patients. **a** Corpus callosum splenium (CCS) hyperintensity with leukoencephalopathy and cerebral atrophy (FLAIR). **b** Diffuse corpus callosum hyperintensity with periventricular white matter hyperintensities (axial FLAIR). **c** Corpus callosum splenium (CCS) hyperintensity with marked cerebral and corpus callosum atrophy, moderate cerebellar atrophy (sagittal FLAIR). **d** Bilateral middle cerebellar peduncle hyperintensities (axial FLAIR). **e** Pons hyperintensities (axial FLAIR). **f** Bilateral dentate nuclei hyperintensities and moderate cerebellar atrophy (coronal T2-weighted)

were negative for MCP hyperintensity yet were authentic FXTAS cases (23 % of patients in our series).

Equally, using this radiological sign as a major criterion, 3 more premutation carriers were diagnosed with definite FXTAS which led to the initiation of genetic counselling for these supplementary patients as well as their relatives, thus improving their management in clinical practice. The presence of CCS and/or MCP hyperintensity appears to be a sensitive morphological marker for FXTAS. The combination of MCP and CCS hyperintensities, not at all uncommon, could be very specific for FXTAS (almost pathognomonic). The CCS hyperintensity is suggestive of FXTAS in patients with chronic neurological signs such as ataxia, tremor, Parkinsonism and/or cognitive decline since the sign has been less frequently identified in neurodegenerative diseases such as MSA-C, ET, PD and AD, respectively. *FMR1* premutation was excluded in the ET control group as well as in MSA-C but not in PD and AD, even if it has been reported that *FMR1* premutation were particularly scarce in all these diseases. Regarding the frequency of CCS hyperintensity, only a trend towards a difference between FXTAS and stroke has been found probably due to the small size of the cohort. Moreover, both patients with FXTAS and stroke shared frequent vascular risk factors (although hypertension was more frequent in stroke). Vascular risk factors are common in FXTAS due to the mean age at onset, the sex ratio and the frequent combination with hypertension, sleep apnoea and cardiac arrhythmia [8]. Whether vascular risk factors contribute to the appearance of neurological signs in FXTAS remains to be studied. Finally, CCS hyperintensity may also be seen in some patients with stroke in the same way that MCP hyperintensity may be encountered in MSA-C. However, stroke is not a differential diagnosis of FXTAS in clinical practice, which is not true for MSA-C. Taken together, these findings strongly support the addition

Table 2 Results of the comparative study between MRI findings in FXTAS and in essential tremor, multiple system atrophy of cerebellar type, Parkinson's disease, Alzheimer's disease and stroke

	Age, years, mean (range)	Age at onset, years, mean (range)	Clinical scores (mean, range)	PWM hyperintensities, %	Brainstem hyperintensity, %	MCP hyperintensities, %	CCS hyperintensity, %	MCP + CCP hyperintensities, %
FXTAS n = 22 patients (17 M/ 5 F)	**68 (50–79)**	**63 (46–84)**	**SARA: 5 (0–13)**	**82 (*n* = 18)**	**41 (*n* = 9)**	**64 (*n* = 14)**	**64 (*n* = 14)**	**41 (*n* = 9)**
ET $n = 24$ patients (10 M/14 F)	69 (60–79)	54 (12–77)	TETRAS score performance subscale: 8.04 (1–18)	33 ($n = 8$) **$p \leq 0.001$**	13 ($n = 3$) **$p = 0.04$**	0 **$p \leq 0.001$**	4 ($n = 1$) **$p \leq 0.001$**	0 **$p \leq 0.001$**
MSA-C $n = 19$ patients (9 M/10 F)	63 (47–78)	60 (44–77)	SARA score: 13 (6–22.5)	47 ($n = 9$) **$p = 0.02$**	26 ($n = 5$) $p = 0.354$	16 ($n = 3$) **$p = 0.002$**	26 ($n = 5$) **$p = 0.017$**	0 **$p = 0.001$**
PD $n = 30$ patients (17 M/13 F)	68 (64–72)	56 (49–62)	UPDRS III score: 29 (10–48)	40 ($n = 12$) **$p = 0.003$**	17 ($n = 5$) $p = 0.06$	0 **$p \leq 0.001$**	0 **$p \leq 0.001$**	0 **$p \leq 0.001$**
AD $n = 22$ patients (12 M/17 F)	70 (63–75)	NA	MMSE score: 24 (20–26)	14 ($n = 3$) **$p \leq 0.001$**	0 **$p = 0.002$**	0 **$p \leq 0.001$**	23 ($n = 5$) **$p = 0.006$**	0 **$p \leq 0.001$**
Stroke $n = 30$ patients (20 M/ 10 F)	69 (61–75)	69 (61–75)	HTN: 53 % ($n = 16$) **$p = 0.026$** DB: 14 % ($n = 4$) $p = 0.975$ Dyslipidemia: 33 % ($n = 10$) $p = 0.64$ Smoking: 40 % ($n = 12$) $p = 0.09$ Migraine: 3 % ($n = 1$) $p = 0.774$ CHD: 10 % ($n = 3$) $p = 0.913$	33 ($n = 10$) **$p \leq 0.001$**	7 ($n = 2$) **$p = 0.004$**	3 ($n = 1$) **$p \leq 0.001$**	44 ($n = 11$) $p = 0.131$	3 ($n = 1$) **$p \leq 0.001$**

Bold values indicate statistical significance ($p < 0.05$)

CCS corpus callosum splenium, *CHD* coronary heart disease, *DB* diabetes mellitus, *ET* essential tremor, *HTN* hypertension, *NA* not available, *MCP* middle cerebellar peduncle, *MMSE* mini-mental state examination, *MSA-C* multiple system atrophy of cerebellar type, *PD* Parkinson's disease, *AD* Alzheimer's disease, *PWM* periventricular white matter, *SARA* Scale for the Assessment and Rating of Ataxia, *TETRAS* The Essential Tremor Rating Assessment Scale (performance subscale), *UPDRS III* Unified Parkinson's Disease Rating Scale part III (motor evaluation)

of CCS hyperintensity to the diagnosis criteria as a new major MRI criterion [10].

In our cohort, MCP hyperintensity was found only in 16 % of MSA-C possibly because patients had a mean evolution of only 3 years. Thus, MCP was found to be more frequent in FXTAS than in MSA-C which needs to be confirmed by further larger prospective studies. Whereas MCP hyperintensity was observed in only 13 % of FXTAS women in a previous series [16], no women in our study presented MCP hyperintensity. Alternately, 3 of the 5 women of our series had CCS hyperintensity. This finding further strengthens the interest of the CCS sign in the clinical practice and especially for the diagnosis of women with FXTAS. Interestingly, in our series MCP hyperintensity was correlated with tremor as the first sign of FXTAS. As the MCP carry the cortico-pontine afferences to the cerebellum, they could be responsible for action tremor when damaged.

Herein, brainstem hyperintensities was found to be equally frequent in FXTAS and in MSA-C and PD which may be considered as differential diagnosis of FXTAS. This is consistent with the fact that brainstem hyperintensity is only a minor radiological criterion.

Tremor may be lacking in FXTAS. Among our patients, 23 % had initial isolated ataxia and 18 % initial isolated mild cognitive impairment. So, FXTAS should be searched for in the case of mild cognitive impairment in the elderly especially if brain MRI demonstrates CCS and/or MCP hyperintensity.

In our series, 55 % of patients had polyneuropathy, which is close to other studies [17] and supports polyneuropathy as minor FXTAS clinical criteria [11]. Indeed, polyneuropathy may be the first sign of the disease [17]. Herein, CGG repeat number was correlated with Parkinsonism as the first sign of the disease, which further highlights the value of Parkinsonism in FXTAS [1, 10].

Our series is limited because it was retrospective, multicentric, non exhaustive and based on only 22 new patients who underwent standard brain MRI though it should enhance support for the relevance of CCS hyperintensity as well as its inclusion in the diagnostic criteria as a novel major radiological criterion. A further and broader prospective study, using FXTAS motor rating scale [18], is required to strengthen and render more accurate these data. Moreover, more precise MRI investigations [19] are needed to learn whether some CCS, MCP and/or brainstem MRI abnormalities are due to normal ageing, or if a sub-group of these abnormalities are particularly suggestive of FXTAS. Indeed, the diagnosis of FXTAS is crucial in clinical practice for an optimum management of the patient and the provision of genetic counselling for the relatives.

Conflicts of interest The final manuscript has been read and approved by all authors who accepted full responsibility for the design and undertaking of the original article, had access to the data and controlled the decision to publish. MA declares conflict of interest with Novartis, Abbvie, TEVA/Lundbeck and Actelion. WM declares conflict of interest with TEVA/Lundbeck, Novartis, UCB, Affiris and GSK. The other authors have no conflict of interest to declare.

Ethical standard Local ethics committees approved the study and all participants being informed gave written consent.

References

1. Hagerman RJ, Leehey M, Heinrichs W, Tassone F, Wilson R, Hills J, Grigsby J, Gage B, Hagerman PJ (2001) Intention tremor, Parkinsonism, and generalized brain atrophy in male carriers of fragile X. Neurology 57:127–130
2. Jacquemont S, Hagerman RJ, Leehey M, Grigsby J, Zhang L, Brunberg JA, Greco C, Des Portes V, Jardini T, Levine R, Berry-Kravis E, Brown WT, Schaeffer S, Kissel J, Tassone F, Hagerman PJ (2003) Fragile X premutation tremor/ataxia syndrome: molecular, clinical, and neuroimaging correlates. Am J Hum Genet 72:869–878
3. Berry-Kravis E, Abrams L, Coffey SM, Hall DA, Greco C, Gane LW, Grigsby J, Bourgeois JA, Finucane B, Jacquemont S, Brunberg JA, Zhang L, Lin J, Tassone F, Hagerman PJ, Hagerman RJ, Leehey MA (2007) Fragile X-associated tremor/ataxia syndrome: clinical features, genetics, and testing guidelines. Mov Disord 22:2018–2030
4. Hall DA, Berry-Kravis E, Jacquemont S, Rice CD, Cogswell J, Zhang L, Hagerman RJ, Hagerman PJ, Leehey MA (2005) Initial diagnoses given to persons with the fragile X associated tremor/ataxia syndrome (FXTAS). Neurology 65:299–301
5. Leehey MA (2009) Fragile X-associated tremor/ataxia syndrome: clinical phenotype, diagnosis, and treatment. J Investig Med 57:830–836
6. Greco CM, Berman RF, Martin RM, Tassone F, Schwartz PH, Chang A, Trapp BD, Iwahashi C, Brunberg J, Grigsby J, Hessl D, Becker EJ, Papazian J, Leehey MA, Hagerman RJ, Hagerman PJ (2006) Neuropathology of fragile X-associated tremor/ataxia syndrome (FXTAS). Brain 129:243–255
7. Tassone F, Hagerman RJ, Taylor AK, Hagerman PJ (2001) A majority of fragile X males with methylated, full mutation alleles have significant levels of FMR1 messenger RNA. J Med Genet 38:453–456
8. Hagerman R, Hagerman P (2013) Advances in clinical and molecular understanding of the FMR1 premutation and fragile X-associated tremor/ataxia syndrome. Lancet Neurol 12:786–798
9. Ngai S, Tang YM, Du L, Stuckey S (2006) Hyperintensity of the middle cerebellar peduncles on fluid-attenuated inversion recovery imaging: variation with age and implications for the diagnosis of multiple system atrophy. AJNR Am J Neuroradiol 27:2146–2148
10. Apartis E, Blancher A, Meissner WG, Guyant-Marechal L, Maltete D, De Broucker T, Legrand AP, Bouzenada H, Thanh HT, Sallansonnet-Froment M, Wang A, Tison F, Roue-Jagot C, Sedel F, Charles P, Whalen S, Heron D, Thobois S, Poisson A, Lesca G, Ouvrard-Hernandez AM, Fraix V, Palfi S, Habert MO, Gaymard B, Dussaule JC, Pollak P, Vidailhet M, Durr A, Barbot JC, Gourlet V, Brice A, Anheim M (2012) FXTAS: new insights and the need for revised diagnostic criteria. Neurology 79:1898–1907
11. Hall DA, Birch R, Anheim M, Jonch AE, Pintado E, O'Keefe J, Troller J, Hagerman RJ, Fahn S, Berry-Kravis E, Leehey MA (2014) Emerging topics in FXTAS. J Neurodev Disord 6:31

12. Schmitz-Hubsch T, du Montcel ST, Baliko L, Berciano J, Boesch S, Depondt C, Giunti P, Globas C, Infante J, Kang JS, Kremer B, Mariotti C, Melegh B, Pandolfo M, Rakowicz M, Ribai P, Rola R, Schols L, Szymanski S, van de Warrenburg BP, Durr A, Klockgether T, Fancellu R (2006) Scale for the assessment and rating of ataxia: development of a new clinical scale. Neurology 66:1717–1720
13. Dubois B, Slachevsky A, Litvan I, Pillon B (2000) The FAB: a frontal assessment battery at bedside. Neurology 55:1621–1626
14. Folstein MF, Folstein SE, McHugh PR (1975) "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res 12:189–198
15. Fu YH, Kuhl DP, Pizzuti A, Pieretti M, Sutcliffe JS, Richards S, Verkerk AJ, Holden JJ, Fenwick RG Jr, Warren ST et al (1991) Variation of the CGG repeat at the fragile X site results in genetic instability: resolution of the Sherman paradox. Cell 67:1047–1058
16. Juncos JL, Lazarus JT, Graves-Allen E, Shubeck L, Rusin M, Novak G, Hamilton D, Rohr J, Sherman SL (2011) New clinical findings in the fragile X-associated tremor ataxia syndrome (FXTAS). Neurogenetics 12:123–135
17. Baba Y, Uitti RJ (2005) Fragile X-associated tremor/ataxia syndrome and movements disorders. Curr Opin Neurol 18:393–398
18. Leehey MA, Berry-Kravis E, Goetz CG, Zhang L, Hall DA, Li L, Rice CD, Lara R, Cogswell J, Reynolds A, Gane L, Jacquemont S, Tassone F, Grigsby J, Hagerman RJ, Hagerman PJ (2008) FMR1 CGG repeat length predicts motor dysfunction in premutation carriers. Neurology 70:1397–1402
19. Battistella G, Niederhauser J, Fornari E, Hippolyte L, Gronchi Perrin A, Lesca G, Forzano F, Hagmann P, Vingerhoets FJ, Draganski B, Maeder P, Jacquemont S (2013) Brain structure in asymptomatic FMR1 premutation carriers at risk for fragile X-associated tremor/ataxia syndrome. Neurobiol Aging 34:1700–1707

2.1.3. Apport de notre étude dans le diagnostic de FXTAS

Notre première étude avait pour objectif de montrer l'importance de l'hypersignal du splénium du corps calleux (SCC) dans le FXTAS, notamment chez les femmes atteintes et de confirmer que ce signe devait être considéré comme un critère radiologique majeur pour le diagnostic de cette pathologie.

Nous avons inclus dans cette étude rétrospective, multicentrique, française, 22 patients dont 5 femmes, porteurs de la prémutation et présentant des symptômes évocateurs du FXTAS. L'hypersignal des pédoncules cérébelleux moyens (PCM) était noté chez 64% des patients, tout comme l'hypersignal du splénium du SCC. L'hypersignal des PCM pouvait être absent particulièrement chez les femmes. L'association d'un hypersignal des PCM à un hypersignal du SCC, présente chez 41% des patients, apparaît très spécifique, voire quasi-pathognomonique du FXTAS. Vu l'importance de l'hypersignal du SCC chez les patients FXTAS dans notre étude, nous en avons conclu qu'il devait être considéré comme un nouveau critère IRM majeur.

Notre étude rappelle également que le FXTAS est hétérogène, différent chez les hommes et les femmes et vraisemblablement sous-diagnostiqué, peut-être du fait d'un spectre phénotypique plus large qu'on ne le pensait initialement.

2.2. Ataxie récessive lentement progressive liée au gène *PEX 10* impliqué dans la biogénèse du peroxysome

2.2.1. Les maladies de la biogénèse du peroxysome

Le peroxysome est un organite omniprésent dans toutes les cellules eucaryotes jouant un rôle important dans le fonctionnement du système nerveux comme en témoignent les nombreuses atteintes neurologiques dans les maladies peroxysomales. L'intégrité des fonctions cérébelleuses repose sur un fonctionnement peroxysomal normal au cours du développement et à l'âge adulte (De Munter et al., 2015). Le peroxysome héberge d'importantes voies métaboliques des lipides: l'α-oxydation de l'acide phytanique, la β-oxydation de l'acide pristanique et des acides gras à très longues chaînes (AGTLC), la synthèse des plasmalogènes et la conversion du cholestérol en acides biliaires. Le peroxysome prend en charge de manière préférentielle les AGTLC, les acides gras polyinsaturés, les acides gras ramifiés (acide pristanique). Les acides gras à courtes, moyennes et longues chaînes sont plutôt les substrats de la mitochondrie. L'acide phytanique est un acide gras d'origine végétale apporté par l'alimentation. Il possède un groupement 3-méthyl l'empêchant de subir directement la β-oxydation. Il doit préalablement être transformé par α-oxydation en un acide 2-méthylé: l'acide pristanique (Wanders and Waterham, 2006).

La biogenèse des peroxysomes comprend la constitution de la bicouche lipidique, l'insertion des protéines membranaires dans cette bicouche et l'importation des protéines dans la matrice (Sacksteder and Gould, 2000). Les peroxines sont les protéines de la membrane peroxysomale. Elles sont codées par 14 gènes *PEX* (*PEX1, 2,*

4, 6, 7, 8, 10, 12, 13, 14, 15, 17, 22, 26) et interviennent dans l'importation des protéines matricielles.

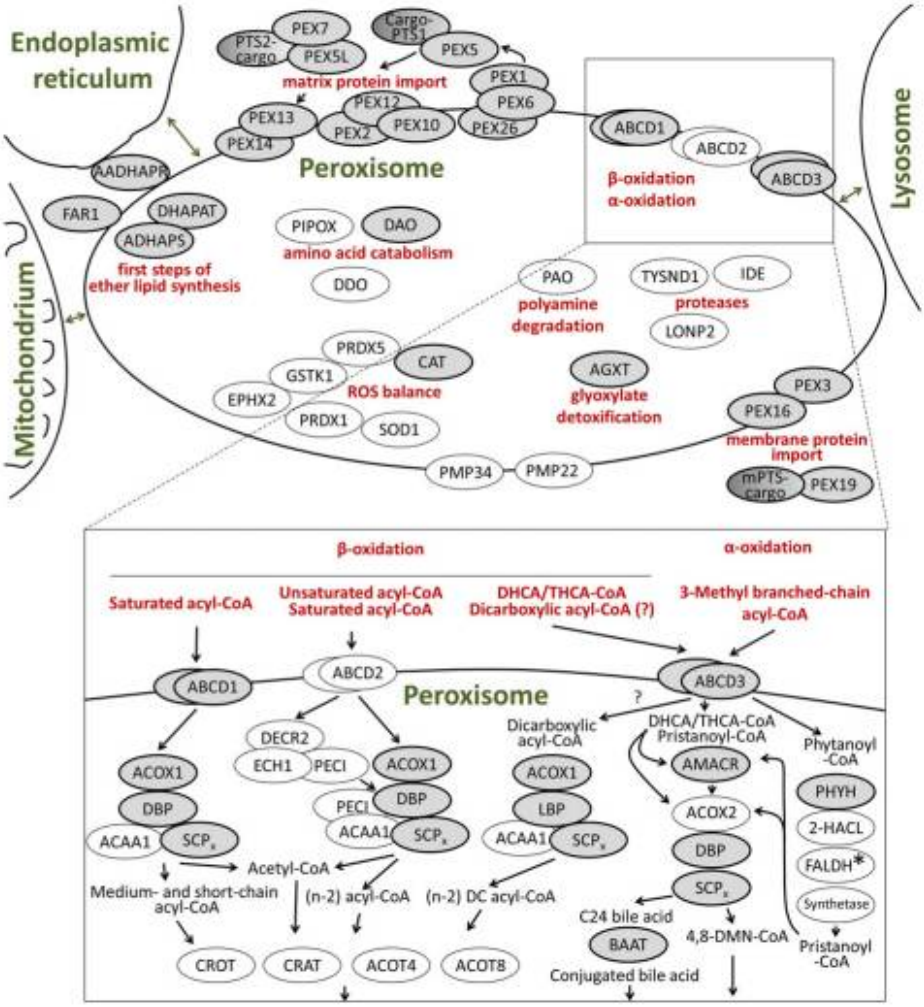

Figure 6: Schéma des principales protéines et voies peroxysomales.

Les protéines sont regroupées selon leur fonction dans les voies métaboliques: biosynthèse, transport des métabolites à travers la membrane peroxysomale (protéines ABCD et PMP), importation de protéines matricielles et membranaires (protéines PEX), dégradation des différents acides gras activés (acyl-CoA saturés, insaturés, dicarboxyliques, à chaîne ramifiée) par l' α-oxydation ou la β-oxydation. Les ovales gris représentent des protéines pour lesquelles des mutations ont été mises en évidence dans des pathologies chez l'homme. (Berger et al, 2015)

Les maladies peroxysomales sont classées en troubles de la biogenèse du peroxysome (PBD) impliquant l'un des 14 gènes codant pour les peroxines (Braverman et al., 2013) et en déficits isolés d'enzymes de la matrice peroxysomale impliquées dans le métabolisme des lipides.

Peroxisomal disorders
A. Peroxisome biogenesis disorders (PBDs)
1. Zellweger syndrome (ZS)
2. Neonatal adrenoleukodystrophy (NALD)
3. Infantile Refsum disease (IRD)
4. Rhi_omelic type chondrodysplasia punctata (RCDP)
5. Zellweger-like syndrome
B. Isolated en_yme deficiencies
1. Adrenoleukodystrophy (ALD)
2. Acyl-CoA oxidase deficiency
3. D-Bifunctional protein deficiency
4. 3-Ketoacyl-CoA thiolase deficiency
5. Dihydroxyacetone phosphate (DHAP) acyltransferase deficiency
6. Alkyl-DHAP synthase deficiency
7. Refsum disease (phytanoyl-CoA hydroxylase deficiency)
8. Alpha-methylacyl-CoA racemase deficiency
9. Acatalasemia
10. Hyperoxaluria type I (alanine glyoxylate aminotransferase deficiency)
11. Mavalonate kinase deficiency
12. Glutaric aciduria type 3 (glutaryl-CoA oxidase deficiency)

Tableau 2: Liste des maladies peroxysomales. (Berger et al., 2015)

Les PBD sont des maladies rares et graves, à transmission autosomique récessive. Leur incidence est estimée à 1/50 000 naissances. La sévérité des mutations dans les gènes codant pour les peroxines est corrélée à la sévérité du phénotype. On distingue au sein des PBD: la maladie de Refsum infantile, le syndrome de Zellweger et l'adrénoleucodystrophie néonatale. (Braverman et al., 2013; Steinberg et al., 2006)

Le syndrome de Zellweger est considéré comme la forme la plus sévère, se caractérisant par des anomalies congénitales multiples: dysmorphie crânio-faciale, anomalies de la substance blanche et de la migration neuronale, hypotonie néonatale sévère, convulsions dès les premières heures de vie, cataracte, rétinite pigmentaire, atteinte hépatique, atteinte rénale et anomalies osseuses. Le pronostic est sombre avec un décès rapide.

Dans l'adrénoleucodystrophie néonatale, la dysmorphie est modérée voire absente. L'hypotonie néonatale est présente, les convulsions sont plus tardives. La leucodystrophie est toujours présente. Les patients ne survivent généralement pas au-delà des 10 premières années de vie.

La maladie de Refsum infantile est caractérisée par un retard staturo-pondéral, une rétinopathie pigmentaire conduisant à une cécité précoce, une surdité et des anomalies du développement psychomoteur. La dysmorphie faciale est modérée voire absente et il n'y a ni anomalie de la substance blanche, ni anomalie de la migration neuronale. La forme infantile de la maladie de Refsum est causée par des mutations dans le gène *PEX7*. (van den Brink et al., 2003).

Certaines investigations biochimiques peuvent explorer les principales fonctions du peroxysome. On peut doser dans le sang: les AGTLC (C26:0, C24:0, C22:0), l'acide phytanique et l'acide pristanique. En cas de suspicion d'un déficit de biogénèse du peroxysome, des tests complémentaires sur fibroblastes de patients sont indiqués. Dans

le cas d’un déficit de biogénèse du peroxysome, il existe un défaut d'incorporation des protéines PEX qui resteront dans le cytosol et seront rapidement dégradées. Les peroxysomes sont absents ou très diminués en nombre. On apporte alors in vitro la protéine suspecte d’être déficitaire avec un moyen de visualisation. Si la protéine en question est réellement déficitaire, il y aura restauration de l’assemblage des peroxysomes du patient: quand on observera les cellules on visualisera un marquage punctiforme. Au contraire si une autre protéine que la protéine suspecte est déficitaire, l'assemblage des peroxysomes ne peut s’effectuer et il n'y a pas de visualisation des peroxysomes.

2.2.2. Manuscrit 2

Expanding the spectrum of PEX10 related peroxisomal biogenesis disorders: slowly progressive recessive ataxia.

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ORIGINAL COMMUNICATION

Expanding the spectrum of PEX10-related peroxisomal biogenesis disorders: slowly progressive recessive ataxia

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Abstract Peroxisomal biogenesis disorders (PBDs) consist of a heterogeneous group of autosomal recessive diseases, in which peroxisome assembly and proliferation are impaired leading to severe multisystem disease and early death. PBDs include Zellweger spectrum disorders (ZSDs) with a relatively mild clinical phenotype caused by *PEX1*, (MIM# 602136), *PEX2* (MIM# 170993), *PEX6* (MIM# 601498), *PEX10* (MIM# 602859), *PEX12* (MIM# 601758), and *PEX16* (MIM# 603360) mutations. Three adult patients are reported belonging to a non-consanguineous French family affected with slowly progressive cerebellar ataxia, axonal neuropathy, and pyramidal signs. Mental retardation and diabetes mellitus were optional. The age at onset was in childhood or in adolescence (3–15 years). Brain MRI showed marked cerebellar atrophy. Biochemical blood analyses suggested a mild peroxisomal defect. With whole exome sequencing, two mutations in *PEX10* were found in the three patients: c.827G>T (novel) causing the missense change p.Cys276Phe and c.932G>A causing the missense change p.Arg311Gln. The phenotypic spectrum related to *PEX10* mutations includes slowly progressive, syndromic recessive ataxia.

Keywords Recessive ataxia · NGS · PEX10 · Peroxisomal biogenesis disorders

Introduction

Autosomal recessive cerebellar ataxias (ARCAs) consist of a heterogeneous group of inherited neurodegenerative disorders of the cerebellum often associated with peripheral nervous system involvement and systemic abnormalities, including ophthalmologic disturbances [1]. Peroxisomal biogenesis disorders (PBDs) associated with the loss of multiple peroxisomal metabolic functions are a

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rare cause of ARCAs. PBDs are due to mutations in *PEX* genes encoding peroxisome biogenesis factors involved in the import of peroxisomal membrane and matrix proteins [2]. PBDs include the Zellweger spectrum disorders (ZSDs), a clinical and biochemical spectrum of recessively inherited diseases caused by mutations in one of 13 different *PEX* genes [3]. The clinical spectrum ranges from patients presenting in the neonatal period who die within the first year of life to patients presenting in adulthood with only minor symptoms. ZSDs with a relatively mild clinical phenotype caused by *PEX1*, (MIM# 602136), *PEX2* (MIM# 170993), *PEX6* (MIM# 601498), *PEX10* (MIM# 602859), *PEX12* (MIM# 601758), and *PEX16* (MIM# 603360) mutations have been reported [4–7]. Here, we report three patients with ataxia and unusually prolonged survival caused by missense mutations in the RING Zinc finger domain of *PEX10*.

Patients and methods

Clinical and biological analysis

Age at onset of the disease, disease duration, and age at last examination were noted. Spinocerebellar degeneration functional score (SDFS) was used to evaluate the disability stage from 1 to 7 (0: no functional handicap; 1: no functional handicap but signs at examination; 2: mild, able to run; 3: moderate, unable to run; 4: severe, walking with one stick, walking unlimited; 5: walking with two sticks; 6: unable to walk, requiring wheelchair, limited walking without aid; and 7: confined to bed) [8].

Patients underwent neurological examination, motor and sensory nerve conduction studies, and brain MRI. Biochemical determinations of plasma amino acids, urinary organic acids, serum alpha-fetoprotein, vitamin E, lactate, very long-chain fatty acids (VLCFA), as well as pristanic, phytanic, and pipecolic acids have been performed. Several peroxisomal parameters were studied in cultured skin fibroblasts, including catalase immunofluorescence microscopy [9], VLCFA profile [10], and dihydroxyacetonephosphate-acyltransferase (DHAPAT) activity [11].

Written informed consent has been obtained from all individuals providing the biological samples for molecular and biochemical investigations and local ethics committee approved the study.

Genetic studies

DNA was extracted by the standard procedures. Whole exome sequencing was performed by exon capture with the Agilent SureSelect kit and high throughput sequencing with an Illumina HiSeq 2500 sequencer [IGBMC Microarray and Sequencing platform, a member of the "France Génomique" consortium (ANR-10-INBS-0009)]. Reads were mapped to the human reference genome (hg19). The total number of mapped reads was 106 242 472, the median read depth over exons was 82, total exons covered by 10 reads or more was 91 %, and exon coverage by at least one sequence 96 %. Variations were annotated, ranked, and analyzed with an in-house pipeline which combine splice site prediction and protein coding changes [12] and the VaRank program [13]. In particular, based on dbSNP (137), the 1000 Genomes project [14], and the Exome Variant Server (NHLBI GO Exome Sequencing Project), single nucleotide polymorphisms with a frequency of 1 % or above were filtered out. Pathogenicity of the variations were assessed for the several type of effects, including splice site alterations (thanks to the Alamut Batch annotations included in VaRank), and missenses using SIFT and PolyPhen-2 [15, 16]. Protein domain annotation was retrieved from the Pfam database (version 29.0) [17].

Results

Clinical data

Two sisters and one brother from a French non-consanguineous family were affected by a slowly progressive cerebellar ataxia. Age at onset was between 3 and 15 years, but disease progression was slow despite SDFS was above 5 at last examination in at least two patients. Neurological examination showed sensorimotor axonal polyneuropathy, pyramidal signs with no spasticity, and mental retardation (in 2 out of 3 patients), in addition to cerebellar signs. Neuropsychological tests revealed intellectual deficiency with difficulties for reasoning, judgment, and structural constructive skills for both patients II.2 and II.4. Patient II.4 had verbal IQ of 56, performance IQ of 52, and limited memory abilities. Intellectual deficiency was marked and stable. Brain MRI showed marked cerebellar atrophy and cerebral white matter hyperintensities (Fig. 1). No motor evoked potentials were available. Diabetes mellitus was diagnosed in two patients. The main clinical data are summarized in Table 1.

Biological data

In patients II.2 and II.4, plasma analysis revealed normal C26:0 concentration but increased C26/C22 and C24/C22 ratios and increased phytanic, pristanic, and pipecolic acid levels (Table 2). Almost no biochemical abnormalities could be observed in cultured fibroblasts of patients II2 and II4 (Table 2): fibroblasts had normal C26/C22 and C24/

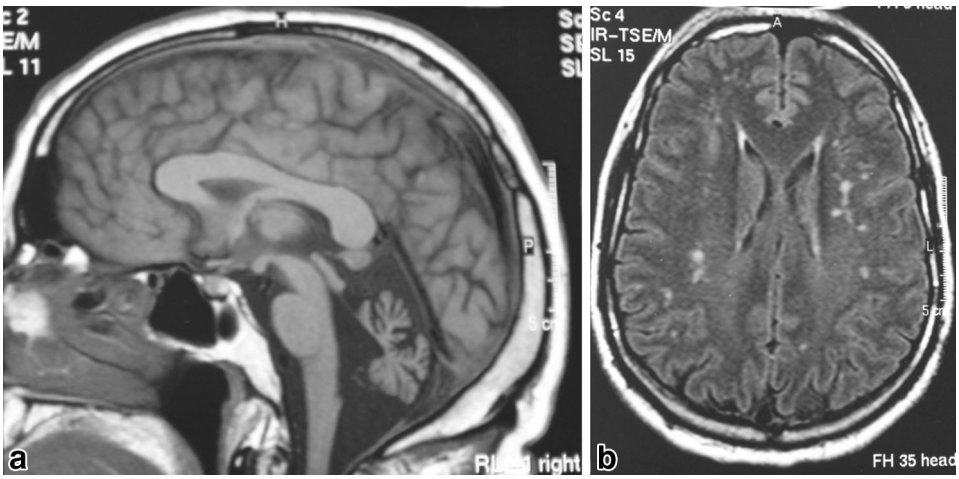

Fig. 1 Brain MRI abnormalities in patient II.4. **a** Cerebellar atrophy (sagittal T1). **b** Periventricular white matter hyperintensities (axial FLAIR)

Table 1 Phenotypic characteristics of the three patients

Patient	Patient II.2	Patient II.3	Patient II.4
Sex	F	F	M
Age at onset (years)	5	15	3
Age at examination (years)	61	59	57
Disease duration (years)	56	44	54
SDFS score	6	na	5
Duration until wheelchair use	29	na	na
Mental retardation	+	0	+
Babinski sign	+	+	+
Tendons reflexes	Abolished	Diminished	Abolished
Nystagmus	+	+	+
Hypermetric saccades	+	na	+
Pes cavus	+	0	+
Diabetes mellitus	+	0	+
Electroencephalography	Normal	na	na
Retinal fundus	Normal	na	normal
Alpha-feto-protein ($N \leq 7$ ng/ml)	1.7 ng/ml	na	2.2 ng/ml
Cerebellar atrophy	+	na	+
Other MRI abnormalities	Midbrain atrophy	na	White matter hyperintensities
EMG	Axonal motor neuropathy	Undetailed neuropathy	Axonal sensorimotor neuropathy

0 absent, + present, *na* not available, *SDFS* spinocerebellar degeneration functional score

C22 ratios, normal DHAPAT activity (Dihydroxyacetonephosphate-acyltransferase, the first enzyme in the plasmalogen biosynthesis pathway and located in the peroxisome), normal very long-chain fatty acid profile, and normal immunoblot profile both for peroxisomal Acyl-CoA oxidase 1 (ACOX1) and peroxisomal 3-ketoacyl-CoA thiolase. Only a very small number of cells had absent peroxisomal staining when performing immunofluorescence microscopy analyses with antibodies against catalase (a peroxisomal matrix protein) in patient II4 (Table 2) both at 37 and 40 °C.

Mutation analysis

DNA sample of patient II.4 was analyzed by the whole exome sequencing. Because the reported family was non-consanguineous, we analyzed both homozygous and compound heterozygous mutations on autosomes. After selection of unique variants out of a set of 12 independent exomes and exclusion of variants found in more than 1 % of the population as well as of variants with a VaRank score lower than 55, we identified 19 homozygous variants and 44 compound heterozygous variants. Among

Table 2 Peroxisomal test results

Peroxisomal test	Patient II.2	Patient II.4	Reference range
Plasma			
C26:0 (μmol/L)	0.70	1.03	0.43–1.6
C24:0 (μmol/L)	23	32	33–84
C22:0 (μmol/L)	23	26	40–119
C26:0/C22:0	0.031	0.040	0.006–0.019
C24:0/C22:0	1.00	1.23	0.69–0.99
Pristanic acid (μmol/L)	na	23.5	0–4.0
Phytanic acid (μmol/L)	25.5	36.4	0–9.0
Pipecolic acid (μmol/L)	27.5	28.1	0.5–3.0
Cultured fibroblasts			
C22:0 (nmol/mg)	4.61	6.46	3.84–10.20
C24:0 (nmol/mg)	na	11.43	7.76–17.66
C26:0 (nmol/mg)	0.20	0.30	0.18–0.38
Ratio C24:0/C22:0	na	1.77	1.55–2.30
Ratio C26:0/C22:0	0.05	0.05	0.03–0.07
DHAPAT activity (nmol/mg/2 h)	na	7.6	5.4–10.6
Thiolase immunoblot			
41 kDa	na	+	+
44 kDa	na	−	−
ACOX1 immunoblot			
70 kDa	na	+	+
50 kDa	na	+	+
20 kDa	na	+	+
Immunofluorescence catalase	na	Abnormal	Normal

ACOX acyl-coenzyme A oxidase1, *DHAPAT* dihydroxyacetonephosphate-acyltransferase, *na* not available

them, two different mutations were found in *PEX10* (NM_002617): c.827G>T causing the missense change p.Cys276Phe and c.932G>A causing the missense change p.Arg311Gln.

Both missenses are part of the RING Zinc finger domain of *PEX10* (PFAM: PF13920 positions 272–311). Looking at the multiple sequence alignment of the 8919 sequences available in the PFAM database, both amino acids are highly conserved amino acids that help defining the motif of this domain. In addition, SIFT and PolyPhen-2 predict both changes to be very probably pathogenic (SIFT score: 0.00 and Polyphen2 score: 1.00 for both mutations) [15, 16].

We confirmed the presence of the two heterozygous *PEX10* mutations, p.Cys276Phe and p.Arg311Gln, in all three patients by direct sequencing. Only one heterozygous mutation, p.Arg311Gln, was found in the youngest healthy sister (Fig. 2), demonstrating that the two mutations are allelic and that the three patients are compound heterozygous for the mutations. DNA samples from the parents and the older healthy sister were not available.

Discussion

Herein, we report the identification of an unusual cause of recessive ataxia, achieved by the exome sequencing analysis. In our family, affected members were clinically characterized by early-onset, slowly progressive cerebellar ataxia associated with axonal polyneuropathy, pyramidal signs, mental retardation, and diabetes mellitus. In the three siblings, phytanic, pristanic, and pipecolic acid plasma levels were increased which was consistent with PBD. Interestingly, C26:0/C22:0 and C24:0/C22:0 ratios were increased in plasma but normal in fibroblasts. Immunoblot analysis of ACOX1 and 3-ketoacyl-CoA thiolase revealed normal peroxisomal processing of these proteins. The only abnormality observed in cultured skin fibroblasts of patient II.3 was the absence of peroxisomal staining in a very small number of cells with the catalase immunofluorescence microscopy analysis both at 37 and 40 °C. In this family, the mutations were not truncating, but missense mutations located in the RING Zinc finger domain of PEX10 which is a crucial domain for its function.

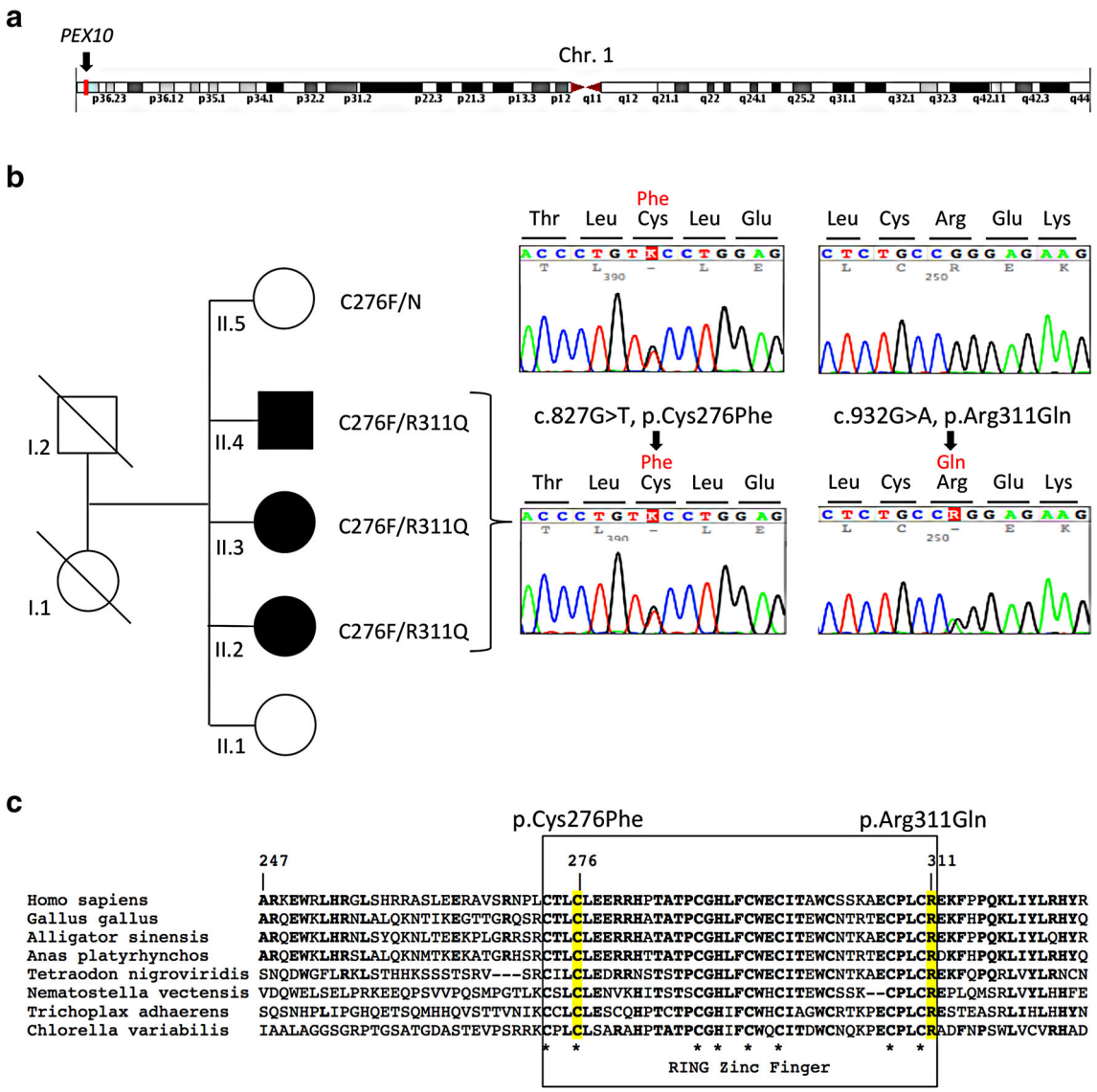

Fig. 2 Identification of *PEX10* mutations in the 3 patients. **a** Localization of *PEX10* on the map of chromosome 1 is indicated by an *arrow*. **b** Pedigree showing segregation of the disease with heterozygous compound mutation: c.827G>T causing the p.Cys276Phe missense change (C276F) and c.932G>A causing the p.Arg311Gln missense change (R311Q). Sanger sequencing results are indicated on the *right* of the pedigree: the three affected are compound heterozygous for C276F and R311Q (for the sake of clarity, electropherograms of only one patient are shown), and the healthy sister is heterozygous for C276F only. **c** Sequence comparison of amino acids in PEX10 and of orthologous proteins from different species. Amino acids that are identical to the human PEX10 sequence are shown in *bold*. The compound heterozygous mutations (on the *top* of the mutated amino acid cysteine, C, and arginine, R, *highlighted in yellow*) are located in a region coding for the RING Zinc finger domain (273–311, *boxed*). The mutated amino acids are conserved in all eukaryotes, including plants. Positions defining the RING motif are shown with a "*"

PEX10 is a 326 amino acid peroxisomal membrane protein, part of the PEX2–PEX10–PEX12 ubiquitin ligase complex [18, 19]. More than 30 different mutations have been identified in the *PEX10* gene, including missense, nonsense, deletion, insertion, splice site mutations, and disruption of the start codon [20]. As recently reported [15], we used the shorter (most abundant) isoform of *PEX10* (NM_002617) as reference and relabeled previously published mutations accordingly. The most common *PEX10* mutation, c.814_815delCT, is found mainly in the Japanese population [21], and the second most common mutation is c.704_705insA [22]. Both mutations cause the occurrence of a truncated protein missing the RING Zinc finger domain. The phenotype is characterized clinically by severe global neurological involvement, dysmorphy, retinitis pigmentosa, sensorineural deafness, and other systemic features (Fig. 1).

Three patients with milder presentations of PBDs due to *PEX10* mutations have been previously reported [5]. Two

of them had cerebellar ataxia, axonal motor neuropathy, and posterior column dysfunction, but neither had mental retardation nor pyramidal signs or diabetes mellitus. The first one had an age of onset at 5 years and was compound heterozygous for the c.704_705insA and p.Arg311Gln mutations. The second had an age of onset at 6 years and was compound heterozygous for a c.730C>T mutation, causing the nonsense change p.Arg244* (again resulting in a truncation before the RING Zinc finger domain) and a c.2>T mutation which abolishes the initiation codon [5]. This latter mutation is assumed to cause re-initiation at the next in-frame ATG codon and production of an N-terminally truncated PEX10 protein, but still containing the RING Zinc finger domain. Similar to our cases, no biochemical abnormalities could be observed in the cultured fibroblasts of both patients. Brain MRI showed severe cerebellar atrophy with normal white matter.

The third previously reported patient [23] developed unsteady gait around 3 years of age, then obvious isolated ataxia with dysarthria, severe cerebellar atrophy, and cerebral white matter changes on brain MRI. Again, he was compound heterozygous for a frame-shift mutation (c.337delC) and a de novo missense mutation in a highly conserved amino acid of the RING Zinc finger domain (p.Leu277Pro).

Our three additional cases expand the phenotypic spectrum of patients with *PEX10* mutations with cerebellar ataxia, peripheral neuropathy, pyramidal signs with no spasticity, and mental retardation. Age at onset is in childhood or in adolescence and the course is very slowly progressive with disease duration of up to 56 years [1]. Whether diabetes mellitus is part of the phenotype or is incidental finding remains unclear. Brain MRI shows obvious cerebellar atrophy without cerebellar white matter changes. All *PEX10* cerebellar ataxia cases have at least one missense mutation in the RING Zinc finger domain or one mutation that produces a truncated protein, but that preserves the RING Zinc finger domain. All these mutations are predicted to be hypomorphic, and therefore, presumably explain the mild presentation of the patients compared to patients at the other end of the PBD spectrum with, for example, homozygous or compound heterozygous mutations that completely abolish the RING Zinc finger domain [2]. These results support the growing concept of spinocerebellar ataxia by mildly affected metabolic pathway, due to the exquisite sensitivity of cerebellar/spinocerebellar neurons to even modest metabolic insult [1].

In conclusion, our data suggest searching for PBDs mutations in patients with unexplained early-onset, slowly progressive ARCA with peripheral neuropathy and pyramidal signs, particularly, since early diagnosis should prompt evaluation of appropriate treatments, such as bile acid supplements or dietary restriction of phytanic acid.

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Compliance with ethical standards

Conflicts of interest The final manuscript has been read and approved by all authors who accepted full responsibility for the design and undertaking of the original article, had access to the data and controlled the decision to publish.

Ethical standards The study was approved by the local ethics committee.

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Web resources UCSC Genome Browser: http://genome.ucsc.edu/index.html [April, 2015].
Ensembl Genome Browser: http://www.ensembl.org/index.html [April, 2015].
Exome Variant Server (EVS), NHLBI GO Exome Sequencing Project (ESP), Seattle, WA: http://evs.gs.washington.edu/EVS [June, 2013].
Human Splicing Finder version 2.4.1: http://www.umd.be/HSF/ [June, 2013].
VaRank: http://www.lbgi.fr/VaRank/ [April, 2015].
PEX database: http://www.dbpex.org/ [April, 2015].

References

1. Anheim M, Tranchant C, Koenig M (2012) The autosomal recessive cerebellar ataxias. N Engl J Med 366(7):636–646
2. Steinberg SJ, Dodt G, Raymond GV, Braverman NE, Moser AB, Moser HW (2006) Peroxisome biogenesis disorders. Biochim Biophys Acta 1763(12):1733–1748
3. Waterham HR, Ebberink MS (2012) Genetics and molecular basis of human peroxisome biogenesis disorders. Biochim Biophys Acta 1822(9):1430–1441
4. Sevin C, Ferdinandusse S, Waterham HR, Wanders RJ, Aubourg P (2011) Autosomal recessive cerebellar ataxia caused by mutations in the PEX2 gene. Orphanet J Rare Dis. BioMed Central Ltd 6(1):8
5. Régal L, Ebberink MS, Goemans N, Wanders RJA, de Meirleir L, Jaeken J et al (2010) Mutations in PEX10 are a cause of autosomal recessive ataxia. Ann Neurol 68(2):259–263
6. Zeharia A, Ebberink MS, Wanders RJA, Waterham HR, Gutman A, Nissenkorn A et al (2007) A novel PEX12 mutation identified as the cause of a peroxisomal biogenesis disorder with mild clinical phenotype, mild biochemical abnormalities in fibroblasts and a mosaic catalase immunofluorescence pattern, even at 40 °C. J Hum Genet 52(7):599–606
7. Guissart C, Drouot N, Oncel I, Leheup B, Gershoni-Barush R, Muller J et al (2015) Genes for spinocerebellar ataxia with blindness and deafness (SCABD/SCAR3, MIM# 271250 and SCABD2). Eur J Hum Genet EJHG. Nature Publishing Group 1–6
8. Anheim M, Fleury M, Monga B, Laugel V, Chaigne D, Rodier G et al (2010) Epidemiological, clinical, paraclinical and molecular study of a cohort of 102 patients affected with autosomal recessive progressive cerebellar ataxia from Alsace, Eastern France: implications for clinical management. Neurogenetics 11(1):1–12

9. Wanders RJ, Wiemer EA, Brul S, Schutgens RB, van den Bosch H, Tager JM (1989) Prenatal diagnosis of Zellweger syndrome by direct visualization of peroxisomes in chorionic villus fibroblasts by immunofluorescence microscopy. J Inherit Metab Dis 12(Suppl 2):301–304
10. Dacremont G, Vincent G (1995) Assay of plasmalogens and polyunsaturated fatty acids (PUFA) in erythrocytes and fibroblasts. J Inherit Metab Dis 18(Suppl 1):84–89
11. Wanders RJ, Ofman R, Romeijn GJ, Schutgens RB, Mooijer PA, Dekker C et al (1995) Measurement of dihydroxyacetone-phosphate acyltransferase (DHAPAT) in chorionic villous samples, blood cells and cultured cells. J Inherit Metab Dis 18(Suppl 1):90–100
12. DePristo MA, Banks E, Poplin R, Garimella KV, Maguire JR, Hartl C et al (2011) A framework for variation discovery and genotyping using next-generation DNA sequencing data. Nat Genet 43(5):491–498
13. Geoffroy V, Pizot C, Redin C, Piton A, Vasli N, Stoetzel C et al (2015) VaRank: a simple and powerful tool for ranking genetic variants. Peer J. 3:e796
14. Abecasis GR, Altshuler D, Auton A, Brooks LD, Durbin RM, Gibbs RA et al (2010) A map of human genome variation from population-scale sequencing. Nature 467(7319):1061–1073
15. Adzhubei IA, Schmidt S, Peshkin L, Ramensky VE, Gerasimova A, Bork P et al (2010) A method and server for predicting damaging missense mutations. Nat Methods 7(4):248–249
16. Kumar P, Henikoff S, Ng PC (2009) Predicting the effects of coding non-synonymous variants on protein function using the SIFT algorithm. Nat Protoc 4(7):1073–1081
17. Finn RD, Bateman A, Clements J, Coggill P, Eberhardt RY, Eddy SR et al (2014) Pfam: the protein families database. Nucleic Acids Res 42(Database issue):D222–D230
18. Okumoto K, Itoh R, Shimozawa N, Suzuki Y, Tamura S, Kondo N et al (1998) Mutations in PEX10 is the cause of Zellweger peroxisome deficiency syndrome of complementation group B. Hum Mol Genet 7(9):1399–1405
19. Chang CC, Warren DS, Sacksteder KA, Gould SJ (1999) PEX12 interacts with PEX5 and PEX10 and acts downstream of receptor docking in peroxisomal matrix protein import. J Cell Biol 147(4):761–774
20. Ebberink MS, Mooijer PAW, Gootjes J, Koster J, Wanders RJA, Waterham HR (2011) Genetic classification and mutational spectrum of more than 600 patients with a Zellweger syndrome spectrum disorder. Hum Mutat 32(1):59–69
21. Shimozawa N, Nagase T, Takemoto Y, Suzuki Y, Kondo N (2003) Genetic heterogeneity in Japanese patients with peroxisome biogenesis disorders and evidence for a founder haplotype for the most common mutation in PEX10 gene. Adv Exp Med Biol 544:71
22. Warren DS, Wolfe BD, Gould SJ (2000) Phenotype-genotype relationships in PEX10-deficient peroxisome biogenesis disorder patients. Hum Mutat 15(6):509–521
23. Steinberg SJ, Snowden A, Braverman NE, Chen L, Watkins PA, Clayton PT et al (2009) A PEX10 defect in a patient with no detectable defect in peroxisome assembly or metabolism in cultured fibroblasts. J Inherit Metab Dis 32(1):109–119

2.2.3. Elargissement du spectre phénotypique des mutations de *PEX10*

Nous avons vu que les PBD constituent un groupe hétérogène de pathologies autosomiques récessives rares et graves menant au décès précoce. Le syndrome de Zellweger peut se présenter avec un phénotype clinique modéré causé par des mutations dans *PEX1*, *PEX2, PEX6, PEX10, PEX12* et *PEX16*.

Dans cette étude, l'ADN de 3 patients adultes issus d'une famille française, alsacienne, non consanguine avait été étudié. Pour résumer, le phénotype clinique des 3 atteints associait ataxie cérébelleuse lentement progressive, neuropathie axonale sensitive, signes pyramidaux, retard mental, diabète de type II (inconstant) avec un âge de début dans l'enfance ou à l'adolescence (de 3 à 15 ans). L'IRM cérébrale montrait une atrophie cérébelleuse marquée.

Les analyses biochimiques sanguines suggéraient des anomalies peroxysomales modérées avec élévation des acides phytanique, pristanique et pipécolique. Les acides gras à très longue chaîne étaient normaux. L'exploration des fonctions peroxysomales sur fibroblastes cultivés de 2 des 3 patients mettait en évidence des activités enzymatiques peroxysomales quasi-normales.

Après le séquençage à haut débit de l'exome d'un des patients atteints, deux mutations faux sens dans *PEX10* ont été retrouvées: c.827G> T, p.Cys276Phe et c.932G> A, p.Arg311Gln. Ces mutations ont également été retrouvées par séquençage Sanger chez les 2 autres atteints de la fratrie. Ces mutations faux sens concernent un acide aminé hautement conservé dans le domaine Zinc finger de *PEX10* ce qui suggère leur pathogénicité.

Nos 3 cas contribuent à décrire le spectre phénotypique des patients avec mutations *PEX10* et ataxie cérébelleuse d'évolution lentement progressive. Seuls 3 autres cas avec

un phénotype clinique proche (ataxie, aréflexie, atrophie cérébelleuse) et mutations *PEX10* ont été rapportés. (Régal et al, 2010)

Nos données et celles des autres articles de la littérature suggèrent de rechercher des mutations *PEX10* chez les patients atteints d'ataxie cérébelleuse de début précoce, et présumée récessive, lorsque le bilan étiologique reste négatif. Enfin, notre étude rappelle de manière plus globale qu'un grand nombre d'ataxies héréditaires résultent de mutations hypomorphes dans des gènes connus pour donner d'autres pathologies en général plus graves.

2.3. Ataxie avec apraxie oculomotrice de type 1: étude clinique, paraclinique et corrélation génotype-phénotype chez 80 patients

2.3.1. L'ataxie avec apraxie oculo-motrice de type 1(AOA1)

L'ataxie avec apraxie oculomotrice de type 1 (AOA1) est une ataxie cérébelleuse autosomique récessive liée à des mutations dans le gène de l'aprataxine (*APTX*). La maladie débute habituellement entre 4 et 8 ans, dominée par une ataxie cérébelleuse progressive associée à une polyneuropathie axono-myélinique sensitivo-motrice sévère et à des mouvements anormaux choréo-dystoniques. Dans l'enfance les patients ont souvent une apraxie oculomotrice ou un défaut d'initiation des saccades. Un léger retard mental peut être observé. L'imagerie par résonance magnétique cérébrale met en évidence une atrophie cérébelleuse, l'électromyogramme confirme la polyneuropathie. (Aicardi et al., 1988; Barbot et al., 2001; Moreira et al., 2001; Shimazaki et al., 2002). Les patients sont confinés au fauteuil roulant après une durée d'évolution de la maladie de 5 à 20 ans (Le Ber et al., 2003). Un début plus tardif à l'âge adulte a été décrit chez certains patients (Criscuolo et al., 2005; Tranchant et al., 2003). L'évolution se fait vers l'aggravation progressive et la perte de la marche. Le bilan biologique révèle une diminution de l'albumine et une augmentation du LDL cholestérol souvent après plusieurs années d'évolution.

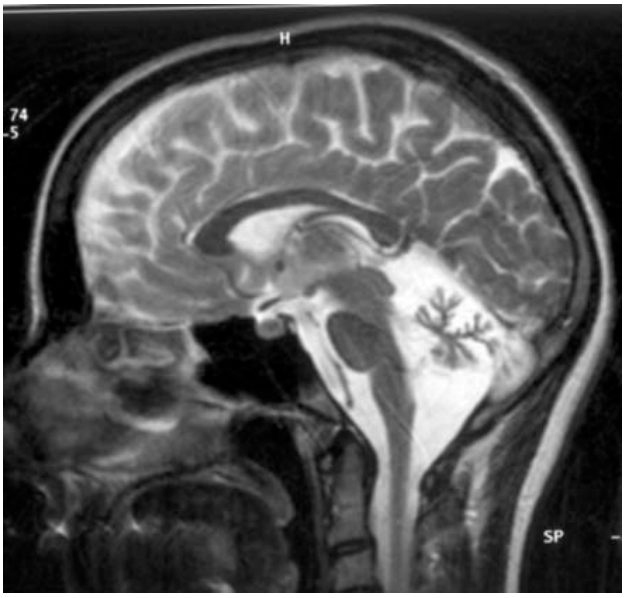

Figure 7: IRM cérébrale en séquence T2, coupe saggitale d'un patient AOA1: atrophie cérébelleuse majeure prédominant sur le vermis. (Le Ber et al., 2003)

Le gène *APTX* a été identifié par l'étude de liaison génétique de 13 familles portugaises (Moreira et al., 2001). Il comprend 36 exons codants et se caractérise par un épissage alternatif dans l'exon 3 entraînant l'utilisation d'un codon ATG alternatif dans l'exon 4 responsable de la production d'un isoforme court de la protéine aprataxine (Moreira et al., 2001).

APTX code pour l'aprataxine, protéine nucléaire exprimée de manière ubiquitaire faisant partie de la superfamille des protéines à domaine HIT (histidine-triad protein). Elle est constituée de 3 domaines :

- un domaine amino terminal similaire à celui d'une polynucléotide kinase phosphatase
- un domaine intermédiaire similaire aux protéines de la famille HIT (nucleotide binding proteins)
- un domaine carboxy terminal contenant un motif «DNA binding Zinc Finger».

Ce domaine C-terminal en doigt de zinc constitue un domaine de liaison à l'ADN/ARN (Caldecott, 2003; Moreira et al., 2001; Sano et al., 2004). Les protéines de la

superfamille des protéines à domaine HIT sont des nucléotides hydrolases transférases. L'aprataxine est potentiellement impliquée dans la réparation des cassures simple brin de l'ADN en facilitant l'excision de bases (Sano et al., 2004).

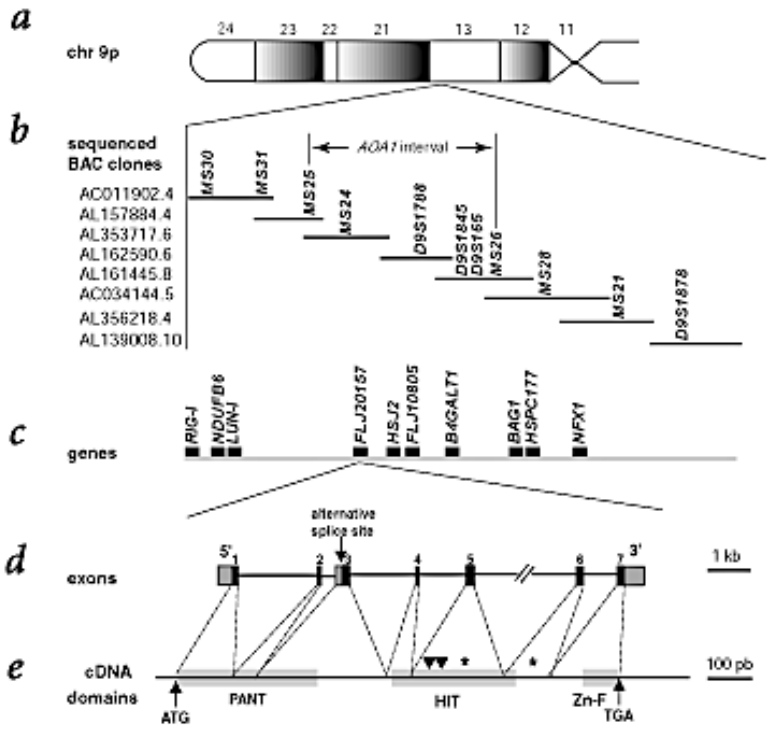

Figure 8: Organisation génomique du gène *APTX* (9p13.3) et de l'aprataxine.

a, bras court du chromosome 9.

b, région du linkage d'AOA1 et localisation des marqueurs microsatellites.

c, gènes présents dans la région du linkage d'AOA1.

d, structure génomique d'*APTX*: les exons sont représentés en noir. L'exon 3 possède un site d'épissage alternatif.

e, cDNA et localisation des premières mutations décrites dans AOA1. Les mutations faux-sens sont décrites par des triangles et les mutations stop et frameshift par des étoiles. Les segments en gris correspondent aux domaines PANT, HIT et Zn-finger. (Moreira et al., 2001)

Les mutations retrouvées dans AOA1 se situent majoritairement au niveau des domaines HIT ou Znf, touchant ainsi à la fois l'isoforme long et l'isoforme court de l'aprataxine. La mutation fondatrice européenne p.Trp279* (Moreira et al., 2001; Tranchant et al., 2003) semble être la plus fréquente dans la population caucasienne.

2.3.2. Manuscrit 3

Ataxia with oculomotor apraxia type 1: a clinical, biomarker and genotype-phenotype study of 80 patients.

M. Renaud, M.-C. Moreira, B. Monga, D. Rodriguez, R. Debs, P. Charles, M. Chaouch, F. Ferrat, C. Laurencin, L. Vercueil, M. Mallaret, A. M'Zahem, L. Ali Pacha, M. Tazir, C. Tilikete, E. Ollognon, F. Ochsner, T. Kuntzer, H. Jung, J.-M. Beis, J.-C. Netter, A. Djamshidian, M. Bower, A. Bottani, S. Murphy, R. Walsh, T. Reiley, E. Bieth, F. Roelens, B.T. Poll-The, C. Marques Lourenço, L. Bannach Jardim, R. Straussberg, P. Landrieu, S. Thobois, J. Pouget, C. Guissart, C. Goizet, A. Dürr, C. Tranchant, M. Koenig, M. Anheim.

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Ataxia with oculomotor apraxia type 1:

A clinical, biomarker and genotype-phenotype study of 80 patients

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Keywords: recessive ataxia, oculomotor apraxia, peripheral neuropathy, aprataxin, mutations

Key points:

Alpha-fetoprotein slightly increased in a substantial fraction of patients, is a new biomarker of ataxia with oculomotor apraxia type 1 (AOA1). Oculomotor apraxia is an optional finding in AOA1 and correlated with more severe disease. The p.Trp279* mutation is the most frequent *APTX* mutation in the Caucasian population.

Abstract

Objective:

Ataxia with oculomotor apraxia type 1 (AOA1) is an autosomal recessive cerebellar ataxia due to aprataxin (*APTX*) mutations, combining early-onset cerebellar ataxia, oculomotor apraxia (OMA), axonal motor neuropathy and eventual decrease of albumin serum level. We aimed at improving the clinical, biomarker and molecular delineation of AOA1 and providing genotype-phenotype correlations.

Methods:

We compiled a broad, range of patients consecutively diagnosed with AOA1 in our laboratory.

Results:

AOA1 was confirmed in 80 patients from 51 families: 57 new (including 8 new mutations) and 23 previously reported patients. Elevated alpha-fetoprotein was found in 41% of patients and hypoalbuminemia in 62%. Median alpha-fetoprotein level was higher in AOA1 patients compared with non-ataxic control patients ($p<0.01$). Decreased albumin and elevated alpha-fetoprotein correlated with disease duration.

The p.Trp279* mutation, initially reported as restricted to the Portuguese founder haplotype, was discovered in 66% of AOA1 patients with broad Caucasian origins. OMA was found in 61% of patients, polyneuropathy in 93%, cerebellar atrophy in 98%. OMA correlated with the severity of ataxia and mutation type, being more frequent with deletion or truncating mutations than with missense variants ($p<0.01$). Mean age at onset was higher for patients with at least one missense mutation ($p<0.01$).

Conclusions:

Alpha-fetoprotein, slightly increased in a substantial fraction of patients, is a new biomarker for AOA1. OMA is an optional finding in AOA1 and correlated with more severe disease. The p.Trp279* mutation is by far the most frequent *APTX* mutation in the Caucasian population. *APTX* missense mutations were strongly associated with a milder phenotype.

Abbreviations:

AFP: alpha-fetoprotein

AOA: Ataxia with oculomotor apraxia

AOA1: Ataxia oculomotor apraxia type 1

AOA2: Ataxia oculomotor apraxia type 2

AOA4: Ataxia oculomotor apraxia type 4

APTX: Aprataxin gene

ARCAs: Autosomal recessive cerebellar ataxias

AT: Ataxia-telangectasia

ATLD: AT-like disorder

BER: Base excision repair

CK: Creatine kinase

DD: Disease duration

DNA: Deoxyribonucleic acid

FHA domain: Forkhead associated domain

HIT domain: Histidine triad domain

LDL cholesterol: Low-density lipoprotein (LDL) cholesterol,

MRI: Magnetic resonance imaging

OMA: Oculomotor apraxia

PANT domain: PNKP-AOA1 N-Terminal domain

PCR: Polymerase chain reaction

RER: Ribonucleotide excision repair

RNA: Ribonucleic acid

SARA score: Scale of assessment and rating of ataxia

SAS: Statistical Analysis System

SDFS: Spinocerebellar degeneration functional score

SLOCA: sporadic late-onset cerebellar ataxia

Introduction

Autosomal recessive cerebellar ataxias (ARCAs), consist of a heterogeneous group of neurodegenerations [1], including ataxia with oculomotor apraxia type 1 (AOA1), due to aprataxin mutations [2,3]. AOA1 was described as an early-onset ataxia with cerebellar atrophy, oculomotor apraxia (OMA), choreo-dystonia and peripheral neuropathy [4]. Though remaining an issue of controversy, OMA may be defined as horizontal saccades of elevated latencies commonly associated with hypometric staircase saccades, oculo-cephalic dissociation and/or head thrust [5,6]. AOA1 the most frequent ARCA in Japan [7-9], has also been found in other countries [2,5,10,11]. During the course of AOA1, decreased albumin serum level and elevated total cholesterol serum level may be observed [2,5,9,10].

The prevalence of OMA in AOA1 rests a matter for debate, whether alpha-foetoprotein (AFP) serum level is a biomarker of AOA1 has not been elucidated and genotype–phenotype correlations need to be strengthened. In this study, we sought to better define the clinical, biological - especially regarding biomarkers of the disease - and molecular spectrum of AOA1 and to provide genotype-phenotype correlations.

Patient and Methods

We analyzed retrospectively the clinical, biological, electrophysiological, imaging and molecular data of all patients consecutively diagnosed with AOA1 in our genetics laboratory between 2002 and 2014.

Sequencing of *APTX* was performed upon request for cases of cerebellar ataxia with hypoalbuminaemia and/or early-onset cerebellar ataxia combined with peripheral neuropathy and/or cerebellar atrophy using brain magnetic resonance imaging (MRI).

Written informed consent was obtained from all participants and the local ethics committee approved the study.

Clinical analysis

Age at onset of the disease, disease duration (DD) and age at last examination were recorded as well as gender, consanguinity and geographic origin. Patients underwent neurological examination, motor and sensory nerve conduction studies and brain MRI. Exhaustive clinical examination abnormalities included ocular motor signs (strabismus, saccadic pursuit, OMA, oculocephalic dissociation and head thrust), movement disorders (chorea, dystonia, parkinsonism, tremor, myoclonus), pyramidal signs, areflexia, muscle wasting, ataxia and intellectual disability.

OMA was defined as described above. Oculocephalic dissociation was defined as dissociation between tandem head and eye movement, the eyes lagging during head rotation. Peripheral neuropathy was defined by reduced deep tendon reflexes or areflexia, distal loss of vibration sense and electrophysiological evidence of nerve conduction abnormalities. Cerebellar atrophy was considered on MRI sagittal and axial slides by both a neuroradiologist and a neurologist.

Spinocerebellar degeneration functional score (SDFS), was used to evaluate the disability stage from 1 to 7, with higher scores representing severe impairment [13]. SDFS corrected for DD (SDFS/DD ratio), was used to evaluate the progression rate of

the disease. Scale of assessment and rating of ataxia (SARA) was used to evaluate spino-cerebellar degeneration [14].

Biological analysis

Measurement of albumin, total cholesterol, low-density lipoprotein cholesterol (LDL), creatine kinase (CK) and AFP serum levels were performed. For some patients, several measurements of AFP and albumin were taken during the course of the disease.

Mutational analysis

DNA samples of patients were obtained from total blood using standard procedure. Genetic analysis of *APTX* was performed by conventional Sanger sequencing. Exons 1 to 6 of *APTX* were amplified as previously described [2]. For exon 7, a new pair of intronic flanking primers was designed (forward, 5'-TAT TGG TTT TCC AGA GTA GC-3'; reverse, 5'-GAA TAG GTG CCA GCA GTT T-3') and used for polymerase chain reaction (PCR), amplification (10" denaturation at 94°C, 10" annealing at 64°C and 10" elongation at 72°C for 35 cycles). PCR products were purified with the NucleoSpin® Extract 2 in 1 kit (Macherey-Nagel GmbH) and sequenced from both the forward and reverse strands. Sequencing was performed using a Taq cycle sequencing kit (Applied Biosystems). Reaction products were run on an automated DNA Sequencer (model 3100 from Applied Biosystems). Homozygous exon deletions were readily detected by absence of PCR amplification product and were verified PCR tests with a different set of primers. In one case, a single heterozygous mutation was identified and copy number variation was excluded by multiplex ligation-dependent probe amplification

(MLPA), analysis (SALSA MLPA kit P316-A1 Recessive Ataxias; MRC-Holland). This case was considered heterozygous carrier by chance and a diagnosis of AOA1 was excluded.

One patient was diagnosed using targeted exon capture strategy coupled with multiplexing and high throughput sequencing of 57 genes causing ataxia when mutated, including *APTX* [25]. When available, parents were sequenced in order to verify segregation *in trans* of the mutations. For consistency with the vast majority of publications on *APTX* mutation identification, the RefSeq gene reference NM_175073, encoding a 342 amino-acid isoform of APTX, was used for mutation report.

AFP serum level measurement in control groups

AFP serum level measurements in controls were performed using the immunoanalysis Kryptor Brahms method as established in the Laboratoire de Biochimie Générale et Spécialisée in the University Hospital of Strasbourg, France. The AFP level assessment using this method with 100 healthy control subjects provided a median value of 3.2 µg/L with a 97.5 percentile at 7 µg/L, which was retained as the upper limit of 'normal' in our study. All healthy subjects had an AFP level between 0.5 and 15.7 µg/L. AFP levels were assessed in 102 patients who had no cerebellar ataxia but were affected with Parkinson's disease (n=37), atypical parkinsonism (n=13), Huntington's disease (n=3), dystonia (n=1), Alzheimer's disease (n=11), multiple sclerosis (n=11), amyotrophic lateral sclerosis (n=2), peripheral neuropathy (n=16) and myopathy (n=6) [17]. AFP levels were assessed in 74 AOA2 control patients already published [17] and 76 patients with sporadic late-onset cerebellar ataxia (SLOCA), with a large variety of cerebellar variants of multiple system atrophy (n=29), dominant or recessive ataxia

(n=9), immune-mediated causes, malformations, toxic or other acquired (n=6) or undetermined (n=32).

Genotype-phenotype correlation studies

For genotype-phenotype correlations, two different classification analyses were employed. The AOA1 patients were first divided into two groups according to the type of the *APTX* mutation: i) at least one missense mutation; ii) two truncating mutations. Then patients were divided into four groups according to the type of mutation *and* its location in *APTX*: i) homozygous p.Trp279*; ii) two truncating mutations with at least one different from p.Trp279*; iii) at least one missense mutation in the first part of HIT domain, before the Histidine triad motif (p.Lys197Gln, p.Ala198Val, Pro206Leu, Val230Gly); iv) at least one missense mutation in the second part of HIT domain at, or after, the Histidine triad motif (p.Leu261Phe, p.Asp267Gly, p.Ser270Phe). Both series of groups were then compared regarding their clinical, electrophysiological, imaging and biochemical features.

Statistical analysis

All statistical analyses were performed with the software package Statistical Analysis System (SAS), for Windows, release 9.3 (SAS Institute Inc., Cary, NC, USA). The Chi-square and the Fisher's exact test were applied to reveal differences in proportions between groups or association between category variables. We used non-parametric statistical tests for the analysis of quantitative data as variable distribution was not normal. We applied Kruskall-Wallis test for comparisons of quantitative variables across three or more independent groups. In case of statistically significant results,

groups were pairwise compared and the p values were adjusted using the Bonferroni-Holm method. The linear relationships between quantitative variables were assessed using the Spearman correlation procedure. For all statistical tests, we considered p values < 0.05 as statistically significant.

Results

Eighty patients from 51 families were diagnosed with AOA1 including 57 new patients and 23 previously reported [2,5,10].

Clinical data

Table 1 summarizes the main features of the AOA1 patients. Patients originated from 18 different countries: France (n=23), Algeria (n=11), Morocco (n=8), Tunisia (n=4), Brazil (n=3), Switzerland (n=3), India (n=2), Belgium (n=2), United States (n=2), Ireland (n=2), Israel (n=2), Italy (n=2), Lebanon (n=2), Rwanda (n=2), Turkey (n=2), Netherland (n=1), Syria (n=1), Portugal (n=1). OMA correlated positively with the SARA score: the higher the SARA score, the more frequent the OMA (p=0.04). The progression rate of the disease (SDFS/DD), correlated positively with the presence of movement disorder: the faster the disease progression, the more intense the movement disorder (p=0.03).

Biological data

Biological data regarding the biomarkers are presented in Table 1.

Longer DD was correlated with lower albumin serum levels (p<0.01). The longer the DD, the higher the AFP serum level (p<0.01) and the higher the total cholesterol serum

level ($p<0.01$). The biomarkers measured with lower levels of albumin, correlated with increased total cholesterol and increased AFP ($p=0.02$ and $p<0.01$, respectively).

Mutation analysis

Eight new mutations were identified (Table 2 and Figure 1). Among these, seven were predicted to result in a major disruption of aprataxin and included: two nonsense (p.Gln187* and p.Tyr195*), a large in-frame deletion (del exon4) and four frame-shift mutations (c.336_337delCA, c.774_775insCTTTCAACTA, c.940_956del17 and c.596delG). Only one was a missense mutation (p.Ser270Phe), resulting in non-conservative substitution affecting an amino-acid residue conserved in all eukaryotes and was predicted pathogenic by two different algorithms (Sift score=0.02, Polyphen2 score=1.00).

The p.Trp279* mutation, initially only in association with the Portuguese founder haplotype, has been found in 53/80 (66.3%) patients. Forty-one patients (51.2%) were homozygous for p.Trp279*.

Genotype-phenotype correlation

AOA1 patients were divided into two groups according the type of the mutation in *APTX*: i) at least one missense mutation (n=15), ii) two truncating mutations (n=65). Patients with at least one missense mutation had a higher mean age at onset (17.7 years versus 5.2, $p<0.0001$) and a less severe SDFS score (mean 2.7 versus 4.4, $p<0.01$). OMA, oculocephalic dissociation and head thrust were correlated with the group ii) type of mutations, being more frequent in patients with deletion or truncating mutations than in patients with missense mutations (respectively $p<0.0001$, $p=0.02$,

p=0.01). There was no difference between the two groups regarding other qualitative or quantitative variables.

In the second analysis, the AOA1 patients were divided into four groups: i) 41 patients (51.2%), with homozygous p.Trp279*, ii) 24 patients (30%), with two truncating mutations with at least one different from p.Trp279*, iii) 10 patients (12.5%), with at least one missense mutation in the first part of the HIT domain, iv) 5 patients (6.3%), with at least one missense mutation in the second part of the HIT domain. Mean age at onset was higher for patients in group iv) (25 years) compared with group iii) (13.4 years, p=0.03), group i) (5.8 years, p<0.01) and group ii) (2 years, p<0.01).

Comparison of AFP level with control groups

AFP levels for control non-ataxic patients, control AOA2 patients, control SLOCA patients and AOA1 patients are presented in Table 3 and Figure 2.

Median AFP was significantly higher in AOA1 patients compared with non-ataxic patients (p<0.01) and SLOCA patients (p <0.01). Median AFP was significantly higher in AOA2 patients compared with AOA1 patients (p< 0.01), non-ataxic patients (p<0.01) and SLOCA patients (p<0.0001). There were no significant differences between non-ataxic patients and SLOCA (p=1).

Discussion

We describe the clinical, biomarker and molecular findings of the largest international cohort of AOA1 patients reported so far. The most frequent clinical findings were:

early-onset cerebellar ataxia with cerebellar atrophy, axonal sensory-motor neuropathy and eventual OMA (2/3 of the cases). Overall, clinical, electrophysiological and imaging features were in accordance with previously reported smaller series [5,8,11].

Strikingly, the nonsense mutation p.Trp279*, first reported associated with the Portuguese founder haplotype [2], was by far the most frequent mutation, representing 66.3% of all mutated alleles. This mutation was found in patients from a large number of countries, including The Netherlands, Belgium, Ireland, Morocco, Algeria and Israel, indicating an ancient origin. This implies that mutation p.Trp279* is the most frequent *APTX* mutation in the Caucasian population.

We found that the mean age at onset was higher for patients having at least one missense mutation and therefore manifesting a less severe presentation. Saccades of elevated latencies, oculocephalic dissociation and head thrust were more frequent in patients with deletion or truncating mutations than in patients with missense mutations. These results are consistent with a recessively inherited disease due to the loss of protein function even if such findings were not observed in a large cohort of patients affected with AOA2 due to senataxin loss of function [17]. In our study OMA, though not a universal finding, was correlated with the SARA score suggesting that OMA is a marker of AOA1. The absence of OMA however, was frequently observed in a significant number of patients which reinforces the notion that the diagnosis of AOA1 should still be considered in patients with autosomal recessive cerebellar ataxias lacking OMA.

Some missense mutations (p.Lys197Gln, p.Ala198Val, p.Pro206Leu, p.Val230Gly), appeared to be associated with a severe phenotype while others (p.Leu261Phe, p.Asp267Gly, p.Ser270Phe), seemed to be linked with a milder presentation. Aprataxin comprises three domains: i) the PANT domain (PNKP-AOA1 N-Terminal domain), also designated as putative forkhead associated (FHA) domain, corresponding to the N-terminal region of aprataxin, ii) a HIT (histidine triad) domain and iii) a C-terminal domain containing a divergent zinc-finger motif [2]. Aprataxin is involved in: i) DNA single-strand break repair, ii) interactions with several proteins involved in the base excision repair (BER) and ribonucleotide excision repair (RER) pathways and iii) actions in RNA-DNA damage response to protect the genome from the accumulation of adenylyl ribosylated 5' termini [19]. Notably, the milder missense mutations are located within or just downstream of the Histidine triad motif HVHLH of the HIT domain, while the other missense mutations are located in the first part of the HIT domain. The Histidine triad motif is the catalytic site for adenosine monophosphate removal activity from the 5' terminus of aborted ligation products [20,21]. Missense mutations localized in the HIT domain lead to APTX destabilization with a severe quantitative reduction and alteration of the subcellular distribution, being perinuclear (cytoplasmic) instead of nuclear and nucleolar [22]. Some genotype-phenotype correlations have been previously reported in AOA1 patients. Date *et al.* reported that patients carrying p.Pro206Leu and p.Val263Gly mutations appear to have a later onset and a milder phenotype than those carrying c.689_690insT or c.del840delT frame-shift mutations [3]. Criscuolo *et al.* reported that patients carrying p.His201Gln, p.Pro206Leu and p.Leu223Pro presented a later age at onset, ranging from 28 to 40 years [23]. In 2011, Yokoseki *et al.* demonstrated that AOA1 patients homozygous for

c.689-690insT had a more severe phenotype than those with a p.Pro206Leu or p.Val263Gly mutation [9]. In our study, the milder presentation associated with the p.Pro206Leu mutation did not reach significance, presumably because the effect on severity is small and our p.Pro206Leu patients were few. However, we present for the first time, probably due to the large size of the sample, a genotype-phenotype correlation for the missense *APTX* mutations according to position relative to the HIT domain.

Results regarding biomarkers in AOA1 were interesting. Strikingly, AFP serum level was elevated in 40% of our patients. Previous studies have only reported three AOA1 cases with slightly raised AFP levels (7.8, 14 and 14.5 ng/ml). For the first time, we reveal that serum AFP level is a biomarker of AOA1 that increases with disease duration. Our results further suggest that a slightly increased AFP in a patient with early-onset ataxia is potentially compatible with AOA1 [18]. Hypoalbuminemia was found in 62.3%, which is comparable with other series [9,18]. Our biological data confirm that serum albumin levels decrease and cholesterol levels increase during the course of the disease [5,8]. Hypoalbuminemia and hypercholesterolemia are the most characteristic biochemical findings in AOA1 while elevated AFP level is typical of patients with AT or AOA2 and may be encountered in AOA4 [24] and to some extent in ARCA3 [1,25]. Modifications of both albumin and AFP serum levels were identified in our AOA1 patients though we were not able to detect any alteration of AFP serum level during the clinical course of the disease in a large series of 90 AOA2 patients [17]. Even if AFP is a biomarker of recessive ataxias with peripheral neuropathy and optional OMA (such as AOA1, AOA2, AT and AOA4), the underlying biological link between AFP and cerebellar ataxia,

peripheral neuropathy and oculomotor abnormalities is still unknown. In contrast to AT, to our knowledge there is neither an increased sensitivity to ionizing radiation [26], nor susceptibility to cancer in either AOA1 and AOA2 [27]. AOA1 patients presented both normal protein intake and rate of albumin degradation: decreased synthesis of albumin in the liver is suspected to be the cause of hypoalbuminemia [28]. Elevated AFP and hypoalbuminemia are on either side of transcriptional impairment in the liver. Indeed, in hepatocytes, AFP and albumin genes have opposite transcriptional regulatory mechanisms [29]. It is likely that elevated AFP levels in AT and AOA2 proceed also from liver transcriptional dysregulation [1]. In the current era of searching for biomarkers, the importance of AFP must be highlighted, especially as its serum level increases progressively during the course of AOA1, in the same way that serum albumin levels decrease progressively. This could be of interest for designing further clinical trials devoted to ARCA with elevated AFP.

Movement disorders reported in our series included: chorea, dystonia, tremor and less frequently myoclonus. Proportions of movement disorders were similar to the Yokoseki *et al.* study: 40 % chorea, 16 % dystonia, 8% myoclonus (respectively 39.6%, 24.5% and 10.5% in our study) [9]. Chorea is classically described in AOA1 [5,9,18,30]. Mild to severe dystonia [5,9,31] and intentional tremor [30,32], have already been described in some cases. Interestingly, we found that movement disorders are more frequent in patients with rapid disease progression. Whether this is due to the disability induced by the movement disorders themselves or by a more severe disease, characterized by marked cerebellar ataxia and movement disorders, remains to be elucidated even if presently it is well-known that the cerebellum is closely connected with basal ganglia.

In conclusion, our study describes the largest series of patients with AOA1 and strengthens the delineation of the clinical, molecular and biomarker characteristics of this rare form of ARCA. We show that OMA may be lacking despite the misleading name of AOA1; that AFP serum level is a new biomarker of AOA1; that p.Trp279* mutation is the most frequent *APTX* mutation in the Caucasian population and that missense *APTX* mutations are associated with a milder phenotype.

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References

1. Anheim M, Tranchant C, Koenig M. The autosomal recessive cerebellar ataxias. N Engl J Med. 2012 Feb 16;366(7):636–46.

2. Moreira MC, Barbot C, Tachi N, Kozuka N, Uchida E, Gibson T, et al. The gene mutated in ataxia-ocular apraxia 1 encodes the new HIT/Zn-finger protein aprataxin. Nat Genet. 2001 Oct;29(2):189–93.

3. Date H, Onodera O, Tanaka H, Iwabuchi K, Uekawa K, Igarashi S, et al. Early-onset ataxia with ocular motor apraxia and hypoalbuminemia is caused by mutations in a new HIT superfamily gene. Nat Genet. 2001 Oct;29(2):184–8.

4. Aicardi J, Barbosa C, Andermann E, Andermann F, Morcos R, Ghanem Q, et al. Ataxia-ocular motor apraxia: a syndrome mimicking ataxia-telangiectasia. Ann Neurol. 1988 Oct;24(4):497–502.

5. Le Ber I, Moreira M-C, Rivaud-Péchoux S, Chamayou C, Ochsner F, Kuntzer T, et al. Cerebellar ataxia with oculomotor apraxia type 1: clinical and genetic studies. Brain. 2003 Dec;126(Pt 12):2761–72.

6. Panouillères M, Frismand S, Sillan O, Urquizar C, Vighetto A, Pélisson D, et al. Saccades and eye-head coordination in ataxia with oculomotor apraxia type 2. Cerebellum. 2013 Aug;12(4):557–67.

7. Uekawa K, Yuasa T, Kawasaki S, Makibuchi T, Ideta T. [A hereditary ataxia associated with hypoalbuminemia and hyperlipidemia--a variant form of Friedreich's disease or a new clinical entity?]. Rinshō Shinkeigaku Clin Neurol. 1992 Oct;32(10):1067–74.

8. Shimazaki H, Takiyama Y, Sakoe K, Ikeguchi K, Niijima K, Kaneko J, et al. Early-onset ataxia with ocular motor apraxia and hypoalbuminemia: the aprataxin gene mutations. Neurology. 2002 Aug 27;59(4):590–5.

9. Yokoseki A, Ishihara T, Koyama A, Shiga A, Yamada M, Suzuki C, et al. Genotype-phenotype correlations in early onset ataxia with ocular motor apraxia and hypoalbuminaemia. Brain. 2011 May;134(Pt 5):1387–99.

10. Tranchant C, Fleury M, Moreira MC, Koenig M, Warter JM. Phenotypic variability of aprataxin gene mutations. Neurology. 2003 Mar 11;60(5):868–70.

11. Amouri R, Moreira M-C, Zouari M, El Euch G, Barhoumi C, Kefi M, et al. Aprataxin gene mutations in Tunisian families. Neurology. 2004 Sep 14;63(5):928–9.

12. Moreira MC, Barbot C, Tachi N, Kozuka N, Mendonça P, Barros J, et al.

Homozygosity mapping of Portuguese and Japanese forms of ataxia-oculomotor apraxia to 9p13, and evidence for genetic heterogeneity. Am J Hum Genet. 2001 Feb;68(2):501–8.

13. Anheim M, Fleury M, Monga B, Laugel V, Chaigne D, Rodier G, et al. Epidemiological, clinical, paraclinical and molecular study of a cohort of 102 patients affected with autosomal recessive progressive cerebellar ataxia from Alsace, Eastern France: implications for clinical management. Neurogenetics. 2010 Feb;11(1):1–12.

14. Schmitz-Hübsch T, du Montcel ST, Baliko L, Berciano J, Boesch S, Depondt C, et al. Scale for the assessment and rating of ataxia: development of a new clinical scale. Neurology. 2006 Jun 13;66(11):1717–20.

15. Redin C, Le Gras S, Mhamdi O, Geoffroy V, Stoetzel C, Vincent M-C, et al. Targeted high-throughput sequencing for diagnosis of genetically heterogeneous diseases: efficient mutation detection in Bardet-Biedl and Alström syndromes. J Med Genet. 2012 Aug;49(8):502–12.

16. Mallaret M, Renaud M, Redin C, Drouot N, Muller J, Severac F, et al. Validation of a clinical practice-based algorithm for the diagnosis of autosomal recessive cerebellar ataxias based on NGS identified cases. J Neurol. 2016 Jul;263(7):1314-22

17. Anheim M, Monga B, Fleury M, Charles P, Barbot C, Salih M, et al. Ataxia with oculomotor apraxia type 2: clinical, biological and genotype/phenotype correlation study of a cohort of 90 patients. Brain Oct;132(Pt 10):2688–98.

18. Castellotti B, Mariotti C, Rimoldi M, Fancellu R, Plumari M, Caimi S, et al. Ataxia with oculomotor apraxia type1 (AOA1): novel and recurrent aprataxin mutations, coenzyme Q10 analyses, and clinical findings in Italian patients. Neurogenetics. 2011 Aug;12(3):193–201.

19. Tumbale P, Williams JS, Schellenberg MJ, Kunkel TA, Williams RS. Aprataxin resolves adenylated RNA-DNA junctions to maintain genome integrity. Nature. 2014 Feb 6;506(7486):111–5.

20. Ahel I, Rass U, El-Khamisy SF, Katyal S, Clements PM, McKinnon PJ, et al. The neurodegenerative disease protein aprataxin resolves abortive DNA ligation intermediates. Nature. 2006 Oct 12;443(7112):713–6.

21. Seidle HF, Bieganowski P, Brenner C. Disease-associated mutations inactivate AMP-lysine hydrolase activity of Aprataxin. J Biol Chem. 2005 Jun 3;280(22):20927–31.

22. Gueven N, Becherel OJ, Kijas AW, Chen P, Howe O, Rudolph JH, et al. Aprataxin, a novel protein that protects against genotoxic stress. Hum Mol Genet. 2004 May 15;13(10):1081–93.

23. Criscuolo C, Mancini P, Saccà F, De Michele G, Monticelli A, Santoro L, et al. Ataxia with oculomotor apraxia type 1 in Southern Italy: late onset and variable phenotype. Neurology. 2004 Dec 14;63(11):2173–5.

24. Bras J, Alonso I, Barbot C, Costa MM, Darwent L, Orme T, et al. Mutations in PNKP cause recessive ataxia with oculomotor apraxia type 4. Am J Hum Genet. 2015 Mar 5;96(3):474–9.

25. Renaud M, Anheim M, Kamsteeg E-J, Mallaret M, Mochel F, Vermeer S, et al. Autosomal Recessive Cerebellar Ataxia Type 3 Due to *ANO10* Mutations: Delineation and Genotype-Phenotype Correlation Study. JAMA Neurol. 2014 Oct 1;71(10):1305.

26. Nahas SA, Duquette A, Roddier K, Gatti RA, Brais B. Ataxia-oculomotor apraxia 2 patients show no increased sensitivity to ionizing radiation. Neuromuscul Disord. 2007 Dec;17(11-12):968–9.

27. Crimella C, Cantoni O, Guidarelli A, Vantaggiato C, Martinuzzi A, Fiorani M, et al. A novel nonsense mutation in the APTX gene associated with delayed DNA single-strand break removal fails to enhance sensitivity to different genotoxic agents. Hum Mutat. 2011 Apr;32(4):E2118–33.

28. Fukuhara N, Nakajima T, Sakajiri K, Matsubara N, Fujita M. Hereditary motor and sensory neuropathy associated with cerebellar atrophy (HMSNCA): a new disease. J Neurol Sci. 1995 Nov;133(1-2):140–51.

29. Onodera O. Spinocerebellar ataxia with ocular motor apraxia and DNA repair. Neuropatholl. 2006 Aug;26(4):361–7.

30. Habeck M, Zühlke C, Bentele KHP, Unkelbach S, Kress W, Bürk K, et al. Aprataxin mutations are a rare cause of early onset ataxia in Germany. J Neurol. 2004 May;251(5):591–4.

31. Sekijima Y, Hashimoto T, Onodera O, Date H, Okano T, Naito K, et al. Severe generalized dystonia as a presentation of a patient with aprataxin gene mutation. Mov Disord. 2003 Oct;18(10):1198–200.

32. Tsao CY, Paulson G. Type 1 ataxia with oculomotor apraxia with aprataxin gene mutations in two American children. J Child Neurol. 2005 Jul;20(7):619–20.

Table 1: A. Main qualitative variables of AOA1 patients.

	AOA1 (N=80) (%)
Consanguinity	60.3
Oculomotor apraxia	60.6
Hypometric saccades	74.1
Nystagmus	61.4
Saccadic pursuit	71.4
Horizontal strabismus	25
Oculocephalic dissociation	50
Head-thrust	44.7
Pes cavus	37.4
Scoliosis	33.3
Pyramidal signs	11.3
Intellectual disability	52.5
Current chorea	39.6
Chorea disappearing with course of the disease	7.6
Dystonia	24.5
Parkinsonism	2.2
Tremor	64.7
Myoclonus	10.5
Peripheral neuropathy	93.3
Cerebellar atrophy	98.2
Elevated AFP (N < 7ng/ml)	41.2
Decreased Albumin (N= 35-48 g/l)	62.3
Elevated total cholesterol (N= 0.5 -2 g/l)	56.5
Elevated LDL cholesterol (N < 1.60 g/l)	42.3
Elevated CK (N = 10 – 200 UI/l)	28.6

Abbreviations : AFP = alpha fetoprotein, CK = creatine kinase, LDL = low-density lipoprotein

B. Main quantitative variables of AOA1 patients.

AOA1 patients (n=80)	Min	Med	Mean (SD)	Max
Age at onset, years	2	6	7.7 (7.4)	40
Age at exam, years	9	26	28.5 (13.7)	59
Disease duration, years	4	17	20.9 (12.6)	52
Age at wheelchair (n=40), years	4	18	20 (11.4)	57
Duration until wheelchair use (n=40), years	2	11.8	13.6 (8.6)	38
SARA score at last examination	6	28	26.4 (7.6)	37
SDFS score at last examination	3	6	5.5 (0.9)	7
Progression rate (SDFS/DD)	0.1	0.3	0.3 (0.2)	1.2
AFP, ng/mL	1.1	6	6.6 (4)	17
Albumin, g/L	17.8	32.5	33.2 (6.5)	45.9
Total cholesterol , g/L	1	2.1	2.2 (0.7)	3.8
LDL cholesterol , g/L	0.8	1.7	1.7 (0.6)	3.2
CK, UI/L	32	185	168.4 (101.5)	360

Abbreviations: AFP = alpha fetoprotein, CK = creatine kinase, DD = disease duration, LDL = low-density lipoprotein, Max = maximum, Med = median, Min = minimum, SARA = scale for the assessment and rating of ataxia, SD = standard deviation.

Table 2: Description of the 8 new mutations.

Patient number (geographic origin)	Nucleotide change (exon)	Amino acid change	Mutation status
8 (Tunisia)	c.809C>T (exon 6)	p.Ser270Phe	Compound heterozygous (c.875-1G>A (exon 7))
19 (France)	c.336_337delCA (exon 3)	p.His112Glnfs*10	Compound heterozygous (c.617C>T (exon 5) p.Pro206Leu)
30 (France)	c.774_775insCTTTCAACTA (exon 6)	p.Val259Leufs*14	Compound heterozygous (c.837G>A (exon 6) p.Trp279*)
32 (Turkey)	c.940_956del17 (exon 7)	p.Lys314Serfs*2	Homozygous
41 and 42 (Brazil)	del exon 4	p.Glu162_Gln181del20	Homozygous
45 (Netherland)	c.559C>T (exon 5)	p.Gln187*	Compound heterozygous (c.837G>A (exon 6) p.Trp279*)
46 et 47 (Morocco)	c.585C>A (exon 5)	p.Tyr195*	Homozygous
	c.596delG (exon 5)	p.Arg199Leufs*15	
48,49 and 50 (India)			Homozygous

Abbreviations: del = deletion, ins = insertion, fs = frameshift, * = stop

Table 3: AFP study in AOA1 patients and control groups.

AFP, ng/mL	Min	Med	Mean (SD)	Max
AOA1 patients (n=80)	1.1	6.0	6.6 (4)	17
AOA2 patients (n=74)	5	32.2	42.1 (34.6)	185
sLOCA patients (n=76)	1.4	3.5	4.1 (2.4)	12.7
Non-ataxic patients (n=102)	0.8	3.4	4.1 (2.8)	17.2

Abbreviations: AFP: alpha-fetoprotein, sLOCA = sporadic late-onsets cerebellar ataxia

Figure 1: Position of the mutations with respect to the APTX protein domains.

The HIT motif (HxHxH) is highlighted in green. The deletion, insertion and splice site mutations are translated into predicted protein consequence: correspondence for novel mutations (in red) is indicated in Table 3; c.544-2A>G and c.770+1G>A, respectively acceptor and donor, exon 5 splice site mutations are both predicted to result in frame-shift exon 5 skipping p.Val182Profs*12; c.875-1G>A (last) exon 7 acceptor splice site mutation is predicted to result in usage of a minor alternative exon 7' (isoform coded by transcript NM_175069) p.Ala292Glufs*2. The position of the in-frame exon 4 deletion (p.Glu162_Gln181del20) is indicated by a double arrow that represents the extent of internal peptide deletion. The large deletion of exons 1 to 4 is not represented since it is predicted to result in complete absence of *APTX* transcription.

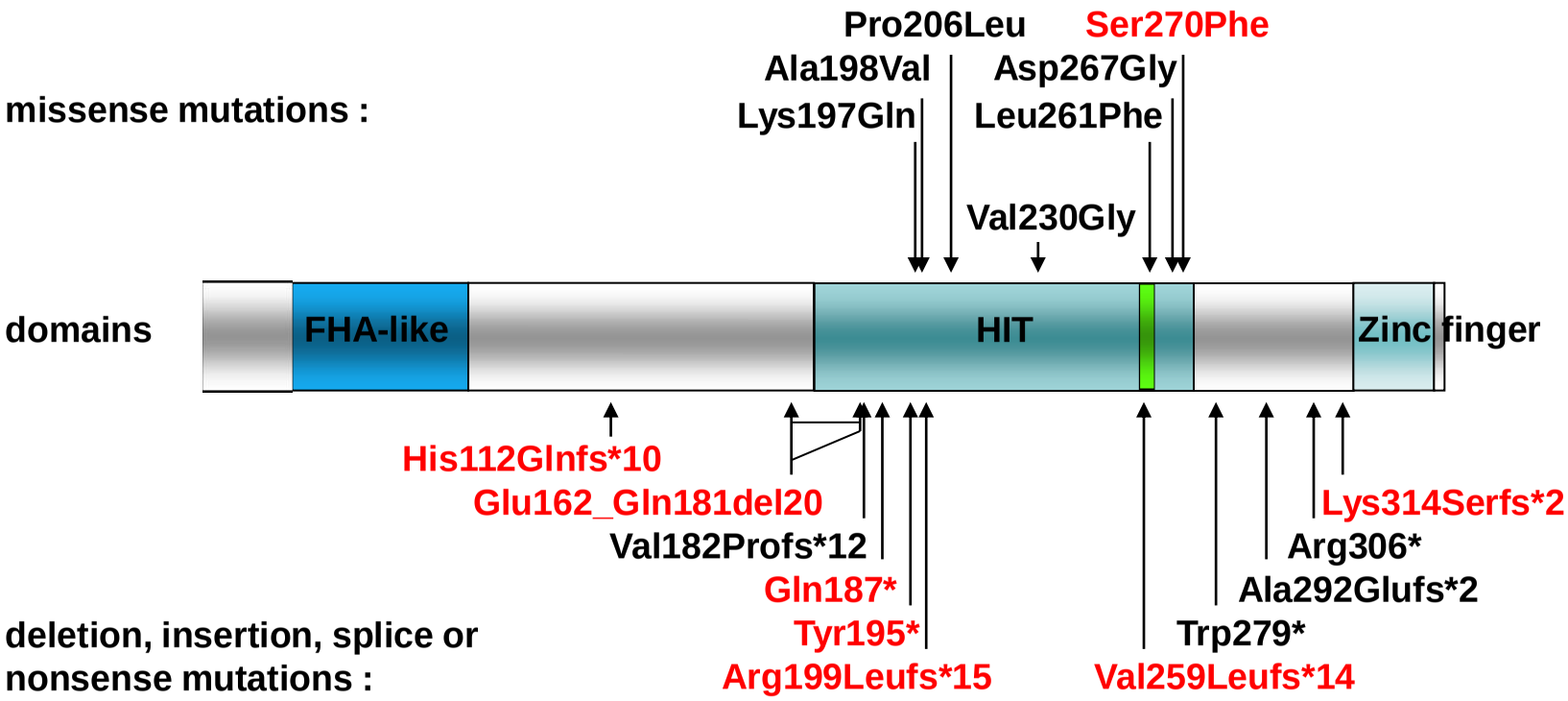
Figure 2: AFP studies in patients.

Distribution of AFP levels in 102 non-ataxic control patients (no ataxia), in 76 patients with sporadic late-onset cerebellar ataxia (SLOCA), 74 AOA2 control patients already published [17] and AOA1 patients of our study. The density of points is presented as log10 of the AFP serum level.

Supplemental data:

Video 1: Patient 72, homozygous for the Ala198Val mutation in *APTX* gene at 28 years: dysmetria during the finger-nose test and oculo-cephalic dissociation (dissociation of eyes-head when looking toward a lateral target) with hypometric saccades during the head movement task.

Video 2: Patient 76, heterozygous for the Trp279* and Lys197Gln mutation in *APTX* at 28 years: cerebellar ataxia at gait and dysmetria during the finger-nose test.



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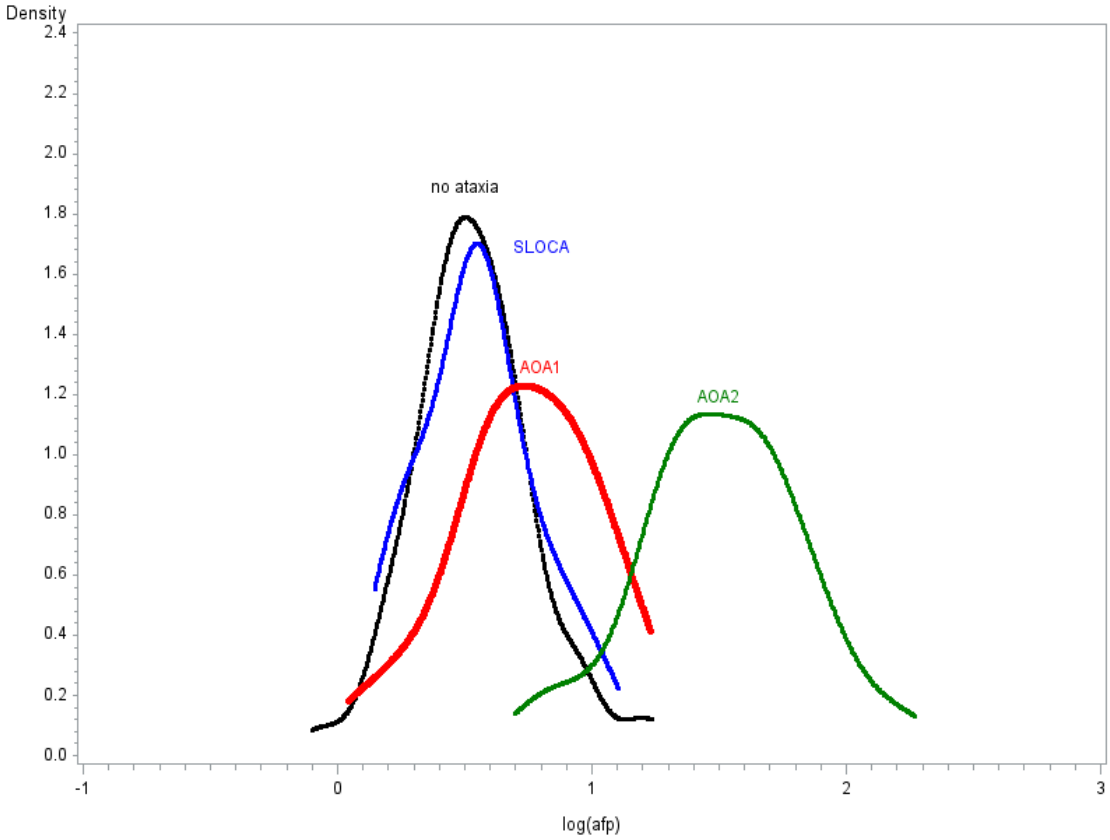

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2.3.3. Corrélation génotype/phénotype dans AOA1

Dans notre troisième étude, le diagnostic d'AOA1 avait été confirmé chez 80 patients issus de 51 familles différentes: 57 nouveaux patients (dont 8 nouvelles mutations) et 23 déjà publiés.

La mutation p.Trp279 * était retrouvée chez 66% des patients AOA1 ce qui confirme qu'il s'agit de la mutation d'*APTX* la plus fréquemment retrouvée dans la population caucasienne.

Nous avons réussi à établir une corrélation génotype/phénotype avec un âge moyen de début plus élevé et une pathologie moins sévère chez les patients avec au moins un faux sens ($p < 0,01$) par rapport aux patients avec deux mutations tronquantes.

2.3.4. L'AFP dans AOA1

Dans notre étude, l'hypoalbuminémie, classique dans AOA1, était observée chez 62% des patients tandis que l'AFP était élevée dans 41% des cas avec un maximum de 17ng/ml. Nous montrions également que le taux d'AFP était plus élevé chez les patients AOA1 que dans une cohorte de patients non ataxiques ($p < 0,01$) et dans une cohorte de patients atteints d'ataxie cérébelleuse tardive ($p < 0,01$). Pour la première fois nous montrions de manière claire que l'AFP pouvait être augmentée de manière modérée et inconstante dans AOA1 mais à des taux néanmoins bien plus faible que dans d'autres ataxies récessives comme l'ataxie avec apraxie oculomotrice de type 2 (AOA2).

A noter que l'élévation de l'AFP tout comme la diminution de l'albumine étaient corrélées à la durée de la maladie**.**

2.4. Création et validation d'un algorithme d'aide au diagnostic moléculaire des ataxies récessives

2.4.1. Manuscrit 4

Validation of a clinical practice-based algorithm for the diagnosis of recessive cerebellar ataxias.

M. Mallaret, M. Renaud, C. Redin, N. Drouot, Jean Muller, F. Severac, J. L. Mandel, W. Hamza, T. Benhassine, L. Ali-Pacha, M. Tazir, A. Durr, ML. Monin, C. Mignot, P. Charles, L. Van Maldergem, L. Chamard, C. Thauvin, V. Laugel, L. Burglen, P. Calvas, MC. Fleury, C. Tranchant, M. Anheim, M. Koenig.

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ORIGINAL COMMUNICATION

Validation of a clinical practice-based algorithm for the diagnosis of autosomal recessive cerebellar ataxias based on NGS identified cases

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Abstract Establishing a molecular diagnosis of autosomal recessive cerebellar ataxias (ARCA) is challenging due to phenotype and genotype heterogeneity. We report the validation of a previously published clinical practice-based algorithm to diagnose ARCA. Two assessors performed a blind analysis to determine the most probable mutated gene based on comprehensive clinical and paraclinical data, without knowing the molecular diagnosis of 23 patients diagnosed by targeted capture of 57 ataxia genes and high-throughput sequencing coming from a 145 patients series. The correct gene was predicted in 61 and 78 % of the cases by the two assessors, respectively. There was a high inter-rater agreement [$K = 0.85$ (0.55–0.98) $p < 0.001$] confirming the algorithm's reproducibility. Phenotyping patients with proper clinical examination, imaging, biochemical investigations and nerve conduction studies remain crucial for the guidance of molecular analysis and to interpret next generation sequencing results. The

M. Anheim and M. Koenig equally contributed to this work.

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proposed algorithm should be helpful for diagnosing ARCA in clinical practice.

Keywords Recessive ataxia · Next generation sequencing · Neurogenetics · Electromyography

Introduction

Autosomal recessive cerebellar ataxias (ARCAs) are heterogeneous and complex inherited neurodegenerative disorders that affect the cerebellum, the spinal cord and the peripheral nerves. Molecular diagnosis of the ARCAs is challenging due to both phenotypic and genetic heterogeneity [1]. It was formerly a step by step approach with serial sequencing of several genes by the Sanger technique. The advent of affordable next generation sequencing (NGS) technologies allows now sequencing of exomes or large gene panels for diagnosis of rare neurological diseases such as ARCAs [2]. However, massive amount of data with multiple rare variants in several genes in a single patient increases the complexity of the analysis. A collaborative cross-talk between molecular geneticists and clinicians is even more necessary than before for NGS diagnosis validation in clinical practice. Age of onset, ataxia progression rate and careful clinical examination combined with laboratory, morphological and neurophysiological investigations [including vitamin E, alpha-fetoprotein (AFP), albumin, cholestanol, brain MRI, nerve conduction studies (NCS)] are useful for reaching a diagnostic conclusion. A new classification of ARCAs also has been established comprising 3 groups: ARCA with pure sensory neuropathy, ARCA with sensorimotor axonal neuropathy and ARCA without neuropathy. An algorithm for the diagnosis of ARCAs based on these items has been proposed by our group according to personal experience and literature data [3] (Fig. 1). We conducted a blind study to validate this clinical practice-based algorithm on a series of patients with molecular diagnosis of ARCA. These patients were part of a cohort of patients consecutively investigated with targeted capture sequencing of a panel of 57 ataxia genes.

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Patients and methods

Between 2010 and 2012, 145 unrelated index patients were recruited in 12 tertiary centers for movement disorders: 130 in France and 15 in Algeria. Inclusion criteria for the NGS analysis were the combination of: (1) progressive cerebellar ataxia; (2) age at onset before 60 years; (3) molecular analysis negative for Friedreich ataxia and other investigations depending on clinical assessment; (4) recessive inheritance or sporadic cases. Written informed consent was obtained from all participants and local ethics committee approved the study. Hundred and forty-five consecutive patients were analyzed by a targeted exon-capture strategy coupled with multiplexing and high-throughput sequencing of 57 genes causing ataxia when mutated (listed in supplementary file-A). Library preparation, targeted capture and sequencing were realized as previously reported [4]. NGS analysis is detailed in supplementary file-B [5].

Hundred and thirty-four patients were presenting ataxia starting before 40 years and 11 patients had late onset ataxia, starting after 40 years. Our cohort was mainly comprised of sporadic cases: 85 patients (59 %) had neither familial history of ataxia nor consanguinity. Fifty-four patients (37 %) had a recessive pedigree (2 or more affected in the kindred or isolated case with parental consanguinity). A molecular diagnosis was made in 27/145 patients (19 %) with mutations in ARCA genes. Among the 27 patients with ARCA molecular diagnosis, 4 were excluded due to the lack of available data. Molecular data of these 4 patients and clinical data of 118 patients without diagnosis are summarized in supplementary file C [6] and D, respectively.

We selected the remaining 23 ARCA patients with an established molecular diagnosis to assess the validity of the clinical practice-based algorithm. Two movement disorders specialists (MA, CT) performed independently a blind analysis based on the clinical (age at onset, current age, current disability based on scale of assessment and rating of ataxia (SARA) [7] score and/or spinocerebellar degeneration functional score (SDFS) [1], exhaustive clinical examination abnormalities including ocular motor signs, movements disorders, pyramidal signs, mental retardation) and paraclinical (biomarkers—especially vitamin E, AFP, albumin, cholestanol-, brain MRI, NCS findings) but molecular data: each patient had to be categorized in one of the three ARCA groups as described above and ranking of

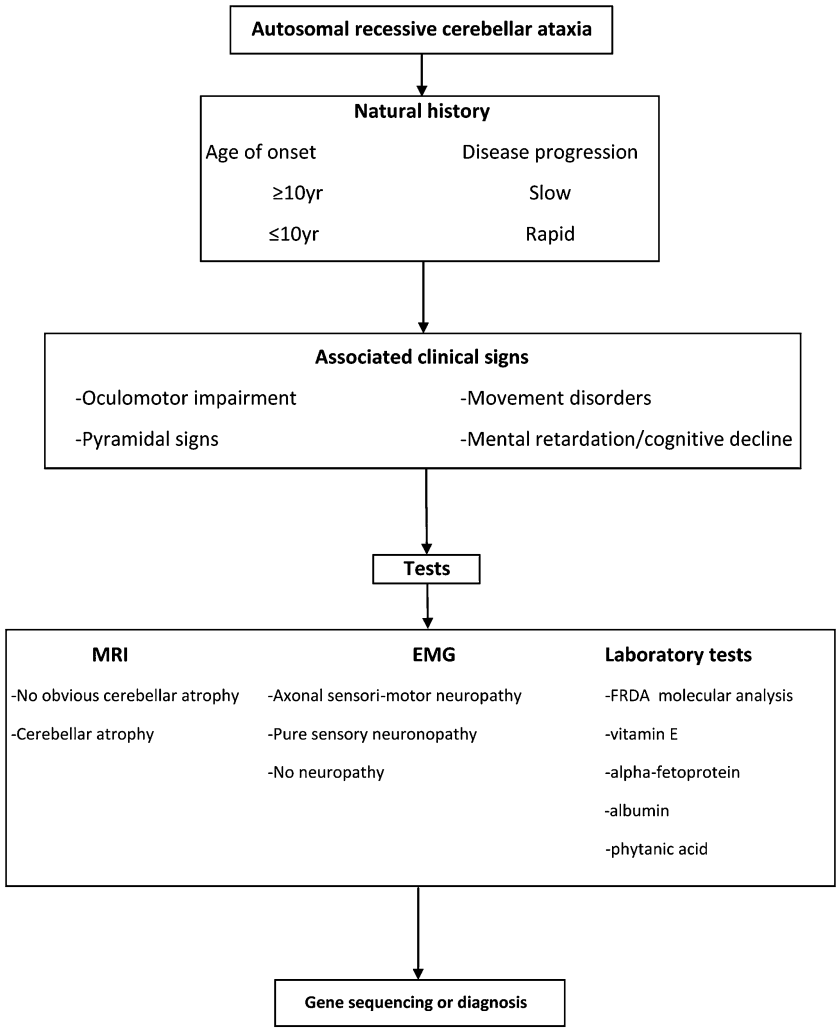

Fig. 1 Algorithm for the diagnosis of autosomal recessive cerebellar ataxias (simplified and adapted [3]). The combination of natural history, associated clinical signs and paraclinical data (such as brain MRI, nerve conduction studies and several biomarkers) leads to one or a few diagnosis. *yr* years, *EMG* electroneuromyography

the two most probable disease causing genes was achieved. Inter-rater agreement was assessed by computing a weighted Kappa coefficient (K) with '"squared"' weights. All mutated genes affecting the 23 patients were ordered according to the data of the literature as followed on a ordinal scale to take account of the degree of disagreement [group without neuropathy: (1) *SYNE1* (2) *ANO10*, (3) *ADCK3*; group with pure sensory neuronopathy: (1) *POLG*, (2) *TTPA*; group with axonal sensorimotor neuropathy (1) *APTX*, (2) *SETX*, (3) *CYP27A1*, (4) *SACS*]. Genes that showed larger number of associated phenotypic differences were separated by greater distance on the ordinal scale. Confidence interval was calculated using the adjusted bootstrap percentile (BCa) method based on 10,000 replicates. A "z" test was performed to assess if the classification which produced the Kappa statistic is significantly better than a random result ($K = 0$). A p value <0.05 was considered statistically significant. Analyses were performed using R software version 3.1.0 (R Project for Statistical Computing) with the "irr" package.

Results

Twenty-three patients were investigated with the clinical practice-based algorithm. The age of onset ranged from 1 to 47 years (median 16). Genetic analysis identified two pathogenic mutations in *ANO10* (6 patients), in *SETX* (4), in *SYNE1* and *ADCK3* (3 each), in *SACS* and *APTX* (2 each) and in *TTPA*, *CYP27A1*, *POLG* (1 each) (Table 1). The correct ARCA group was found in all patients by the two assessors. The gene ranked first by the first assessor (MA) was correct in 14/23 cases (61 %) and 18/23 cases (78 %) for the second assessor (CT). The most frequent error (11 errors/14) was misdiagnosis within the ARCA group without neuropathy probably due to the closely overlapping phenotypes of pure cerebellar ataxias (especially ARCA1, ARCA3 and to some extent ARCA2), with a wide range of age at onset. Five errors were shared by both experts. Considering the two most probable genes according to the assessors, the correct diagnosis was identified in 18/23 (78 %) and 21/23 (91 %), respectively.

Table 1 Gene ranking based on the clinical practice-based algorithm

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8
Geographical origin	French	French	Algerian	French	French	Portuguese	French	French
Gender	F	M	F	M	M	M	M	F
Age of onset (years)	27	27	7	10	5	15	33	37
Age (years)	62	43	32	41	18	46	44	44
SDFS (/7)	3	3	3	2	2	3	4	3
SARA (/40)	18	13	21	12	ND	10	14	6,5
Upper motor neuron dysfunction	−	−	−	−	−	−	+	−
Others symptoms	Scoliosis	Square wave jerks, horizontal ophtalmoparesis, unilateral ptosis	Slow evolution, pes cavus	Mild developmental delay	Dysarthria, seizures, delayed growth	Mild developmental delay	Left hypoacousia	Tongue fasciculations, gaze evoked nystagmus
Others individuals affected in the family	−	+	−	−	−	−	−	−
Reference			[6]			[17]	[8]	[8]
Nerve conduction studies	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Cerebellar atrophy	+	NA	+	+	+	+	+	+
Other MRI findings	–	NA	–	Global brain atrophy	–	–	–	–
Biomarkers	–	–	–	–	Lactate, pyruvate, CK, AFP normal	–	–	–
Mutated gene	*SYNE1*	*SYNE1*	*SYNE1*	*ADCK3*	*ADCK3*	*ADCK3*	*ANO10*	*ANO10*
Zigosity	homo	homo	homo	homo	het	het	het	het
Variant n°1/ cDNA variant/protein change	Nonsense c.6978G>A p.Trp2326* [exon 47]	Donor splice site c.12315+1G>A p.Ile4047_Lys 4105del59 (predicted) [intron 76]	Nonsense c.3736G>T p.Glul246* [exon 30]	Missense c.911C>T p.Ala304Val [exon 7]	Missense c.8950T p.Arg299Trp [exon 7]	Missense c.895C>T p.Arg299Trp [exon 7]	Missense c.685G>T p.Gly229Trp [exon 6]	Frameshift c.l214delT p.Leu405* [exon 7]
Variant n°2/ cDNA variant/protein change					Missense C.1651G>A p.Glu551Lys [exon 14]	Frameshift c.l358delT p.Leu453Arg fs*24[exon 11]	Nonsense c.1291C>T p.Gln431* [exon 8]	Donor splice site c.1476+1 G>T; p.Ser432_Leu49 2del61 [exon 9]
Results first assessor/gene n°1	***SYNE1***	***SYNE1***	*ANO10*	*ANO10*	***ADCK3***	*ANO10*	*SYNE1*	*SYNE1*
Results first assessor/gene n°2	*ANO10*	*ANO10*	*ADCK3*	*SYNE1*	*ANO10*	*SYNE1*	*ANO10*	*ANO10*
Results second assessor/gene n°1	***SYNE1***	***SYNE1***	*ADCK3*	*ANO10*	***ADCK3***	*SYNE1*	***ANO10***	***ANO10***
Results second assessor/gene n°2	*ANO10*	*ANO10*	*ANO10*	*ADCK3*	*ANO10*	*ANO10*	*SYNE1*	*SYNE1*

Table 1 continued

	Patient 9	Patient 10	Patient 11	Patient 12	Patient 13	Patient 14	Patient 15	Patient 16
Geographical origin	French	French	French	French	Algerian	French	Lebanese	Algerian
Gender	F	F	F	M	M	M	M	M
Age of onset (years)	30	32	32	30	17	47	16	7
Age (years)	33	61	37	34	23	60	51	15
SDFS (/7)	3	4	3	4	3	3	5	4
SARA (/40)	10	ND	10.5	16	ND	11.5	ND	ND
Upper motor neuron dysfunction	−	−	+	+	−	+	+	−
Others symptoms	Hypermetric saccades, ankle clonus	Diplopia, dysphagia, gaze evoked nystagmus, increased reflexes	–	Focal epilepsy since 18 with secondary generalization, mild dysexecutive syndrome	Head tremor, cerebellar and proprioceptive ataxia, abolished reflexes	Cerebellar and proprioceptive ataxia, external ophtalmoplegia, abolished reflexes, absence of vibration sense	Decreased vibration sense, abolished reflexes, intellectual deterioration, abnormal ocular movements	Oculomotor apraxia
Others individuals affected in the family	−	+	−	+	−	+	+	−
Reference	[8]	[8]	[8]		[6]		[20]	[6]
Nerve conduction studies	ND, no clinical signs of neuropathy	Normal	Normal	Normal	Normal	Axonal sensory neuropathy	Mild axonal sensorimotor neuropathy with sensory predominance	Axonal sensory neuropathy
Cerebellar atrophy	+	+	+	+	−	+	+	NA
Other MRI findings	–	–	–	Left parietooccipital porencephalic cyst	−	−	Vermis atrophy	−
Biomarkers	–	AFP : 12ng/ml	–	–	NA	–	Hypercholesterolemia	–
Mutated gene	*ANO10*	*ANO10*	*ANO10*	*ANO10*	*TTPA*	*POLG*	*APTX*	*APTX*
Zigosity	homo	het	het	het	homo	het	homo	homo
Variant n°1/ cDNA variant/protein change	Deletion of exon 12	Missense c.1009T>G p.Phe337Val [exon 6]	Missense c.512T>C p.Phel71Ser [exon 5]	Missense c.1009T>G p.Phe337Val [exon 6]	Frame shift c.744delA p.Glu249Asnfs* 15 [exon 5]	Missense c.l880G>A pArg627Gln [exon 10]	Missense c.781C>T p.Leu261Phe [exon 6]	Nonsense c.837G>A p.Trp279* [exon 6]
Variant n°2/ cDNA variant/protein change		Frameshift c.l32dupA, p.Asp45Argfs*9 [exon 2]	Frameshift c.l32dupA, pAsp45Argfs*9 [exon 2]	Frameshift c.l32dupA, p.Asp45Argfs*9 [exon 2]		Missense c.2243G>C p.Trp748Ser [exon 13]		
Results first assessor/gene n°1	*SYNE1*	***ANO10***	***ANO10***	*ADCK3*	***TTPA***	***POLG***	*POLG*	*ATM*
Results first assessor/gene n°2	*ANO10*	*ATM*	*SYNE1*	*POLG*	*POLG*	*TTPA*	*ANO10*	*APTX*
Results second assessor/gene n°1	***ANO10***	***ANO10***	***ANO10***	*ADCK3*	***TTPA***	***POLG***	*POLG*	***APTX***
Results second assessor/gene n°2	*SYNE1*	*SYNE1*	*SYNE1*	*ANO10*	*POLG*	*SETX*	*APTX*	*ATM*

Table 1 continued

	Patient 17	Patient 18	Patient 19	Patient 20	Patient 21	Patient 22	Patient 23
Geographical origin	French	French	French	French	French	French	Algerian
Gender	M	M	M	M	M	F	F
Age of onset (years)	15	13	Adolescence	12	29	1	3
Age (years)	25	26	67	33	33	42	39
SDFS (/7)	4	4	6	3	5	5	4
SARA (/40)	ND	21,5	ND	ND	7, 5	ND	12
Upper motor neuron dysfunction	−	−	−	−	+	+	−
Others symptoms	Oculomotor apraxia	Oculomotor apraxia	–	Oculomotor apraxia	Developmental delay, epilepsy since early childhood	–	Cataract at 37, spasticity in lower limbs
Others individuals affected in the family	+	–	+	−	−	−	−
Reference					[21]		
Nerve conduction studies	Axonal sensorimotor neuropathy with sensory predominance	Axonal sensorimotor neuropathy	Axonal sensorimotor neuropathy	Axono–myelinic sensorimotor neuropathy	Axonal sensorimotor neuropathy	Axonal sensorimotor neuropathy	Demyelinating sensorimotor neuropathy
Cerebellar atrophy	+	+	NA	+	−	+	NA
Other MRI findings	−	−	NA	−	Cerebellar white matter abnormalities	−	NA
Biomarkers	AFP: 50ng/ml	AFP: 13ng/ml	AFP: 32ng/ml	AFP : 90ng/ml	Increased cholestanol	–	–
Mutated gene	*SETX*	*SETX*	*SETX*	*SETX*	*CYP27A1*	*SACS*	*SACS*
Zigosity	het	het	homo	het	homo	het	homo
Variant n°1/ cDNA variant/protein change	Nonsense C.4075C>T p.Glnl359* [exon 10]	Missense c.986G>C p.Arg329Pro [exon 6]	Frameshift c.5075delT p.Leul 692Cysfs* 15 [exon 8]	Nonsense C.4087C>T p.Argl363* [exon 8]	Missense C.1016C>T p.Thr339Met [exon 5]	Nonsense C.12973C>T p.Arg4325* [exon 10]	Missense C.12220G>C p.Ala4074Pro [exon 10]
Variant n°2/ cDNA variant/protein change	Missense C.6694C>T pArg2232Cys [exon 21]	Missense c7331G>A p.Arg2444His [exon 24]		Nonsense c.5617G>T p.Glu1873* [exon 11]		Frameshift c.l358delG p.Gly453Valfs* 25 [exon 8]	
Results first assessor/gene n°1	***SETX***	***SETX***	***SETX***	***SETX***	***CYP27A1***	***SACS***	***SACS***
Results first assessor/gene n°2	*ATM*	*ATM*	*ATM*	*ATM*	*POLG*	*ATM*	*CYP27A1*
Results second assessor/gene n°1	***SETX***	***SETX***	***SETX***	***SETX***	***CYP27A1***	***SACS***	***SACS***
Results second assessor/gene n°2	*ATM*	*ATM*	*ATM*	*ATM*	*POLG*	*APTX*	*CYP27A1*

The clinical features of the 23 ataxic patients presented in Table 1 were available for the two assessors for a blinded study, in a random way. The first selected gene is *bolded* when correctly ranked by the assessor. Some patients were already published: patients 3, 13 and 16 [6], patient 6 (patient 4 [17]), patients 7-8-9-10-11 (case 2, 4, 5, 6, 9, respectively [8]), patient 15 (case 28 [20]), patient 21 (case 13 [21]) and patient 1, 2, 3 and 25 (case 16-1, 15-1, 17-1 and 20-1 [22]). In biomarkers section, "–" means no abnormalities in biomarkers tested. Biochemical tests were performed prior to NGS analysis

het compound heterozygous, *homo* homozygous, *M* male, *F* female, *SARA* scale for the assessment and rating of ataxia, *SDFS* spinocerebellar degeneration functional score, *AFP* alpha-fetoprotein (normal range is less than 7 ng/ml), *NA* not available

There was a high inter-rater agreement [$K = 0.85$ (0.55–0.98) $p < 0.001$] on the first gene ranked by the assessors confirming the algorithm's reproducibility.

Discussion

We report on the validation of a clinical practice-based algorithm in a series of patients, based on a blind analysis of clinical and paraclinical data. Given the high percent of correct diagnosis in our study (the assessors were able to find the good molecular diagnosis in 2/3 and 3/4 of cases, respectively), it is expected that this algorithm will be useful in clinical practice for neurologists and geneticists. Moreover, the algorithm allowed us to identify nine distinct entities, including entities that belong to the three different groups of the new classification of ARCAs [3]. One hundred forty-five patients suspected with ARCA were included in the study which is a high number since Friedreich ataxia was previously excluded. Therefore, the evaluation of the 23 patients with a molecularly confirmed diagnosis is relevant.

Confirmation of diagnosis by NGS can be straightforward in presence of clear-cut mutations, even with few clinical data. However, NGS data analysis frequently reveals several variants of unknown significance especially missense mutations in one or more ARCA-causing genes. In these difficult cases, proper phenotyping of ARCA patients, including precise clinical examination, biochemical investigations, brain imaging and NCS, is still necessary for guidance of genetic analysis and interpretation of the NGS data: these data have to be in agreement with an already described phenotype in order to confirm the variant's pathogenicity. Similarly, appropriate knowledge of the several entities and of their description in the literature is recommended for the best management of such scarce diseases.

Despite the relevance of the algorithm, it is not infallible since few errors were made. *ANO10* and *SYNE1* mutations are responsible for close phenotypes [8, 9] with pure cerebellar ataxia and slow progression and may be difficult to distinguish without genetic analysis especially because there is no biomarker. Some errors were therefore done during the blinded assessment regarding these entities. Such algorithm is limited by the presence of atypical phenotypes associated with mutations in known genes. For instance, most patients with autosomal recessive spastic ataxia of Charlevoix Saguenay (ARSACS) experience their first symptoms before 5 years of age, but in very few patients the first signs may occur after 20 or 30 years. Atypical phenotypes have also been reported regarding peripheral neuropathy which was lacking in 1/11 patient in a recent series of ARSACS [10]. Lack of neuropathy is also a rare feature in ataxia with oculomotor apraxia type 2 (AOA2), representing only 2.5 % of cases in a series of 90 patients [11]. Only one patient with *ADCK3* confirmed mutations presented a mild axonal neuropathy (out of 34 patients described in the litterature [12–18]). In the same way, few patients with AOA2 or ataxia telangiectasia presented with the very unusual lack of elevated AFP serum level [11, 19]. However, the overall clinical and paraclinical assessment mostly lead to only a few possible diagnoses, confirming that the phenotype in one subtype of ARCA is relatively homogeneous. Few clinical findings (such as oculomotor apraxia, vertical supranuclear gaze palsy, spastic paraplegia, telangiectasia) as well as reliable biomarkers (such as vitamin E, AFP and albumin serum level) may also be suggestive of one or few diseases. The good results of our blinded assessment support this statement.

Herein, the percentage of positive diagnosis (19 %) is similar to previous studies on NGS in cerebellar ataxias [2] but remains low for several reasons including selection of patients with onset before 60 years of age (whereas ARCAs mostly occur before 30), exclusion of patients with Friedreich ataxia, absence of important ataxia genes in the gene panel, such as *WFS1* and *SPG7*, and the fact that many genes have not been identified yet. It is also possible that a few sporadic cases have in fact polyglutamine SCA due to marked anticipation, particularly for SCA2 and SCA7. Polyglutamine expansions should therefore also be tested, along with Friedreich ataxia expansions.

Web resources

UCSC Genome Browser: http://genome.ucsc.edu/index.html

Ensembl Genome Browser: http://www.ensembl.org/index.html

Exome Variant Server (EVS), NHLBI GO Exome Sequencing Project (ESP), Seattle, WA: http://evs.gs.washington.edu/EVS (June, 2013)

http://www.lbgi.fr/VaRank/.

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Compliance with ethical standards

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References

1. Anheim M, Fleury M, Monga B, Laugel V, Chaigne D, Rodier G, Ginglinger E, Boulay C, Courtois S, Drouot N, Fritsch M, Delaunoy JP, Stoppa-Lyonnet D, Tranchant C, Koenig M (2010) Epidemiological, clinical, paraclinical and molecular study of a cohort of 102 patients affected with autosomal recessive progressive cerebellar ataxia from Alsace. Eastern France: implications for clinical management Neurogenetics 11:1–12
2. Németh AH, Kwasniewska AC, Lise S, Parolin Schnekenberg R, Becker EB, Bera KD, Shanks ME, Gregory L, Buck D, Zameel Cader M, Talbot K, de Silva R, Fletcher N, Hastings R, Jayawant S, Morrison PJ, Worth P, Taylor M, Tolmie J, O'Regan M; UK Ataxia Consortium, Valentine R, Packham E, Evans J, Seller A, Ragoussis J (2013) Next generation sequencing for molecular diagnosis of neurological disorders using ataxias as a model. Brain 136:3106–3118
3. Anheim M, Tranchant C, Koenig M (2012) The autosomal recessive cerebellar ataxias. N Engl J Med 366:636–646
4. Redin C, Le Gras S, Mhamdi O, Geoffroy V, Stoetzel C, Vincent MC, Chiurazzi P, Lacombe D, Ouertani I, Petit F, Till M, Verloes A, Jost B, Chaabouni HB, Dollfus H, Mandel JL, Muller J (2012) Targeted high-throughput sequencing for diagnosis of genetically heterogeneous diseases: efficient mutation detection in Bardet-Biedl and Alström syndromes. J Med Genet 49:502–512
5. Geoffroy V, Pizot C, Redin C, Piton A, Vasli N, Stoetzel C, Blavier A, Laporte J, Muller J (2015) VaRank: a simple and powerful tool for ranking genetic variants. PeerJ 3:e796. doi:10.7717/peerj.796
6. Hamza W, Ali Pacha L, Hamadouche T, Muller J, Drouot N, Ferrat F, Makri S, Chaouch M, Tazir M, Koenig M, Benhassine T (2015) Molecular and clinical study of a cohort of 110 Algerian patients with autosomal recessive ataxia. BMC Med Genet 16:36
7. Schmitz-Hübsch T, du Montcel ST, Baliko L, Berciano J, Boesch S, Depondt C, Giunti P, Globas C, Infante J, Kang JS, Kremer B, Mariotti C, Melegh B, Pandolfo M, Rakowicz M, Ribai P, Rola R, Schöls L, Szymanski S, van de Warrenburg BP, Dürr A, Klockgether T, Fancellu R (2006) Scale for the assessment and rating of ataxia: development of a new clinical scale. Neurology 66:1717–1720
8. Renaud M, Anheim M, Kamsteeg EJ, Mallaret M, Mochel F, Vermeer S, Drouot N, Pouget J, Redin C, Salort-Campana E, Kremer HP, Verschuuren-Bemelmans CC, Muller J, Scheffer H, Durr A, Tranchant C, Koenig M (2014) Autosomal recessive cerebellar ataxia type 3 due to ANO10 mutations: delineation and genotype-phenotype correlation study. JAMA Neurol 71:1305–1310
9. Gros-Louis F, Dupré N, Dion P, Fox MA, Laurent S, Verreault S, Sanes JR, Bouchard JP, Rouleau GA (2007) Mutations in SYNE1 lead to a newly discovered form of autosomal recessive cerebellar ataxia. Nat Genet 39:80–85
10. Synofzik M, Soehn AS, Gburek-Augustat J, Schicks J, Karle KN, Schüle R, Haack TB, Schöning M, Biskup S, Rudnik-Schöneborn S, Senderek J, Hoffmann KT, MacLeod P, Schwarz J, Bender B, Krüger S, Kreuz F, Bauer P, Schöls L (2013) Autosomal recessive spastic ataxia of Charlevoix Saguenay (ARSACS): expanding the genetic, clinical and imaging spectrum. Orphanet J Rare Dis 8:41
11. Anheim M, Monga B, Fleury M, Charles P, Barbot C, Salih M, Delaunoy JP, Fritsch M, Arning L, Synofzik M, Schöls L, Sequeiros J, Goizet C, Marelli C, Le Ber I, Koht J, Gazulla J, De Bleecker J, Mukhtar M, Drouot N, Ali-Pacha L, Benhassine T, Chbicheb M, M'Zahem A, Hamri A, Chabrol B, Pouget J, Murphy R, Watanabe M, Coutinho P, Tazir M, Durr A, Brice A, Tranchant C, Koenig M (2009) Ataxia with oculomotor apraxia type 2: clinical, biological and genotype/phenotype correlation study of a cohort of 90 patients. Brain 132:2688–2698
12. Lagier-Tourenne C, Tazir M, López LC, Quinzii CM, Assoum M, Drouot N, Busso C, Makri S, Ali-Pacha L, Benhassine T, Anheim M, Lynch DR, Thibault C, Plewniak F, Bianchetti L, Tranchant C, Poch O, DiMauro S, Mandel JL, Barros MH, Hirano M, Koenig M (2008) ADCK3, an ancestral kinase, is mutated in a form of recessive ataxia associated with coenzyme Q10 deficiency. Am J Hum Genet 82:661–672
13. Mollet J, Delahodde A, Serre V, Chretien D, Schlemmer D, Lombes A, Boddaert N, Desguerre I, de Lonlay P, de Baulny HO, Munnich A, Rötig A (2008) CABC1 gene mutations cause ubiquinone deficiency with cerebellar ataxia and seizures. Am J Hum Genet 82:623–630
14. Gerards M, van den Bosch B, Calis C, Schoonderwoerd K, van Engelen K, Tijssen M, de Coo R, van der Kooi A, Smeets H (2010) Nonsense mutations in CABC1/ADCK3 cause progressive cerebellar ataxia and atrophy. Mitochondrion 10:510–515
15. Horvath R, Czermin B, Gulati S, Demuth S, Houge G, Pyle A, Dineiger C, Blakely EL, Hassani A, Foley C, Brodhun M, Storm K, Kirschner J, Gorman GS, Lochmüller H, Holinski-Feder E, Taylor RW, Chinnery PF (2012) Adult-onset cerebellar ataxia due to mutations in CABC1/ADCK3. J Neurol Neurosurg Psychiatry 83:174–178
16. Blumkin L, Leshinsky-Silver E, Zerem A, Yosovich K, Lerman-Sagie T, Lev D (2014) Heterozygous Mutations in the ADCK3 Gene in Siblings with Cerebellar Atrophy and Extreme Phenotypic Variability. JIMD Rep 12:103–107
17. Mignot C, Apartis E, Marques Durr A, Lourenço C, Charles P, Devos D, Moreau C, de Lonlay P, Drouot N, Burglen L, Kempf N, Nourisson E, Chantot-Bastaraud S, Lebre AS, Rio M, Chaix Y, Bieth E, Roze E, Bonnet I, Canaple S, Rastel C, Brice A, Rötig A, Desguerre I, Tranchant C, Koenig M, Anheim M (2013) Phenotypic variability in ARCA2 and identification of a core ataxic phenotype with slow progression. Orphanet J Rare Dis 8:173
18. Liu YT, Hersheson J, Plagnol V, Fawcett K, Duberley KE, Preza E, Hargreaves IP, Chalasani A, Laurá M, Wood NW, Reilly MM, Houlden H (2014) Autosomal-recessive cerebellar ataxia caused by a novel ADCK3 mutation that elongates the protein: clinical, genetic and biochemical characterisation. J Neurol Neurosurg Psychiatry 85:493–498
19. Méneret A, Ahmar-Beaugendre Y, Rieunier G, Mahlaoui N, Gaymard B, Apartis E, Tranchant C, Rivaud-Péchoux S, Degos B, Benyahia B, Suarez F, Maisonobe T, Koenig M, Durr A, Stern MH, Dubois d'Enghien C, Fischer A, Vidailhet M, Stoppa-Lyonnet D, Grabli D, Anheim M (2014) The pleiotropic movement disorders phenotype of adult ataxia-telangiectasia. Neurology 83:1087–1095
20. H'mida-Ben Brahim D, M'zahem A, Assoum M, Bouhlal Y, Fattori F, Anheim M, Ali-Pacha L, Ferrat F, Chaouch M, Lagier-Tourenne C, Drouot N, Thibaut C, Benhassine T, Sifi Y, Stoppa-Lyonnet D, N'Guyen K, Poujet J, Hamri A, Hentati F, Amouri R, Santorelli FM, Tazir M, Koenig M (2011) Molecular diagnosis of known recessive ataxias by homozygosity mapping with SNP arrays. J Neurol 258:56–67
21. Lionnet C, Carra C, Ayrignac X, Levade T, Gayraud D, Castelnovo G, Besson G, Androdias G, Vukusic S, Confavreux C, Zaenker C, De Seze J, Collongues N, Blanc F, Tranchant C, Wallon D, Hannequin D, Gerdelat-Mas A, Brassat D, Clanet M, Zephir H, Outteryck O, Vermersch P, Labauge P (2014) Cerebrotendinous xanthomatosis: a multicentric retrospective study of 15 adults, clinical and paraclinical typical and atypical aspects. Rev Neurol (Paris) 170:445–453

22. Synofzik M, Smets K, Mallaret M, Di Bella D, Gallenmüller C, Baets J, Schulze M, Magri S, Sarto E, Mustafa M, Deconinck T, Haack T, Züchner S, Gonzalez M, Timmann D, Stendel C, Klopstock T, Durr A, Tranchant C, Sturm M, Hamza W, Nanetti L, Mariotti C, Koenig M, Schöls L, Schüle R, de Jonghe P, Anheim M, Taroni F, Bauer P (2016) SYNE1 ataxia is a common recessive ataxia with major non-cerebellar features: a large scale multi-centre study. Brain 139:1378–1393

2.4.2. Séquençage à haut débit avec capture ciblée

La stratégie diagnostique de capture d'exons couplée au séquençage à haut débit, mise au point à l'IGBMC à partir de 2012, permettait une analyse des séquences exoniques et introniques flanquantes de 57 gènes d'ataxie. A l'issue des analyses bioinformatiques, la découverte de mutations non connues avait nécessité pour chacune d'entre elles une corrélation entre les connaissances sur la fonction du gène muté, la conservation de l'acide aminé impliqué, et le type de mutation pour obtenir une prédiction de la pathogénicité de la mutation.

La caractérisation précise de manifestations cliniques (échelles SARA, fiche SPATAX, vidéo), neurophysiologiques (EMG) et morphologiques (IRM cérébrale) de tous les patients passés dans la capture d'exons d'ataxie avait été effectuée au préalable pour chaque patient.

L'algorithme proposé par Anheim et *al*. en 2012 pour le diagnostic des ataxies héréditaires a ensuite été testé chez 21 des patients atteints d'ataxie autosomique récessive. Il s'agissait de trouver le gène responsable de la maladie (identifié grâce au panel) en se basant sur l'algorithme et sur les seules données cliniques et paracliniques (c'est-à-dire en aveugle des résultats de l'analyse moléculaire). Le gène correct a été prédit dans 62% et 76% des cas par deux cliniciens évaluateurs indépendants, experts en ataxie. La concordance entre les évaluateurs était forte ($p < 0.00007$) confirmant la reproductibilité de l'algorithme.

Tableau 3: Liste des 57 gènes et des maladies correspondantes inclus dans la capture d'exons de gènes d'ataxie.

Nom de la maladie	Gène
Abétalipoprotéinémie	MTTP
ADCK3, déficit en coenzyme Q10	CABC1
Déficit en alpha-methylacyl-coA racemase	AMACR
Anémie sidéroblastique et ataxie spino-cérébelleuse	ABCB7
ARCA3	ANO10
ARCA1	SYNE1
ARSACS	SACS
Ataxie télangectasie	ATM
Ataxie télangectasie-like (ATLD)	MRE11A
Ataxie, neuropathie axonale, hypoalbuminémie	TDP1
Ataxie avec apraxie oculo-motrice type 2 (AOA2)	SETX
Ataxie avec apraxie oculo-motrice type 1 (AOA1)	APTX
Ataxie de Salih	KIAA0226
AVED	TTPA
Ataxie de Cayman	ATCAY
Ataxie cérébelleuse et retard mental avec ou sans locomotion quadrupédale type 3	CA8
Ataxie cérébelleuse et retard mental avec ou sans locomotion quadrupédale type 1	VLDLR
Xanthomathose cérébrotendineuse	CYP27A1
Ataxie-épilepsie	WWOX
Désordre de glycosylation type 1a (CDG1A)	PMM2
Ataxie de Friedreich	FXN
Gangliosidose 1 (GM1)	GLB1
IOSCA	C10orf2
Syndrome de Joubert type 1	INPP5E
Syndrome de Joubert type 10	OFD1
Syndrome de Joubert type 12	KIF7
Syndrome de Joubert type 13	TCTN1

Nom de la maladie	Gène
Syndrome de Joubert type 3	AHI1
Syndrome de Joubert type 6	TMEM67
Syndrome de Joubert type 8	ARL13B
Syndrome de Joubert type 9	CC2D2A
Maladie de Karak	PLA2G6
Maladie de Krabbe (leucodystrophie globoïde)	GALC
Acidurie L-2-hydroxyglutarique	L2HGDH
Syndrome de Marinesco-Sjögren	SIL1
Maladie de Niemann-Pick type C1	NPC1
Maladie de Niemann-Pick type C2	NPC2
Polyneuropathie, hypoacousie, ataxie, rétinite pigmentaire, cataracte (PHARC)	ABHD12
Ataxie cordonale postérieure, rétinite pigmentaire (PCARP)	FLVCR1
Maladie de Refsum	PHYH
Maladie de Refsum, forme infantile	PEX2
Maladie de Refsum like	PEX7
Maladie de Sandhoff (GM2)	HEXB
SCA 5	SPTBN2
SCA11	TTBK2
SCA13	KCNC3
SCA14	PKCG
SCA15	ITPR1
SCA23	PDYN
SCA27	FGF14
SCA28	AFG3L2
Ataxie sensitive, dysarthrie, ophtalmoplégie, (SANDO)	POLG
Ataxie spastique de type 4	MTPAP
ARCA11	SYT14
Maladie de Tay-Sachs (GM2)	HEXA
Maladie de Unverricht Lundborg type 1A	CSTB
Maladie de Unverricht Lundborg type1B	PRICKLE1
Syndrome de Wolfram 2	CISD2

2.4.3. Manuscrit 5

Recessive ataxia diagnosis ranking algorithm: a new paradigm for the next-generation sequencing era

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Running Title: Novel Ataxia diagnosis ranking algorithm

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Abstract

Diagnosis of autosomal recessive cerebellar ataxias (ARCAs) is challenging due to their complex and heterogeneous nature. Next-generation sequencing (NGS) has markedly improved the genetic diagnosis of autosomal recessive cerebellar ataxias, with the identification of new genes and phenotypic variants. However, the large volume of novel genetic variants, in combination with complex, often overlapping clinical phenotypes, poses a substantial challenge for interpretation of data generated by NGS approaches. A ranking algorithm predicting the molecular diagnosis of autosomal recessive cerebellar ataxia based on the clinical phenotype would therefore be of great value to guide genetic testing and to align genetic findings with the clinical context.

An automated algorithm that follows clinical practice, including patient history (age at onset and severity of disease progression), clinical, brain MRI, electromyography and biomarker features, was developed based on the results of a literature search on autosomal recessive cerebellar ataxias and personal clinical experience. The frequency and specificity of each feature was defined for each autosomal recessive cerebellar ataxia, and corresponding prediction scores assigned. Clinical and paraclinical features of patients are entered into the algorithm and a patient's total score for each autosomal recessive cerebellar ataxia is calculated producing a ranking of possible diagnoses. The features of 67 autosomal recessive cerebellar ataxias, equating to 124 clinical and paraclinical features, defined the algorithm. Sensitivity and specificity of the algorithm were assessed by blinded analysis of a multinational cohort of 834 patients with molecularly confirmed autosomal recessive cerebellar ataxia, representing 45 different autosomal recessive cerebellar ataxias. The performance of the algorithm versus a blinded panel of ARCA experts was assessed.

The correct diagnosis was ranked within the top 3 highest-scoring diagnoses at a sensitivity or specificity of >90% for 84% and 91% of the evaluated genes, respectively. Mean sensitivity and specificity of the top 3 highest-scoring diagnoses were 92% and 95%, respectively. The algorithm outperformed the panel of autosomal recessive cerebellar ataxia experts (P=0.001).

Our algorithm is highly sensitive and specific, accurately predicting the underlying molecular diagnoses of autosomal recessive cerebellar ataxias, thereby guiding targeted sequencing or facilitating interpretation of next-generation sequencing data.

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List of abbreviations

0	Not present
ARCA	Autosomal recessive cerebellar ataxia
DD	Differential diagnosis
H	High frequency
L	Low frequency
LOFA	Late-onset Friedreich's ataxia
NGS	Next-generation sequencing
NK	Not known
RADIAL	RAnking differential DIagnosis ALgorithm
S	Specific
v-LOFA	Very late-onset Friedreich's ataxia
VUS	Variants of unknown significance

Introduction

Clinical heterogeneity and rarity of some neurological disorders may challenge the ability of clinicians to make timely diagnoses. This is true for autosomal recessive cerebellar ataxia (ARCA), a complex group of rare disorders (Fogel and Perlman, 2007; Anheim *et al.*, 2010; Anheim *et al.*, 2012; van de Warrenburg *et al.*, 2014). The revolution in molecular genetics, especially next-generation sequencing (NGS) (Koboldt *et al.*, 2013), has led to the identification of new ARCA-causing genes or novel phenotypes of known ARCA-causing genes, improving our understanding of these disorders and our ability to diagnose them (Nemeth *et al.*, 2013; Marelli *et al.*, 2016). However, NGS generates a huge amount of data, including variants of unknown significance (VUS) that may be difficult and time-consuming to correctly interpret and establish relationships between potential pathogenic variants and observed phenotypes (Precone *et al.*, 2015).

There is therefore an unmet need for a tool to assist physicians and geneticists in providing a comprehensive and balanced differential diagnosis (DD) of ARCAs. A DD tool that predicts the gene responsible for a phenotype by converting clinical and paraclinical data into a shortlist of likely molecular diagnoses could significantly increase the speed and yield of diagnosis for ARCAs, subsequently improving patient care, particularly in cases where treatments are available.

Our study aimed to create and validate a tool for the DD of patients with suspected ARCA.

Materials and methods

The Recessive Ataxias ranking differential DIagnosis ALgorithm (RADIAL)

A diagnostic algorithm for ARCA based on literature and expert opinion has been produced to guide neurologists who may encounter patients with ataxia in clinical practice (**Fig. 1**). Recessive disorders (herein referred to as entities, each defined by mutations in specific gene(s)) with ataxia as a common, but not necessarily an initial or prominent feature, were identified from the literature according to previously published recommendations (Anheim *et al.*, 2010; Anheim *et al.*, 2012).

Publications were identified by PubMed search for English language articles between January 1995–January 2016, using the following terms: “recessive cerebellar ataxia”, “recessively inherited cerebellar ataxia”, or “inherited cerebellar ataxia”, and screened for reports of molecularly confirmed cases of recessively inherited disorders with cerebellar ataxia in order to include the majority of the most common ARCA; 281 manuscripts were used to describe the entities included in the algorithm (**Supplementary Table 1**). To describe entities, 124 individual clinical and paraclinical findings including routine biomarkers (so-called features), were identified and refined by expert opinion.

The relationship between each feature and each entity was defined by frequency and/or specificity according to the literature and clinical experience of CT and MA, as follows:

- High frequency (H) – feature occurs in ≥50% of patients with the entity
- Low frequency (L) – feature occurs in <50% of patients with the entity. Where not clearly definable, low frequency is assumed

- Specific (S) – feature presents in <10% of entities
- Not present (0) – feature not considered to be associated with the entity
- Not known (NK) – feature not reported with the entity, but with insufficient evidence to exclude association

These classifications were combined as necessary, e.g. HS, high frequency and specific; LS, low frequency and specific.

Scores were assigned to the frequency and specificity of features, and weighted based on the importance for DD. NK, 0, L, H, LS, and HS were scored 0, −1, 1, 3, 7 and 9, points respectively, for clinical features, neuroimaging and electromyography, whereas scores were doubled for age of onset, severity of disease progression and biomarkers (**Supplementary Table 2**). For example, if a patient with suspected ARCA has a *cataract*, they would score +3 points for cerebrotendinous xanthomatosis, in which *cataract* is high frequency (H), but would score -1 points for Friedreich's ataxia, in which *cataract* is not reported (0). A second patient who does not present with *cataract* would score 0 points for both cerebrotendinous xanthomatosis and Friedreich's ataxia.

The outcome of this process was the RADIAL, comprising a knowledgebase that defines the association of each clinical and paraclinical feature with each entity, with a corresponding score assigned to each feature-entity association (**Supplementary Table 3**).

ARCA patient clinical features data collection

Diagnostic performance of RADIAL was assessed by applying it blindly to a population of patients with molecularly confirmed ARCA, for whom a description of features at the time of molecular diagnosis was available.

The features of a worldwide patient cohort were collected by retrospective chart review between February–May 2016. The persons responsible for data collection from collaborating centres were blinded to the scoring and weighting of features. All contributors of data are listed in **Supplementary Table 4.**

All patient data were blinded by the contributor so that names, addresses or other identifying information were not available to any other party involved in the analysis or review of the data. All investigators adhered to local privacy laws and regulations to ensure patient confidentiality. Patients gave written consent and ethical approval for the study was provided by the local ethics committee of the Strasbourg University Hospital, France.

Statistical analysis

Performance assessment of RADIAL

Features of each patient were assessed against the knowledgebase according to the aforementioned calculation method. For each patient, the sum of scores for each feature was calculated for each entity. The total score for each entity defined its position on a ranked list of most likely (highest score) to least likely (lowest score) molecular diagnoses. Algorithm performance was a measurement of sensitivity, and specificity. Correct patient classification was defined as a ranking of the correct diagnosis within

the first, third, and fifth highest scores. The primary outcome of the performance assessment was the ability of RADIAL to correctly predict entities within the Top 3 highest scores. Other outcomes were: correctly predicting entities within the Top 1 and Top 5 highest scores; average sensitivity and specificity for all patients; the ability of RADIAL to correctly identify an entity compared to that of a panel of ARCA experts and correlation between performance and the total number and/or number of specific clinical features per entity.

Blind evaluation against an ARCA expert panel

The ability of RADIAL to correctly identify an entity within the Top 3 highest scores was compared against a blind evaluation by a panel of five ARCA experts (FM, MP, MS, CT and BW) in a sample of 100 patients for each expert, randomly selected from the patient cohort. The experts were given the same list of features provided by the investigators for the algorithm assessment, and produced a ranked shortlist of the three most likely diagnoses for each patient. The experts were able to consult the literature (e.g. PubMed) and were allowed as much time as required to complete the task. The three most likely diagnoses were compared against the Top 3 diagnoses provided by RADIAL for the same 100 patients. For mutations in *FXN* gene, the diagnosis was considered correct when Friedreich's ataxia, LOFA and v-LOFA was proposed. Statistical differences were assessed by McNemar's exact test.

Correlation was tested using Pearson correlation coefficient. All statistical analyses were performed using the statistical package SAS v9.3.

Results

The RADIAL

The algorithm knowledgebase contains 67 individual ARCAs (**Supplementary Table 5**). The well-described variants LOFA and v-LOFA were considered a single entity distinct from Friedreich's ataxia within the knowledgebase and these analyses (Reetz *et al.*, 2015; Lecocq *et al.*, 2016). The RADIAL knowledgebase contains 8,308 individual correlations between 67 entities and 124 features (**Supplementary Table 3**).

Details of the patient cohort

Data of 834 patients from 18 different countries and representing 45 distinct entities were collected (**Table 1**).

A broad symptomatology was reported in the patient cohort; a summary of the symptomatology observed in the entire cohort and in the 8 entities with ≥30 patients is presented in **Supplementary Table 6.**

Algorithm performance

Of the 45 entities tested, 91% (41/45) were ranked within the Top 3 highest-scoring diagnoses at a specificity of >90% and 84% (38/45) at a sensitivity of >90% (**Table 2**). Among these entities, the correct diagnosis was the highest scoring in 23 entities (51%), and was always found within the Top 16 highest scoring entities. The Top 3 highest scoring diagnoses had an average sensitivity and specificity of 92.2% and 95.4%, respectively (**Table 3 and Fig. 2**). The highest scoring diagnosis had an average sensitivity and specificity of 77.1% and 99.3% respectively. The Top 5 highest scoring

diagnoses had an average sensitivity and specificity of 96.8% and 91.1%. Average sensitivity and specificity plots including all tested disorders are shown in **Fig. 2**. *ADCK3* (Mignot *et al.*, 2013), *OPA1* (Bonifert *et al.*, 2014), *PNPLA6* (Synofzik *et al.*, 2014), *STUB1* (Shi *et al.*, 2013) and *SYNE1* (Synofzik *et al.*, 2016) and LOFA/vLOFA (Reetz *et al.*, 2015; Lecocq *et al.*, 2016) were not identified within the Top 3 scores with a sensitivity >90%.

Assessment of the correlation between the number of specific features associated with each entity and RADIAL performance, and between the total number of features associated with each entity and RADIAL performance, showed that neither the number of total signs (r=0.052, *P*=0.730) nor specific signs (r=−0.033, *P*=0.830) influence discriminatory ability (**Fig. 3**).

RADIAL versus expert panel challenge

RADIAL performed well compared with a panel of 5 ARCA experts. In five series of 100 patients randomly selected from the patient cohort, RADIAL placed the correct diagnosis in the Top 3 highest scoring entities in 95.2% of patients versus 77.8% identified by the experts (*P*<0.001; McNemar's exact test) and as the highest scoring entity in 80.6% of patients versus 67.6% identified by the experts (*P*<0.001; McNemar's exact test). The experts required an average of 7.2 hours [range: 6–10] to complete their 100 cases.

Discussion

This study describes RADIAL, an algorithm that aims to improve the DD approach towards ARCA by using patients' features to predict the underlying responsible gene. Sensitivity and specificity of the algorithm in correctly identifying the diagnosis within

the Top 3 highest scoring entities was excellent, even outperforming a panel of ARCA experts.

In clinical practice, use of RADIAL should be considered in all patients suspected with ARCA. The diagnostic workup outlined in **Fig. 1** takes a stepwise approach to the patient with ataxia (Anheim *et al.*, 2010; Durr, 2010; Klockgether, 2010; Anheim *et al.*, 2012), also indicating how the point of suspecting ARCA is reached.

Performance of RADIAL depends on accurate identification of the patient's features, and could be impaired in the absence of sufficiently detailed information. In such cases, the algorithm could be used to guide clinical investigations based on the features of the highest scoring entities from the knowledgebase. The knowledgebase could also clarify the clinical phenotype in a 'genotype-first' (Mefford, 2009; Stessman *et al.*, 2014) (i.e. genotyping before phenotyping), or 'reverse phenotyping' (i.e. phenotyping following genotyping according to genetic results) method (Uliana and Percesepe, 2016). However, due to the risk of missing a correct diagnosis, we would not recommend a 'genotype-first' approach, and 'reverse phenotyping' should be considered with caution. Regardless of where in the diagnostic workup it occurs, a sufficiently detailed phenotypic evaluation is always mandatory. RADIAL could also be used to identify one high likelihood entity which can be confirmed by single gene sequencing. Thus, in this 'phenotype-first' approach, the algorithm could also guide molecular analyses. Whether the 'phenotype-first' or 'genotype-first' approach is followed, the entity ranking provided by RADIAL supports identification of the gene responsible for the phenotype. Indeed, RADIAL should be considered as an interface between phenotype and genotype, enabling each one to complement the other.

Beyond its efficacy to predict the underlying molecular defect based on clinical data, RADIAL will provide guidance on best-practice for the diagnostic work-up of patients with suspected ARCA, including medical history, clinical examination, and paraclinical signs, serving as a reminder to assess many features that may otherwise be overlooked.

Given the performance of RADIAL, it could facilitate the interpretation of large volumes of data provided by NGS (panel gene sequencing, whole exome or whole genome sequencing), and pathogenicity of VUS could be more easily determined (Cooper, 2015). For instance, the probability of a VUS being pathogenic when the affected gene is not within the Top 16 entities should be very low, whereas a VUS in a high-ranking gene, especially one within the Top 3, is much more likely, facilitating interpretation of NGS data and guiding searches for a second mutation in the same gene. The good sensitivity of RADIAL is important to avoid a missed diagnosis. Conversely, the good specificity provides confidence in the identified top scoring genes, especially when use of RADIAL follows molecular analyses. However, in such cases, identification of many VUS may return misleading false-positive results.

RADIAL also represents an up-to-date knowledgebase comprising clinical descriptions of 67 individual ARCAs based on the integration of numerous references and expert clinical experience. The 67 entities described in the knowledgebase include the majority of the most common ARCAs, but are not an exhaustive list since there are several other recessively inherited diseases that may include cerebellar ataxia. Some of these diseases have not been included in the current knowledgebase as they lack sufficiently detailed information on their clinical features (e.g. *KIAA0226)* (Assoum *et al.*, 2010; Mallaret *et al.*, 2014). Several other entities included in the knowledgebase could be viewed as controversial, as cerebellar ataxia is not the initial or most prominent feature of the

disease. However, the entities were included in the knowledgebase because cerebellar ataxia was sufficiently well-described as a clinical feature. Following this initial study, periodic updates of the knowledgebase as the literature grows could add or redefine associations between features and entities further improving performance of RADIAL, particularly for entities that are currently poorly characterised, and allowing addition of new entities. One may hypothesize that increasing the number of entities covered by the algorithm, may increase its superiority to the experts.

RADIAL performance is limited by the quality and completeness of data published in the literature. The knowledgebase should be helpful to any physician facing ataxia, or interested in becoming more specialised within this field. The extensive list of features allows a very precise description of each entity (124 features equates to over 2.1 $x10^{37}$ pairwise combinations) leading to good differentiation between entities, even those with similar but non-identical phenotypes. The performance and flexibility of RADIAL means that an exhaustive assessment of all the signs in patients suspected with ARCA is not required for good performance, and that accurate diagnoses for many patients are possible with few clinical features. A second class of entities, including *ADCK3* (Mignot *et al.*, 2013), *OPA1* (Bonifert *et al.*, 2014), *PNPLA6* (Synofzik *et al.*, 2014), *STUB1* (Shi *et al.*, 2013) and *SYNE1* (Synofzik *et al.*, 2016), are still poorly recognised by RADIAL. These entities are unlikely to appear near the top of the ranked lists, but should be considered as DD when genetic analyses are inconclusive and the clinical phenotype does not clearly match the highest scoring entities. The poorer performance of RADIAL in recognising these entities might be attributable to an intrinsic difficulty in their identification due to pleiotropic, overlapping clinical phenotypes (e.g. pure cerebellar ataxia in *ADCK3* and *SYNE1*) (Mignot *et al.*, 2013; Synofzik *et al.*, 2016), the lack of helpful biomarkers, or limitations in our classification of their specific clinical

features due to their novelty (e.g. *PNPLA6* and *STUB1*) (Hakonen *et al.*, 2007; Mignarri *et al.*, 2014). RADIAL was not able to recognise vLOFA and LOFA as effectively as Friedreich's ataxia. However, given that the former are variants of Friedreich's ataxia, the performance of RADIAL is successful at identifying *FXN* gene mutations. Moreover, Friedreich's ataxia, including LOFA and v-LOFA, is not diagnosed by NGS, therefore validating a VUS on the basis of such diagnoses is not pertinent.

The use of real-life clinical data to test the accuracy of RADIAL addresses many of the limitations discussed above, as the excellent diagnostic performance suggests that the knowledgebase provides a good representation of the clinical characteristics of each entity. This is reinforced by the algorithm outperforming an ARCA expert panel in correctly identifying diagnoses in both the Top 1 and Top 3 positions, a method commonly used to validate the performance of diagnostic algorithms, despite recent evidence showing that the superiority of the physician is maintained over several algorithms (Semigran *et al.*, 2016).

The true incidence of ARCAs observed in clinical practice is not reflected in the patient cohort. We were particularly interested in testing the algorithm with a broad sample of entities, especially those that are not well known and/or difficult to diagnose, which is not the case for Friedreich's ataxia, the most frequent ARCA (Anheim *et al.*, 2010). Since the ranking calculation does not take into account the prevalence/incidence of a disease we do not believe that this negatively impacted the validation.

In order to obtain a sufficient quantity of data to robustly validate RADIAL performance, it was necessary to obtain previously reported patient data with clear-cut molecular diagnoses by retrospective chart review. Unfortunately the main limitation of this approach is that many patients whose data were collected would have been reported

in the literature used to construct the knowledgebase; this is more problematic with the rarer entities for which the published cases may represent the majority of the global patient population. Regardless, many more patients whose data were used in construction of the knowledgebase were not assessed in the study. That the expert panel were able to correctly identify the diagnosis in the majority of cases also supports the accuracy of the phenotypic definitions of each entity that were used to construct the knowledgebase. Taken together, these arguments support the genuinely very good diagnostic performance of RADIAL.

Another consideration, is the difficulty in accurately determining the frequency and specificity of clinical features, especially in rare entities where reported patient numbers are low. Future large-scale prospective real-world validation of RADIAL should be undertaken to address these concerns, further validate and, if necessary, refine RADIAL. A prospective study would also allow the opportunity to assess utility of RADIAL for interpretation of NGS-derived data, and assess whether RADIAL can predict the pathogenicity of novel VUS. For this purpose, RADIAL will soon be made available to all healthcare professionals as a free-to-use electronic application, along with an ARCA registry into which physicians can deposit patient data.

This algorithmic approach may be of further interest for many other diseases with inherent diagnostic difficulties, including neurological (e.g. autosomal dominant cerebellar ataxias, hereditary spastic paraplegias, neuropathy, myopathy, complex dystonia, and early dementia) and non-neurological disorders.

In summary, we have developed a tool that facilitates the differential diagnosis of autosomal recessive cerebellar ataxias. RADIAL uses patient's features to produce a list of potential diagnoses ranked by likelihood, and which may be used to inform further

confirmatory clinical or genetic testing, and assist the interpretation of next-generation sequencing data.

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Author contributions

Mathieu Anheim, Stefan Kolb, Christine Tranchant, Mathilde Renaud and Juan Vicente Torres Martin (JVTM) were involved in developing the concept and design of the study.

Mathieu Anheim, Mathilde Renaud and Christine Tranchant conducted the literature review and refined the ARCA knowledgebase. All drafts were written by the corresponding and senior authors, and reviewed by all other authors. Fanny Mochel, Massimo Pandolfo, Matthis Synofzik, Christine Tranchant and Bart van de Warrenburg comprised the panel of ARCA experts who undertook the blinded assessment. Statistical analysis of the data was performed by JVTM. All authors also provided approval for the submission of the manuscript and agreed to be accountable for all aspects of the work.

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Supplementary material

6 Supplementary Tables

References

Anheim M, Tranchant C, Koenig M. The autosomal recessive cerebellar ataxias. N Engl J Med 2012; 366: 636-46.

Anheim M, Fleury M, Monga B, Laugel V, Chaigne D, Rodier G, *et al.* Epidemiological, clinical, paraclinical and molecular study of a cohort of 102 patients affected with autosomal recessive progressive cerebellar ataxia from Alsace, Eastern France: implications for clinical management. Neurogenetics 2010; 11: 1-12.

Assoum M, Salih MA, Drouot N, H'Mida-Ben Brahim D, Lagier-Tourenne C, AlDrees A, *et al.* Rundataxin, a novel protein with RUN and diacylglycerol binding domains, is mutant in a new recessive ataxia. Brain 2010; 133: 2439-47.

Bonifert T, Karle KN, Tonagel F, Batra M, Wilhelm C, Theurer Y, *et al.* Pure and syndromic optic atrophy explained by deep intronic OPA1 mutations and an intralocus modifier. Brain 2014; 137: 2164-77.

Cooper GM. Parlez-vous VUS? Genome Res 2015; 25: 1423-6.

Durr A. Autosomal dominant cerebellar ataxias: polyglutamine expansions and beyond. Lancet Neurol 2010; 9: 885-94.

Fogel BL, Perlman S. Clinical features and molecular genetics of autosomal recessive cerebellar ataxias. Lancet Neurol 2007; 6: 245-57.

Hakonen AH, Isohanni P, Paetau A, Herva R, Suomalainen A, Lonnqvist T. Recessive Twinkle mutations in early onset encephalopathy with mtDNA depletion. Brain 2007; 130: 3032-40.

Klockgether T. Sporadic ataxia with adult onset: classification and diagnostic criteria. Lancet Neurol 2010; 9: 94-104.

Koboldt DC, Steinberg KM, Larson DE, Wilson RK, Mardis ER. The next-generation sequencing revolution and its impact on genomics. Cell 2013; 155: 27-38.

Lecocq C, Charles P, Azulay JP, Meissner W, Rai M, N'Guyen K, *et al.* Delayed-onset Friedreich's ataxia revisited. Mov Disord 2016; 31: 62-9.

Mallaret M, Synofzik M, Lee J, Sagum CA, Mahajnah M, Sharkia R, *et al.* The tumour suppressor gene WWOX is mutated in autosomal recessive cerebellar ataxia with epilepsy and mental retardation. Brain 2014; 137: 411-9.

Marelli C, Guissart C, Hubsch C, Renaud M, Villemin JP, Larrieu L, *et al.* Mini-exome coupled to read-depth based copy number variation analysis in patients with inherited ataxias. Hum Mutat 2016; 37: 1340-53.

Mefford HC. Genotype to phenotype-discovery and characterization of novel genomic disorders in a "genotype-first" era. Genet Med 2009; 11: 836-42.

Mignarri A, Gallus GN, Dotti MT, Federico A. A suspicion index for early diagnosis and treatment of cerebrotendinous xanthomatosis. J Inherit Metab Dis 2014; 37: 421-9.

Mignot C, Apartis E, Durr A, Marques Lourenco C, Charles P, Devos D, *et al.* Phenotypic variability in ARCA2 and identification of a core ataxic phenotype with slow progression. Orphanet J Rare Dis 2013; 8: 173.

Nemeth AH, Kwasniewska AC, Lise S, Parolin Schnekenberg R, Becker EB, Bera KD, *et al.* Next generation sequencing for molecular diagnosis of neurological disorders using ataxias as a model. Brain 2013; 136: 3106-18.

Precone V, Del Monaco V, Esposito MV, De Palma FD, Ruocco A, Salvatore F, *et al.* Cracking the Code of Human Diseases Using Next-Generation Sequencing: Applications, Challenges, and Perspectives. Biomed Res Int 2015; 2015: 161648.

Reetz K, Dogan I, Costa AS, Dafotakis M, Fedosov K, Giunti P, *et al.* Biological and clinical characteristics of the European Friedreich's Ataxia Consortium for Translational Studies (EFACTS) cohort: a cross-sectional analysis of baseline data. Lancet Neurol 2015; 14: 174-82.

Semigran HL, Levine DM, Nundy S, Mehrotra A. Comparison of physician and computer diagnostic accuracy. JAMA Intern Med 2016; 176: 1860-1.

Shi Y, Wang J, Li JD, Ren H, Guan W, He M, *et al.* Identification of CHIP as a novel causative gene for autosomal recessive cerebellar ataxia. PLoS One 2013; 8: e81884.

Stessman HA, Bernier R, Eichler EE. A genotype-first approach to defining the subtypes of a complex disease. Cell 2014; 156: 872-7.

Synofzik M, Gonzalez MA, Lourenco CM, Coutelier M, Haack TB, Rebelo A, *et al.* PNPLA6 mutations cause Boucher-Neuhauser and Gordon Holmes syndromes as part of a broad neurodegenerative spectrum. Brain 2014; 137: 69-77.

Synofzik M, Smets K, Mallaret M, Di Bella D, Gallenmuller C, Baets J, *et al.* SYNE1 ataxia is a common recessive ataxia with major non-cerebellar features: a large multi-centre study. Brain 2016; 139: 1378-93.

Uliana V, Percesepe A. Reverse phenotyping comes of age. Mol Genet Metab 2016; 118: 230-1.

van de Warrenburg BP, van Gaalen J, Boesch S, Burgunder JM, Durr A, Giunti P, *et al.* EFNS/ENS Consensus on the diagnosis and management of chronic ataxias in adulthood. Eur J Neurol 2014; 21: 552-62.

Table 1: Number of patients included in the analyses by molecularly confirmed diagnosis.

Gene	Disease name	Number of patients (%)
Total		834 (100)
ABHD12	**Polyneuropathy, hearing loss, ataxia, retinitis pigmentosa, and cataract (PHARC)**	9 (1.1)
ADCK3	**ARCA2**	13 (1.6)
ANO10	**ARCA3**	14 (1.7)
APTX	**Ataxia-oculomotor apraxia 1**	58 (7.0)
ATM	**Ataxia-telangiectasia**	44 (5.3)
ATP7B	**Wilson disease**	9 (1.1)
C10ORF2	**Infantile onset spinocerebellar ataxia (IOSCA)**	24 (2.9)
CP	**Aceruloplasminemia**	1 (0.1)
CTX	**Cerebrotendinous xanthomatosis**	21 (2.5)
DARS2	**Leukoencephalopathy with brain stem and spinal cord involvement and lactate elevation**	8 (1.0)
FXN (FRDA)	**Friedreich's ataxia**	20 (2.4)
FXN (LOFA)	**late-onset Friedreich's ataxia and very late-onset Friedreich's ataxia**	40 (4.8)
GAN	**Giant axonal neuropathy-1**	6 (0.7)
GBE1	**Adult polyglucosan body disease**	7 (0.8)
GOSR2	**Epilepsy, progressive myoclonic 6**	12 (1.4)
GRM1	**Congenital cerebellar ataxia, autosomal recessive, associated with mental retardation**	10 (1.2)
HSD17B4	**Perrault syndrome 1**	3 (0.4)
KCNJ10	**Epilepsy, ataxia, sensorineural deafness and tubulopathy (EAST) syndrome**	1 (0.1)
MRE11	**Ataxia-telangiectasia-like disorder**	10 (1.2)
NEU1	**Sialidosis, type I**	26 (3.1)

NPC1/NPC2	**Niemann-Pick disease Type C**	57	(6.8)
OPA1	**Behr syndrome**	4	(0.5)
OPA3	**3-methylglutaconic aciduria, type III**	10	(1.2)
PEX10	**Peroxisome biogenesis disorder 6A**	3	(0.4)
PEX7	**Peroxisome biogenesis disorder 9B**	10	(1.2)
PHYH	**Refsum disease**	1	(0.1)
PLA2G6	**PLA2G6 associated neurodegeneration (PLAN) Infantile neuroaxonal dystrophy 1**	11	(1.3)
PMM2	**Congenital disorder of glycosylation, type Ia (CDG1a)**	29	(3.5)
PNKP	**Ataxia-oculomotor apraxia 4**	11	(1.3)
PNPLA6	**Boucher-Neuhauser syndrome**	14	(1.7)
POLG	**Sensory ataxic neuropathy with dysarthria and ophthalmoplegia (SANDO)**	15	(1.8)
POLR3A	**Leukodystrophy, hypomyelinating, 7, with or without oligodontia and/or hypogonadotropic hypogonadism**	20	(2.4)
POLR3B	**Leukodystrophy, hypomyelinating, 8, with or without oligodontia and/or hypogonadotropic hypogonadism**	30	(3.6)
SACS	**Autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS)**	38	(4.6)
SCARB2	**Epilepsy, progressive myoclonic 4, with or without renal failure**	15	(1.8)
SETX	**Ataxia-oculomotor apraxia 2**	60	(7.2)
SNX14	**Spinocerebellar ataxia, autosomal recessive 20**	7	(0.8)
SPG7	**Spastic paraplegia 7, autosomal recessive**	23	(2.8)
STUB1	**Spinocerebellar ataxia, autosomal recessive 16**	9	(1.1)
SYNE1	**Spinocerebellar ataxia, autosomal recessive 8**	26	(3.1)
TTPA	**Ataxia with vitamin E deficiency**	69	(8.3)
VLDLR	**Cerebellar hypoplasia and mental retardation with or without quadrupedal**	15	(1.8)

	locomotion 1	
VWA3B	**Spinocerebellar ataxia, autosomal recessive 22**	3 (0.4)
WDR73	**Galloway-Mowat syndrome**	13 (1.6)
ZNF592	**Spinocerebellar ataxia, autosomal recessive 5**	5 (0.6)

Table 2: Size of ranking window required to meet 90% sensitivity for each disorder.

Gene	To reach 90% sensitivity		
	Window size	**Sensitivity**	**Specificity**
ABHD12	3	1.000	0.978
ADCK3	4	0.923	0.961
ANO10	3	0.929	0.966
APTX	3	0.914	0.937
ATM	1	1.000	0.975
ATP7B	1	1.000	0.970
C10ORF2	1	0.958	0.989
CP	1	1.000	0.999
CTX	1	1.000	0.998
DARS2	3	1.000	0.936
FXN (FRDA)	1	0.900	0.989
FXN (LOFA)	10	0.900	0.884
GAN	1	1.000	1.000
GBE1	1	1.000	0.954
GOSR2	1	1.000	0.999
GRM1	2	1.000	0.973
HSD17B4	1	1.000	0.995
KCNJ10	2	1.000	0.986
MRE11	2	1.000	0.999
NEU1	2	0.962	0.985
NPC1/NPC2	2	0.947	1.000
OPA1	4	1.000	0.999
OPA3	1	0.900	0.987
PEX10	1	1.000	1.000
PEX7	1	0.900	1.000
PHYH	5	1.000	0.977
PLA2G6	1	0.909	0.966
PMM2	2	0.966	0.968

PNKP	1	0.909	0.995
PNPLA6	16	0.929	0.894
POLG	1	0.933	0.998
POLR3A	1	1.000	0.962
POLR3B	2	0.900	0.974
SACS	1	1.000	0.992
SCARB2	2	0.933	0.933
SETX	1	0.900	0.994
SNX14	1	1.000	1.000
SPG7	3	0.913	0.959
STUB1	10	1.000	0.692
SYNE1	9	0.962	0.847
TTPA	1	0.913	0.973
VLDLR	2	1.000	0.994
VWA3B	1	1.000	0.999
WDR73	1	1.000	1.000
ZNF592	3	1.000	0.996

Table 3: Sensitivity and specificity of the algorithm for correctly placing disorders into the Top 1, Top 3, or Top 5 places of output lists ranked by total score.

	Top 1		Top 3		Top 5	
Gene	**Sensitivity**	**Specificity**	**Sensitivity**	**Specificity**	**Sensitivity**	**Specificity**
ABHD12	0.889	0.999	1.000	0.978	1.000	0.955
ADCK3	0.538	1.000	0.846	0.987	0.923	0.948
ANO10	0.500	0.995	0.929	0.966	0.929	0.924
APTX	0.793	0.999	0.914	0.937	0.983	0.823
SETX	0.900	0.994	1.000	0.929	1.000	0.854
ATM	1.000	0.975	1.000	0.851	1.000	0.792
ATP7B	1.000	0.970	1.000	0.895	1.000	0.840
TTPA	0.913	0.973	1.000	0.933	1.000	0.907
C10ORF2	0.958	0.989	1.000	0.905	1.000	0.814
CP	1.000	0.999	1.000	0.968	1.000	0.933
CTX	1.000	0.998	1.000	0.956	1.000	0.893
DARS2	0.500	1.000	1.000	0.936	1.000	0.877
FXN (FRDA)	0.900	0.989	0.950	0.953	1.000	0.910
FXN (LOFA)	0.100	0.999	0.625	0.929	0.775	0.908
GAN	1.000	1.000	1.000	0.996	1.000	0.975
GBE1	1.000	0.954	1.000	0.898	1.000	0.827
GOSR2	1.000	0.999	1.000	0.987	1.000	0.959
GRM1	0.800	0.988	1.000	0.956	1.000	0.920
HSD17B4	1.000	0.995	1.000	0.933	1.000	0.890
KCNJ10	0.000	0.998	1.000	0.972	1.000	0.950
MRE11	0.800	1.000	1.000	0.999	1.000	0.995
NEU1	0.769	0.996	1.000	0.967	1.000	0.939
NPC1/NPC2	0.895	1.000	0.965	0.994	0.965	0.988
OPA1	0.000	1.000	0.750	1.000	1.000	0.995
OPA3	0.900	0.987	1.000	0.964	1.000	0.927
PEX10	1.000	1.000	1.000	0.984	1.000	0.966
PEX7	0.900	1.000	0.900	0.994	1.000	0.985
PHYH	0.000	0.999	0.000	0.986	1.000	0.977

PLA2G6	0.909	0.966	0.909	0.770	1.000	0.588
PMM2	0.897	0.998	1.000	0.952	1.000	0.913
PNKP	0.909	0.995	1.000	0.908	1.000	0.781
PNPLA6	0.571	1.000	0.714	1.000	0.714	0.998
POLG	0.933	0.998	1.000	0.965	1.000	0.894
POLR3A	1.000	0.962	1.000	0.923	1.000	0.871
POLR3B	0.733	0.993	0.967	0.970	1.000	0.938
SACS	1.000	0.992	1.000	0.941	1.000	0.886
SCARB2	0.800	0.999	0.933	0.988	0.933	0.980
SNX14	1.000	1.000	1.000	0.998	1.000	0.987
SPG7	0.478	0.991	0.913	0.959	0.913	0.935
STUB1	0.444	0.995	0.556	0.924	0.667	0.827
SYNE1	0.500	0.990	0.692	0.968	0.769	0.936
VLDLR	0.867	0.998	1.000	0.989	1.000	0.975
VWA3B	1.000	0.999	1.000	0.981	1.000	0.936
WDR73	1.000	1.000	1.000	0.937	1.000	0.893
ZNF592	0.600	1.000	1.000	0.996	1.000	0.970
Average	0.771	0.993	0.924	0.954	0.968	0.911

Figure legends

Figure 1: Algorithm outline.

Figure 2: Average sensitivity and specificity for all patients related to the window size defined as the position of the entity in the ranking list.

Figure 3: Relationship between the total number of features (x-axis), the number of specific features (y-axis), and the required window size to reach 90% sensitivity (diameter of bubble increases as performance decreases). The figure shows the lack of association between performance of the algorithm and the number of features. Pearson correlation coefficient for total number of features vs window size: r=0.052, *P*=0.730; specific number of features vs window size: r=−0.033, *P*=0.830.

Figure 1

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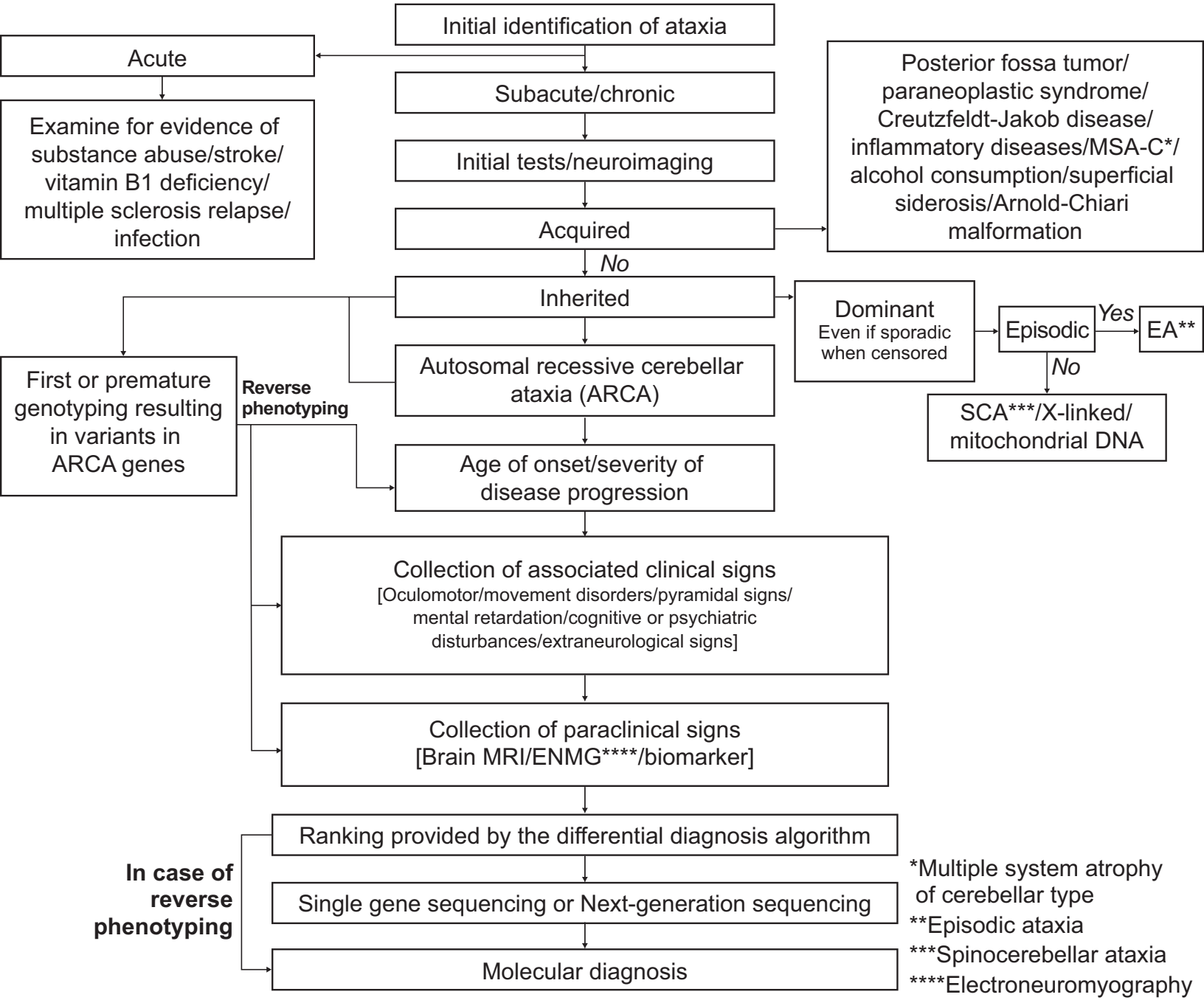

Figure 2

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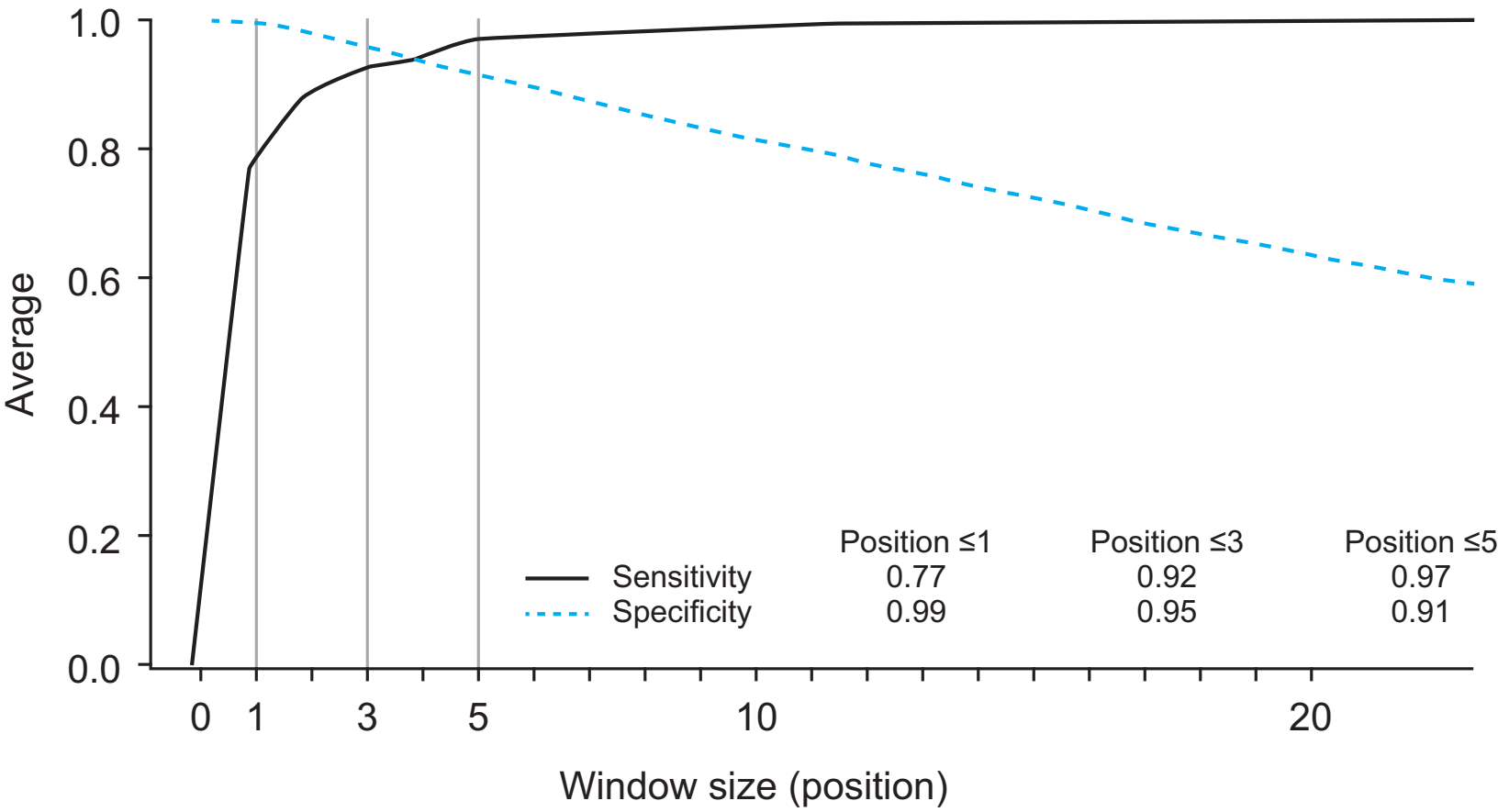

Figure 3

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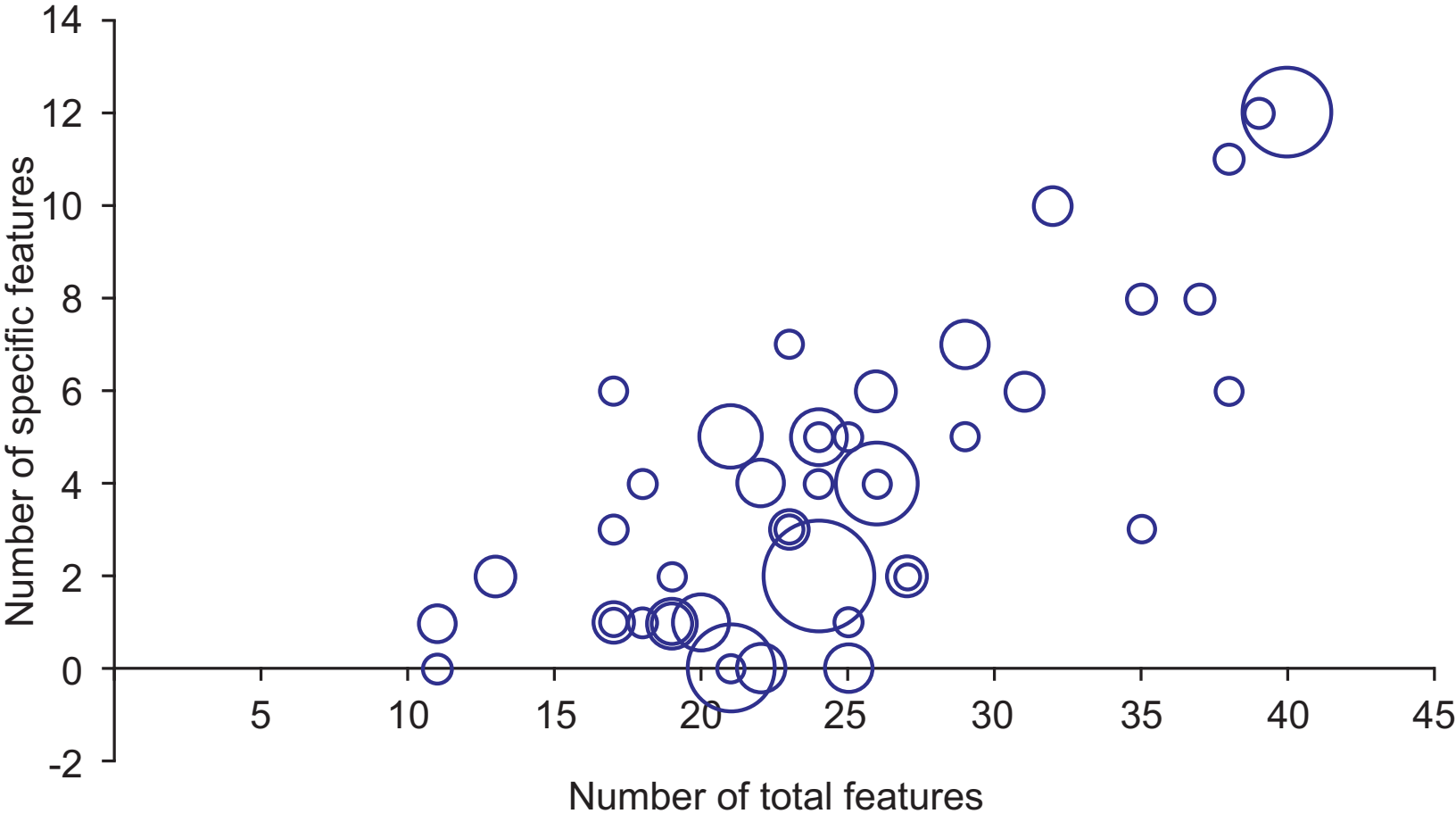

Supplementary Table 1: All articles used in developing the knowledgebase separated by entity.

Entity	Citation
Multiple entities	Abele et al. Brain 2002;125:961–8
	Anderson et al. J Clin Pathol 2005;1305–10
	Anheim et al. Neurogenetics 2010;11:1–12
	Anheim et al. New Eng J Med 2012;366:636–46
	Boddaert et al. J Neuroradiology 2010;37:220–30
	Brusse et al. Clin Genet 2007;71:12–24
	Degardin et al. Cerebellum 2012;11:289–99
	Fasano et al. Brain 2010;133:3635–48
	Fogel and Perlman. Lancet Neurol 2007;6:245–57
	Fogel et al. JAMA Neurol 2014;71:1237–46
	Gasser et al. Eur J Neurol 2010;17:179–88
	Giordano et al. J Neurol 2013;260:2175–6
	Hara et al. Arch Neurol 2007;64:545–51
	Head et al. Ann Neurol 2005 ;58 :234–41
	Jara-Prado et al. Epiliepsy Res 2014 ;108:1501–10
	Jobling et al. Brain 2015;138:1505–17
	Kleta et al. Nature Genet 2004;36:999–1002
	Klockgether. Lancet Neurol 2010;9:94–104
	Klockgether. Curr Opin Neurol 2011;24:339–45
	Koenig. Seminar Ped Neurol 2003:10:183–92
	Le Ber et al. Curr Neurol Neurosci Rep 2005;5:411–7
	Manto & Marmolino. Cur Opin Neurology 2009;22:419–29
	Mégarbané et al. Am J Med Genet 2001;101:135–41
	Nemeth et al. Brain 2013;136:3106–18
	Nia et al. J Neurol 2014;261:S559–68
	Palau and Espinos. Orphanet J Rare Dis 2006;1:47
	Pandolfo. Nat Clin Pract 2008;4:86–96
	Rass et al. Cell 2007;130:991–1004
	Shapira. Lancet 2006;368:70–82
	Taroni & DiDonato. Nat Rev Neurosci 2004;5:641–55
	Tusa & Hove. J Child Neurol 1999;14:621–7
	Van de Warrenburg et al. Eur J Neurol 2014;21:552–62
	Vermeer et al. J Med Genet 2011;48:651–9
	Wagner et al. J Neurol Neurosurg Psychaitry 2008;79:672–7
	Wardle et al. J Neurol 2009;256:343–8
AAAS	Kimber et al. J Neurol Neurosurg Psychiatry 2003;74:654–7
	Vallet et al. J Neurol 2012;259:39–46
ABHD12	Chen et al. Hum Mutat 2013;34:1672–8
	Eisenberger et al. Orphanet J Rare Dis 2012;7:59
	Fiskerstrand et al. Am J Hum Genet 2010;87:410–7
	Yoshimura et al. Ann Otol Rhinol Laryngol 2015;124:77S–83S
ABL	No publications identified dealing specifically with this entity
ACO2	Spiegel et al. Am j Hum Genet 2012;90:518–23
ADCK3	Gerards et al. Mitochondrion 2010;10:510–5
	Horvath et al. J Neurol Neurosurg Psychiatry 2012;83:174–8
	Lagier-Tourenne et al. Am J Hum Genet 2008;82:661–72
	Liu et al. J Neurol Neurosurg Psychiatry 2014;85:493–8
	Mignot et al. Orphanet J Rare Dis 2013;8:173

	Mollet et al. Am J Hum Genet 2008:82;623–30
	Montero et al. Cerebellum 2007;6:118–22
ANO10	Balreira et al. J Neurol 2014;261:2192–8
	Chamova et al. J Neurol 2012;259:906–11
	Minnerop and Bauer. JAMA Neurol 2015;72:238–9
	Muruyama et al. Clin Genet 2014;85:296–7
	Renaud et al. JAMA Neurol 2014;71:1305–10
	Vermeer et al. Am J Hum Genet 2010;87:813–9
APTX	Bohelga et al. BMC Med Genet 2011;12:27
	Castellotti et al. Neurogenetics 2011;12:193–201
	Criscuolo et al. Ann Neurol 2005;57:777
	Hirano et al. Ann Neurol 2007;61:162–74
	Le Ber et al. Brain 2003;126:2761–72
	Moreira et al. Nat Genet 2001;189–93
	Salvatore et al. J Neurol 2008;255:45–48
	Shahwan et al. Dev Med Child Neurol 2006;48:529–32
	Yakoseki et al. Brain 2011;134:1387–99
ATM	Ambrose & Gatti. Blood 2013;121:4036–45
	Chun & Gatti. DNA Repair 2004;1187–96
	Crawford et al. Neurology 2000;54:1505–9
	Crawford et al. Arch Dis Child 2006;91:610–1
	Dar et al. J Neurosci 2006;26:7767–74
	Lavin et al. Br Med Bulletin 2007;81:129–47
	Lewis et al. Ann Neurol 1999;46:287–95
	Lohmann et al. J Neurol 2015;262:1724–7
	Meissner et al. Mov Disord 2013;28:1897–9
	Meneret et al. Neurology 2014;83:1087–95
	Micol et al. J Allergy Clin Immunol 2011;128:392–9
	Oba et al. Acta Neuropathol 2010;119:513–20
	Paull. Annu Rev Biochem 2015;84:711–38
	Silvestri et al. J Neurol 2010;257:1738–40
	Sunders–Pullman et al. Neurology 2012;78:649–57
	Verhagen et al. Neurology 2009;73:430–7
	Verhagen et al. Hum Mut 2012;33:561–71
ATP7B	Bandmann et al. Lancet Neurol 2015 ;14 :103-13
BTD	Hymes et al. Hum Mutat 2001;18:375–81
	Wolf. Mol Genet Med 2011;104:27–34
	Wolf. Genet Med 2012;14:565–75
C10ORF2	Echaniz–Laguna et al. Neurogenetics 2010;11:21–5
	Hakonen et al. Brain 2007;130:3032–40
	Hakonen et al. Hum Mol Genet 2008;17:3822–35
	Nikali et al. Hum Mol Genet 2005;14:2981–90
	Park et al. Neurogenetics 2014;15:171–82
CA8	Kaya et al. Am J Med Genet 2011;156:826–34
CP	McNeill et al. Eur Neurol 2008;60:200–5
	Schneider et al. Mov Disord 2012;27:42–53
CTX	Lagarde et al. Mov Disord 2012;27:1805–10
	Mignarri et al. J Inherit Metab Dis 2014;37:421–9
	Moghadasian et al Arch Neurol 2002;59:527–9
	Nie et al. Orphanet J Rare dis 2014;9:179
	Pilo–de–la–Fuente et al. Eur J Neurol 2011;18:1203–11
	Su et al. Mov Disord 2010;25:452–8
DARS2	Isohanni et al. J Med Genet 2010;47:66–70
	Scheper et al. Nat Genet 2007;39:534–9

	Tzoulis et al. J Neurol 2012;259:292–6
DNAJC19	Davey et al. J Med Genet 2006;43:385–93
	Ojala et al. Pediatr Res 2012;72:432–7
FA2H	Kruer et al. Ann Neurol 2010;68:611–8
	Tonelli et al Eur J Neurol 2012;19:e127–9
	Zaki et al. Clin Gent 2015;88:95–7
FXN	Bayot et al. BMC Med 2011;9:112
	Boesch et al. Ann Neurol 2007;62:521–4
	Burk et al. Mov Disord 2009;24:1779–84
	Coppola et al. Hum Mol Genet 2009;18:2452–61
	Corben et al. Brain Cogn 2011;76:140–5
	De Michele et al. Neurology 1996;47:1260–4
	Durr et al. New Eng J Med 1996;335:1169–75
	Durr. Lancet Neurol 2002;1:370–4
	Evans–Galea et al. Neurogenetics 2011;12:307–13
	Fortuna et al. Brain 2009;132:116–23
	Gottesfeld et al. J Neurochem 2013;126:147–54
	Koeppen et al. Acta Neurologica 2011;122:323–30
	Koeppen. J Neurochem 2013;126:4–10
	Mariotti et al. Neurology 2003;60:1676–9
	Pandolfo et al. Arch Neurol 2008;65:1296–303
	Pandolfo et al. J Neurol 2009;256:3–8
	Pandolfo and Pastore. J Neurol 2009;256:9–17
	Rasmussen et al. J Neurol Neurosurg Psychiatry 2006;77:1370–2
	Reetz et al. Lancet Neurol 2015;14:174–82
	Ribai et al. Arch Neurol 2007;64:558–64
	Sanz–Gallego et al. Cerebellum Ataxias 2014;1:10
	Weidemann et al. J Neurochem 2013;126:88–93
GAN	Buysse et al. Am J Med Genet 2010;152A:2802–4
	Demir et al. J Neurol Neurosurg Psychiatry 2005;76:825–32
GBE1	Billot et al. J Neuro Sci 2013;324:179–82
	Dainese et al. Gene 2013;515:376–9
	Mochel et al. Ann Neurol 2012;72:433–41
GOSR2	Boisse Lomax et al. Brain 2013;136:1146–54
	Corbett et al. Am J Hum Genet 2011;88:657–63
	Van Egmond et al. Mov Disord 2014;29:139–43
GRID2	Coutelier et al. Neurology 2015;84:1751–9
	Hills et al. Neurology 2013;81:1378–86
GRM1	Guergueltcheva et al. 2012;91:553–64
HEXA	Deik and Saunders–Pullman. Muscle Nerve 2014;49:768–71
	Maegawa et al. Pediatrics 2006;118:e1550
HEXB	Delnooz et al. J Neurol Neurosurg Psychiatry 2010;81:968–72
	Maegawa et al. Pediatrics 2006;118:e1550
HSD17B4	Lieber et al. BMC Med Genet 2014;15:30
	Lines et al. Neurology 2014;82:963–8
	Pierce et al. Am J Hum Genet 2010;87:282–8
KCNJ10	Bockenhauer et al. New Eng J Med 2009;360:1960–70
	Cross et al. Dev Med Child Neurol 2013;55:846–56
	Scholl et al. Proc Nat Acad Sci USA 2009;106:5842–7
KCTD7	Blumkin et al. J Neurol 2012;259:2590–8
	Farhan et al. Epilepsia 2014;55:e106–11
	Staropoli et al. Am J Hum Genet 2012;91:202–8
	Van Begaert et al. Ann Neurol 2007;61:579–86
MARS2	Bayat et al. PLoS Biol 2012;10:e1001288

MRE11	Fernet et al. Hum Mol Genet 2005;14:307–18
	Uchisaka et al. J Pediatr 2009;155:435–8
MTPAP	Crosby et al. Am J Hum Genet 2010;87:655–60
NEU1	Canafoglia et al. Neurology 2014;82:2003–6
	Lai et al. Eur J Neurol 2009;16:912–9
NPC1/NPC2	Anheim et al. J Neurol 2014;261:174–9
	Josephs et al. J Neurol Neurosurg Psychiatry 2003;74:528–9
	Klunemann et al. Ann Neruol 2002;52:743–9
	Mengel et al. Orphanet J Rare Dis 2013;8:166
	Patterson et al. Lancet 2007;6:765–72
	Schicks et al. Neurology 2013;80:1169–70
	Sevin et al. Brain 2007;130:120–33
	Shulman et al. Neurology 1995;45:1739–43
	Vanier. Orphanet J Rare Dis 2010;5:16
	Walterfang et al. Orphanet J Rare Dis;7:76
	Wraith et al. J Inherit Dis 2014;37:93–101
OPA1	Bonifert et al. Brain 2014;137:2164–77
	Bonneau et al. Brain 2014;137:1–4
	Marelli et al. Brain 2011;134:e169
OPA3	Yahalom et al. J Neurol 2014;261:2275–82
PEX10	Okumoto et al. Hum Mol Genet 1998;7:1399–405
	Regal et al. Ann Newurol 2010;68:259–63
	Warren et al. Am J Hum Genet 1998;63:347–59
PEX7	Nanetti et al. J Clin Neurol 2015;11:197–9
	Van den Brink et al. Am J Hum Genet 2003;72:471–77
PHYH	Bompaire et al. JIMD Reports 2015;19:7–10
	Fertl et al. J Neurol Neurosurg Psychiatry 2001;70:563–7
	Jansen et al. Nat Genet 1997;17:190–3
	Mihalik et al. Nat Genet 1997;17:185–9
	Weinstein et al. J Clin Apheresis 1999;14:181–4
	Wierzbicki et al. J Neurochem 2002;80:727–35
PIK3R5	Al Tassan et al. Hun Mut 2012;33:351–4
PK1/PRICKLE1	Bassuk et al. Am J Hum Genet 2008;83:572–81
	Berkovic et al. Brain 2005;218:652–8
PLA2G6	Karkheiran et al. Tremor Other Hyperkinet Mov (NY) 2015;5:317
	Kurian et al. Neurology 2008;70:1623–9
	Salih et al. PLoS One 2013;8:e76831
	Schneider et al. Mov Disord 2012;27:42–53
PMM2	Barone et al. J Neurol 2015;262:154–64
	Monin et al. Orphanet J Rare Dis 2014;9:207
	O'Sullivan et al. Mov Disord 2006;21:869–72
	Serrano et al. Orphanet J Rare Dis 2015;10:138
	Vermeer et al. J Neurol 2007:254;1356–8
PNKP	Bras et al. Am J Hum Genet 2015;96:474–9
PNPLA6	Deik et al. J Neurol 2014;261:2411–23
	Synofzik et al Brain 2014;137:69–77
	Tarnutzer et al. J Neurol 2015;262:194–202
POLG	Tchikviladze et al. J Neurol Neurosurg Psychiatry 2015;86:646–54
	Echaniz–Laguna et al. Arch Neurol 2010;67:1140–3
	Farnum et al. Biochim et Biophys Acta 2014;1837:1113–21
	Tzoulis et al. Brain 2006;129:1685–92
	Tzoulis et al. Brain 2010;133:1428–37
POLR3A	Bernard et al. Am J Hum Genet 2011;89:415–23
	Daoud et al. J Med Genet 2013;50:194–7

	Saitsu et al. Am J Hum Genet 2011;89:644–51
	Wolf et al. Neurology 2014;83:1898–1905
POLR3B	Daoud et al. J Med Genet 2013;50:194–7
	Saitsu et al. Am J Hum Genet 2011;89:644–51
	Synofzik et al. Neurology 2013;81:e145
	Tetreault et al. Am J Hum Genet 2011;89:652–5
	Wolf et al. Neurology 2014;83:1898–1905
SACS	Boulal et al. J Mol Neurosci 2009;39:333–6
	Duquette et al. Mov Disord 2013;28:2011–14
	Gazulla et al. J Neurol 2012;259:869–78
	Gerwig et al. Neurology 2010;75:2133
	Girard et al. Proc Natl Acad Sci USA 2012;109:1661–6
	Martin et al. Am J Neuroradiol 2007;28:1606–8
	Pablo et al. Mol Vis 2011;17:1871–6
	Parfitt et al. Hum Mol Genet 2009;18:1556–65
	Synofzik et al. Orphanet J Rare Dis 2013;8:41
	Tzoulis et al. PLoS One 2013;8:e66145
	Vermeer et al. Neurogenetics 2008;9:207–14
SCARB2	Berkovic et al. Am J Hum Genet 2008;82:673–84
	Dibbens et al. Ann Neurol 2009;66:532–36
	Perandones et al. Mov Disord 2014;29:158–9
SETX	Airoldi et al. Neurogenetics 2010;11:91–100
	Anheim et al. Brain 2009;132:2688–98
	Bechere et al. Hum Mol Genet 2015;24:5759–5774
	Bernard et al. Neuropediatrics 2008;39:347–50
	Bohlega et al. BMC Med Genet 2011;12:27
	Frismand et al. Neuroimage Clin 2013;10:542–8
	Lavin et al. Rare Dis 2013;1:e25230
	Nanetti et al. Orphanet J Rare Dis 2013;8:123
	Tazir et al. J Neurol Sci 2009;278:77–81
	Yeo et al. PLoS One 2014;9:e90219
SIL1	Goto et al. Orphanet J Rare Dis 2014;9:58
	Krieger et al. Brain 2013;136:3634–44
	Noreau et al. Neurogenetics 2015;16:315–8
	Senderek et al. Nat Genet 2005;37:1312–4
SLC9A1	Guissart et al. Hum Mol Genet 2015;24:463–70
SNX14	Akizu et al. Nat Genet 2015;47:528–36
	Thomas et al. Am J Hum Genet 2014;95:611–21
SPG7	Klebe et al. Brain 2012;135:2980–93
	Pfeffer et al. Brain 2014;137:1323–36
	Pfeffer et al. Neurology 2015;84:1174–7
	Van Gassen et al. Brain 2012;135:2994–3004
SPTBN2	Elsayed et al. Eur J Hum Genet 2014;22:286–8
	Lise et al. PLoS Genet 2012;8:e1003074
	Schnekenberg et al. Brain 2015;138:1817–32
STUB1	Heimdal et al. Orphanet J Rare Dis 2014;9:146
	Shi et al. PLoS One 2013;8:e81884
	Shi et al. Hum Mol Genet 2014;23:1013–24
	Synofzik et al. Orphanet J Rare Dis 2014;9:57
SYNE1	Dupré et al. Ann Neurol 2007;62:93–8
	Gros–Louis et al. Nat Genet 2007;39:80–5
	Izumi et al. Neurology 2013;80:600–1
	Noreau et al. JAMA Neurol 2013;70:1296–31
	Synofzik et al. Brain 2016;139:1378–93

SYT14	Doi et al. Am J Hum Genet 2011;89:320–7
TDP1	Takashima et al. Nat Genet 2002;32:267–72
TDP2	Gomez-Herreros et al. Nat Genet 2014;46:516–21
TPP1	Dy et al. Neurology 2015;85:1259–61
	Kousi et al. Brain 2009;132:810–19
	Kousi et al. Hum Mutat 2012;33:42–63
	Mole et al. Neurogenetics 2005;6:107–26
	Sun et al. Hum Mutat 2013;34:706–13
TTPA	El Euch–Fayache et al. Brain 2014;137:402–10
VLDLR	Boycott et al. Am J Hum Genet 2005;77:477–83
	Boycott et al. J Child Neurol 2009;24:1310–5
	Moheb et al. Eur J Hum Genet 2008;16:270–3
	Turkmen et al. PLoS Genet 2009;5:e1000487
VWA3B	Kawarai et al. J Neurol Neurosurg Psychiatry 2016;87:656–62
WDR73	Jinks et al. Brain 2015;138:2173–90
	Vodopiutz et al. Hum Mutat 2015;36:1021–8
ZNF592	Nicolas et al. Eur J Hum Genet 2010;18:1107–13

Supplementary Table 2: Convention for notation and scoring for frequency and/or specificity for each category of signs and symptoms included in the algorithm.

Category	Clinical features	Neuroimaging	Electro-myography	Age of onset	Rapid disease progression	Biomarkers
NK	0	0	0	0	0	0
0	-1	-1	-1	-2	-2	-2
L	1	1	1	2	2	2
H	3	3	3	6	6	6
LS	7	7	7	14	14	14
HS	9	9	9	18	18	18

0, No association; L, Low frequency (<50% of patients); LS, Low frequency (<50% of patients) and specific (<10% of entities); H, High frequency (≥50% of patients); HS, High frequency (≥50% of patients) and specific (<10% of entities); NK, extent of association not known.

Supplementary Table 3: Full algorithm knowledgebase including correlations defined for all 124 signs and symptoms to each of the 67 entities.

[See excel spreadsheet]

Ataxia Algorithm knowlegebase_lock

Supplementary Table 4: Full list of the manuscript authors and the additional investigators (those who contributed to patient data collection but did not qualify for full authorship according to ICMJE criteria).

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Supplementary Table 5: Full list of genes and disorders included in the differential diagnosis algorithm with disorders included in the retrospective analyses in bold.

Gene	Disorder
AAAS	Achalasia-addisonianism-alacrimia syndrome (Triple A or Allgrove syndrome)
ABHD12	**Polyneuropathy, hearing loss, ataxia, retinitis pigmentosa, and cataract (PHARC)**
ABL	Abetalipoproteinemia
ACO2	Infantile cerebellar-retinal degeneration
ADCK3	**ARCA2**
ANO10	**ARCA3**
APTX	**Ataxia-oculomotor apraxia 1**
ATM	**Ataxia-telangiectasia**
ATP7B	**Wilson disease**
BTD	Biotinidase deficiency
C10ORF2	**Infantile onset spinocerebellar ataxia (IOSCA)**
CA8	Cerebellar ataxia and mental retardation with or without quadrupedal locomotion 3
CP	**Aceruloplasminemia**
CTX	**Cerebrotendinous xanthomatosis**
DARS2	**Leukoencephalopathy with brain stem and spinal cord involvement and lactate elevation**
DNAJC19	3-methylglutaconic aciduria, type V
FA2H	Spastic paraplegia 35
FXN	**Friedreich ataxia, late-onset Friedreich ataxia and very late-onset Friedreich ataxia**
GAN	**Giant axonal neuropathy-1**
GBE1	**Adult polyglucosan body disease**
GOSR2	**Epilepsy, progressive myoclonic 6**
GRID2	Spinocerebellar ataxia, autosomal recessive 18
GRM1	**Spinocerebellar ataxia, autosomal recessive 13**
HEXA	Tay-Sachs disease
HEXB	Sandhoff disease
HSD17B4	**Perrault syndrome 1**
KCNJ10	**Epilepsy ataxia sensorineural deafness and tubulopathy (EAST) syndrome**
KCTD7	Epilepsy, progressive myoclonic 3, with or without intracellular inclusions
MARS2	Spastic ataxia 3, autosomal recessive
MRE11	**Ataxia-telangiectasia-like disorder**
MTPAP	Ataxia, spastic, 4
NEU1	**Sialidosis, type I**
NPC1/NPC2	**Niemann-Pick disease Type C**
OPA1	**Behr syndrome**
OPA3	**3-methylglutaconic aciduria, type III**
PEX10	**Peroxisome biogenesis disorder 6A**
PEX7	**Peroxisome biogenesis disorder 9B**
PHYH	**Refsum disease**
PIK3R5	Ataxia-oculomotor apraxia 3
PK1/PRICKLE1	Epilepsy, progressive myoclonic 1B

PLA2G6	**PLA2G6 associated neurodegeneration (PLAN) Infantile neuroaxonal dystrophy 1**
PMM2	**Congenital disorder of glycosylation, type Ia (CDG1a)**
PNKP	**Ataxia-oculomotor apraxia 4**
PNPLA6	**Boucher-Neuhauser syndrome**
POLG	**Sensory ataxic neuropathy with dysarthria and ophthalmoplegia (SANDO)**
POLR3A	**Leukodystrophy, hypomyelinating, 7, with or without oligodontia and/or hypogonadotropic hypogonadism**
POLR3B	**Leukodystrophy, hypomyelinating, 8, with or without oligodontia and/or hypogonadotropic hypogonadism**
SACS	**Autosomal recessive spastic ataxia of Charlevoix-Saguenay**
SCARB2	**Epilepsy, progressive myoclonic 4, with or without renal failure**
SETX	**Ataxia-oculomotor apraxia 2**
SIL1	Marinesco-Sjogren syndrome
SLC9A1	Lichtenstein-Knorr syndrome
SNX14	**Spinocerebellar ataxia, autosomal recessive 20**
SPG7	**Spastic paraplegia 7, autosomal recessive**
SPTBN2	Spinocerebellar ataxia, autosomal recessive 14
STUB1	**Spinocerebellar ataxia, autosomal recessive 16**
SYNE1	**Spinocerebellar ataxia, autosomal recessive 8**
SYT14	Spinocerebellar ataxia, autosomal recessive 11
TDP1	Spinocerebellar ataxia plus neuropathy (SCAN1)
TDP2	Spinocerebellar ataxia, autosomal recessive 23
TPP1	Spinocerebellar ataxia, autosomal recessive 7
TTPA	**Ataxia with vitamin E deficiency**
VLDLR	**Cerebellar hypoplasia and mental retardation with or without quadrupedal locomotion 1**
VWA3B	**Spinocerebellar ataxia, autosomal recessive 22**
WDR73	**Galloway-Mowat syndrome**
ZNF592	**Cerebellar Ataxia with Mental retardation, Optic atrophy and Skin abnormalities (CAMOS)**

Supplementary Table 6: Summary of the disease characteristics for the entire patient cohort and for the 8 ARCAs with a sample size of ≥30 patients.

Sign/symptom	Total	*APTX*	*ATM*	*FXN* (LOFA)	*NPC1* *NPC2*	*POLR3B*	*SACS*	*SETX*	*TTPA*
N	**834**	**58**	**44**	**40**	**57**	**30**	**38**	**60**	**69**
Age of onset of ataxia	**821**	**54**	**44**	**40**	**56**	**30**	**38**	**58**	**69**
<10 years	395 (47.4)	50 (86.2)	31 (70.5)	0 (0.0)	16 (28.1)	26 (86.7)	27 (71.1)	3 (5.0)	15 (21.7)
≥10 years	426 (51.1)	4 (6.9)	13 (29.5)	40 (100.0)	40 (70.2)	4 (13.3)	11 (28.9)	55 (91.7)	54 (78.3)
Speed of progression	**641**	**44**	**44**	**40**	**41**	**23**	**30**	**29**	**27**
Slow (can walk unaided 20 years after disease onset)	252 (30.2)	6 (10.3)	17 (38.6)	17 (42.5)	2 (3.5)	1 (3.3)	23 (60.5)	5 (8.3)	19 (27.5)
Rapid (required assistance to walk 20 years after disease onset)	389 (46.6)	38 (65.5)	27 (61.4)	23 (57.5)	39 (68.4)	22 (73.3)	7 (18.4)	24 (40.0)	8 (11.6)
Ocular symptoms	**225**	**0**	**34**	**1**	**0**	**3**	**38**	**0**	**3**
Telangiectasia	34 (4.1)	0 (0.0)	34 (77.3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Cataract	45 (5.4)	0 (0.0)	0 (0.0)	1 (2.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Retinitis pigmentosa / retinal degeneration / chorioretinal dystrophy	43 (5.2)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	3 (4.3)
Optic atrophy	82 (9.8)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	3 (10.0)	0 (0.0)	0 (0.0)	0 (0.0)
Red cherry macula	6 (0.7)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Retinal nerve fibre layer hypertrophy	38 (4.6)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	38 (100.0)	0 (0.0)	0 (0.0)
Ocular motor symptoms	**521**	**52**	**34**	**13**	**55**	**29**	**36**	**38**	**13**
Diplopia / strabismus	117 (14.0)	14 (24.1)	0 (0.0)	0 (0.0)	1 (1.8)	0 (0.0)	0 (0.0)	5 (8.3)	2 (2.9)

Gaze evoked nystagmus	230 (27.6)	29 (50.0)	7 (15.9)	12 (30.0)	0 (0.0)	28 (93.3)	30 (78.9)	0 (0.0)	7 (10.1)
Hypometric saccades	128 (15.3)	12 (20.7)	15 (34.1)	1 (2.5)	0 (0.0)	7 (23.3)	34 (89.5)	0 (0.0)	0 (0.0)
Slow saccades	61 (7.3)	0 (0.0)	10 (22.7)	0 (0.0)	0 (0.0)	0 (0.0)	4 (10.5)	0 (0.0)	0 (0.0)
Saccades of elevated latencies	11 (1.3)	0 (0.0)	5 (11.4)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Oculomotor apraxia/oculocephalic dissociation	136 (16.3)	36 (62.1)	26 (59.1)	0 (0.0)	1 (1.8)	0 (0.0)	0 (0.0)	35 (58.3)	4 (5.8)
Vertical supranuclear gaze palsy	78 (9.4)	0 (0.0)	0 (0.0)	0 (0.0)	55 (96.5)	3 (10.0)	0 (0.0)	0 (0.0)	0 (0.0)
Progressive external ophthalmoplegia	43 (5.2)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Tonic upgaze	1 (0.1)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Movement disorders	**275**	**24**	**24**	**0**	**43**	**6**	**0**	**11**	**0**
Chorea	79 (9.5)	18 (31.0)	4 (9.1)	0 (0.0)	1 (1.8)	0 (0.0)	0 (0.0)	5 (8.3)	0 (0.0)
Dystonia	174 (20.9)	10 (17.2)	19 (43.2)	0 (0.0)	42 (73.7)	6 (20.0)	0 (0.0)	8 (13.3)	0 (0.0)
Myoclonus	104 (12.5)	3 (5.2)	12 (27.3)	0 (0.0)	7 (12.3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Parkinsonism	23 (2.8)	0 (0.0)	5 (11.4)	0 (0.0)	0 (0.0)	1 (3.3)	0 (0.0)	0 (0.0)	0 (0.0)
Cortico-spinal tract	**417**	**6**	**3**	**32**	**33**	**7**	**38**	**6**	**59**
Enhanced/diffused tendon reflexes	267 (32.0)	0 (0.0)	3 (6.8)	17 (42.5)	32 (56.1)	7 (23.3)	33 (86.8)	0 (0.0)	1 (1.4)
Extensor plantar reflexes	293 (35.1)	6 (10.3)	3 (6.8)	26 (65.0)	7 (12.3)	1 (3.3)	38 (100.0)	6 (10.0)	59 (85.5)
Spasticity	198 (23.7)	0 (0.0)	1 (2.3)	13 (32.5)	13 (22.8)	7 (23.3)	32 (84.2)	0 (0.0)	9 (13.0)

Cognitive & psychiatric	**354**	**28**	**3**	**3**	**52**	**28**	**0**	**0**	**0**
Hallucinations and/or delusions	27 (3.2)	0 (0.0)	0 (0.0)	0 (0.0)	12 (21.1)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Cognitive decline	226 (27.1)	0 (0.0)	0 (0.0)	2 (5.0)	51 (89.5)	20 (66.7)	0 (0.0)	0 (0.0)	0 (0.0)
Mental retardation / delayed developmental milestones	228 (27.3)	28 (48.3)	3 (6.8)	0 (0.0)	10 (17.5)	20 (66.7)	0 (0.0)	0 (0.0)	0 (0.0)
Treatment resistant psychiatric symptoms	24 (2.9)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (3.3)	0 (0.0)	0 (0.0)	0 (0.0)
Disruptive/aggressive behaviour in childhood	13 (1.6)	0 (0.0)	0 (0.0)	1 (2.5)	2 (3.5)	2 (6.7)	0 (0.0)	0 (0.0)	0 (0.0)
Neurological	**510**	**58**	**20**	**30**	**35**	**2**	**7**	**58**	**67**
Absence of tendon reflexes	335 (40.2)	58 (100.0)	19 (43.2)	19 (47.5)	0 (0.0)	0 (0.0)	1 (2.6)	58 (96.7)	67 (97.1)
Myopathy (skeletal)	18 (2.2)	0 (0.0)	0 (0.0)	7 (17.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Epilepsy	142 (17.0)	0 (0.0)	1 (2.3)	2 (5.0)	19 (33.3)	1 (3.3)	2 (5.3)	0 (0.0)	0 (0.0)
Deafness	73 (8.8)	0 (0.0)	0 (0.0)	4 (10.0)	12 (21.1)	1 (3.3)	5 (13.2)	0 (0.0)	0 (0.0)
Cataplexia	20 (2.4)	0 (0.0)	0 (0.0)	0 (0.0)	20 (35.1)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Anosmia	4 (0.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Quadrupedal gait	1 (0.1)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Stridor	4 (0.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Stroke-like episodes	23 (2.8)	0 (0.0)	0 (0.0)	1 (2.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Autonomic dysfunction	57 (6.8)	0 (0.0)	0 (0.0)	11 (27.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

Musculo-skeletal	**344**	**27**	**3**	**18**	**5**	**26**	**35**	**29**	**35**
Pes cavus	228 (27.3)	21 (36.2)	0 (0.0)	12 (30.0)	0 (0.0)	0 (0.0)	35 (92.1)	29 (48.3)	31 (44.9)
Scoliosis	128 (15.3)	15 (25.9)	3 (6.8)	10 (25.0)	5 (8.8)	2 (6.7)	1 (2.6)	0 (0.0)	12 (17.4)
Loss of the dorsal kyphosis	2 (0.2)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.4)
Adducted thumbs	4 (0.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Oligodontia	42 (5.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	26 (86.7)	0 (0.0)	0 (0.0)	0 (0.0)
Short fourth metacarpal / metatarsal bones	7 (0.8)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Short fifth metacarpal / metatarsal bones	9 (1.1)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Osteoporosis	53 (6.4)	0 (0.0)	0 (0.0)	1 (2.5)	0 (0.0)	1 (3.3)	0 (0.0)	0 (0.0)	0 (0.0)
Coarsened facial features	17 (2.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Visceral & gastrointestinal	**138**	**0**	**0**	**6**	**39**	**0**	**0**	**0**	**3**
Hepatomegaly	26 (3.1)	0 (0.0)	0 (0.0)	0 (0.0)	22 (38.6)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Splenomegaly	42 (5.0)	0 (0.0)	0 (0.0)	0 (0.0)	37 (64.9)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Cardiomyopathy / pericardial effusions / cardiac arrhythmia	20 (2.4)	0 (0.0)	0 (0.0)	3 (7.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (2.9)
Diabetes	16 (1.9)	0 (0.0)	0 (0.0)	4 (10.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Diarrhoea	19 (2.3)	0 (0.0)	0 (0.0)	0 (0.0)	2 (3.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Malabsorption	1 (0.1)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

Vomiting	24 (2.9)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Renal failure	20 (2.4)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Achalasia	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Cryptorchidism	4 (0.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.4)
Skin	**54**	**0**	**25**	**0**	**0**	**0**	**0**	**0**	**0**
Xanthoma	17 (2.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Telangiectasias	24 (2.9)	0 (0.0)	24 (54.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Café-au-lait spots	18 (2.2)	0 (0.0)	18 (40.9)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Frizzly/kinky hair	6 (0.70	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Alopecia	3 (0.4)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Xeroderma / ichthyosis	3 (0.4)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Miscellaneous	**108**	**0**	**18**	**6**	**7**	**13**	**0**	**0**	**1**
Recurrent infections	15 (1.8)	0 (0.0)	6 (13.6)	0 (0.0)	7 (12.3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Malignancies	13 (1.6)	0 (0.0)	5 (11.4)	3 (7.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Immune system defects	13 (1.6)	0 (0.0)	13 (29.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Obesity	17 (2.0)	0 (0.0)	1 (2.3)	3 (7.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Adrenal insufficiency	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

Alacrimia	1 (0.1)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.4)
Hypogonadotropic hypogonadism	59 (7.1)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	13 (43.3)	0 (0.0)	0 (0.0)	0 (0.0)
Neuroimaging data	**672**	**54**	**20**	**32**	**44**	**30**	**0**	**55**	**65**
Obvious cerebellar atrophy	447 (53.6)	54 (93.1)	18 (40.9)	7 (17.5)	16 (28.1)	30 (100.0)	0 (0.0)	55 (91.7)	6 (8.7)
No cerebellar atrophy	207 (24.8)	0 (0.0)	2 (4.5)	24 (60.0)	28 (49.1)	0 (0.0)	0 (0.0)	0 (0.0)	59 (85.5)
Spinal cord atrophy	33 (4.0)	0 (0.0)	0 (0.0)	1 (2.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Cerebellar white-matter changes	99 (11.9)	0 (0.0)	1 (2.3)	1 (2.5)	0 (0.0)	30 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)
Stroke-like lesions	18 (2.2)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Anterior superior cerebellar atrophy	14 (1.7)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Olivary nuclei hyperintensity	4 (0.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
T2-weighted linear hypointensities in The pons	1 (0.1)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Cerebral leukopathy	96 (11.5)	0 (0.0)	0 (0.0)	1 (2.5)	3 (5.3)	30 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)
Brainstem atrophy	85 (10.2)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	18 (60.0)	0 (0.0)	0 (0.0)	0 (0.0)
Small pituitary	5 (0.6)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Hyperintensities in the pons	39 (4.7)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (3.3)	0 (0.0)	0 (0.0)	0 (0.0)
Hypointensity of basal ganglia	17 (2.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.8)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Bilateral middle cerebellar peduncles hyperintensities	45 (5.4)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	22 (73.3)	0 (0.0)	0 (0.0)	0 (0.0)

Simplified cerebral gyration	15 (1.8)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Thin corpus callosum	61 (7.3)	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.8)	22 (73.3)	0 (0.0)	0 (0.0)	0 (0.0)
Electro-myography	**628**	**55**	**14**	**36**	**46**	**12**	**38**	**57**	**68**
Axonal sensorimotor neuropathy	240 (28.8)	53 (91.4)	9 (20.5)	0 (0.0)	1 (1.8)	0 (0.0)	38 (100.0)	57 (95.0)	2 (2.9)
Pure sensory neuropathy	126 (15.1)	0 (0.0)	0 (0.0)	26 (65.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	41 (59.4)
Demyelinating component of the neuropathy	79 (9.5)	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.8)	0 (0.0)	38 (100.0)	0 (0.0)	0 (0.0)
No peripheral neuropathy	246 (29.5)	2 (3.4)	5 (11.4)	10 (25.0)	44 (77.2)	12 (40.0)	0 (0.0)	0 (0.0)	25 (36.2)
Biomarker evidence	**358**	**44**	**44**	**0**	**2**	**1**	**0**	**54**	**69**
Elevated serum lactic acid level	6 (0.7)	0 (0.0)	1 (2.3)	0 (0.0)	0 (0.0)	1 (3.3)	0 (0.0)	0 (0.0)	0 (0.0)
Decreased serum vitamin e level	69 (8.3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	69 (100.0)
Decreased serum cholesterol &/or triglycerides	11 (1.3)	0 (0.0)	3 (6.8)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Decreased serum vitamin a, d and/or k	14 (1.7)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Abetalipoproteinemia	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Acanthocytosis	1 (0.1)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Elevated serum LDL cholesterol level	44 (5.3)	19 (32.8)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Elevated serum creatine kinase level	28 (3.4)	8 (13.8)	2 (4.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.7)	0 (0.0)
Decreased serum immunoglobulin level	34 (4.1)	0 (0.0)	17 (38.6)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	3 (5.0)	0 (0.0)

Decreased serum albumin level	60 (7.2)	33 (56.9)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Elevated serum alpha-fetoprotein level	126 (15.1)	17 (29.3)	43 (97.7)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	54 (90.0)	0 (0.0)
Hexosaminidase a deficiency	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Hexosaminidase b deficiency	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Elevated serum phytanic acid level	12 (1.4)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Elevated serum cholestanol level	21 (2.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Decreased coenzyme q10 level	2 (0.2)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Decreased ceruloplasmin serum level	10 (1.2)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Decreased iron serum level	2 (0.2)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Decreased copper serum level	10 (1.2)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Elevated 24 hours urinary copper	9 (1.1)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Elevated serum oxysterol level	1 (0.1)	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.8)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Altered urinary oligosaccharides	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Elevated 3-methyglutaconic acid and/or elevated 3-methylglutaric acid	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Elevated aspartate transaminase and/or elevated alanine transaminase	14 (1.7)	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.8)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Anemia	18 (2.2)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

Glycogen branching enzyme activity decreased in leukocytes	7 (0.8)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Serum biotinidase enzyme activity decreased	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Elevated ferritin serum level	6 (0.7)	0 (0.0)	1 (2.3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Decreased neuraminidase activity in fibroblasts	11 (1.3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Karyotype translocations	19 (2.3)	0 (0.0)	19 (43.2)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Type 1 transferrin isoform pattern and/or abnormal serum transferrin glycosylation by isoelectrofocusing or immunofixation	16 (1.9)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Decreased leukocytes phosphomannomutase activity	21 (2.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Elevated pristanic acid level	6 (0.7)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Coagulations anomalies and thrombotic events	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

2.4.4. Mise au point et validation d’un algorithme pour les ataxies cérébelleuses autosomiques récessives

A l’issu de la première étude, nous avons poursuivi le projet de recherche sur l’algorithme d’ataxies cérébelleuses autosomiques récessives avec cette fois ci la mise au point et la validation d’un algorithme informatique à plus grande échelle.

Un algorithme automatisé suivant la pratique clinique, incluant les antécédents du patient (âge de début et gravité de la progression de la maladie), les signes cliniques, l’IRM cérébrale, l’EMG et les principaux biomarqueurs rencontrés dans ce cadre (AFP, cholestérol, albumine, vitamine E, cholestanol, etc.) a été développé par le Pr Anheim à partir des résultats d'une recherche bibliographique sur les ACAR et de son expérience clinique personnelle. Le principe de cet algorithme est de rentrer dans un tableur les données cliniques et paracliniques d’un patient suspect d’avoir une ataxie récessive, et l’ordinateur utilise l’algorithme pour donner une liste de gènes les plus probablement mutés avec un score pour chacun. La fréquence et la spécificité de chaque caractéristique ont été définies pour chaque ACAR, et des scores de prédiction ont été attribués à chaque caractéristique selon leur spécificité et leur fréquence dans la pathologie. Les caractéristiques cliniques et paracliniques des patients sont entrées dans l'algorithme et le score total d'un patient pour chaque ACAR est calculé produisant un classement des diagnostics possibles.

Au total, les caractéristiques de 67 ataxies récessives, correspondant à 124 caractéristiques cliniques et paracliniques (biologiques et radiologiques) ont été rentrées pour établir l'algorithme.

Nous avons ensuite recruté une cohorte internationale de 834 patients avec ACAR confirmée sur le plan moléculaire pour pouvoir tester la sensibilité et la spécificité de l'algorithme. Ces 834 patients représentent 45 ataxies différentes. Nous avons montré dans cette étude que l'algorithme a classé dans le top 3 des scores diagnostiques les plus élevés, le diagnostic correct, avec une sensibilité > 90 % et une spécificité > 90% respectivement pour 84% et 91% des gènes évalués. La sensibilité et la spécificité moyennes des 3 principaux diagnostics les plus élevés ont été respectivement de 92% et 95%.

La performance de l'algorithme par rapport à un panel d'experts dans le domaine des ataxies a été évaluée à l'aveugle. L'algorithme a surpassé le panel d'experts (p = 0,001).

3. Discussion, perspectives et conclusion

3.1. Hétérogénéité du FXTAS et conseil génétique

La première étude de la thèse confirme que les signes radiologiques contribuent à diagnostiquer les patients FXTAS et qu'il est tout à fait pertinent de considérer l'hypersignal du splénium du corps calleux comme critère radiologique majeur. En pratique clinique, il faut penser au FXTAS même si il n'y a pas d'histoire familiale de retard mental, chez une femme, en absence de tremblement, en cas de troubles cognitifs inauguraux et en l'absence d'hypersignal des pédoncules cérébelleux moyens.

Afin d'affiner le phénotype clinico-radiologique du FXTAS, de nouvelles études prospectives, prolongées, à grande échelle seraient nécessaires chez des patients prémutés, avec pour chaque patient une IRM cérébrale, un EMG, un DATscan, des tests neuropsychologiques, etc.

Le conseil génétique reste un élément primordial de la prise en charge multidisciplinaire des patients FXTAS. Il peut s'avérer particulièrement complexe du fait de la nature multi-générationnelle de la maladie, de la multiplicité des phénotypes et des implications pour les différents membres de la famille. L'entrée de la famille dans le conseil génétique peut se faire par le FXTAS chez le grand-père, mais le plus souvent elle s'effectue par le syndrome X-fragile chez l'enfant. Par ailleurs on doit se rappeler que la prémutation prédispose également les femmes à une ménopause précoce, avant 40 ans et parfois avant 30 ans. Cette notion est importante pour le conseil génétique et les femmes prémutées désireuses d'avoir des enfants.

3.2. Fréquence des mutations hypomorphes dans les ataxies cérébelleuses récessives

Nous avons vu que dans les ataxies cérébelleuses autosomiques récessives, un certain nombre des gènes concernés sont des gènes d'ataxies métaboliques, notamment des gènes impliqués dans le métabolisme des lipides, glucides, acides aminés, les fonctions mitochondriales, peroxysomales (comme *PEX10*) ou encore lysosomales. La plupart sont responsables de pathologies graves et mortelles et ce n'est que lorsque la mutation est modérée (mécanisme par perte de fonction partielle) que l'ataxie devient le symptôme prédominant.

On peut penser que la fréquence des mutations hypomorphes dans les ataxies récessives est en partie responsable du faible rendement diagnostique des analyses par séquençage de l'exome de patients atteints, la nature hypomorphe des mutations compliquant les éventuelles études fonctionnelles. Par exemple, dans le cas des mutations faux sens dans *PEX10,* l'effet délétère des deux mutations faux sens était détecté sur le sérum des patients et non sur leurs fibroblastes alors que dans le cas de mutations sévères retrouvées dans le syndrome de Zellweger, les marqueurs sont toujours anormaux quel que soit le prélèvement analysé (sérum et fibroblastes).

Du fait de la multiplicité fonctionnelle des protéines touchées dans les ACAR impliquant des mécanismes physiopathologiques divers, les connaissances actuelles suggèrent qu'aucune voie physiopathologique privilégiée ne permette d'expliquer l'apparition d'une ataxie cérébelleuse. Une des explications seraient que le symptôme « ataxie » pourrait résulter d'une sensibilité particulière des neurones cérébelleux et/ou spinocérébelleux aux désordres métaboliques modérés. La susceptibilité particulière des

cellules de Purkinje pourrait être due à leur grande taille, à leur arborisation dendritique importante et à leur intense activité métabolique (Hekman et Gomez 2015). La susceptibilité particulière des neurones de la couche des grains, quant à elle, pourrait être due à leur très grande densité par mm^3 (la plus importante de tout le système nerveux central). Enfin, la susceptibilité particulière des neurones spino-cérébelleux et sensitifs pourrait s'expliquer de manière plus globale par la très grande taille de leur axone (ce qui est également vrai pour les neurones pyramidaux et expliquerait donc pourquoi ces neurones sont souvent affectés de concert dans les ataxies spastiques). En effet, le type de neurone principalement affecté varie d'une forme d'ataxie à une autre et n'est pas corrélé avec les voies métaboliques. A titre d'exemple, l'ataxie de Friedreich et l'ARCA2 (liée au gène *ADCK3*) sont toutes deux des ataxies mitonchondriales, mais avec une dégénérescence des neurones spino-cérébelleux, sensitifs et pyramidaux pour la première et des neurones de Purkinje et des grains pour la deuxième.

3.3. L'AFP dans les ataxies cérébelleuses récessives

L'AFP est une glycoprotéine oncofoetale de 590 acides aminés comportant 15 ponts disulfures. Le gène codant pour l'AFP se situe comme celui de l'albumine sur le bras long du Chromosome 4. L'AFP joue un rôle important pendant la vie embryonnaire comme transporteur d'ions, de bilirubine et d'acides gras polyinsaturés. Elle est alors synthétisée par la vésicule vitelline puis par le foie. Sa concentration sérique chez le foetus atteint 3 à 4g/l vers la 13ème semaine de gestation puis décroit, l'AFP sérique restant détectable 6 mois après la naissance dans le sang. Chez l'adulte, le taux sérique est susceptible de s'élever lors de la prolifération de cellules ou de la régénération de

tissus dont l'origine embryonnaire est la même que celle des cellules sécrétant l'AFP chez le foetus. Ainsi l'AFP est élevée en cas de pathologie hépatique bénigne ou maligne, et dans les tumeurs d'origine germinale (testicules, ovaires, tératome).

L'AFP est connue pour être un marqueur classique de 2 grandes ataxies cérébelleuses récessives: l'ataxie télangiectasie (AT) et l'ataxie avec apraxie oculo-motrice de type 2 (AOA2). AT débute le plus souvent avant l'âge de 5 ans et associe ataxie, apraxie oculomotrice, télangiectasies oculaires ou cutanées, sensibilité aux radiations ionisantes, risque accru de néoplasies (hémopathies en particulier), déficit immunitaire variable favorisant des infections pulmonaires itératives. Un des éléments biologiques orientant vers le diagnostic est cette élévation d'AFP, présente chez 90 % des patients, bien souvent au-delà de 100 ng/ml. Il s'y ajoute un déficit immunitaire mixte (déficit en lymphocytes CD4 et CD8, déficit en IgA ou G) et la présence de remaniements chromosomiques (translocations en général) sur le caryotype (Chun and Gatti, 2004; Shiloh, 2003). Une mutation du gène *ATM,* codant pour une phosphatidylinositol-3-kinase impliquée dans la progression du cycle cellulaire, la réponse cellulaire aux altérations de l'ADN et le maintien de la stabilité du génome, est retrouvée (Chen and Lee, 1996; Shiloh, 2003).

AOA2 débute dans l'adolescence et associe également ataxie, apraxie oculo-motrice, polyneuropathie axonale sensitivo-motrice (décrite comme moins sévère que dans AOA 1), possible dystonie inaugurale et transitoire. Une élévation de l'AFP est constante au cours de l'évolution de la maladie. (Németh et al., 2000; Watanabe et al., 1998) Le diagnostic est confirmé par la mise évidence de mutations du gène de la senataxine, protéine contenant un domaine ADN/ARN hélicase (Moreira et al., 2004), impliquée dans le processing et la terminaison des ARN par la reconnaissance des structures

boucles-R (R-loop) (Groh M. et al., J Mol Biol 2016). Le taux d'AFP est toutefois moins élevé dans AOA2 que dans l'ataxie-télangiectasie classique, mais est équivalent à celui des formes adultes d'ataxie-télangiectasie. (Méneret et al., 2014)

Récemment le gène de l'AOA4 a été mis en évidence chez des patients avec un phénotype associant ataxie et apraxie oculo-motrice. Il s'agit du gène *PNKP* codant pour la polynucleotide kinase 3'-phosphatase. Dans cette pathologie, l'AFP peut également être augmentée de 1,5 à 4 fois la normale. (Bras et al., 2015)

Nous avions décrit une possible augmentation modérée de l'AFP dans une autre forme d'ataxie récessive ne codant pas cette fois ci pour une protéine impliquée dans la réparation de l'ADN. Il s'agit d'ARCA3 dans laquelle le gène muté, *ANO10* code pour l'anoctamine 10, protéine à 8 domaines transmembranaires, transporteur chlorique activé par le calcium. Dans notre série de 9 patients, nous avions montré que l'AFP pouvait être élevée dans un tiers des cas jusqu'à 16 ng/ml. (Renaud et al., 2014) Ainsi l'AFP ne serait pas seulement augmentée dans les ACAR impliquées dans des altérations de la réplication, de la réparation et de la transcription des acides nucléiques (AT, AOA1, AOA2, AOA4).

Le lien biologique entre AFP et ataxie cérébelleuse reste à l'heure actuelle méconnu. Il est classiquement décrit que l'élévation de l'AFP, marqueur tumoral hépatique comme nous l'avons dit, indique une dédifférenciation des cellules cancéreuses hépatique et un retour à un "programme" embryonnaire. Néanmoins malgré une prédisposition à un nombre important de néoplasmes, il ne semble pas particulièrement exister de cancers hépatiques dans AT et il n'existe pas de prédispositions particulières aux cancers dans AOA1 et AOA2.

Une des hypothèses serait que cette augmentation soit en lien avec une altération de la régulation de la transcription de l'AFP au niveau du foie. (Anheim et al., 2012) L'hypoalbuminémie et l'hypercholestérolémie dans AOA1 pourraient également résulter d'une dérégulation transcriptionnelle hépatique. (Shimazaki et al., 2002) Ainsi, il a été démontré que la senataxine était impliquée dans la terminaison de la transcription mais n'était pas directement impliquée dans la réparation de l'ADN (Groh M et al., J Mol Biol 2016). La kinase ATM, quant à elle, a effectivement de nombreux effets sur la réparation de l'ADN (expliquant la prédisposition aux cancers), mais également sur la transcription ce qui pourrait être responsable des effets neurologiques et hépatiques (augmentation de l'AFP). Ce sujet reste à l'heure actuelle très controversé.

3.4. Nécessité de l'utilisation d'algorithmes et interprétation du séquençage à haut débit

L'élaboration d'un algorithme de diagnostic étiologique est un outil majeur pour guider l'enquête étiologique face à une ataxie cérébelleuse présumée héréditaire et/ou pour interpréter la grande quantité de variants obtenus par séquençage à haut débit. Notre algorithme informatisé s'est révélé très sensible et spécifique, prédisant avec précision les diagnostics moléculaires sous-jacents des ACAR. Ainsi, l'utilisation de l'algorithme permettrait de faire concorder les explorations cliniques et paracliniques au nombre important de résultats obtenus par NGS. On rappelle que l'algorithme repose sur des signes neurologiques classiques mais aussi sur des signes extra-neurologiques comme par exemple l'évaluation de l'audition ou de la vision des patients, les atteintes sensorielles étant souvent associées aux ataxies. Dans les examens paracliniques, l'EMG et l'IRM paraissent nécessaires à l'orientation diagnostique. L'usage

systématique de certains biomarqueurs semble également indispensable avant tout séquençage pour aiguiller l'enquête étiologique. Il s'agit de l'AFP, la vitamine E, les AGTLC, l'acide phytanique, les oxystérols, le cholestanol, les lactates et le pyruvate. (Van de Warrenburg et al., 2014) Les autres analyses métaboliques sont à réaliser en seconde intention selon l'examen clinique et éventuellement le résultat du séquençage à haut débit.

Malgré le développement du NGS, le diagnostic moléculaire des ataxies récessives reste autour de 50% (Németh et al., 2013 ; Pyle et al., 2014) et ce pour plusieurs raisons :

- la difficulté d'interpréter les variants notamment les variants hypomorphes comme cités ci-dessus.

- les limites techniques du séquençage haut débit. En effet, le séquençage d'exome ne permet pas de couvrir l'ensemble des régions d'intérêt sans risque d'erreur et les données obtenues ne permettent pas de détecter tous les types de mutations, notamment les CNV (copy number variations) comme les délétions ou les duplications, ou les expansions de répétitions de triplets. Le séquençage du génome permettrait d'augmenter les capacités de détection de ce type de mutations en évitant les biais de capture et en permettant l'identification des fragments de jonction des réarrangements. Néanmoins, l'interprétation du génome entier devrait s'avérer encore plus difficile du fait de la grande quantité de variants révélés par cette analyse.

- le fait qu'une fraction des ataxies sporadiques, présumées héréditaires (notamment chez l'adulte) ne le soient pas tant il est difficile de distinguer les formes héréditaires et non héréditaires d'ataxie.

- la probabilité que certains gènes responsables ne soient pas encore identifiés.

3.5. Stratégies pour identifier de nouveaux gènes responsables d'ataxies

Le développement du NGS a modifié profondément les anciennes méthodes d'identification de gènes responsables par études de grandes familles consanguines avec étude des régions d'homozygotie et séquençage de gènes candidats.

L'étude en exome de grandes cohortes de patients avec même phénotype pourrait amener à trouver plusieurs mutations dans le même gène après avoir exclu les gènes connus. L'interprétation des résultats dans ce cas reste toujours très délicate car on peut retrouver de manière fortuite des variants de signification inconnue dans le même gène chez plusieurs patients de la cohorte. Dans ce cas, l'analyse fonctionnelle permettra de les incriminer mais là encore, les analyses fonctionnelles peuvent s'avérer difficiles d'interprétation en cas de mutations hypomorphes.

Cependant, en associant phénotypage clinique détaillé et analyse complète de l'exome voire du génome, nous pensons encore pouvoir identifier des mutations dans des gènes non connus. Pour cela, il est nécessaire de constituer des groupes homogènes de patients ataxiques (non diagnostiqués sur le plan génétique) avec un phénotype clinique et paraclinique proche. Deux stratégies sont particulièrement efficaces pour l'identification de nouveaux gènes: la recherche de mutation homozygote dans les familles consanguines pour les maladies récessives et la recherche de néomutation dans les formes sporadiques sévères et congénitales (mutation dominante). L'identification de gènes de maladie dominante tardive est limitée par la nécessité d'identifier des familles avec au moins 10 individus atteints, surtout si la mise en évidence fonctionnelle d'un mécanisme gain de fonction est techniquement difficilement réalisable.

Du fait de la rareté de ces pathologies, un recrutement international des patients et une collaboration avec les différents experts du monde entier semblent nécessaires pour avancer sur l'identification de nouveaux gènes.

A noter que l'identification d'un nouveau gène responsable d'ataxie cérébelleuse héréditaire pourrait être l'occasion de découvrir une voie physiopathologique nouvelle, à l'origine d'une thérapeutique ciblée et/ou innovante.

3.6. Conclusion

Au cours de ce travail de thèse, nous avons élargi le spectre phénotypique clinique, biologique, radiologique d'ataxies cérébelleuses héréditaires connues (FXTAS, ataxie liée à *PEX10,* AOA1) et mis en évidence des corrélations du génotype au phénotype (AOA1).

Nous avons réussi à valider un algorithme facilitant le diagnostic des ACAR et l'interprétation du NGS, les données seules du séquençage à haut débit étant insuffisantes pour statuer sur l'implication d'un variant.

Les stratégies diagnostiques actuelles (panel ou mini-exome) ne sont pas encore fixées. A moyen terme, l'objectif est la généralisation de l'exome pour chaque patient suspect d'ataxie héréditaire, une fois les grandes causes génétiques infirmées (Friedreich, SCA par répétitions de triplets, FXTAS).

Il reste important dans ce type de pathologies rares de pouvoir établir au maximum un diagnostic moléculaire afin de guider au mieux le conseil génétique (exemple de

FXTAS) et mettre en évidence les ataxies accessibles à une thérapeutique (ataxie avec déficit en vitamine E (AVED), maladie de Niemann Pick de type C, xanthomatose cérébro-tendineuse, maladie de Refsum). L'aboutissement du diagnostic permettra également d'envisager un pronostic et d'inclure les patients dans d'éventuels protocoles de recherche et d'essais thérapeutiques.

Malgré la découverte ces dernières années d'une multitude de gènes responsables, la prise en charge thérapeutique des ataxies génétiques reste dans la plupart des cas limitée. Une prise en charge symptomatique, pluri-disciplinaire, associant kinésithérapie, orthophonie, prise en charge des complications orthopédiques et de la spasticité, est nécessaire pour chaque patient. Ces mesures permettent une amélioration de la qualité de vie en tentant de limiter au maximum les symptômes dans la vie quotidienne.

Au total, les ataxies génétiques sont des pathologies rares et hétérogènes, mais une bonne description phénotypique ainsi qu'une bonne compréhension des résultats du NGS facilitent le diagnostic positif d'ataxies déjà connues. L'analyse de l'exome voire du génome de chaque patient suspect d'ataxie génétique représente l'avenir pour faciliter le diagnostic et l'identification de nouveaux gènes.

Bibliographie

Aicardi, J., Barbosa, C., Andermann, E., Andermann, F., Morcos, R., Ghanem, Q.,et al. Ataxia-ocular motor apraxia: a syndrome mimicking ataxia-telangiectasia. Brain 1988 Oct;24(4):497-502.

Anheim, M., Monga, B., Fleury, M., Charles, P., Barbot, C., Salih, et al. Ataxia with oculomotor apraxia type 2: Clinical, biological and genotype/phenotype correlation study of a cohort of 90 patients. Brain 2009 Oct;132(Pt 10):2688-98.

Anheim M, Tranchant C, Koenig M. The autosomal recessive cerebellar ataxias. N Engl J Med. 2012 Feb 16;366(7):636-46.

Apartis E, Blancher A, Meissner WG, Guyant-Maréchal L, Maltête D, De Broucker T et al. FXTAS: new insights and the need for revised diagnosis criteria. Neurology. 2012 Oct 30;79(18):1898-907.

Barbot, C., Coutinho, P., Chorão, R., Ferreira, C., Barros, J., Fineza, I., et al. Recessive ataxia with ocular apraxia: review of 22 Portuguese patients. Arch. Neurol. 2001 Feb;58(2):201-5.

Berger J., Dorninger F., Forss-Petter S., Kunze M. Peroxisomes in brain development and function. Biochim Biophys Acta. 2016 May;1863(5):934-55.

Berry-Kravis E, Abrams L, Coffey SM, Hall DA, Greco C, Gane LW, et al. Fragile X-associated tremor/ataxia syndrome: clinical features, genetics, and testing guidelines. Mov Disord. 2007 Oct 31;22(14):2018-30.

Bras J., Alonso I., Barbot C., Costa M., Darwent L., Orme T., et al. Mutations in PNKP cause recessive ataxia with oculomotor apraxia type 4. Am J Hum Genet. 2015 Mar 5;96(3):474-9.

Braverman N.E., D'Agostino, M.D., and Maclean, G.E. Peroxisome biogenesis disorders: Biological, clinical and pathophysiological perspectives. Dev. Disabil. Res. 2013;17(3):187-96.

Caldecott K.W. DNA single-strand break repair and spinocerebellar ataxia. Cell. 2003 Jan 10;112(1):7-10.

Chen, G., and Lee, E.Y.-H.P. The product of the ATM gene is a 370-kDa nuclear phosphoprotein. J. Biol. Chem. 1996 Dec 27;271(52):33693-7.

Chun, H.H., and Gatti, R. Ataxia-telangiectasia, an evolving phenotype. DNA Repair (Amst). 2004 Aug-Sep;3(8-9):1187-96.

Criscuolo, C., Mancini, P., Menchise, V., Saccà, F., De Michele, G., Banfi, S., and Filla, A. Very late onset in ataxia oculomotor apraxia type I. Ann. Neurol. 2005 May;57(5):777.

De Munter S., Verheijden S., Régal L., Baes M. Peroxisomal Disorders: A Review on Cerebellar Pathologies. Brain Pathol. 2015 Nov;25(6):663-78.

Hagerman RJ, Leehey M, Heinrichs W, Tassone F, Wilson R, Hills J, et al. Intention tremor, parkinsonism, and generalized brain atrophy in male carriers of fragile X. Neurology. 2001 Jul 10;57(1):127-30.

Hagerman R, Hagerman P. Advances in clinical and molecular understanding of the FMR1 premutation and fragile X-associated tremor/ataxia syndrome. Lancet Neurol. 2013 Aug;12(8):786-98.

Groh M, Albulescu LO, Cristini A, Gromak N. Senataxin: Genome Guardian at the Interface of Transcription and Neurodegeneration. J Mol Biol 2016 Oct 19.

Jacquemont S, Hagerman RJ, Leehey M, Grigsby J, Zhang L, Brunberg JA, et al. Fragile X premutation tremor/ataxia syndrome: molecular, clinical, and neuroimaging correlates. American journal of human genetics. 2003 Apr;72(4):869-78.

Jacquemont S, Hagerman RJ, Leehey MA, Hall DA, Levine RA, Brunberg JA, et al. Penetrance of the fragile X-associated tremor/ataxia syndrome in a premutation carrier population. Jama. 2004 Jan 28;291(4):460-9.

Harding, A.E. Classification of the hereditary ataxias and paraplegias. Lancet 1983 May 21;1(8334):1151-5.

Harding, A.E. (1993). Clinical features and classification of inherited ataxias. Adv. Neurol. 61, 1–14.

Hekman, K.E., and Gomez, C.M. The autosomal dominant spinocerebellar ataxias: emerging mechanistic themes suggest pervasive Purkinje cell vulnerability. J. Neurol. Neurosurg. Psychiatry 2015 May;86(5):554-61.

Klein C.J., Foroud T.M. Neurology Individualized Medicine: When to Use Next-Generation Sequencing Panels. Mayo Clin Proc. 2017 Feb;92(2):292-305.

Koenig, M. Rare forms of autosomal recessive neurodegenerative ataxia. Semin. Pediatr. Neurol. 2003 Sep;10(3):183-92.

Le Ber I, Moreira M-C, Rivaud-Péchoux S, Chamayou C, Ochsner F, Kuntzer T, et al. Cerebellar ataxia with oculomotor apraxia type 1: clinical and genetic studies. Brain. déc 2003;126(Pt 12):2761‑72.

Lecocq, C., Charles, P., Azulay, J.-P., Meissner, W., Rai, M., N'Guyen, K., et al. (2015). Delayed-onset Friedreich's ataxia revisited. Mov. Disord. 2016 Jan;31(1):62-9.

Leehey MA. Fragile X-associated tremor/ataxia syndrome: clinical phenotype, diagnosis, and treatment. J Investig Med. 2009 Dec;57(8):830-6.

Mertes F., Elsharawy A., Sauer S., van Helvoort J.M., van der Zaag P.J., Franke A., ett al. Targeted enrichment of genomic DNA regions for next-generation sequencing. Brief Funct Genomics. 2011 Nov;10(6):374-86.

Moreira, M.C., Barbot, C., Tachi, N., Kozuka, N., Uchida, E., Gibson, T., et al. The gene mutated in ataxia-ocular apraxia 1 encodes the new HIT/Zn-finger protein aprataxin. Nat. Genet. 2001 Oct;29(2):189-93.

Moreira, M.-C., Klur, S., Watanabe, M., Németh, A.H., Le Ber, I., Moniz, J.-C., et al. Senataxin, the ortholog of a yeast RNA helicase, is mutant in ataxia-ocular apraxia 2. Nat. Genet. 2004 Mar;36(3):225-7.

Méneret, A., Ahmar-Beaugendre, Y., Rieunier, G., Mahlaoui, N., Gaymard, B., Apartis, E., et al. (2014). The pleiotropic movement disorders phenotype of adult ataxia-telangiectasia. Neurology 83, 1087–1095.

Németh, A.H., Bochukova, E., Dunne, E., Huson, S.M., Elston, J., Hannan, M.A., et al. Autosomal recessive cerebellar ataxia with oculomotor apraxia (ataxia-telangiectasia-like syndrome) is linked to chromosome 9q34. Am. J. Hum. Genet. 2000 Nov;67(5):1320-6.

Németh A.H., Kwasniewska A.C., Lise S., Parolin Schnekenberg R., Becker E.B., Bera K.D., et al. Next generation sequencing for molecular diagnosis of neurological disorders using ataxias as a model. Brain. 2013 Oct;136(Pt 10):3106-18.

Pyle A., Smertenko T., Bargiela D., Griffin H., Duff J., Appleton M., et al. Exome sequencing in undiagnosed inherited and sporadic ataxias. Brain. 2015 Feb;138(Pt 2):276-83.

Régal L., Ebberink M.S., Goemans N., Wanders R.J., De Meirleir L., Jaeken J., et al. Mutations in PEX10 are a cause of autosomal recessive ataxia. Ann Neurol. 2010 Aug;68(2):259-63.

Ramnani N. The primate cortico-cerebellar system: anatomy and function. Nat Rev Neurosci. 2006 Jul;7(7):511-22.

Renaud M., Anheim M., Kamsteeg E.-J., Mallaret M., Mochel F., Vermeer S., et al. Autosomal recessive cerebellar ataxia type 3 due to ANO10 mutations: delineation and genotype-phenotype correlation study. JAMA Neurol. 2014 Oct;71(10):1305-10.

Ruano L., Melo C., Silva M.C., Coutinho P. The global epidemiology of hereditary ataxia and spastic paraplegia: a systematic review of prevalence studies. Neuroepidemiology 2014 ;42(3) :174-83.

Sacksteder, K.A., and Gould, S.J. The genetics of peroxisome biogenesis. Annu. Rev. Genet. 2000;34:623-652.

Sano, Y., Date, H., Igarashi, S., Onodera, O., Oyake, M., Takahashi, T., et al. Aprataxin, the causative protein for EAOH is a nuclear protein with a potential role as a DNA repair protein. Ann. Neurol. 2004 Feb;55(2):241-9.

Schmitz-Hübsch T., du Montcel S.T., Baliko L., Berciano J., Boesch S., Depondt C., et al. (2006). Scale for the assessment and rating of ataxia: development of a new clinical scale. Neurology 2006 Jun 13;66(11):1717-20.

Sevin M, Kutalik Z, Bergman S, Vercelletto M, Renou P, Lamy E, et al. Penetrance of marked cognitive impairment in older male carriers of the FMR1 gene premutation. Journal of medical genetics. 2009 Dec;46(12):818-24.

Shiloh Y. ATM and related protein kinases: safeguarding genome integrity. Nat. Rev. Cancer 2003 Mar;3(3):155-68.

Shimazaki, H., Takiyama, Y., Sakoe, K., Ikeguchi, K., Niijima, K., Kaneko, J., et al. Early-onset ataxia with ocular motor apraxia and hypoalbuminemia: the aprataxin gene mutations. Neurology 2002 Aug 27;59(4):590-5.

Soontarapornchai K, Maselli R, Fenton-Farrell G, Tassone F, Hagerman PJ, Hessl D, et al. Abnormal nerve conduction features in fragile X premutation carriers. Archives of neurology. 2008 Apr;65(4):495-8.

Steinberg S.J., Dodt G., Raymond G. V, Braverman N.E., Moser A.B., and Moser H.W. Peroxisome biogenesis disorders. Biochim. Biophys. Acta 2006 Dec;1763(12):1733-48.

Subramony S.H. SARA-a new clinical scale for the assessment and rating of ataxia. Nat. Clin. Pract. Neurol.2007 Mar;3(3):136-7.

Tranchant, C., Fleury, M., Moreira, M.C., Koenig, M., and Warter, J.M. Phenotypic variability of aprataxin gene mutations. Neurology 2003 Mar 11;60(5):868-70.

Van den Brink D.M., Brites P, Haasjes J., Wierzbicki A.S., Mitchell J., Lambert-Hamill M., et al. Identification of PEX7 as the second gene involved in Refsum disease. Am J Hum Genet. 2003 Feb;72(2):471-7.

Van de Warrenburg B.P.C., van Gaalen, J., Boesch, S., Burgunder, J.-M., Dürr, A., Giunti, P., et al. EFNS/ENS Consensus on the diagnosis and management of chronic ataxias in adulthood. Eur. J. Neurol. 2014 Apr;21(4):552-62.

Wanders R.J., and Waterham H.R. Biochemistry of mammalian peroxisomes revisited. Annu. Rev. Biochem. 75, 295–332.

Watanabe M., Sugai Y., Concannon P., Koenig M., Schmitt M., Sato M., et al. Familial spinocerebellar ataxia with cerebellar atrophy, peripheral neuropathy, and elevated level of serum creatine kinase, gamma-globulin, and alpha-fetoprotein. Ann. Neurol. 1998 Aug;44(2):265-9.

Mathilde HODILLE épouse RENAUD

Ataxies cérébelleuses héréditaires: identification de gènes responsables, description clinique et stratégie diagnostique

Résumé

Les ataxies cérébelleuses héréditaires sont des pathologies neuro-dégénératives rares, hétérogènes, complexes affectant le cervelet et parfois la moelle épinière et/ou les nerfs périphériques. Elles se transmettent sur le mode autosomique récessif (ARCA), dominant (SCA) ou lié à l'X. Les objectifs de cette thèse de sciences étaient la description phénotypique d'ataxies cérébelleuses héréditaires, la mise en évidence de corrélations du génotype au phénotype et la description de stratégies diagnostiques pour mettre en évidence ces pathologies rares.

Grâce à nos résultats, nous avons pu élargir le spectre phénotypique clinique, biologique, radiologique d'ataxies cérébelleuses héréditaires connues : Fragile X Tremor Ataxia Syndrome (FXTAS), ataxie récessive lentement progressive liée au gène *PEX 10* impliqué dans la biogénèse du peroxysome, ataxie avec apraxie oculomotrice de type 1 (AOA1). Nous avons pu mettre en évidence des corrélations du génotype au phénotype dans AOA1 et montré que l'âge moyen de début était plus élevé et que la pathologie était moins sévère chez les patients avec au moins un faux sens ($p < 0,01$) par rapport aux patients avec deux mutations tronquantes. Nous avons réussi également à établir un algorithme pour faciliter le diagnostic des ataxies cérébelleuses autosomiques récessives et aider à l'interprétation du séquençage à haut débit.

Il est important dans ce type de pathologies rares de pouvoir établir au maximum un diagnostic moléculaire afin de guider le conseil génétique et mettre en évidence les ataxies accessibles à une thérapeutique.

Mots clefs: ataxie, FXTAS, AOA1, *PEX 10*, algorithme diagnostique

Résumé en anglais

Hereditary cerebellar ataxias are a group of rare and heterogeneous neurodegenerative diseases. The transmission mode is recessive, dominant or X-linked. Our objectives were to better describe the phenotype of some inherited ataxias, to provide genotype-phenotype correlations and to improve the diagnostic strategies for these rare diseases.

We enlarged the clinical, biological, radiological phenotype of Fragile X Tremor Ataxia Syndrome (FXTAS), recessive ataxia due to *PEX10* related peroxisomal biogenesis disorders, ataxia with oculomotor apraxia type 1 (AOA1). We showed genotype-phenotype correlations in AOA1 patients: mean age at onset was higher with at least one missense mutation. A ranking algorithm has been created to predicting the molecular diagnoses of recessive cerebellar ataxia in order to guide the diagnosis and facilitate interpretation of next generation sequencing.

The establishment of a molecular diagnosis is important in this type of rare pathologies to guide the genetic counseling and to diagnosis the ataxias accessible to a treatment.

Key words: ataxia, FXTAS, PEX 10, algorithm