HHS Public Access

Author manuscript

*Neurodegener Dis*. Author manuscript; available in PMC 2018 May 31.

Published in final edited form as:

*Neurodegener Dis*. 2017 ; 17(4-5): 208–212. doi:10.1159/000464445.

# SLC25A46 mutations associated with Autosomal Recessive Cerebellar Ataxia in North African families

**Monia B. Hammer**[1], **Jinhui Ding**[1], **Fanny Mochel**[2,3], **Ghada Eleuch-Fayache**[4], **Perrine Charles**[2], **Marie Coutelier**[3], **J. Raphael Gibbs**[1], **Sampath K. Arepalli**[1], **Sean B. Chong**[1], **Dena G. Hernandez**[1], **Elisa Majounie**[1], **Steven Clipman**[1], **Yosr Bouhlal**[5], **Houda Nehdi**[4], **Alexis Brice**[2,3], **Faycal Hentati**[4], **Giovanni Stevanin**[3,6], **Rim Amouri**[4], **Alexandra Durr**[2,3], and **Andrew B. Singleton**[1]

[1]Molecular Genetics Section, Laboratory of Neurogenetics, National Institute on Aging, National Institutes of Health, Bethesda, MD 20892, USA

[2]APHP, Genetic department, Pitié-Salpêtrière University Hospital, Paris, France

[3]ICM Institut du Cerveau et de la Moelle épinière, Inserm U 1127, CNRS UMR 7225, Sorbonne Universités, UPMC University Paris 06 UMR S 1127, Paris, France

[4]Department of Molecular Neurobiology and Neuropathology, National Institute of Neurology, La Rabta, Tunis 1007, Tunisia

[5]Institute of Human Genetics, UCSF, 513 Parnassus Avenue, Box 0793, San Francisco, CA 94143, USA

[6]EPHE, PSL Research University, 75014 Paris, France

## Abstract

**Background—**Autosomal recessive cerebellar ataxias (ARCA) is a complex group of neurodegenerative disorders with high clinical and genetic heterogeneity. In most cases, the cerebellar ataxia is not pure and complicating clinical features such as pyramidal signs or extra-neurological features are found.

**Objective—**To identify the genetic origin of the cerebellar ataxia for three consanguineous North African families presenting with ARCA.

**Methods—**Genome-wide high density SNP genotyping and whole-exome sequencing were performed followed by Sanger sequencing for mutation confirmation.

**Results—**Two variants were identified in *SLC25A46*. Mutations in this gene have been previously associated with Charcot-Marie-Tooth type 2 and optic atrophy. While the previously reported variant p.Arg340Cys seems to be associated with consistently the same clinical features

Correspondence: Andrew B. Singleton. Address: Laboratory of Neurogenetics, NIA, NIH, Building 35, Room 1A1014, 35 Convent Drive, Bethesda, MD 20892, USA. Phone 001-301 451-6079; Fax 001-301-451-5466; singleta@mail.nih.gov.

**Institute where the work was conducted:** Laboratory of Neurogenetics, National Institute on Aging, National Institutes of Health, Bethesda, MD, USA

**Declaration of interest**

The authors declare no conflict of interest.

with childhood onset, optic atrophy, gait and speech difficulties and wasting of the lower limbs, the patient with the novel mutation p.Trp160Ser did not present with optic atrophy and his ocular abnormalities were limited to nystagmus and saccadic pursuit.

**Conclusion—**In this study, we report a novel variant (p.Trp160Ser) in *SLC25A46* and we broaden the phenotypic spectrum associated with mutations in *SLC25A46*.

### Keywords

Ataxia; SLC25A46; mutation; North Africa

## Introduction

Autosomal recessive cerebellar ataxias (ARCA) is a large group of neurodegenerative disorders highly heterogeneous, clinically and genetically. Phenotypic manifestations commonly consist of cerebellar dysarthria, gait ataxia and dysmetria. Clinical features may be complicated with gait spasticity, peripheral neuropathy, hearing impairment or optic atrophy. There are at least five forms of ARCA associating cerebellar ataxia with visual impairment: (i) CAMOS (Cerebellar Ataxia associated with Mental retardation, Optic atrophy and Skin abnormalities), a rare non-progressive cerebellar ataxia syndrome, originally identified in a Lebanese family [1] and caused by a mutant zinc-finger protein, *ZNF592* [2]; (ii) IOSCA (Infantile Onset SpinoCerebellar Ataxia), a severe neurodegenerative disorder that manifests at the age of 9–18 months in previously healthy infants, due to mutations in genes encoding mitochondrial proteins Twinkle and Twinky [3]; (iii) Refsum's disease, a hereditary motor sensory neuropathy associated with mutations in *PHYH* causing the accumulation of phytanic acid in plasma and lipid-containing tissues [4]; (iv) Boucher-Neuhäuser syndrome, or more broadly the spectrum of *PNPLA6*-related diseases [5] and (v) Nyssen-van Bogaert syndrome with a still unknown genetic origin. Recently, Abrams et al [6] reported four mutations in *SLC25A46* associated with Charcot-Marie-Tooth type 2 and optic atrophy. In this study, we describe three consanguineous North African families with mutations in the same gene but presenting mainly with recessive cerebellar ataxia with/or without optic atrophy.

## Methods

### Patients

Three consanguineous families, one of Tunisian (G) and two of Algerian descent (AAR-322 and AAR-404) were studied (pedigrees shown in Figure 1). All individuals gave informed consent. The work was approved by the local ethics committee and by the Office of Human Subjects Research at the National Institutes of Health.

### Molecular analysis

Genotyping was performed with the OmniExp-12, v1.0 DNA Analysis BeadChip (Illumina Inc., San Diego, CA) according to the manufacturer's instructions. SNP array data were subjected to homozygosity mapping with the Homozygosity Mapper software using only homozygous stretches of 15 alleles or longer [7]. Whole exome sequencing was

accomplished according to the Nimblegen (Nimblegen v2.0, Roche Nimblegen, Indianapolis, IN) and the Extended Nextera Rapid-Capture Exome kit (Illumina, San Diego, CA, USA) protocols on an Illumina HiSeq 2000. Paired end sequence reads were aligned against the reference human genome (UCSC hg19). Exome data analysis was accomplished as previously described [8a, 8b]. Variants were visually inspected with the Integrative Genomics Viewer (IGV) [9]. Mutation confirmation was done with Sanger sequencing using an ABI BigDye Terminator Cycle Sequencing Kit on an ABI 3730 sequencer. Sequence traces were analyzed via Sequencher (version 4.2; Gene Codes Corporation, Ann Arbor, MI, USA).

Nucleotide and protein positions are based on the following accession numbers from the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/) *SLC25A46*, NM_138773 and NP_620128. Variant positions within the cDNA are numbered using the A of the translation initiation codon as position 1.

## Results

### Clinical study

**Family G—**We describe two siblings (G2 and G3) born from a consanguineous marriage. At age 1, G2 experienced an outbreak of anterior poliomyelitis causing the amyotrophy and the shortening of the left lower limb. Later on, he presented with dysarthria and decrease in visual acuity that forced him to quit school by the age of 10. At 14, the patient showed gait instability. At 22, he was wheelchair-bound. Neurological examination revealed bilateral plantar extensor responses, abolished Achilles reflexes and finger to nose dysmetria. Nystagmus, divergent strabismus (right eye), blindness and bilateral optic nerve atrophy were found at the eye exam. Cerebral tomodensitometry showed a cerebellar mega cisterna without atrophy. Nerve conduction study (NCS) revealed severe sensorimotor demyelinating neuropathy. Nerve biopsy exhibited hypertrophic neuropathy with segmental demyelination, giant axons and onion bulb formations. His sister G3 presented at age 6 with a decrease in visual acuity especially on the left side. At 8, she showed walking difficulties. At 19, walking became impossible. Neurological examination revealed a mild dysarthria, lower limbs wasting, a Babinski sign, finger to nose dysmetria and abolished knee and Achilles reflexes. Skeletal abnormalities such as scoliosis and hollow feet were found. The eye exam revealed bilateral divergent strabismus, a decrease in visual acuity and an optic atrophy in both eyes. NCS showed a severe sensorimotor axonal neuropathy predominantly in lower limbs. Superficial peroneal nerve biopsy indicated the presence of onion fibers and a decrease in myelinated fibers.

**Patient AAR-322-001—**The patient was born from a consanguineous union. He was diagnosed with bilateral optic atrophy at 2 years of age. He started developing gait difficulties when he was 17 years old with an ataxic gait due to sensory deficit. Distal motor deficit and amyotrophy in his lower limbs led to the diagnosis of a sensorimotor axonal polyneuropathy. The patient also presented with mild pyramidal features with increased tendon reflexes, except for the Achilles reflexes that were absent, a positive Hoffmann's sign but no Babinski's sign. Muscle biopsy showed abnormal sub-sarcolemnic accumulations

without typical mitochondrial features. Subtle white matter changes in the cerebellum were noticed on cerebral MRI at age 31 (Figure 2). Other clinical features include arrhythmia and dyspnea.

**Patient AAR-404-009—**This patient also originates from a consanguineous family with first cousin parents. He presented with gait unsteadiness at age 23 and complaint of “not feeling the ground”. At 26, examination revealed moderate gait ataxia and mild lower limb ataxia, with a disability stage of 2 (mild, able to run). Plantar reflexes were indifferent. No motor deficit was noted. Vibration sense at ankles was abolished. The patient had mild scoliosis and presented with nystagmus and saccadic pursuit. EMG revealed axonal peripheral neuropathy. Alphafeto protein, cholesterol and vitamin E were normal. An older sister of the proband had a clinical diagnosis of multiple sclerosis (MS).

### Molecular study

Family G was initially studied. Genome wide genotyping of both affected individuals and their mother was conducted. Homozygosity mapping revealed a total of 15 homozygous regions across 9 different chromosomes. The exome data showed that, within these regions, only two variants were shared between the patients. The first region located on chromosome 1, (chr1: 235,523,555-241,183,893) includes a variant ([T131S]) in *NID1*. The second region, on chromosome 5 spanning from rs4704382 (chr5: 76,318,206bp) to rs10149476 (chr5: 107,287,663bp), comprises a substitution of arginine for cysteine at residue 340 (c. 1018C>T, p.Arg340Cys in *SLC25A46*. While this study was ongoing, Abrams et al. reported the same variant in a Sardinian family [6]. Sanger sequencing confirmed that the variant was homozygous in both siblings and heterozygous in the mother. Subsequently, we looked for mutations in *SLC25A46* in our exome data of similar cases and identified the same variant, p.Arg340Cys and a potentially novel variant in two unrelated Algerian families. The novel variant consists of a homozygous substitution c.479G>C that changes the tryptophan residue at position 160 to a serine (p.Trp160Ser). Public databases searches (dbSNP138, NHLBI ESP6500and ExAC) revealed that this variant was not reported. It is predicted to be damaging by Polyphen2 (score=1), SIFT (score=0) and MutationTaster (score=1). Moreover, tryptophan 160 is a highly conserved amino acid residue across species.

## Discussion

By sequence similarity analysis, SLC25A46 encodes a mitochondrial carrier protein. It locates in the mitochondrion inner membrane and is a multi-pass membrane protein. As are many other members in mitochondrial carrier family [10, 11] SLC25A46 is predicted to consist of six transmembrane alpha-helices TM1-TM6 (Figure 3). Both mutations, p.Arg340Cys and p.Trp160Ser, are on the loops of the repeat sequence facing inside matrix space. p.Trp160Ser is on the matrix loop between transmembrane helix TM1 and TM2 while p.Arg340Cys is on the matrix loop between TM5 and TM6. The arginine at position 340 is a highly conserved residue in mitochondrial carrier family (NCBI Conserved Domains Database), and it is adjacent to the highly conserved signature motif Px[D/E]xx[K/R], which is characteristic of all mitochondrial carriers. It is pointing to the mitochondria matrix space.

The PSSM score (log-odds score, basically calculated as the log (base 2) of the observed substitution frequency at a given position divided by the expected substitution frequency at that position) for p.Arg340Cys is -9, suggesting that the substitution is pathogenic especially in structures of mitochondrial carrier protein family. p.Trp160Ser is a change from nonpolar to polar decreasing hydrophobicity and is pathogenic based upon the PSSM scores. Slc25a46 is important for both the growth and maintenance of neuronal processes and these mutations may affect the normal function of the protein.

Abrams et al [6] reported a wide phenotypic range associated with mutations in *SLC25A46* from mild with patients well and fit until their 40s to lethal. In fact, different mutations seem to trigger different phenotypes. The p.Arg340Cys mutation seems to be associated with consistently the same clinical features across families (the Tunisian and Algerian families reported in this study and the Sardinian family from Abrams paper) with childhood onset, optic atrophy, cerebellar or sensitive ataxia, speech difficulties and wasting of the lower limbs due to polyneuropathy. The clinical description associated with the p.Trp160Ser mutation surprisingly lacked the presence of optic atrophy and ocular abnormalities were limited to nystagmus and saccadic pursuit. Also, the age at onset was later in life (23 years) in comparison with all of the other families (below 15 years). Ataxia was observed in two families (Palestinian and Sardinian) out of four reported by Abrams while it was the striking feature in our patients. In this study, we broadened the phenotypic spectrum associated with mutations in *SLC25A46*. This gene seems to be linked to neurological disorders worldwide.

## Acknowledgments

The authors thank J. Hammer for his contribution in the correction of the manuscript and his help with the figures. This work was supported in part by the Intramural Research Programs of the National Institute on Aging and the National Institutes of Neurological Disorders and Stroke, within the National Institutes of Health, Department of Health and Human Services. Project number ZO1 AG000958″ and the National Ataxia Foundation. We are also grateful to the European Union (7th PCRD, Omis Call, grant NEUROMICS), the VERUM foundation and Connaitre Les Syndromes Cérébelleux.

## References

1. Mégarbané A, Delague V, Ruchoux MM, Rizkallah E, Maurage CA, Viollet L, Rouaix-Emery N, Urtizberea A. A new autosomal recessive cerebellar ataxia disorder in a large inbred Lebanese family. Am J Med Genet. 2001 Jun 15; 101(2):135–41. [PubMed: 11391656]
2. Nicolas E, Poitelon Y, Chouery E, Salem N, Levy N, Mégarbané A, Delague V. CAMOS, a nonprogressive, autosomal recessive, congenital cerebellar ataxia, is caused by a mutant zinc-finger protein, ZNF592. Eur J Hum Genet. 2010 Oct; 18(10):1107–13. [PubMed: 20531441]
3. Nikali K, Suomalainen A, Saharinen J, Kuokkanen M, Spelbrink JN, Lönnqvist T, Peltonen L. Infantile onset spinocerebellar ataxia is caused by recessive mutations in mitochondrial proteins Twinkle and Twinky. Hum Mol Genet. 2005 Oct 15; 14(20):2981–90. [PubMed: 16135556]
4. Wierzbicki AS, Lloyd MD, Schofield CJ, Feher MD, Gibberd FB. Refsum's disease: a peroxisomal disorder affecting phytanic acid alpha-oxidation. J Neurochem. 2002 Mar; 80(5):727–35. [PubMed: 11948235]
5. Synofzik M1, Gonzalez MA, Lourenco CM, Coutelier M, Haack TB, Rebelo A, Hannequin D, Strom TM, Prokisch H, Kernstock C, Durr A, Schöls L, Lima-Martínez MM, Farooq A, Schüle R, Stevanin G, Marques W Jr, Züchner S. PNPLA6 mutations cause Boucher-Neuhauser and Gordon Holmes syndromes as part of a broad neurodegenerative spectrum. Brain. 2014 Jan; 137(Pt 1):69–77. [PubMed: 24355708]

6. Abrams AJ, Hufnagel RB, Rebelo A, Zanna C, Patel N, Gonzalez MA, Campeanu IJ, Griffin LB, Groenewald S, Strickland AV, Tao F, Speziani F, Abreu L, Schüle R, Caporali L, La Morgia C, Maresca A, Liguori R, Lodi R, Ahmed ZM, Sund KL, Wang X, Krueger LA, Peng Y, Prada CE, Prows CA, Schorry EK, Antonellis A, Zimmerman HH, Abdul-Rahman OA, Yang Y, Downes SM, Prince J, Fontanesi F, Barrientos A, Németh AH, Carelli V, Huang T, Zuchner S, Dallman JE. Mutations in SLC25A46, encoding a UGO1-like protein, cause an optic atrophy spectrum disorder. Nat Genet. 2015 Aug; 47(8):926–32. [PubMed: 26168012]

7. Li H, Durbin R. Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics. 2009 Jul 15; 25(14):1754–60. [PubMed: 19451168]

8a. Hammer MB, Eleuch-Fayache G, Gibbs JR, Arepalli SK, Chong SB, Sassi C, Bouhlal Y, Hentati F, Amouri R, Singleton AB. Exome sequencing: an efficient diagnostic tool for complex neurodegenerative disorders. Eur J Neurol. 2013 Mar; 20(3):486–92. [PubMed: 23043354]

8b. Hammer MB, Eleuch-Fayache G, Schottlaender LV, Nehdi H, Gibbs JR, Arepalli SK, Chong SB, Hernandez DG, Sailer A, Liu G, Mistry PK, Cai H, Shrader G, Sassi C, Bouhlal Y, Houlden H, Hentati F, Amouri R, Singleton AB. Mutations in GBA2 cause autosomal-recessive cerebellar ataxia with spasticity. Am J Hum Genet. 2013 Feb 7; 92(2):245–51. [PubMed: 23332917]

9. Robinson JT, Thorvaldsdottir H, Winckler W, Guttman M, Lander ES, Getz G, Mesirov JP. Integrative genomics viewer. Nat Biotechnol. 2011 Jan; 29(1):24–6. [PubMed: 21221095]

10. Pebay-Peyroula E1, Dahout-Gonzalez C, Kahn R, Trézéguet V, Lauquin GJ, Brandolin G. Structure of mitochondrial ADP/ATP carrier in complex with carboxyatractyloside. Nature. 2003 Nov 6; 426(6962):39–44. [PubMed: 14603310]

11. Berardi MJ, Shih WM, Harrison SC, Chou JJ. Mitochondrial uncoupling protein 2 structure determined by NMR molecular fragment searching. Nature. 2011 Jul 24; 476(7358):109–13. [PubMed: 21785437]

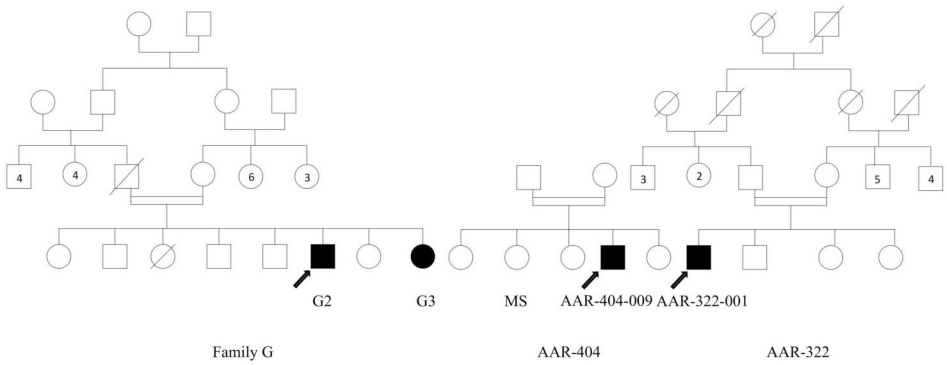


**Figure 1.**

Family pedigrees

Affected family members are shaded. Males are represented by squares, females are represented by circles

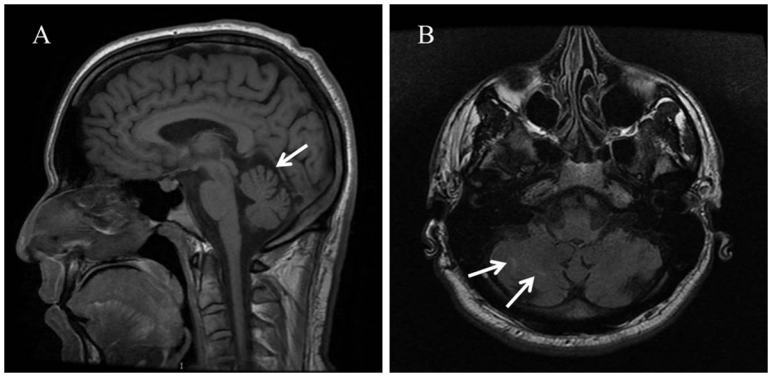


**Figure 2.**

Brain MRI of patient AAR-322-001 at age 31.

Sagittal view of T1-weighted image (A) showing a very mild upper vermis atrophy of the cerebellum (arrow) and an axial FLAIR section (B) showing very subtle white matter abnormalities in the cerebellar hemisphere (arrows).

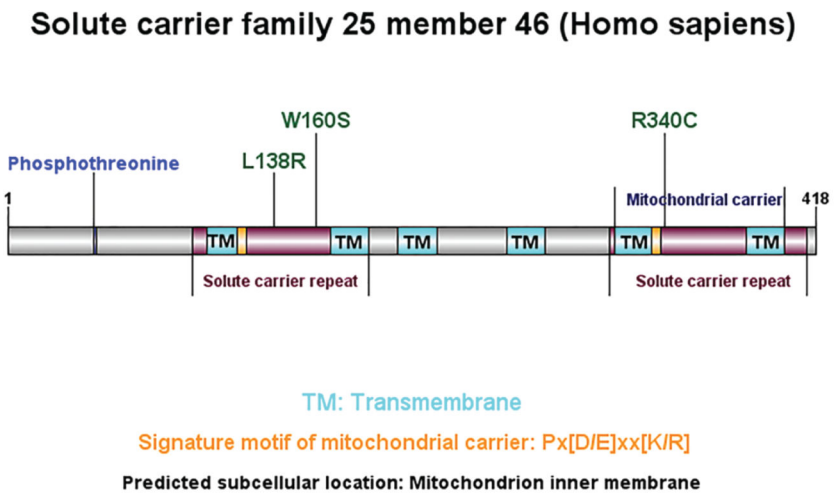


**Figure 3.**
SLC25A46 domains and functional sites