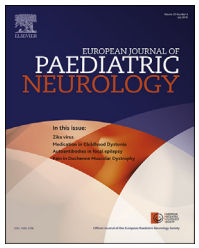
EUROPEAN JOURNAL OF PAEDIATRIC NEUROLOGY XXX (2017) 1–9

Official Journal of the European Paediatric Neurology Society



Original article

Mutations in *AIFM1* cause an X-linked childhood cerebellar ataxia partially responsive to riboflavin

G. Heimer [a,b,c,**,l], E. Eyal [d,l], X. Zhu [e], E.K. Ruzzo [f], D. Marek-Yagel [g], Doron Sagiv [c,h], Y. Anikster [c,g], H. Reznik-Wolf [i], E. Pras [c,i], D. Oz Levi [j], D. Lancet [j], B. Ben-Zeev [a,c,m], A. Nissenkorn [a,c,k,*,m]

[a] Pediatric Neurology Unit, Edmond and Lilly Safra Children Hospital, Chaim Sheba Medical Center, 52621 Ramat Gan, Israel
[b] Pinchas Borenstein Talpiot Medical Leadership Program, Chaim Sheba Medical Center, 52621 Ramat Gan, Israel
[c] The Sackler School of Medicine, Tel Aviv University, Tel Aviv, Israel
[d] Cancer Research Center, Pediatric Hemato/oncology Unit, Edmond and Lily Children's Hospital, Chaim Sheba Medical Center, 52621 Ramat Gan, Israel
[e] Institute for Genomic Medicine, Columbia University Medical School, Columbia University Medical Center, New York, NY 10032, USA
[f] Semel Institute for Neuroscience and Human Behavior, University of California Los Angeles, USA
[g] The Metabolic Disorder Unit, Edmond and Lilly Safra Children Hospital, Chaim Sheba Medical Center, 52621 Ramat Gan, Israel
[h] Department of Otolaryngology Head and Neck Surgery, Chaim Sheba Medical Center, 52621 Ramat Gan, Israel
[i] Danek Gertner Institute of Human Genetics, Chaim Sheba Medical Center, 52621 Ramat Gan, Israel
[j] Department of Molecular Genetics, Weizmann Institute of Science, Rehovot, 76100, Israel
[k] The Service for Rare Disorders, Edmond and Lilly Safra Children Hospital, Chaim Sheba Medical Center, 52621 Ramat Gan, Israel

ARTICLE INFO

Article history:
Received 4 June 2017
Received in revised form
16 August 2017
Accepted 11 September 2017

Keywords:
Cerebellar atrophy
Auditory neuropathy
Axonal neuropathy

ABSTRACT

Background: AIFM1 encodes a mitochondrial flavoprotein with a dual role (NADH oxidoreductase and regulator of apoptosis), which uses riboflavin as a cofactor. Mutations in the X-linked *AIFM1* were reported in relation to two main phenotypes: a severe infantile mitochondrial encephalomyopathy and an early-onset axonal sensorimotor neuropathy with hearing loss. In this paper we report two unrelated males harboring *AIFM1* mutations (one of which is novel) who display distinct phenotypes including progressive ataxia which partially improved with riboflavin treatment.

Methods: For both patients trio whole exome sequencing was performed. Validation and segregation were performed with Sanger sequencing. Following the diagnosis, patients

Abbreviations: WES, whole exome sequencing; OXPHOS, oxidative phosphorylation.

* *Corresponding author*. Pediatric Neurology Unit, Edmond and Lily Children's Hospital The Chaim Sheba Medical Center, 52621 Ramat Gan, Israel.
** *Corresponding author*. Pediatric Neurology Unit, Edmond and Lily Children's Hospital The Chaim Sheba Medical Center, 52621 Ramat Gan, Israel.
E-mail addresses: galih.md@gmail.com (G. Heimer), Andreea.nissenkorn@sheba.health.gov.il (A. Nissenkorn).
[l] Gali Heimer and Eran Eyal are to be considered first authors; they contributed equally to this work.
[m] Andreea Nissenkorn and Prof. Bruria Ben Zeev are to be considered last authors; they contributed equally to this work.

http://dx.doi.org/10.1016/j.ejpn.2017.09.004

Myoclonus
Riboflavin

were treated with up to 200 mg riboflavin/day for 12 months. Ataxia was assessed by the ICARS scale at baseline, and 6 and 12 months following treatment.

Results: Patient 1 presented at the age of 5 years with auditory neuropathy, followed by progressive ataxia, vermian atrophy and axonal neuropathy. Patient 2 presented at the age of 4.5 years with severe limb and palatal myoclonus, followed by ataxia, cerebellar atrophy, ophthalmoplegia, sensorineural hearing loss, hyporeflexia and cardiomyopathy. Two deleterious missense mutations were found in the *AIFM1* gene: p. Met340Thr mutation located in the FAD dependent oxidoreductase domain and the novel p. Thr141Ile mutation located in a highly conserved DNA binding motif. Ataxia score, decreased by 39% in patient 1 and 20% in patient 2 following 12 months of treatment.

Conclusion: *AIFM1* mutations cause childhood cerebellar ataxia, which may be partially treatable in some patients with high dose riboflavin.

1. Introduction

The extensive use of whole exome sequencing (WES) in the last decade, leads to discovery of seemingly unassociated phenotypes related to the same genetic etiology. Mutations in the X-linked *AIFM1* (OMIM: 300169) have been initially described in relation to a severe neonatal mitochondrial encephalomyopathy.[1–3] A second very different presentation, including sensorineural hearing loss and axonal neuropathy was subsequently described.[4,5] A third intermediate "mitochondrial" phenotype, of variable severity and symptoms was documented,[6,7] as well as other unique presentations as motor neuron disease[8] or skeletal dysplasia.[9]

Cerebellar ataxia is not a constant symptom in AIFM1 deficiency, but it accompanies especially the intermediate severity phenotypes.[6,7,9] It is associated with variable unspecific symptoms suggestive of oxidative phosphorylation (OXPHOS) defect as hearing loss,[6,7] neuropathy[6,9] or external ophthalmoplegia.[7] Moreover, onset of ataxia may vary from childhood[7,9] to adulthood,[6] therefore the recognition of a specific phenotype associated with *AIFM1* related ataxia is challenging.

The AIFM1 protein functions both as regulator of apoptosis[10,11] and a NADH oxidoreductase, which uses riboflavin as a cofactor.[1,12,13]

In this paper we describe two patients, one of which with a novel *AIFM1* mutation, presenting with distinct phenotypes associated with cerebellar ataxia, including previously undescribed status myoclonicus. In both patients, ataxia was partly improved with riboflavin supplementation, emphasizing the importance of reaching a molecular diagnosis in this clinically heterogeneous syndrome.

2. Material and methods

2.1. Ethical approval

The molecular studies were approved by the ethical committee of the Sheba Medical Center and the Israeli Ministry of Health, IRB number 7786-10. Written informed consent was obtained from all participants or their respective legal guardians.

2.2. Exome sequencing and bioinformatics

DNA was extracted from peripheral blood and trio whole-exome sequencing was performed at the Center for Human Genome Variation, Duke University School of Medicine, Durham, North Carolina, USA as previously described (Zhu, Petrovski et al., 2015). To capture the coding regions, the 65-Mb Illumina TruSeq Exome Enrichment Kit (Illumina, San Diego, CA) was used in patient 1 and the 64-Mb Roche NimbleGen SeqCap EZ Exome Library Kit (Roche NimbleGen, Madison, WI) was used in patient 2. The captured regions were sequenced using the HiSeq 2000 platform (Illumina Inc, San Diego, CA, USA). The resulting reads were aligned to the reference genome (GRCh37/hg19) using the Burrows-Wheeler Alignment (BWA-0.5.10).[14] Polymerase chain reaction (PCR) duplicates were removed using picard-tools-1.59 (http://picard.sourceforge.net). Genetic differences relative to the reference genome were called using UnifiedGenotyper of the Genome Analysis Toolkit (GATK-1.6–11)[15] and annotated using SnpEff-3.3 (Ensembl 73 database).[16]

Variants which were called less than 8X coverage and with a quality score of <20 were excluded. In terms of functional annotation, only protein-altering variants (stop gain/loss, start loss, frameshift, missense, splice-site) were included. The dbNSFP database was used to access the functional prediction of non-synonymous SNPs. We primarily focused on genotypes absent in control data sets including the dbSNP138, the 1000 Genomes Project, NHLBI GO Exome-sequencing Project (http://evs.gs.washington.edu/EVS/), the ExAc browser http://exac.broadinstitute.org, 240 in-house controls of different Israeli ethnic origins and the internal control cohort comprised of 3027 subjects enrolled in the Center for Human Genome Variation through Duke institutional review board-approved protocols. Among all heterozygous variants only de novo or compound heterozygous variants were kept. The available protein predicting datasets such as PolyPhen2[17], SIFT,[18] Mutation Assessor[19] Mutation Taster,[20] LRT[21] and FATHMM[22] were used to predict mutations deleteriousness.

Please cite this article in press as: Heimer G, et al., Mutations in *AIFM1* cause an X-linked childhood cerebellar ataxia partially responsive to riboflavin, European Journal of Paediatric Neurology (2017), http://dx.doi.org/10.1016/j.ejpn.2017.09.004