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Short communication

Ataxia and dysarthria due to an *ABCA2* variant: Extension of the phenotypic spectrum

Faiza Aslam[a], Sadaf Naz[a,*]

[a] School of Biological Sciences, University of the Punjab, Lahore, FA, Pakistan

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ABSTRACT

Introduction: Ataxias are heterogeneous disorders that are caused by variants in a large number of genes. The study was conducted to identify the molecular basis of a movement disorder in a consanguineous Pakistani family.

Methods: We performed clinical assessments and magnetic resonance imaging of the older of two siblings affected with a movement disorder. Molecular analyses included whole-exome sequencing in order to delineate the underlying pathology of the disorder. Segregation of variants with the phenotype was checked by Sanger sequencing.

Results: Symptoms of the two affected subjects were consistent with cerebellar ataxia with dysarthria. Magnetic resonance imaging did not reveal brain abnormalities. The levels of low density lipid proteins were elevated in blood samples of both affected individuals. Whole-exome sequencing data analyses identified a frameshift variant, c.4993delG:p.(Val1665TyrfsTer36) in *ABCA2* (NM_212533.2) which segregated with the disorder and was absent from all publicly available databases and ethnically matched controls. Although recessively inherited *ABCA2* variants have been reported in two patients who had intellectual disability with global developmental delays, our study demonstrates the role of an *ABCA2* variant in the pathogenesis of ataxia with dysarthria. The phenotype observed in our patients shows high concordance with that observed in *Abca2* knockout mice.

Conclusion: Our research links an *ABCA2* variant with a distinct form of ataxia with dysarthria in humans and demonstrates pleiotropic effects due to the gene mutation. The findings further delineate the importance of low density lipid metabolism and intracellular sterol trafficking in brain function.

1. Introduction

Cerebellar ataxias involve gait, balance and limb incoordination. Onset can be in childhood or adulthood. Ataxia is transmitted as an autosomal dominant, recessive, X-linked or a mitochondrial disorder. Extreme genetic and phenotypic heterogeneity of hereditary cerebellar ataxias makes precise diagnosis difficult. Mutations in many of the genes implicated in ataxia cause cerebellar degeneration accompanied by central and peripheral neurological signs, such as dysarthria [1]. Some of these encoded proteins play a critical role in the normal function of brain such as maintenance of synapse function and neurons structures. Others participate in DNA repair, or mitochondrial structure maintenance.

We report a biallelic frameshift mutation, c.4993delG:p.(Val1665TyrfsTer36) in *ABCA2,* in two members of a Pakistani family who presented ataxia, impaired limb coordination and dysarthria.

2. Materials and methods

This study was approved by Institutional Review Board of School of Biological Sciences, University of the Punjab, Lahore, Pakistan. Written informed consent was taken from all participating individuals or guardians in case of minor children. Guardians provided consent for online publication of the videos.

2.1. Family

A consanguineous family RDFA01, with two affected individuals suffering from a movement disorder was identified from Lahore. Clinical history was obtained which included age of onset, disease progression and other relevant information. Blood lipid profiling was completed for both affected individuals. Patients were videotaped according to a standard videotaping protocol for the assessment of their neurological condition [2].

* Corresponding author. School of Biological Sciences, University of the Punjab, Quaid-i-Azam Campus, Lahore, 54590, Pakistan.
E-mail addresses: faizaaslam.phd.sbs@pu.edu.pk (F. Aslam), naz.sbs@pu.edu.pk (S. Naz).

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2.2. Molecular analysis

Blood samples were collected from parents, normal sibling and patients. One child, VI:4 was eight months old, and could not be included in the study as her blood was not obtained even by a phlebotomist at a hospital. Genomic DNA was extracted according to a standard protocol [3]. DNA from one affected individual (VI:1) was subjected to whole-exome sequencing using Agilent SureSelect Human All Exon V6 capture library and illumina Hiseq 2000 sequencer with a coverage of 100X (Macrogen Inc, South Korea). Data was analyzed according to standard procedures [4]. Co-segregation of prioritized variants with the phenotype was checked by PCR amplification of respective exons from DNA of all participants and Sanger sequencing. The variant segregating with disease was similarly checked in 100 ethnically matched control samples.

RNA was extracted from whole blood samples of the family members on the day of sampling. Trizol (Molecular research center Inc., Cincinnati, OH, USA) was used to extract RNA by following the prescribed protocol. First strand cDNA synthesis was carried out from RNA using oligo dT or hexamer primers in separate reactions by using RevertAid™ premium first strand cDNA synthesis kit (Fermentas, Thermo Fisher Scientific Inc., Pittsburgh, PA, USA). Two sets of *ABCA2* cDNA specific primers were designed spanning exon-exon boundaries to amplify part of the gene using PCR (Supplementary Table 1).

3. Results

3.1. Clinical findings

Family RDFA01 (Fig. 1A) consists of two affected males. The 11 years old proband, VI:1, was born after normal delivery. He started walking after two years of age and exhibited a wide based ataxic and staggered gait with recurrent falls. These walking difficulties were mild in the beginning which became worse with the passage of time (Video 1). Lack of appendicular coordination was more severe on the left side. He had delayed cognitive, language and speech development with prominent dysarthria. His weight and height were reduced as compared to age and sex matched controls (Table 1). He manifested mild

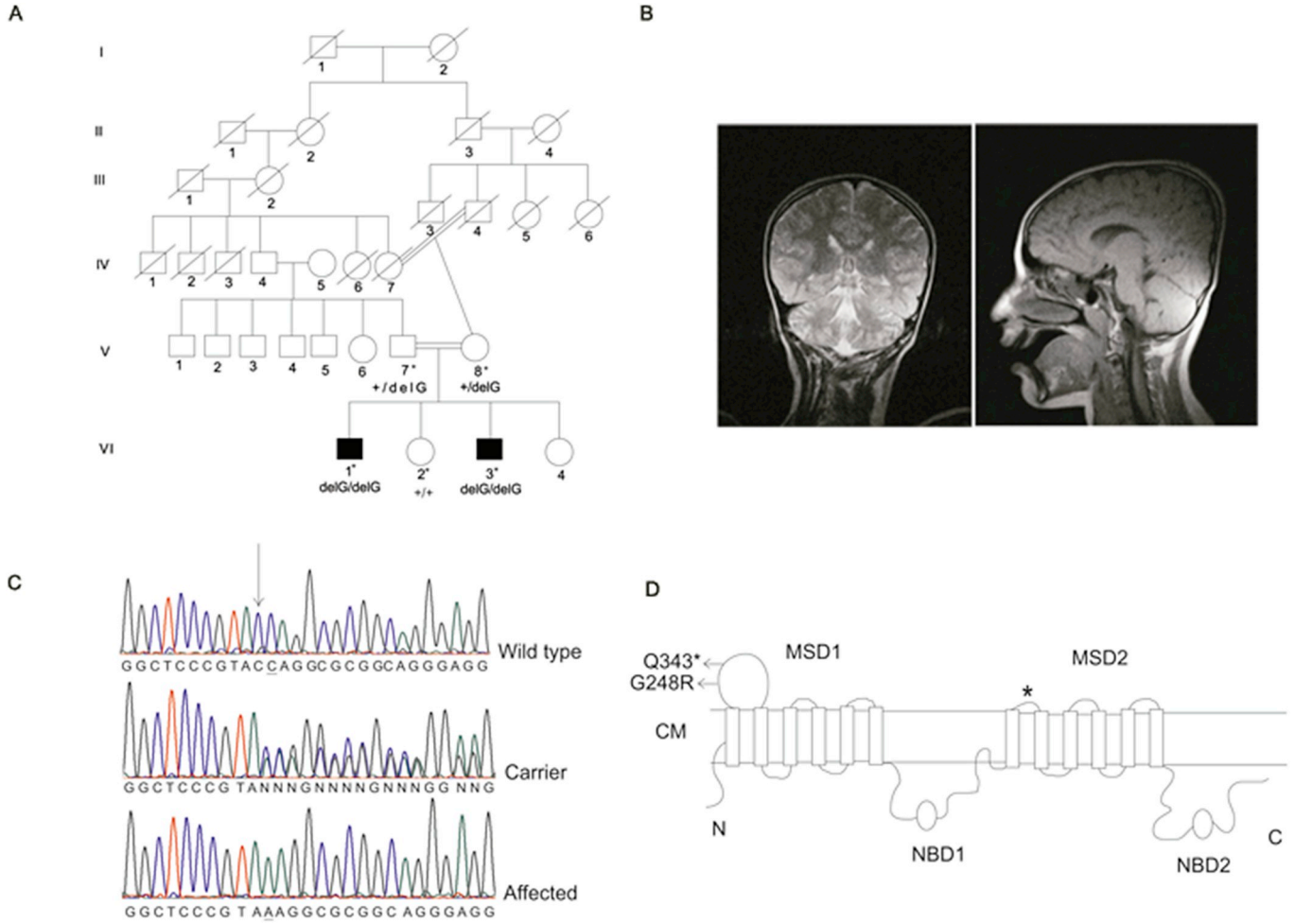

Fig. 1. Family RDFA01 with MRI data, variant analyses and ABCA2 structure A. Pedigree of RDFA01. Asterisks indicate individuals who participated in the study. DNA of individual VI:1 was used for whole-exome sequencing. Genotypes for *ABCA2* deletion variant for the participants are shown below their respective symbols.B. Brain MRI of affected individual # VI:1. Coronal and sagittal view of brain revealed no cerebellar and other lesions in the brain. Distribution of white and grey matter was also normal. C. Chromatograms of *ABCA2* for normal, carrier and affected individuals. Sequences of the reverse complement strands are shown in all three cases. Arrow indicates the base which is deleted from the *ABCA2* sequence. The affected individuals were homozygous for the deletion c.4993delG, and the transversion of an adjacent nucleotide, c.4992G > T, (Underlined). D. Diagrammatic representation of the ATP binding cassette transporter subfamily A member 2 (ABCA2) protein. ABCA2 transporter has two symmetric halves each with six transmembrane helices and one nucleotide binding domain. The transporter has two membrane spanning domains (MSD) and two nucleotide binding domains (NBD). Arrows at the beginning of the protein represent the variants described to date in ABCA2. Note that individuals due to previously described biallelic variants had intellectual disability and developmental delay. The p.(Met255Lys) variant associated with ALS in a sporadic case is monoallelic. Asterisk denotes the region of transporter, which will be affected due to the frameshift deletion identified in family RDFA01.

Table 1
Clinical details of patients with *ABCA2* variants.

Subject	VI:1 This study	VI:3 This study	Steinberg et al., 2015	Maddirevula S et al., 2018	
Current age	11 yrs	6 yrs	Not reported	10 yrs	13 yrs
Age at onset	2 yrs	2 yrs	Late onset	By birth	9 months
Sex	Male	Male	Male	Male	Female
Weight Kg (SD)	25 (−2.34)	18 (−1.12)	Not reported	33.1 (0.17)	37.9 (−1.03)
Height Inches (SD)	53 (−1.32)	42 (−1.32)	Not reported	140 (0.18)	131.5 (−3.21)
Movement	Delayed ambulation Ataxic gait	Delayed ambulation Ataxic gait	Not reported	Diffuse hypotonia No ataxia	Delayed ambulation No ataxia
Limb incoordination	Present	Present	Not reported	Not present	Not present
Speech	Dysarthria	Dysarthria	Not reported	Speech delay	Speech delay
Intellectual disability	Present (slight)	Absent	Not reported	Present	Present
Behavior	Aggressive	Normal	Not reported	Hyperactive	ADHD
Additional findings	High LDL	High LDL	Not reported	Dysmorphic features with prominent forehead	Microcephaly and internal rotation of hip muscles
MRI	Unremarkable	Not Assessed	Not reported	Unremarkable	Small pituitary gland
Variant	p.(Val1665TyrfsTer36) (Homozygous)	p.(Val1665TyrfsTer36) (Homozygous)	p.(Met255Lys) (Heterozygous)[a]	p.(Gly248ArgfsTer38) (Homozygous)	p.(Gln343Ter) (Homozygous)
Diagnosis	Ataxia with dysarthria	Ataxia with dysarthria	ALS	ID, DD and epilepsy	ID, DD and epilepsy

SD; standard deviations (SD), ADHD; Attention Deficit Hyperactivity Disorder, ALS; Amyotrophic Lateral Sclerosis, ID; Intellectual Disability, DD; Developmental Delay.

[a] The second ABCA2 variant, p.(Val1453Phe) reported by Steinberg et al., 2015 in the same patient, has a minor allele frequency of 0.01 in 1000 genomes. Therefore, due to its high frequency, this second variant cannot be considered as pathogenic.

intellectual disability and displayed aggressive behavior by becoming angry when asked to perform any activity. His brain MRI indicated normal spinal cord and cerebra. Distribution of brain white and grey matter was also normal (Fig. 1B, Supplementary Fig. 1). The disorder in both affected individuals was diagnosed as cerebellar ataxia by the neurologists. However, in absence of further testing, sensory origin of the ataxia cannot be excluded.

Supplementary video related to this article can be found at https://doi.org/10.1016/j.parkreldis.2019.04.017.

The second affected individual VI:3 was a six years old child, born by Caesarean section. He displayed the same phenotype as his brother, though with reduced severity. He walked with a wide based ataxic gait but had reduced frequency of falls (Video 2). He also had appendicular coordination problems which were more severe on the left side of the body. His cognition was normal and he attended school. He exhibited mild dysarthria. Both individuals had higher than average values of low density lipoproteins, LDL (126 and 130 mg/dl-normal range < 100 mg/dl) while cholesterol, triglycerides, high density lipoproteins and very low density lipoproteins were within normal range (Supplementary Table 2). Patients did not have dysmorphic features.

Supplementary video related to this article can be found at https://doi.org/10.1016/j.parkreldis.2019.04.017.

The parents V:7 and V:8, and the ten year old sibling VI:2, of the two affected individuals, did not have movement disorders or intellectual disability. The eight months old child VI:4 could not be evaluated due to her young age.

3.2. Data analysis

Filtration of the exome data yielded 50 variants for analysis (Supplementary Table 3). Examination of the data reduced the variants to three (Supplementary Table 4) after eliminating those which were homozygous or hemizygous in a high number of unaffected controls, were predicted to be benign by multiple software, or the amino acids were not conserved in vertebrates. The *ABCA2* (OMIM:600047) variant c.4993delG:p.(Val1665TyrfsTer36) (NM_212533.2), segregated in the family with the disorder (Fig. 1A), while the others did not (Fig. 1C, Supplementary Table 4). Affected individuals were homozygous for the variant, obligate carriers were heterozygous while one individual was homozygous for the wild type allele (Fig. 1C).

The variant affected an exon present in all *ABCA2* isoforms. This variant was absent from public population databases including gnomAD, ExAC, 1000 genomes, Exome variant server and from 200 ethnically matched chromosomes. Products specific to *ABCA2* were obtained from cDNA library prepared from RNA extracted from patients' blood samples (Supplementary Fig. 2A). Sanger sequencing of PCR products confirmed the presence of the variant in the cDNA (Supplementary Fig. 2B).

4. Discussion

ABCA2 is expressed in brain and a few other tissues [5]. Impaired transport of lipids by *ABCA* family members is associated with many disorders. For example, *ABCA1* variants cause Tangier disease (OMIM:600046) and *ABCA4* has pathological role in Stargardt disease (OMIM:601691).

We describe a homozygous frameshift variant in *ABCA2* as cause of staggered gait ataxia, limb incoordination, and dysarthria in humans and elevated LDL levels. Previously, a monoallelic variant of *ABCA2* was described in a sporadic case with amyotrophic lateral sclerosis [6], while biallelic variants have been reported in two patients with intellectual disability, developmental delay, speech delay and epilepsy [7]. In the latter study, dysmorphic features or microcephaly with few other symptoms were present in the two patients (Table 1) (Dr. Satish Maddirevula, Dr. Fowzan Alkuraya, personal communication). This lack of concordance of phenotypes in humans with *ABCA2* variants suggests pleiotropy. However, it could also indicate that perhaps c.4993delG:p.(Val1665TyrfsTer36) variant is not the cause of the disorder, and is in linkage disequilibrium with the disease causing mutation.

Two independent *Abca2* knockout mice models have been generated. One of these model mice had hyperactivity, a lower body weight and exhibited shaking motion of the body, ears and tail while walking in microisolation cage as well. They also had non-ataxic tremors. The mice were unable to counterbalance their weight on a balance beam [8]. Hyperactivity observed in this case was more pronounced in female mice than in males. The second group of *Abca2* knockout mice exhibited shaking behavior, and had reduced body weights. They were sensitive to external factors, being frightened by sudden sound, and body touch [9]. This study demonstrated that *Abca2* absence affects intracellular lipid metabolism in brain.

In one of the *Abca2* mouse model, altered myelin compaction in

cerebellum and increased myelin membrane thickness in spinal cord was observed with the help of transmission electron microscope (TEM). However, these pathologies were not observed in the other mouse model [9]. The brain MRI of patient VI:1 in our family also revealed appropriate distribution of grey and white matter. This difference is perhaps due to better resolution of TEM as compared to MRI.

In our study, both patients had staggered ataxic gait, limb incoordination and dysarthria, with or without reduced body weight or aggressive behavior. This difference in phenotype between the two siblings may be due to progressive nature of the disease. Other phenotypes such as dysarthria and limb incoordination observed in our study which were not present in *Abca2* knockout mice, can be due to inherent differences between humans and mice.

These studies in mice and humans show that, the effects of variants affecting ABCA2 can be diverse and may result in different clinical presentation of the disease. The *Abca2* mice models have complete loss of the gene, so differences of phenotype among mice and our patients may be due to the complete loss of *Abca2* in mice and partial loss of function of *ABCA2* in humans. In this regard it can be noted that transcript specific to *ABCA2* with frameshift variant could be detected in the patients' blood samples. Since the mutation c.4993delG:p.(Val1665TyrfsTer36) was in exon 31 of the 49 *ABCA2* exon gene, we hypothesized that nonsense mediated decay (NMD) will occur and consequently no *ABCA2* mRNA will be recovered. However, the amplification of *ABCA2* specific product from patient blood samples suggests that mRNA escapes NMD, at least in blood. If translated, the mutant protein will be missing half of the 2436 amino acids. ABCA2 has two symmetric halves (Fig. 1D). One half of the transporter consists of six transmembrane domains and one ATP binding cassette [10]. The c.4993delG:p.(Val1665TyrfsTer36) variant of *ABCA2,* disrupts the second half of the transporter (Fig. 1-D). The three previously reported variants are predicted to affect the first half of the protein.

Our description of ataxia with dysarthria, together with previous description of defects due to *ABCA2* variants supports pleiotropic effects of this gene. Continued research will determine the contribution of *ABCA2* variants to ataxia and other symptoms in different patients. It will also indicate whether the different phenotypes are due to type of mutations or location within the protein. This will be helpful in determining genotype-phenotype correlations.

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Ethical approval

This study is carried out according to ethical standards of Helsinki and is approved by Institutional Review Board of School of Biological Sciences, University of the Punjab, Lahore. Written informed consent was obtained from all the participants.

Disclosure of conflict of interest

SN reports no conflict of interest.
FA reports no conflict of interest.

Contributions of authors

SN designed the study, helped with the analyses and wrote the manuscript.

FA carried out the experimental work, data analyses and wrote the manuscript.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.parkreldis.2019.04.017.

References

[1] N. Mendoza-Ferreira, M. Coutelier, E. Janzen, S. Hosseinibarkooie, H. Löhr, S. Schneider, J. Milbradt, M. Karakaya, M. Riessland, C. Pichlo, Biallelic CHP1 mutation causes human autosomal recessive ataxia by impairing NHE1 function, Neurol. Genet. 4 (1) (2018) e209.
[2] C. Moskowitz, C. Stewart, Proposed guidelines for videotaping individuals with movement disorders, J. Neurosci. Nurs. 23 (3) (1991) 191–193.
[3] S. Miller, D. Dykes, H. Polesky, A simple salting out procedure for extracting DNA from human nucleated cells, Nucleic Acids Res. 16 (3) (1988) 1215.
[4] H. Tariq, S. Naz, TFG associated hereditary spastic paraplegia: an addition to the phenotypic spectrum, Neurogenetics 18 (2) (2017) 105–109.
[5] W.E. Kaminski, A. Piehler, K. Püllmann, M. Porsch-Özcürümez, C. Duong, G.M. Bared, C. Büchler, G. Schmitz, Complete coding sequence, promoter region, and genomic structure of the human ABCA2 gene and evidence for sterol-dependent regulation in macrophages, Biochem. Biophys. Res. Commun. 281 (1) (2001) 249–258.
[6] K.M. Steinberg, B. Yu, D.C. Koboldt, E.R. Mardis, R. Pamphlett, Exome sequencing of case-unaffected-parents trios reveals recessive and de novo genetic variants in sporadic ALS, Sci. Rep. 5 (2015) 9124.
[7] S. Maddirevula, F. Alzahrani, M. Al-Owain, M.A. Al Muhaizea, H.R. Kayyali, A. AlHashem, Z. Rahbeeni, M. Al-Otaibi, H.I. Alzaidan, A. Balobaid, Autozygome and high throughput confirmation of disease genes candidacy, Genet. Med. (2018) 1.
[8] J.T. Mack, V. Beljanski, A.M. Soulika, D.M. Townsend, C.B. Brown, W. Davis, K.D. Tew, "Skittish" Abca2 knockout mice display tremor, hyperactivity, and abnormal myelin ultrastructure in the central nervous system, Mol. Cell Biol. 27 (1) (2007) 44–53.
[9] H. Sakai, Y. Tanaka, M. Tanaka, N. Ban, K. Yamada, Y. Matsumura, D. Watanabe, M. Sasaki, T. Kita, N. Inagaki, ABCA2 deficiency results in abnormal sphingolipid metabolism in mouse brain, J. Biol. Chem. 282 (27) (2007) 19692–19699.
[10] N. Aberuyi, S. Rahgozar, A. Moafi, The role of ATP-binding cassette transporter A2 in childhood acute lymphoblastic leukemia multidrug resistance, Iran, J. Ped. Hematol. Oncol. 4 (3) (2014) 118–126.