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SLC52A2 mutations cause SCABD2 phenotype: A second report

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ABSTRACT

Introduction: Autosomal recessive cerebellar ataxias (ARCAs) are a large group of neurodegenerative disorders that manifest mainly in children and young adults. Most ARCAs are heterogeneous with respect to age at onset, severity of disease progression, and frequency of extracerebellar and systemic signs.
Methods: The phenotype of a consanguineous Iranian family was characterized using clinical testing and pedigree analysis. Whole-exome sequencing was used to identify the disease-causing gene in this family.
Results and conclusion: Using whole exome sequencing (WES), a novel missense mutation in SLC52A2 gene is reported in a consanguineous Iranian family with progressive severe hearing loss, optic atrophy and ataxia. This is the second report of the genotype-phenotype correlation between this syndrome named spinocerebellar ataxia with blindness and deafness type 2 (SCABD2) and SLC52A2 gene.

1. Introduction

The hereditary ataxias represent a mixed group of conditions that, according to their mode of inheritance, can be classified into autosomal dominant, autosomal recessive, X-linked, and mitochondrial ataxias [1,2]. Autosomal recessive cerebellar ataxias (ARCAs) correspond to a heterogeneous group of inherited neurodegenerative disorders which manifest mainly in children and young adults so the signs and symptoms of the disorder first appear in early to mid-adulthood. They affect both the central and peripheral nervous system, and in some cases, other systems and organs, and range from isolated ataxia to syndromic forms. Based on a classification of ARCA, there are three groups: ARCA with pure sensory neuropathy, ARCA with sensorimotor axonal neuropathy, and ARCA without neuropathy. Malfunctions of five main mechanisms have been proposed as pathogenic: damaged DNA repair mechanisms, misfolded proteins and degradation, channelopathies, and mitochondrial and metabolic defects. Although, to date, a growing list of rare molecular defects has been identified associated with ARCA, the cause of the disease is unknown in many of the affected families [3,4].

In 1974, van Bogaert and Martin as well as Spoendlin described a recessively inherited spinocerebellar ataxia with optic and cochlear degeneration leading to blindness and deafness which does not fit in any classification [5,6]. In 2000, Bomont et al. concluded that the same disorder was present in a consanguineous Arab Israeli family in whom the uncle and niece were affected by early-onset recessive ataxia. They subsequently developed hearing impairment and optic atrophy so the authors named the syndrome spinocerebellar ataxia autosomal recessive 3 [7]. More recently, using whole exome sequencing (WES), the *SLC52A2* gene (OMIM#607882) was identified as responsible for this type of ataxia and this syndrome was named spinocerebellar ataxia with blindness and deafness type 2 (SCABD2) [8]. This syndrome is rare and affects less than 1 in 1 000 000 people worldwide and its onset has been reported to be in childhood [9]. *SLC52A2* produces a 1900-bp transcript, four coding exons of 445 amino acids and since riboflavin metabolites are critical components of the mitochondrial electron transport chain (ETC), it is assumed that reduced riboflavin transport would result in impaired mitochondrial activity.

Mutations in *SLC52A2* gene can also cause Brown-Vialetto-Van Laere Syndrome (BVVLS) (OMIM#211530). This is an autosomal recessive progressive neurologic disorder characterized by early childhood onset of sensorineural deafness, bulbar dysfunction, and severe diffuse muscle weakness of which ataxia is presented as one of the potential additional features [10].

In this report, using WES, we describe a consanguineous Iranian family with progressive severe hearing loss, optic atrophy and ataxia with a novel missense mutation in *SLC52A2* gene. To the best of our knowledge, this is only the second report to describe an association between SCABD2 and *SLC52A2* gene with genotype-phenotype correlation.

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Table 1
Clinical findings for the three affected individuals in the family.

Clinical findings	Affected individuals		
	III-2	III-3	III-4
Age at examination, years	35	40	27
Age of onset, years	9	8	9
Hearing loss	Postlingual Severe Progressive	Postlingual Severe Progressive	Postlingual Severe Progressive
Optic atrophy	+	+	+
Visual loss	+	+	+
Facial muscle weakness	–	–	–
Dysphagia	–	–	–
Tongue fasciculations	–	–	–
Neck muscle weakness	–	–	–
Kyphoscoliosis	–	–	–
Respiratory insufficiency	–	–	–
Upper limb muscle weakness	–	–	–
Lower limb weakness	–	–	–
Hypotonia	–	–	–
Muscle atrophy	–	–	–
Cranial nerve palsies	–	–	–
Ataxia	+	+	+
Ambulation	–	–	–
Axonal sensorimotor neuropathy	–	–	–
Sensory impairment	–	–	–
Cognition	Preserved	Preserved	Preserved
Seizure	–	–	+
Progression	+	+	+
Passed away	–	+	–

Table 2
Details of coverage depth for the patient who underwent WES.

Average depth (X)	Coverage with depth > 10X (%)	Coverage with depth > 20X (%)	Coverage with depth > 30X (%)
68	96.5	92.1	84.6

2. Materials and methods

2.1. Subjects and clinical evaluation

The family evaluated in this study included three affected individuals and six unaffected members with first cousins parents. A clinical description of the three affected members is shown in Table 1. The patients III: 2, III: 3 and III: 4 were all born at term with unremarkable pregnancies and neonatal periods. They all had normal neurodevelopment during infancy. Their parents reported that they considered their hearing was impaired at age 8–9 years. They developed visual loss at age 13–15 years. Patient III-4 had a history of recurrent seizures which were controlled by medication. All three patients developed an unbalanced gait at age 14–16 years. They had normal facial muscle movement. There was no history of dysphagia, respiratory insufficiency, hypotonia or upper or lower muscle weakness. All three had normal cognition and III-4 recently showed restlessness. At 35 (III-2), 40 (III-3) and 27 (III-4) years, they had severe progressive postlingual hearing impairment (Table 1). They could build multi-word phrases with normal articulation. Fundoscopic examination showed optic atrophy in all affected members (Table 1). From neurological examinations, they had normal extraocular movement, normal sensation in their upper and lower extremities and there was no muscle weakness or atrophy in the neck or upper and lower extremities. They did not show any kyphosis or scoliosis. Cognitive evaluation at age 35 (III-2), 40 (III-3) and 27 (III-4) years (WAIS-IV) showed Intelligence Quotients (IQs) in the normal range (over 90).

2.2. Whole exome sequencing

Approximately 5 ml of blood was collected from the affected individuals, healthy siblings and parents after obtaining signed informed consent, and approval from the Ethics Committee of the University of Social Welfare and Rehabilitation Sciences, Tehran, Iran. Genomic DNA was isolated using the salting out method. About 50 ng of high-quality DNA from patient III-3 was used for whole exome sample preparation using the Agilent SureSelect Human All Exon Kit (V5) according to the manufacturer's instructions. The exome was sequenced using the Illumina Hiseq2500 sequencing system according to the manufacturer's protocols. Paired-end reads of 150 bases were generated, which were quality and adapter trimmed at a Phred quality score of 20. The average coverage depth and percentage of targeted coding regions covered by 10, 20 or 30 reads are shown in Table 2. Sequence alignment and variant calling were performed against the reference human genome (UCSC hg19) using Burrows-Wheeler Aligner (BWA) software. The Genome Analysis Toolkit (GATK) and Annovar tool were then used to recalibrate base quality scores, call and filter the variants, and annotate called variants, respectively. Mutations fitting autosomal recessive mode were filtered against some databases such as Exome Variant Server (ESP), dbSNP, 1000 Genome Project, Exome Aggregation Consortium (ExAC), and Kaviar. The pathogenicity of variants was predicted using bioinformatics databases such as SIFT, Polyphen, Combined Annotation Dependent Depletion (CADD), and MutationTaster. Thereafter, the final candidate variants in this family were validated by ethnic-specific minor allele frequency (MAF) filtering using 600 Iranian control chromosomes from 300 normal hearing control subjects. Flowchart of tertiary analysis of WES data in the family studied has been shown in Fig. 3.

In addition, we used the ACMG (American College of Medical Genetics and Genomics) guidelines to interpret the selected variant and predict its pathogenicity.

Finally, to exclude technical artifacts and conduct segregation analysis, the candidate variant was confirmed by Sanger sequencing, which was performed using the ABI PRISM Big Dye Terminator Cycle Sequencing Kit and ABI PRISM 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). The primers used for sequencing of detected variant were as follows: forward: ACCTCGCTTCTTACGGT CAT, reverse: GTGAGCGAGCAGAATGTCAG.

3. Results

Whole exome sequencing (WES) of patient III-3 in the studied family revealed a homozygous novel missense mutation (c.T973G, p.C325G) in exon 3 of *SLC52A2* gene and this was confirmed in the family by Sanger sequencing. This variation, which has not been reported in databases and predicted as pathogenic by bioinformatics software, was compatible with the phenotype of the family. In addition, this variant is predicted to be likely pathogenic based on ACMG guidelines. Information and in silico prediction of the variant found in *SLC52A2* gene are presented in Table 3.

Table 3
Information and in silico prediction of variant found in *SLC52A2* gene.

Chr	Transcript	HGVS c.	HGVS p.	cytoBand	SIFT	Polyphen2	Mutation Taster	LR	CADD Score	Mutation Assessor	PROVEAN	MetaSVM	MetaLR	GERP++
8: 145584125	NM_001253815	c.T973G	p.C325	8q24.3	D	P	D	D	20.6	M	D	D	D	3.54

(A)

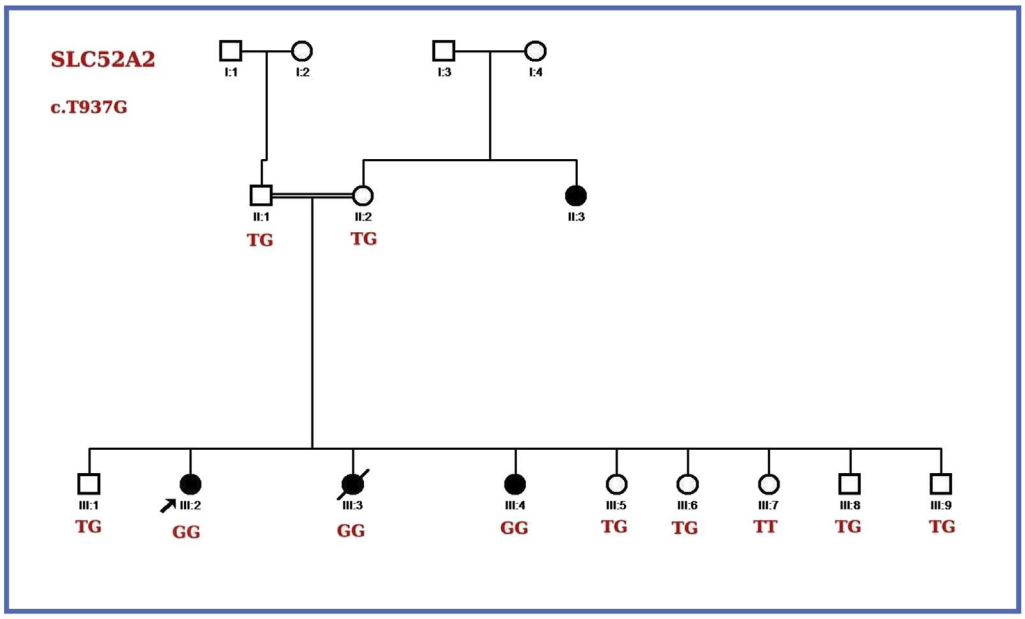
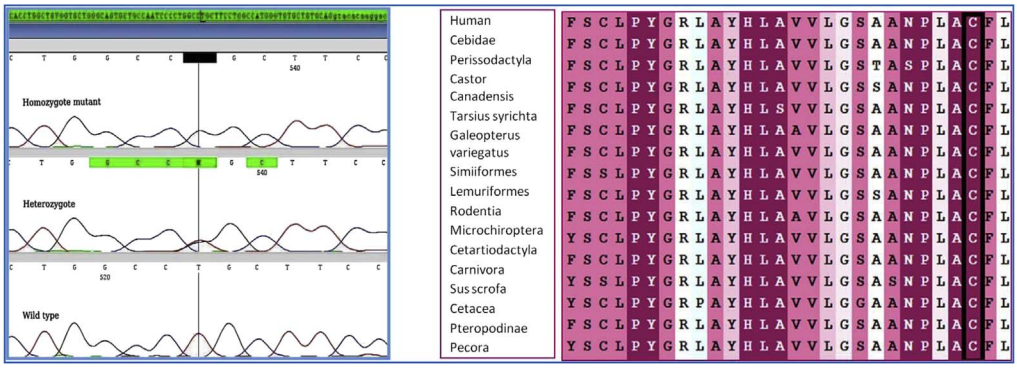


Fig. 1. A) Pedigree of the family, B) Electropherogram of c.T973G in *SLC52A2* gene in the family, C) Conservation of p.Cys325 amino acid residues in *SLC52A2* across species.

(B) (C)



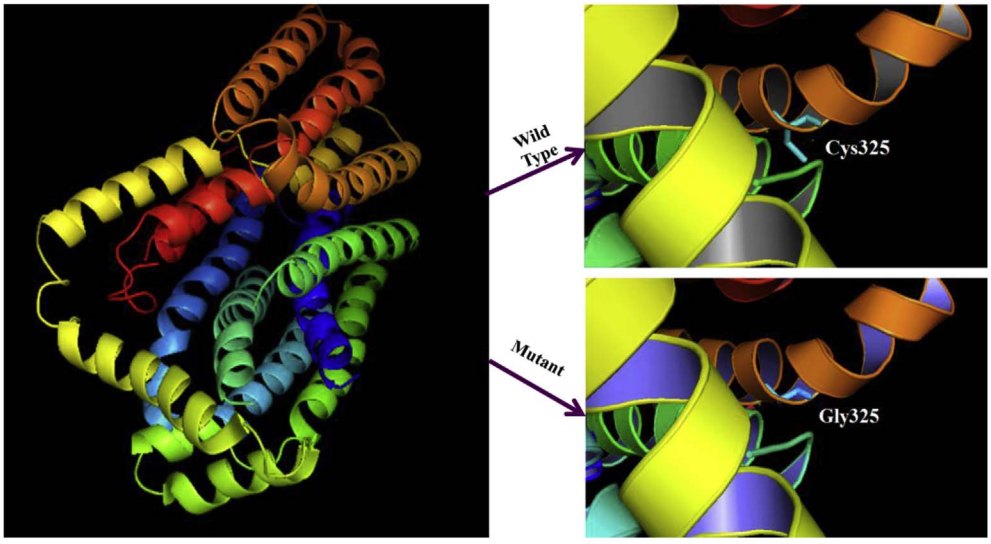


Fig. 2. 3D modeling of protein, wild type and mutant residue using Pymol.

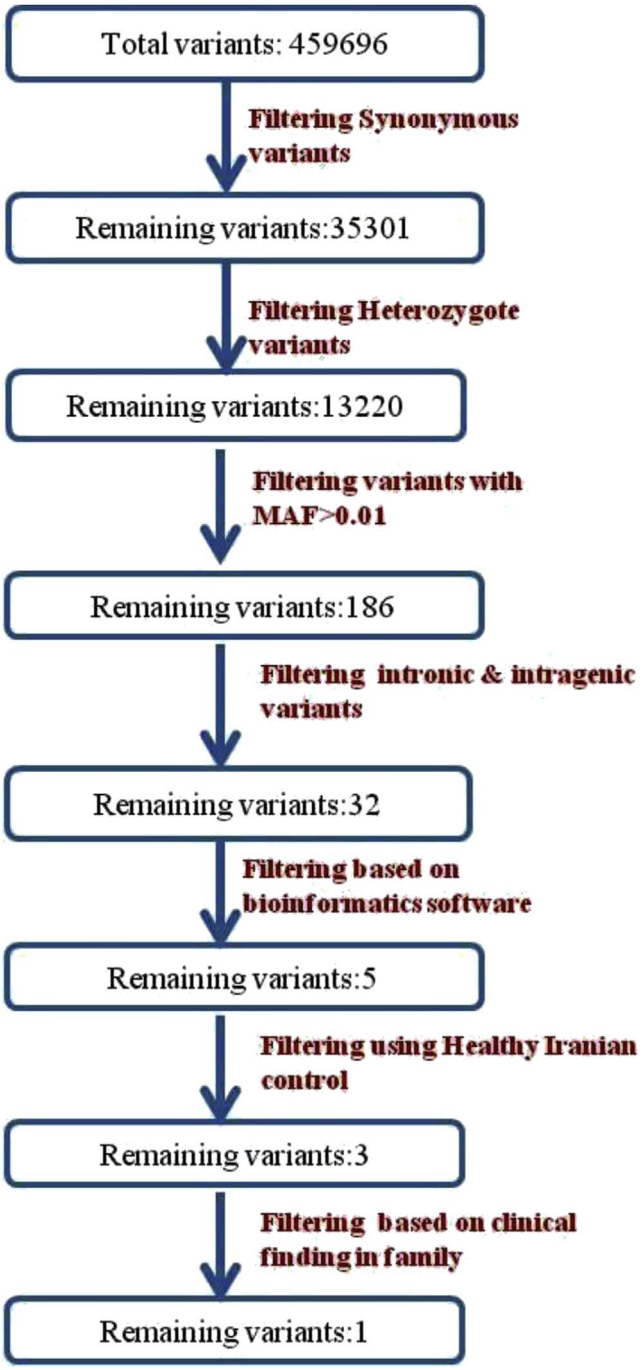


Fig. 3. Flowchart of tertiary analysis of WES data in the family studied.

Using the SOSUI and TMHMM programs, it was predicted that *SLC52A2* protein has 11 putative membrane domains of which the variation detected in this study is located at the eighth transmembrane domain. The p.C325G residue lies in a highly conserved region in exon 3 (Fig. 1C). We used Pymol to perform 3D modeling of wild-type and mutant amino acid in protein structure. The mutant residue is smaller than the wild-type residue and so this size difference can affect the contacts with the lipid membrane [11] (Fig. 2). On the other hand, the wild-type residue is more hydrophobic than the mutant residue and the differences in hydrophobicity can affect the hydrophobic interactions with the membrane lipids. It should be noted that the Glycines are very flexible and can disturb the required rigidity of the protein at this position.

4. Discussion

SLC52A2 belongs to a family of genes encoding for mitochondrial riboflavin. In humans, riboflavin must be obtained by intestinal absorption since it cannot be synthesized by the body. The water-soluble vitamin riboflavin is processed to form the coenzymes flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD) which function as cofactors for a number of redox enzymes and play essential roles in the transfer of electrons in biological oxidation-reduction cycles [12,13].

In 2000, Bomont et al. [7] considered an Arab Israeli family with two affected individuals in the pedigree having early-onset recessive ataxia, hearing impairment and optic atrophy using homozygosity mapping. They demonstrated a linkage over a 17-cM region at 6p21–p23 with a logarithm of odds (LOD) score of 3.25 and suggested a locus for ataxia; however, they were not able to identify the causal gene in this interval. By whole exome sequencing of the same family, Guissart et al. [8] revealed a homozygous missense mutation (p.Gly306Arg) in *SLC52A2*, located in 8qter. They inferred that the false linkage result was perhaps due to the high recombination rate of telomeric regions and the absence of 8qter polymorphic markers in the initial Linkage Mapping Set utilized for whole-genome analysis. They also found a novel missense mutation in *SLC52A2* gene (p.Pro134Leu) in an independent family with exactly the same phenotype, and so they named the syndrome SCABD2, which belongs to the group of cerebellar ataxias with sensorimotor neuropathy [7,8].

SCABD2 is allelic to Brown-Vialleto-Van Laere syndrome type 2 (BVVLS2), which is also caused by a mutation in *SLC52A2*. It has been suggested that the overlap between BVVLS2 syndrome and SCABD type 2 is through partial loss of function in mitochondria. Brown-Vialleto-Van Laere syndrome, which belongs to a spectrum of diseases caused by riboflavin transporter deficiencies, is a neurodegenerative disorder characterized by sensorineural deafness, respiratory difficulty, pontobulbar palsy and muscle weakness due to the involvement of cranial nerves VII, IX and XII. The genes responsible for this syndrome are *SLC52A2* (BVVL type 2) and *SLC52A3* (BVVL type 3). It has been demonstrated that there is a genotype-phenotype correlation between *SLC52A2*, *SLC52A3* mutations and clinical phenotypes of patients with Brown-Vialleto-Van Laere syndrome in which ataxia was presented as one of the potential additional features of individuals with *SLC52A2* gene mutation [14,15].

It has been shown that, in most people with BVVL syndrome, riboflavin treatment (high-dose oral supplementation of riboflavin between 10 mg and 50 mg/kg per day) can normalize the metabolic abnormalities and lead to clinical stabilization and improvement. Because oral riboflavin supplementation is effective (and possibly lifesaving) and it is clear that earlier initiation of therapy may lead to a better prognosis, supplementation should begin as soon as a riboflavin transporter deficiency neuronopathy is suspected. With regard to this, identifying patients with ataxia as a result of *SLC52A2* mutations will be important and can be ameliorated by riboflavin supplementation [16–18].

In conclusion, in our ongoing study to find the genetic basis of hearing loss in Iran using targeted and whole exome sequencing, we discovered a family with phenotypic presentation of hearing loss, vision loss and ataxia. Whole exome sequencing in this family led to the identification of a novel homozygous c.T973G missense mutation (p.Cys325Gly) in the riboflavin transporter *SLC52A2* gene. This variation, which has not been reported in any public databases, was predicted to be pathogenic by different bioinformatics software and is likely pathogenic using the ACMG scoring guidelines. It was also compatible with the phenotypic presentation in the family, strongly suggesting that it is a disease-causing mutation in this family.

This is the second report of an association between *SLC52A2* gene and SCABD2 syndrome and confirms that various mutations in different parts of the protein can cause an overlapping or distinct phenotype. It

also emphasizes once again that, in rare diseases, next-generation sequencing provides a valuable differential diagnostic tool indicating marked genetic and phenotypic heterogeneity due to partial loss of function of the mutated genes and also helps to initiate the proper treatment.

Conflicts of interest

None.

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