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Novel Homozygous Mutation in the MYO15A Gene in Autosomal Recessive Hearing Loss

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Abstract

Background: Hearing loss is one of the most common sensory disorders, which can be syndromic and non-syndromic. So far, 61 genes and more than 100 genetic loci have been identified in ARNSHL.
Materials and Methods: In this case report study, in order to verify the ARNSHL in a patient with several clinical symptoms and study the variations of the MYO15A gene for the first time in Southwest Iran, the MYO15A gene was entirely sequenced. Coding exons analysis of MYO15A gene was performed by exon direct sequencing.
Results: A novel homozygous missense mutation, c.9698T > G in exon 59 of the MYO15A gene was founded by Molecular genetic testing in the patient.
Conclusions: This novel mutation results in substituting a Leusin for an Arginin (p.L3233A). It seems that this change is predicted to affect the function of the myosin XVa protein negatively, maybe by disturbing its interaction with whirlin.

Keywords: Hearing Loss, MYO15A, Novel Mutation, Sequencing

1. Introduction

Hearing loss is one of the most common sensory disorders that can significantly affected quality of life [1]. Syndromic and non-syndromic hearing loss display in autosomal dominant, autosomal recessive, Y-linked, X-linked or mitochondrial pattern of inheritance [2]. Autosomal recessive nonsyndromic hearing loss (ARNSHL) accounts for up to 80% of cases of NSHL [3]. To date, 61 genes and more than 100 genetic loci have been identified in ARNSHL (http://hereditaryhearingloss.org/).

The most frequently genes involved in ARNSHL are those encoding myosin XVA (MYO15A, MIM# 602666), gap junction protein beta 2 (GJB2, MIM# 121011), solute carrier family 26 (anion exchanger) member 4 (SLC26A4, MIM# 605646), transmembrane channel- like 1 (TMC1, MIM# 606706), otoferlin (OTOF, MIM# 603681) and cadherin-related 23 (CDH23, MIM#605516), each of which has been contained more than 20 various mutations that most of them have been detected in consanguineous families [4]. In present study, we report a case of novel mutation discovered from the direct mutation screening of all exons in the MYO15A gene in an Iranian patient with hearing loss disorder (HLD).

2. Case Presentation

The patient was a 24-years-old male, the only-child of Iranian consanguineous couple (Figure 1A). He was diagnosed with congenital hearing loss and had no dysmorphic features. There was no significant history of hearing loss in this family and no history of systemic disease in the patient.

2.1. Molecular Analysis

Genomic DNA was extracted from peripheral leukocytes of the patient by the standard salting out protocol, and the PCR was conducted under the following conditions: 200 μM deoxyribonucleotide triphosphates (dNTPs), 100 ng genomic DNA, 2.5 units supertaq polymerase, 1.5 mm MgCl, and 25 pmoL each primer (Table 1). Amplification Carried out in 25 μL volumes and 35 cycles: 94°C for 1 minute, 64°C for 35 seconds and 72°C for 45 seconds. Direct sequencing of the 66 exons performed by using the big dye terminator cycle sequencing ready reaction kit on an ABI Prism 3700 automated genetic analyzer. Finally, the sequencing reactions were carried out and the sequences were compared to the reported gene sequence using the BLASTN program.

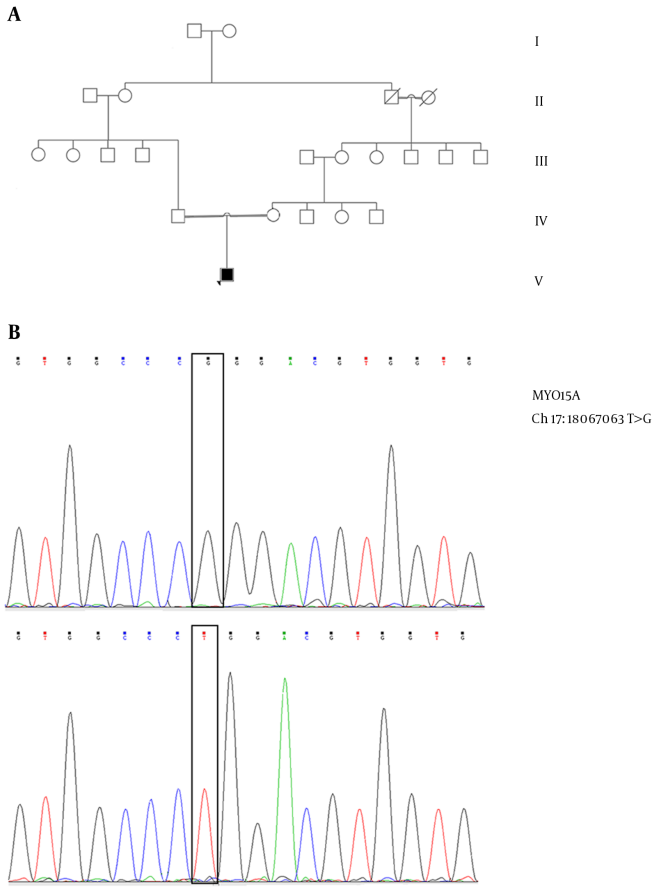

Figure 1. A, Pedigree of the family; B, The result of genetic sequencing, showing the homozygous missense mutation CTG > CGG in exon 59 of the patient's MYO15A gene

The effect of Candidate variant in protein structure and phylogenetic conservation was predicted by using bioinformatic tools like PolyPhen-2 (PolymorphismPhenotyping v2).

3. Results

Sequencing analysis of the patient, after comparison with MYO15A reference sequence in 1000 Genomes database, demonstrated a novel mutation, a homozygous missense mutation, c.9698T > G in exon 59 of the MYO15A gene (Figure 1B). The c.9698T > G mutation is novel and has not been previously described in HLD.

The novel homozygous missense mutation was predicted to be possibly damaging by in silico prediction of the recognized variant, Polyphen 2 (probably damaging, score 1.00).

4. Discussion

We analyzed an affected person with HLD with PCR and direct sequencing of coding exons of the MYO15A gene. As a result we identified a genetic variant of MYO15A in ARNSHL patient. The mutation identified in our patient involved a novel homozygous mutation, c.9698T > G in exon 59, which results in substituting a Leusin to an Arginin (p.L3233A) in the ferm domain and tail region of the Myosin protein. So, this exchange amino acid results in alter a nonpolar amino acid to polar positively charged that can modify interaction of tail region of myosin with membranous compartments and change its movement to actin filaments. This change is predicted to negatively affect the usual function of the myosin XVa protein by in silico. Overall, it seems that this amino acid has an important role in the myosin-XV protein, and mutation at this site results in pathogenicity and deafness.

MYO15A has 66 exons and its coding protein, myosin XVa, has a critical role in formation of stereocilia in hair

cells of the cochlea [5]. Myosin XVa in the organ of Corti is localized completely at the tips of stereocilia and is anactin-activated ATPase that uses the hydrolysis of ATP to move on actin filaments. The tip of a stereocilium is one of the proposed sites of mechano-electrical transduction and the site of stereocilia growth [6]. Myosin XVa is required for proper function and formation of the mechanotransduction machinery. All myosins are composed of one or two heavy chains and several light chain. The tails of the myosins presumed to bind to membranous compartments, which would be moved relative to actin filaments [7].

Twenty-nine mutations have been described in MYO15A in HGMD (http:// www.hgmd.org).

In summary, this is the first case with hearing loss in south-west of Iran confirmed by genetic analysis involving a novel MYO15A gene mutation. Further studies are required to understand the structural and functional changes of proteins involved in this disorder and their relations with phenotypic spectrum. Genotype-phenotype relations of MYO15A mutations and degree of hearing loss suggest that mutations in all 66 exons cause intense deafness.

Footnotes

Authors' Contribution: The first two authors contributed equally to this work. All authors had equal role in design, work, statistical analysis and manuscript writing.

Conflict of interest: The authors declare no conflict of interest.

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Table 1. Primers used for amplification of the MYO15A gene

Exon	Forward Primers (5' - 3')	Reverse Primer (5' - 3')	Amplicon Size, bp
2	Multiple - available upon request		
3	ATG ACC AAG CCA GGG GTC	CTC TGG CTG GGA GGG TG	223
4	GAC CCA TGC CAG AAC CAG	AGA AAT CTG TGC GTC CCA CC	204
5	ATC TGT CCG GAT GGA AAC AG	TCT GAC TCA TGG CTC AGG TG	311
6 - 7	GGG AGG TGT GGG AGC TTA G	TCG GGA GTA CAT GAG GTG TG	499
8	TCC TGG AGA GAG TGG TGG TC	CTA GGA CAG GCC TTT GGA TG	239
9 - 10	GGG TGT CCC CAG CTA TGC	TAT CTG TAC CTC CCA CCC CG	435
11	GTT CTC ATC TGC AGC CCA CT	AAA CTC ACC CTC CCC AAA TC	365
12	CAA CTC AGG CCA CCA CAC TA	AAA ACA GGA ACA AGT GAT ATG TGC	381
13	GAC TAC TGG CAT GAG CCA CA	TGA CCC AGG GAC AGA GAG AG	335
14 - 15	GCT TTC CGG AGG CAG AG	GAG GGA GGC GAG ACC TTG	385
16	AGG GAA GGT AGG GGC AAA	CTG TCT CCA AGG AGG TCC AC	231
17	ATT CAA CAT GGG AGG GAG G	TGA GGA CAT GAG GCT GAG AG	269
18	ATA GTG AGG TTG CCA CCA GG	TCT CCA ACA GCT AGC AGC AC	262
19	TCC CTC CTA GGA TAG ACA GAG AG	AAG GCA GGC TGG GTG TG	212
20	TTC CTC CTC ATT TCG GTC TC	CAA GGT CAC ACA GCA TGG G	441
21-22	TTC CTC CTC ATT TCG GTC TC	CAA GGT CAC ACA GCA TGG G	441
23	TAG CAG ACA CCT CGG GTA GG	GAC TCA GTA GTT GTG GAC CCC	241
24	CTT AGT CCA GCC TCC TGG C	TTC AGG CGT GAC CTC TCC	297
25	AGG GCC TCT CTA CCT TTT GG	CTA AGT GCC CTT TCC CCT TC	219
26 - 28	GTG CCG GTC GTC ACC TC	CCC AGG GCA AGG ACA ATG	569
29	CAC AGA GCA GTG GGT CCA G	CTC ATG GCC CAG TTT CAG G	231
30	GGG GAC TGG AAG GAA CAA C	CTT TAA GAC CCT GCC TTG GG	368
31	CAG CCC TCA GCC CCA AG	ACT GGG CCC TGC TGA CTC	300
32	GCA CAG CCA AAC TGG ACT C	CCT TCT GCC TGG GAG TGG	566
33	TCT GTT CAT GTT TAG GGT CTG G	CTC AGC CTG TCC CAG CAG	396
34 - 35	GGA GAA AGC CAC TGA ATA CCA G	GAG AAG CTC TCA GGT CAC CC	553
36 - 38	AGT GTC AGG TGC CTG TTG C	TCC TCT TTA CAG CTT GTG TCT CC	620
39 - 40	TCT GGA GTC CCA GAG AGC AG	GGG CCA TGA TGG ACA CTC	549
41 - 42	ATG TGA TGG GAA AGG GAG AC	CTG TGC CCA CAG ACT TCC TC	460
43	ACT CTA GCC TGG GGG ACA AC	CCC AAG TCC TAG ACC CTC CT	320
44	CCC AGG AGG ACA GAA AAA GG	GGG AGG GGG AGA TTC AAT AA	356
45	AGT ATA GTC CAG CCT GGG TCC	CTG GCT GTG CCT CTG ACT G	202
46	TGG CCA TCT CAT CCA TTT CT	CAC AGC TAG GAG CTG CAC AC	397
47	GAA CCA GCT GGA CAC ACA GA	AAA TGG GTT TGC TTC AAT GG	458
48	GGG CAG GAC AGG ATC AGA AG	AGG GAG ATC CCT GTT GCT G	291
49 - 50	CTA GGC CTC TGG GAG TGG	CAC CAC GAG TGG GTG AAA C	400
51	CCC CTT AGT CAC AAG ACA AGA C	TTA TCC CCA CTC GCC TCA C	319
52	CTA GGG GTT CGC TTG TCA GT	AGT GGG GCC TCC GAG ACT	295

53	TGT GAG GCT CAT TTC AGT GC	AGG GTG CTG AGA ATC AGA GG	352
54 - 55	TGT GTC CCC TTT CTG TTC TG	TGA TAG ATG GGG AAA CTG AAC C	534
56	GTG CCC ACC CTG TTC TTA TG	CCT CCT GGA GCA TGG ACA C	222
57	TCT CAG CTC AAT CCC AGG AG	TCC ACC CAG TCC CCA AG	271
58	ATG GGG GAG TAA ATG CCT TC	GGC TTG TGT CTC CCA TTC AT	594
59	CAG GAG ACA AGG GCT GTC C	CTG GAG CCT GGG CTG TC	214
60	AGA AGG ACA GAG GTC AAG CC	AAA TCT GGG TGG AGG GC	236
61	AAG CTG TGT CCC AGA ACA GG	ACA GGG CCT GAA TCA TGA AC	418
62	TGA GAG GGC AGG GTT GC	CAT GCA TGT CCC CAG GTC	271
63	ACA GTG AGG ATT GCC TGA GC	TAC CCA TCC TCC ATG ACC AC	269
64	AGC CCA GAG AAG CTA TGC AG	AGG CTC AGA GGA GGG AAG AG	374
65	TGG TTG AGA CTA TCC TCG CC	GAC CTG ACC TAT CTT GGA GCC	271
66	CAA GGT AAG AGC TGG GGA AG	TTG ATC CTG AGA GGT TCA GTG	240