Yano et al., J Clin Case Rep 2013, 3:12
DOI: 10.4172/2165-7920.1000319

Journal of Clinical Case Reports

Case Report Open Access

A Novel Mutation of *MYO15A* Associated with Hearing Loss in a Japanese Family

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Abstract

Mutations in the *MYO15A* gene located on chromosome 17p11.2, are responsible for non-syndromic autosomal recessive profound hearing loss (DFNB3). Direct sequencing of 96 Japanese families with profound congenital hearing loss revealed one family with a novel homozygous mutation in *MYO15A*, a T to A transition at the nucleotide of 9413 (c.9413T>A) that encodes the MyTh4 domain of the protein (p. L3138Q). This is the first report of an East Asian hearing loss patient with a *MYO15A* mutation.

Keywords: DFNB3; MYO15A; Mutation; Hearing loss

Introduction

Hearing loss is one of the most common communication disorders in humans, affecting millions of individuals worldwide. To date, 95 loci for autosomal recessive sensorineural hearing loss (ARSNHL) have been reported and at 41 of these loci, the causative genes have been identified (Hereditary Hearing Loss Homepage: http://hereditaryhearingloss.org/). *MYO15A* is comprised of 66 exons distributed across 71 kbp of DNA on chromosome 17p11.2. The *MYO15A* mRNA transcript encodes a 3530 amino acid protein in its longest form. *MYO15A* has MyTh4 (Myosin-Tail like Homology region 4) domains, FERM (4.1 protein, Ezrin, Radixin, and Moesin) motifs, a SH3 (Src Homology 3) domain, and the PDZ domain.

In humans, 36 different *MYO15A* mutations have been reported and 35 of these cause congenital profound ARSNHL. The remaining *MYO15A* mutation was a heterozygous missense mutation detected in a Smith-Magenis syndrome patient who had moderate sensorineural hearing loss.

In this report, we describe the first identified novel missense *MYO15A* mutation in a Japanese ARSNHL patient together with a review of the previous literature. This mutation is located in a MyTh4 domain and is thought to disrupt normal *MYO15A* function, resulting in congenital hearing loss.

Subjects

DNA samples from 96 independent subjects who had profound congenital ARSNHL were collected from 33 ENT departments nationwide in Japan. All subjects gave prior written informed consent for participation in the project, which was approved by each hospital's ethical committee. Anamnestic and physical examinations were performed to exclude those with syndromic symptoms, outer and/or middle ear diseases, and environmental factors such as premature birth, or newborn meningitis. Controls were 192 Japanese healthy individuals with normal hearing confirmed by pure tone audiometry.

Mutation Analysis

All of the *MYO15A* exons were amplified using gene-specific primers described elsewhere [1]. PCR reactions were performed with 25 μl in 1.5 mM $MgCl_2$, 100 mM of each dNTP, 1U of Taq DNA polymerase, and 2 mM forward and reverse primers. After an initial denaturation at 95°C for 90 seconds, amplification was performed for 35 cycles of 95°C for 45 seconds, 60°C for 45 seconds, and 72°C for 2 minutes. Then, a final extension was performed at 72°C for 5 minutes. Sequencing was performed with a BigDye™ v1.1 Terminator Cycle Sequencing kit (Applied Biosystems, Foster City, CA), according to the manufacturer's instructions. Sequencing products were analyzed by an ABI PRISM® 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA).

Computer analysis to predict the effect of missense variants on *MYO15A* protein function was performed with Sorting Intolerant from Tolerant (SIFT; http://sift.jcvi.org/), and Polymorphism Phenotyping (PolyPhen2; http://genetics.bwh.harvard.edu/pph2/).

Results

Direct sequencing revealed a novel homozygous mutation of *MYO15A* at exon 57 (c.9413T>A) in one patient (Figure 1). This mutation (p. L3138Q) is located in the MyTh4 domain of the myosin 15a protein, and is predicted to be pathologic by prediction programs (Table 1). We also confirmed that the patient's father and mother had heterozygous mutations and that the mutation was absent in the controls. The patient had no mutations in *GJB2*, the gene most frequently involved with hearing impairment in Japanese, nor in mitochondrial 1555A>G.

In detail, the patient was a female with congenital severe to profound sensorineural hearing loss. At age one, her mother became aware of her hearing impairment because she did not speak. The patient visited the hospital for genetic testing the age of 17 (Figure 1). Computed Tomography examination indicated that she did not have any malformations, such as ossicular anomalies, cochlear hypoplasia, vestibular dilation or enlarged vestibular aqueduct. In addition, she had no history of vertigo. Her sister also had severe congenital hearing loss, but her parents, brother, and other relatives did not have hearing impairment (Figure 1). DNA samples were not obtained from her siblings.

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Received July 13, 2013; **Accepted** November 28, 2013; **Published** November 30, 2013

Citation: Yano T, Ichinose A, Nishio S, Kobayashi Y, Sato H, et al. (2013) A Novel Mutation of *MYO15A* Associated with Hearing Loss in a Japanese Family. J Clin Case Rep 3: 319. doi:10.4172/2165-7920.1000319

Exon	Domain	Nucleotide change	Amino acid change	Frequency	Control	Hereditary	Age of onset	Hearing level	Functional prediction	
									Poly Phen2 score	SIFT score
3	N-terminal extension	3658G>A	G1220R	1/96	0/192	Sporadic	Congenital	Severe	0	0.09
12	Motor	4322G>T	G1441V	1/96	0/192	Autosomal recessive	Congenital	Profound	0.785	0.01
30	MyTH4	6486delG	A2153fs	1/96	0/192	Sporadic	Congenital	Profound	-	-
57	MyTH4	9413T>A	L3138Q	1/96	0/192	Autosomal recessive	Congenital	Profound	0.791	0
65	-	10420A>G	S3474G	1/96	0/192	Sporadic	Congenital	Severe	0.427	-

Table 1: *MYO15A* variants found in this study.

Exon	Domain	Nucleotide change	Amino acid change	Mutation type	Age of onset	Hearing level	Origin of family	References
Exon 2	N-terminal extension	373delCG	R125VfsX101	Frameshift	-	-	Ashkenazi Jewish	12
Exon 2	N-terminal extension	867C>G	Y289X	Nonsense	Congenital or prelingual	Moderate to severe	Turkey	11
Exon 2	N-terminal extension	1185dupC	E396fsX431	Frameshift	10-14 yrs	Moderate to severe	Pakistan	13
Exon 2	N-terminal extension	1387A>G	M463V	Missense	-	Severe to profound	Iran	14
Exon 2	N-terminal extension	3313G>T	E1105X	Nonsense	-	Profound	Pakistan	7, 13
Exon 2	N-terminal extension	3334delG	G1112fsX1124	Frameshift	-	Severe to profound	Pakistan	7, 13
Exon 3	Motor	4023C>T	Q1229X	Nonsense	Congenital	Profound	Pakistan	6
Intron 4	Motor	IVS4+1G>T	D1232fsX1241	Splice donor site	Congenital	Profound	Pakistan	6
Exon 5	Motor	3758C>T	T1253I	Missense	-	Severe to profound	India	7
Intron 5	Motor	IVS5+1G>A	T1253fsX1277	Splice donor site	-	Severe to profound	Pakistan	7
Exon 10	Motor	4176C>A	Y1392X	Nonsense	-	Severe to profound	Pakistan	7
Exon 10	Motor	4198G>A	V1400M	Missense	Congenital or prelingual	Severe to profound	Turkey	11
Exon 11	Motor	4240G>A	E1414K	Missense	-	-	Palestinian Arab	12
Exon 11	Motor	4273C>T	Q1425X	Nonsense	-	-	Turkey	15
Exon 12	Motor	4351G>A	D1451N	Missense	-	Severe to profound	India	7
Exon 12	Motor	4441T>C	S1481P	Missense	Congenital or prelingual	Severe to profound	Turkey	11,15
Exon 14	Motor	4652C>A	A1551D	Missense	-	-	Turkey	15
Exon 15	Motor	4669A>G	K1557E	Missense	-	Severe to profound	Pakistan	7
Exon 17	Motor	4904-4907delGAG	E1637del	Frameshift	-	Severe to profound	Iran	14
Exon 17	Motor	4998C>A	C1666X	Nonsense	-	Severe to profound	Tunisia	10
Exon 18	Motor	5117_5118GC>TT	L1706V	Missense	-	Severe to profound	Pakistan	7
Exon 19	Motor	5189T>C	G1730P	Missense	-	Severe to profound	Pakistan	7
Exon 20	Motor	5305A>G	T1769A	Missense	-	Severe to profound	Iran	14
Exon 22	Motor	5419-21delT	F1807L fsX6	Frameshift	-	Severe to profound	Iran	14
Exon 22	Motor	5492G>T	G1831V	Missense	-	Profound	Turkey	8
Exon 24	Motor	5810G>A	R1937H	Missense	-	Severe to profound	Iran	14
Exon 24	Motor	5807_5813delCCCGTGG	R1937TfsX10	Frameshift	Congenital or prelingual	Severe to profound	Turkey	11
Exon 26	IQ Motif	5925G>A	W1975X	Nonsense	-	Severe to profound	Iran	14
Exon 28	-	6061C>T	Q2021X	Nonsense	-	Severe to profound	Pakistan	7
Exon 29	MyTH4	6217C>T	P2073S	Missense	Congenital	Profound	Iran	1
Exon 30	MyTH4	6331A>T	N2111Y	Missense	Congenital	Profound	India	5
Exon 30	MyTH4	6337A>T	I2113F	Missense	Congenital	Profound	Indonesia	5
Exon 30	MyTH4	6371G>A	R2124Q	Missense	Congenital	Profound	Iran	1
Exon 31	MyTH4	6952C>T	T2205I	Missense	Congenital	Moderate	North America*	6
Exon 32	-	6731G>A	G2244E	Missense	-	Severe to profound	Pakistan	7
Exon 33	-	6796G>A	V2266M	Missense	-	Severe to profound	Pakistan, Turkey	7

Intron 37	-	IVS37 + 3G>C	-	Splice donor site	-	Severe to profound	Tunisia	10
Exon 41	-	7801A>T	K2601X	Nonsense	Congenital	Profound	India	5
Exon 44	FERM	8486G>T	Q2716H	Missense	Congenital	Profound	Pakistan	6
Exon 45	FERM	8158G>C	D2720H	Missense	-	Severe to profound	Pakistan	7
Exon 45	FERM	8183G>A	R2728H	Missense	-	-	Ashkenazi Jewish	12
Exon 48	FERM	8467G>A	D2823N	Missense	-	Severe to profound	Iran	14
Intron 50	-	IVS50-1G>C	-	Splice donor site	-	Profound	Turkey	8
Exon 51	SH3	8821_8822insTG	V2940fsX3034	Frameshift	-	Severe to profound	Pakistan	7
Intron 54	-	IVS54+1G>A	-	Splice donor site	-	Severe to profound	Tunisia	10
Exon 57	**MyTH4**	**9413T>A**	**L3138Q**	**Missense**	**Congenital or prelingual**	**Profound**	**Japan**	**This case**
Exon 57	MyTH4	9478C>T	L3160F	Missense	-	Severe to profound	Pakistan	7
Exon 62	FERM	9957_9960delTGAC	D3320fs	Frameshift	Frameshift	Severe to profound	Brazil**	9
Exon 62	FERM	9995_10002dupGCCG-GCCC	S3335AfsX121	Frameshift	Congenital or prelingual	Severe to profound	Turkey	11
Exon 65	-	10474C>T	Q3492X	Nonsense	-	Severe to profound	Pakistan	7
Exon 66	-	10573delA	S3525fs	Frameshift	Prelingual	Severe to profound	Brazil	9
*Mutation was found in a patient heterozygous at the DFNB3 locus with Smith-Magenis Syndrome.					**Mutation was found in a heterozygous individual.			

Table 2: DFNB3-causing *MYO15A* mutations.

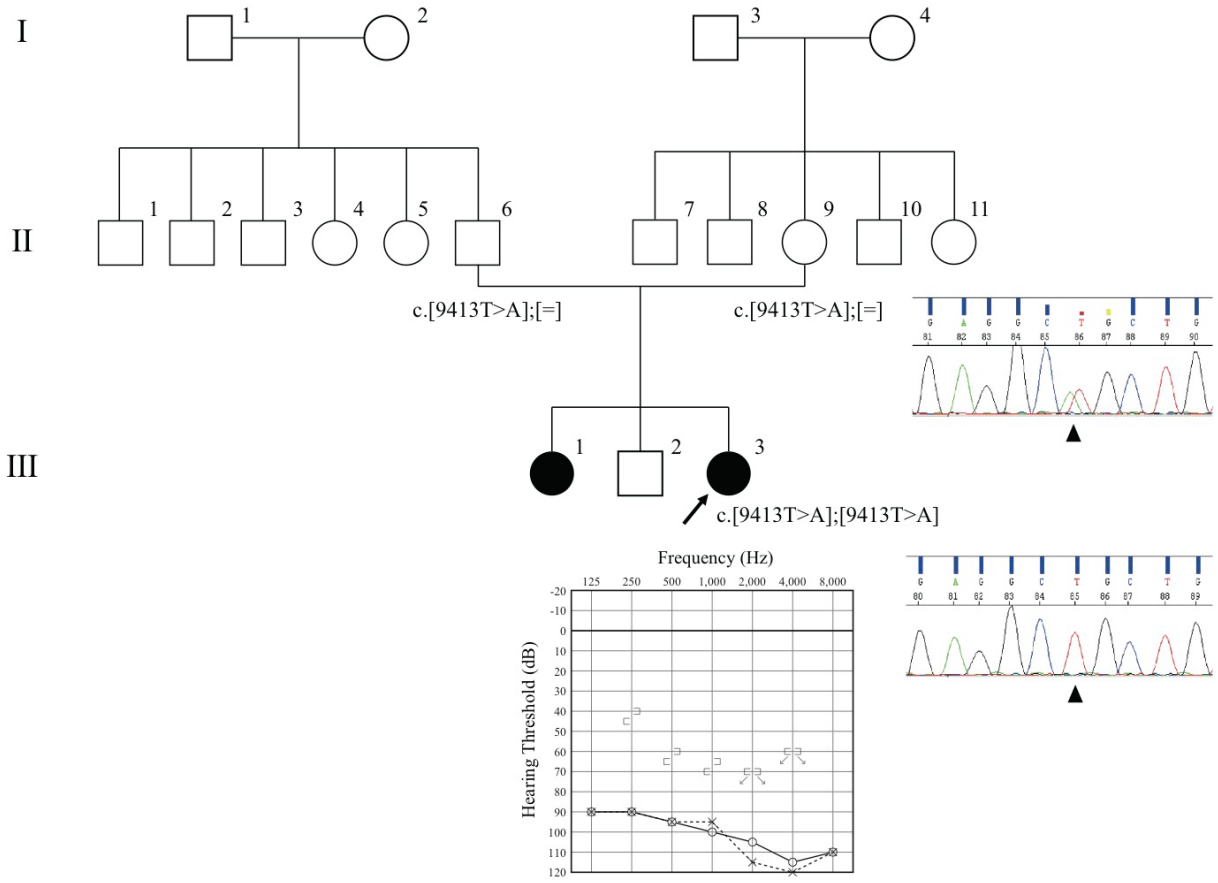

Figure 1: Family pedigree, audiogram of the patient, and lectropherogram analysis of MYO15A in the family. A novel homozygous mutation of MYO15A at exon 57 (c.9413T>A) is found in a patient with congenital severe to profound sensorineural hearing loss.

We also found other heterozygous variants: c.6824delG, p.G1441V, p.G1220R, and p.S3474G, each in a different independent patient, and none being found in the controls (Table 1).

Discussion

Myosin 15a protein is required for normal auditory function,

therefore *MYO15A* mutations cause ARSNHL. Mutations in this gene also cause the shaker 2(sh2) phenotype in mice. Sh2 mice are characterized by a vestibular defect and profound hearing loss [2,3] but such vestibular defects are not found in human carriers of *MYO15A* mutations. The stereocilia of hair cells of the sh2 mice are short and lack the characteristic staircase-like pattern [4].

In our patient, the novel *MYO15A* mutation located in the MyTH4 domain caused sensorineural hearing loss. In addition, this is the first *MYO15A* mutation found in an East Asian population. To date, 43 mutations in *MYO15A* were reported. Type of mutations, domains, and clinical features are summarized in Table 2 [1,5-15]. All *MYO15A* mutations previously reported were found in prelinguistic or congenital hearing loss patients, except for one Smith-Meganis syndrome patient [6]. Our patient had prelingual profound hearing loss, consistent with previous reports.

Of the 43 reported *MYO15A* mutations, six were missense mutations in the MyTH4 domains. Five of those six were found in homozygous state: p.N2111Y in Indians [5]; p.I2113F in Indonesians [5]; p.R2124Q and p.P2073S in Iranians [1]; and p. L3160F in a Pakistani family [7]. The sixth missense mutation was a heterozygous mutation, p. T2205I, in a North American family affected by Smith-Magenis syndrome [6] (Table 2).

Furthermore, based on the prediction programs, two missense mutations, p. G1441V, p. L3138Q, are predicted to be pathologic variants (Table 1). However, except for p. L3138Q, all variants found in this study were identified as heterozygous and no associated mutation was found in the other allele.

The structure of the MyTH4 domain has not been fully characterized. In other myosins, it has been implicated in microtubule binding as well as actin binding to the plasma membrane. Some data suggest that the MyTH4/FERM domains are required for localization of Myosin15a to stereocilia tips. The co-localization of Myosin15a and whirlin proteins appears essential to form the complex at the stereocilia tips [16]. From our data combined with previous reports, the MyTH4 domain mutations interfere with the interaction between Myosin15a and whirlin, preventing the formation of the complex required for normal hearing [1]. *MYO15A* mutations have been found in each domain (Motor, MyTH4, N-terminal extension, FERM, and SH3) and caused similar clinical features including hearing level, implying the overall importance of *MYO15A* protein in cochlear function.

Acknowledgements

We thank all the families that participated in the present study. We would also like to thank Ms. S. Matsuda for technical assistance, and Ms. A. C. Apple-Mathews for help in preparing the manuscript. This study was supported by a Health Sciences Research Grant from the Ministry of Health and Welfare of Japan

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