RESEARCH NOTE

A novel mutation of the USH2C (*GPR98*) gene in an Iranian family with Usher syndrome type II

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Introduction

Usher syndrome (USH) is an autosomal recessive disease characterized by bilateral sensorineural hearing impairment (ARSNHL), and progressive visual loss due to retinitis pigmentosa (RP) with the present or absent of vestibular dysfunction. USH is classified into three types based on the severity and progression of the clinical periods of the disease. Usher syndrome type 1 (USH1) which is manifested by severe to profound congenital hearing loss, vestibular dysfunction and prepubertal onset of RP is the most severe form of the USH (Millan *et al*. 2011; Le Quesne Stabej *et al*. 2012). Patients with Usher type 2, presented at the second decade of the life, are characterized by moderate to severe hearing loss (HL), normal vestibular function and RP. For Usher type 3 (USH3) clinically, postlingual progressive HL, RP and variable vestibular function are the most distinguished features (Dai *et al*. 2008; Millan *et al*. 2011; Le Quesne Stabej *et al*. 2012). Genetically, USH is highly heterogeneous (Dai *et al.* 2008). To date, underlying mutations in 15 loci and 11 genes have been identified to be responsible for USH (http://hereditaryhearingloss.org).

While two-thirds of patients with the key features of USH phenotype were associated with Usher type II, only 30% of the patients were affected by Usher type 1 (OMIM: 605472) (Dai *et al.* 2008). Using fluorescence *in situ* hybridization (FISH), the mouse USH IIA gene pertaining to chromosome 1 has been mapped syntenically to chromosome 1q41 within the human population (Huang *et al*. 2002). In 2004, it was determined that the prevalent Usherin gene contains 72 exons (Weston *et al*. 2000; van Wijk *et al*. 2004). Then, mutations of the *GPR98* (NM_032119) gene were known to be the second common cause of USH2, accounting for upto 15% of the phenotype. It was mapped to chromosome 5q14 (Skradski *et al.* 2001) and was clarified that the *GPR98* gene should have 90 exons and span at least 600 kb (McMillan *et al.* 2002). However, in a recent study performed by the UK National Collaborative Usher Study in 2012, *GPR98* mutations consisted 6.6% of Usher families (Le Quesne Stabej *et al.* 2012).

Until 2009, several mutations had been named for *GPR98*, such as c.6901C>T (p.Gln2301*), c.8713_8716dup (p.Ile2906Lysfs*6), c.8790del (p.Met2931Trpfs*11), c.18732_18750del (p.Tyr6244*) and c.18131A>G (p.Tyr6044Cys) (Hilgert *et al*. 2009; Hmani-Aifa *et al*. 2009). Hilgert *et al*. (2009) reported a large deletion, g.371657-507673 del, in exons 84 and 85 which presumably lead to a frameshift mutation in an affected Iranian family. García-García *et al.* (2013) reported some new mutations, namely, c.17368_17369 delinsTTAT (p. Ser5790Leufs*6), c.18261delA (p.Gln6088Serfs*20), c.17204+4_17204+7del, c.6932_6939dup (p.Glu2314 Thrfs*13) and c.12528-1G>T. Here, we intent to report a novel missense mutation associated with the *GPR98* gene in an Iranian family with two affected individuals compatible with USH2 phenotype.

Methods

Clinical data

In a comprehensive study on families with affected individuals of hearing impairments, we studied a first cousin consanguineous Iranian family with two deaf siblings (both sexes) associated with prelingual bilateral hearing loss and night blindness which had started in adolescence and loss of vision field since age 15 and 7, referred to Genetics Research Center, Tehran, Iran (figure 1a). Visual deterioration was progressive in both the patients. Written informed consent was obtained and the study was approved by the Ethical

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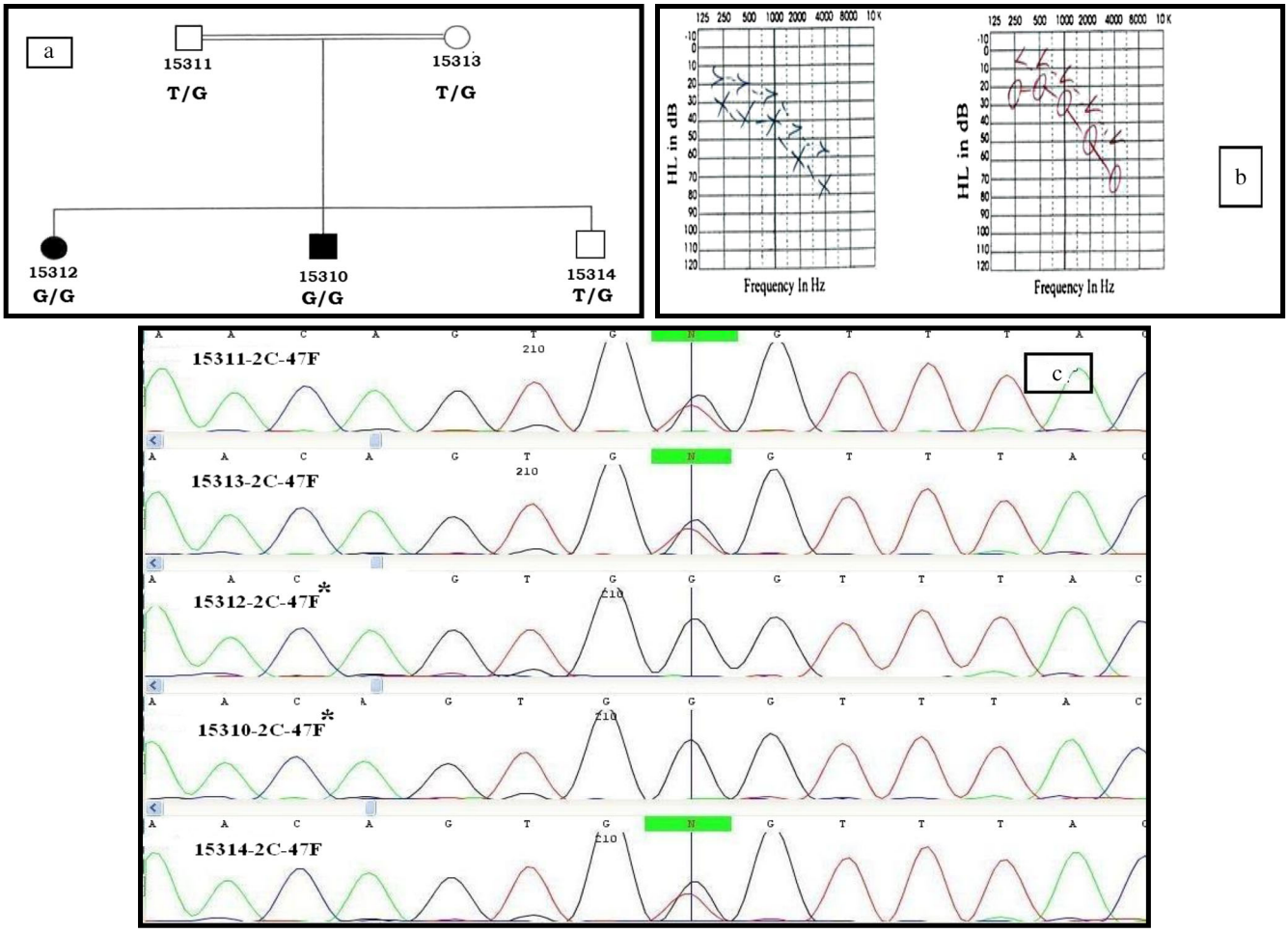

Figure 1. Clinical and molecular data relating to the family are shown. (a) Family pedigree with two affected individuals. (b) Bilateral down-sloping moderate sensorineural HL across all frequencies in this family. (c) Segregation of c.10019T>G (p.V3340G) in family members. (*Affected siblings.)

Committees of University of Social Welfare and Rehabilitation Sciences, Tehran, Iran. Clinical assessment included ophthalmologic and fundoscopic examination of the subjects as well as audiologic evaluation.

Molecular testing

Genomic DNA was extracted from the peripheral blood samples according to a standard procedure (Miller *et al.* 1988). A panel of eight microsatellite markers were designed from Marshfield chromosome-3 map (http://research.marshfieldclinic.org/genetics) for haplotype analyses of the *GPR98* and *USH2A* genes in two USH2 loci (USH2C, USH2A). A minimum of four microsatellite markers per locus (D5S1452, D5S2044, D5S1725, D5S1463, D1S2141, D1S2827, D1S395 and D1S229) were selected to genotype of the two affected children, parents and one healthy sibling within the family. Parameters of the PCR amplification of the microsatellite markers are available upon request. Polyacrylamide gel electrophoresis (PAGE) and a standard silver stain protocol were used to visualize the results. If the haplotype at a given locus was found to be homozygous in all affected individuals then it could be considered for screening of the disease-causing mutations.

We have therefore used whole-exome sequencing (WES) for one of the affected individual to prioritize the mutation and detected variant in *GPR98* gene conducted by Sanger sequencing (Applied Biosystems 3130 Genetic Analyzer, Foster City, USA) for all family members to segregate the findings. *In silico* analyses were used to predict the impact of missense variants on the protein structures, pathogen severity and also sequence conservation on the basis of the existing guidelines.

Results

In a first cousin consanguineous Iranian family with autosomal recessive pattern of inheritance, the parents of the two affected individuals were asymptomatic. The affected children had prelingual bilateral hearing impairment and

Table 1. STR markers close to *GPR98* and *USH2A* genes used for linkage analysis.

STR	Primer sequence	Repeated unit	PCR product size (bp)
D5S1452	5′-AAGCTAAAAATGCCCCAGTT-3′ 5′-CACAAGCCTGGAAGCCTG-3′	Tetranucleotide	102–117
D5S2044	5′-AGTCCCAGCTACTCGG-3′ 5′-TTGTCTGCCAAATCTCTTA-3′	Dinucleotide	180–210
D5S1725	5′-TGTACTTCAGGCTACCCTGC-3′ 5′-CCAGAGAAAGAAAACCAATAGG-3′	Tetranucleotide	188–212
D5S1463	5′-ATTAGCCAGTCATTTAAAAATCG-3′ 5′-ATTAAATACATACAGGTGTGTGCG-3′	Tetranucleotide	168–196
D1S2141	5′-AGACTTACAGCACTGGCTGC-3′ 5′-TGCTCCTAGGAAAGGAAACA-3′	Tetranucleotide	236–263
D1S2827	5′-GCTTCTGGCCTCTGTCA-3′ 5′-AATTTTGCGTGTGTGTGC-3′	Dinucleotide	142–152
D1S395	5′-AACAGGTCAAAGTAAGAAAGG-3′ 5′-CCAGCTAAGCTAGCAATGG-3′	Tetranucleotide	205
D1S229	5′-GCTTGTTTCCATTTATGGTG-3′	Dinucleotide	191–207

night blindness, started since adolescence and loss of visual field since age 15 and 7. Pure tone audiometry testing showed bilateral down-sloping moderate sensorineural hearing impairment in affected children (figure 1b). There was no history of imbalance gait or vertigo. Family history revealed a maternal female cousin with symptoms. Fundoscopic examination showed pigmentary changes in the entire retina (RP). Electroretinography also revealed a cone dystrophy in the affected female which was symmetric in both eyes.

Homozygosity mapping using short tandem repeat (STR) markers was performed as the first step of analysis of *GPR98* and *USH2A* genes due to consanguineous marriage. Homozygosity was revealed at the *GPR98* gene in the family but the results excluded linkage to *USH2A*. All three STR markers showed linkage to *GPR98* gene in this family and two affected sibs were homozygous for all markers except D5S1463 (table 1).

Using WES approach, a novel homozygous missense mutation c.10019T>G of the *GPR98* (NM_032119) gene was identified that was located within exon 47 leading to p.Val3340Gly. Synchronically, this variation was mapped into the domain EAR 2 of the vlgr1 protein and the residue was predicted to belong to a β strand with probability 0.978 (figure 2, a&b).

This change segregated in parents and in one healthy brother as heterozygote status (figure 1c). The detected missense variant was excluded in 100 Iranian normal controls or 200 normal alleles. *In Silico* analysis (PolyPhen-2 biobyte solutions GmbH, Bothestrasse 14269126 Heidelberg, Germany) that was used for the prediction of functional effects of human SNPs showed that the mutation was probably damaging with a score of 0.976 (sensitivity, 0.76; specificity, 0.96). SIFT result supports the prediction provided by PolyPhen-2 as this substitution at position 3340 from V to G is predicted to affect protein function with a score of 0.00 and median sequence conservation of 2.72. After using Usher syndrome missense analysis website (https://neuro-2.iurc.montp.inserm.fr/USMA/), outcomes showed alignment average percentage identity (AAPI) and alignment average percentage identity of the region (AAPIR) (20 residues surrounding position 3340) of 65.35 and 39.48% for 20 sequences, respectively. Moreover, it presented a 60% conservation of V3340 among sequences. Figure 3 a&b represents the valine conservation among alignments of 20 species. Venn diagram of alignment is also shown (figure 3c).

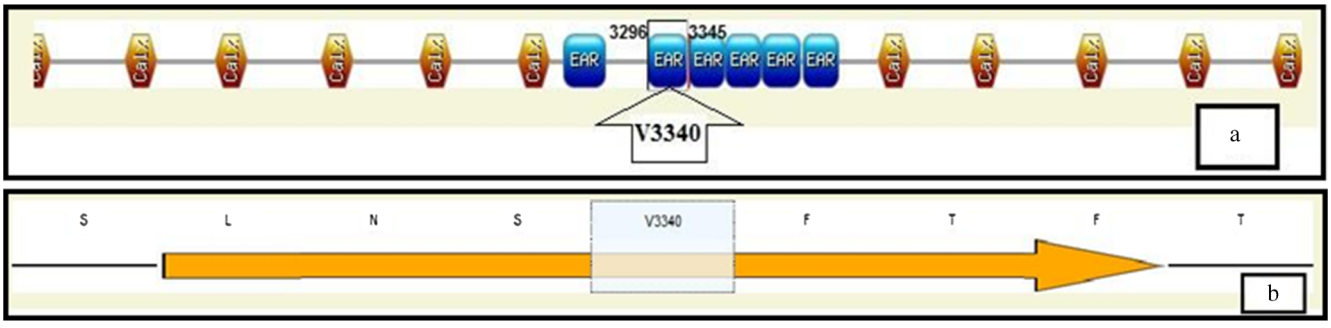

Figure 2. EAR2 domain of vlgr1 protein. (a) The p.V3340G missense mutation occurrs in EAR 2 domain of vlgr1 protein among 3296–3345 amino acids. AAPI of the domain among 125 sequences is 21.07%. (b) Variant p.V3340G is predicted to belong to a β strand. Mutant residue (glycine) is less observed in this type of structure (https://neuro-2.iurc.montp.inserm.fr/USMA/).

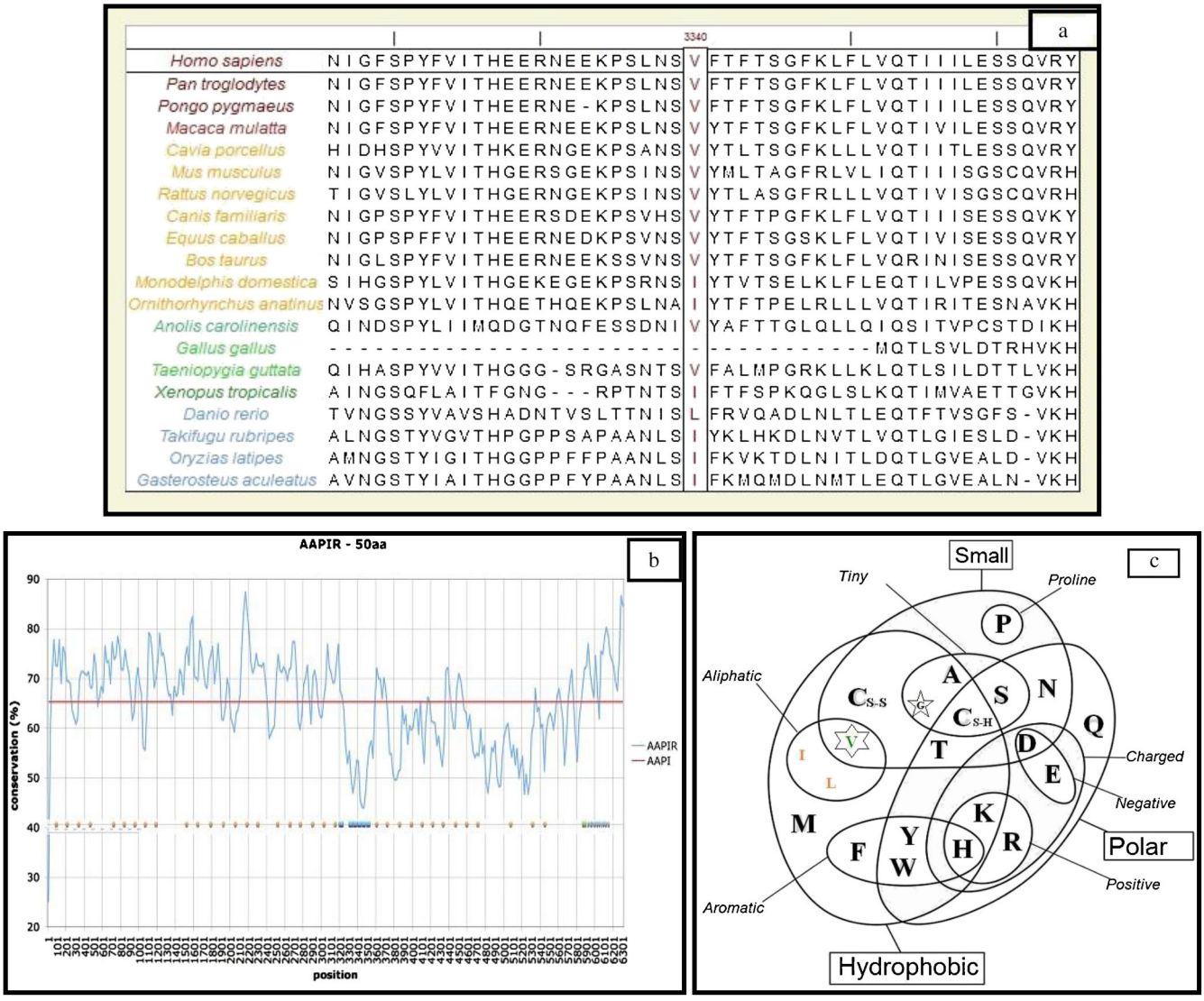

Figure 3. Amino acid conservation. (a) Valine conservation among alignments of 20 species. AAPIRs (calculated on 20 residues around the variant position) compared with AAPI of the whole alignment. (b) The local conservation has been calculated every 20 positions using 50 residues. Venn diagram of alignment. * Wild-type and mutant residues. (c) Glycine and mutant amino acids are not found as normal in the alignment (https://neuro-2.iurc.montp.inserm.fr/USMA/).

Discussion

Usher type 2 accounts for two-thirds of USH patients and the mutations of the *GPR98* gene is known to be the second common cause of USH2. Molecular analysis of *GPR98* has remained scarce because of the number of exons ($n = 90$) to screen by conventional sequencing. In contrast to mutations of usherin, the mutational spectrum of *GPR98* predominantly results in a truncated protein product (Besnard *et al.* 2012).

In this study, we examined an Iranian family with clinically diagnosed USH type 2 and screened the *GPR98* gene. Here, we could utilize WES to screen the entire coding regions of *GPR98* to define a novel missense mutation in the second family with USH2C. This mutation is a change in position of valine of 3340 to glycine, which occurred in EAR2 (for epilepsy-associated repeat) domain. It has located nearby to the centre of the ectodomain protein which contains seven copies of a short ~50 amino acid repeat named epitempin (EPTP) and consists of the folded β-sheets (Staub *et al.* 2002).

There is an unproven hypothesis that the EPTP domain of VLGR1 interacts functionally with ligands during neurogenesis (Scheel *et al.* 2002). Besides the two variants in *GPR98* gene, two other different heterozygote variants in EAR2 domain which led to the USH2C manifestations have been reported (Le Quesne Stabej *et al.* 2012). Indeed our variant is the first defect in homozygote status that resulted in change inside the EAR2 domain. Previous studies described that the role of the EAR domain primarily is to come back to its structure and organize in the ectodomain receptor as a ligand binding. All disease-causing variants in the genes (*VLGR1/MASS1* and *LGI1*) which had truncating or

missense defects occur at highly conserved sites and result in significant changes in polarity or charge of the EAR repeats and likely has an impact on both EAR and entire membrane domains (Scheel *et al.* 2002). According to PolyPhen and (SIFT) (http://sift-dna.org, Fred Hutchinson Cancer Research Center, 1100 Fairview Avenue N A1-162, Seattle, WA 98109, USA) prediction online software, a probably damaging effect of the related variant has been recognized. After using USMA site that is specifically designed for Usher correlated variants, alignment average percentage identity of the domain among 125 sequences were distinguished. As shown in figure 2b, our variant belongs to β strand and is less observed in this type of structure.

In conclusion, in this study one missense change of the *GPR98* gene was identified to be associated with the EAR domain. It is the second detailed variant among Iranian affected individuals who have key features of the USH2C phenotype. It probably has an impact on the protein function as a ligand binding form. Functional study on this domain and its related ligands can more clearly clarify the effects of the variant and its pathogencities. Our findings also expand the spectrum of *GPR98* mutations in USH and demonstrate that the long isoform of *GPR98* might carry even more mutations of the *GPR98* gene.

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