ONLINE MUTATION REPORT

MYO7A mutation screening in Usher syndrome type I patients from diverse origins

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Usher syndrome (USH) (OMIM 276901) is an autosomal recessive disorder characterised by hearing impairment associated with retinitis pigmentosa and in some cases vestibular dysfunction. This disease accounts for approximately 50% of individuals with combined deafness and blindness in developed countries. The estimated prevalence of USH ranges from 3.8 to 6.2/100 000.[1–3]

Phenotypically, three clinical types of Usher syndrome have been defined according to the severity of hearing impairment, age of retinitis pigmentosa onset and the presence or absence of vestibular response. Usher syndrome type I (USH1) is the most serious type, characterised by severe to profound congenital sensorineural hearing loss, balance deficiency and prepubertal onset of retinitis pigmentosa leading to blindness. USH2 is characterised by moderate to severe hearing impairment, normal vestibular function and later onset of retinal degeneration than USH1. USH3 displays progressive hearing loss, retinitis pigmentosa and variable vestibular phenotype.

Six loci for USH1 (USH1B–USH1G) have been mapped and, to date, five genes have been identified.[4 5] The *MYO7A* gene was found to be responsible for USH1B[6] and is the most common subtype of USH1, accounting for approximately 50% of cases.[7–9] Defects in *MYO7A* also cause autosomal dominant non-syndromic sensorineural hearing impairment (DFNA11) (MIM 601317),[10] autosomal recessive deafness (DFNB2) (MIM 600060)[11 12] as well as atypical types of Usher syndrome which are clinically similar to USH3.[13]

The *MYO7A* gene has 49 exons, of which 48 are coding, and spans approximately 87 kb of genomic sequence on chromosome 11q13.5. The encoded protein is an unconventional myosin, the myosin VIIA,[14] predicted to consist of 2215 amino acids and has a molecular mass of 254 kDa. This protein contains three typical domains: the N terminal head or motor; the neck or regulatory domain consisting of five IQ motifs; and the tail which begins with a short coiled coil domain followed by two large repeats, each containing a MyTH4 and a FERM domain, separated by a SH3 domain.[15]

In humans, myosin VIIA is expressed in a variety of tissues: the inner ear, retina, testis, lung and kidney.[16] Different roles have been postulated for myosin VIIA in the inner ear, such as participation in signal transduction in hair cells and differentiation and organisation of hair cell stereocilia.[17] In the human retina, myosin VIIA displays an active function in the migration of RPE melanosomes, phagocytosis of photoreceptor cell outer segment tips and opsin transport through the cilium of these photoreceptors.[18 19]

The *MYO7A* gene has been studied in USH1 patients from different origins. Results indicate there is a high diversity of mutations[4] distributed along the gene with no indication of a hot spot of mutations. In the present study, we report the results of screening for mutations in the *MYO7A* gene in 40 probands with USH1 from Spain, Italy, Turkey, Morocco and the Czech Republic.

MATERIALS AND METHODS

Subjects

In total, 40 individuals affected by USH1 were collected as part of a large study on the genetics of Usher syndrome. Twenty five Spanish families were recruited from the Federación de Asociaciones de Afectados de Retinosis Pigmentaria del Estado Español (FAARPEE) and also from Ophthalmology and ENT Services of several Spanish Hospitals. Similarly, 10 families were studied from Italy, two from Turkey, one from the Czech Republic and two from Morocco.

Patients were considered as suffering from USH1 on the basis of profound and congenital hearing loss, vestibular areflexia and retinitis pigmentosa. Clinical confirmation of this diagnosis was not available for nine of these patients.

Fifty unrelated healthy Spanish individuals were screened as controls to evaluate the presence and frequency of novel changes found in the patient samples.

Mutation screening

Genomic DNA was extracted from peripheral blood of affected individuals and their family members by standard methods. Polymerase chain reaction (PCR) was performed for all 49 exons, including intron–exon boundaries of the *MYO7A* gene (primer sequence and PCR conditions are available from the authors on request).

Single strand conformational polymorphism (SSCP) analysis was used to screen all amplified fragments from all affected individuals on polyacrylamide gel electrophoresis. DNA samples with different known polymorphisms were available. Those fragments with a migration pattern different from normal and polymorphic controls were directly sequenced in both forward and reverse modes on an automated sequencer (ABI-PRISM, model 3130x1) to confirm sequence change location.

After SSCP analysis, in an attempt to detect missed mutations, the whole gene was sequenced directly in patients found to be heterozygous for only one pathogenic variant.

To discard the possible known deletion of exons 47–49,[20] one PCR was performed in patients who were not heterozygous for any variation in this region, as described by Jaijo *et al*.[8]

Prediction of splice scores

Isocoding changes in coding regions and intronic variants were analysed to assess their likelihood of creating or eliminating splice sites using the BDGP splice site prediction program (available at http://www.fruitfly.org/seq_tools/splice.html) and the Splice View program (http://bioinfo.itb.cnr.it/oriel/

Abbreviations: PCR, polymerase chain reaction; SSCP, single strand conformational polymorphism; USH, Usher syndrome

Table 1 Summary of myosin VIIA mutations detected in this study

Nucleotide change	Codon change	Exon	Domain	No. of families	Origin
Missense					
c.77C>A*	p.A26E	3	Motor	1	Italy
c.395C>T	p.P132L	5	Motor	1	Spain
c.721C>G	p.R241G	7	Motor	3	Italy
c.1097T>C	p.L366P	11	Motor	1	Italy
c.3134T>C/ c.5507T>C	p.I1045T/p.L1836P	25/40	Tail	1	Italy
c.3652G>A	p.G1218R	29	Tail	1	Italy
c.3719G>A†	p.R1240Q	29	Tail	1	Italy
c.4475C>T	p.A1492V	34	Tail	1	Spain
c.6610G>C	p.A2204P	49	Tail	1	Spain
Nonsense					
c.1884C>A‡	p.C628X	16	Motor	1	Spain
c.5581C>T§	p.R1861X	40	Tail	1	Spain
Insertion/deletion					
c.655_660del	p.I219_H220del	7	Motor	1	Morocco
c.986dupG	p.N330QfsX5	9	Motor	1	Italy
c.3764delA	p.K1255RfsX8	30	Tail	1	Spain
c.4297delC	p.Q1433SfsX116	32	Tail	1	Spain
c.5835_5838delCTTT	p.F1946SfsX23	42	Tail	1	Italy
c.6025delG*	p.A2009PfsX32	44	Tail	1	Italy
Putative splice site mutation					
c.2283-1G>T¶	—	20	Neck	1	Morocco

Reference sequence for the *MYO7A* gene: NM_000260.
*Mutation previously reported by Bharadwaj *et al*.[21]
†Mutation previously described by Janecke *et al*.[22]
‡Mutation first described by Cuevas *et al*.[23]
§Mutation reported by Adato *et al*.[20]
¶Mutation first reported by Roux *et al*.[9]

splice-view.html). Intronic variant sequences were considered pathological when loss of splicing acceptor or donor activity was predicted and they were not present in control samples. Exonic variants were analysed using the RESCUE-ESE program (http://genes.mit.edu/burgelab/rescue-ese) to detect a possible alteration in predicted ESE.

RESULTS

The *MYO7A* gene was screened for mutations in 40 patients with USH1 from different countries. In this study, 19 likely pathogenic changes were detected: two nonsense mutations, one putative splice site mutation, one insertion and five deletions (one of them in frame) and 10 missense mutations (table 1). Thirteen of these changes have not been described previously. None of the novel missense mutations, the putative splice site mutation or deletion inframe were found in the control sample.

On SSCP analysis three patients were found to be carriers of a single missense mutation. The remaining exons were then directly sequenced to detect any possible second mutation missed by the first technique. A second mutation was only found in one patient, who was compound heterozygous for p.R241G and the p.G1218R change, detected by sequencing. Furthermore, the 2 kb deletion reported by Adato *et al*[20] was screened but was not detected in any of the patients.

Mutations in both alleles were identified in 12 families (six homozygous and six compound heterozygous) whereas only one putative pathogenic variant was found in two families. Genotypes of the USH1 patients are shown in table 2.

One putative splice site mutation was observed in this study. This change comprised a variant in the −1 position at intron 19.

Table 2 Genotypes of patients with Usher syndrome type 1B

Genotype/patient	Mutation 1	Mutation 2	Origin
Homozygous			
RP-1264	c.5835_5838delCTTT	c.5835_5838delCTTT	Italy
RP-1283*	p.P132L	p.P132L	Spain
RP-1301	c.2283-1G>T	c.2283-1G>T	Morocco
RP-1340*	p.I219_H220del	p.I219_H220del	Spain
RP-1356	p.I1045T/p.L1836P	p.I1045T/p.L1836P	Italy
RP-1462*	p.R241G	p.R241G	Italy
Compound heterozygous			
RP-1252*	c.3764delA	p.R1861X	Spain
RP-1266*	p.A26E	p.L366P	Italy
RP-1268	p.N330QfsX5	p.R1240Q	Italy
RP-1269	p.R241G	c.6025delG	Italy
RP-1271	p.R241G	p.G1218R	Italy
RP-1436	p.C628X	c.4297delC	Spain
Heterozygous			
RP-1293	p.A1492V	ND	Spain
RP-1426	p.A2204P	ND	Spain

ND, not determined.
*Patients in whom segregation analysis was performed.

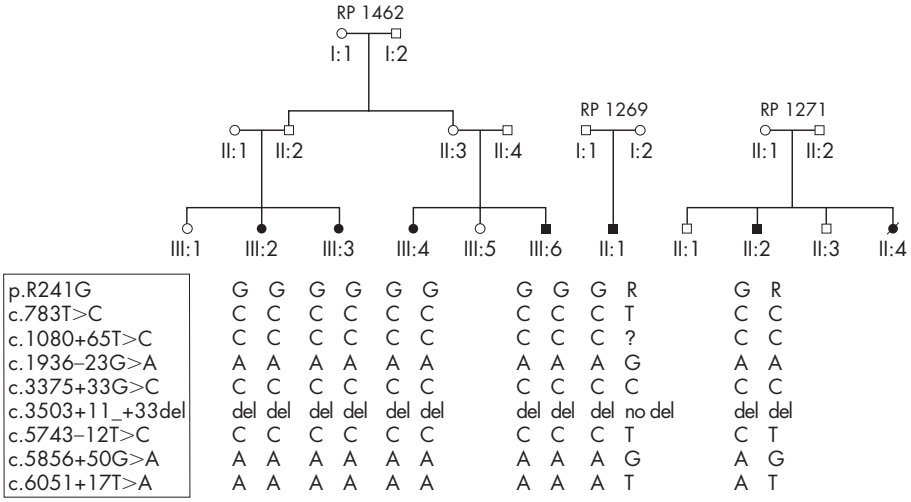

Figure 1 Pedigree of the Italian families. Haplotypes linked to the common mutation p.R241G are shown.

An in silico analysis was performed to predict whether c.2283-1G>T could affect the normal splicing of this region. Analysis of the normal allele with the Splice View program gave a score of 0.84 for the acceptor splice site; however, the splice sequence was not recognised for the mutant allele.

One patient was found to carry two missense changes in the homozygous state, p.I1045T in exon 25 and p.L1836P in exon 40. These were novel changes that were not found in the 100 control chromosomes, making it difficult to predict the pathogenic significance of these variations.

All mutations were private with the exception of the novel variation p.R241G found in three Italian families. Intragenic haplotypes were constructed using the polymorphic variants detected in these patients and the same haplotype linked to the mutation was observed (fig 1).

A non-described variant was found in exon 33. It consisted of a tandem repetition of a 70 base sequence comprising the last 17 bases of exon 33 and the flanking 53 intronic nucleotides (sequence: AGC CTA CAA ATT CTC AGg tac ccc gca gcc tgc aat gct ccc agt ccc ttg ctc tgt agc tcc agc cca c). The consensus sequence (NM_000260; UCSC Genome Browser) shows that this region is repeated three times but in USH1 patients, three, four and five copies have been found in variable proportions: 42%, 4% and 54%, respectively. Screening for this variant in the control population detected a similar rate for the number of repetitions.

Furthermore, previously unreported non-pathological intronic or exonic base substitutions were found. Novel variants that do not fall into the likely pathogenic category are summarised in table 3.

Table 3 Novel non-pathological variants found in this study

Exon	Polymorphism	Frequency
14	c.1555-71G>A	1/80
14	c.1555-42T>C	1/80
31	c.4023C>T	1/80
33	3 Tandem repetition	34/80
33	4 Tandem repetition	3/80
33	5 Tandem repetition	54/80
40	c.5636+53C>T	1/80
40	c.5598C>A	1/80
41	c.5637-27C>G	1/80
49	c.6439-11C>T	2/80

Allele frequency refers to patient sample.

DISCUSSION

Mutation screening of the human *MYO7A* gene in 40 unrelated patients with USH1 showed a total of 19 pathogenic variants in the coding sequence and flanking intronic sequences. Mutations were detected in 14 of the 40 families studied, accounting for 35%. No mutation was detected in those patients where a diagnosis of USH1 could not be confirmed. If we considered only patients for whom the USH1 diagnosis was unequivocal, the detection rate increased to 45%.

Thirteen different novel mutations were found in addition to the six previously reported ones. Seven variants were found to be located in the motor domain, one in the first IQ motif of the neck and the other 11 in the tail. Previous studies have reported that the majority of mutations in the *MYO7A* gene are located within the head domain[20]; however, mutations reported here display regular distribution along the gene. Furthermore, results described in this work confirm the high diversity of mutations responsible for USH1B already reported in previous studies. Most pathological variants found in this screening proved to be private and no mutational hotspots in *MYO7A* were observed.

The missense variants p.I1045T and p.L1836P were found in a homozygous state in the same patient. Both changes are located in MyTH4 domains which contain four highly conserved regions (MGD, LRDE, RGW and ELEA) present in different myosins. The Ile1045 precedes the MGD region of the first MyTH4 domain and is a quite commonly conserved residue among other species. On the other hand, the Leu1836 is an amino acid found in the RGW region of the second MyTH4 domain (fig 2). The implication of both amino acids in important regions of the MyTH4 domains together with the fact that neither of these missense variants were found in control samples implies that we cannot discard the possibility that these changes may exert a pathological effect.

One novel mutation, the p.R241G, was found in three USH1 Italian families who came from the same region in southern Italy. One was homozygous for this mutation and two families were compound heterozygous with another mutation, the c.6025delG, described in the Spanish population,[24] and the previously unreported p.G1218R variant. Haplotypes were constructed using the intragenic variants observed in these

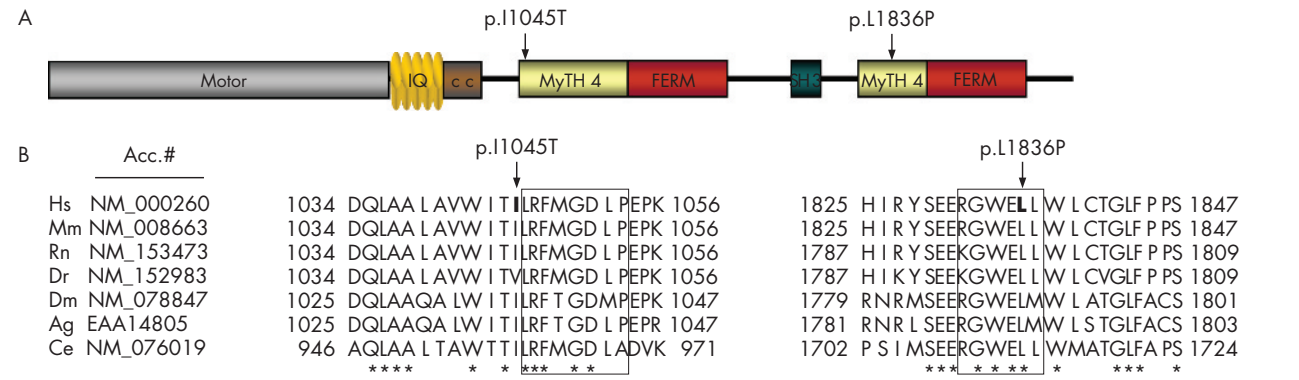

Figure 2 (A) Myosin VIIA protein structure, where the different domains and mutations p.I1045T and p.L1836P are indicated. (B) Amino acid sequence alignment of myosin VIIA orthologues derived from seven different species: *Homo sapiens* (*Hs*), *Mus musculus* (*Mm*), *Rattus norvegicus* (*Rn*), *Danio rerio* (*Dr*), *Drosophila melanogaster* (*Dm*), *Anopheles gambiae* (*Ag*) and *Caenorhabditis elegans* (*Ce*). Blocks show location of the MGD and RGW regions. Bold indicates the substituted residue in the USH1 patient. * Indicates the conserved amino acid in the seven species. Consensus MGD region: (K/R)(F/Y)MGDhP; consensus RGW region: RGWxLH; where x is any residue and h is an hydrophobic amino acid.

patients. The same haplotype linked to the mutation was observed in these families, suggesting a common origin.

The variation c.2283-1T>G was found in the homozygous state in one USH1 patient. This change has recently been found in another study in the heterozygous state with a missense mutation.[9] In silico analysis of the efficiency of the splice site indicated a pathogenic effect, given the lack of acceptor splice site recognition. Nevertheless, the effect of this mutation should be confirmed by in vitro analysis. Roux *et al*[9] found this sequence variant in a family from Algeria and in our study it was found in a patient from Morocco. As both families come from Magreb, it is very tempting to speculate a common origin for this mutation; however, only haplotype analysis can elucidate this question.

The change p.Y1719C has been described previously in several studies.[22 25] This variant was considered pathological because the region where it is located is highly conserved and because it was not found in control samples. This variant was found by Nájera *et al*[24] in a control population and thus its pathological significance was called into doubt. Jaijo *et al*[8] reported this variant in three USH1 probands. These patients were directly sequenced for mutations in the *MYO7A* gene but a second mutation was not found. Finally, Roux *et al*[9] also found this change in control samples and considered it as a non-pathogenic variant. In this report, the variant p.Y1719C has been found in two patients and designated as a polymorphic variant without pathological significance.

USH1 patients were found to carry different numbers of copies for a tandem repetition of 70 bp in exon/intron 33. A high proportion of three and five copies were observed while four repeats were only found in three patients. This region comprises the donor splice site of exon 33 and therefore the control population was screened in order to discard the possible pathological implication of the number of repeats in splicing. The frequency observed in control samples for three, four and five copies was the same as in the USH1 population, and thus this variant has been considered as non-pathogenic.

Two patients were found to carry only one mutation: p.A1492V and p.A2204P. Although we directly sequenced the remaining exons and the 2 kb deletion was not found, a second mutation could not be identified. The probability that these patients are carriers of a *MYO7A* mutation and that Usher syndrome is caused by mutations in another USH gene is low (estimated at approximately 1.5%).[26] Possible explanations are that the second mutation is located deep in an intronic region or in the promoter, which we did not screen. One should also bear in mind that with the screening method used in the present study, large deletions or inversions would also escape detection.

Screening for USH1 genes in patients with USH1 has recently been performed in different populations and the prevalence observed for *MYO7A* ranges from 39% to 55%.[7 9] In our series (Jaijo *et al*[8] and the present study), the *MYO7A* gene is implicated in approximately 45% of USH1 patients. It is worth noting that we were able to find 14 of the 20 expected mutations in the Italian patients, yielding a 70% detection rate in *MYO7A*. This would represent the highest percentage ever detected but because of the small sample size and the fact that three of them share a common mutation, a greater number of families must be studied to determine the real rate of *MYO7A* mutations in the Italian population.

In spite of the major implication of *MYO7A* in USH1, recent studies have reported the importance of other USH1 genes,

Key points

- Usher syndrome type I (USH1) is the most severe form of Usher syndrome and is characterised by profound sensorineural hearing loss, prepubertal onset of retinitis pigmentosa and vestibular dysfunction. Different genes are causative for USH1; however, the *MYO7A* gene bears the main responsibility.
- Our aim was to identify *MYO7A* gene mutations in Usher syndrome type I patients from diverse origins. Therefore, mutation screening was performed in 40 unrelated patients in all 49 exons of the gene.
- 19 different mutations were identified, 13 of which were novel. These variants include two nonsense mutations, one putative splice site mutation, one insertion and five deletions in coding sequence and 10 missense mutations.
- All mutations were private, with the exception of p.R241G. It is likely that this variant shares a common origin in the southern Italian population.
- A wide spectrum of *MYO7A* mutations have been observed, with this gene being responsible for USH1 in approximately 45% of the families studied.

such as *CDH23*, *PCDH15* or *USH1C*.[9] Screening for mutations in these genes in patients without *MYO7A* mutations must be carried out to determine the extent of the involvement of other genes in our series.

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