ORIGINAL ARTICLE

Ala397Asp mutation of myosin VIIA gene segregating in a Spanish family with type-Ib Usher síndrome

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Abstract

In the current study, 12 Spanish families affected by type-I Usher syndrome, that was previously linked to chromosome 11q, were screened for the presence of mutations in the *N*-terminal coding portion of the motor domain of the myosin VIIA gene by single-strand conformation polymorphism analysis of the first 14 exons. A mutation (Ala397Asp) segregating with the disease was identified, and several polymorphisms were also detected. It is presumed that the other USH1B mutations in these families could be located in the unscreened regions of the gene.

Introduction

Usher syndrome is an autosomal-recessive disorder, char acterized by hearing loss and retinitis pigmentosa (RP). Three clinical forms have been described according to the severity of the deafness and the presence or absence of vestibular response. The most severe form of the disease, Usher syndrome type I (USH1) is characterized by a severe-to-profound congenital sensorineural hearing impairment and vestibular abnormalities. Type II (USH2) exhibits a moderate-to-severe hearing loss and normal vestibular response. Finally, type III (USH3) exhibits a progressive loss of hearing and variable vestibular responses. At least seven loci have been located: USH1A on 14q (Kaplan et al. 1992; Larget-Piet et al. 1994), USH1B on 11q (Kimberling et al. 1992; Bonné-Tamir et al. 1994), USH1C on 11p (Smith et al. 1992; Keats et al. 1994), USH1D on 10q (Wayne et al. 1996); USH1E on 21q (Chaïb et al. 1997), USH2A on 1q (Kimberling et al. 1990; Lewis et al. 1990), and USH3 on 3q (Sankila et al. 1995). In addition, there are USH1 and USH2 families that fail to show linkage to any of these loci (Larget-Piet et al. 1994; Chaïb et al. 1997).

To date, only one gene, that consists of 49 exons, is known to be responsible for an Usher phenotype (USH1b). This gene encodes for the protein myosin VIIA (MyVIIA), a member of the superfamily of actin-based motors (Bement et al. 1994; Solc et al. 1994; Cheney et al. 1993) that is expressed in cochlea, retina, testis, lung, and kidney (Hasson and Mooseker 1995). Mutations in the myosin VIIA gene (MYO7A) have been shown to segregate with Usher syndrome type Ib (Weil et al. 1995; Weston et al. 1996; Lévy et al. 1997), and the cell-specific localization of this protein suggests that the blindness and deafness associated with USH is due to a defective MyVIIA within cochlear hair cells and the retinal pigmented epithelial cells.

Moreover, mutations in the MYO7A gene cause a nonsyndromic recessive deafness (DFNB2) (Weil et al.
1997) and a nonsyndromic dominant deafness (DFNA11) (Liu et al. 1997).

Materials and methods

Twelve Spanish families clinically diagnosed as USH1 with a total of 34 affected patients (23 males and 11 females) have been studied. Most of these families have multiple affected children and four present consanguinity.

Clinical tests were performed on all affected subjects. The ophthalmologic studies included determination of the visual acuity, fundus appearance, ophthalmoscopy, visual field examination, electroretinography, and registration of visually evoked potentials. The diagnostic criteria used to recognize RP were nightblindness, loss of visual-field and visual-acuity, and altered or nonrecordable electroretinogram. The otolaryngologic evaluation included audiological examination, neurootological exploration, electronystagmography and posturography.

The first 14 exons of MYO7A were amplified from genomic DNA by means of the polymerase chain reaction (PCR), using the intronic primers reported by Weston et al. (1996), with the exception of the primers for exon 5, taken from Lévy et al. (1997). The amplification conditions were 96°C for 5 min, then 37 cycles of 95°C for 30 s, 60°C for 30 s and 72°C for 45 s, with a final extension for 5 min at 72°C. For exons 11 and 5, the annealing temperature was decreased to 58°C.

Single-strand conformation polymorphism (SSCP) analysis after PCR amplification of the first 14 exons was carried out on samples from all the affected members of the families studied. The amplified DNA was electrophoresed on 10–12% polyacrylamide gels with 5% glycerol (except for PCR products of exons 1, 5, 11, 12, and 14, which

were without glycerol) at room temperature (except for exons 1, 2, 13, and 14 which were electrophoresed at 4°C). Bands were detected by means of staining with silver nitrate. Those samples showing an anomalous pattern of mobility in SSCP were sequenced by means of the fluorescent dideoxy-terminator method using fluorescent dideoxynucleotides and were analysed using an Abiprism DNA sequencer.

Finally, an analysis was carried out using the programs PEPTIDESTRUCTURE and PLOTSTRUCTURE (GCG version 5). For a peptide sequence, the program calculates measurements of antigenicity, flexibility, hydrophobicity, and surface probability; to predict the secondary structure, including helices, sheets, and turns, the original method by Chou and Fasman (1978) and a slightly modified method of Garnier et al. (1978) were used.

Results

Of 68 USH1B chromosomes surveyed for mutations by means of SSCP, one mutation and seven polymorphisms, two of which have been described previously, were found; these are discussed below.

Mutation

In exon 11, a GCC®GAC transversion in codon 397 was observed in the two affected siblings (aged 47 years old and 30 years old) of an USH1 nonconsanguineous family, their mother being the carrier of the mutation (Fig. 1). This condition had previously been linked to chromosome 11q (Espinós et al. 1998). This change led to a substitution of an alanine by an aspartic acid. The DNA sequences of the 3’ end of the MYO7A exon 11 of a control individual and a heterozygous patient are shown in Fig. 2. This mutation can be typed by digestion of the exon 11 PCR product with the restriction enzyme *Hga*I, being the Asp allele refractory to digestion.

Clinically, this mutation led to the onset of RP in the third decade and the patient presented with a profound congenital sensorineural deafness and areflexia since birth.

Polymorphisms

Two polymorphisms described by Weston et al. (1996) were observed in our sample. One of them was the TCG®TTG transition which caused the amino acid substitution Ser16®Leu in exon 3. This polymorphism was found in 22 chromosomes from 12 patients. According to our results, and those from Weston et al. (1996), this polymorphism is probably the most common in the simples analysed. To date, however, studies in healthy populations have not been carried out.

The other polymorphism was the silent mutation GGT®GGC which does not imply an amino acid change in exon 8. Fifteen chromosomes from 11 individuals presented this polymorphism.

Five novel polymorphisms were detected using primers of the exons 4, 7, 10 and 11, all of them in intronic sequences. In intron 3, two nucleotide changes were observed; a C®T and a C®G substitution, 51 and 127 bases upstream of the beginning of exon 4, respectively. The former was found in five chromosomes from five patients, and the latter in three from three. A C®T change was seen five bases upstream of the beginning of exon 7, in two chromosomes from two individuals. The same substitution (C®T) was found in intron 10, 69 bases downstream of the end of exon 10. It was present in 19 chromosomes from 14 patients. The last polymorphism found, a G®C change, was detected 80 bases upstream of the initiation of exon 11, in intron 10, in eight chromosomes of six individuals.

Discussion

MyVIIA belongs to one of at least 11 different classes of unconventional myosins. These proteins are actin-based motor molecules that hydrolyze ATP and transduce this energy to the production of a force, enabling them to move along actin filaments (Mooseker and Cheney, 1995). Unconventional myosins are characterized by a conserved *N*terminal motor domain (head domain), a calmodulin-based regulatory domain (neck region), and a unique tail domain that varies from one class of myosin to another and is supposed to be membrane binding (Cheney and Mooseker, 1992). MyVIIA consists of a 729-amino acid motor domain that includes a 9-amino acid ATP-binding site (residues 158–166; an 8-amino acid actin-binding site (residues 622–629); a 126-amino acid neck region that is composed of five consecutive repeats of the IQ motif, which are thought to be associated with calmodulin and calmodulin-like proteins, and a long tail (1360 residues) that is composed of an a-helical segment that forms a coiled-coil structure; and two internal repeated domains, similar to those in other proteins, the MyTH4 and the talin homology domains (Chen et al. 1996; Weil et al. 1996).

Mutation Ala397Asp lies in the motor domain between the ATP-binding site and the actin-binding site. Such amino acid substitution would be expected to alter dramatically the charge of the surrounding motor region. This alteration might affect a communication pathway between the ATP- and actin-binding sites (Rayment et al. 1993). It is suggested that the binding sites are potentially composed of different types of interaction: an ionic interaction involving a flexible loop, and a stereospecific interaction involving hydrophobic residues. According to the PEPTIDESTRUCTURE and PLOTSTRUCTURE programs, the secondary-structure prediction has shown that the mutated protein might present higher hydrophilicity than the normal protein in the residues surrounding the substituted amino acid. Moreover, the likelihood of formation

of an a-helical secondary structure is increased with respect to the normal protein. Furthermore, amino acid sequences of *Sus scrofa*, *Mus musculus* and *Homo sapiens* were fetched from the GDB (Genome Data Base), and compared using the GCG (Genetics Computer Group) package. We found that alanine at position 397 and the residues around it are evolutionarily conserved. Altogether, the results obtained suggested an important conformational change of the secondary structure in the vicinity of the mutation point. It is hard to predict whether this mutation is pathologic or not, since there is not a comprehensive biochemical picture of the role of MyVIIA in cellular function; however, this change might implicate that the mutated peptide is not able to carry out its function correctly.

Only one mutant allele is known in each of the two affected brothers. It is presumed that the other mutant allele is located in some of the remaining exons not yet analysed. The fact that only the affected members of the family have the mutation and that this mutational event has not been observed in any of the 66 alleles screened in our sample makes the possibility of a pathological change more likely.

The primary structure of the *N*-terminal motor protein of the myosin superfamily has been highly conserved throughout evolution. Watkins et al. (1995) found that most of the mutations in the MyH7 causing autosomal dominant familial hypertrophic cardiomyopathy lay on the motor domain, indicating that mutations in the motor domain are more likely to produce a pathological phenotype than tail mutations (Vikstrom and Leinwand 1996).

The study of mutations in MYO7A and their relationship to the Usher-syndrome phenotype is just beginning. The identification of additional mutations in families with Usher syndrome will provide further information for detailed genotype–phenotype correlations that will help elucidate the function of MyVII in the ear and the eye.

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Figure Legends

Fig. 1 Pedigree of the USH1B family studied. Both affected siblings present the Ala397Asp in a heterozygous state.

Fig. 2 DNA sequencing of the 3' end of the MYO7A exon 11 of a control individual and an affected individual showing a C®A transversion. The *arrows* show the nucleotide change that is only present in the heterozygous patient. The *vertical black bar* in the sequence corresponds to the 3' end of this exon.

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