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Detection of a novel Cys628STOP mutation of the myosin VIIA gene in Usher syndrome type Ib

J. M. Cuevas,[1,2] C. Espinós,[1] J. M. Millán,[2] F. Sánchez,[1] M. J. Trujillo,[3] B. García-Sandoval,[4] C. Ayuso,[3] C. Nájera[1] and M. Beneyto[2]*

[1]*Departamento de Genética, Universidad de Valencia, Valencia, Spain,*
[2]*Unidad de Genética y Diagnóstico Prenatal, Hospital La Fe, Valencia, Spain,*
[3]*Servicio de Genética, Fundación Jiménez Díaz, Madrid, Spain and*
[4]*Servicio de Oftalmología, Fundación Jiménez Díaz, Madrid, Spain*

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A Spanish family with three Usher I syndrome-affected members was linked to markers located on chromosome 11q. A search for mutations on the myosin VIIA gene revealed a novel mutation (Cys628STOP) on exon 16 segregating with the disorder in a homozygous state. This nonsense mutation could be responsible for the disease since it leads to a truncated protein that presumably has no function.

KEYWORDS: Usher syndrome, USH1B, myosin VIIA.

INTRODUCTION

The Usher syndrome (USH) is an autosomal recessive disease characterized by the association of a hearing impairment with retinitis pigmentosa (RP). It is clinically and genetically heterogeneous. According to the phenotype, three types of Usher can be distinguished.[1] Type I (USH1) presents a severe-to-profound congenital deafness and vestibular areflexia. Type II (USH2) has a moderate-to-severe hearing loss and normal vestibular response. Finally, type III (USH3) shows a progressive hearing impairment and variable vestibular problems. Children with type I usually begin to have impaired night vision during the first decade of life. Patients with type II experience the onset of night blindness in the second decade of life.[2] In both types an ERG shows an abnormal pattern as early as 2–3 years of age, although type I often shows extinction of ERG before 10 years. With regard to genetic aspects, seven different Usher loci were known to date: USH1A on 14q,[3,4] USH1B on 11q,[5,6] USH1C on 11p,[7,8] USH1D on 10q,[9] USH1E on 21q,[10] USH2A on 1q[11,12] and USH3 on 3q.[13] Moreover, it is reasonable to suppose that there are more Usher loci, because there are USH1 and USH2 families that fail to show linkage to any of these loci.[4,10]

To date, the MYO7A gene is the only defective gene identified that causes an Usher phenotype, the USH1b.[14,15] This gene, which consists of 49 exons, encodes for an unconventional myosin, myosin VIIA, which presents the three typical domains in all myosins: the head or motor domain, which includes an ATP-binding site and an actin-binding site; the neck or regulatory domain, composed of five consecutive repeats of the IQ motif which seems to be associated with calmodulin and calmodulin-like proteins; and the tail, which has an α-helical segment forming a coiled-coil structure, and an internal repeated domain, similar to domains in other proteins, the MyTH4 domain and the talin homology domain. This tail

* Author to whom all correspondence should be addressed at: Unidad de Genética y Diagnóstico Prenatal, Hospital Universitario La Fe, Avenida de Campanar, 21, 46009 Valencia, Spain. Tel: +34 96 398 73 70; Fax: +34 96 386 87 89.

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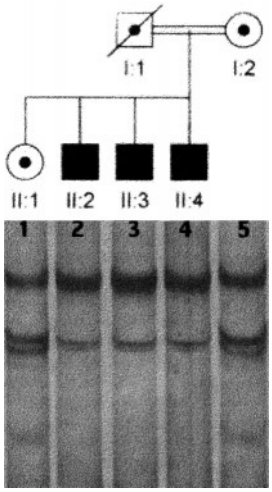

Fig. 1. Pedigree and polyacrilamide gel of the family studied. The three central lanes correspond to the three affected brothers (individuals II:2, II:3 and II:4). The first lane corresponds to their sister, who is a carrier of the mutation in a heterozygous state, and the last one corresponds to their mother, who is an obligate carrier.

segment participates in dimerization and subcellular location.

Mutations associated with families segregating the disorder have been described along the MYO7A gene, and show a great diversity of mutations responsible for USH1B (missense, nonsense, deletion, frameshift and splice-site mutations).[16–21] Moreover, both non-syndromic recessive (DFNB2) and dominant (DFNA11) deafness are caused by mutations on the MYO7A gene.[22–24] These mutations are located in the motor domain in DFNB2 patients belonging to three unrelated families, and on the tail domain in the only DFNA11 family reported.

Three USH1b patients belonging to a consanguineous family showed a homozygous nonsense mutation in the head domain of the MYO7A gene. Genetic and related clinical findings are described.

MATERIAL AND METHODS

A consanguineous family with three siblings (Fig. 1) aged 48–54 years is presented who had been previously diagnosed as having USH syndrome type I. A complete ophthalmologic and audiologic examination was performed on all of them. The patients showed a profound congenital sensorineural hearing loss and progressive symptoms of retinal degeneration. The three patients developmental history was marked by a substantial delay in motor development. Vestibular testing had been carried out in the fourth decade of life in one of them, and anomalous function had been observed. The onset of retinitis pigmentosa was early in patient II:2, with night blindness and peripheral visual constriction at 4 years of age, while in patients II:3 and II:4 the RP symptoms began in the second decade of life (15 years). However, the decrease in visual acuity was earlier in patient II:3 than in patient II:2 (51 and 54 years of age, respectively). At the present time, the visual fields are constricted to 10 central degrees or less in the affected patients, and visual acuity is severely impaired. In all three patients funduscopy reveals narrowed retinal vessels and bone spicule pigmentation, mainly in peripheral retina and pale discs. ERG rod, mixed and cone responses have been abolished.

The results of the segregation analysis were consistent with the involvement of the USH1B gene in this family's disease.[25] Therefore, a search for mutations was carried out in the MYO7A gene in all the members of this family, and in unaffected unrelated controls. DNA was amplified by the polymerase chain reaction (PCR) using the intronic primers reported by Weston *et al.*[17] for exons 1–14, and by Lévy *et al.*[18] for exons 15–49 (which, in the study by Lévy *et al.*, are referred to as exons 14–48, respectively). The conditions were optimized for each exon, and the amplification conditions for exon 16 were 95°C for 5 min, then 35 cycles of 95°C for 45 s, 55°C for 45 s and 72°C for 30 s, without final extension. Single-strand conformational polymorphism (SSCP) was carried out on 12% non-denaturing polyacrylamide gel. The electrophoresis conditions were: room temperature and 700 V for 16–20 h. Single-strand conformational polymorphism bands were detected using a silver staining method. Samples showing an anomalous pattern of mobility in SSCP were sequenced by the fluorescent dideoxy terminator method using fluorescent dideoxynucleotides, and analysed using an ABIPRISM DNA sequencer.

RESULTS AND DISCUSSION

The amplified DNA of exon 16 showed a different band on SSCP in all the affected members with respect to the unaffected family members (Fig. 1). Direct sequencing of this fragment revealed a homozygous transversion in codon 628 (TGC→TGA) causing a Cys→STOP change (Fig. 2). This mutation was not detected in the healthy controls. The remaining unaffected family members were heterozygous for this mutation, which results in a truncated protein whose amino acid sequence is encoded by the first 16 exons. Therefore, mutated myosin VIIA only consists of part of the motor domain, which begins within exon 2 and extends through exon 19. In spite of the fact that

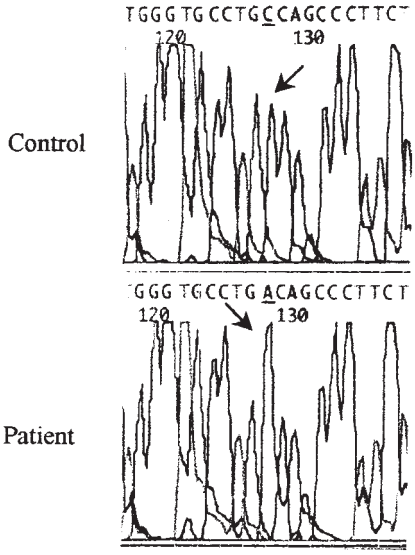

Fig. 2. Direct sequencing of one of the affected brothers and of a healthy control individual. The arrow shows the mutation C→A, leading to a substitution of a Cysteine by a STOP codon (Cys628STOP).

alterations in the amino acid sequence of the myosin VIIA polypeptide are not always pathological, it is presumed that the existence of a premature interruption of the protein due to this change in the homozygous state leads to a severe loss of function, that is responsible for the USH1b phenotype in this family. The comparison of the amino acid sequences of *Sus scrofa*, *Mus musculus* and *Homo sapiens* reveals that cysteine at position 628 and the residues around it are conserved throughout evolution.

In humans, myosin VIIA is expressed in the retinal pigmented epithelial and photoreceptor cells, as well as in the cochlear and vestibular sensory cells.[15,26] The lack of a functional myosin VIIa protein could explain the clinical features of the Usher 1B patients because of its nature and cellular expression. The motor domain of the MYO7A gene, mutated in yet another way, causes the non-syndromic recessive deafness DFNB2.[22,23] Other mutations on the same domain result in cochlear and vestibular disorders without retinal degeneration in shaker-1 mice.[14]

Different roles for myosin VIIA have been proposed. Maintaining the rigidity of the stereocilia during the dynamic movements of the bundle might be one of the most important.[21] Several mutations affecting this function more or less severely could be responsible for the variability in the onset and degree of hearing loss.[27] However, the exact mechanism of the retinal degeneration in USH1b patients with mutations on MYO7A remains unclear. Weston *et al.*,[17] suggest that tissue-specific differences in function, could explain the different effects that a specific mutation in the MYO7A gene can have on the eye and on the ear. On the other hand, Liu *et al.*[23] suggest a relationship between the stability of myosin VIIA and the absence/presence of retinitis pigmentosa in DFNB2 and USH1b patients, respectively. Neither of these theories, however, can explain the intrafamilial variations in the manifestations of RP in this family. According to the theory of Liu *et al.*, the retinal degeneration could be explained in our patients if the Cys628STOP mutation produced an unstable molecule; this is, in fact, what probably occurs, in other pathologies like cystic fibrosis, where an important decrease in mRNA as a consequence of nonsense mutations has been reported.[28] However, the difference in the age of onset of nyctalopia in the affected patients, two of whom showed a later onset of this symptom than the other brother, as well as the fact that other mutations thought to be more stable than Cys628STOP have been described in USH1b patients with prepubertal onset of RP suggests that unknown factors or genetic background could influence the effect that a given mutation has on retinal cells. These unknown interacting elements could also influence the evolution of the retinal degeneration, and this would explain why one of the patients (II-3), with a later onset of the night-blindness and reduction of visual field showed earlier impairment visual acuity than his brother (II-2) who began to experience RP symptoms in the first decade. Independently of the onset of RP symptoms, all our patients showed a severe impairment of visual function during the third and fourth decades of life, and this is a typical feature in USH1 patients according to Edwards *et al.*[29]

A mechanism that accounts for the effect of the MYO7A gene on retinal cells is still not clear, nor the previously reported hypothesis that the initial characteristics of RP are not a discriminatory parameter between USH1 and USH2.[30,31] It may be, as several studies have suggested,[32] that ERG testing is the most suitable method for identifying children with USH1 before the onset of signs of visual abnormalities. Such early diagnosis could identify children who would benefit from cochlear implantation. Unfortunately, in our family the ERG tests were not carried out in childhood before the onset of RP symptoms. Further studies are necessary in order to establish the genotype–phenotype correlations.

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