Linkage analysis in Usher syndrome type I (USH1) families from Spain

Carmen Espinós[1], Carmen Nájera[1], Jose María Millán[2], Carmen Ayuso[3], Montserrat Baiget[4], Herminio Perez-Garrigues[5], Onofre Rodrigo[5], Concepción Vilela[6], Magdalena Beneyto[2]

1. Departamento de Genética, Facultad de CC Biológicas, Universidad de Valencia, Valencia, Spain

2. Unidad de Genética y Diagnóstico Prenatal, Hospital La Fe, Valencia, Spain

3. Departamento de Genética, Fundación Jiménez Díaz, Madrid, Spain

4. Unitat de Genètica, Hospital de la Santa Creu i Sant Pau, Barcelona, Spain

5. Servicio de Otorrinolaringología, Hospital La Fe, Valencia, Spain

6. Unidad de Neurofisiología, Hospital La Fe, Valencia, Spain

Correspondence to:

Professor Carmen Nájera

Departamento de Genetica, Facultad de Ciencias Biológicas, Universidad de Valencia

Calle Dr. Moliner 50, 46100 Burjassot, Valencia. SPAIN

Abstract

Usher syndrome (USH) is an autosomal recessive hereditary disorder characterised by congenital sensorineural hearing loss and gradual visual impairment secondary to retinitis pigmentosa (RP). The disorder is clinically and genetically heterogeneous. With regard to Usher type I (USHI), several subtypes have been described, the most frequent being USHIB located on chromosome IlqI3.5. Of 18 USH1 families studied by linkage analysis, 12 (67%) showed significant lod score values for locus DllS527 (Zmax=14.032, 0=0.000) situated on chromosome 11q. Our findings suggest considerable genetic heterogeneity in the Spanish USH1 population. It is important to note that one of our families linked to the USHIB locus shows interesting intrafamilial clinical variability. As regards the remaining six USHI families, the linkage analysis did not provide conclusive data, although two of them show slight linkage to markers located on chromosome 3q (Zmax=1.880, 0=0.000 for D3S1279), the same location that had previously been assigned to some USH3 families.

INTRODUCTION

Usher syndrome (USH), which is recognised as the most common cause of combined deafness and blindness in adults, is an autosomal recessive hereditary disorder characterised by congenital sensorineural hearing loss and gradual visual impairment secondary to retinitis pigmentosa (RP).[1,2]

The disorder is clinically heterogeneous and, at present, three types can be distinguished on the basis of clinical findings principally related to audiological and vestibular parameters. [3-7] Usher type I (USHl) patients suffer severe to profound congenital hearing loss and abnormal or absent vestibular response, whereas Usher type II (USH2) is characterised by moderate to severe congenital hearing loss and normal vestibular function. Usher type III (USH3), which is clinically similar to USH2, is characterised by the progressive nature of its hearing loss and by the mild vestibular dysfunction. Although retinitis pigmentosa has been accepted as occurring earliest in type I, the ocular data are insufficient for distinguishing between types. It is interesting to note that a number of heterozygous carriers have shown significant audiological and Ophthalmological abnormalities.[8,9]

Genetic heterogeneity for USH1 and USH2 has been verified by linkage analysis. At least six different loci have been located: USHlA on 14q,[10,11] USHIB on llq, [12,13] USH1C on 11p, [14,15] USHlD on 10q,[16] USH1E on 2lq21,[17] and USH2A on lq.[18,19] There are, however, families of both types which fail to show linkage to any of these regions.[11,17] As far as USH3 is concerned, a notable clustering has been observed in eastern Finland. Sankila et al [20] assigned an USH3 locus to chromosome 3q. Furthermore, some USH2 families have been linked to the USH3 locus.[21] To date, only one gene related to USH is known, myosin VIIA, an unconventional myosin, involved in the USHIB phenotype.[22,24]

In the present study, the results of linkage analysis in 18 Spanish USH1 families are reported.

MATERIAL AND METHODS

Families and clinical studies

Eighteen Spanish families clinically diagnosed as having USHI 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 show consanguinity. Patient II.4 of family 11 (fig 1) shows peculiar clinical features, since he suffers prelingual profound deafness and vestibular dysfunction; however, to date (39 years old), he has not shown any symptons of RP. Moreover, he is also severely mentally retarded. In the remaining patients, mental retardation or physical dysmorphology were not noted.

Clinical tests were performed on all affected subjects. The ophthalmological studies included determination of visual acuity, fundus ophthalmoscopy, visual field examination, electroretinography, and visually evoked potentials. The diagnostic criteria used to recognise RP were night blindness, loss of visual field and acuity, and altered or unrecordable electroretinogram. The otolaryngological evaluation included audiological examination, neurootological exploration, electronystagmography, and posturography.

DNA studies

DNA was extracted from peripheral blood leucocytes from each person.[25] PCR amplifications were performed in a total volume of 50 ±l containing 200 ng of human

genomic DNA, 50 mmol/l KC1, 10 mmol/l Tris-HCl, pH 8.3, 1.5 mmol/l MgCl,2 100

,ug/ml gelatin, 200 gmol/l dNTP, 1 jmol/l of each primer (50 pm), and 1 unit of Taq

DNA polymerase. The polymorphic microsatellite markers and their PCR conditions are

given in tables 1 and 2. [26-33] The amplified DNA was electrophoresed on a 8-12%

polyacrylamide gel and stained with silver. The marker ROM1/2 was analysed by SSCP

with electrophoresis on a polyacrylamide gel containing 5% glycerol.

Linkage analysis

All the markers used in the present study were microsatellite markers, except for

ROM1/2 which detects a polymorphism within intron 1 of the ROMI gene.[34 35] Pairwise

and multipoint linkage analysis was performed using the MLINK and LINKMAP

subroutines of version 5.1 of the LINKAGE program.[36] The linear order of markers on

chromosomes 1lq[37] and 3q,[20] and distances between them, are shown in figs 2 and 3.

Penetrance was assumed to be 0.99 and the mode of inheritance was assumed to be

autosomal recessive.

RESULTS

Haplotype analysis (fig 1) suggested that in 12 of the 18 families (67%), linkage for the

USHlB locus existed. Pairwise lod scores for chromosome 11 marker loci are presented

in table 3. Taking all 12 families together, pairwise linkage analysis gives lod scores of

14.032, 7.259, and 13.524 for D11S527, OMP, and D11S911 respectively, at zero

recombination distance.

Family 11 shows linkage to markers placed on chromosome 1 lq if we assume that patient II.4 is affected with USH 1. If only the two females are considered as affected, this family fails to show linkage to any of the USH loci described.

In the 12 families linked to 1 q, none of the alleles that closely flank USHIB displayed significant differences between gene frequencies of the parental USH1B chromosomes and the normal parental chromosomes, which indicates that linkage disequilibrium is not present in this Spanish sample.

The remaining six USH 1 families were studied with markers located in other Usher candidate regions: 14q32, llpl5.1, l0q, 21q21, 3q21-q25, and 1q42. For two of the families (fig 4), slightly positive lod scores were observed for five markers located on chromosome 3q (Zmax=1.880, 0=0.000 for D3S1279) (table 4). For the remaining four USHI families (fig 5), no conclusive evidence of linkage to any loci previously described was obtained.

With respect to family 15, which includes three sibs with clinical symptoms of USHI, it is important to note that the mother suffers profound bilateral sensorineural deafness, difficult but intelligible speech, normal vestibular response, and absence of RP. The father had no ocular or otological symptoms. The dead grandmother, who was not investigated, also had a hearing impairment of unknown degree. Taking this family as USH1, segregation of the disease with markers of the loci described was not observed.

DISCUSSION

Even though a high percentage of the 18 USH1 Spanish families studied are linked to chromosome 11q (67%), there are an important number of cases (33%) that fail to show linkage to either 11q or other known regions for USH1 (11p, 14q, 10q, or 21q). The results indicate considerable genetic heterogeneity in these USH1 families. For the 11q linked cases, lod scores obtained here are in agreement with those previously reported elsewhere with respect to the physical mapping and localisation of USH1B. These new data, thus, place the candidate gene in the region between D1S527 and D1S911, which is compatible with other reports[12 13] and which, although we have not yet analysed mutations in the myosin gene, supports the role of this gene in the pathogenesis of USH1B families.[22-24 38 39] These results also discount the possible involvement in the USH1 syndrome of other genes formerly considered candidates, such as ROMI and INT2, [12 35] since recombinant events have been found with these loci.

It is important to emphasise that at least two of the USH1 families in this study show positive lod scores for markers located on chromosome 3q. This chromosomal region is exactly the same as that in which Sankila et al[20] and, to a lesser extent, Kimberling et al[21] reported linkage for USH3 families from Finland and for some Swedish USH2 families, respectively. To date, families classified as USH1 have not been found to be related to this locus, although the possibility that some USH3 patients show a phenotype similar to USH1 or USH2 has already been suggested.[40] Lod scores are not high, but they contrast with the negative results for markers located on chromosome 3q shown by the other USH1 families linked to 1q or unlinked to the other loci described, and also with the data obtained for markers placed in other candidate regions in these two families.

Vestibular function is a reliable and consistent discriminator between Usher types I and II since most, if not all, type I patients lack vestibular function.[41] There are, nevertheless, cases of USH2 and USH3 in which some abnormal vestibular response is reported.[6 42] In this study, families 1 to 18 could not be diagnosed as USH2 or USH3 since they had severely abnormal vestibular function and profound congenital prelingual hearing loss leading to total deaf-mutism. Similarly, the onset of RP is not conclusive for differentiation of the three types.[43] Therefore, according to the present results and to others which are similar,[20 21] families classified as USHI, USH2, and USH3 have all shown linkage to 3q. It would be interesting to re-examine the clinical parameters, owing to the wide range of clinical symptoms in every Usher type, and to perform genotype-phenotype correlations in order to discriminate between the different types and to investigate whether some of them may be the result of allelic manifestations of a single gene.

It is important to stress the special clinical aspects of family 11, which has in the same sibship two affected females who show a typical USH1 phenotype and one male who suffers profound hearing impairment and absence of RP. This family has been considered as USH1, mainly because of linkage to 11q markers. Moreover, since allelic variants of the myosin VIIA gene may be involved in both USH1B syndrome and an isolated form of deafness (DFNB2),[44 45] the possibility that a mutation in this gene is responsible for the phenotypic variability within this family cannot be discarded. It is also interesting to note that the same genomic region is involved in a dominant form of non-syndromic deafness (DFNA1 1).[46] This phenomenon has already been observed in other chromosome regions (DFNA3 and DFNB1 to 13q11, [47 48] DFNA9 and DFNB5 to 14q12[49]).

Family 15 may represent a similar example to family 1 1, since it also presents intrafamilial clinical variability if we consider members of different generations. This family remains unlinked to any Usher locus described to date. It could be possible that one mutation in an unknown gene is responsible for syndromic and non-syndromic forms of hearing loss within this family. If this supposition were correct, each family would represent an example of a single mutation responsible for syndromic and isolated forms of deafness.

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