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ARTICLE

A novel locus for Usher syndrome type II, USH2B, maps to chromosome 3 at p23–24.2

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Usher type II syndrome is defined by the association of retinitis pigmentosa, appearing in the late second to early third decade of life, with congenital moderate to severe non-progressive hearing loss. This double sensory impairment is not accompanied by vestibular dysfunction. To date, only one Usher type II locus, USH2A, at chromosome band 1q41, has been defined. Here, we demonstrate by linkage analysis, that the gene responsible for Usher type II syndrome in a Tunisian consanguineous family maps to chromosome 3 at position p23–24.2, thus providing definitive evidence for the genetic heterogeneity of the syndrome. A maximum lod score of 4.3 was obtained with the polymorphic microsatellite markers corresponding to loci D3S1578, D3S3647 and D3S3658. This maps the gene underlying USH2B to a chromosomal region which overlaps the interval defined for the non-syndromic sensorineural recessive deafness DFNB6, raising the possibility that a single gene underlies both defects. However, the audiometric features in the patients affected by USH2B and DFNB6 are very different.

Keywords: Usher syndrome type II; homozygosity mapping; DFNB6

Introduction

Usher syndrome represents a group of autosomal recessive disorders characterised by retinitis pigmentosa associated with congenital sensorineural hearing loss. The prevalence of this syndrome among profoundly deaf children may be as high as 8%.[1] Three clinical forms have been reported:

(i) type I which is defined by congenital severe to profound, hearing loss, vestibular dysfunction, and development of retinitis pigmentosa during the first to early second decade;

(ii) type II which is characterised by congenital, moderate to severe, stable hearing loss, normal vestibular function, and appearance of retinitis pigmentosa in the late second to early third decade;

(iii) type III which associates progressive hearing loss and retinitis pigmentosa with a variable age of onset;[1] vestibular troubles may occur in parallel to loss of auditory function.

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Clinical heterogeneity is accompanied by genetic heterogeneity. Six loci for Usher syndrome type I, USH1 (A to F), have been reported mapping to chromosomes 14q32,[2] 11q13,[3,4] 11p15,[4] 10q,[5] 21q21[6] and 10,[7] respectively. Only the gene responsible for USH1B has been identified.[8]

The prevalence of Usher type II syndrome in the general population in Europe and the USA has been estimated at 1/100 000. Evidence for genetic heterogeneity of this syndrome was reported several years ago,[9] but only one locus, USH2A, has been identified to date. This locus maps to chromosome band 1q41[10–12] and the corresponding gene has recently been identified.[13] According to a study performed on populations from England, Ireland, Italy, The Netherlands, Spain, Sweden, Columbia and the USA, approximately 12.5% of families affected with Usher type II, fail to show linkage to chromosome band 1q41 (USH2A).[12]

Here, we report on the study of a consanguineous family originating from the south of Tunisia, affected with a typical type II form of Usher syndrome. Genetic linkage analysis using homozygosity mapping led us to identify a novel locus referred to as USH2B.

Results and Discussion

Clinical Features

All members of family Us, affected and unaffected, underwent a general audiological and ophthalmological clinical evaluation. A complete medical history was obtained for each affected individual. Air-conduction pure-tone average (ACPTA) threshold in the conversational frequencies (0.1, 1 and 2 kHz) was calculated for each deaf ear, and was used to define the severity of the hearing loss according to the better hearing ear: mild (20 dB ACPTA 39 dB), moderate (40 dB ACPTA 69 dB), severe (70 dB ACPTA 89 dB), and profound (ACPTA ≥ 90 dB). Audiometry tests detected a moderate to severe sensorineural hearing loss in the affected individuals. Very similar audiometric sloping curves were observed in each patient, showing that the hearing loss predominates in the high frequencies; the values ranged from 20–50 dBHL at 250 Hz to 70–95 dBHL at 8000 Hz, for the seven affected individuals. Ophthalmological examination detected the development of a retinitis pigmentosa in all deaf persons, with the appearance of night blindness occuring in the late second to early third decade. No vestibular dysfunction was noted using the caloric test, nor had a delay in the age of walking been noticed in any of the affected individuals. On the whole, the clinical signs observed in affected individuals in family Us, were indicative of a typical type II form of Usher syndrome. All heterozygous carriers exhibited normal results for the audiometric tests, and for the ophthalmological examination.

Linkage Analysis

In the first instance, we tested the possible involvement of the sole locus already known to be associated with a type II form of Usher syndrome, USH2A.[12] Upon its elimination, given the possibility that a single locus might be responsible for different clinical forms of Usher syndrome, we investigated the possible involvement of the other known USH loci: USH1 (A to F)[2–7] and USH3.[14] No linkage could be detected between the disease and the polymorphic markers associated with these loci. We then tested the possibility of linkage with the DFNB (DFNB1 to DFNB15) loci, which underlie isolated forms of autosomal recessive deafness. Indeed, it has been shown that *MYO7A*, the gene encoding myosin VIIA, is responsible for both USH1B[15] and an isolated form of recessive deafness, DFNB2.[8,16] During this exclusion mapping, linkage was observed with marker AFM198yf2 (locus D3S1289) which is located within the candidate interval defined for DFNB6 at 3p24.[17] Eight microsatellite markers were used to map the USH2 locus involved in Usher syndrome in family Us. Pairwise haplotypes and lod scores are shown in Figure 1. A maximum lod score of 4.3 was obtained at loci D3S1578 and D3S3658. Haplotypes are presented such as to minimise the recombination events occuring during meiosis. From the analysis of the data, we conclude that the gene responsible for the type II form of Usher syndrome in family Us is located in a chromosomal interval spanning a maximum distance of 23 cM, between D3S1289 and D3S1266. The haplotypes of the three affected siblings, IV-8, IV-9 and IV-10, in the absence of the possible analysis of the genotype of their mother (III-4), deserves further discussion. The Usher syndrome in III-4 might involve a different locus, and the Usher syndrome in the three affected children might result from a digenism. However, the haplotypes of these patients in the chromosomal regions of the other Usher loci do not argue for their involvement, ie no evidence for homozygosity in these chromosomal regions could be obtained in the reconstituted genotype of III-4. Moreover, the phenotypes of the three affected individuals in branch III and in the other affected members are very similar. Alternatively, the mother (III-4) and the father (III-3) might carry the same

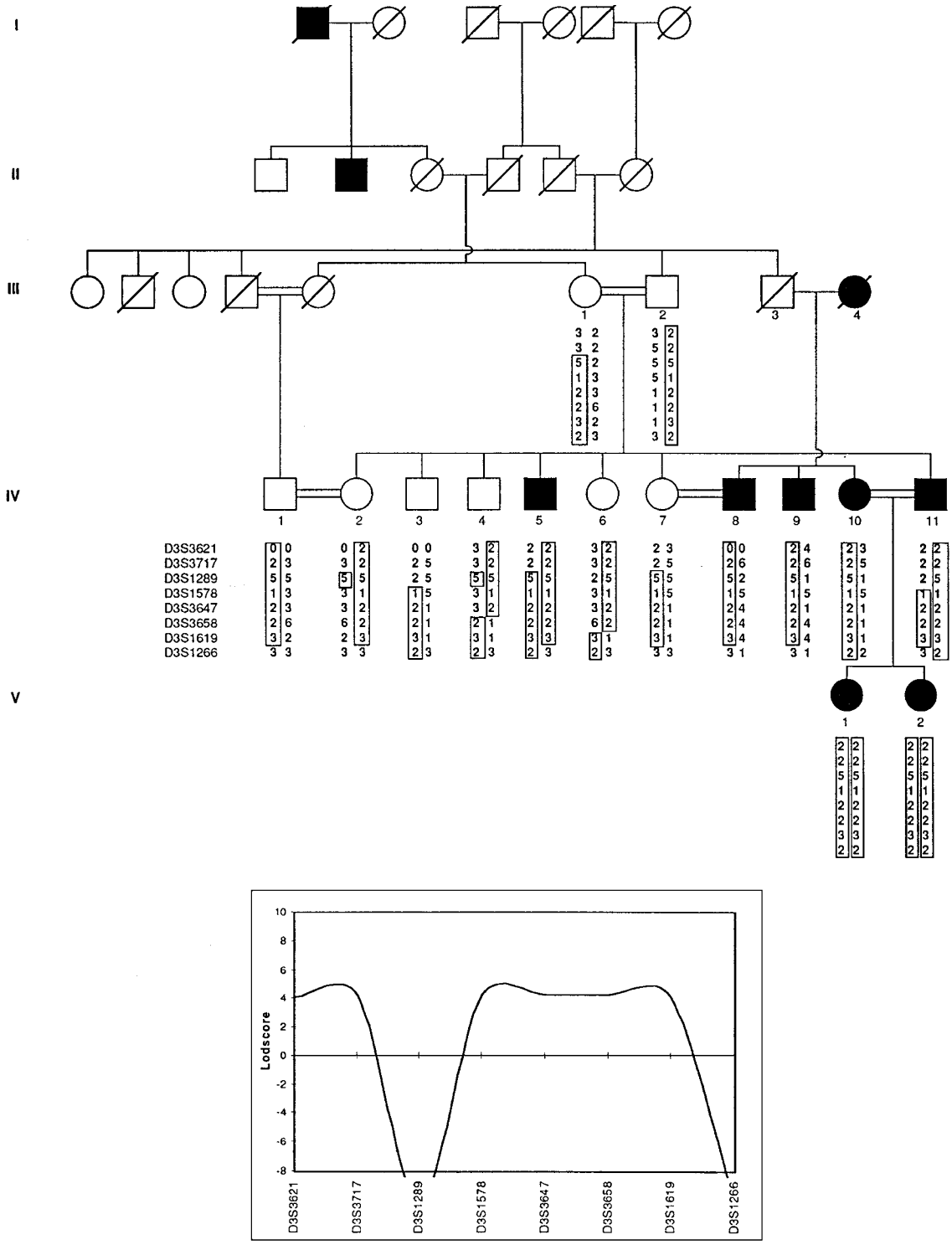

Figure 1 *Homozygosity by descent in a consanguineous Tunisian family affected with a form of Usher type II syndrome (USH2B). Dark symbols correspond to affected individuals. The haplotype associated with USH2B is boxed. The genotypes of individuals IV-4, IV-6 and IV-11 can be explained by maternal double recombination events in the 27 cM region between D3S3717 and D3S1266. The* USH2B *gene is localised between D3S1289 and D3S1266*

mutation. Since affected individuals share only a maternal allele, an allele of D3S1578, this would leave a very small chromosomal region as candidate interval for the responsible gene. Several ancestral recombination events would have reduced the size of the homozygosity region. In this line, the recombinations flanking locus D3S1578 observed in four out of seven siblings in generation IV support the idea of a high recombination activity in this particular chromosomal region.

The USH2B interval defined here overlaps over 14 cM (between D3S1289 and D3S1619) the distal part of the previously defined chromosomal interval for the isolated deafness DFNB6, a 23 cM interval extending between D3S1766 and D3S1619[17] (see Figure 2). Therefore, the possibility that a single gene underlies both USH2B and DFNB6 should be considered. Such a situation has already been shown for USH1B and DFNB2,[8,15,16] and may also apply to USH1C and DFNB18[4,18] or USH3 and DFNB15,[14,19] which colocalise. In the frame of this hypothesis, a more deleterious mutation for USH2B than for DFNB6 would be expected. However, in the single family affected with DFNB6 described so far, affected individuals suffer from profound congenital deafness,[17] whereas the reported USH2B family has the typical moderate to severe hearing loss characteristic of Usher type II syndrome. These audiometric data are more in favour of the existence of two different genes underlying USH2B and DFNB6. A large number of genes and ESTs (up to 324 in Genome DataBase) have been mapped in the USH2B interval. In the absence of any potentially attractive candidate genes in the region, the search for *USH2B* itself will only be undertaken when the localisation interval has been narrowed down to an amenable size.

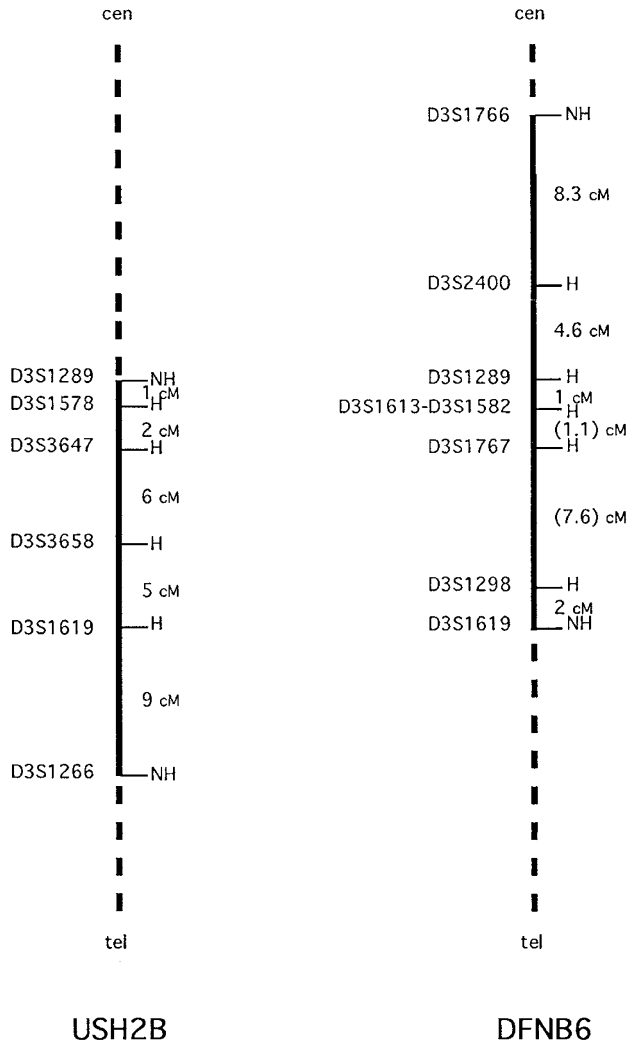

Figure 2 *Schematic representation of the part of chromosome 3 showing USH2B and DFNB6 localisations.* USH2B *and* DFNB6 *candidate intervals overlap on a 14 cM distance, between D3S1289 and D3S1619. H and NH represent respectively the homozygous and non-homozygous status of the microsatellite markers in the affected individuals (except individuals IV-8, IV-9 and IV-10)*

Methods

Clinical Examination

A consanguineous family, family Us, with marriages between first cousins (see Figure 1) living in the south of Tunisia, was studied. It comprises ten affected individuals, among whom eight are alive.

The diagnosis of Usher syndrome in this family was established by an ophthalmological examination motivated by the appearance of night vision problems. Retinitis pigmentosa was diagnosed in affected individuals, based on concentric loss of visual field and granular accumulation of pigment at the ocular fundus. Audiometry tests were performed with a Belltone 2000 clinical audiometer on all family members. They consisted of pure-tone audiometry with air and bone-conduction at 250, 500, 1000, 2000, 4000 and 8000 Hz. In addition, bilateral vestibular function was investigated by caloric stimulation.

Genotyping

Genomic DNA was extracted from 10 ml blood samples using the standard phenol–chloroform technique. PCR amplifications on 60 ng genomic DNA were performed using a Techne thermocycler, in a final volume of 50 µl containing 2 µM primers, 125 µM dNTP, 1.5 mM $MgCl_2$, 50 mM KCl, 10 mM Tris-HCl pH 8.8 and 1U Taq DNA polymerase. PCR products were run on a 6% polyacrylamide sequencing gel, transferred to N^+-Hybond membrane (Amersham, UK) and hybridised with a poly AC probe labelled with α^{32}-P-dCTP.

Polymorphic microsatellite markers from the Généthon collection were used.[20] For the exclusion of linkage to

previously identified loci, markers were chosen according to the panel indicated by the authors. The USH2A locus has been excluded using AFM205xd8 (locus D1S237), AFM280ycl (locus D1S474) and AFM196xh4 (locus D1S229).[12] A putative linkage to the Usher type I (A to F) and type III loci was then investigated. In parallel, we tested a possible linkage to the loci corresponding to autosomal recessive forms of isolated deafness (DFNB forms).

Linkage Analysis

Lod scores were calculated using the MAPMAKER/HOMOZ program[21] (version 0.9), a computer package based on an algorithm especially designed for homozygosity mapping. Usher type II syndrome was assumed to be inherited in a recessive manner and coded as fully penetrant with a disease allele frequency estimated to 10^{-3}. The lod score value was 4.3; changing the disease allele frequency to 10^{-2} only slightly modified the lod score value (4.2). The allele frequencies of the polymorphic markers and the meiotic recombination frequencies for males and females were assumed to be equal.

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