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Uniparental disomy of chromosome 13q causing homozygosity for the 35delG mutation in the gene encoding connexin26 (*GJB2*) results in prelingual hearing impairment in two unrelated Spanish patients

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Inherited hearing impairment is a highly heterogeneous group of disorders with an overall incidence of about 1 in 2000 newborns.[1] In approximately 70% of cases, the auditory impairment is not associated with other clinical features, that is, it is non-syndromic. The most frequent condition is a severe or profound hearing loss of prelingual onset, which is inherited mainly as an autosomal recessive trait.[1] To date, 31 different DFNB loci for autosomal recessive non-syndromic hearing loss have been reported, and 16 genes have been identified.[2] Among these loci, DFNB1 in 13q12 stands out because of its complexity and clinical relevance. It contains the gene *GJB2*, which encodes connexin26, a component of intercellular gap junctions. Mutations in the *GJB2* gene are responsible for up to 50% of all cases of autosomal recessive hearing impairment in most of the populations tested so far, with a frequent mutation (35delG) accounting for up to 86% of the *GJB2* mutant alleles in white populations.[3 4] However, not all the DFNB1 mutations affect the *GJB2* gene. Recently, several research teams found a deletion in the 13q12 region which is frequently inherited in double heterozygosity with mutant *GJB2* alleles in affected subjects,[5–7] but it was also found in homozygosity.[6 7] Molecular characterisation of this deletion, termed del(*GJB6*-D13S1830), showed that it encompasses 342 kb and it does not affect the *GJB2* gene, but it truncates the gene encoding connexin30 (*GJB6*), another gap junction protein expressed in the inner ear.[6] The existence of this deletion was first suspected by the finding of inconsistencies in the segregation of genetic markers distal to *GJB2*.

Key points

- Mutations in the gene encoding the gap junction protein connexin26 (DFNB1 locus on 13q12) are responsible for up to 50% of all cases of autosomal recessive hearing impairment in most populations, the 35delG mutation being the most frequent in white populations.
- Here we report two unrelated cases of homozygotes for 35delG whose biological fathers were not carriers of the mutation. The study of the segregation of polymorphic genetic markers showed uniparental (maternal) disomy of chromosome 13, causing homozygosity for the mutation. In both cases, the disomic maternal gamete may have resulted from non-disjunction of chromosome 13 in meiosis II.
- These two cases represent the first description of UPD(13) with an abnormal phenotype, and they are also the first cases of UPD resulting in non-syndromic hearing impairment.

Here we report another inconsistency in the segregation of markers in the 13q12 region in two unrelated cases of subjects with prelingual hearing impairment. In these two cases, uniparental disomy of chromosome 13 caused homozygosity for

the 35delG mutation in the *GJB2* gene, resulting in the hearing defect.

MATERIALS AND METHODS

Informed consent was obtained from all the subjects that were enrolled in this study. Peripheral blood samples were obtained, and DNA extraction was performed by standard procedures.

Screening for the 35delG mutation was carried out by PCR amplification of a 122 bp DNA fragment including the first 34 codons of the *GJB2* gene, using the following primers: forward, 5′-CAAACCGCCCAGAGTAGAAG-3′; reverse, 5′-CATAATGCGAAAAATGAAGAGG-3′. The forward primer was 5′ end labelled with a fluorescent dye (TET, 6-FAM, or HEX, which allows us to mix three samples per tube in the separation procedure). The PCR reaction was carried out in a total volume of 15 µl including 20-40 ng of genomic DNA from the patient, 10 pmol of each primer, 2.5 nmol of each dNTP, $MgCl_2$ at a final concentration of 1.5 mmol/l, and 0.75 units of Ampli*Taq* Gold DNA polymerase (Perkin-Elmer), in the buffer provided by the manufacturer. PCR was performed using standard conditions with an annealing temperature of 59°C. Protruding tails of adenine nucleotides, which are added by the Ampli*Taq* Gold DNA polymerase to the 3′ ends of the DNA product during the PCR, were eliminated by treatment with T4 DNA polymerase (Roche), under the conditions recommended by the manufacturer. Then, samples were resolved by capillary electrophoresis in an Abi Prism 310 Genetic Analyzer (Applied Biosystems). The rationale of this procedure is to detect the loss of one nucleotide in the mutant allele. Then, the presence of the mutation was confirmed by DNA sequencing of a second PCR product.

Primers and PCR conditions for the amplification of the microsatellite markers used in this study have been previously reported.[8 9]

RESULTS

During the routine screening of subjects with non-syndromic prelingual hearing impairment for the 35delG mutation, we found two unrelated cases, E112-3 and E232-3, who were homozygous for 35delG, and had a relevant characteristic in common. In both cases, the mother of the patient carried the mutation, but the father did not. Both cases were sporadic, not having any other affected relative. In family E112, there was also a brother with normal hearing who was a carrier of the 35delG mutation (fig 1). Both patients and their participating relatives were genotyped for seven microsatellite markers flanking the *GJB2* gene within an approximate 2 cM interval. These included D13S175, D13S1275, and D13S292,[8] and D13S1830, D13S1831, D13S1832, and D13S1835.[9] The marker order is indicated in fig 1. Subject E112-3 was homozygous for all these markers except for the most distal (D13S292). In addition, haplotype analysis showed that the subject had not inherited any allele from his father for five of these markers (fig 1). His brother had inherited the 35delG mutation from their mother, and there was no segregation inconsistency in the alleles he had received from his parents, as expected. Subject E232-3 was homozygous for the seven markers, and she did not share any allele with her father for six of them, as shown by haplotype analysis (fig 1). False paternity was investigated by genotyping the patients and their parents for a series of highly polymorphic microsatellite markers in other chromosomes. Ten markers were completely informative, namely D1S220, D1S234, D1S425, D7S2420, D7S2459, D14S288, D15S153, D15S205, D16S404, and D21S1252.[8] In both cases E112 and E232, the non-maternal alleles of all these markers in the child fitted those of the alleged father, the residual probability of a false paternity being 10^{-7}. This suggested that the anomalous inheritance would be confined to chromosome 13.

Therefore we genotyped the patients and their parents for a set of 13 additional markers from the whole long arm of chromosome 13, evenly distributed at intervals of about 10 cM (fig 1). In patient E112-3, there were two markers with homozygosity for an exclusively maternal allele (D13S217 and D13S265). There was heterozygosity for all the other markers. For six of them, the patient did not share any allele with his father (maternal heterodisomy). For the remaining five markers, although the patient did share at least one allele with his father, his genotypes were also consistent with maternal heterodisomy. As regards patient E232-3, there were four markers with homozygosity for a exclusively maternal allele (D13S156, D13S265, D13S158, and D13S1265). There was also homozygosity for two other markers (D13S173 and D13S285) for which the patient did share an allele with her father, but the genotypes were also consistent with maternal disomy. There was heterozygosity for the remaining seven markers, all the genotypes being consistent with maternal heterodisomy (for two of these markers, the patient did not share any allele with her father).

DISCUSSION

Altogether, our data indicate that the anomalous segregations of the 35delG mutation in the two cases reported here are the result of uniparental disomy (UPD) of chromosome 13. UPD is defined as the inheritance of both homologues of a pair of chromosomes from only one parent.[10–12] This includes isodisomy (two copies of the same parental chromosome), heterodisomy (one copy of each homologue from the same parent), or a mixture of both.[11 12] In case E112-3, maternal isodisomy seems to be confined to 13q11-q12, with maternal heterodisomy in the rest of the long arm. Conversely, in case E232-3, maternal isodisomy alternates with maternal heterodisomy along 13q. Depending on the affected chromosome and on the resulting homozygosities, UPD can produce no clinical manifestations or a diversity of abnormal phenotypes. In our two cases, maternal UPD of chromosome 13 results in homozygosity for the 35delG mutation, which causes profound, prelingual non-syndromic hearing impairment. Similar cases of uniparental disomy creating homozygosity for autosomal recessively inherited mutations have been reported in over 20 disorders.[12 13] No other clinical signs or symptoms were observed in our two patients, who were aged 15 years (E112-3) and 2 years (E232-3) at the time of examination. In the last few years, several cases of either maternal or paternal UPD of chromosome 13 (UPD13) have been reported.[14–20] All of these cases were phenotypically normal, indicating that there are no maternally imprinted genes in chromosome 13.[14 16] Our data further support this conclusion.

Mechanisms leading to UPD include (1) gamete complementation, when the zygote arises from the union of a disomic gamete and a nullisomic gamete; (2) trisomy rescue, when a trisomic zygote loses one chromosome in an early mitotic division (one third of the cases result in UPD); (3) monosomy rescue, by mitotic duplication of the monosomic chromosome, which leads to isodisomy for the whole chromosome; and (4) postzygotic errors, when in a normal zygote one chromosome is lost and it is replaced by duplication of its homologue (isodisomy for the whole chromosome).[11 21] In our two cases of UPD(13), regions of maternal isodisomy and heterodisomy are observed, which excludes monosomy rescue and postzygotic errors. Although we cannot distinguish which of the two remaining mechanisms (gamete complementation or trisomy rescue) led to UPD(13) in the cases reported here, we can conclude that in both cases a disomic maternal gamete was involved. Formation of abnormal gametes is the result of meiotic non-disjunction events. When non-disjunction errors occur in meiosis I, they result in heterozygosity for centromeric genetic markers (primary heterodisomy), whereas if they occur in meiosis II, they result in homozygosity for the

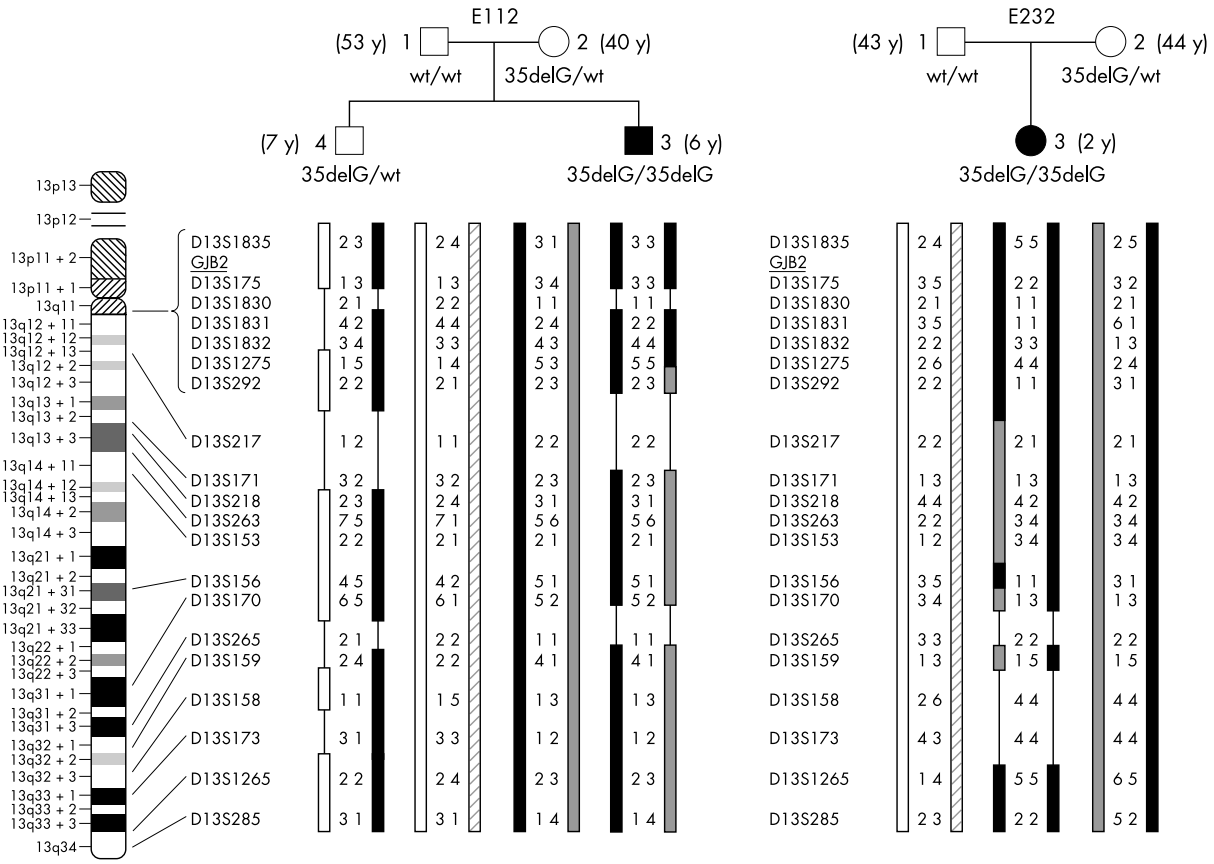

Figure 1 Pedigrees of families E112 and E232, showing the genotypes for a set of genetic markers from chromosome 13. Ages in years (y) are indicated between parentheses. The carrier status for the 35delG mutation in the *GJB2* gene is shown below each subject symbol; wt, wild type allele. The location of the genetic markers, as well as that of the *GJB2* gene, is indicated to the right of a schematic drawing of chromosome 13. Currently, the most centromeric sequence contig from chromosome 13 is NT_009917.11 (NCBI database, http://www.ncbi.nlm.nih.gov). In this contig, marker D13S1835 is approximately 1.55 Mb from the centromeric end. Alleles are represented by numbers. Vertical bars represent chromosomes. Regions of non-informativeness (that is, when it was not possible to determine unambiguously the parental chromosome from which an allele in a child was being inherited) are indicated by thin lines.

centromeric markers (primary isodisomy). Recombination events can introduce regions of homozygosity in a situation of primary heterodisomy (secondary isodisomy) and, conversely, they can introduce regions of heterozygosity in a situation of primary isodisomy (secondary heterodisomy).[22] In both E112-3 and E232-3, there are regions of primary isodisomy for centromeric markers, whereas secondary heterodisomy is observed in other regions. This suggests that the non-disjunction event leading to the formation of the disomic maternal gamete took place in meiosis II. The formation of abnormal gametes leading to UPD is frequently found in association with chromosomal rearrangements. In fact, all of the published cases of UPD(13) were associated with Robertsonian translocations or isochromosomes.[14–20] However, the karyotypes of both patients E112-3 and E232-3 were normal, as well as that of subject E232-1 (father of E232-3) (data not shown). No karyotyping data from the other parents could be obtained.

The two cases reported here are the first cases of UPD(13) with an abnormal phenotype, and they also represent the first cases of UPD resulting in non-syndromic hearing impairment. They were found among a total of 115 unrelated affected subjects who were homozygous for the 35delG mutation (1.7%). Given the high incidence of inherited hearing impairment, this frequency should be kept in mind when performing large screenings of patients for recessively inherited mutations, and UPD should be considered a possibility when anomalous segregation patterns are found in routine genetic testing.

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