A rare event of maternal UPD in a proband with congenital non-syndromic hearing impairment with homozygosity for GJB2 p.W24X mutation

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Accepted 17 June, 2010

In the present study, the authors reported a family of a male proband with prelingual, profound non-syndromic hearing impairment homozygous for p.W24X mutation in GJB2 gene, arising as a result of maternal uniparental disomy of a part of chromosome 13q.

Key words: Hearing impairment, p.W24X mutation, uniparental disomy.

INTRODUCTION

Hearing impairment is the most common sensory disorder affecting 1 in 1000 newborns worldwide (Cohen and Gorlin, 1995). More than 100 loci have been associated with the nonsyndromic hearing loss, with majority of the cases (~80%) being autosomal recessive in inheritance (Cryns et al., 2004). DFNB1 at 13q12 is the first locus identified for hearing impairment and is subsequently focused more because of its complexity and clinical relevance. It harbors the gene GJB2 which encodes for connexin 26, a component of intercellular gap junction. It plays a vital role in auditory signal transduction mechanism by recycling the potassium ions in the cochlea. It contains two exons separated by a large intron. Mutations in the GJB2 gene responsible for DFNB1, account for up to 50% of prelingual recessive nonsyndromic deafness (Denoyelle et al., 1997; Green et al., 1999). In Indian population, p.W24X (c.71G > A) mutation was found to be the most frequent allele causing autosomal recessive non-syndromic hearing impairment (Padma et al., 2009; Joseph et al., 2009; Ramchander et al., 2005; Ramshanker et al., 2003; Maheswari et al., 2003) and haplotype analysis of markers flanking the GJB2 gene suggested a possible founder effect for this mutation in Indian population (Ramshanker et al., 2003; Maheswari et al., 2003). The mutation affects the first transmembrane domain of the connexin26 protein (Figure 1) and results in the formation of a protein that is one-tenth the length of the wild type protein (Kelsell et al., 1997). However, not all the mutations at DFNB1 locus affect the GJB2 gene as several researchers found a deletion in the 13q12 region which is frequently inherited in double heterozygosity with mutant GJB2 alleles in affected subjects (Lerer et al., 2001; del Castillo et al., 2002; Pallarez-Ruiz et al., 2002). Molecular characterization 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 (del Castillo et al., 2002). The existence of this deletion was first suspected by the finding of inconsistencies in the segregation of genetic markers distal to GJB2 gene.

Another inconsistency in the segregation of markers in the 13q12 region was reported by Alvarez et al. (2003) and Yan et al. (2007). Alvarez et al. (2003) screened parents of 115 unrelated Spanish patients with homozygous 35delG mutation and pre-lingual hearing impairment and found aberrant inheritance of the mutation in two families: one with a 15 year old affected boy and the other with a 2 year old affected girl. In both families, the mothers of the patients carried the mutation, but the fathers did not. Genotyping of microsatellite markers flanking the GJB2 gene in the two patients and their
parents indicated that mutations in both the patients resulted from maternal uniparental disomy for chromosome 13 (mat UPD13). In another attempt made by Yan et al. (2007) who screened parents of 50 unrelated patients with homozygous 35delG mutation, there was a family in which the father was heterozygous for the mutation but the mother was not. The proband was a Hispanic boy with profound bilateral sensorineural hearing loss and genotyping of microsatellite markers flanking the $GJB2$ gene in the proband as well as the parents revealed paternal isodisomy in three segments and paternal heterodisomy in two segments. Thus in all the three cases reported so far, non-disjunction of chromosome13 leading to UPD 13 could have occurred in meiosis II, with distal recombinational events resulting in heterodisomic segments.

In Indian population p.W24X mutation is found to be the most frequent cause of hearing defect and not c.35delG which is commonly found in Caucasian population. Here we report another case of UPD wherein maternal disomy of chromosome 13q resulted in homozygosity for p.W24X mutation (unlike 35delG mutation in other three cases) in the $GJB2$ gene resulting in hearing defect. This is the first case of UPD reported in a proband with p.W24X mutation of $GJB2$ gene.

**MATERIALS AND METHODS**

In the present study, 303 profound, non-syndromic sensorineural hearing impaired patients including family members of 25 probands were tested for the presence of p.W24X mutation in the $GJB2$ gene with their consent to participate in the study. They were referred at the Government Ear, Nose and Throat (ENT) Hospital, Hyderabad, India and Schools for Deaf in Hyderabad, India. All the probands were examined by the ENT specialists for the degree of hearing impairment and also pediatricians to rule out the presence of mental retardation and other syndromes in which hearing loss occurs as one of the symptoms. The loss of hearing among the probands of the present study was graded based on the Pure Tone Audiometry. Criteria for inclusion of cases in this study was deafness graded as profound (≥90 decibles) sensorineural hearing loss with no associated acquired etiology or syndromes. Also 200 healthy controls with normal hearing and without the history of deafness in their family were selected at random from the same population to compare with the data generated on the probands.

DNA from all the patients and controls was isolated from the whole blood using rapid non-enzymatic method (Lahiri and Nurnberger, 1991) and screened for p.W24X mutation in $GJB2$ gene using PCR-RFLP. Primers 1F: 5'-TCT TTT CCA GAG CAA ACCGC-3') and 1R: 5'- GAC ACG AAG ATC AGC TGC AG-3') were used for amplification and the reaction was performed with 50 ng genomic DNA, 2.5 pmol primers, 200 μM dNTPs, and 0.25 U Taq DNA polymerase in a total reaction volume of 10 μl for 40 cycles (each cycle of 94°C for 30 s, 60°C for 30 s and 72°C for 60 s). The PCR products were digested with Alu1 restriction enzyme.
Figure 2. Pedigree of family DF-436a, showing the genotypes for five genetic markers on chromosome 13. The carrier status for p.W24X mutation in the GJB2 gene is shown below each member; wt-wild type allele; mt-mutant for p.W24X mutation. The order of the genetic markers is indicated below the pedigree. The alleles are represented in base pairs. Region 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) is indicated by thin line.

and genotyped by electrophoresis on 8% polyacrylamide gels and the results were confirmed by sequencing. Primers and PCR conditions for the amplification of the microsatellite markers for haplotype analysis have been previously reported (Dib et al., 1996; Kibar et al., 1999). PCR for the markers was performed using fluorescent labeled (5' Fam labeled) forward primers. Genotyping was done using an ABI 3730 sequencer and the allele sizes were determined using gene mapper software.

RESULTS AND DISCUSSION

Screening of 303 probands with congenital, profound, non-syndromic hearing impairment for p.W24X mutation in GJB2 gene, revealed homozygosity in 24 probands and heterozygosity in four probands with no other detected mutation in Cx26. These four cases were considered as compound heterozygotes with the second mutation causing hearing impairment yet to be discovered. Of the 25 families studied, two families were compatible with mendelian segregation, one family showed co-segregation of p.W24X mutation (GJB2 gene) along with mitochondrial c.1555A > G mutation in Mt-RNR1 gene. One family however showed inconsistent segregation wherein a male proband (DF-436a) born to p.W24X carrier mother and normal father was homozygous for p.W24X mutation. The parents were first cousins with normal phenotype and there was positive family history with the first cousin of the proband being affected. To explore the cause for the inconsistent inheritance of the mutation, the proband and his parents were genotyped for five highly polymorphic microsatellite markers flanking the GJB2 gene within an approximate 2 cM interval. The order of markers is indicated in Figure 2. Other members of the family did not participate in the study. The proband was found to be homozygous for all markers except for D13S1835. Haplotype analysis showed that the proband had inherited alleles on both the homologous chromosomes only from the mother. As the parents were first cousins they had one haplotype in common. Screening of the samples for other mutations in GJB2 (c.35deG, c.167delT, c.231G-A, c.235delC, p.R127H, p.M163V etc..), GJB6 gene including deletion (GJB6-D13S1830) and mitochondrial genes MT-RNR1 and MT-TS1 did not reveal any mutation in the proband and his parents supporting UPD as the origin of homozygosity for p.W24X mutation in the proband.

UPD is defined as the inheritance of both homologues of a pair or part of chromosomes from only one parent (Engel, 1980; Robinson, 2000; Kotzot, 2001). 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 (Robinson, 2000; Kotzot, 2001). In the present study, there is evidence for maternal UPD of chromosome 13q that resulted in homozygosity for the p.W24X mutation in the proband wherein a non-disjunctional event leading to UPD could have occurred during second meiosis in the
mother. The origin of the UPD can be explained as due to a) gamete complementation when the abberant oocyte with disomic 13 might have been fertilized by a spermatic cell without chromosome 13 producing a balanced zygote or b) trisomic rescue when the abberant (disomic) oocyte would have been fertilized by a normal haploid spermatozoa and the resulting trisomic 13 zygote was rescued by the loss of the paternal chromosome 13 leading to isodisomic state.

The case reported here is the first case of UPD (13) causing homozgyosity of p.W24X mutation which is frequently observed in Indian patients resulting in non-syndromic hearing impairment. This case of UPD was found in a total of 303 unrelated affected subjects. Other studies conducted on Indian patients however did not find/report the event of UPD (Joseph et al., 2009; Ramchander et al., 2005; Ramshanker et al., 2003; Maheswari et al., 2003). Keeping in view the high incidence of hearing impairment, the present study suggests the need to consider UPD as a possibility of anomalous segregation in routine genetic testing which helps in better counseling of the patients.

REFERENCES


