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Mapping of the otogelin gene (OTGN) to mouse Chromosome 7 and human Chromosome 11p14.3: a candidate for human autosomal recessive nonsyndromic deafness DFNB18

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Identifying the genes responsible for isolated deafness in man is an important challenge. Nearly one in every 1000 children is affected by hearing impairment at birth or before 2 years of age, that is, in the prelingual period. Of these cases, approximately 65% are genetically determined, and the vast majority of these (80%) are inherited in an autosomal recessive mode (DFNB forms). To date, 22 DFNB loci have been identified. With the exception of the DFNB1 form, which accounts for about half of the cases of prelingual deafness (Denoyelle et al. 1997; Estivill et al. 1998), most of the other deafness loci are represented with only one or few affected families. As a result, most of the localization intervals are too large to undertake the identification of the corresponding genes by a positional cloning strategy. To circumvent these difficulties, we recently developed a candidate gene approach based on the isolation of genes specifically expressed in the cochlea. The products of these genes are indeed likely to play a crucial role in the development and/or the function of the inner ear, and could underlie deafness (Petit 1996). We therefore undertook the construction of a mouse cochlea subtracted cDNA library, following the representational difference analysis method (Hubank and Schatz 1994). By this approach, we first identified a gene encoding a protein related to mucin, which we named otogelin (*Otgn*), which is specifically expressed in the inner ear. Immunofluorescence studies demonstrated that this protein is a component of all the acellular membranes of the inner ear (Cohen-Salmon et al. 1997).

The acellular membranes of the inner ear, that is, the tectorial membrane in the cochlea, the otoconial membrane in the utricule, the saccule, and the cupula of the semicircular canal in the vestibule, are acellular gelatinous structures covering the neuroepithelia. Their displacement relative to the neuroepithelia, induced either by the sound in the cochlea or by movements of the head or gravity in the vestibule, leads to the deflection of the sensory hair cell stereocilia bundle, which in turn opens the mechanotransduction canal (Hudspeth and Corey 1977; Denk and Holt 1995). These membranes have been reported to be composed of collagenase-sensitive (collagens type II, V, IX), and -insensitive proteins (Richardson et al. 1987; Thalmann et al. 1987). Belonging to this last category, three proteins specific to the inner ear have been identified up to now in mouse, the α- and β-tectorins (Legan et al. 1997) and otogelin (Cohen-Salmon et al. 1997). Recently, mutations in the human α-tectorin gene TECTA have been shown to cause an autosomal dominant form of nonsyndromic deafness, DFNA12 (Verhoeven et al. 1998).

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In order to investigate whether otogelin could be involved in hearing impairment in mouse and/or human, we mapped the corresponding genes on mouse and human chromosomes. Localization on the mouse chromosomes was performed by fluorescence in situ hybridization on 50 metaphase spreads prepared from a WNP male mouse, in which all the autosomes except Chr 19 were in the form of metacentric Robertsonian translocations (Bonhomme and Guénet 1989). A 4.2-kb *Otgn* cDNA fragment (position 3174 to 7467) was used as probe. Specific labeling of the two Chr 7, in the 7B4-7C region, was observed in 44 metaphases (Fig. 1). So far, no deafness locus has been mapped to this chromosomal region (Steel 1995).

In order to map the human OTGN gene, we first hybridized a Southern blot containing *Eco*RI-digested DNAs from a human/rodent somatic cell hybrid panel (Dubois and Naylor 1993) with a mouse *Otgn* cDNA fragment (position 3174–3466). It revealed a unique 3-kb band on human Chr 11 (Fig. 2). This result also confirmed that OTGN is a single-copy gene. In order to isolate OTGN, the same *Otgn* probe was used to screen a human BAC genomic library (Genome Systems). The positive BAC clone 202p23 was subsequently subcloned and sequenced. Sequence comparison analysis with GenBank database revealed a homology of 98% with the sequence of the human genomic PAC clone 6-106f23 (accession number AC005137), which has been localized on the human Chr 11p14.3, between markers D11S1310 and 1115A14. This sequence could, therefore, be considered as the human ortholog of *Otgn*. This result is also consistent with the existence of a known synteny between mouse Chr 7 and the short arm of human Chr 11.

Very recently, an autosomal recessive form of nonsyndromic deafness, segregating in a large consanguineous Indian family, has been mapped to Chr 11p14-15.1, defining a new locus, DFNB18 (Jain et al. 1998). The candidate region covered 1.6 cM from markers D11S1307 to D11S2368. As this region includes the OTGN locus, we propose that this gene could be responsible for the nonsyndromic deafness DFNB18.

The DFNB18 interval (Jain et al. 1998) also encompasses the Usher syndrome type 1C (deafness associated with blindness) candidate region (Marietta et al. 1997). Jain and associates postulated that DFNB18 and USH1C could be allelic variants of the same gene. This situation has also been reported for the isolated deafness DFNB2 and DFNA11 and for USH1B, which are all due to mutations in the gene encoding the unconventional myosin VIIA (MYO7A) (Weil et al. 1995, 1997; Liu et al. 1997a, 1997b). However, as previously reported, *Otgn* was shown to be specifically

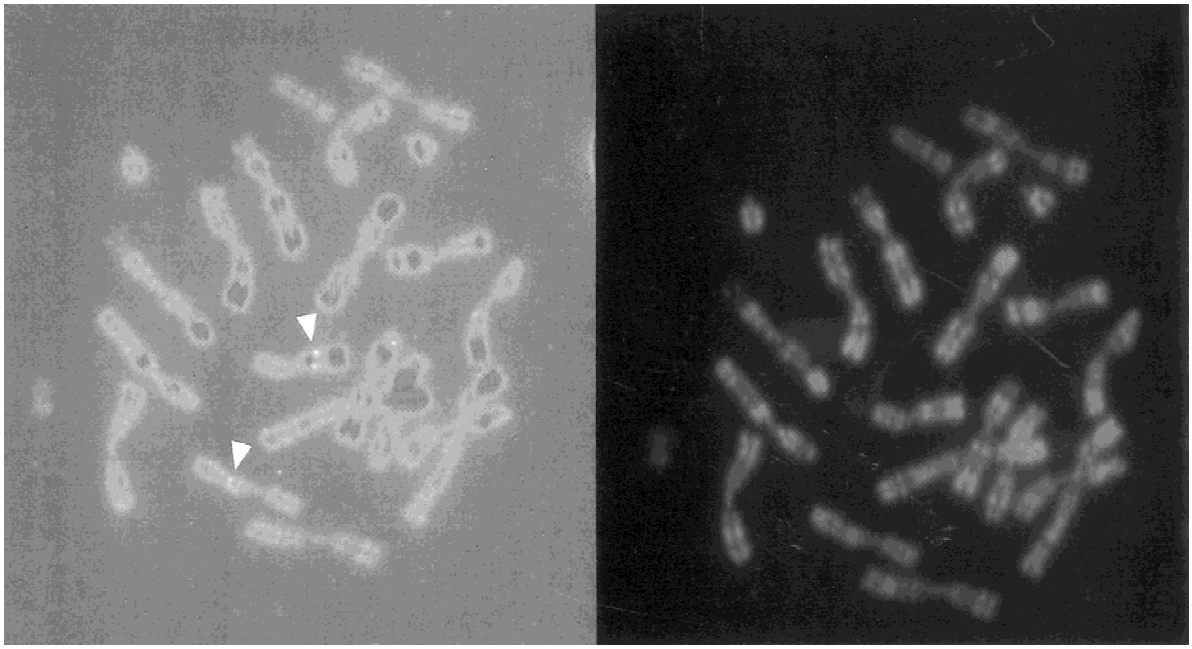

Fig. 1. Localization of *Otgn* to mouse Chr 7 by fluorescence in situ hybridization. Left, rhodamine labeling; right, FITC labeling. The arrow indicates the labeled site on Chr 7 belonging to the Robertsonian translocation Rob (7;8) of the WMP mouse. An *Otgn* cDNA fragment (position 3174 to 7467) was biotinylated by nick translation with biotin-16-dUTP, as outlined by the Boehringer Mannheim protocol. Hybridization to chromosome spread was performed according to standard protocols (Pinkel et al. 1986; Matsuda et al. 1992). Biotin-labeled DNA was added to the hybridization solution at a final concentration of 10 μg/ml (300 ng per slide). The hybridized probe was detected by means of fluorescence isothiocyanate-conjugated avidin (Vector Laboratories). Chromosomes were counterstained and R-banded with propidium iodide diluted in antifade solution pH 11.0 as described by Lemieux and colleagues (1992).

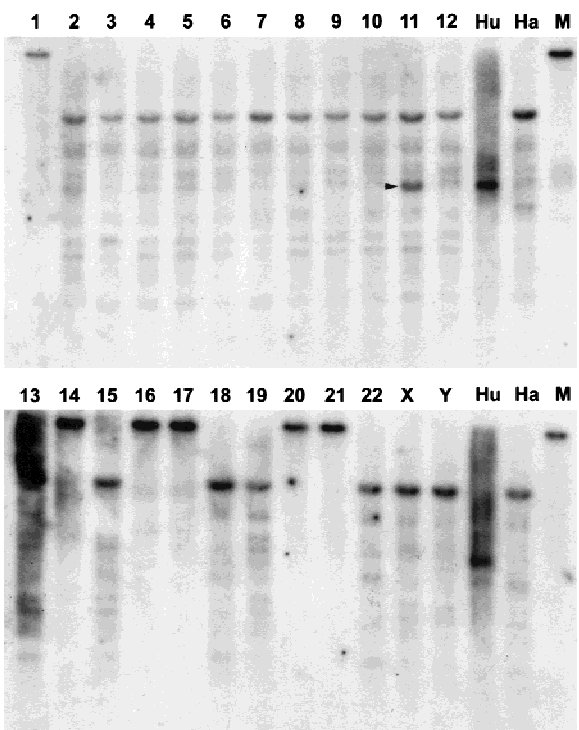

Fig. 2. Localization of OTGN to the human Chr 11 by hybridization on a human/rodent somatic cell hybrid panel. The arrow indicates the human specific band. HU, human; Ha, hamster; M, mouse. α^{32}P-dCTP-labeled *Otgn* fragment (position 3174 to 3466) was hybridized to a Southern blot containing the *Eco*RI-digested DNA from the NIGMS human/rodent somatic cell hybrid mapping panel #2 (Coriell; Dubois and Naylor 1993), at 65°C in a 7% SDS medium.

expressed in the inner ear (Cohen-Salmon et al. 1997). In particular, no expression could be detected in the eye. Therefore, it is improbable that a mutation in OTGN could cause USH1C, unless a difference in the expression pattern of the otogelin genes in mouse and human is postulated.

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