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# Physical fine mapping of genes underlying X-linked deafness and non fra(X)-X-linked mental retardation at Xq21

**I. Bach[1], D. Robinson[2], N. Thomas[3], H.-H. Ropers[1], and F. P. M. Cremers[1]**

[1]Department of Human Genetics, University Hospital Nijmegen, P.O. Box 9101, NL-6500 HB Nijmegen, The Netherlands
[2]Wessex Regional Genetics Laboratory, General Hospital Salisbury, Wiltshire SP2 7SX, UK
[3]Institute of Medical Genetics, University Hospital of Wales, Heath Park, Cardiff CF4 4XN, UK

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**Summary.** Linkage studies and cytogenetically visible deletions associated with nonspecific X-linked mental retardation (XLMR) and a specific form of deafness (DFN3) have indicated that the genes responsible for these disorders are located at Xq21. Using DNA probes from this region, we have studied several overlapping deletions spanning different parts of Xq21. This has enabled us to assign the DFN3 gene and a gene for non-specific XLMR to an interval that encompasses the locus DXS232 and that is flanked by DXS26 and DXS121.

## Introduction

Microscopically visible male-viable deletions are clustered in three regions of the human X-chromosome, i.e. band Xp22.3, band Xp21 carrying the gene for Duchenne muscular dystrophy and band Xq21 on the proximal long arm. Most of the Xq21-deletions give rise to a complex phenotype including choroideremia (CHM), mental retardation (MR), and deafness (Tabor et al. 1983; Hodgson et al. 1987; Nussbaum et al. 1987; Rosenberg et al. 1987; Wells et al. 1991).

Evidence that several independent loci are involved in these complex syndromes has come from linkage studies. A gene responsible for progressive mixed deafness with stapes fixation and perilymphatic gusher during stapes surgery (DFN3, MIM 304400) was mapped by different groups to the Xq13–q21 region (Brunner et al. 1988; Wallis et al. 1988; Reardon et al. 1991). At least one locus for non-fragile X nonspecific X-linked mental retardation [non fra(X)-XLMR] could be assigned to proximal Xq by means of linkage studies in different families (Arveiler et al. 1988; Sutherland et al. 1988; Stevenson et al. 1991). The CHM gene was mapped to Xq21 by means of linkage studies, translocations and deletions (Davies et al. 1987; Cremers et al. 1989a, b; Merry et al. 1989). Physical characterization of these deletions allowed the fine mapping of the CHM locus within the Xq21-band and paved the way for the cloning of this gene (Cremers et al. 1990b). Simultaneously, these studies indicated that the relevant deafness and XLMR genes are located proximal to CHM (Cremers et al. 1989b).

Here, we report molecular studies of additional overlapping deletions, two of which are associated with a complex phenotype, including deafness and mental retardation. This has enabled us to refine the localization of the DFN3 gene and the gene for non-specific XLMR.

*Correspondence to:* I. Bach

## Materials and methods

### *Patients*

The clinical and cytogenetic data of the patients are listed in Table 1.

### *Southern blot analysis*

Chromosomal DNA from peripheral blood and Epstein-Barr virus immortalized B-cells was isolated by standard methods (Miller et al. 1988) and digested with the appropriate enzymes (Biolabs, BRL) according to the instructions of the manufacturer. After separation of DNA fragments by gel electrophoresis (0.8% Seakem agarose, FMC), blotting onto GeneScreen Plus membranes (NEN) was performed either using an alkaline Southern transfer (Sambrook et al. 1989) or an alternative "dry blot transfer". The latter method is performed in the same way as the standard Southern blotting procedure except for the omission of transfer buffer. The gel is placed directly onto a glass plate and is covered with the nylon membrane, 3 sheets of Whatman 3MM (soaked in 0.5 *M* NaOH/1.5 *M* NaCl) and several layers of disposable tissues. Radioactive probes were prepared according to Feinberg and Vogelstein (1983) by random oligonucleotide priming of DNA inserts resolved in and isolated from low-gelling-temperature agarose. Probes containing repetitive sequences (pX104f, pF1 and pF8) were pre-associated with sonicated human DNA as published elsewhere (Litt and White 1985). (Pre)hybridization and washing procedures were performed by standard methods (Sambrook et al. 1989).

### *DNA markers*

The anonymous DNA markers from Xq21 used in this study have been described previously (Goodfellow et al. 1985; Nussbaum et

**Table 1.** Clinical and cytogenetic data of patients studied

| Patient | Age (years) | Clinical features | Cytogenetic examination | Reference |
|---|---|---|---|---|
| LGL 2905 | 37 | CHM | Not investigated (ni) | Sankila et al. (1990) |
| MBU | ≈35 | CHM, seizures, (MR) | 46,Y,del(X)q21) approximately 50% | Hodgson et al. (1987) Merry et al. (1989) |
| 25.6 | 38 | CHM | ni | F. Brunsmann, personal communication |
| 33.1 | 43 | CHM | ni | F. Brunsmann, personal communication |
| 3.5 | 31 | CHM | 46,XY | F. Brunsmann, personal communication |
| D20[a] | 2 | Retinal abnormalities, mixed deafness, seizures, MR | 46,Y,del(X)(q21.1–q21.2) | Wells et al. (1991) D. Robins, personal communication |
| SD[a] | 9 | Retinal pigmentary disturbances, sensorineural deafness, urinary abnormalities, delayed mental development | 46,Y,del(X)(q21) only part of Xq21 | Reardon et al. (1992) |
| DM | 26 | CHM, MR, profound sensorineural deafness, myopia, epilepsy | 46,Y,del(X)(q21.1–q21.3) | Rosenberg et al. (1987) Schwartz et al. (1988) |
| XL45 | 40 | CHM, MR, profound sensorineural deafness, obesity | 46,Y,del(X)(q21) approximately 50% | Ayazi et al. (1981) Nussbaum et al. (1987) |
| XL62 | 18 | CHM, MR, moderately severe, mixed deafness with stapes fixation and perilymphatic gusher, short stature | 46,Y,del(X)(q21) | Nussbaum et al. (1987) |
| NP | 11 | CHM, MR, cleft lip and palate, hypertelorism, macrocephaly, agenesis of the corpus callosum | 46,Y,del(X)(q21.1–q21.33) | Tabor et al. (1983) Rosenberg et al. (1986) |

[a] Too young to show the characteristics of CHM

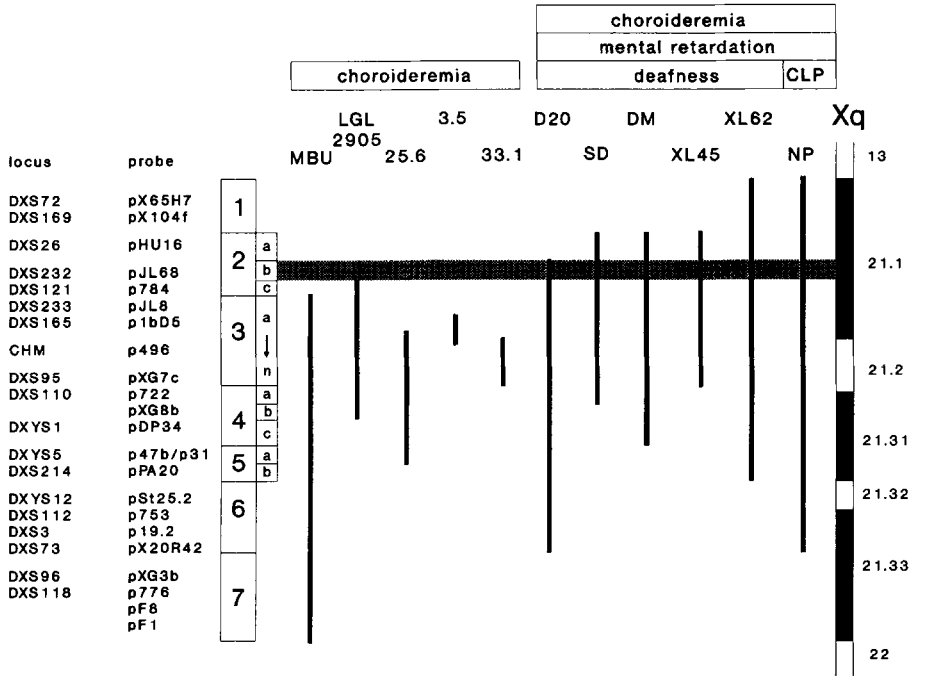


**Fig. 1.** Deletion map of the Xq21-region. Deleted segments are indicated by *vertical lines*. The *horizontal bar* represents the DFN3/XLMR region. Probe order for intervals 6 and 7 are arbitrary except for probes pX20R42 (DXS73) and pF1, which have been positioned employing an X/autosome-translocation panel (C. Philippe, personal communication). The intervals and banding pattern of this chromosomal fragment are not drawn to scale. Patients SD and D20 are included in the group of CHM patients because their ophthalmological findings are consistent with the pre-status of choroideremia. *CLP*, Cleft lip and palate

al. 1987; Cremers et al. 1990a; Sankila et al. 1990). Probe p496 (CHM) corresponds to the cloned cDNA of the choroideremia gene (Cremers et al. 1990b).

## Results

Two recently identified large deletions (Wells et al. 1991; Reardon et al. 1992) were tested with all relevant probes from the Xq21-region, including a new anonymous marker from interval 2, pHU16 (DXS26) (Sankila et al. 1990). Based on previous mapping data and the results presented in this study, we could divide the previously defined intervals 2 to 5 of Xq21 (Cremers et al. 1990a) into subsegments, as depicted in Fig. 1. The relative order of all probes from these intervals could be established.

The proximal breakpoint of the deletion in patient SD was mapped between intervals 1 and 2. Thus, with the available probes, the proximal boundary of this deletion could not be distinguished from those of two previously studied patients, XL45 and DM.

Locus DXS26 (probe pHU16) was found to be present in the DNA of patient D20 but absent from DNAs of patients SD, DM and XL45. This implies that the DXS26 locus must be proximal to the genes involved in deafness and XLMR.

The distal boundary of the region carrying the DFN3 gene could be inferred from a study of a deletion found in the DNA of a patient with classical CHM. In this patient (LGL2905), who is neither deaf nor mentally impaired, the deletion includes the DXS121 locus (p784) but not the DXS232 locus (pJL68, see Fig. 1). Therefore, the DFN3 and a putative XLMR gene locus must be located proximal to the DXS121 locus and distal to DXS26 (interval 2b).

## Discussion

Isolated-X-linked deafness is a relatively rare and clinically heterogeneous trait (Konigsmark and Gorlin 1976). Often, deafness is also part of a variety of other well-defined disorders (e.g. albinism-deafness syndrome, or mucopolysaccharidosis type II) that map to different regions on the X chromosome.

Among the 4 types of nonsyndromic X-linked deafness distinguished by McKusick (1990), progressive mixed deafness with perilymphatic gusher during stapes surgery (DFN3) is the most frequent form, accounting for about half of all X-linked deafness cases (Nance et al. 1971). Phelps and coworkers (1991) have recently suggested that the true proportion of DFN3 may be even higher because the sensorineural component in this type of deafness can progress very rapidly (Glasscock 1973), thereby masking the conductive component.

Linkage analyses in large kindreds in which DFN3 is segregating have allowed the assignment of this disorder to the Xq13–q21 region (Brunner et al. 1988; Wallis et al. 1988). Further evidence for a deafness locus in this chromosomal region came with the identification, in male patients, of cytogenetically visible deletions that comprise at least part of Xq21, and that are associated with a complex phenotype including deafness, MR, CHM and other features (Nussbaum et al. 1987; Rosenberg et al. 1987; Schwartz et al. 1988; Wells et al. 1991; Reardon et al. 1992).

Molecular analyses have enabled us and others to assign the genes for deafness and XLMR to the same small interval of the Xq21 band (interval 2 in Cremers et al. 1989b, 1990a; Merry et al. 1989). In this study, we have characterized two additional deletions that were found in patients suffering from deafness, MR and other features. Furthermore, an overlap with a large deletion that has been found previously in a patient with classical CHM (Sankila et al. 1990) enabled us to exclude the deafness and XLMR genes from the distal segment of interval 2 and to map it between the DXS121 and DXS26 loci.

Recent radiological studies in several unrelated families with X-linked deafness have shed more light on the classification and differential diagnosis of this disorder. Employing high resolution computer tomography, characteristic structural abnormalities of the inner ear could be demonstrated in patients with the mixed type of deafness and in others with pure sensorineural deafness. These abnormalities included bulbous internal auditory meatus (IAM), dilated facial canals, incomplete separation of the coils of the cochlea from the IAM, and wide first and second parts of the intratemporal nerve canal with a less acute angle between them. Since these characteristic alterations give rise to an abnormal communication between the cerebrospinal and the perilymphatic fluid, it seems most likely that these structural changes are responsible for the gusher observed frequently at stapes surgery of patients with DFN3 (Phelps et al. 1991).

Segregation studies, in families with these cochlear and temporal bone abnormalities, have consistently shown linkage with Xq21 markers. It is of note, however, that linkage with Xq21 loci was also found in a family that did not show these specific alterations. This indicates, that there is more than one deafness gene on the proximal long arm of the X-chromosome (Reardon et al. 1991), or alternatively, that allelic mutations cause different phenotypes.

Of the patients described in this study, only probands XL62 and D20 exhibited mixed hearing loss, supporting the diagnosis of DFN3. Surgical treatment was performed in patient XL62 only; this resulted in a perilymphatic gusher when the stapedial footplate was opened (Merry et al. 1989). All other patients had profound sensorineural hearing loss, indicating either the involvement of different genes, allelic variation or "masking" of the conductive defect by the profound sensorineural hearing loss. The latter has been established for patient SD, who was shown to exhibit the same structural inner ear abnormalities as seen in DFN3 patients (Reardon et al. 1992). Similarly, patient XL45 has sensorineural deafness, whereas mixed deafness was diagnosed in his younger brother (Ayazi 1981). This indicates that the DFN3 gene is also involved in the hearing impairment in this family. To establish diagnosis in the other patients, radiological and audiological (re)examination will be necessary.

The observation that one of the previously studied patients (NP) does not show any signs of deafness, despite his gross deletion spanning interval 1 to 6 of Xq21 (Fig. 1) has hitherto been unexplained (Cremers et al. 1989b). This finding indicates that the manifestation of deafness depends not only on the absence of the DFN3 gene but also on other genetic or environmental factors. Alternatively, the chromosomal rearrangement of patient NP may be more complex than previously thought; however, since all markers from intervals 1 to 7 have been tested on the DNA of the patient (Fig. 1), there is no indication for this so far. Examples for such complex rearrangements have been previously described in a sex-reversed patient (Page et al. 1990) and in a patient with adrenoleukodystrophy and colour blindness (Feil et al. 1991). Isolation of new markers from the critical Xq21-

region and long range restriction mapping will be useful for ruling out complex rearrangements in our deletion panel and will enable us to complete the physical map of interval 2 of Xq21 (Cremers et al. 1989b; Merry et al. 1989). Based on flow karyotype analysis, the deletion of patient XL45 has been estimated to comprise about 5.3 Mbp (Merry et al. 1989). Long range restriction mapping around the TCD locus (i.e. the distal part of the XL45 deletion) gives a minimum size of 3.6 Mbp for the DXS95-DXS232 region (W. Berger, unpublished results). Consequently, the chromosomal region between DXS232 and the proximal endpoint of the XL45 deletion, which encompasses the DFN3 and XLMR loci, should not exceed 2–3 Mbp.

It has been estimated that mutations in loci distinct from the fra(X) locus account for about 60% of all X-linked mental retardation cases (Mikkelsen 1987). Linkage studies in families with non fra(X)-XLMR and deletion studies have defined at least one region on proximal Xq that may be involved in the etiology of this disorder (Arveiler et al. 1988; Sutherland et al. 1988; Cremers et al. 1989b, 1990a; Stevenson et al. 1991; Schwartz et al. 1991). Moreover, several complex X-linked disorders involving mental retardation have been assigned to this chromosomal region (Wieacker et al. 1985; Schwartz et al. 1990; Arena et al. 1991). These observations indicate that there is more than one gene in this region giving rise to mental retardation, or alternatively, that there is single gene defect that is relatively frequent.

Recently, a balanced t(X:13) (q21.1;q34) in a girl with profound mental retardation and minor additional symptoms, such as asymmetry of the face, clinodactyly of the index finger and a mild scoliosis, was described by Teboul et al. (1989). Assuming that the mental retardation in this girl is caused by a translocation within the XLMR locus, molecular studies in this patient should enable us to refine the localization of this locus and may significantly contribute to its isolation.

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