Letters to the Editor

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Deletion Mapping of X-Linked Mixed Deafness (DFN3) Identifies a 265–525-kb Region Centromeric of DXS26

To the Editor:

Nonsyndromic X-linked deafness is a rare cause of hereditary deafness accounting for ~5% of all congenital deafness (Reardon 1990). The DFN3 locus (MIM 304400) has previously been mapped to the Xq13-21 region by linkage analyses (Brunner et al. 1988; Reardon et al. 1991) and was further substantiated by the observation of deafness segregating with deletions involving this region (Nussbaum et al. 1987; Rosenberg et al. 1987; Merry et al. 1989). Molecular characterization of the deletions have indicated that the DFN3 gene is located in proximal Xq21, centromeric of the choroideremia locus (CHM) (Cremers et al. 1989; Bach et al. 1992*a*, 1992*b*). Several marker loci at Xq21 are located within the different deletions, and a critical region has been suggested around DXS232 (Bach et al. 1992*a*, 1992*b*), giving the likely order Xqcen-DXS169-DXS26-(DXS232, DFN3)-DXS121. The lack of critical deletions and physical maps and clones covering the region has hampered a more accurate mapping of DFN3. In addition, the clinical heterogeneity of nonsyndromic X-linked deafness has been confirmed by linkage studies, suggesting the existence of two distinct loci in Xq13-q21 (Reardon et al. 1991, 1992). A recent report based on studies of several deletions estimates the DFN3 candidate region to ~400 kb around DXS26 (Huber et al. 1994). We report here physical cloning of a region associated with DFN3 in a YAC. Detailed mapping of three deletions associated with DFN3 has enabled us to further map a region centromeric of DXS26 involved in the disease.

Three nonrelated male probands were included in the study. One is from a newly characterized French family where an X-linked mode of inheritance was established (proband ML). The two other DFN3 deletions included in our study correspond to patients with a more complex phenotype, including choroideremia and mental retardation. They have been described elsewhere (patients XL-45 and SD in Ayazi et al. 1981; Nussbaum et al. 1987; Reardon et al. 1992; Bach et al. 1992*b*).

One novel deletion was identified in the French family segregating for DFN3. In the DNA of the proband ML, no hybridization was found at DXS169, whereas DXS26 was present. For the two other probands (XL-45 and SD), deletions were detected for DXS26, whereas DXS169 was present, consistent with a previous report (Bach et al. 1992*b*). Based on these results, we hypothesized that a common deleted region between the two loci should involve at least part of the DFN3 gene.

In order to build up a physical map of the region, probes pX104f (DXS169) (Nussbaum et al. 1987) and pHU16 (DXS26) (Sankilä et al. 1990) were sequenced using the appropriate vector primers. Selected sequences were converted into sequence-tag sites (STSs) (table 1) and used to screen YAC libraries constructed at the CEPH (Albertsen et al. 1990; Chumakov et al. 1992). One YAC (813A2) was identified that spans both DXS26 and DXS169 (fig. 1). Restriction mapping of the YAC was performed after partial digestion with *Mlu*I, *Nae*I, *Nar*I, *Eag*I, or *Sfi*I, followed by hybridization of pBR322 fragments specific for the right and left arm of the pYAC4 vector. Pulsed-field gel electrophoresis (PFGE) analyses was performed in 1% agarose gels with a Pharmacia-CHEF system. DNA fragments in a 50–600-kb range were separated for 60 h at 100 V in 15°C, with a switch time of 40 s. For size estimation of the entire YAC, migration was performed for 50 h at 120 V, with a switch time of 90 s for 30 h and 60 s for 20 h.

Table 1

STSs Derived from the DXS26-DXS169 Interval

Designation	Sequence	Size (bp)	Annealing Temperature
DXS169	5′-TAATGTAACCGTATGTACTACAC 5′-ATGTCCCTGATACCTAATGATG	390	60°C
DXS26	5′-TGACTCAGGAATCTCACTTCTG 5′-CTGTTAATGGCCATTTAGTGCC	140	62°C
71:15	5′-ATATACTCTCCCAAATGGATGC 5′-TGTCCTTTGTGTGACCTCTGTG	280	62°C
24:5	5′-TACTCTCCCAAATGGATGCCTG 5′-CATCCAAGAGGTAACTCAGTTAG	212	62°C
34/71:2	5′-AAGCACTCCACAATTAGCAGA 5′-TATGTGATGAGGAACTTCTCAC	249	66°C
24:17	5′-TCTAGCCCATTTGTTGAATAGG 5′-GAAGTGGAAGAAATAATTCTTAG	227	60°C
71:2	5′-CTAACTGGATGAATGTGGTAGC 5′-GAGAATCTAATAGCCTCATAGTC	208	64°C
71:21	5′-CTCTCTAAATCGAGGCACAAATC 5′-CTTTTAAGATTCTGTGCCTTGCC	185	62°C
71:3	5′-ATCAGTAATATTCACCTGAGTC 5′-GTATGAAGTGGAAGAATAATTC	165	55°C
34(2)	5′-CCAACAATATGACACTCTGGAG 5′-GGGATAACTCCACTGAGACATC	280	63°C

NOTE.—Primer sequences for DXS26 and DXS169 were derived from cosmids positive for the two corresponding probes.

Alu consensus sequences (Nelson et al. 1991) were used on DNA from YAC 813A2 to generate human-specific PCR products that were blunt-end ligated in pBS (Stratagene). Individual clones were size-estimated and mapped on PFGE blots of the YAC.

Eight clones were selected from their map position, were converted into STSs, and were used on DNA from the three probands (table 1). For proband ML, PCR products were successfully generated using the five more-telomeric STSs, whereas the five more-centromeric STSs were consistently negative. This allowed us to map the telomeric breakpoint between STSs 24:17 and 71:2 located on the same 95-kb *Eag*I-*Nar*I fragment. The restriction fragment is positioned at a minimum distance of 265 kb centromeric of DXS26.

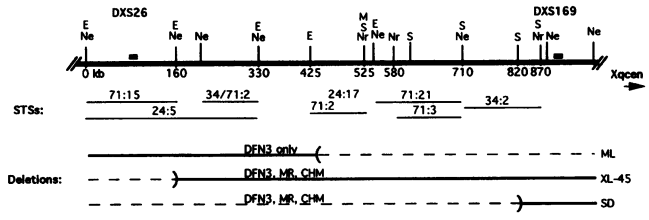

Figure 1 Physical map of the DXS26-DXS169 region. The upper bar corresponds to YAC 813A2. The position of the *Alu*-PCR–derived STSs used are assigned to their corresponding restriction fragment. Deleted segments in genomic DNA from three DFN3 patients are indicated as dashed lines. Restriction sites indicated are E, *Eag*I; Ne, *Nae*I; M, *Mlu*I; S, *Sfi*I; and Nr, *Nar*I. CHM indicates choroideremia; and MR indicates mental retardation.

In DNA from patient XL-45, all STSs except the one derived from DXS26 generated specific PCR products, which means that the deletion breakpoint is relatively close to DXS26. The nonoverlapping deletions in DNA from ML and XL-45 are thus separated by 265–525 kb.

In contrast, DNA from the proband SD was successfully PCR-amplified only with the DXS169-derived STSs, whereas all other STSs failed to generate any PCR products. The results shows that the proximal deletion boundary is close to DXS169 and the deletion overlaps with the one in patient ML.

The previous assignment of the DFN3 candidate region close to DXS232 and telomeric of DXS26 was based on the analysis of one large deletion (Bach et al. 1992*b*). The patient (D20) has a complex phenotype including sensorineural deafness, choroideremia, and mental retardation. A more recent report including additional DFN3-associated deletions shows that the gene extends ≥150 kb centromeric of DXS26 spanning a

minimum region of 400 kb (Huber et al. 1994). One possible explanation for this observation would be the existence of a very large gene for the DFN3 phenotype. The results presented here extend the critical region to ≥265 kb centromeric of DXS26, indicating that the gene spans ≥500 kb. Another possible explanation is nonallelic heterogeneity at Xq21 in recessive deafness. This is supported by clinical and genetic heterogeneity in families with X-linked deafness (Cremers et al. 1989; Reardon et al. 1991, 1992). Our observations of the hitherto most centromeric DFN3-associated deletion could fit with either explanation. The centromeric breakpoint of the new deletion has not been determined, but it extends ≥100 kb centromeric of DXS169 and spans a minimum of 550 kb. Since the affected family members show no major associated symptoms, the deleted region can be expected to be relatively depleted of (vital) genes. This is in accordance with our findings of only one putative CpG island (fig. 1). Several previously described patients have had deletions associated with more complex phenotypes, such as mental retardation, retinal abnormalities, chorioderemia, seizures, or epilepsia. These deletions are very large and extend in a different direction, i.e., telomeric.

Our observations and physical mapping have added important information for the localization of a gene underlying DFN3 at Xq21.1. We present evidence for the localization of at least part of a gene at a minimum distance of 265 kb centromeric of DXS26. A detailed and continued characterization of the region at Xq21 will provide a powerful tool in the ongoing search for the DFN3 gene.

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Diverse Mutations in the Aldolase B Gene That Underlie the Prevalence of Hereditary Fructose Intolerance

To the Editor:

Hereditary fructose intolerance (HFI), first recognized in 1956 by Chambers and Pratt, is an autosomal recessive disorder with a wide ethnic distribution. It is caused by deficiency of liver aldolase (aldolase B) (Hers and Joassin 1961) and is characterized by vomiting, failure to thrive, liver disease, and metabolic disturbances—including hypoglycemia—that occur on exposure to fructose and related sugars, at weaning (Gitzelmann et al. 1989). Diverse mutations have been identified in the human aldolase B gene in association with fructose intolerance, several of which are sufficiently widespread to be of diagnostic utility (Cross et al. 1990; Cox 1994). The frequency of HFI has not been determined with precision in any population, and many pediatricians consider it to be rare (Cornblath and Schwartz 1991). Nonetheless, recognition of HFI is of critical importance, since it responds favorably to dietary exclusion of fructose, sucrose, and sorbitol; survival to adulthood is associated with the development of strong aversions to sweet-tasting foods and drinks.

There is mounting evidence that mutant alleles of aldolase B are more frequent in the population than was first recognized: (1) there are multiple reports of parent-to-offspring transmission of fructose intolerance in nonconsanguineous pedigrees (Cox. et al. 1982); (2) after surviving the stormy period of infancy, individuals adjust their dietary habits and escape formal diagnosis—they may later come forward in response to articles in the public domain (Cross and Cox 1989); and (3) the effects of administration of fructose-based solutions have resulted in at least 16 deaths of patients not known to have been suffering from HFI but in whom the diagnosis has been subsequently confirmed (Gitzelmann et al. 1989; Ali et al. 1993; Cox 1993). We have investigated key members of a large kindred from the United States who are affected by HFI that vividly illustrates all these features.

The pedigree is depicted in figure 1. The original fructose-intolerant subjects came from two families, of maternal Swiss-German descent, within a large kindred and were first cousins (Landau et al. 1971). Diagnosis was based on characteristic symptoms and the induction of hypophosphatemia and hypoglycemia after controlled fructose challenge (Froesch et al. 1963). Since then, one patient has died at age 10 years, from acute hepatorenal failure after intravenous infusion of invert sugar during a minor surgical procedure. There have been additions to the pedigree—notably the marriage of the fructose-intolerant subject III-22 to an unrelated woman, γ, with the birth of offspring IV-16 and IV-17. Their son, IV-16, and his female cousin, IV-1, the oldest member of generation IV, avoid sugary foods and fruit and have dietary preferences that, combined with their immediate family history, are highly suggestive of fructose intolerance (Chambers and Pratt 1956; Froesch et al. 1963; Gitzelmann et al. 1989). IV-1 had suffered severe nutritional disturbances, including adolescent scurvy, since early infancy. Thus, because IV-1 and IV-16 questioned, as adults, whether they suffered from HFI, first-degree and collateral relatives of the original fructose-intolerant subjects requested diagnostic confirmation and clarification of the mode of transmission of disease within their kindred. Accordingly, blood samples were obtained from key members, for DNA extraction and aldolase B genotyping based on the PCR (Cross et al. 1990). Detailed inquiry has failed to reveal consanguinity in the kindred: subjects α, β, γ, and δ are of English, German, French, and Anglo-American descent, respectively.

To screen systematically for aldolase B gene mutations that are associated with HFI and widespread in the European population, exon 5 sequences were first amplified in the PCR for study. Aliquots were digested with the restriction enzyme *Bsa*HI (GPu↓CGPyC), an isoschizomer of *Aha*II, that cleaves the products at the site of the common missense mutation, A149P (Ala^{149}→Pro; G→C) (Cross et al. 1988). One copy of this allele was detected in DNA samples from subjects α, III-22, and IV-17. To identify other alleles segregating in this kindred, DNA obtained from the brother of the child who had suffered lethal fructose intoxication (III-36) was examined as the index case. Direct genomic sequencing revealed two distinct mutations. In exon 4, the 4-bp deletion elsewhere described in a patient of British ancestry (Cox et al. 1983; Dazzo and Tolan 1990) was identified. To screen for the presence of this mutation, heteroduplex analysis—which facilitates detection of small deletions in PCR products that also contain wild-type sequence (Nagamine et al. 1989)—was undertaken. The hybrid amplified exon 4 sequences were identified by their anomalous mobility during electrophoresis through polyacrylamide. Heteroduplex analysis con-