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Further mutations in *Brain 4* (POU3F4) clarify the phenotype in the X-linked deafness, DFN3

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X-linked non-syndromic deafness accounts for a small proportion of non-syndromic deafness. Clinical and radiological parameters have now identified a group of patients in whom the deafness gene maps either by linkage or by deletion mapping to the same region of the X chromosome (1–3). Patients with a history of perilymphatic gusher at stapes surgery or a radiological abnormality of the cochlea on CT scan (4), which may be the cause of the 'stapes gusher', map to Xq13-q21 and affected males may have either mixed deafness, or less commonly, sensorineural deafness. Some families in whom the radiology is normal have also been found to map to Xq21 although there is locus heterogeneity (2,5,6).

Recently, point mutations and small deletions have been described in the human homologue of mouse *Brain 4* gene (now called POU3F4) in patients with X-linked deafness in whom there is a history of gusher or who have the classical abnormality on CT scan (7). POU3F4 is a member of the POU family of genes which contain DNA binding motifs and act as transcription factors. Using SSC analysis and sequencing of genomic DNA we analyzed the homeodomain of human POU3F4 in two families in whom X-linked deafness was segregating.

Family 1 (Fig. 1b), consists of a mother and two sons of Finnish origin. The proband (LGL3673) has a mixed hearing loss of 40–50 dB and his brother (LGL3675) a loss of 75–95 dB. One brother (LGL3673) underwent stapedectomy and was found to have a perilymphatic gusher at surgery. Family 2 (Fig. 2a) is a four generation British family in which there are three affected males. All affected males have profound sensorineural deafness diagnosed in infancy with no suggestion of a conductive component. Individual III_1 has a moderately severe learning difficulty of unknown cause but III_2 and IV_1 are of normal intelligence. One of the males underwent high resolution CT scanning of the cochlea and was found to have the characteristic bony abnormality of deficiency of bone between the basal turn of the cochlea and the internal auditory meatus.

Genomic DNA isolated from lymphocytes was amplified by PCR using primers described previously, (Va and Vb) and subjected to SSC analysis by electrophoresis in a 6% polyacrylamide gel with 5% glycerol at 300 V overnight at 4°C. PCR products were gel purified and the sequence determined using the Circumvent Thermal Cycle Dideoxy DNA sequencing kit (New England Biolabs) using ^{32}P end-labelled primers as directed. Mutations were confirmed by restriction enzyme digestion (*Hph*I in family 1 and *Acc*I in family 2) and separation of products on a 4% agarose gel.

In family 1, sequencing of the SSC/PCR product revealed a 4 bp deletion at bases 862–866 located in the homeodomain which results in a frameshift (Fig. 1a). Mutation at this site abolishes a cutting site for the enzyme *Hph*I with consequent production of a large band of 300 bp seen in the mother and two deaf sons (Fig. 1b). This change was not seen in 40 normal X chromosomes (data not shown).

In family 2, altered bands on SSC analysis appear in individual II_2 as a *de novo* phenomenon and are inherited by the deaf boys in the family (Fig. 2a). The mutant band is not present in this individual's mother I_1, or her sister II_1. Furthermore, linkage analysis with the marker DXS1002, which we have shown previously to have a two point lod score of 9.8 at $\theta = 0$ with the disease gene, (5) shows that the mutation is carried on the grandmaternal X chromosome, inherited from I_1 and thus represents a new mutation in II_2 (Fig. 3). Direct sequencing has shown this to be a C to T

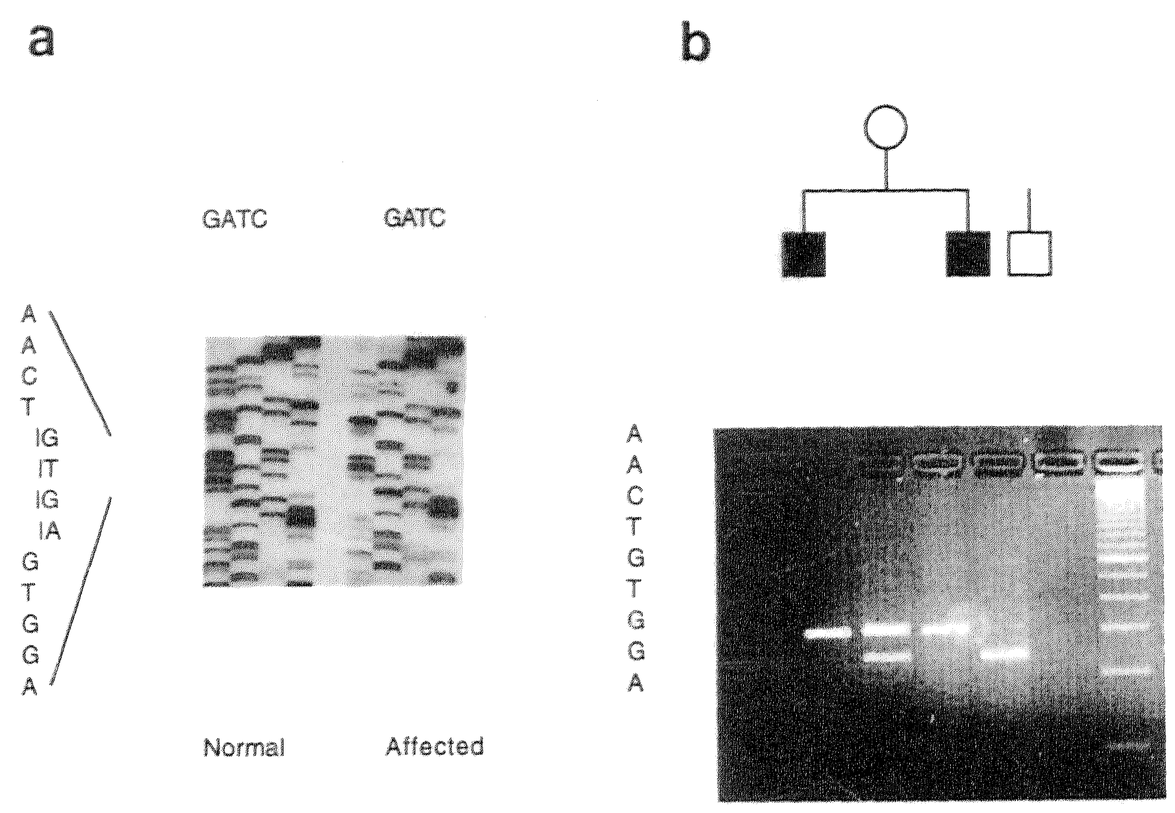

Figure 1. (**a**) In family 1, sequencing shows a 4 bp deletion in the affected male compared with a normal male. (**b**) The 4 bp deletion abolishes a cutting site for the enzyme *Hph*I with consequent retention of a band at 300 bp seen in the mother (LGL3676) and 2 deaf sons (LGL3673 and LGL3675), but not in the control. Size marker is 100 bp ladder.

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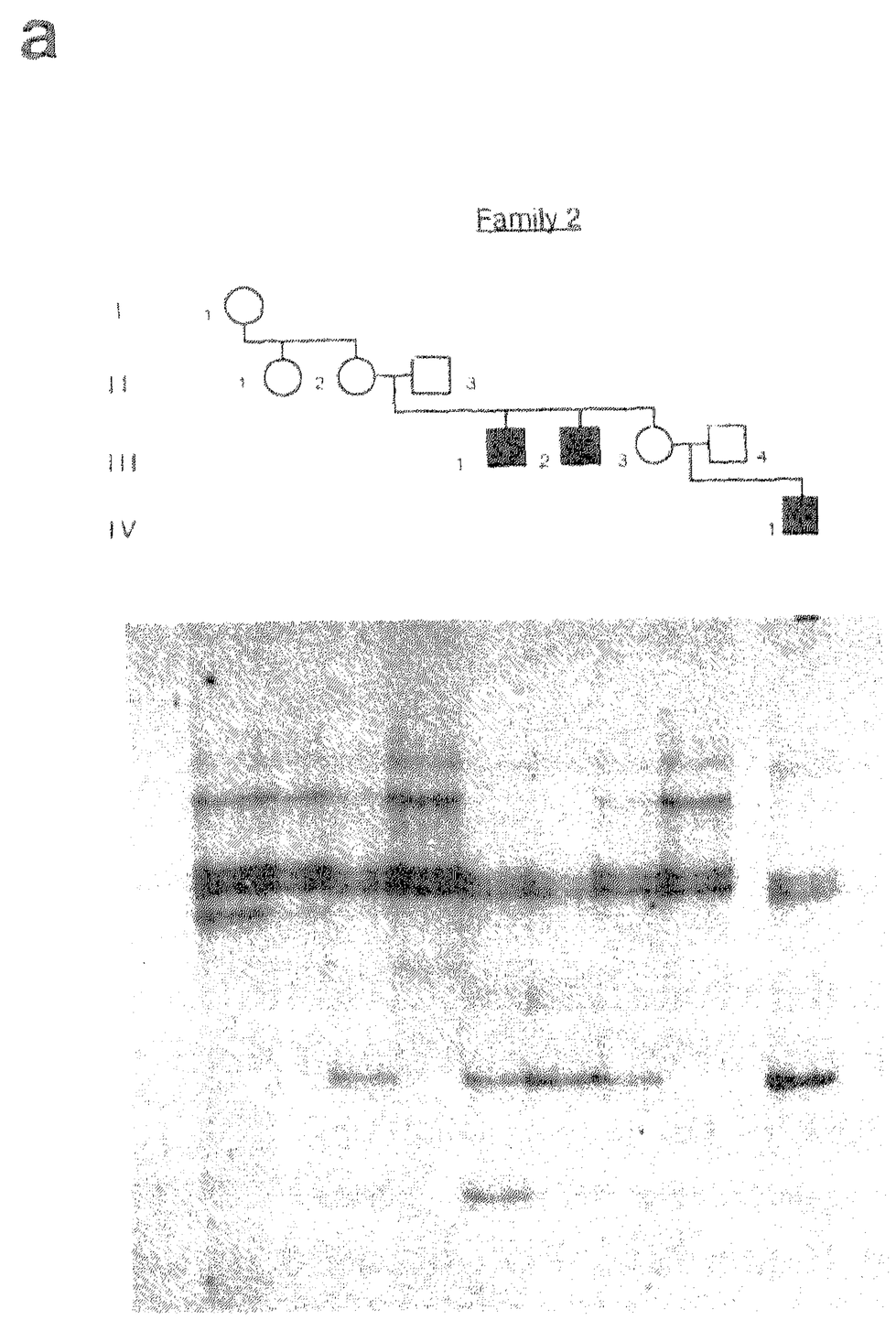


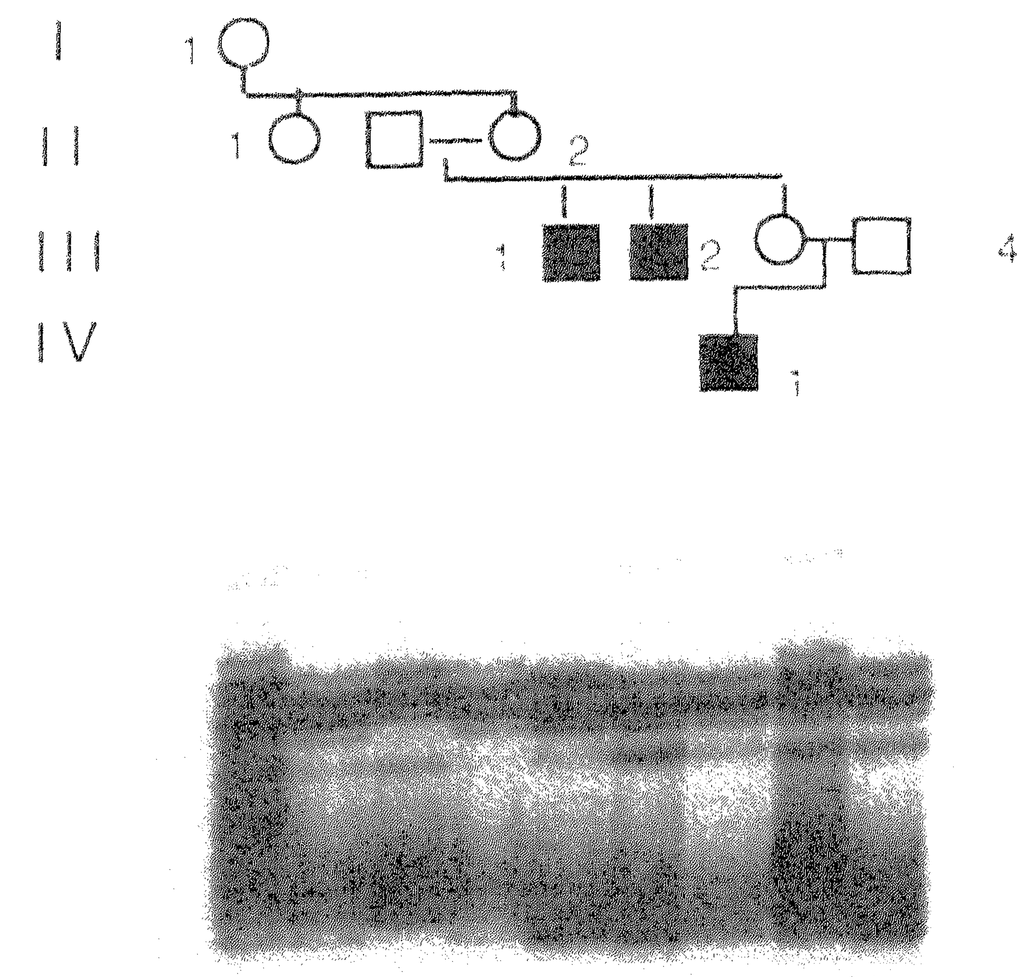
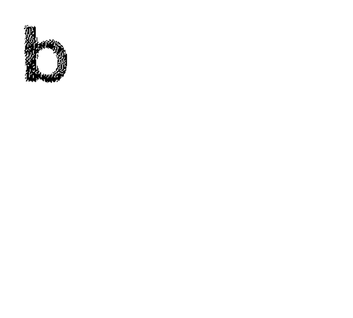


Figure 3. Inheritance of alleles for the marker DXS1002 in family 2. Both II_1 and II_2 have inherited the same maternal X chromosome. Affected boys (III_1 and III_2) have also inherited the grandmaternal X chromosome from I_1, as has IV_1. Thus the mutant SSC band observed in II_2, not present in her sister, demonstrates that this is a *de novo* change.



restricted manner. They have gained their name from sequence homology with three mammalian transcription factors *Pit-1*, *Oct-1* and *Oct-2*, and a *C.elegans* developmental control gene *unc-86*. Developmental mutants in two of these genes indicate that they have a role in determining cell fate and phenotype: mutations in the gene *Pit-1* have been shown to cause pituitary

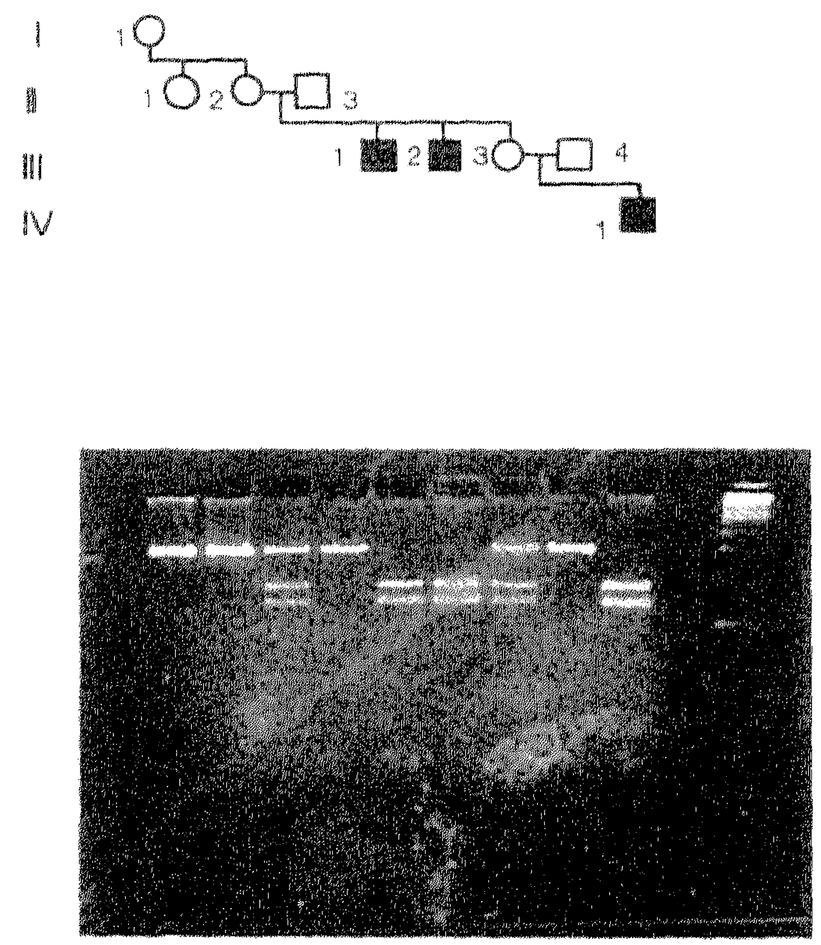


Figure 2. (**a**) In family 2, autoradiograph of SSCP gel in which shifted bands appear in II_2 as a *de novo* phenomenon and are inherited by the deaf boys and the obligate carrier III_3. (**b**) Gain of an *Acc*I cutting site as a result of the C to T transition which cosegregates with the deafness and appears in II_2 as a *de novo* change.

transition at nucleotide 935 resulting in an alanine to valine substitution in a highly conserved residue of the homeodomain of the predicted protein. This mutation gives rise to a novel *Acc*I restriction site (Fig. 2b), which also arises *de novo* in individual II_2 and cosegregates with the deafness in this family.

The POU domain family of genes are transcription factors, expressed early in development in a temporally and tissue-dwarfism in the Snell mouse and in humans due to failure of development of mature thyrotrophs, lactotrophs and somatotrophs (8,9); mutation in the gene *unc-86* in *C.elegans* prevents mother cells from differentiating into daughter cells and subsequent failure of development of specific types of sensory neurons. The POU domain is bipartite and consists of a POU-specific domain (POU_S), found only in this class of genes, and a POU-homeodomain (POU_{HD}) which has homology to other classical homeodomain containing proteins. *In vitro* studies have shown that both POU_S and POU_{HD} contact DNA and that POU_S is required for high affinity, site-specific binding. The POU domain is highly conserved across species and exhibits remarkable amino acid conservation at critical residues between organisms as diverse as drosophila, zebrafish and man. Point mutations leading to non-conservative amino acid substitutions in conserved regions of the homeodomain (helix 3) have been shown to greatly reduce or abolish DNA binding in *Pit-1* in one instance (8) and to preserve its DNA binding ability but to inhibit its transcriptional activating ability in another (9).

The mutation described here in family 1 is predicted to cause a frameshift mutation from amino acid 13 of the homeodomain onwards which is likely to severely reduce or abolish DNA binding. Functional DNA binding studies should indicate whether or not this is the case. In family 2 the single base change resulting in an alanine to valine substitution occurs in helix 2 of the homeodomain in a residue conserved in all members of the POU gene family from mammals to yeast, and also in the 'classic' homeodomain gene, Antennapedia. The observation that this is a *de novo* point mutation formally confirms that mutations in POU3F4 cause deafness. This is the first gene known to cause non-syndromic deafness in

humans and is only the second POU gene which has been confirmed to underlie a human disease, namely a form of X-linked deafness in which there is a developmental abnormality of the cochlea, seen on CT scan as deficient or absent bone between the lateral end of the internal auditory meatus and the basal turn of the cochlea together with a dilated internal auditory meatus. Other members of this gene family are numerous and are scattered throughout the genome (10).

POU3F4 is the first gene known to cause non-syndromic deafness in humans. Identification of mutations in the gene in a subset of patients has helped towards a reclassification of non-syndromic X-linked deafness. To date only patients with the bony abnormality of the cochlea have mutations in POU3F4 whereas families that map to Xq13-q21 but have normal cochlear morphology do not. Furthermore, this study confirms that families with mixed deafness and families with sensorineural deafness both have mutations in POU3F4 (7). Thus McKusick's classification which distinguishes types of non-syndromic X-linked deafness on the audiometric findings alone would appear to need updating based on the results of this work (11). Consequently, DFN3 is not characterized by mixed conductive and sensorineural deafness associated with perilymphatic gusher at stapes surgery, but by profound sensorineural deafness with or without a conductive component associated with a unique developmental abnormality of the ear.

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