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Mapping and cloning hereditary deafness genes

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In the past two years, considerable progress has been made in the mapping and cloning of human deafness genes. Highlights are the chromosomal localization of at least five genes for autosomal forms of non-syndromic deafness and, more recently, the cloning of an X-linked deafness gene, *DFN3*, and the Usher syndrome type IB gene. This last gene encodes a myosin-like protein and was identified as the human homolog of the mouse *shaker-1* gene. The *DFN3* gene *Brain 4* encodes a POU domain containing transcription factor that is involved in the development of the inner ear.

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Introduction

Deafness is the most common inherited human sensory defect, affecting one in 2000 children [1]. In 70% of cases, the deafness is non-syndromic, i.e. not associated with other clinical features [2]. Progress in mapping deafness genes has been relatively slow for several reasons. Firstly, ~75% of cases are of the recessive type [3] which, because of the inherently small pedigree sizes, renders linkage analysis difficult. Secondly, several forms of hearing loss are age-dependent; in others, disease manifestation and clinical course depend on environmental factors. Thirdly, the nosologic classification of hearing loss is complicated by the inaccessibility of the inner ear for clinical examination. Fourthly, our understanding of the auditory signal transduction cascade in the sensory hair cells of the organ of Corti is still in its infancy, so we know of only a few candidate genes for hereditary forms of sensorineural deafness that can be tested for mutations. Fifthly, only recently have inner ear specific cDNA libraries been established [4,5,6•]. Finally, when compared to the funding of eye research, financial support for research into genetic forms of deafness has been relatively meager. Still, remarkable progress has been made in this field during the past few months. Molecular studies have recently shed more light on the etiology and pathogenesis of various syndromes in which deafness is an inconsistent or minor symptom.

In this review, we have chosen, somewhat arbitrarily, to confine ourselves to two syndromes in which early-onset deafness is a major symptom, Usher syndrome (USH) and Waardenburg syndrome (WS). The central focus of the review will be the human genes involved in non-syndromic deafness that have been mapped and cloned recently, and on the nosologic implications of this work. In

another review, Steel and Brown [7] have dealt with various forms of mammalian deafness on the basis of their pathologic features.

Usher syndrome

USH comprises a group of autosomal recessive disorders associated with congenital sensorineural deafness and progressive visual loss caused by retinitis pigmentosa. Clinically, at least three types of Usher syndrome (USH1–3) can be distinguished. Patients with USH1 have congenital, severe to profound hearing loss and absence of vestibular function, whereas in USH2, the hearing loss is congenital but moderate to severe and the vestibular function is normal.

Linkage studies suggest the presence of causal genes for USH1 at 14q32.1–q32.3 (*USH1A*), 11q13.5 (*USH1B*), and 11p15.1–p14 (*USH1C*) [8–12]. Recently, a novel type VII myosin gene at 11q13.5 has been shown to be homologous to the murine deafness gene *shaker-1* and is responsible for USH1B [13•,14•]. The phenotypes of the original *shaker-1* mutant and five other *shaker-1* mutants seem to be different from that of USH1 patients in that no retinal degeneration has so far been reported in these mouse mutants. In another study, the autosomal recessive deafness gene *DFNB2* has been localized to the same interval of chromosome 11 [15]. A major locus for USH2 (*USH2A*) is situated at 1q41 [16–18], but evidence exists for a further USH2 locus, as one large USH2 family does not show linkage to 1q [19]. The choroideremia-like gene *CHML* is a candidate for *USH2A* because it maps to the same chromosomal in-

Abbreviations

DFN3—X-linked deafness; USH—Usher syndrome; WS—Waardenburg syndrome.

terval, but the search for *CHML* mutations in *USH2A* patients has been unsuccessful [20,21].

The third type of Usher syndrome (*USH3*) differs from *USH2* by the progressive nature of its hearing loss. Linkage studies have assigned the underlying gene to 3q21–q25 [22]. A clear clinical distinction between *USH2A* and *USH3* is difficult, as exemplified by the observed linkage of one 'USH3' family to 1q41 [22] and the linkage of five 'USH2A' families to 3q21–q25 (WS Kimberling, personal communication).

Waardenburg syndrome

Waardenburg syndrome (WS) is an auditory pigmentary syndrome in which hearing loss is associated with abnormal pigmentation of hair, skin and eyes. Clinically, WS has been divided into two types, WS1 and WS2, which are distinguished by the presence or absence of mild facial dysmorphic features such as dystopia canthorum, a lateral displacement of the inner canthi of the eyes, which is defined by the W index. Redefinition of the W index now allows a better classification of WS families. Patients with WS1 have been shown to have mutations in the *PAX3* gene [23,24], whereas WS2 patients have mutations in other genes. In a putative mouse model for WS, mutations were found in the *microphthalmia* gene, encoding a novel basic helix-loop-helix zipper protein [25]. Subsequently, the human homologue of this gene, *MITF*, and a locus for WS2 were mapped at 3p14.1–p12.3 [26,27], and mutations were found in the *MITF* gene in two families with WS2 [28]. As a result of improved clinical classification, families can now be reliably defined as WS1 or WS2 and mutations sought in the appropriate genes.

Mitochondrially inherited deafness

In recent years, mutations in the mitochondrial genome have been reported to be associated with a variety of multisystem disorders [29], many of which include sensorineural deafness as a symptom. In four unrelated families, a maternally transmitted susceptibility to non-syndromic sensorineural hearing loss was associated with a mitochondrial DNA mutation at nucleotide 1555 in the 12S rRNA gene [30•]. In three of these families, the disease became manifest after aminoglycoside antibiotic treatment; in the fourth family, it was associated with a mutation in an (as yet) unknown nuclear gene. Early-onset sensorineural hearing loss in another maternal pedigree has been found to be associated with a mutation at nucleotide 7445, converting the 3' terminal thymine residue of tRNA-ser(UCN) to a cytosine [31].

Autosomal recessive deafness

Non-syndromic autosomal recessive deafness is extremely heterogeneous, as documented by the fact that most marriages between unrelated deaf individuals do not result in affected offspring. Estimates of the number of genes involved in recessive deafness do not include the possibility that affected individuals are non-complementary double heterozygotes, as shown recently in retinitis pigmentosa [32••]. After a systematic genome-wide search using highly polymorphic microsatellite repeats, the first gene for autosomal recessive deafness (*DFNB1*) was localized to the pericentromeric region of chromosome 13. In this study, homozygosity by descent was observed in affected individuals from consanguineous Tunisian families for two genetic markers [33]. The *DFNB1* locus may also be involved in other populations, as homozygosity for this region has also been found in a consanguineous family of Pakistani origin [34]. Using the same approach, a second locus for autosomal recessive deafness (*DFNB2*) was mapped to chromosome 11q13.5 [15], a region which also harbours the human *USH1B* gene [14••] (see above). A third autosomal recessive gene (*DFNB3*) was assigned to the pericentromeric region of chromosome 17 by a strategy termed allele frequency dependent homozygosity mapping [35•]. Evolutionary conserved synteny with other markers indicates that the murine deafness gene *shaker-2* may be the homologue of *DFNB3* [36].

Autosomal dominant deafness

In isolated populations, a single or very few deafness genes may prevail. This was illustrated in a Costa Rican and an Indonesian pedigree in which genes for non-syndromic autosomal dominant deafness mapped to 5q31 (*DFNA1*) and 1p (*DFNA2*), respectively [37,38]. In the latter study, the locus on 1p was confirmed in an American family. Using a large French family, a third locus for autosomal dominant deafness has been localized recently to human chromosome 13 [39]. Possibly the same locus is involved as in *DFNB1*, the recessive form of deafness described above. As this region has now been implicated in families from three different populations, it may be a major locus for deafness. Dominant and recessive forms of deafness may result from different mutations in the same gene, as shown recently for mutations in the *COL11A2* gene which can be associated with autosomal dominant and recessive forms of Stickler syndrome, a syndromic form of sensorineural deafness [40••].

X-linked deafness

The most frequent form of X-linked deafness, DFN3 (X-linked deafness with perilymphatic gusher during stapes surgery), has been mapped to the Xq13–q22 region by linkage studies [41–43]. In patients with DFN3, computerized tomography has revealed specific structural defects in the temporal bone resulting in an abnormal wide communication between the cerebrospinal fluid and the perilymph, which appears to account for the 'gusher' observed during stapes surgery. In families with X-linked sensorineural deafness without the bony defect, linkage studies suggest the association of one locus at or near Xq21 and another at Xp21 [44,45]. Detailed analysis of Xq21 deletions associated with DFN3, choroideremia and mental retardation, and others found in patients with non-syndromic DFN3, has localized the underlying gene to a 500 kb segment of Xq21.1 [46–50].

These data, and the assignment of the mouse POU domain encoding gene *Brain 4* to the murine X-chromosome in a region homologous to human Xq13–q22 [51], have been instrumental in the recent isolation of the human DFN3 gene [52**]. The human homologue of *Brain 4*, *POU3F4*, could be mapped to the physical interval carrying the DFN3 locus, and *POU3F4* mutations were found in five unrelated DFN3 patients [52**]. Although these observations demonstrate that mutations in the *POU3F4* gene cause DFN3, they do not explain the association of DFN3 with four microdeletions and a duplication that map up to 400 kb proximal to *POU3F4*. Although the involvement of a second DFN3 gene in the Xq21.1 band cannot be excluded, it is equally possible that *cis*-acting regulatory sequences proximal (5') to *POU3F4* are involved.

POU3F4 is a member of a multigene family encoding transcription factors (with >25 members) [53]. At least five POU domain genes have been found to be expressed in different parts of the rat cochlea [54]. In the light of our data [52**], it is now tempting to speculate that these genes are implicated in other forms of deafness. In the mouse genome, several POU domain genes have been mapped [55], and the precise chromosomal localization of their human counterparts is eagerly awaited.

Conclusions

Hereditary deafness is extremely heterogeneous. Most cases show an autosomal recessive mode of inheritance. Through the widespread use of a dense array of microsatellite markers, the introduction of novel genetic mapping techniques and improved clinical classification, several deafness genes have been mapped in the human genome. Although in man, precise regional assignment of the underlying gene defects is frequently hampered by small family sizes, comparison of their map locations with those of corresponding defects in the mouse will

greatly facilitate their isolation. Recently, this has been illustrated by the identification of the human *USH1B* gene on the basis of its murine homologue, *shaker-1*. Positional cloning and candidate gene approaches have led to the identification of the gene for X-linked deafness, DFN3. Future efforts in cloning deafness genes will benefit from the availability of an increasing number of candidate genes which are being isolated from inner ear specific cDNA libraries.

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
 - of outstanding interest
1. McKusick VA: *Mendelian inheritance in man*, edn 10. Baltimore: Johns Hopkins University Press; 1992.
 2. Bergström L, Hemenway WG, Downs MP: **A high risk registry to find congenital deafness.** *Otolaryngol Clin N Am* 1971, **4**:369–399.
 3. Rose SP, Conneally PM, Nance WE: **Genetic analysis of childhood deafness.** In *Childhood deafness*. Edited by Bess FH. New York: Grune and Stratton; 1977:19–36.
 4. Wilcox E, Fex J: **Construction of a cDNA library from microdissected guinea pig organ of Corti.** *Hearing Res* 1992, **62**:124–126.
 5. Ryan AF, Batchelor S, Lin L, Brumm D, O'Driscoll K, Harris JP: **Cloning genes from an inner ear cDNA library.** *Arch Otolaryngol Head Neck Surg* 1993, **119**:1217–1220.
 6. Robertson NG, Khetarpal U, Gutiérrez-Espeleta GA, Bieber FR, Morton CC: **Isolation of novel and known genes from a human fetal cochlear cDNA library using subtractive hybridization and differential screening.** *Genomics* 1994, **23**:42–50.
- A human fetal cochlear (membranous labyrinth) cDNA library was subtracted against total human fetal brain RNAs by an avidin-biotin-based procedure. Analysis of a subset of cDNAs yielded several known genes that serve structural or regulatory functions in the inner ear and some novel cochlear genes.
7. Steel KP, Brown SDM: **Genes and deafness.** *Trends Genet* 1994, **10**:428–435.
 8. Kaplan J, Gerber S, Bonneau D, Rozet JM, Delrieu O, Briard ML, Dollfus H, Ghazi I, Dufier JL, Frézal J, Munnich A: **A gene for Usher syndrome type I (USH1A) maps to chromosome 14q.** *Genomics* 1992, **14**:979–987.
 9. Smith RJH, Lee EC, Kimberling WJ, Daiger SP, Pelias MZ, Keats BJB, Jay M, Bird A, Reardon W, Guest M *et al.*: **Localization of two genes for Usher syndrome type I to chromosome 11.** *Genomics* 1992, **14**:995–1002.
 10. Kimberling WJ, Möller CG, Davenport S, Priluck IA, Beighton PH, Greenberg J, Reardon W, Weston MD, Kenyon JB, Crunkemeyer JA *et al.*: **Linkage of Usher syndrome type I gene (USH1B) to the long arm of chromosome 11.** *Genomics* 1992, **14**:988–994.

11. Bonn -Tamir B, Korostishevsky M, Kalinsky H, Seroussi E, Beker R, Weiss S, Codel V: **Genetic mapping of the gene for Usher syndrome: linkage analysis in a large Samaritan kindred.** *Genomics* 1994, 20:36-42.
12. Keats BJB, Nouri N, Pelias MZ, Deininger PL, Litt M: **Tightly linked flanking microsatellite markers for the Usher syndrome type I locus on the short arm of chromosome 11.** *Am J Hum Genet* 1994, 54:681-686.
13. Gibson F, Walsh J, Mburu P, Valera A, Brown KA, Antonio M, •• Beisel KW, Steel KP, Brown SDM: **A type VII myosin encoded by the mouse deafness gene *shaker-1*.** *Nature* 1995, 374:62-64.
Shaker-1 homozygotes show vestibular dysfunction and progressive degeneration of the organ of Corti. Three mutations are described in the type VII myosin gene, two missense mutations and a splice acceptor site mutation, all in the region encoding the myosin head.
14. Weil D, Blanchard S, Kaplan J, Guilford P, Gibson F, Walsh J, •• Mburu P, Valera A, Levilliers J, Weston MD *et al.*: **Defective myosin VIIA gene responsible for Usher syndrome type 1B.** *Nature* 1995, 374:60-61.
Two different premature stop codons, a 6 bp deletion, and two different missense mutations were detected in five unrelated USH1B families. As USH1B appears to represent a primary cytoskeletal protein defect, other unconventional myosins and their interacting proteins are candidates for other forms of USH.
15. Guilford P, Ayadi H, Blanchard S, Chaib H, Le Paslier D, Weissenbach J, Drira M, Petit C: **A human gene responsible for neurosensory, non-syndromic recessive deafness is a candidate homologue of the mouse *sh-1* gene.** *Hum Mol Genet* 1994, 3:989-993.
16. Lewis RA, Otterud B, Stauffer D, Lalouel J-M, Leppert M: **Mapping recessive ophthalmic diseases: linkage of the locus for Usher syndrome type II to a DNA marker on chromosome 1q.** *Genomics* 1990, 7:250-256.
17. Kimberling J, Weston MD, M ller C, Davenport SLH, Shugart YY, Priluck IA, Martini A, Milani M, Smith RJ: **Localization of Usher syndrome type II to chromosome 1q.** *Genomics* 1990, 7:245-249.
18. Kimberling WJ, Weston MD, M ller C, Van Aarem A, Cremers CWRJ, Sumegi J, Ing PS, Connolly C, Martini A, Milani M *et al.*: **Gene mapping of Usher syndrome type IIa: localization of the gene to a 2.1-cM segment on chromosome 1q41.** *Am J Hum Genet* 1995, 56:216-223.
19. Pieke Dahl S, Weston MD, Kimberling WJ, Gorin MB, Shugart YY, Kenyon JB: **Genetic heterogeneity of Usher syndrome type II.** *J Med Genet* 1993, 30:843-848.
20. Cremers FPM, Molloy CM, Van de Pol TJR, Van den Hurk JAJM, Bach I, Geurts van Kessel AHM, Ropers H-H: **An autosomal homologue of the choroideremia gene colocalizes with the Usher syndrome type II locus on the distal part of chromosome 1q.** *Hum Mol Genet* 1992, 1:71-75.
21. Van Bokhoven H, Van Genderen C, Molloy CM, Van de Pol TJR, Cremers CWRJ, Van Aarem A, Schwartz M, Rosenberg T, Geurts van Kessel AHM, Ropers H-H, Cremers FPM: **Mapping of the choroideremia-like (CHML) gene at 1q42-qter and mutation analysis in patients with Usher syndrome type II.** *Genomics* 1994, 19:385-387.
22. Sankila E-M, Pakarinen L, K ari inen H, Aittom ki K, Karjalainen S, Sistonen P, De la Chapelle A: **Assignment of an Usher syndrome type III (USH3) gene to chromosome 3q.** *Hum Mol Genet* 1995, 4:93-98.
23. Baldwin CT, Hoth CF, Amos JA, Da-Silva EO, Milunsky A: **An exonic mutation in the *HuP2* paired domain gene causes Waardenburg's syndrome.** *Nature* 1992, 355:637-638.
24. Tassabehji M, Read AP, Newton VE, Harris R, Balling R, Gruss P, Strachan T: **Waardenburg's syndrome patients have mutations in the human homologue of the Pax-3 paired box gene.** *Nature* 1992, 355:635-636.
25. Hodgkinson CA, Moore KJ, Nakayama A, Steingrimsson E, Copeland NG, Jenkins NA, Arnheiter H: **Mutations at the mouse microphthalmia locus are associated with defects in a gene encoding a novel basic-helix-loop-helix-zipper protein.** *Cell* 1993, 74:395-404.
26. Hughes A, Newton VE, Liu XZ, Read AP: **A gene for Waardenburg syndrome type 2 maps close to the human homologue of the *microphthalmia* gene at chromosome 3p12-p14.1.** *Nature Genet* 1994, 7:509-512.
27. Tachibana M, Perez-Jurado LA, Nakayama A, Hodgkinson CA, Li X, Schneider M, Miki T, Fex J, Francke U, Arnheiter H: **Cloning of *MITF*, the human homolog of the mouse *microphthalmia* gene and assignment to chromosome 3p14.1-p12.3.** *Hum Mol Genet* 1994, 3:553-557.
28. Tassabehji M, Newton VE, Read AP: **Waardenburg syndrome type 2 caused by mutations in the human microphthalmia (*MITF*) gene.** *Nature Genet* 1994, 8:251-255.
29. Wallace DC: **Mitochondrial diseases: genotype versus phenotype.** *Trends Genet* 1993, 9:123-128.
30. Prezant TR, Agapian JV, Bohlman MC, Bu X,  ztas S, Qiu W-Q, Arnos KS, Cortopassi GA, Jaber L, Rotter JI *et al.*: **Mitochondrial ribosomal RNA mutation associated with both antibiotic-induced and non-syndromic deafness.** *Nature Genet* 1993, 4:289-294.
This study offers the first description of a mitochondrial rRNA mutation leading to disease, the first case of non-syndromic deafness caused by a mitochondrial DNA mutation, and the first molecular genetic study of antibiotic-induced ototoxicity. The A1555→G mutation occurs at a highly conserved region of the 12S rRNA gene, in which aminoglycosides are known to bind and in which aminoglycoside resistance mutations have been described in other species.
31. Reid FM, Vernham GA, Jacobs HT: **A novel mitochondrial point mutation in a maternal pedigree with sensorineural deafness.** *Hum Mutat* 1994, 3:243-247.
32. Kajiwara K, Berson EL, Dryja TP: **Digenic retinitis pigmentosa due to mutations at the unlinked peripherin/*RDS* and *ROM1* loci.** *Science* 1994, 264:1604-1608.
Three families were identified with mutations in unlinked photoreceptor-specific genes *ROM1* and peripherin/*RDS*, in which only double heterozygotes develop retinitis pigmentosa. This study is the first example of digenic inheritance in human disease.
33. Guilford P, Arab SB, Blanchard S, Levilliers J, Weissenbach J, Belkahlia A, Petit C: **A non-syndromic form of neurosensory, recessive deafness maps to the pericentromeric region of chromosome 13q.** *Nature Genet* 1994, 6:24-28.
34. Brown KA, Karbani G, Nobel A, Janjua AH, Newton V, Markham AF, Mueller RF: **Autozygosity mapping of autosomal recessive non-syndromic sensorineural hearing loss (*ARNSSNHL*).** *Am J Hum Genet* 1994, 55:1051.
35. Friedman TB, Liang Y, Weber JL, Hinnant JT, Barber TD, Winata S, Arhya IN, Asher JH: **A gene for congenital, recessive deafness *DFNB3* maps to the pericentromeric region of chromosome 17.** *Nature Genet* 1995, 9:86-91.
By comparing the frequency of short tandem repeat homozygotes among 13 apparently unrelated deaf individuals and 48 unrelated unaffected individuals of an isolated population, allelic disequilibrium was detected between *DFNB3* and short tandem repeats in the pericentromeric region of chromosome 17.
36. Lyon MF, Kirby MC: **Mouse chromosome atlas.** *Mouse Genome* 1994, 92:16-61.
37. Leon PE, Raventos H, Lynch E, Morrow J, King M-C: **The gene for an inherited form of deafness maps to chromosome 5q31.** *Proc Natl Acad Sci USA* 1992, 89:5181-5184.
38. Coucke P, Van Camp G, Djoyodiharjo B, Smith SD, Frants RR, Padberg GW, Darby JK, Huizing EH, Cremers CWRJ, Kimberling WJ *et al.*: **Linkage of autosomal dominant hearing loss to the short arm of chromosome 1 in two families.** *N Engl J Med* 1994, 331:425-431.
39. Chaib H, Lina-Granada G, Guilford P, Plauchi H, Levilliers J, Morgon A, Petit C: **A gene responsible for a dominant form of neurosensory non-syndromic deafness maps to the *NSRD1* recessive deafness gene interval.** *Hum Mol Genet* 1994, 3:2219-2222.

40. Vikkula M, Mariman ECM, Lui VCH, Zhidkova NI, Tiller GE, Goldring MB, Van Beersum SEC, De Waal Malefijt MC, Van den Hoogen FHJ, Ropers H-H et al.: **Autosomal dominant and recessive osteochondrodysplasias associated with the COL11A2 locus.** *Cell* 1995, **80**:431-437.

In an autosomal dominant form of Stickler syndrome characterized by mild spondyloepiphyseal dysplasia, osteoarthritis, sensorineural hearing loss, but without eye involvement, a splice site mutation resulting in 'exon skipping' within the COL11A2 gene was found. In an autosomal recessive form of Stickler syndrome with more severe characteristics, a Gly→Arg substitution was found which is predicted to destabilize the heterotrimeric collagen XI molecules.

41. Brunner HG, Van Bennekom CA, Lambermon EMM, Oei TL, Cremers CWRJ, Weirnga B, Ropers H-H: **The gene for X-linked progressive mixed deafness with perilymphatic gusher during stapes surgery (DFN3) is linked to PGK.** *Hum Genet* 1988, **80**:337-340.
42. Wallis C, Ballo R, Wallis G, Beighton P, Goldblatt J: **X-linked mixed deafness with stapes fixation in a Mauritian kindred: linkage to Xq probe pDP34.** *Genomics* 1988, **3**:299-301.
43. Reardon W, Middleton-Price HR, Sandkuijl L, Phelps P, Bellman S, Luxon L, Pembrey ME, Malcolm S: **A multi-pedigree linkage study of X-linked deafness: linkage to Xq13-q21 and evidence for genetic heterogeneity.** *Genomics* 1991, **11**:885-894.
44. Phelps PD, Reardon W, Pembrey ME, Bellman S, Luxon LM: **X-linked deafness, stapes gusher and a distinctive defect of the inner ear.** *Neuroradiology* 1991, **33**:326-330.
45. Lalwani AK, Brister JR, Fex J, Grundfast KM, Pikus AT, Ploplis B, San Agustin T, Skarka H, Wilcox ER: **A new nonsyndromic X-linked sensorineural hearing impairment linked to Xp21.2.** *Am J Hum Genet* 1994, **55**:685-694.
46. Bach I, Robinson D, Thomas N, Ropers H-H, Cremers FPM: **Physical fine mapping of genes underlying X-linked deafness and non fra(X)-X-linked mental retardation at Xq21.** *Hum Genet* 1992, **89**:620-624.
47. Bach I, Brunner HG, Beighton P, Ruvalcaba RHA, Reardon W, Pembrey ME, Van der Velde-Visser SD, Bruns GAP, Cremers CWRJ, Cremers FPM, Ropers H-H: **Microdeletions in patients with gusher-associated, X-linked mixed deafness (DFN3).** *Am J Hum Genet* 1992, **50**:38-44.
48. Huber I, Bitner-Glindzicz M, De Kok YJM, Van der Maarel SM, Ishikawa-Brush Y, Monaco AP, Robinson D, Malcolm S, Pembrey ME, Brunner HG et al.: **X-linked mixed deafness (DFN3): cloning and characterization of the critical region allows the**

identification of novel microdeletions. *Hum Mol Genet* 1994, **3**:1151-1154.

49. Bitner-Glindzicz M, De Kok Y, Summers D, Huber I, Cremers FPM, Ropers H-H, Reardon W, Pembrey ME, Malcolm S: **Close linkage of a gene for X linked deafness to three microsatellite repeats at Xq21 in radiologically normal and abnormal families.** *J Med Genet* 1994, **31**:916-921.
50. Piussan C, Hanauer A, Dahl N, Mathieu M, Kolski C, Biancalana V, Heyberger S, Strunski V: **X-linked progressive mixed deafness: a new microdeletion that involves a more proximal region in Xq21.** *Am J Hum Genet* 1995, **56**:224-230.
51. Douville PJ, Atanasoski S, Tobler A, Fontana A, Schwab ME: **The brain-specific POU-box gene *Brn4* is a sex-linked transcription factor located on the human and mouse X chromosomes.** *Mammalian Genome* 1994, **5**:180-182.
52. De Kok YJM, Van der Maarel SM, Bitner-Glindzicz M, Huber I, Monaco AP, Malcolm S, Pembrey ME, Ropers H-H, Cremers FPM: **Association between X-linked mixed deafness and mutations in the POU domain gene POU3F4.** *Science* 1995, **267**:685-688.
- This paper describes the first cloning of a nuclear gene involved in non-syndromic deafness (DFN3). Three mutations in the POU3F4 gene result in carboxy-terminal truncation of the predicted proteins, and two missense mutations affect highly conserved amino acid residues. No mutations were identified in patients with X-linked sensorineural deafness lacking the temporal bone defect.
53. Verrijzer CP, Van der Vliet PC: **POU domain transcription factors.** *Biochim Biophys Acta* 1993, **1173**:1-21.
54. Ryan AF, Crenshaw EB III, Simmons DM: **Gene expression in normal and abnormal inner ears.** *Ann NY Acad Sci* 1991, **630**:129-132.
55. Xia Y-R, Andersen B, Mehrabian M, Diep AT, Warden CH, Mohandas T, McEvilly RJ, Rosenfeld MG, Lusk AJ: **Chromosomal organization of mammalian POU domain factors.** *Genomics* 1993, **18**:126-130.

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