Dominant modifier *DFNM1* suppresses recessive deafness *DFNB26*

Saima Riazuddin[1,2], Caley M. Castelein[3], Zubair M. Ahmed[1,2], Anil K. Lalwani[3], Mary A. Mastroianni[4], Sadaf Naz[2], Tenesha N. Smith[1], Nikki A. Liburd[1], Thomas B. Friedman[1], Andrew J. Griffith[1,4], Sheikh Riazuddin[2] & Edward R. Wilcox[1]

More than 50% of severe childhood deafness is genetically determined, approximately 70% of which occurs without other abnormalities and is thus termed nonsyndromic[1,2]. So far, 30 nonsyndromic recessive deafness loci have been mapped and the defective genes at 6 loci, *DFNB1*, *DFNB2*, *DFNB3*, *DFNB4*, *DFNB9* and *DNFB21*, have been identified, encoding connexin-26 (ref. 3), myosin VIIA (ref. 4), myosin XV (ref. 5), pendrin[6], otoferlin[7] and α-tectorin[8], respectively. Here we map a new recessive nonsyndromic deafness locus, *DFNB26*, to a 1.5-cM interval of chromosome 4q31 in a consanguineous Pakistani family. A maximum lod score of 8.10 at θ=0 was obtained with *D4S1610* when only the 8 affected individuals in this family were included in the calculation. There are seven unaffected family members who are also homozygous for the *DFNB26*-linked haplotype and thus are non-penetrant. A dominant modifier, *DFNM1*, that suppresses deafness in the 7 nonpenetrant individuals was mapped to a 5.6-cM region on chromosome 1q24 with a lod score of 4.31 at θ=0 for *D1S2815*.

Large consanguineous families are a powerful resource for mapping and identifying additional deafness loci and genes that modify deafness phenotypes. We identified 141 members of a large consanguineous Pakistani family (PK2) segregating deafness, and our study of this family was approved by Institutional Review Boards at the National Institutes of Health and at the Center of Excellence in Molecular Biology (Lahore, Pakistan). After obtaining written informed consent, medical and family histories and pure-tone audiograms were collected from a subset of study participants. Physical examinations were performed by one of the co-authors (A.J.G.). The pedigrees shown (Figs 1 and 2) include only the PK2 sibships segregating profound, congenital, nonsyndromic, sensorineural hearing loss.

After exclusion of linkage to loci known to cause nonsyndromic deafness, we carried out a genome-wide scan using 358 microsatellite markers (ABI Prism-linkage v1) on affected and unaffected members of the family. We found evidence of linkage with marker *D4S1625* on chromosome 4q31. Haplotype analyses

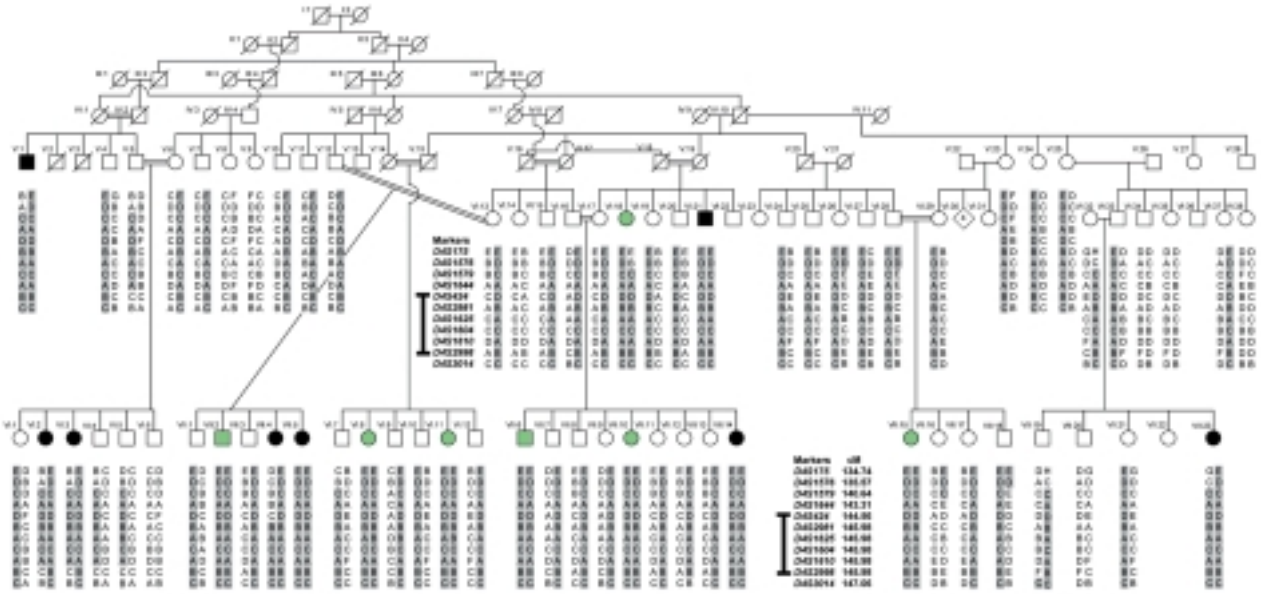

Fig. 1 *DFNB26*-linked haplotypes of PK2 family members on chromosome 4q31. Black symbols, affected individuals; green symbols, normal-hearing, nonpenetrant individuals homozygous for the *DFNB26*-linked haplotype. The grey-shaded haplotype is linked to *DFNB26*. Deaf individuals from the five sibships of family PK2 are all offspring of consanguineous matings. Individual VI:32 married into the PK2 family, but she may also be distantly related to PK2 because she carries the *DFNB26*-linked haplotype and comes from the same caste. Individuals VI:8 and VI:11 are homozygous for alleles of 11 markers of the *DFNB26*-linked haplotype, except *D4S1625*. Heterozygosity for the marker *D4S1625* in two individuals suggests the occurrence of a *de novo* mutation in the father V:15. Allele B of *D4S1625* is present in individual VI:5 and is also segregating in his offspring (data not shown; ref. 30). Seventy-two individuals of family PK2 are not shown, as none of them were homozygous for the *DFNB26*-linked haplotype and none had meiotic recombinations that helped to refine the *DFNB26* interval.

[1]*Laboratory of Molecular Genetics, NIDCD/NIH, Rockville, Maryland, USA.* [2]*Center of Excellence in Molecular Biology, Punjab University, Lahore, Pakistan.* [3]*Laboratory of Molecular Otology, Department of Otolaryngology, University of California, San Francisco, California, USA.* [4]*Neuro-Otology Branch, NIDCD, NIH, Bethesda, Maryland, USA. Correspondence should be addressed to E.R.W. (e-mail: wilcoxe@nidcd.nih.gov).*

Table 1 • Maximum 2-point lod scores for *DFNB26* on chromosome 4q31

	Affected individuals only		Unaffected and affected individuals	
Marker	Lod	θ	Lod	θ
D4S1576	1.41	0.08	2.34	0.09
D4S1579	0.82	0.07	0.51	0.15
D4S1644	1.97	0.06	1.16	0.08
D4S424	3.31	0.03	2.70	0.10
D4S1625	6.20	0	5.87	0.03
D4S1604	3.62	0	3.89	0
D4S2981	7.59	0	6.10	0.07
D4S1610	8.10	0	6.53	0.07
D4S2998	2.40	0.04	2.31	0.10
D4S3014	2.53	0.04	1.95	0.08

of additional markers refined *DFNB26* to a 1.5-cM interval defined by recombinations with *D4S424* and *D4S2998* in individuals VI:2, VI:3 and VII:4. All of the eight affected individuals were homozygous for the *DFNB26*-linked markers on chromosome 4q31 (Fig. 1). A maximum lod score of 8.10 at θ=0 was obtained for *D4S1610* when only affected individuals were included in the calculation, whereas a lod score of 6.53 was obtained at θ=0.07 for *D4S1610* when 60 unaffected PK2 individuals were included in the calculation (Table 1).

We found that seven unaffected individuals (VI:8, VI:11, VI:18, VII:2, VII:6. VII:10 and VII:15) from five different sibships within the family were homozygous for the *DFNB26*-linked haplotype (Fig. 1). This observation indicated either the existence of a modifier gene suppressing the development of *DFNB26* deafness or that the *DFNB26* is located elsewhere. We therefore performed a second genome-wide linkage analysis of both affected and nonpenetrant individuals using 348 markers from the Weber 8 panel (Research Genetics). We found no additional regions of homozygosity linked to *DFNB26* deafness or to the nonpenetrance trait in the seven unaffected individuals (Fig. 3).

We next tested a model for a dominant modifier of *DFNB26*, and found that the nonpenetrance trait linked to markers on chromosome 1q24. Haplotype analysis of the markers linked to this locus (*DFNM1*, for deafness, nonsyndromic, modifier 1) demonstrated meiotic recombinations with markers *D1S2658* and *D1S2790* in individuals VII:15 and VI:18, which reduced the critical interval to 5.6 cM (Fig. 2). A lod score of 4.31 at θ=0 with *D1S2850* was obtained for *DFNM1* by assigning phenotypic status as affected for the nonpenetrant *DFNB26* homozygotes, the deaf as unaffected, and all others as unknown (Table 2). The *DFNM1*-linked haplotype was not inherited by any deaf individuals. As further support for the correct genetic map localization of *DFNB26* and *DFNM1*, we used a multipoint linkage analysis to exclude other regions of the genome. We excluded 71.5% of the genome with a lod score of less than or equal to –2 and 27.4% with a lod score of less than or equal to –1. We calculated the lod scores under a model of recessive inheritance with 100% penetrance and a mutant allele frequency of 0.0001. We also excluded the common mitochondrial mutations that cause deafness in this family by sequencing mitochondrial DNA for the 12S rRNA mutation A1555G, and $tRNA^{ser}$ mutations A7445G and 7472insC (refs 9–11). Audiometric and otoacoustic emissions testing revealed no differences between family members with normal hearing and nonpenetrant *DFNB26* homozygotes (ranging in age from 18 to 51 years).

The map location of *DFNM1* is within the 22-cM *DFNA7* interval[12], indicating that the *DFNM1* suppressor phenotype and *DFNA7* deafness may be two phenotypic variants of the same gene. Within the *DFNM1* interval, *PMX1* (paired mesoderm homeobox) is a potential candidate gene that is expressed in the cochlea[13]. There are no reported cochlear ESTs within the 1.5-cM interval of *DFNB26*, and there are no known deafness loci located within the predicted region of conserved linkage on mouse chromosome 8. Candidate genes identified within the *DFNB26* interval encode a ribosomal protein (*RPS2*; ref. 14), GRB2-associated binding protein-1 (*GAB1*; ref. 15), SWI/SNF-related, matrix-associated, actin-dependent regulator of chromatin (*SMARCA5*; ref. 16), and two ESTs (AJ243229 and DKFZp432C2112) with no sequence similarity to known genes. We are carrying out mutational analyses of these genes from PK2 individuals.

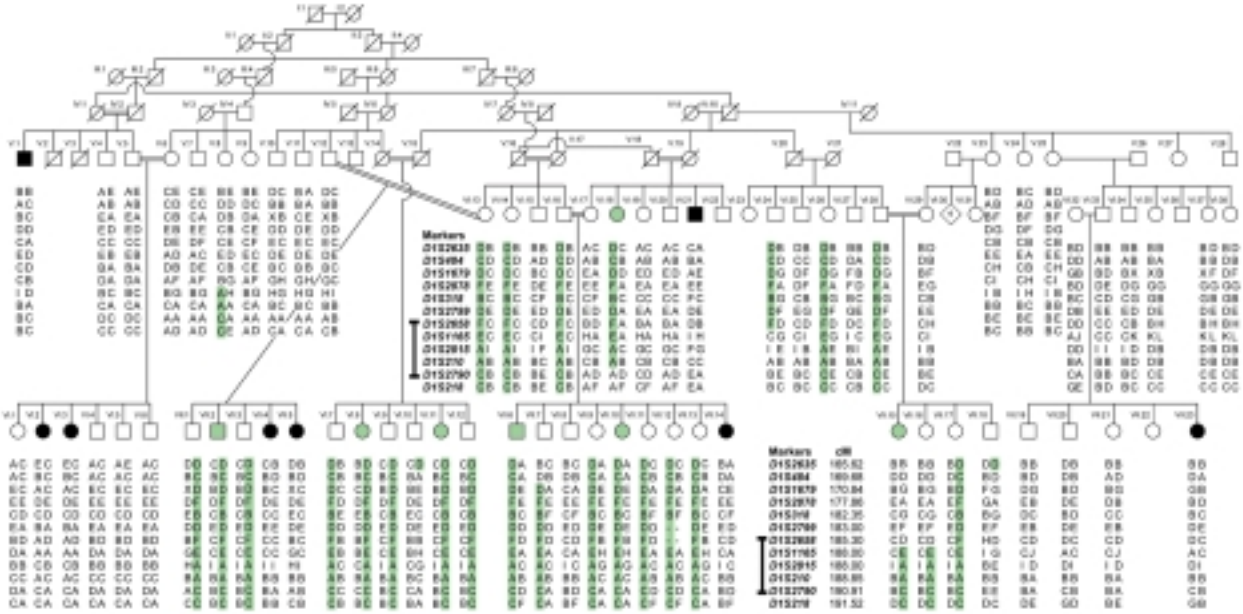

Fig. 2 *DFNM1*-linked haplotypes of PK2 family members on chromosome 1q24. Green symbols, nonpenetrant individuals homozygous for the *DFNB26*-linked haplotype; black symbols, phenotypically affected individuals. The green-shaded haplotype is linked to *DFNM1*-mediated nonpenetrance for *DFNB26* deafness.

Table 2 • Maximum 2-point lod scores for *DFNM1* on chromosome 1q24

Marker	Lod	θ
D1S2635	1.59	0.07
D1S484	2.45	0.05
D1S1679	2.38	0.05
D1S2878	2.41	0.06
D1S318	2.42	0.06
D1S2658	2.64	0.06
D1S1165	4.18	0
D1S2815	4.31	0
D1S210	1.84	0
D1S2790	1.21	0.06
D1S218	0	0.09

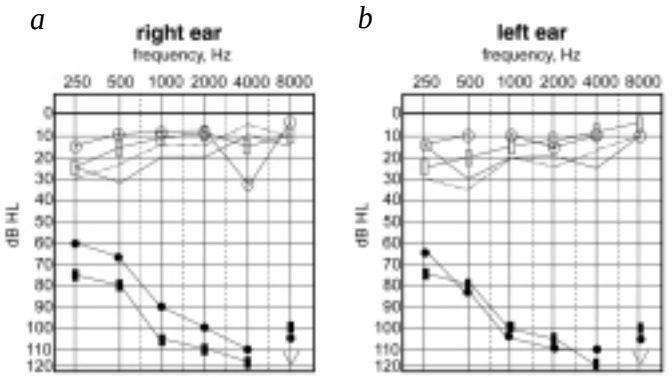

Fig. 3 Representative pure-tone audiograms for PK2 family members. Pure-tone response thresholds are shown for right ears (***a***) and left ears (***b***) of unaffected individuals VII:11 (solid line) and VII:3 (dashed line), nonpenetrant individuals VII:10 (open square) and VII:2 (open circle), and affected individuals VII:14 (filled square) and VII:4 (filled circle). Unaffected individuals, including nonpenetrant individuals, had pure-tone audiometric thresholds at approximately 30 dB HL (Figs 1 and 2). These slightly elevated thresholds may be due to ambient noise present during audiological testing, which was not performed in a soundproof booth. Some individuals may also have slightly elevated thresholds due to prior exposure to aminoglycosides or noise, which are common in this population. Deaf individuals who are homozygous for the linked *DFNB26* haplotype, but do not carry the *DFNM1* modifier, have severe to profound congenital sensorineural hearing loss.

The existence of modifier genes has been known for almost 100 years. In 1919, Bridges reported *Drosophila melanogaster* genes that by themselves produce little or no effect, yet modify the eye colour of the sex-linked mutant *eosin* (ref. 17). There are well-documented examples of intrafamilial variable expressivity for deafness in humans, which are usually attributed to environmental factors or genetic background effects due to modifier genes[18]. A nuclear-encoded modifier seems to cause deafness in association with the mitochondrial mutation A1555G in the absence of exposure to aminoglycosides[19]. The mouse provides several examples of interactions of genetic modifiers with deafness genes. The *mdfw* locus modifies the hearing loss phenotype in *dfw*/+ heterozygotes[20], whereas a dominant allele of *moth1* protects *tubby* mice from hearing loss[21]. Modifier genes can therefore act to suppress or enhance the mutant phenotype[22–27], and *DFNM1* would thus be classified as a suppressor of *DFNB26* deafness.

The genetics of nonpenetrance of *DFNB26* deafness seems to be understood. It is possible that *DFNM1* will prove to be a more general suppressor of a specific class of mutant alleles of a variety of different genes. The elucidation of the underlying mechanism of suppression, however, awaits the identification of the *DFNB26* and *DFNM1* genes. Functional analysis of *DFNB26* and *DFNM1* should provide new insights into the molecular mechanisms of auditory function and facilitate the rational design of therapies for hearing loss.

Methods

Genotyping. We extracted genomic DNA from blood according to a standard protocol[28] and performed genotypic analysis of polymorphic markers to exclude linkage to known *DFNB* loci. We performed a genome-wide linkage analysis by genotype analyses of 674 microsatellite markers, with a resolution of less than 10 cM. PCR products were separated and detected on 4.25% acrylamide gels with an ABI 377 DNA sequencer, and alleles were assigned with Genotyper software (v 2.0 Applied Biosystems).

Statistical analysis. We calculated lod scores using FASTLINK (v 4.1p; ref. 29) and modelled deafness as an autosomal recessive trait. We carried out computations of two-point lod scores with MLINK on the full pedigree as described in Figs 1 and 2 and analysed multipoint exclusion mapping with LINKMAP on a simplified pedigree containing only three loops. To calculate the initial lod score for the *DFNB26* locus, we ascribed all deaf individuals as affected and all others as unknown. For the *DFNM1* locus, a dominant model was assumed and subjects with normal hearing homozygous for the *DFNB26*-linked haplotype were assigned as affected, deaf individuals homozygous for *DFNB26*-linked haplotype were assigned as unaffected, and all others were assigned as unknown. We determined marker allele frequencies by genotype analysis of genomic DNA from 90 unrelated Pakistani subjects.

Note added in proof: Three additional nonsyndromic recessive deafness loci, *DFNB10*/*B8*, *DFNB12* and *DFNB29*, were recently identified, encoding transmembrane serine protease-3 (Hereditary Hearing Loss Home Page, http://dnalab-www.uia.ac.be/dnalab/hhh/), cadherin-23 (ref. 31) and claudin-14 (Hereditary Hearing Loss Home Page, http://dnalab-www.uia.ac.be/dnalab/hhh/), respectively.

Acknowledgements

We thank the PK2 family members for their participation, which is supported by the National Institute on Deafness and Other Communication Disorders, Intramural Research Project Z01DC00035; B.U. Amjad for help in initiating this study; R. Morell, D. Drayna, J. Bork, T. Ben-Yosef and K. Kurima for comments; and S. Khan and B. Ploplis for technical support. University Grants Commission supported part of this study in Pakistan. This study used the computational capabilities of the Biowoulf Center at the CIT, NIH.

Received 12 September; accepted 18 October 2000.

1. Morton, N.E. Genetic epidemiology of hearing impairment. *Ann. NY Acad. Sci.* **630**, 16–31 (1991).
2. Willems, P.J. Genetic causes of hearing loss. *N. Engl. J. Med.* **342**, 1101–1109 (2000).
3. Kelsell, D.P. *et al*. Connexin 26 mutations in hereditary non-syndromic sensorineural deafness. *Nature* **387**, 80–83 (1997).
4. Liu, X.Z. *et al*. Mutations in the myosin VIIA gene cause non-syndromic recessive deafness. *Nature Genet.* **16**, 188–190 (1997).
5. Wang, A. *et al*. Association of unconventional myosin *MYO15* mutations with human nonsyndromic deafness *DFNB3*. *Science* **280**, 1447–1451 (1998).
6. Li, X.C. *et al*. A mutation in *PDS* causes non-syndromic recessive deafness. *Nature Genet.* **18**, 215–217 (1998).
7. Yasunaga, S. *et al*. A mutation in *OTOF*, encoding otoferlin, a FER-1-like protein, causes *DFNB9*, a nonsyndromic form of deafness. *Nature Genet.* **21**, 363–369 (1999).
8. Mustapha, M. *et al*. An α-tectorin gene defect causes a newly identified autosomal recessive form of sensorineural pre-lingual non-syndromic deafness, *DFNB21*. *Hum. Mol. Genet.* **8**, 409–412 (1999).
9. Prezant, T.R. *et al*. Mitochondrial ribosomal RNA mutation associated with both antibiotic-induced and non-syndromic deafness. *Nature Genet.* **4**, 289–294 (1993).
10 Tiranti, V. *et al*. Maternally inherited hearing loss, ataxia and myoclonus associated with a novel point mutation in mitochondrial tRNASer(*UCN*) gene. *Hum. Mol. Genet.* **4**, 1421–1427 (1995).
11. Verhoeven, K. *et al*. Hearing impairment and neurological dysfunction associated with a mutation in the mitochondrial tRNASer(*UCN*) gene. *Eur. J. Hum. Genet.* **7**, 45–51 (1999).
12. Fagerheim, T. *et al*. Identification of a new locus for autosomal dominant non-syndromic hearing impairment (*DFNA7*) in a large Norwegian family. *Hum. Mol. Genet.* **5**, 1187–1191 (1996).
13. Kern, M.J., Argao, E.A., Birkenmeier, E.H., Rowe, L.B. & Potter, S.S. Genomic organization and chromosome localization of the murine homeobox gene Pmx. *Genomics* **19**, 334–340 (1994).
14. Slynn, G. *et al*. Human cDNA sequence homologous to the mouse *LLRep3* gene family. *Nucleic Acids Res.* **18**, 681 (1990).
15. Holgado-Madruga, M., Moscatello, D.K., Emlet, D.R., Dieterich, R. & Wong, A.J. *Grb2*-associated binder-1 mediates phosphatidylinositol 3-kinase activation and the promotion of cell survival by nerve growth factor. *Proc. Natl Acad. Sci. USA* **94**, 12419–12424 (1997).
16. Aihara, T. *et al*. Cloning and mapping of *SMARCA5* encoding *hSNF2H*, a novel human homologue of Drosophila *ISWI*. *Cytogenet. Cell. Genet.* **81**, 191–193 (1998).
17. Bridges, C.B. Specific modifiers of eosin eye color in *Drosophila melanogaster*. *J. Exp. Zool.* **28**, 337–384 (1919).
18. Friedman, T.B. *et al*. Modifier genes of hereditary hearing loss. *Curr. Opin. Neurobiol.* **10**, 487–493 (2000).
19. Bykhovskaya, Y. *et al*. Candidate locus for a nuclear modifier gene for maternally inherited deafness. *Am. J. Hum. Genet.* **66**, 1905–1910 (2000).
20. Noben-Trauth, K., Zheng, Q.Y., Johnson, K.R. & Nishina, P.M. *mdfw*: a deafness susceptibility locus that interacts with deaf waddler (*dfw*). *Genomics* **44**, 266–272 (1997).
21. Ikeda, A. *et al*. Genetic modification of hearing in tubby mice: evidence for the existence of a major gene (*moth1*) which protects tubby mice from hearing loss. *Hum. Mol. Genet.* **8**, 1761–1767 (1999).
22. Bejjani, B.A. *et al*. Multiple *CYP1B1* mutations and incomplete penetrance in an inbred population segregating primary congenital glaucoma suggest frequent de novo events and a dominant modifier locus. *Hum. Mol. Genet.* **9**, 367–374 (2000).
23. Cheng, N.C. *et al*. A human modifier of methylation for class I *HLA* genes (*MEMO-1*) maps to chromosomal bands 1p35–36.1. *Hum. Mol. Genet.* **5**, 309–317 (1996).
24. Marin, M.C. *et al*. A common polymorphism acts as an intragenic modifier of mutant p53 behavior. *Nature Genet.* **25**, 47–54 (2000).
25. Wu, X. *et al*. Evidence for regulation of the *PTEN* tumor suppressor by a membrane-localized multi-PDZ domain containing scaffold protein *MAGI-2*. *Proc. Natl Acad. Sci. USA* **97**, 4233–4238 (2000).
26. Zielenski, J. *et al*. Detection of a cystic fibrosis modifier locus for meconium ileus on human chromosome 19q13. *Nature Genet.* **22**, 128–129 (1999).
27. Sertie, A.L., Sousa, A.V., Steman, S., Pavanello, R.C. & Passos-Bueno, M.R. Linkage analysis in a large Brazilian family with van der Woude syndrome suggests the existence of a susceptibility locus for cleft palate at 17p11.2–11.1. *Am. J. Hum. Genet.* **65**, 433–440 (1999).
28. Grimberg, J. *et al*. A simple and efficient non-organic procedure for the isolation of genomic DNA from blood. *Nucleic Acids Res.* **17**, 8390 (1989).
29. Schaffer, A.A., Gupta, S.K., Shriram, K. & Cottingham, R.W. Jr Avoiding recomputation in linkage analysis. *Hum. Hered.* **44**, 225–237 (1994).
30. Weber, J.L. & Wong, C. Mutation of human short tandem repeats. *Hum. Mol. Genet.* **2**, 1123–1128 (1993).
31. Bork, J.M. *et al.* Usher syndrome 1D (*USH1D*) and nonsyndromic recessive deafness *DFNB12* are caused by allelic mutations of the novel cadherin-like gene *CDH23*. *Am. J. Hum. Genet.* (in press).