Human Molecular Genetics, 1996, Vol. 5, No. 7 **1061–1064**

Mapping of *DFNB12*, a gene for a non-syndromal autosomal recessive deafness, to chromosome 10q21–22

Hassan Chaïb[1], Christophe Place[2,+], Nabiha Salem[2], Catherine Dodé[1], Sébastien Chardenoux[1], Jean Weissenbach[3], Elie El Zir[4], Jacques Loiselet[2] and Christine Petit[1,*]

[1]Unité de Génétique Moléculaire Humaine, URA CNRS 1968, Institut Pasteur, 25 rue du Docteur Roux, 75724 Paris cedex 15, France, [2]Département de Biochimie, Faculté de Médecine, Université St Joseph, Beyrouth, Liban, [3]Généthon, rue de l'Internationale, 91000 Evry, France and [4]Clinique d'Audiologie, Hôpital du Sacré-Coeur, POB 116, Baabda, Brazilia, Liban

Received March 6, 1996; Revised and Accepted April 18, 1996

We report here, the localization of a new recessive non-syndromal deafness gene (*DFNB12*) to 10q21–22 by linkage analysis, of a Sunni family. Affected individuals suffer from congenital profound sensorineural hearing loss. A maximum LOD score of 6.40 (θ = 0.00) was obtained with locus D10S535. Analysis of patients carrying recombinations mapped the gene distal to D10S529 and proximal to D10S532, delineating an interval between 11 and 15 cM. Three deaf mouse mutants *Jackson circler* (*jc*), *Waltzer* (*v*) and *Ames waltzer* (*av*) have been localized to the homologous murine region on chromosome 10. Each of these mouse mutants is a candidate mouse model for the *DFNB12*-associated hearing impairment.

INTRODUCTION

Deafness is the most frequent sensorineural nonsyndromic defect in humans, affecting one in 1000 children at birth or in infancy in the United States (1). About 75% of the inherited forms of congenital isolated deafness have an autosomal recessive mode of transmission (2). This frequency is higher in geographic regions where there exists a high level of consanguinity (3). Localization of the genes responsible for autosomal recessive forms of deafness encounters serious difficulties due to the combination of: (i) the extreme genetic heterogeneity of this defect, with an estimated number of 40 genes segregating in Western populations (1,4); (ii) the absence of clinical criteria allowing differentiation between the inner ear defects due to the various genes; and (iii) the high proportion of marriages between deaf people, at least in developed countries, leading to the coexistence of several defective genes responsible for clinically indistinguishable phenotypes within the same family.

Three different strategies have been used for mapping deafness genes. The first is based on the study of large affected consanguineous families living in isolated regions for several generations. In such families, the probability of having more than one deafness gene segregating is minimized. Using this approach, LOD score linkage genetic analysis of two large families living in northern and in southern Tunisia allowed us to localize the first two genes involved in autosomal recessive deafness, *DFNB1* to the pericentromeric region of chromosome 13q11 (5) and *DFNB2* to 11q13.5 (6), respectively. Once a chromosomal interval is defined, the localization of the gene can then be refined by homozygosity mapping. Subsequently, the same strategy was used to map several other *DFNB* genes: *DFNB4* (7), *DFNB8* (8) and *DFNB9* (9) to 7q31, 21q22 and 2p22–23, respectively. The second approach is the analysis of an affected isolated population with a high proportion of deafness cases. This analysis, based on a direct search for homozygosity regions with allele frequency disequilibrium, has allowed the mapping of the *DFNB3* gene to 17q11.2–12 (10). Finally, direct homozygosity involving the identification of homozygous regions shared by affected inbred children, enables the mapping of rare recessive deafness genes in small consanguineous families (11). Three *DFNB* genes, *DFNB5* (12), *DFNB6* (13) and *DFNB7* (14) were mapped to chromosomes 14q12, 3p14–21 and 9q13–21, respectively, in small consanguineous families originating from western and southern India. None of the localized *DFNB* genes has yet been cloned.

To date, we have essentially mapped *DFNB* genes segregating in consanguineous families living around the Mediterranean sea. We report here on the localization of a new gene for a non-syndromal recessive deafness (*DFNB12*) to chromosome 10 (10q21–22). This gene is segregating in a consanguineous Sunni family, living in an isolated village in Syria, which is affected by a profound prelingual sensorineural hearing impairment.

RESULTS

Family members underwent a general, as well as an otoscopic, clinical evaluation. A complete medical history was obtained from each affected individual to exclude the possibility of

*To whom correspondence should be addressed
+Present address: Unité de Physicochimie des Macromolécules, Institut Pasteur, 25 rue du Docteur Roux, 75724 Paris cedex 15, France

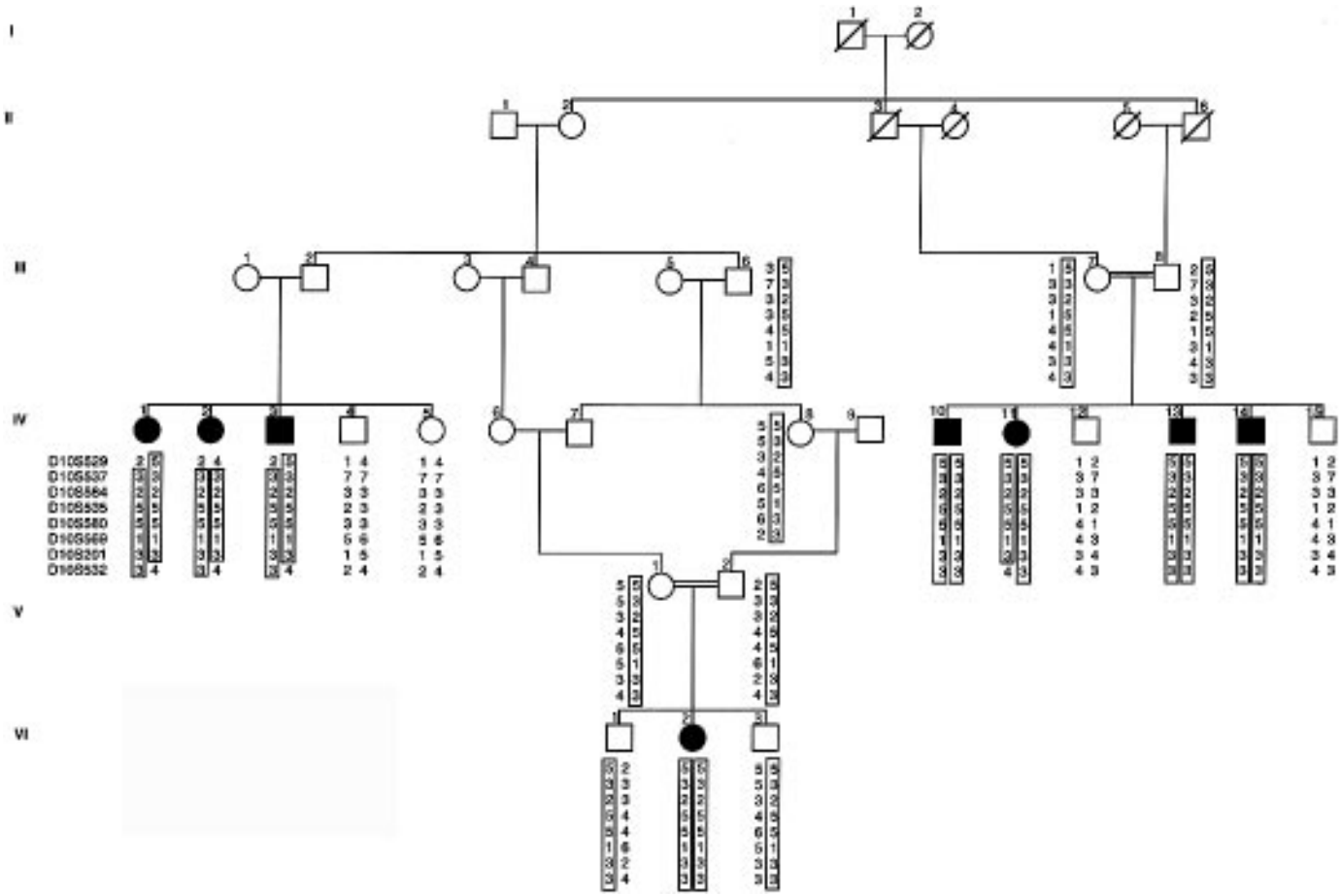

Figure 1. Segregation analysis of the Sunni Syrian family using eight polymorphic markers in the 10q21–22 region. Dark symbols represent deaf individuals and slashed symbols, deceased members. Boxes indicate the haplotypes linked to the deafness. The order of the microsatellite markers was obtained from the Généthon database (38).

infectious or environmental causes of hearing impairment. These individuals showed no evidence of external ear abnormality, mental retardation, loss of vision, renal anomaly or integumentary disorder. In addition, no apparent balance problems or vertigo and no delay in the age of walking was noted (although no further assessment of vestibular function was conducted). Audiometry tests showed no response at 100 dB for frequencies superior to 1000 Hz in all affected individuals, and no response at 80 dB for frequencies up to 1000 Hz. In the affected children (4–15 years of age), no auditory brainstem response (ABR) was observed up to 100 dB. The parents, who are heterozygous carriers, showed normal results for the audiometric tests.

Before initiating linkage analysis, we excluded the involvment of the loci already known to be responsible for autosomal recessive deafness (see Introduction). In addition, given that we have previously shown that a single locus can be responsible for both dominant and recessive forms of deafness (DFNA3 and *DFNB1*) (15), we also excluded the involvement of the following three loci, *DFNA1* (16), *DFNA2* (17) and *DFNA4* (18), reported as responsible for dominant forms of sensorineural deafness. Analysis of 80 polymorphic markers from the Généthon collection, approximately spaced each 20 cM, allowed us to exclude about 35% of the genome. Significant linkage was found with the marker AFM200wf4 (locus D10S535) on chromosome 10 (Fig. 1), which resulted in a maximum LOD score of 6.64 at $\theta = 0.00$. The analysis was repeated assuming a penetrance of 80% and a maximum LOD score of 6.40 at $\theta = 0.00$ was found confirming linkage.

The markers adjacent to D10S535 were then tested: on the proximal side—locus D10S529 (3 cM), D10S537 (3 cM) and D10S584 (1 cM); and on the distal side—D10S532 (12 cM), D10S201 (8 cM), D10S569 (5 cM) and D10S580 (3 cM) (Fig. 2). Analysis of individuals carrying recombinations mapped the gene involved distal to the D10S529 locus (individuals IV1, IV2, IV3) and proximal to D10S532 (individuals IV1, IV2, IV3, IV11), thus delineating an interval of 11–15 cM for the *DFNB12* gene. Moreover, the recombination event in individual IV2 allowed us to localize D10S529 proximal to D10S537. All affected individuals are homozygous for the polymorphic markers located within the D10S529–D10S532 interval.

DISCUSSION

We here report on the mapping of a gene responsible for an autosomal recessive form of deafness, *DFNB12*, to 10q21–22 by LOD score analysis of a Sunni family living in Syria.

There exist a number of genes already mapped to the 10q21–22 chromosomal interval defined for *DFNB12*, as well as some other genes assigned to the region but which are less precisely mapped, which could be considered as candidate genes. Among them,

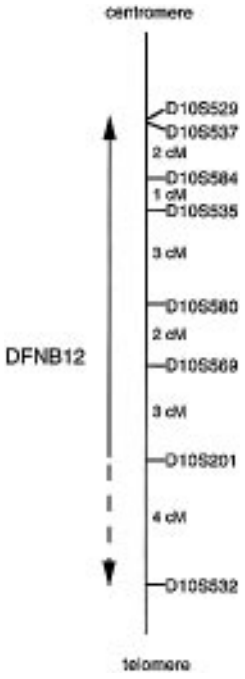

Figure 2. A simplified human chromosome 10q genetic map showing the interval containing *DFNB12*. Distances between adjacent markers are in centimorgans.

however, a more likely candidate for *DFNB12* may be the gene encoding the mitochondrial transcription factor 6-like 2 (19), since mitochondrial deficits have already been shown to be responsible for deafness (20,21).

The isolation of a gene by a positional cloning strategy first requires refinement of the localization interval. This step, which is based on linkage analysis, encounters a particular difficulty in the case of *DFNB* genes, as, due to the extreme genetic heterogeneity of deafness, only large families that provide a significant LOD score can be used to narrow down the gene interval. Alternatively, cloning of *DFNB* genes can greatly benefit from the numerous deaf mouse mutants which have been identified (22). Some of them have already been shown to involve genes responsible for hearing impairment in humans, the phenotypic expression often being different from one species to the other (22–28). In each case where the comparative human and murine maps lead to the hypothesis that the mouse homologue of a *DFNB* gene may be mutated in a deaf mouse mutant, cloning this *DFNB* gene will be more efficiently accomplished by first isolating the murine homologue. At present, for all mapped *DFNB* genes, with the exception of *DFNB1* and *DFNB5*, the homologous murine region has been defined. For these loci, the possible existence of a deaf mouse mutant within the homologous region has been investigated. However, for only four of these—*DFNB2* (6,29), *DFNB3* (10,30), *DFNB6* (13,31) and *DFNB7* (14,32)—does the phenotype of the candidate mouse mutant seem consistent with the phenotype of the human defect. Interestingly, the homologous murine region for *DFNB12*, which extends at least 28–37 cM from the centromere of chromosome 10, contains three deaf mouse mutants, *Jackson circler* (*jc*), *Waltzer* (*v*) and *Ames Waltzer* (*av*) (33,34). These three recessive mutants show the typical circling, head-tossing, deafness and hyperactivity of the circling mutants. Homozygous *jc* mutants show erratic circling behaviour and hyperactivity which become more pronounced with age (35,36). Most of the homozygous *v* mutants are deaf from birth and display anomalies of the inner ear including degeneration of the organ of Corti, spiral ganglion, stria vascularis and saccular macula (29). In *av* mutants (37), the development of the cochlea is initially normal and degrades in the postnatal period. Thus it is tempting to speculate that one of these mutants involves the *DFNB12* orthologous murine gene.

MATERIALS AND METHODS

Auditory tests

Audiometry testing was performed on all family members. Affected children (4–15 years of age) showed profound prelingual sensorineural hearing impairment. Pure-tone audiometry with air and bone conduction at 250, 500, 1000, 2000, 4000 and 8000 Hz were systematically performed (with a Belton 2000 clinical audiometer), as well as otoscopic examination of each individual over 5 years of age. For the youngest children (less than 4 years of age), auditory brainstem responses (ABR) were tested upon click stimulation using the Lindar apparatus (RACIA).

Genotyping

Genomic DNA was prepared from 5 to 10 ml of blood by standard techniques. Microsatellite markers were amplified by the polymerase chain reaction (PCR) and analysed on polyacrylamide gels. The primer sequences of the Généthon markers AFM162xa1 (D10S529), AFM203xc5 (D10S537), AFM294wh9 (D10S584), AFM200wf4 (D10S535), AFM284vf5 (D10S580), AFM265zg5 (D10S569), AFM155zc3 (D10S201) and AFM192xa5 (D10S532) have previously been reported (38).

Linkage analysis

LOD scores were calculated using the LINKAGE 5.1 program package (39). Two-point LOD scores were calculated with MLINK and ILINK programs. Deafness was assumed to be inherited in a recessive manner and coded as fully penetrant. The gene frequency was set at 10^{-4} and the variation of this frequency between 10^{-3} and 10^{-5} resulted in negligible alteration of the two-point LOD score. Recombination frequencies were assumed to be equal for both males and females. The allele frequencies of the polymorphic markers were assumed to be equal. A 100-fold change in the frequency of the linked allele resulted in only minor variation of the maximum two-point LOD score. The 90% confidence limits were calculated using the '1 LOD down' method (40).

ACKNOWLEDGEMENTS

We are grateful to the family who participated in this work. We thank J-P. Hardelin, V. Kalatzis, J. Levilliers and G. Lévy for critically reading the manuscript. We thank K. P. Steel and S. D. M. Brown for information concerning mouse homologues. This work was supported by grants from the AFM (Association Française contre les Myopathies) and EEC.

REFERENCES

1. Morton, N. E. (1991). Genetic epidemiology of hearing impairment. In *Genetics of hearing impairment.* The New York Academy of Science, New York, 630, 16–31
2. Newton, V. E. (1985). Aetiology of bilateral sensorineural hearing loss in young children. *J. Laryngol. Otol.* (Suppl.), **10,** 1–57.
3. Majumder, P. P., Ramesh, A. and Chinnappan, D. (1989). On the genetics of prelingual deafness. *Am. J. Hum. Genet.*, **44,** 86–99.
4. Brownstein, Z., Friedlander, Y., Peritz, E. and Cohen, T. (1991). Estimated number of loci for autosomal recessive severe nerve deafness within the Israeli Jewish population, with implications for genetic counseling. *Am. J. Med. Genet.*, **41,** 306–312.
5. Guilford, P., Ben Arab, S., Blanchard, S., Levilliers, J., Weissenbach, J., Belkahia, A. and Petit, C. (1994). A non-syndromic form of neurosensory, recessive deafness maps to the pericentromeric region of chromosome 13q. *Nature Genet.*, **6,** 24–28.
6. Guilford, P., Ayadi, H., Blanchard, S., Chaïb, H., Le Paslier, D., Weissenbach, J., Drira, M. and Petit, C. (1994). A human gene responsible for neurosensory, nonsyndromic recessive deafness is a candidate homologue of the mouse sh-1 gene. *Hum. Mol. Genet.*, **3,** 989–993.
7. Baldwin, C. T., Weiss, S., Farrer, L. A., De Stefano, A. L., Adair, R., Franklyn, B., Kidd, K. K., Korostishevsky, M. and Bonné-Tamir, B. (1995). Linkage of congenital, recessive deafness (DFNB4) to chromosome 7q31 and evidence for genetic heterogeneity in the Middle Eastern Druze population. *Hum. Mol. Genet.*, **4,** 1637–1642.
8. Veske, A., Oehlmann, R., Younus, F., Mohyuddin, A., Müller-Myhsok, B., Mehdi, S. Q. and Gal, A. (1996). Autosomal recessive non-syndromic deafness locus (DFNB8) maps on chromosome 21q22 in a large consanguineous kindred from Pakistan. *Hum. Mol. Genet.*, **5,** 165–168.
9. Chaïb, H., Place, C., Salem, N., Chardenoux, S., Vincent, C., Weissenbach, J., El-Zir, E., Loiselet, J. and Petit, C. (1996). A gene responsible for a sensorineural nonsyndromic recessive deafness maps to chromosome 2p22–23. *Hum. Mol. Genet.*, **5,** 155–158.
10. Friedman, T. B., Liang, Y., Weber, J. L., Hinnant, J. T., Barber, T. D., Winata, S., Arhya, I. N. and Asher, J. H. (1995). A gene for congenital, recessive deafness DFNB3 maps to the pericentromeric region of chromosome 17. *Nature Genet.*, **9,** 86–91.
11. Lander, E. S. and Botstein, D. (1987). Homozygosity mapping: a way to map human recessive traits with the DNA of inbred children. *Science*, **236,** 1567–1570.
12. Fukushima, K., Ramesh, A., Srisailapathy, C. R. S., Ni, L., Chen, A., O'Neil, M., Van Camp, G., Coucke, P., Smith, S. D., Kenyon, J. B., Jain, P., Wilcox, E. R., Zbar, R. I. S. and Smith, R. J. H. (1995). Consanguineous nuclear families used to identify a new locus for recessive non-syndromic hearing loss on 14q. *Hum. Mol. Genet.*, **4,** 1643–1648.
13. Fukushima, K., Ramesh, A., Srisailapathy, C. R. S., Ni, L., Wayne, S., O'Neil, M. E., Van Camp, G., Coucke, P., Jain, P., Wilcox, E. R., Smith, S. D., Kenyon, J. B., Zbar, R. I. S. and Smith, R. J. H. (1995). An autosomal recessive non-syndromic form of sensorineural hearing loss maps to 3p-DFNB6. *Genome Res.*, **5,** 305–308.
14. Jain, P. K., Fukushima, K., Deshmukh, D., Ramesh, A., Thomas, E., Lalwani, A. K., Kumar, S., Ploplis, B., Skarka, H., Srisailapathy, C. R. S., Wayne, S., Zbar, R. I. S., Verma, I. C., Smith, R. J. H. and Wilcox, E. R. (1995). A human recessive neurosensory nonsyndromic hearing impairment locus is a potential homologue of the murine deafness (dn) locus. *Hum. Mol. Genet.*, **4,** 2391–2394.
15. Chaïb, H., Lina-Granade, G., Guilford, P., Plauchu, H., Levilliers, J., Morgon, A. and Petit, C. (1994). A gene responsible for a dominant form of neurosensory non-syndromic deafness maps to the NSRD1 recessive deafness gene interval. *Hum. Mol. Genet.*, **3,** 2219–2222.
16. Leon, P. E., Raventos, H., Lynch, E., Morrow, J. and King, M.-C. (1992). The gene for an inherited form of deafness maps to chromosome 5q31. *Proc. Natl Acad. Sci. USA*, **89,** 5181–5184.
17. Coucke, P., Van Camp, G., Djoyodiharjo, B., Smith, S. D., Frants, R. R., Padberg, G. W., Darby, J. K., Huizing, E. H., Cremers, C. W. R. J., Kimberling, W. J., Oostra, B. A., Van de Heyning, P. H. and Willems, P. J. (1994). Linkage of autosomal dominant hearing loss to the short arm of chromosome 1 in two families. *N. Engl. J. Med.*, **331,** 425–431.
18. Chen, A. H., Ni, L., Fukushima, K., Marietta, J., O'Neil, M., Coucke, P., Willems, P. and Smith, R. J. H. (1995). Linkage of a gene for dominant non-syndromic deafness to chromosome 19. *Hum. Mol. Genet.*, **4,** 1073–1076.
19. Tiranti, V., Rossi, E., Ruiz-Carrillo, A., Rossi, G., Rocchi, M., Di Donato, S., Zuffardi, O. and Zeviani, M. (1995). Chromosomal localization of mitochondrial transcription factor A (TCF6), single-stranded DNA-binding protein (SSBP), and endonuclease G (ENDOG), three human housekeeping genes involved in mitochondrial biogenesis. *Genomics*, **25**, 559–64.
20. Prezant, T. R., Agapian, J. V., Bohlman, M. C., Bu, X., Öztas, S., Qiu, W.-Q., Arnos, K. S., Cortopassi, G. A., Jaber, L., Rotter, J. I., Shohat, M. and Fischel-Ghodsian, N. (1993). Mitochondrial ribosomal RNA mutation associated with both antibiotic-induced and non-syndromic deafness. *Nature Genet.*, **4,** 289–294.
21. Reid, F. M., Vernham, G. A. and Jacobs, H. T. (1994). A novel mitochondrial point mutation in a maternal pedigree with sensorineural deafness. *Hum. Mutat.*, **3,** 243–247.
22. Steel, K. P. and Smith, R. J. H. (1992). Normal hearing in Splotch (Sp/+), the mouse homologue of Waardenburg syndrome type 1. *Nature Genet.*, **2,** 75–79.
23. Epstein, D. J., Vekemans, M. and Gros, P. (1991). splotch (Sp2H), a mutation affecting development of the mouse neural tube, shows a deletion within the paired homeodomain of Pax-3. *Cell*, **67,** 767–774.
24. Baldwin, C. T., Hoth, C. F., Amos, J. A., da-Silva, E. O. and Milunsky, A. (1992). An exonic mutation in the HuP2 paired domain gene causes Waardenburg's syndrome. *Nature*, **355,** 637–638.
25. Tassabehji, M., Read, A. P., Newton, V. E., Patton, M., Gruss, P., Harris, R. and Strachan, T. (1993). Mutations in the PAX3 gene causing Waardenburg syndrome type 1 and type 2. *Nature Genet.*, **3,** 26–30.
26. Tassabehji, M., Newton, V. E., Leverton, K., Turnbull, K., Seemanova, E., Kunze, J., Sperling, K., Strachan, T. and Read, A. P. (1994). PAX3 gene structure and mutations: close analogies between Waardenburg syndrome and the Splotch mouse. *Hum. Mol. Genet.*, **3,** 1069–1074.
27. Gibson, F., Walsh, J., Mburu, P., Varela, A., Brown, K. A., Antonio, M., Beisel, K. W., Steel, K. P. and Brown, S. D. M. (1995). A type VII myosin encoded by the mouse deafness gene Shaker-1. *Nature*, **374,** 62–64.
28. Weil, D., Blanchard, S., Kaplan, J., Guilford, P., Gibson, F., Walsh, J., Mburu, P., Varela, A., Levilliers, J., Weston, M. D., Kelley, P. M., Kimberling, W. J., Wagenaar, M., Levi-Acobas, F., Larget-Piet, D., Munnich, A., Steel, K. P., Brown, S. D. M. and Petit, C. (1995). Defective myosin VIIA gene responsible for Usher syndrome type 1B. *Nature*, **374,** 60–61.
29. Deol, M. S. (1956). The anatomy and development of the mutants pirouette, shaker-1 and waltzer in the mouse. *Proc. R. Soc. London Ser. B*, **145,** 206–213.
30. Deol, M. S. (1954). The anomalies of the labyrinth of the mutants Varitint-wadler, Shaker-2 and Jerker in the mouse. *J. Genet.*, **52,** 562–594.
31. Deol, M. S. and Robbins, M. W. (1962). The spinner mouse. *J. Hered.*, **53,** 133–136.
32. Deol, M. S. and Kocher, W. (1958). A new gene for deafness in the mouse. *Heredity*, **12,** 463–466.
33. Burmeister, M., Sufalko, D., Kapfhamer, D. and Raphael, Y. (1994). Genetic mapping of the mouse mutation *Ames Waltzer* (*av*). in Mouse Genome Conference (eds, London)
34. Taylor, B. A., Burmeister, M. and Bryda, E. C. (1994). Mouse chromosome 10. *Mamm. Genome*, **5,** S154–163.
35. Kocher, W. (1960). Untersuchungen zur Genetik und Pathologie des Entwicklung von 8 Labyrinth-Mutanten (*deaf-waltzer-shaker*-Mutanten) der Maus (Mus musculus). *Z. Vererbn.*, **91,** 114–140.
36. Southard, J. L. and Dickie, M. M. (1970). Jackson circler. *Mouse Newslett.*, **42,** 30.
37. Osako, S. and Hilding, D. A. (1971). Electron microscopic studies of capillary permeability in normal and *Ames waltzer* deaf mice. *Acta Otolaryngol. (Stockh.)*, **71,** 365–376.
38. Gyapay, G., Morissette, J., Vignal, A., Dib, C., Fizames, C., Millasseau, P., Marc, S., Bernardi, G., Lathrop, M. and Weissenbach, J. (1994). The 1993–94 Généthon human genetic linkage map. *Nature Genet.*, **7,** 246–339.
39. Lathrop, G. M., Lalouel, J. M., Julier, C. and Ott, J. (1985). Multilocus linkage analysis in humans: detection of linkage and estimation of recombination. *Am. J. Hum. Genet.*, **37,** 482–498.
40. Conneally, P. M., Edwards, J. H., Kidd, K. K., Lalouel, J-M., Morton, N. E., Ott, J. and White, R. (1985). Report of the committee on the methods of linkage analysis and reporting. *Cytogenet. Cell Genet.*, **40,** 356–359.