Genetic Mapping Refines *DFNB3* to 17p11.2, Suggests Multiple Alleles of *DFNB3,* and Supports Homology to the Mouse Model *shaker-2*

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Summary

The nonsyndromic congenital recessive deafness gene, *DFNB3,* first identified in Bengkala, Bali, was mapped to a ~12-cM interval on chromosome 17. New short tandem repeats (STRs) and additional DNA samples were used to identify recombinants that constrain the *DFNB3* interval to ≤6 cM on 17p11.2. Affected individuals from Bengkala and affected members of a family with hereditary deafness who were from Bila, a village neighboring Bengkala, were homozygous for the same alleles for six adjacent STRs in the *DFNB3* region and were heterozygous for other distal markers, thus limiting *DFNB3* to an ~3-cM interval. Nonsyndromic deafness segregating in two unrelated consanguineous Indian families, M21 and I-1924, were also linked to the *DFNB3* region. Haplotype analysis indicates that the *DFNB3* mutations in the three pedigrees most likely arose independently and suggests that *DFNB3* makes a significant contribution to hereditary deafness worldwide. On the basis of conserved synteny, mouse deafness mutations *shaker-2* (*sh2*) and *sh2^J* are proposed as models of *DFNB3.* Genetic mapping has refined *sh2* to a 0.6-cM interval of chromosome 11. Three homologous genes map within the *sh2* and *DFNB3* intervals, suggesting that *sh2* is the homologue of *DFNB3.*

Introduction

Individuals homozygous for *DFNB3* (MIM 600316) are profoundly deaf from birth, with no other associated clinical features. *DFNB3* was initially identified in a population of 2,200 villagers of Bengkala, Bali, in which 45 individuals are deaf (Friedman et al. 1995; Winata et al. 1995). As an adaptation to the large proportion of deaf individuals who have lived in Bengkala for at least seven generations (fig. 1), the villagers have developed a unique and complex sign language that is known by most of the Bengkala community. Given the history and geography of Bengkala, we hypothesized that the gene causing deafness should exhibit a founder effect. Accordingly, a genomewide screen to identify an interval of excess homozygosity (homozygosity mapping) among affected individuals placed the *DFNB3* locus between *D17S122,* on the p arm, and *D17S783,* on the q arm, of chromosome 17 (Friedman et al. 1995). On the basis of genetic maps available at the time, the size of this interval was estimated to be 5.3 cM, but more recent data suggest that it is likely to be ~12 cM (Dib et. al. 1996).

The data in the present article reduce the *DFNB3* interval to ~3 cM within the Smith-Magenis syndrome (SMS [MIM 182290]) region of 17p11.2. This was accomplished by the development of new polymorphic markers (short tandem repeats [STRs]) in the *DFNB3* region; by evaluation of several recently reported STRs on chromosome 17; and by procurement of DNA samples from recently ascertained affected individuals from Bengkala and neighboring villages, one of which, Bila, is segregating *DFNB3.* In addition, we report that, in two unrelated consanguineous families from India, nonsyndromic hereditary deafness is likely due to *DFNB3*
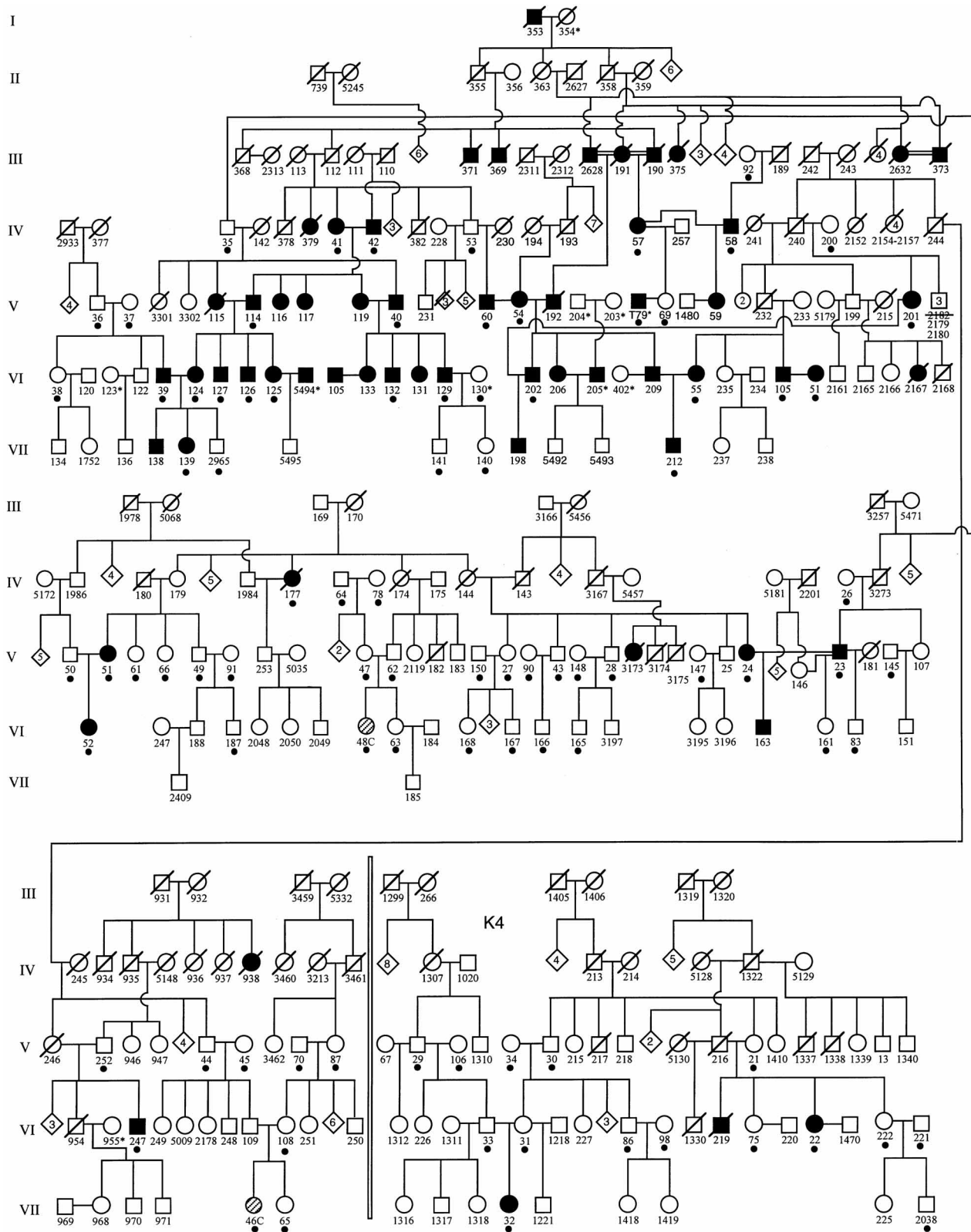
Figure 1 Genealogy of families with deaf individuals from Bengkala, Bali. Blackened symbols denote that the subject is profoundly deaf; unblackened symbols denote that individuals have hearing; and hatched symbols (for Bengkala villagers 46C and 48C) denote deafness that likely was acquired and not genetic. A dot below a symbol indicates that genomic DNA was obtained from the subject. An asterisk to the right of the subject's identification number denotes that the individual or his or her parents are not from Bengkala. With the exception of individuals 22 and 32, who are homozygous for the *DFNB3* haplotype (fig. 3), and deaf individuals from other villages in Bali (subjects T79*, 205*, and 5494*), there are familial connections between all the other deaf individuals in Bengkala.

mutations, each with unique 17p11.2 haplotypes for *DFNB3,* suggesting that there are two independently arising mutations of *DFNB3.*

On the basis of conserved synteny and similar phenotypes, we proposed that the murine autosomal recessive mutation *shaker-2* (*sh2*) is the homologue of *DFNB3* (Friedman et al. 1995). Affected mice are deaf and exhibit circling and head-tossing behaviors, presumably as a result of vestibular defects (Dobrovolskaia-Zavasdkaia 1928). Classical genetic mapping studies placed *sh2* on mouse chromosome 11, proximal to *Trembler* (*Pmp22^{Tr}*) (Falconer and Sobey 1953; Suter et al. 1992) and very close to *vestigial tail* (*Wnt3a^{vt}*) (Michie 1955; Greco et al. 1996). In the present study, genetic analyses and colocalizations of human and mouse expressed sequence tags (ESTs) near *DFNB3* and *sh2* strengthen the hypothesis that *sh2* is likely to be the homologue of *DFNB3.*

Subjects and Methods

This study was approved by the institutional review boards of the National Institute of Neurological Disorders and Stroke/National Institute on Deafness and Other Communication Disorders (IRB approval numbers OH97-DC-N006 and OH93-DC-016), Michigan State University (93-547 and 93-349), Udayana University, Denpasar, Bali (1997-1), the Indonesian Institute of Sciences (LIPI, 2403/V3/KS/96), and the All India Institute of Medical Sciences (S-8066-01).

Families from India

Large consanguineous families with probable hereditary hearing impairment were ascertained in schools for the deaf from the district of Kolhapur, Maharashtra State, in western India. A medical history was obtained, to exclude environmental causes of hearing impairment. Physical examinations were performed to eliminate syndromic causes of hearing impairment. There were no stigmata of syndromic hearing impairment in either family M21 or family I-1924. Audiology was performed with a pure-tone, portable audiometer, and bone conductance was also tested. Affected individuals demonstrated severe-to-profound sensory hearing impairment. Obligate carriers had normal hearing. Genomic DNA was isolated from venous blood samples (Grimberg et al. 1989).

Families from Bali

Methods of collecting DNA samples and clinical data from villagers of Bengkala have been described elsewhere (Friedman et al. 1995; Winata et al. 1995). A complete genealogy of the population of Bengkala village was constructed, and additional deaf individuals

from Bengkala were ascertained, with the goal of revealing historical recombinants of markers flanking *DFNB3* more closely.

We considered the possibility that congenital recessive deafness in families in other Balinese villages might be due to mutations in *DFNB3*. In northern Bali, we surveyed five villages—Anturan, Bila, Sinabun, Suwug, and Tamblang—and discovered families with apparent nonsyndromic recessive deafness. Unlike the situation in Bengkala, where 2.2% of the population is profoundly deaf, in five other Balinese villages the total number of deaf individuals was <0.2% of the population. Bila is 2 km from Bengkala and has a long history of close ties with Bengkala. The vital *Subak* system of rice-irrigation channels and water allocation (Lansing 1991) has its administrative and religious headquarters at the higher elevation of Bila, but ~75% of the *Subak* members are from Bengkala. Rice fields and gardens of members of both villages are interspersed, suggestive of past marriages between communities, since most land is obtained through inheritance. One of the 12 Bengkala clans segregating for *DFNB3* traces its origin to Bila. When the historical and geographic relations between these two villages are taken into consideration, it is reasonable to hypothesize that deafness segregating in Bila is due to *DFNB3.*

Tamblang (population 5,009 as of May 1995) is ~4 km from Bengkala and has two families with a total of four congenitally deaf individuals who were typed for STRs in the *DFNB3* region. Two other villages were surveyed because of their historical connection to Bengkala, which was chartered in the 12th century and has moved twice, once from an area now occupied by Sinabun (population 4,980 as of May 1997) and then from the region where Suwug (population 6,075 as of May 1997) is now located. There are still ancestral Bengkala temples in Sinabun and Suwug that are attended by people from Bengkala. Sinabun has three families with five congenitally deaf individuals, and Suwug has three families with four congenitally deaf individuals, all of whom were typed for the *DFNB3* markers. We also typed five deaf members of three families from Anturan, a large village with no obvious historical connection to Bengkala.

Pure-tone audiological exams were conducted in each village, with a portable Belltone model 120 with headphones that had ambient sound-attenuating audio cups that do not entirely remove the sound of the ubiquitous Balinese crowing game cocks. Affected individuals had sound pressure–level thresholds >90 decibels, uniformly at 250–8,000 hertz.

Isolation of Genomic DNA, and Marker Typing

A few drops of blood from a finger puncture from individuals in the villages of Anturan, Bila, Sinabun, Su-

wug, and Tamblang were collected on Isocode cards (Schleicher & Schuell). DNA was isolated according to the manufacturer's instructions and was used as template to amplify markers *D17S799, D17S122, D17S261, D17S740, D17S620, D17S2202,* and *D17S783.* A $\frac{1}{16}$-inch circle of the Isocode filter paper, from which some but not all DNA previously had been extracted according to Schleicher & Schuell instructions, was used as template to amplify markers *D17S953, D17S71, D17S805, D17S2201,* and *D17S842,* since DNA in the supernatant from blood collected on the Isocode card did not permit amplification of these five markers. Nested PCR was performed for markers *D17S805, D17S2202,* and *D17S2201.* None of the five STRs that required solid-state amplification from the Isocode card were problematic when DNA isolated from venous blood cells was used.

PCR amplifications were performed under conditions described elsewhere (Weber and Wong 1993; Friedman et al. 1995), with one exception: the PCR reaction for *D17S2201* (95°C for 1 min, 55°C for 1 min, and 72°C for 1 min, for 30 cycles) was performed with a buffer that contained 75 mM KCl, 10 mM Tris-HCl, and 1.5 mM $MgCl_2$ (pH 8.8). For DNA samples isolated from Isocode cards that were difficult to amplify by PCR, the following primers were developed: *D17S805*—forward, 5'-GAG GCA GGA GAA TCA CTT GAA C-3'; and reverse, 5'-CCA AAT GTG GTG TGT CCT AAA C-3'; *D17S2202*—forward, 5'-ATC TTG CTC AGG CTG GTA AAG C-3'; and reverse, 5'-CCT GCA CTT AAA TTC AAA TGG TTC-3'; and *D17S2201*—forward, 5-CAG ACT GGG TGA CAG GGT GAG AC-3'; and reverse, 5'-AGT GGA GGA GAT GGC CAT GGA G-3'.

Three New 17p11.2 STRs

Inter-*Alu* PCR products from YACs were synthesized by means of different combinations of two *Alu* primers (Tagle and Collins 1992) and YAC-vector end primers (catalog number 95060; Research Genetics). *Alu*-PCR products were digested with *Sau3*AI, were subcloned into *Bam*HI-restricted pBluescript II KS$^+$, and were screened at high stringency with an alkaline phosphatase–linked $(CA)_{12-16}$ oligonucleotide (Quick-Light™; Lifecodes). Three new STRs (*D17S2201, D17S2202,* and *MSU3*) were isolated from YACs 797g1, 828b9, and 965d9, respectively (fig. 2).

Linkage Analysis

Linkage analyses were performed by means of Fastlink version 3.0P (Cottingham et al. 1993). The frequency and penetrance of *DFNB3* were estimated to be 1/1,000 and 100%, respectively. Genetic distances are based on Cooperative Human Linkage Center (http://www.chlc.org/chlcmarkers.html) and Généthon maps (Dib et al. 1996).

Physical Map of 17p11.2: Human/Rodent Somatic Cell–Hybrid Lines and YACs

PCR primer pairs for some of the STRs and ESTs believed to map to the pericentromeric region of chromosome 17 were identified from the Genome Database (http://gdbwww.gdb.org), the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov), the Whitehead Institute for Biomedical Research/MIT Center for Genome Research (http://wwwgenome. wi.mit.edu), The Institute for Genomic Research (http://www.tigr.org/tigr_home/index.html), the Center for Medical Genetics (http://www.marshmed.org/genetics), and the XREF (http://www.ncbi.nlm.nih.gov/XREFdb) resource. References for other PCR primers used herein are as follows: Nicholson et al. (1994), *PMP22;* Townsend-Nicholson et al. (1995), *ADORA2B;* Potocki et al. (1996), *HIBBE73;* Chen et al. (1997), *LLGL;* Hua et al. (1995), *SREBF1;* Elsea et al. (1995), *cSHMT;* Zhao et al. (1995), *MFAP4;* Chen et al. (1997), *ZNF179;* Bloch et al. (1995), *NOS2A, NOS2B,* and *NOS2C;* Takahashi et al. (1994), *Kcnj12* (*IRK2*); and Greco et al. (1996), *Wnt3a.* The following mouse and human primers were developed for this study: *FL1*—forward, GAG AGT GAG GAC AAC CAG GG; and reverse, AGT TCA TCT TGG CAA AAG TC (490 bp); *ALDH3*−forward, ACT GAG GAG GGG TGG CTC; and reverse, CAG GTC AGC AGA GGA GTG (141 bp); *Aldh3*—forward, GCT GCT GGA GAG GAC TGT GT; and reverse, AGT CTG GAC TAG TTG CCC TCA (150 bp); and *Aldh4*—forward, CCT AGT GTG GCT AAG CAG GG; and reverse, CTT CAG ATA GCC TCA ACG GC (203 bp).

Human/rodent somatic cell–hybrid lines were used to determine the relative positions of STRs and uncharacterized ESTs within intervals defined by breakpoints of overlapping natural deletions of 17p11.1-11.2 (fig. 2). DNA was isolated from the following cell lines obtained from the Coriell Institute for Medical Research: NA10498, a human/mouse somatic cell hybrid retaining only human chromosome 17; NA10889, a human/mouse somatic cell hybrid retaining the del(17)(p11.2p11.2) as the only intact human chromosome; NA10501, a human/Chinese hamster somatic cell hybrid retaining der(X)t(X;17)(p11.21;p11.1); NA12517, a human/mouse somatic cell hybrid with human isochromosome (17q) and other human chromosomes; and NA12510, a human/Chinese hamster somatic cell hybrid with der(17)t(X;17)(q22;p11.2) and other human chromosomes. Four other cell lines (117-4D, 254-1D, 357-2D, and 484-2D) have been reported elsewhere (Chen et al. 1995*b*). Along with the 17p11.2

Figure 2 *DFNB3*-region integrated map of human/rodent somatic cell hybrids, STRs, YACs, and ESTs. The black vertical lines represent those regions of chromosome 17 that remain in each somatic cell hybrid; and the horizontal dashed lines indicate the breakpoints in each hybrid, demarcating 11 bins. STRs and ESTs were assigned to bins by PCR assay. The short vertical lines represent YAC clones mapped either within or adjacent to the *DFNB3* critical region. YAC clones denoted by dashed lines are either chimeric or have rearranged elements. The unblackened circle at the top denotes an STR hit; and the blackened circle at the top denotes an EST hit on a YAC clone. A dot adjacent to either a blackened or an unblackened circle indicates that the data were obtained from the physical map either published by Chen et al. (1997) or from the Whitehead Institute for Biomedical Research /MIT Center for Genome Research database. Note that there are two copies of STR MSU3, one on 567a12 in bin VI and the other on 797h1 in bin VII, and that there are two copies of EST T52530, one on YAC 915c12 in bin V and the other on 907e8 in bin VIII. In parentheses, following "stSG2083," "NIB1041," and "stSG8339," is a YAC address. An asterisk (*) indicates that a YAC that includes the STR or EST is not available.

somatic cell hybrids, 26 YACs from the 17p11.2 region were used to determine the order of STRs and ESTs in the *DFNB3* interval.

Genetic Mapping of sh2

All mice were obtained from the Jackson Laboratory (Bar Harbor, ME) and were cared for according to National Institutes of Health guidelines, in experiments approved by the University of Michigan Committee on the Use and Care of Animals (protocol number A3314-01). The *sh2* mutation arose in 1928, among the progeny of an x-ray–irradiated mouse. Histological studies have documented a progressive degeneration of the inner ear in *sh2* homozygotes (Deol 1954). Both a backcross ([*sh2*/*sh2* × CAST/Ei]F_1 × *sh2*/*sh2*) and an intercross (F_1 × F_1), between *sh2* homozygous mice and *Mus musculus castaneus* (CAST/Ei) mice, have been established in our laboratory. Mutants were distinguished from normal mice by both the absence of a Preyer reflex and the presence of circling and head-tossing behaviors. DNA was isolated from the tail of each mouse (Miller et al. 1988) and was typed, by PCR, with markers on mouse chromosome 11.

Results

We have reported elsewhere that there are six kindreds in Bengkala that contain deaf individuals (Friedman et al. 1995; Winata et al. 1995), but at that time we were unaware of ancestors who connected five of the six kindreds. By 1995 the genealogy of the ~2,200 living and 834 deceased Bengkala villagers was completed. A portion of this genealogy, including seven generations with 45 living and 19 deceased deaf Bengkala villagers, is shown in figure 1. Relatives have not been identified who link 2 affected individuals (individuals 22 and 32) in kindred 4 with the 43 other extant deaf individuals from Bengkala.

Not all of the deafness in Bengkala has a genetic etiology. We were told by several relatives that individuals 46C and 48C (fig. 1, *cross-hatched symbols*) had been able to hear at birth, as evidenced by a startle response to loud noise, but had lost their hearing in childhood, after intractable high fevers, and are now profoundly deaf. There are many possible infections that could have caused their profound hearing impairment (Estrada 1997). Suspecting that 46C and 48C were *DFNB3* phenocopies, we excluded them from our original mapping strategy (Friedman et al. 1995).

With regard to the statement that two deaf parents in Bengkala always have deaf children (Winata et al. 1995), there are three exceptions in Bengkala. The first exception is a case of nonpaternity, as revealed by marker genotypes, and is therefore not shown in figure 1. The remaining two exceptions appear to be instances of genetic complementation, since they involve marriages between deaf individuals from Bengkala and deaf individuals who have recently moved to Bengkala from other areas in Bali. Some of the deaf people of Bengkala have indicated their desire to have hearing children and have discovered that marrying deaf individuals from other villages usually produces hearing children. In particular, marriages between individual 125 from Bengkala and individual 5494* from Banjar Jawa (22 km from Bengkala) and between individual 206 from Bengkala and individual 205* from Desa Banjar (45 km from Bengkala) have produced children who were able to hear at birth.

Refining the DFNB3 Interval

DNA from 24 affected individuals from Bengkala were typed for two new STRs (*D17S2201* and *D17S2202*), five STRs in the databases (*D17S953, D17S740, D17S71, D17S620,* and *D17S842*), and five markers that have been typed elsewhere (*D17S799, D17S122, D17S261, D17S805,* and *D17S783*) (Friedman et al. 1995). The most likely disease haplotype for these 12 STRs was constructed. As shown in figure 3, there are two historical recombinations, between markers *D17S953* and *D17S740,* represented in individuals 32 and 51, and there are five historical recombinations, between markers *D17S805* and *D17S2201,* in individuals 23, 40, 42, 126, and 127. Individual 42 is also heterozygous for *D17S842,* which is likely to be more proximal than *D17S2201*. Both *D17S2201* and *D17S842* are in somatic cell–hybrid bin IX (fig. 2), but they are on different YACs, with 797h1 more distal than 714a10 (Chen et al. 1997).

Bila Family Segregating DFNB3

In Bila there is a family with 11 siblings—4 congenitally deaf and 7 hearing (fig. 4). The parents are deceased but were said to have been able to hear. The hearing grandmother was also genotyped. For five STRs in the 17p11.2 region (*D17S260, D17S740, D17S2202, D17S805,* and *D17S783*) a LOD score of 1.58 at a recombination fraction (θ) of 0 was obtained for this small family, by means of two-point linkage analysis. Although, by itself, this LOD score is not significant, this family appears to have the 17p11.2 Bengkala *DFNB3* haplotype, with the exception of *D17S71* and four distal STRs for which the three affected individuals are heterozygous (fig. 4). In contrast, deaf individuals in Anturan, Tamblang, Sinabun, and Suwug have, for 17p11 markers, genotypes that are entirely different from the Bengkala haplotype (data not shown). Deafness in individuals from these four villages, if genetic in origin, is not due to *DFNB3*.
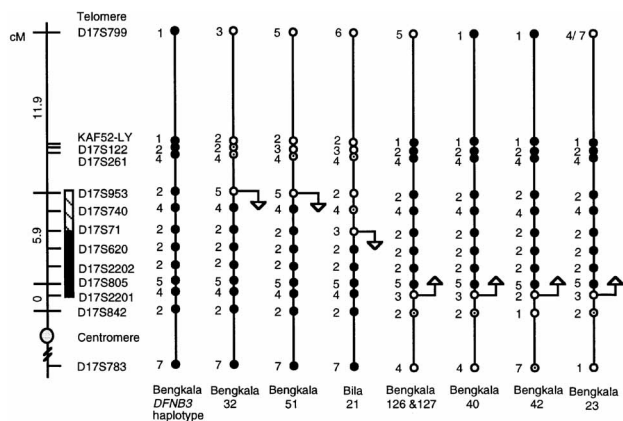
Figure 3 Map of genetic markers flanking *DFNB3* and of historical recombinations that define the *DFNB3* region. The *DFNB3* critical region between *D17S953* and *D17S2201* is represented by a rectangle. A likely historical recombination between *D17S71* and *D17S2201* is indicated by the blackened rectangle. Horizontal demarcations on the right represent STRs for which a genetic distance has not been reported. The placement of these markers is based on their position on the physical map of 17p (fig. 2; also see Chen et al. 1997). Horizontal bars that span the vertical line are markers for which a genetic distance (in cM) has been reported. Chromosomes with historical recombinations are depicted with arrows indicating the location of the *DFNB3* interval. Blackened circles denote the region that contains *DFNB3;* and unblackened circles denote the region from which *DFNB3* has been excluded. The number(s) next to each circle denotes the marker allele. When a haplotype cannot be determined, both alleles are shown. Alleles assumed to be identical by state are denoted unblackened circles containing a dot. The identification numbers of Bengkala individuals with recombinant chromosomes are also shown in figure 1. The individuals from Bengkala who have the *DFNB3* haplotype are 24, 54, 58, 60, 125, 201, 202, and 247 (fig. 1). Bila individual 21 is shown in the pedigree in figure 4.

DFNB3 *Segregating in Two Unrelated Families from India*

M21 and I-1924, two unrelated families from India that have nonsyndromic congenital recessive deafness, were screened for linkage to 10 *DFNB* loci (*DFNB1–DFNB8, DFNB10,* and *DFNB11*), by means of STRs from the Hereditary Hearing Loss (http://hgins.uia.ac.be/dnalab/hhh) screening panel and some additional markers. With the exception of *DFNB3,* linkage to all of the reported *DFNB* loci was excluded in these two Indian families. For families M21 and I-1924, positive LOD scores of 4.45 and 4.32, at $\theta = 0$, for markers *D17S805* and *D17S71,* respectively, were found at the *DFNB3* locus (table 1). Twelve *DFNB3* markers in the *DFNB3* region were then typed, and haplotypes were constructed (fig. 4). The 17p11.2 haplotypes were different in the two Indian families and were distinct from the Bengkala *DFNB3* haplotype.

It is possible that there may be more than one *DFNB* gene in this region, since 17p11 is gene rich (fig. 2; also

see Saccone et al. 1996). However, a parsimonious explanation for our data is that nonsyndromic congenital recessive deafness in Bengkala and in the two unrelated families from India is caused by independently arising allelic mutations of *DFNB3,* suggesting that *DFNB3* may make a contribution to hereditary deafness worldwide. Moreover, the existence of three independently arising and possibly different molecular defects of *DFNB3* should make it easier to causally connect a candidate gene to *DFNB3.*

Physical Map of the 17p11.2 Region

A total of 24 microsatellltes (i.e., STRs) and 72 ESTs were typed against our somatic cell–hybrid panel and the 17p11 YACs (fig. 2). Eleven bins are defined by overlapping natural deletion breakpoints of aberrant human chromosome 17/rodent somatic cell hybrids and by their position on a partial YAC contig of 17p11 (fig. 2). Nine STRs and 20 ESTs represent new assignments of genes to the SMS common-deletion region, schematically represented by bins IV–X (fig. 2). Eight STRs and 29 ESTs map within the *DFNB3* interval defined by *D17S71* and *D17S2201.*

Of these 72 ESTs, 43 were excluded as *DFNB3* candidates, on the basis of their physical map positions being either distal to *D17S71* or proximal to *D17S2201.* Among the 43 genes excluded as *DFNB3* candidates on the basis of their genetic and/or physical map position are the gene for peripheral myelin protein (*PMP22*), the gene for the A_{2b} adenosine receptor (*Adora2b*), and *KCNJ12,* an inward-rectifying potassium-channel gene. Included among the 29 ESTs mapped within the *DFNB3* interval are those for the following: the gene for the fatty-acyl-phospholipid synthase–like protein (stSG2083), the gene for the sodium/glucose cotransporter–like protein (WI-14642), the gene for the estradiol 17 beta-dehydrogenase 3–like protein (WI-6778), and diphenol oxidase *HIBBE73.* There are in the *DFNB3* interval several other genes that will be examined as candidates for *DFNB3,* including that for ZNF179, a brain finger protein (*BFP* [Chen et al. 1997]), that for sterol regulatory element–binding protein-1 (*SREBF1* [Hua et al. 1995]), that for cytosolic hydroxymethltransferase (*c-SHMT* [Elsea et al. 1995]), and that for microfibril-associated glycoprotein (*MFAP4* [Zhao et al. 1995]), an extracellular matrix protein involved in cell adhesion or intercellular interactions. *LLGL,* the human homologue of the *Drosophila melanogaster* lethal(2) giant larva, is thought to be involved in brain development (Campbell et al. 1997) and is in the *DFNB3* interval. However, a mouse knockout of *Llglh* complemented *sh2* (Wakabayashi et al. 1997). If *sh2* is the murine orthologue of *DFNB3,* then *LLGL* is not *DFNB3.*
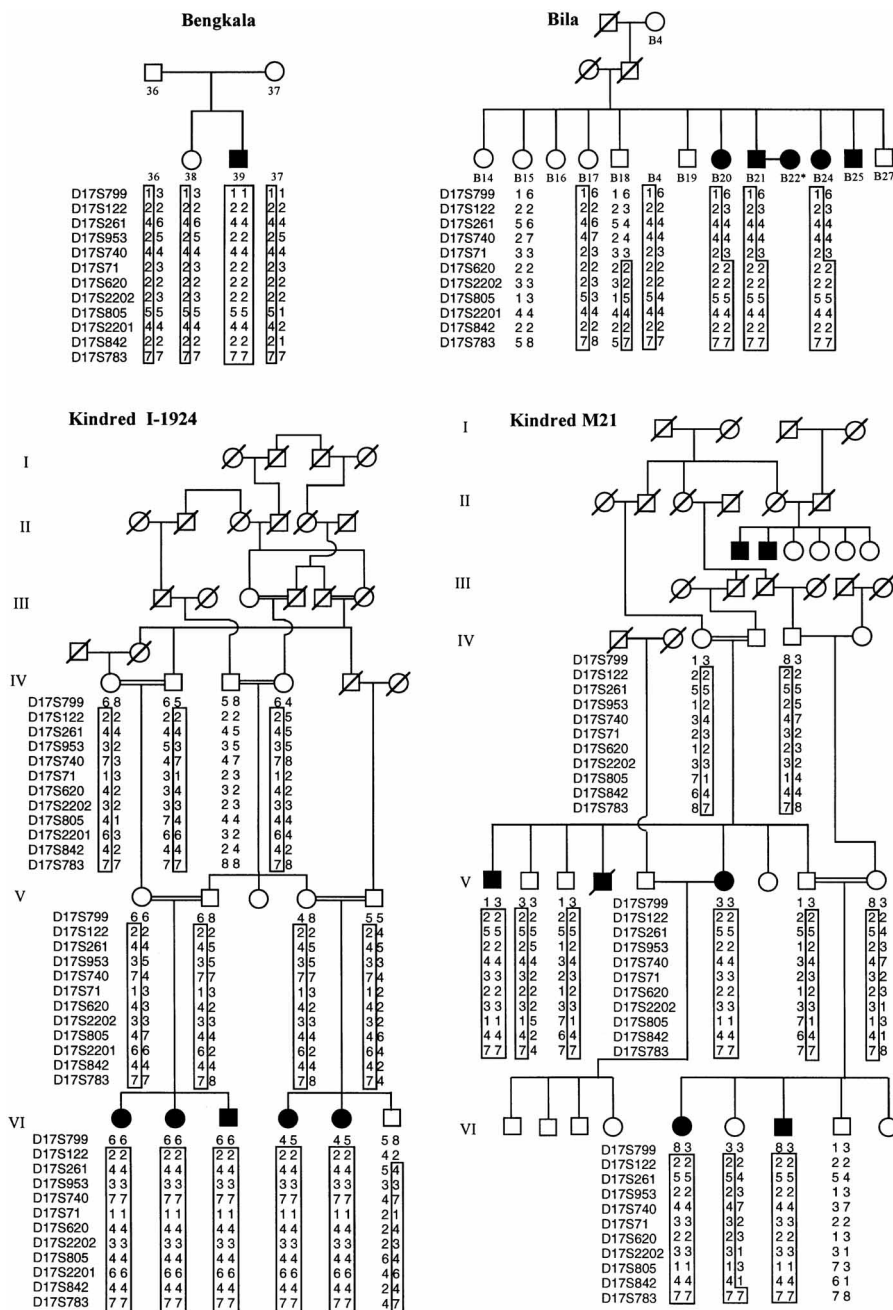
Figure 4   Haplotypes of a Bengkala nuclear family, a Bila family, and two unrelated Indian pedigrees (I-1924 and M21). The most likely haplotypes for the *DFNB3* region markers are shown. The *DFNB3*-linked haplotype is boxed.

Phenotypic Characterization and Allelism of sh2 *and* sh2[J]

Auditory brain-stem response (ABR) analyses were performed on 1-mo-old *sh2/sh2* and *sh2[J]/sh2[J]* mice, with wild-type litter mates serving as controls. Homozygous *sh2* and *sh2[J]* mice showed no ABR response to sound-pressure levels (SPL) at ⩽90 decibels, at frequencies of 4, 10, and 20 kilohertz, indicating that *sh2/sh2* and *sh2[J]/* *sh2[J]* mice are profoundly deaf. Control mice showed responses at all frequencies tested, at SPL values comparable to those seen in other strains of normal mice (Yamasoba and Dolan 1997).

An allelism test between *sh2* and *sh2[J]* homozygotes yielded eight affected individuals among eight total progeny, confirming the lack of complementation (Cook and Davisson 1993). A small intercross was made with *sh2[J]*

Table 1

Two-Point LOD Score for Linkage between Deafness and STRs on Chromosome 17 in Two Different Families

| Family and Locus	LOD score at $\theta =$						
	.00	.01	.05	.10	.20	.30	.40
I-1924:							
D17S799	$-\infty$	−.68	.47	.73	.87	.63	.43
D17S122	2.15	1.99	1.87	1.65	1.34	.75	.33
D17S261	2.77	2.67	2.43	2.05	1.34	.66	.17
D17S953	1.53	1.48	1.27	.99	.46	.04	−.10
D17S740	1.43	1.38	1.19	.95	.51	.18	.03
D17S71	4.32	4.14	3.67	3.23	2.51	1.39	.57
D17S620	2.86	2.63	2.37	2.01	1.30	.79	.28
D17S2202	1.64	1.30	1.17	1.02	.71	.42	.18
D17S805	.93	.89	.76	.58	.26	.04	−.04
D17S2201	3.53	3.28	2.99	2.74	1.99	1.29	.53
D17S842	2.83	2.65	2.43	2.14	1.73	.91	.38
D17S783	3.68	3.57	3.26	2.86	2.13	1.38	.66
M21:							
D17S799	$-\infty$	−1.06	.08	.46	.37	.31	.09
D17S122	1.13	1.04	.94	.80	.53	.29	.11
D17S261	2.03	1.97	1.71	1.51	1.08	.67	.30
D17S953	3.65	3.32	2.87	2.62	1.78	1.25	.35
D17S740	2.71	2.62	2.48	2.31	1.53	1.09	.28
D17S71	3.68	3.36	3.10	2.66	1.78	.94	.29
D17S620	3.25	3.01	2.76	2.35	2.08	1.35	.33
D17S2202	2.52	2.43	2.22	1.95	1.40	.86	.38
D17S805	4.45	4.13	3.67	3.38	2.11	1.45	.57
D17S2201	1.25	1.05	1.01	.97	.68	.49	.23
D17S842	4.14	3.92	3.65	3.18	2.23	1.25	.57

homozygous mice and CAST/Ei mice. None of the 10 deaf progeny had CAST/Ei alleles at markers <1 cM from *sh2*. Thus, *sh2ᴶ* maps to the same location as does *sh2* ($P < .01$), ruling out the possibility of nonallelic noncomplementation (Deol 1956; Kocher 1960). The existence of two alleles of *sh2* will be valuable in identification of *sh2* and, in turn, *DFNB3.*

Genetic Mapping of sh2

Analysis of 500 meioses allowed the localization of *sh2* to a 0.6-cM region on mouse chromosome 11 (fig. 5). On the basis of our genetic maps, *Ocp2-rs2,* the organ of Corti protein 2 (Chen et al. 1995*a;* Liang et al. 1997), and *Myo7b-rs1,* the gene for an unconventional myosin, which is similar to other genes associated with the deafness mutants (Avraham et al. 1995; Hasson et al. 1996), can be excluded as candidate genes for *sh2.* *Aldh3, Aldh4, Kcnj12,* and *Wnt3aᵛᵗ,* four genes with homologues on human chromosome 17, have been mapped on the *sh2* cross via SSCP analyses, but they are excluded from the *sh2* region (fig. 5). A similar cross has been constructed by Wakabayashi et al. (1997). However, many of the markers in the critical region of their cross are anonymous, and none of the four genes that we report herein were present on their map.

Discussion

DFNB3, a gene for nonsyndromic congenital profound deafness, previously had been mapped to an ~12-cM region near the centromere of chromosome 17 (Friedman et al. 1995). To further refine the *DFNB3* region, we obtained DNA samples from additional affected individuals from Bengkala who were not ascertained in 1993, and we also surveyed five villages in northern Bali, for families with hereditary deafness. Seven historical recombinations occurring among affected individuals from Bengkala refined the *DFNB3* region to an ~4-cM interval flanked by marker *D17S953,* on the distal side of 17p11.2, and marker *D17S2201,* on the proximal side of 17p11.2, within the SMS critical-deletion region (fig. 3). In the village of Bila, the *DFNB3* Bengkala haplotype from *D17S783* to *D17S620* was associated with deafness. The three deaf individuals in this Bila family are heterozygous for *D17S71,* with a 167-bp/171-bp genotype, whereas affected individuals from Bengkala are homozygous for the 171-bp allele. A historical mutation of *D17S71,* from 171 bp to 167 bp, is an unlikely explanation, given that 86% of STR mutations result in one repeat change (Weber and Wong 1993). Heterozygosity for *D17S71* is more likely to be
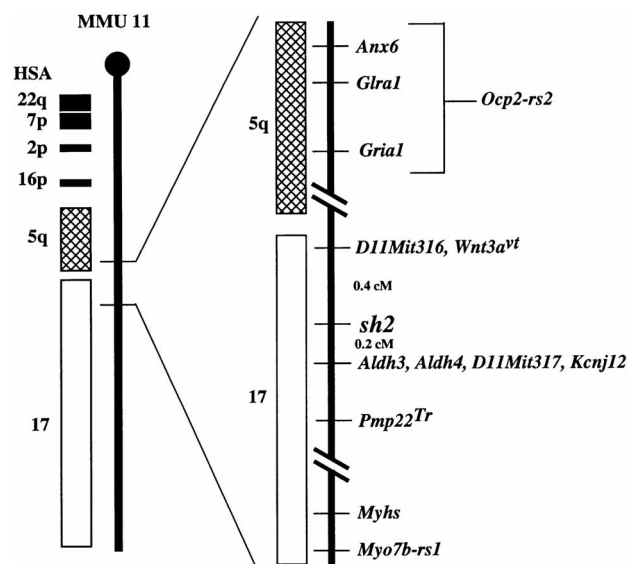
Figure 5 High-resolution genetic map of the *sh2* critical region on mouse chromosome 11. *Aldh3, Aldh4, D11Mit316, D11Mit317, Kcnj12, sh2,* and *Wnt3aᵛᵗ* were all mapped in our cross. The relative positions of *Anx6, Glra1, Grai1, Pmp22ᵀʳ, Myhs,* and *Myo7b-rs1* are placed unambiguously, on the basis of previous mapping (Watkins-Chow et al. 1997; Wakabayashi et al. 1997). The position of *Ocp2-rs2* is relative to *Anx6, Glra1,* and *Gria1* and has not been refined (Chen et al. 1995*a;* Liang et al. 1997). Regions of conserved synteny between mouse and human chromosomes (HSA) are indicated on the left (Watkins-Chow et al. 1997).

the result of a historical recombination event, since alleles of three of the five flanking markers distal to the 167-bp allele of *D17S71* are also different from the Bengkala haplotype (fig. 3). Recombination with marker *D17S71* limits the *DFNB3* region to ~3 cM within the interval between *D17S71* and *D17S2201* (fig. 2).

The *DFNB3* critical region, defined by *D17S71,* on the distal side of 17p11, and by *D17S2201,* on the proximal side of 17p11, is included within the SMS common deletion, del(17)p11.2, which is estimated to be ~5 Mb (Chen et al. 1997). SMS is a highly pleiotropic contiguous-gene syndrome characterized by mental retardation, sleep abnormalities, and minor craniofacial and skeletal dysmorphology, as well as by short stature, behavioral abnormalities, and cardiac and renal malformations (Greenberg et al. 1996). Audiological evaluations of 78 SMS patients indicated that 49 (63%) have conductive, sensorineural, or mixed hearing impairment (Chen et al. 1997). Sensorineural hearing impairment of some SMS individuals may be due to a mutation of *DFNB3* in the trans-configuration to the SMS deletion. Such SMS individuals might allow for a more comprehensive analysis of the relationship between the deafness phenotype and the molecular genetics of *DFNB3.*

Clusters of repeated genes in 17p11 appear to be responsible for homologous but unequal recombination events that give rise to 17p11.2 microdeletions (Chen et al. 1997). We considered the possibility that *DFNB3* might be a null mutation caused by a deletion of part of 17p11. Prophase spreads of chromosomes from an obligate *DFNB3* heterozygote and two *DFNB3* homozygotes from Bengkala were examined at the 700+ band-resolution level and were normal (data not shown). Moreover, no submicroscopic deletions of 17p11 in affected individuals from Bengkala or Bila or in the two Indian families (M21 and I-1924) segregating for *DFNB3* were detected by PCR analysis of 28 ESTs and 8 STRs in the *DFNB3* interval (fig. 2).

At present, there is not a published gap-free YAC, bacterial artificial chromosome (BAC), or cosmid contig spanning the *DFNB3* region. Our physical map of 17p11.2 also has a gap (bin VI; see fig. 2). Different sources of the same YAC address and different growths of some 17p11.2 YACs, such as 797g1 and 797h1, appear to include or exclude particular ESTs and STRs. The instability or absence of YAC clones from other gene-rich regions has been noted (Saccone et al. 1996). The inconstancy of YACs in the 17p11 region may also reflect the multiple low-copy repeats within this genomic region (Chen et al. 1997).

Without additional large *DFNB3* families, further refinement of the *DFNB3* critical region to ≤3 cM seems unlikely. Two interrelated strategies to clone *DFNB3* in a 3-cM region are being pursued. One relies on the identification of *sh2* in the mouse, by positional cloning, and

on BAC rescue (Probst et al. 1997). The other involves the screening of candidate genes in the *DFNB3* and *sh2* intervals (fig. 2). To this end, we and others (Chevillard et al. 1993; Chen et al. 1997; Wilgenbus et al. 1997) have assigned ≥47 ESTs to 17p11 (39 ESTs in bins IV–X; see fig. 2). There are also additional ESTs and genes in the 17p11.2 region that are not shown in figure 2, which include *SRP, TRE, KER,* and *CLP* (Chen et al. 1997), *CHRNB* (Beeson et al. 1990), the gene for small nuclear RNA *U3* (Chevillard et al. 1993), topoisomerase III (Elsea et al., in press), and *UbB* (Webb et al. 1990).

WI-13499, a GTP-binding protein, is expressed in the human cochlea (data not shown) and was evaluated as a candidate for *DFNB3.* DNA sequence analysis of reverse transcriptase–PCR products of the full-length coding region of this GTP-binding protein from affected individuals from Bengkala and from family M21 (fig. 4) failed to reveal a mutant allele (data not shown), and thus this gene is unlikely to be *DFNB3. ALDH3* and *FALDH,* two members of the human aldehyde dehydrogenase family, are on the proximal side but within the *DFNB3* region. The in vivo function of *ALDH3* is unknown. An important role for *FALDH* in the normal functioning of the human nervous system and epidermal tissue was established when mutations of *FALDH* were shown to cause Sjogren-Larsson syndrome (De Laurenzi et al. 1996). However, the murine homologues of *FALDH* and *ALDH3* have been excluded from the *sh2* critical region (fig. 5; also see Probst et al. 1997). *KCNJ12* was also eliminated as a candidate for *DFNB3,* on the basis of the genotypes of 3 of 16 Bengkala deaf individuals who are heterozygous for a *KCNJ12* polymorphic marker (Hugnot et al. 1997). The murine homologues of *KCNJ12* (*Kcnj12* or *Irk2*) and *ADORA2B* were also excluded as candidate genes for *sh2* (fig. 5 and data not shown).

The presence of three genes closely linked to *sh2* (*Aldh3, Aldh4,* and *Kcnj12;* see fig. 5), also found in the *DFNB3* critical region, provides compelling evidence that *sh2* is the mouse homologue of *DFNB3.* The combination of all of our *DFNB3* and *sh2* data indicates that *DFNB3* is probably located, on the physical map, between D17S71 (bin VI; see fig. 2) and *ALDH3* (bin VIII; see fig. 5). Both the potential for high-resolution genetic mapping in mice and the ability to correct the deafness and vestibular defects in *sh2*-transgenic animals with a murine BAC from the *sh2* region (F. J. Probst, unpublished data) suggest that *sh2* will be invaluable in identification of *DFNB3.*

Acknowledgments

References

Avraham KB, Hasson T, Steel KP, Kingsley DM, Russell LB, Mooseker MS, Copeland NG, et al (1995) The mouse *snell's waltzer* deafness gene encodes an unconventional myosin required for structural integrity of inner ear hair cells. Nat Genet 11:369–375

Beeson D, Jeremiah S, West LF, Povey S, Newsom-Davis J (1990) Assignment of the human nicotinic acetylcholine receptor genes: the alpha and delta subunit genes to chromosome 2 and the beta subunit gene to chromosome 17. Ann Hum Genet 54:199–208

Bloch KD, Wolfram JR, Brown DM, Roberts JD, Zapol DG, Lepore JJ, Filippov G, et al (1995) Three members of the nitric oxide synthase LL gene family (NOS2A, NOS1B, NOS2C) colocalize to human chromosome 17. Genomics 27:526–530

Campbell HD, Fountain S, Young IG, Claudianops C, Hoheisel JD, Chen K-S, Lupski JR (1997) Genomic structure, evolution, and expression of human *FLII,* a gelsolin and leucine-rich-repeat family member: overlap with *LLGL.* Genomics 42:46–54

Chen H, Thalmann I, Adams JC, Avraham KB, Copeland NG, Jenkins NA, Beier DR, et al (1995*a*) cDNA cloning, tissue distribution, and chromosomal localization of Ocp2, a gene encoding a putative transcription-associated factor predominantly expressed in the auditory organs. Genomics 27: 389–398

Chen K-S, Gunaratne PH, Hoheisel JD, Young IG, Miklos GLG, Greenberg F, Shaffer LG, et al (1995*b*) The human homologue of the *Drosophila melanogaster* flightless-I gene (*fliI*) maps within the Smith-Magenis microdeletion critical region in 17p11.2. Am J Hum Genet 56:175–182

Chen K-S, Manian P, Koeuth T, Potocki L, Zhao Q, Chinault AC, Lee CC, et al (1997) Homologous recombination of a flanking repeat gene cluster is a mechanism for a common gene deletion syndrome. Nat Genet 17:154–163

Chevillard C, Paslier DL, Passage E, Ougen P, Billault A, Boyer S, Mazan S, et al (1993) Relationship between Charcot-Marie-Tooth 1A and Smith-Magenis regions: snU3 may be a candidate gene for the Smith-Magenis syndrome. Hum Mol Genet 2:1235–1243

Cook SA, Davisson MT (1993) Re-mutation to shaker-2 (sh2). Mouse Genome 91:312

Cottingham RW Jr, Idury RM, Schäffer AA (1993) Faster sequential genetic linkage computations. Am J Hum Genet 53: 252–263

De Laurenzi V, Rogers GR, Hamrock DJ, Marekov LN, Steinert PM, Compton JG, Markova N, et al (1996) Sjogren-Larsson syndrome is caused by mutations in the fatty aldehyde dehydrogenase gene. Nat Genet 12:52–57

Deol MS (1954) The anomalies of the labyrinth of the mutants *varitint-waddler*, *shaker-2* and *jerker* in the mouse. J Genet 52:562–588

——— (1956) The anatomy and development of the mutants pirouette, shaker-1 and waltzer in the mouse. Proc R Soc Lond [Biol] 145:206–213

Dib C, Faure S, Fizames C, Samson D, Drouot N, Vignal P, Millasseau P (1996) A comprehensive genetic map of the human genome based on 5264 microsatellites. Nature 380: 152–154

Dobrovolskaia-Zavasdkaia N (1928) L'irradiation des testicules et l'hérédité chez la souris. Arch Biol 38:457–501

Elsea SH, Fritz E, Schoener-Scott R, Meyn MS, Patel PI (1998) The gene for topoisomerase III maps within the Smith-Magenis syndrome critical region: analysis of cell-cycle distribution and radiation sensitivity. Am J Med Genet 75: 104–108

Elsea SH, Juyal RC, Jiralerspong S, Finucane BM, Pandolfo M, Greenberg F, Baldini A, et al (1995) Haploinsufficiency of cytosolic serine hydroxymethyltransferase in the Smith-Magenis syndrome. Am J Hum Genet 57:1342–1350

Estrada B (1997) Infectious causes of hearing loss beyond otitis media. Infect Med 14: 239–244

Falconer DS, Sobey WR (1953) The location of Trembler in linkage group VII. J Hered 44:159–160

Friedman TB, Liang Y, Weber JL, Hinnant JT, Barber TD, Winata S, Arhya IN, et al (1995) A gene for congenital, recessive deafness DFNB3 maps to the pericentromeric region of chromosome 17. Nat Genet 9:86–91

Greco Tl, Takada S, Newhouse MM, McMahon JA, McMahou AP, Camper S (1996) Analysis of the vestigial tail mutation demonstrates that *WNt-3a* gene dosage regulates mouse axial development. Genes Dev 10:313–324

Greenberg F, Lewis RA, Glaze D, Parke J, Killian J, Murphy MA, Williamson D, et al (1996) Multi-disciplinary clinical study of Smith-Magenis syndrome (deletion 17p11.2). Am J Med Genet 62:247–254

Grimberg J, Nawoschik S, Belluscio L, McKee R, Eisenberg A (1989) A simple and efficient non-organic procedure for the isolation of genomic DNA from blood. Nucleic Acid Res 17:8390

Hasson T, Skowron J, Gilbert D, Avraham K, Perry W, Bement W, Anderson B, et al (1996) Mapping of unconventional myosins in mouse and human. Genomics 36:431–439

Hua X, Wu J, Goldstein JL, Brown MS, Hobbs HH (1995) Structure of the human gene encoding sterol regulatory element binding protein-1 (SREBF1) and localization of SREBF1 and SREBF2 to chromosomes 17p11.2 and 22q13. Genomics 25:667–673

Hugnot JP, Pedeutour F, Calvez CL, Grosgeorge J, Passage E, Fontes M, Lazdunski M (1997) The human inward rectifying K^+ channel Kir 2.2 (KCNJ12) gene: gene structure, assignment to chromosome 17p11.1, and identification of

a simple tandem repeat polymorphism. Genomics 39: 113–116

Kocher W (1960) Untersuchungen zur Genetik und Pathologie der Enkwicklung von 8 labyrinth-mutanten (deaf-waltzer-shaker-Mutanten der Maus (Mus musculus)). Z Vererbung 91:114–140

Lansing JS (1991) Priests and programmers: technologies of power in the engineered landscape of Bali. Princeton University Press, Princeton

Liang Y, Chen H, James H, Asher J, Chang C-C, Friedman TB (1997) Human inner ear OCP2 cDNA maps to 5q22-5q35.2 with related sequences on chromosomes 4p16.2-4p14, 5p13-5q22, 7pter-q22, 10 and 12p13-12qter. Gene 184:163–167

Michie D (1955) Genetical studies with 'vestigial tail' mice. II. The position of vestigial in the seventh linkage group. J Genet 53:280–284

Miller S, Dykes D, Polesky H (1988) A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 16:1215

Nicholson GA, Valentijn LJ, Cherryson AK, Kennerson ML, Bragg TL, DeKroon RM, Ross DA, et al (1994) A frame shift mutation in the *PMP22* gene in hereditary neuropathy with liability to pressure palsies. Nat Genet 6:263–266

Potocki L, Chen K-S, Lupski JR (1996) Identification of a highly conserved diphenol oxidase gene within the Smith-Magenis syndrome critical region of 17p11.2. Am J Hum Genet Suppl 59:A310

Probst FJ, Wang A, Friedman TB, Camper SA (1997) Genetic and physical map of the *shaker-2* critical region, a mouse model for nonsyndromic deafness. Am J Hum Genet Suppl 61:A242

Saccone S, Caccio S, Kusuda J, Andreozzi L, Bernardi G (1996) Identification of the gene-richest bands in human chromosomes. Gene 174:85–94

Suter U, Welcher A, Ozcelik T, Snipes G, Kosaras B, Francke U, Billings-Gagliardi S, et al (1992) Trembler mouse carries a point mutation in a myelin gene. Nature 356:241–243

Tagle DA, Collins FS (1992) An optimized Alu-PCR primer pair for human-specific amplification of YACs and somatic cell hybrids. Hum Mol Genet 1:121–122

Takahashi N, Morishige K, Jahangir A, Yamada M, Findlay I, Koyama H, Kurachi Y (1994) Molecular cloning and functional expression of cDNA encoding a second class of inward rectifier potassium channels in the mouse brain. J Biol Chem 269:23274–23279

Townsend-Nicholson A, Baker E, Sutherland GR, Scofield PR (1995) Localization of the adenosine A2b receptor subtype gene (ADORA2B) to chromosome 17p11.2-p12 by FISH and PCR screening of somatic cell hybrids. Genomics 25: 605–607

Wakabayashi Y, Kikkawa Y, Matsumoto Y, Shinbo T, Kosugi S, Chou D, Furuya M, et al (1997) Genetic and physical delineation of the region of the mouse deafness mutation shaker-2. Biochem Biophys Res Commun 234:107–110

Watkins-Chow DE, Buckwalter MS, Newhouse MM, Lossie AC, Brinkmeier ML, Camper SA (1997) Genetic mapping of 21 genes on mouse chromosome 11 reveals disruptions in linkage conservation with human chromosome 5. Genomics 40:114–122

Webb GC, Baker RT, Fagan K, Board PG (1990) Localizations of the human UbB polyubiquitin gene to chromosome band 17p11.2-17p12. Am J Hum Genet 46:308–315

Weber JL, Wong C (1993) Mutation of human short tandem repeats. Hum Mol Genet 2:1123–1128

Wilgenbus KK, Seranski P, Brown A, Luechs B, Mincheva A, Lichter P, Poustka A (1997) Molecular characterization of a genetically unstable region containing the SMS critical area and a breakpoint cluster for human PNETs. Genomics 42: 1–10

Winata S, Arhya IN, Moeljopawiro S, Hinnant JT, Liang Y, Friedman TB, Asher JH (1995) Congenital non-syndromal autosomal recessive deafness in Bengkala, an isolated Balinese village. J Med Genet 32:336–343

Yamasoba T, Dolan DF (1997) Chronic strychnine administration into the cochlea potentiates permanent threshold shift following noise exposure. Hearing Res 112(1–2): 13–20

Zhao Z, Lee C-C, Jiralerspong S, Juyal RC, Lu F, Baldini A, Greenberg F, et al (1995) The gene for a human microfibril-associated glycoprotein is commonly deleted in Smith-Magenis syndrome patients. Hum Mol Genet 4:589–597