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Genetic and Physical Delineation of the Region of the Mouse Deafness Mutation *shaker-2*

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A total of 951 backcross progeny have been obtained from a backcross segregating for the mouse deafness mutation, *shaker-2*(*sh-2*). Linkage analysis provides a detailed genetic map in the vicinity of *sh-2* which comprises 40 backcross mice identified as recombinant within a 4 cM region. This allows construction of a contig consisting of 21 BAC clones across an approximately 700-kb region of *sh-2*. This covers the entire nonrecombinant region of *sh-2* and is therefore useful to facilitate the identification of genes in the *sh-2* region. © 1997 Academic Press

Mutations at many different loci in both humans and mice are known to cause hearing impairments (1). About one in every 1,000 children is born with a significant hearing impairment, and about half of these are attributed to genetic defects. Mouse mutants exhibiting deafness may be useful in identifying some of genes involved. At present approximately 25 deafness mutations are known, including the *shaker-2* (*sh-2*) mutation. Homozygous *sh-2* mice are deaf and lack the Preyer reflex, a startle response to sound (2,3). They also show the circling, headtossing and hyperactivity due to vestibular dysfunction of the inner ear. By light microscopy, the stria vascularis of the inner ear appears normal at two weeks but begins to show degenerative changes shortly thereafter (4,5).

The *sh-2* mutation is autosomal recessive and shows complete penetrance. It has been mapped to the central region between the *Camb* and *Pmp22* loci on mouse chromosome 11 (2). In this paper we report the establishment of a large intersubspecific backcross segregating for *sh-2* and positioning of recombination breakpoints relative to the flanking markers in the vicinity of *sh-2*. We also describe a physical map in the region of *sh-2* that has been generated using the panel of informative breakpoints. This consists of 21 BAC clones that cover the entire nonrecombinant region of *sh-2*. The map is indispensable for the identification of the *sh-2* gene, because it facilitates the isolation of genes in the region of *sh-2*.

MATERIALS AND METHODS

Genetic cross. The *sh-2* mice were purchased from the Jackson laboratory. Homozygotes were mated with MSM and F_1 progeny were backcrossed to *sh-2* homozygotes. MSM is an inbred strain derived from the Japanese wild mouse, *Mus. m. molossinus.* Nine hundred fifty-one backcross progeny were recovered and homozygotes for *sh-2* were identified from their heterozygous sibs using the phenotypic head shaking, circling, and loss of Preyer reflex characteristics of these mice.

PCR analysis. Genomic DNA was extracted from liver and subjected to polymerase chain reaction (PCR). Reaction was carried out by standard protocols. Aliquots of 5 μl of product were separated by electrophoresis on 4% NuSieve-agarose gel or on 8% polyacrylamide gel. Microsatellite markers used were synthesized according to sequences reported (6,7).

Isolation of YACs and BACs and analysis of their linkage. YAC and BAC clones were isolated by PCR screening. Their libraries were purchased from *Research Genetics, Inc.* The size of clones was determined by pulsed-field gel electrophoresis. YAC ends were recovered using vectorette PCR (8) and subjected to sequence analysis. Each end of BAC inserts was directly sequenced. PCR primers for the ends were synthesized from sequences obtained.

Pulsed-field gel electrophoresis of DNA. DNA was prepared in agarose plugs as described (9). The plugs were digested with restriction enzymes under the manufacture's recommended conditions, and subjected to electrophoresis using a Beckman Gene Line apparatus.

RESULT

An intersubspecific backcross segregating for the *sh-2* mutation [(MSM+/+ × *sh-2*/*sh-2*)F_1 × (*sh-2*/*sh-2*)]

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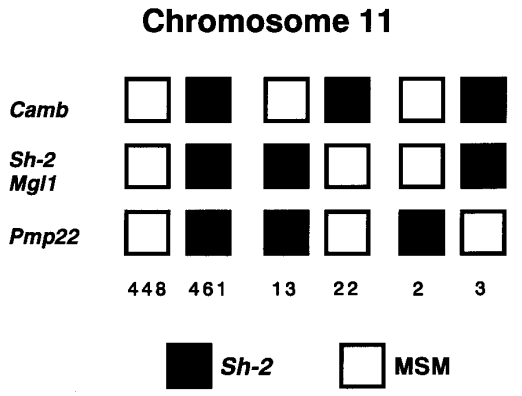

FIG. 1. Distribution of the haplotypes for 951 progeny from intersubspecific backcross mice that were obtained by mating *sh-2* homozygous females to (*sh-2* × MSM)F_1 males. The loci followed in the cross are indicated on the left. The filled squares represent the *sh-2* allele, and open squares represent the MSM allele. Each column represents the chromosome identified in the progeny. The numbers of the progeny carrying each type of chromosome are listed at the bottom. The lod scores between *Camb* and *sh-2* and between *sh-2* and *Pmp22* are 221 and 272, respectively.

yielded a total of 951 progeny mice. *sh-2*/*sh-2* mutant mice could be easily distinguished from phenotypically normal littermates by observation of their head shaking, circling, and loss of Preyer reflex. Scoring the *sh-2* phenotype provided that 487 were found to be heterozygous, whereas 464 were homozygous for *sh-2*. The ratio of genotypes did not significantly diverge from a 1:1 ratio (X^2 (1) = 0.232 with Yates's continuity correction, $P > 0.5$), consistent with that *sh-2* is an autosomal recessive mutation with complete penetrance (2,3).

Genetic mapping studies have positioned *sh-2* around the *Pmp22* region of mouse chromosome 11 (2). Accordingly, DNA typing of the backcross progeny was carried out for the *Camb*(*Anx6*) and *Pmp22* loci which were approximately 4 cM apart. Thirty-five were found to be recombinant between *Camb* and *sh-2* giving a genetic distance of 3.68 +/− 0.61 cM, and 5 were found to be recombinant between *sh-2* and *Pmp22* indicating a genetic distance of 0.53 +/− 0.23 cM. No recombinant was observed of the *Mgl1* gene located between the two gene loci (10). Fig. 1 summarizes the haplotype analysis giving the order of three loci in the vicinity of *sh-2*. This order was deduced by a simple multipoint analysis, taking into account the minimum number of crossovers for a given probe order.

The panel of 40 backcross mice identified as recombinant in the *sh-2* region can be used to generate a detailed genetic map of this region. Twelve Mit and one Jpk microsatellite markers were chosen and subjected to DNA typing of the backcross mice. The result is summarized in Fig. 2. This shows the order of the markers and their relative distance. Nine Mit markers clustered in a position are segregated into five groups in this backcross panel, and *D11Mit155* and *D11Mit143/112* show inverse position. The result also identifies the closet recombination breakpoints flanking the *sh-2* locus. *D11Mit26* is nonrecombinant with *sh-2*, whereas *D11Mit316* and *D11Mit261* display two and three recombinants with *sh-2*, respectively.

On the basis of this genetic data, we started the construction of a physical map in the region of *sh-2*. Five probes of *D11Mit316*, *Mgl1*, *D11Mit26*, *D11Mit261* and *D11Mit260* were used as start points for screening a YAC library. Six YAC clones were isolated, and their centromeric and telomeric ends were recovered using vectorette polymerase chain reaction. Sequence-tagged sites (STSs) obtained from the end fragments were subjected to linking the YACs and mapping closer to *sh-2*. This revealed the presence of a gap between *D11Mit316* and a centromeric fragment (Mgl1L) of the 1YMgl1-1 YAC (see Fig. 3). To bridge this gap and to extend a contig both distally and proximally, we isolated 30 BAC clones using the STSs and the five marker probes. Analysis of the ends of these BAC clones for physical linkage showed that they were grouped into two clusters. Fig. 3 shows two tiling paths consisting of 21 and 9 non-chimaeric BACs identified.

To position *sh-2* more precisely, we searched polymorphisms in the end fragments. Two STSs, *D11Jpk14* and *D11Jpk15*, showing variation between the *sh-2*

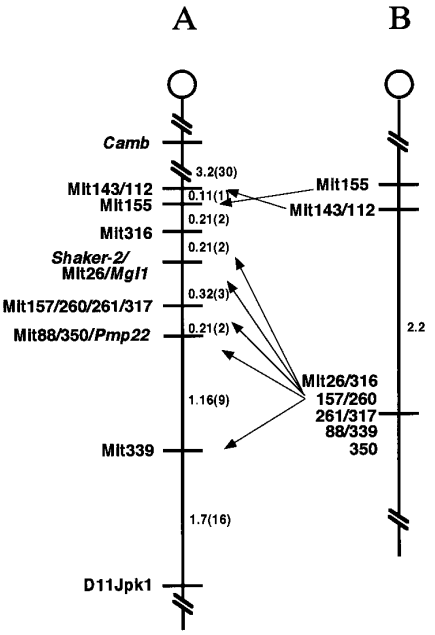

FIG. 2. Chromosome linkage map showing the locations of three genes, twelve *Mit* and one *Jpk* markers in the vicinity of *sh-2* on chromosome 11 (A). The number of recombinant N2 mice among the 951 backcross mice scored and the recombination frequencies are shown for each pair of loci on the map. The numbers in parentheses indicate the numbers of mice scored as recombinant. The MIT map is displayed in the right (B). Nine markers mapped in a position were segregated into five groups and the *D11Mit155* and *D11Mit143/112* showed inverse location in our backcross mice.

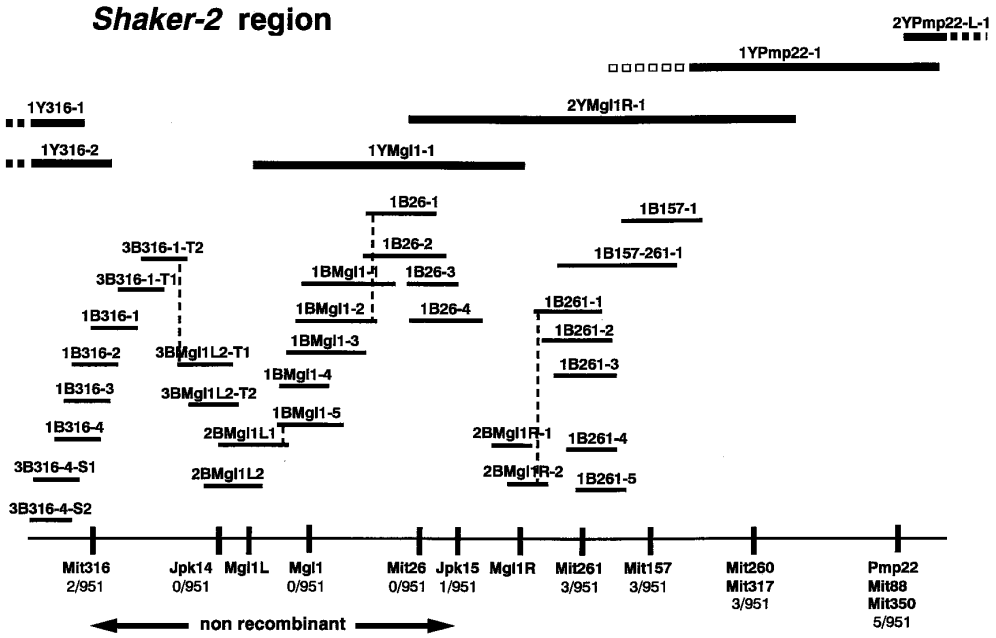

FIG. 3. Physical map of the mouse *sh-2* locus. A chromosome walk across the *sh-2* locus was completed in BACs using the flanking markers, *D11Mit316, Mgl1, D11Mit26,* and *D11Mit261* as start points. Bold and thin bars represent YACs and BACs, respectively. Filled squeres adjacent to the bold bars show regions not displayed in this figure. Open squares indicate chimeric YAC region. Each clone name consists of three parts. Y and B indicate YACs and BACs, respectively. The number left Y or B indicates kinds of probes used for isolation: 1 shows the clone isolated using gene or marker probe; 2 represents the clone obtained by the YAC-end probe; 3 indicates the clone by the BAC-end probe. The remaining is the indication of original gene or marker used for isolation. Numbers in the bottom indicate designation of recombinant mice in the region of *sh-2* among 951 meioses scored. The nonrecombinant region is marked by two arrows.

strain and MSM were obtained from the two BACs, 2BMgl1-L1 and 1B26-3, respectively. Genotyping the backcross mice revealed that *D11Jpk14* did not show recombination to *sh-2* but *D11Jpk15* gave one recombinant. This delimited the non-recombinant region around *sh-2* to the end of a BAC (1B26-3). This region was estimated to span approximately 700 kb by sizing the clones on PFGE (data not shown) and was entirely covered by the contig of 21 overlapping BAC clones (Fig. 3). The exact size of the critical region could not be determined until the points of recombination in these mice were precisely localized.

DISCUSSION

In this paper we provide the genetic and physical mapping of a region comprising the *sh-2* gene on mouse chromosome 11 which causes autosomal recessive deafness in the mouse (2-5). A total of 951 backcross mice are used for positioning the *sh-2* locus. *sh-2* is likely to be located between *D11Mit316* and *D11MitJpk15* marker loci that is approximately 700-kb apart. There were there recombination events in this region among 951 meioses. In the region of *sh-2*, therefore, one cM of genetic distance corresponds to about 2200 kb, a rate of recombination nearly equal to average for the entire mouse genome. Twenty-one BAC clones are used to construct a contig across the 700-kb region in the vicinity of *sh-2*. This physical map covers the entire nonrecombinant region of *sh-2* and therefore is helpful to facilitate the identification of genes in the *sh-2* region. Also, several experimental approaches such as candidate gene testing can be performed to identify the *sh-2* gene.

We have demonstrated that the *Mgl1* gene is located within the nonrecombinant region. Since the gene is expressed in central nervous system (11), it can be a suitable candidate for *sh-2*. The *Mgl1*-deficient mice have been developed by T. Noda *et al.* at Cancer Institute in Tokyo (unpublished), and hence complementation was examined by mating *sh-2/sh-2* to *mgl1/+*. No mouse exhibited the *sh-2* phenotype, of eighteen progeny examined. This suggests that the *Mgl-1* gene is not responsible for *sh-2*.

An unconventional myosin gene, *Myo7b-rs,* is mapped near the *sh-2* locus (12). This gene may be another candidate for *sh-2,* because mutations in two genes coding for myosin-VIIa and myosin-VI belonging to the same unconventional myosin family have been demonstrated to cause hereditary hearing loss. Myosin-VIIa is encoded by the mouse *shaker-1* (*sh-1*) locus (13). Homozygous *sh-1* mice are similar in behavior and pathology to *sh-2,* with exception that the abnormalities are observed a little later. Myosin-VI is encoded by the mouse Snell's waltzer (*sv*)

locus (14). Homozygous *sv* mice exhibit an inner ear phenotype that is very similar to that observed in *sh-1* mice (14). The expression of both proteins in the inner ear is restricted to the hair cells, thus indicating that both forms of deafness result from primary sensory cell defects (13,14). However, *Myo7b-rs* was mapped about one cM distal to *Pmp22*. This suggests it unlikely that *Myo7b-rs* is the gene for *sh-2*.

Autosomal recessive forms of hearing loss (*DFNB*) account for almost all congenital profound deafness in humans (1,15). A form, *DFNB2*, is characterized by profound neurosensory deafness, vestibular dysfunction, and blindness due to retinitis pigmentosa. Recently, Myosin-VIIa defects have been identified in *DFNB2* patients (16). The number of *DFNB* genes is estimated to between 30 and 100 (1). One such locus, *DFNB3*, has been mapped by genetic linkage analysis to the region on 17p11.2-17q12 that is syntenic to the mouse *sh-2* locus (17). Interestingly, the human *Mgl1* homolog exists in that region (18). This linkage conservation between the mouse *sh-2* and human *DFNB3* loci strongly suggests that *sh-2* is a mouse homologue of the *DFNB3* gene.

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