Mouse homeobox-containing gene, *Otx2*, maps to mouse Chromosome 14

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Species: Mouse
Locus name: orthodenticle-related homeobox-2
Locus symbol: *Otx2*
Map position: centromere–*D14Mit3*–8.4 cM–*Otx2*, *D14Mit101*–4.5 cM–*D14Mit5*–telomere
Method of mapping: Intersubspecific backcross mice were used for genetic mapping: (C57BL/6 × MSM)F_1 females × MSM males. Haplotype analysis is summarized in Fig. 1.
Molecular reagents: The primer sequences used for typing the backcross mice are 5′-GAGAACTCAGTCTTGTATCCG-3′ and 5′-GGCAATGTGTGTAAGGCTGG-3′.
Methods: Sequence analysis of genomic clones of the *Otx2* gene revealed that an $(AG)_{19}$ repeat was present in a region 5′ to the transcription start site. A set of two primers flanking the AG repeat detected a polymorphism between C57BL/6J and MSM by conventional polyacrylamide gel electrophoresis. With the primers, 131 intersubspecific backcross mice were typed that were obtained by mating (C57BL/6J × MSM)F_1 females to MSM males. Each strain distribution pattern was compared with that of 155 anchor loci. The markers used for anchors were 85 *Mit* and 51 *Jpk* microsatellites and 19 authentic genes [1,2]. As summarized in Fig. 1, the results of typing showed a clear linkage of the *Otx2* locus to *D14Mit101* on Chromosome (Chr) 14.
Previously identified homolog: The human OTX2 gene was mapped to 14q21-22 with hybrid panel and in situ hybridization techniques [3]. The region is slightly apart from the human chromosome region 14q11-13 syntenic to this mouse *Otx2* locus where TCRA/*Tcra* and NP/*Np* are mapped.
Discussion: The *Otx2* mutation displays craniofacial malformations designated as otocephaly (agnathia-haoprosencephaly) by haplo-insufficiency [4]. Three developmental mouse mutations have been mapped near the *Otx2* locus; pugnose (*pn*) and disorganization (*Ds*) are about 3 cM and 4 cM apart, respectively, while waved coat (*Wc*) is very close to *Otx2*. In the *Wc* mutation, homozygous mutants die in utero; embryonic ectoderm is aberrant, while extraembryonic structures develop normally [5]. The phenotype is quite similar to *Otx2* homozygous mutant phenotype [4,6,7]. The *Wc* heterozygous phenotype is, however, found in coat and whiskers and quite distinct from *Otx2* heterozygous one [4,5]. The *pn* and *Ds* mutations also bear several phenotypes similar to *Otx2* mutation. The *pn* homozygotes display a shorter head; the parietal, frontal, nasal bones and the mandibular bones are shorter and wider than normal [8]. Shortening of nasal bone and mandible is frequent in *Otx2* heterozygotes [9]. The *Ds* mutation disrupts the orderly process of development; most of the homozygous mutants die after implantation, and some of heterozygotes are monstrous with multiple defects not only in rostral head but also in posterior parts of bodies [9]. Of particular interest is that *Otx2*, *Wc*, and *Ds* mutations are all semidominant, and the heterozygous phenotype displays variable phenotypes being affected by the genetic background of mice.

Many agnathia-holoprosencephaly syndromes have been reported in humans [10]. Although most of them are not assigned for their chromosomal locations, a holoprosencephaly syndrome is known to map to Chr 2 [11]. It is also shown that the proximal 14 trisomy causes holoprosencephaly with craniofacial abnormalities [12]. Several otocephaly mutants have also been reported in the rodent: a semidominant one in guinea pig [13] and two recessive ones in mouse designated as jaw (*j*) and otocephaly (*oto*) [14]. The *oto* locus maps to Chromosome 1 [14]. Thus, there are at least

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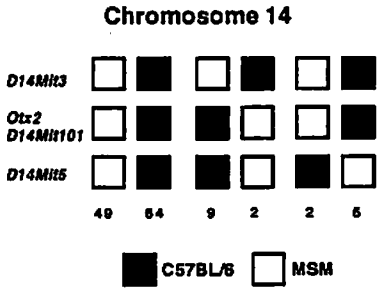

Fig. 1. Distribution of the haplotypes for 131 progeny from intersubspecific backcross mice that were obtained by mating (C57BL/6 × MSM)F_1 females to MSM males. The loci followed in the cross are indicated on the right. The filled squares represent the C57BL/6 allele, and open squares represent the 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.

several genes involved in otocephaly in mammals, and these may play crucial roles in patterning of rostral head in vertebrate [4].

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