SHORT COMMUNICATION

Localization of Insulin-2 (Ins-2) and the Obesity Mutant Tubby (tub) to Distinct Regions of Mouse Chromosome 7

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A DNA mapping panel derived from an interspecific backcross was used to position the mouse insulin-2 locus (Ins-2) on Chromosome 7, near H19 (0/114 recombinants) and Th (1/114 recombinants). Ins-2 is part of a human–mouse conserved linkage group that includes Th, H19, and Igf-2. Analysis of segregation in the F2 generation from the cross C57BL/6J-tub/tub × CAST/ Ei demonstrated that Ins-2 and the obesity mutant tubby (tub) are distinct loci, thus eliminating Ins-2 as a candidate gene for tub. These results also refine the estimated genetic distance between tub and Hbb to 2.4 ± 1.4 cM. The predicted location for a human homolog of tubby is HSA 11p15. © 1992 Academic Press, Inc.

The rodent genome contains two insulin genes, insulin-1 (Ins-1) and insulin-2 (Ins-2), that are expressed at similar levels in the beta cells of the pancreas (12). Ins-2 contains two introns and is the homolog of the active insulin gene in human and other vertebrates. Ins-1 contains only the first intron and appears to be derived by reverse transcription of a partially processed transcript (10). Ins-1 and Ins-2 are assigned to mouse Chromosomes 6 and 7, respectively (6), but subchromosomal localizations have not been reported. A new autosomal recessive obesity mutation tubby was also recently mapped to mouse Chromosome 7 (2). The mouse obesity mutants are of particular interest in the context of identifying the genes involved in control of body weight in mice contained a mutant Ins-2 gene, the potential identity of Ins-2 and tub was tested genetically by analysis of 39 F2 tub/tub offspring produced by intercrossing (C57BL/6J-tub/tub × CAST/ Ei) F1 mice. The F2 mice were maintained for at least 14 weeks, and obese mice with fat evenly distributed throughout the body cavity were classified as tub/tub. Hemoglobin β-chain (Hbb) was typed by electrophoresis of blood lysates (13). Ins-2 was typed by Southern blotting using the PCR-amplified genomic fragments (Fig. 1) as hybridization probes. A 4.3-kb HindIII fragment was observed in C57BL/6J-tub/tub DNA, and a 7.6-kb HindIII fragment was observed in CAST/Ei DNA. These fragments segregated as alleles in the F2 generation (Fig. 1B). Minor fragments of 8.4 kb (C57BL/6-tub/ tub) and 15 kb (CAST/Ei) segregated independently of the major fragments and may represent cross-hybridization with Ins-1. Of the 39 F2 tub/tub mice typed, 20 were homozygous for the C57BL/6-tub/tub-derived Ins-2 allele, 6 were homozygous for the CAST/Ei derived allele, and 13 were heterozygous. These results indicate that Ins-2 and tub are not allelic and suggest that they are loosely linked (25 recombinant chromosomes out of 78 chromosomes tested; 32 ± 5 cM). These results clearly eliminate Ins-2 as a candidate gene for the tub mutation. We also observed 2 recombinants between tub and Hbb (2/78) in the 39 F2 mice. Combining these data with the earlier observation of 1/47 recombinants (2) gives an estimated distance between Hbb and tub of 2.4 ± 1.4 cM.

To further localize Ins-2 on Chromosome 7, we analyzed an interspecific backcross [(C3H/HeJ-gld/gld × Mus spretus) F1 female × C3H/HeJ-gld/gld male] that has been characterized for more than 400 genetic markers (8, 9, 11). Variation at Ins-2 was detected by PCR amplification of genomic DNA using primers 3 and 4 (Fig. 1), followed by digestion of the amplified fragment with the restriction enzyme Ddel. (The C3H product is cleaved once to generate fragments 340 and 400 bp in length, whereas the M. spretus product is cleaved twice to yield fragments of 340, 280, and 120 bp.) Each backcross DNA displayed either the homozygous C3H pattern (two fragments) or the heterozygous pattern (four fragments). Localization of Ins-2 on Chromosome 7 was determined by a stratified mapping approach. We first typed 9 backcross mice known to have inherited numerous recombinant chromosomes. Comparison with previously typed markers provided a provisional localiza-

tion to distal mouse Chromosome 7. This localization
was confirmed by analysis of 20 additional DNAs from the backcross mapping panel of 114, including all mice with crossovers between Hbb and Th (tyrosine hydroxylase) (8). Among the 29 backcross individuals typed for Ins-2, 16 were recombinant between Ins-2 and Hbb, 1 was recombinant between Ins-2 and Th, and none were recombinant between Ins-2 and H19 (Fig. 2). (The 85 mice not tested contained no recombinations in the region between Hbb and Th.) Assuming no double recombinants occurred between Hbb and Th, the gene order is centromere-Hbb (14 ± 3)–Ins-2, H19 (0.9 ± 0.9)–Th. The recombinant rate between Hbb and Ins-2 was significantly higher in the cross with CAST/Ei (23/78) than in the cross involving M. spretus (16/114) (2 × 2 contingency table, \( \chi^2 = 9; P < 0.003 \)). The basis for this difference will require further analysis.

The mapping data presented here eliminate Ins-2 as a candidate gene for tubby and, unfortunately, do not identify any candidate genes from its map position. Determination of the molecular basis for tub and the other single locus obesity mutants in the mouse has potential for providing insight into related human disorders. Efforts to isolate the diabetes (db) and obesity (ob) genes by positional cloning are already in progress (3). The proximity of tubby to Hbb provides the basis for a similar approach to cloning of the tubby gene.

The human homologs of mouse Ins-2, H19, and Th are located on chromosome 11p15.5. The conserved linkage group also includes Hbb and Igf-2 (insulin-like growth factor-2) (1, 4). On the basis of close linkage of tubby and Hbb, the role of a human homolog of tubby in inherited obesity can be tested using the human hemoglobin beta locus as a linked marker in pedigree studies.

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REFERENCES


