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.

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 man [reviewed by (3)]. In view of the association between diabetes and obesity, we tested *Ins-2* as a candidate for the *tub* mutation and obtained more precise localization of both genes on Chromosome 7.

Ins-2 was amplified from homozygous *tub/tub* genomic DNA, and the product was sequenced (5, 7). The structure of the *Ins-2* gene and the PCR primers are indicated in Fig. 1. The sequence obtained for the region −43 to +981 was identical to the published sequence (12), with the exception of an A to G substitution in the second intron at position +472, a substitution unlikely to influence gene function. Since the sequence did not provide evidence that *tub/tub* 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 *Hin*dIII fragment was observed in C57BL/6J-*tub/tub* DNA, and a 7.6-kb *Hin*dIII 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 *Dde*I. (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 localization to distal mouse Chromosome 7. This localization

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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 recombination 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.

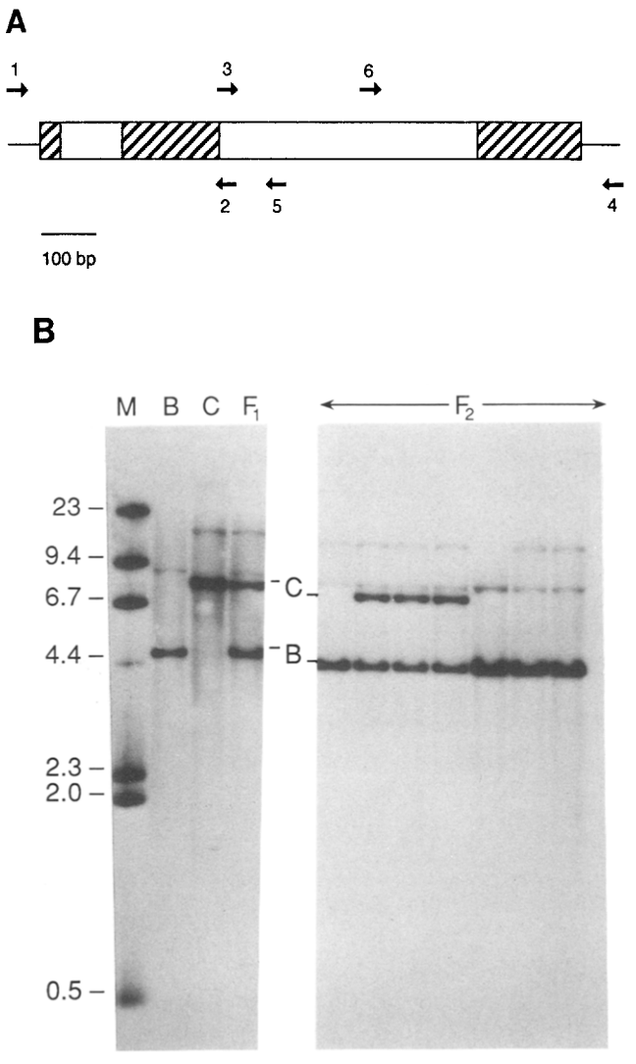

FIG. 1. (**A**) Amplification of the *Ins-2* gene by PCR. The three exons are represented by striped boxes and the two introns by open boxes. Primers were designed from the gene sequence (12); nucleotides are renumbered here from the start site of transcription. Primers 1 and 2 amplify a 479-bp fragment from the 5′ end of the gene. Primers 3 and 4 amplify a 740-bp fragment from the 3′ end of the gene. Primer sequences: (1) $^{-91}$GAGCCCTTAA TGGGTCAAAC AGCAAAGTCC^{-62} (2) $^{+398}$CTGGGGACAG AATTCAGTGG CAGAACTCAC^{+369}, (3) $^{+369}$GTGAGTTCTGCCACTGAATT CTGTCCCCAG^{+398}, (4) $^{+1110}$CACTCATGTC TCCTGGTCCCACATATGCAC^{+1081}, (5) $^{+472}$TTATCCCGAG ACCCTCCACA CCTAGGACAC^{+443}, (6) $^{+639}$GCACTGACTG AAGATGAGTA GG^{+660}. (**B**) RFLP at the *Ins-2* locus. Genomic DNA was digested with *Hin*dIII and analyzed by Southern blotting using, as hybridization probes, the two *Ins-2* fragments obtained by amplification of genomic DNA with primers 1, 2, 3, and 4 (described above). The positions of size markers (kb) are indicated at the left. M, size markers (λ digested with *Hin*dIII; B, C57BL/6J-*tub*/*tub*, C, CAST/Ei.

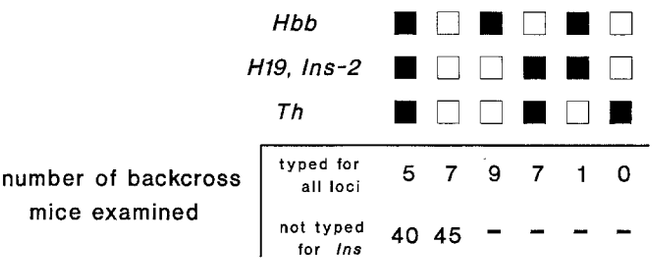

FIG. 2. Segregation of *Ins-2* among progeny from the interspecific backcross (C3H/HeJ-*gld*/*gld* × *M. spretus*)F1 × C3H/HeJ-*gld*/*gld*. Solid boxes represent the homozygous C3H pattern and open boxes the F1 pattern. *Ins-2* was typed by PCR as described in the text. Typing for *Hbb*, *H19*, and *Th* was previously reported (8).

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 *tub* to *Hbb* provides the basis for a similar approach to cloning of the *tub* 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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