

## SHORT COMMUNICATION

# Mapping of the *KHSRP* Gene to a Region of Conserved Synteny on Human Chromosome 19p13.3 and Mouse Chromosome 17

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**The K homology-type splicing regulatory protein, KSRP, activates splicing through intronic splicing enhancer sequences. It is highly expressed in neural cells and is required for the neural-specific splicing of the c-src N1 exon. In this study, we mapped the gene (gene symbols *KHSRP* and *Khsrp*) to human chromosome 19 by using radiation hybrid panels and to mouse chromosome 17 by studying an interspecific backcross panel. Human *KHSRP* is a positional candidate gene for familial febrile convulsion and Cayman type cerebellar ataxia. Comparative analysis of the human and mouse genomes indicates that the *KHSRP* gene is located in regions of conserved synteny between the two species.** © 1999 Academic Press

Alternative splicing is a common mechanism of gene regulation in eukaryotic cells. The mammalian nervous system is especially rich in regulated splicing events, and many important gene transcripts are generated in neural-specific spliced forms. The choice of splice-site usage is thought to be determined by proteins that bind to regulatory sequences in the pre-mRNA and act to enhance or suppress spliceosome assembly at specific sites. The recently discovered K homology-type splicing regulatory protein, KSRP, is required for neural-specific splicing of the c-src N1 exon (8). This 75-kDa protein binds specifically to an intronic splicing enhancer element downstream of the c-src N1 exon where it assembles as a complex with five other proteins. The KSRP protein contains four K homology RNA-binding domains, a proline- and glycine-rich N-terminal domain, and a glutamine-rich C-terminal domain that contains a repeated motif. This domain structure is similar to two other proteins, the mammalian protein FBP (1, 2) and the *Drosophila* splicing regulator PSI (11). FBP-2, a protein identified by cross-hybridization with FBP, is identical to KSRP. Al-

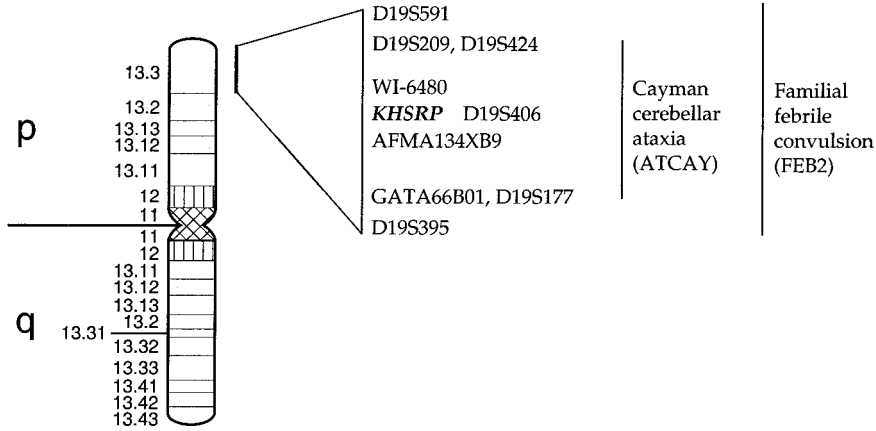
though widely expressed, KSRP is particularly abundant in neural cells where it may be involved in regulating the splicing patterns of many genes. Determining the chromosomal localization of the *KHSRP* gene is the first step in evaluating its potential involvement in inherited disorders.

To map the *KHSRP* gene in human and mouse, we developed gene-specific PCR assays based on known sequence data. GenBank accession numbers are U94832 (human cDNA), AF093745–48 (human genomic), and AF094696 (mouse genomic). The PCR primers used were human *KHSRP*, 5'-AGA TTG ATT GTG ACT GGG TCG-3' and 5'-AGT GGA GTT GGT CAG GGT TG-3', and mouse *Khsrp*, 5'-GAC CCT CCA TCT GTA AAG TTG C-3' and 5'-CTG GAT TCC GTG ACA GGG-3'. Products of the expected size were amplified from human or mouse DNA and were verified by sequencing. No amplification product was obtained with Chinese hamster DNA as template. When multichromosomal somatic cell hybrid panels (3) were analyzed by PCR, the *KHSRP* gene was assigned to human chromosome 19 and mouse chromosome 17.

To define the location of the human *KHSRP* gene further, two radiation hybrid (RH) mapping panels, GeneBridge4 and Stanford G3 (4, 12), were typed by PCR amplification using human *KHSRP*-specific primers. In the Stanford G3 panel, *KHSRP* was concordant with D19S406 in all 83 RH cell lines, while in the GeneBridge 4 panel, *KHSRP* was placed 12.7 cR<sub>3000</sub> from the chromosome 19 markers WI-6480 and AFMA134XB9. The order of markers in this region from pter to centromere is IB1264–WI-6480–*KHSRP*–AFMA134XB9–D19S221. The physical localization of *KHSRP* is at 19p13.3 since D19S406 is in the chromosome 19 bin 1 on the SHGC RH map, and flanking markers D19S424 and D19S177 were previously mapped to 19p13.3 (Genome Data Base) (Fig. 1).

To regionally map the mouse *Khsrp* gene, we performed linkage mapping by genotyping an interspecific

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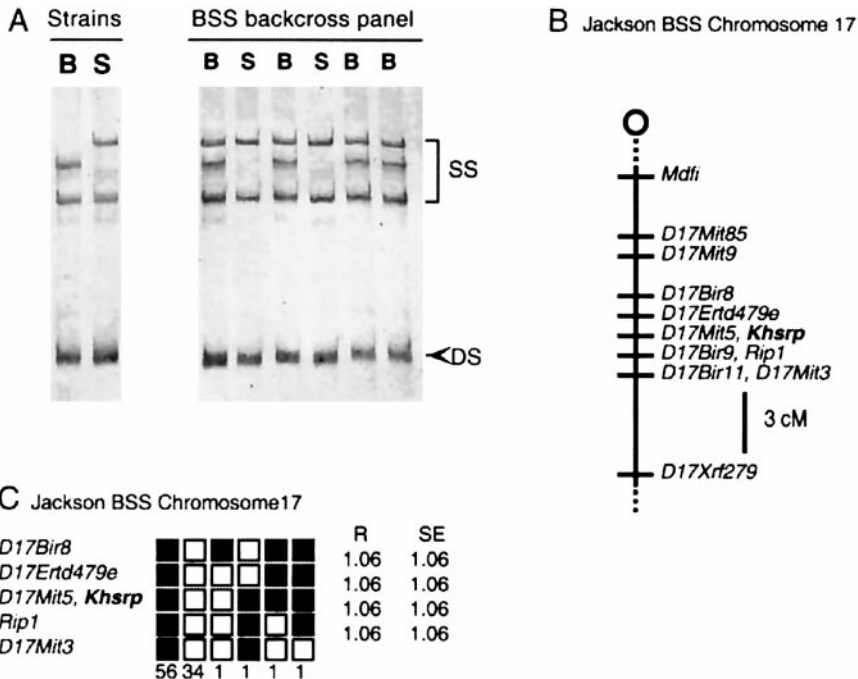


**Human Chromosome 19**

**FIG. 1.** Chromosomal localization of the human *KHSRP* gene: Idiogram of human chromosome 19 with relative position of markers in 19p13.3. Familial febrile convulsion (FEB2) and Cayman cerebellar ataxia (ATCAY) are neurogenetic disorders previously mapped to this region.

backcross panel of 94 progeny of a (C57BL/6JEi × SPRET/Ei)F<sub>1</sub> × SPRET/Ei (BSS) backcross, obtained from The Jackson Laboratory (10). A 187-bp PCR product was amplified with *Khsrp*-specific primers and analyzed by single-strand conformation analysis, which allowed detection of strain-specific variation between parental strains (C57BL/6JEi vs SPRET/Ei) (Fig. 2A). Upon comparison of the strain distribution patterns of the *Khsrp* allele with The Jackson Laboratory's BSS

panel database (<http://www.jax.org/resources/documents/cmdata>), *Khsrp* was found to cosegregate with D17Mit5 in all 94 animals analyzed (Fig. 2C). Thus, the *Khsrp* gene was placed on the BSS linkage map of mouse chromosome 17 at 34.3 cM from the centromere (Fig. 2B). Several known mouse genes in this region, such as *C3*, *Emr1*, *Ptprs*, *Nrtn*, and *Vav*, have human homologues that have been mapped to band 19p13.3. Thus, the mouse *Khsrp* locus is part of the conserved



**FIG. 2.** Chromosomal localization of the mouse *Khsrp* gene. (A) Single-strand conformation analysis of *Khsrp*-specific PCR products from parental C57BL/6JEi (B) and SPRET/Ei (S) mice (left) and BSS backcross panel 2 DNA (right). Single-stranded (SS) and double-stranded (DS) conformers are indicated. Genotyping results of six backcross animals are shown, with four animals scored as heterozygous for the B allele and two scored as homozygous for the S allele. (B) Linkage map of mouse chromosome 17 around the *Khsrp* locus. (C) Haplotypes from The Jackson Laboratory BSS backcross showing part of chromosome 17 with loci linked to *Khsrp*. Loci are listed in order with the most proximal at the top. The black boxes represent the B allele, and the white boxes represent the S allele. The numbers of offspring inheriting each type of chromosome are listed at the bottom of each column. The percentage of recombination (*R*) between adjacent loci is given to the right, with the standard error (SE) for each *R*.

syntenic region on mouse 17 (32–36 cM) and human 19p13.3, indicating that the mouse and human *KHSRP* genes that we have studied are true homologues.

Searching the Mouse Genome Informatics Database (<http://www.informatics.jax.org/locus.html>) for phenotypic mutations near the *Khsrp* locus revealed only one unlikely candidate, *thf*, *thin fur*, at 35.3 cM. Homozygotes for this recessive mutation are normal except for reduced hair formation (7). Genes for human disorders involving the central nervous system that have been mapped near *KHSRP* include familial febrile convulsion 2 (FEB2) (5) and Cayman cerebellar ataxia (ATCAY) (9). Febrile convulsions are a common form of childhood seizure. Predispositions segregating in families are genetically heterogenous. One autosomal dominant febrile convulsion locus (FEB2) was localized to an 11.7-cM, 1- to 2-Mb section of 19p13.3, between loci D19S591 and D19S395 (5). Autosomal recessive Cayman cerebellar ataxia, identified by Johnson *et al.* (6) in a population isolate on Grand Cayman Island, is characterized by marked psychomotor retardation and cerebellar hypoplasia, associated with nonprogressive cerebellar dysfunction including nystagmus, intention tremor, dysarthria, and wide-based ataxic gait. The ATCAY gene was mapped to a 9-cM region between the flanking markers D19S424 and GATA66B01 (9). *KHSRP* falls into the critical regions for both FEB2 and ATCAY loci, and its function as a regulator of neural-specific splicing makes it a good positional candidate gene for these two syndromes (Fig. 1).

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#### REFERENCES

- Davis-Smyth, T., Duncan, R. C., Zheng, T., Michelotti, G., and Levens, D. (1996). The far upstream element-binding proteins comprise an ancient family of single-strand DNA-binding trans-activators. *J. Biol. Chem.* **271**: 31679–31687.
- Duncan, R., Bazar, L., Michelotti, G., Tomonaga, T., Krutzsch, H., Avigan, M., and Levens, D. (1994). A sequence-specific, single-strand binding protein activates the far upstream element of c-myc and defines a new DNA-binding motif. *Genes Dev.* **8**: 465–480.
- Francke, U., Yang, F. T., Brissenden, J. E., and Ullrich, A. (1986). Chromosomal mapping of genes involved in growth control. *Cold Spring Harbor Symp. Quant. Biol.* **51**: 855–866.
- Gyapay, G., Schmitt, K., Fizames, C., Jones, H., Vega-Czarny, N., Spillett, D., Muelet, D., Prud'Homme, J. F., Dib, C., Auffray, C., Morissette, J., Weissenbach, J., and Goodfellow, P. N. (1996). A radiation hybrid map of the human genome. *Hum. Mol. Genet.* **5**: 339–346.
- Johnson, E. W., Dubovsky, J., Rich, S. S., O'Donovan, C. A., Orr, H. T., Anderson, V. E., Gil-Nagel, A., Ahmann, P., Dokken, C. G., Schneider, D. T., and Weber, J. L. (1998). Evidence for a novel gene for familial febrile convulsions, FEB2, linked to chromosome 19p in an extended family from the midwest. *Hum. Mol. Genet.* **7**: 63–67.
- Johnson, W. G., Murphy, M., Murphy, W. I., and Bloom, A. D. (1978). Recessive congenital cerebellar disorder in a genetic isolate: CPD type VII? *Neurology* **28**: 352–353.
- Key, M., and Hollander, W. F. (1972). Thin fur, a recessive mutant on chromosome 17 of the mouse. *J. Hered.* **63**: 97–98.
- Min, H., Turck, C. W., Nikolic, J. M., and Black, D. L. (1997). A new regulatory protein, KSRP, mediates exon inclusion through an intronic splicing enhancer. *Genes Dev.* **11**: 1023–1036.
- Nystuen, A., Benke, P. J., Merren, J., Stone, E. M., and Sheffield, V. C. (1996). A cerebellar ataxia locus identified by DNA pooling to search for linkage disequilibrium in an isolated population from the Cayman Islands. *Hum. Mol. Genet.* **5**: 525–531.
- Rowe, L. B., Nadeau, J. H., Turner, R., Frankel, W. N., Letts, V. A., Eppig, J. T., Ko, M. S. H., Thurston, S. J., and Birkenmeier, E. H. (1994). Maps from two interspecific backcross DNA panels available as a community genetic mapping resource. *Mamm. Genome* **5**: 253–274.
- Siebel, C. W., Admon, A., and Rio, D. C. (1995). Soma-specific expression and cloning of PSI, a negative regulator of P element pre-mRNA splicing. *Genes Dev.* **9**: 269–283.
- Stewart, E. A., McKusick, K. B., Aggarwal, A., Bajorek, E., Brady, S., Chu, A., Fang, N., Myers, R. M., and Cox, D. R. (1997). An STS-based radiation hybrid map of the human genome. *Genome Res.* **7**: 422–433.