SHORT COMMUNICATION

Human Synaptotagmin V (SYT5): Sequence, Genomic Structure, and Chromosomal Location

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We have determined the sequence, genomic structure, and chromosomal location of the human synaptotagmin V (SYTV) gene. The human SYTV gene encodes a 386-amino-acid product which is 91% identical to rat Syt V. The human SYTV open reading frame is interrupted by seven introns which can be alternatively spliced. Human SYTV was found to lie very close to SYTIII on chromosome 19q13.4 by PCR analysis of somatic cell hybrid DNA and by DNA hybridization to arrayed cosmids of the chromosome 19 metric physical map. This provides the first report of linked synaptotagmin genes.

Synaptotagmins constitute a multigene family of evolutionarily conserved vesicle proteins (26). Neuronal isoforms are crucially involved in the calcium-regulated exocytosis of synaptic vesicles (2, 8, 20). It is possible that the necessary functional diversity of synaptic vesicles is increased by targeting different synaptotagmin isoforms to different vesicles and by targeting certain combinations of synaptotagmins to certain vesicles. This view is supported by studies that show that different synaptotagmin isoforms are differentially expressed with overlapping patterns (3, 17–19, 28, 29) and have different biochemical properties (6, 15, 16, 22, 27, 28). Nine mammalian isoforms have been described so far (4, 7, 9, 10, 15, 21, 23, 30), although the sequence of only one human isoform, Syt I/p65, has been determined (24). Further to our discovery of the fifth synaptotagmin isoform in rat, we set out to clone the human homologue and to determine its chromosomal location.

A 721-bp *Msc*I restriction enzyme fragment from rat Syt V clone RB8 (Accession No. X84884) was used to probe a λgt10 human hippocampus cDNA library. The insert of the longest positive clone from this library, hs10, was used to probe another human cDNA library from hippocampus (Clontech), to generate full-length sequence. The longest positive clone from this library, mp2, and clone hs10 were completely sequenced on both strands, giving the sequence in Fig. 1. Clone hs10 includes sequence from positions 1 to 1583 and clone mp2 includes positions 462 to 2062. A typical AATAAA motif together with a poly(A) tail indicates that the 3′ untranslated region is complete. The translation product is 386 amino acids in length, as is the rat Syt V product. It is 91% identical to rat Syt V, with the 34-amino-acid differences clustered in the N-terminal half (Fig. 2).

A Northern blot of human mRNAs from multiple tissues (Clontech) probed with hs10 (Fig. 3) shows a single transcript of approximately 2 kb which is expressed predominantly in brain. The pattern of expression is similar to that in rat (4) and the size of the transcript indicates that our cDNA sequence is likely to be full length.

To aid chromosomal assignment, some genomic sequence was sought. Oligonucleotides a–j (Fig. 1), designed to amplify overlapping regions of SYTV, were used in PCR with human cDNA or human genomic DNA (Clontech), in the hope that at least one pair of oligonucleotides would amplify a relatively small intron. Fortunately, almost all of the genomic PCR products contained intron sequences, and these introns appeared to be relatively small. It was therefore possible to use the genomic PCR products to determine the intron–exon structure as well as to provide sufficient intron sequence for chromosomal assignment. The PCR products were all subcloned into pUC18 and sequenced. An overlapping set of these subclones was compiled and fully sequenced on both strands, giving a genomic sequence contig of 6471 bp (Accession No. X96783). Once the sequence contig was compiled, the intron–exon structure was confirmed by sizing PCR products obtained with numerous oligonucleotide pairs, completely covering the region between positions 43 and 6230 of the genomic sequence.

Sequence data from this article have been deposited with the GenBank Data Library under Accession No. X96783.

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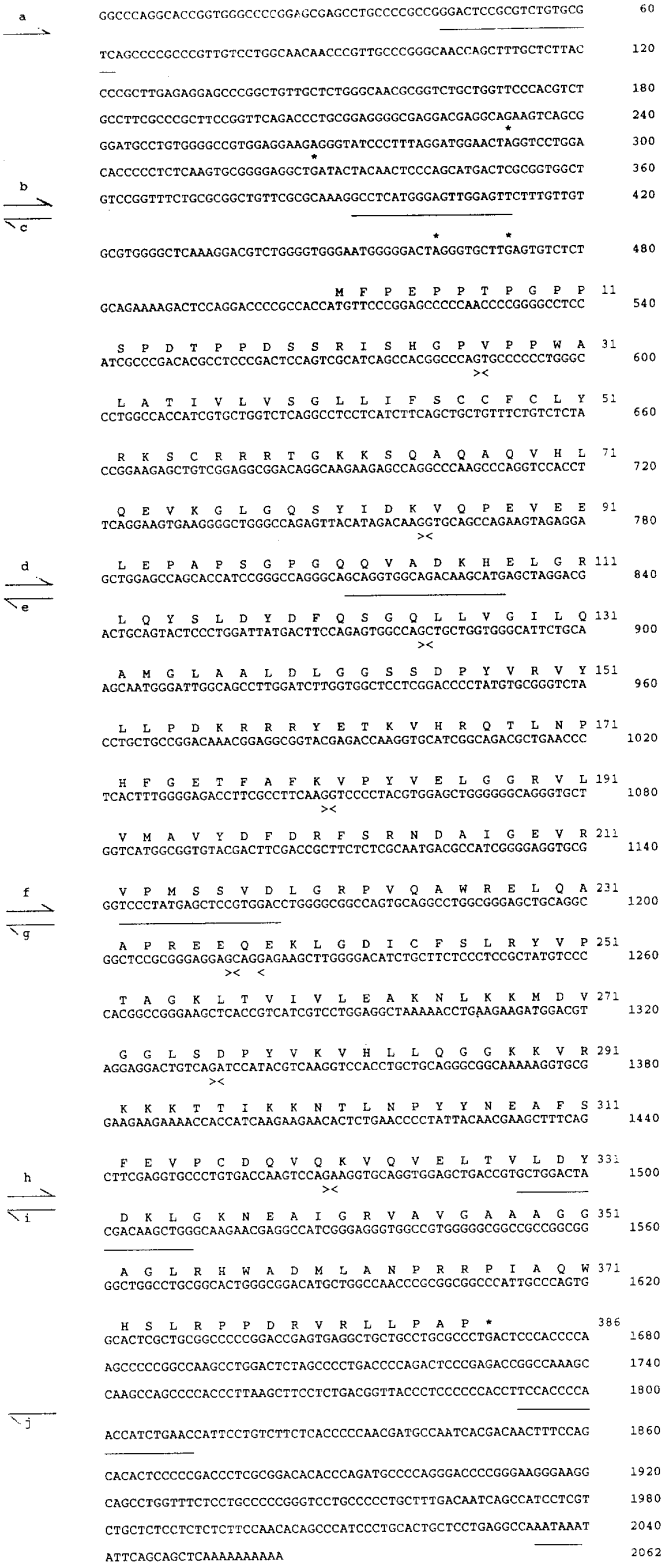

FIG. 1. Nucleotide and predicted amino acid sequence of human SYTV. Nucleotides are numbered in the 5′ to 3′ direction and the amino acids are shown in single-letter code above the nucleotide sequence. In-frame termination codons are marked by asterisks. The AATAAA motif is underlined. The positions of introns are marked with ⟩⟨ below the nucleotide sequence. Oligonucleotide sequences a to j are underlined and their orientation is indicated by arrows on the left margin.

The genomic sequence revealed that the human SYTV gene is composed of eight exons and seven introns. The largest intron (intron 2), of 2070 nt, contains an *Alu* repeat. The second largest intron (intron 7), of 857 nt, contains a series of short repeats of about 26 nt.

Human SYTV was assigned a chromosomal location as follows. Three pairs of oligonucleotides were used in three sets of PCR experiments with a panel of monochromosomal somatic cell hybrid DNAs (12). The oligonucleotide sequences are detailed below, with the numbers referring to the SYTV genomic sequence. Pair 1, 2374–2393 (intron 2) and 2841–2860 (intron 2). Pair 2, 3908–3927 (intron 4) and 4301–4320 (exon 5, g in Fig. 1). Pair 3, 4709–4728 (intron 6) and 5911–5930 (exon 8, i in Fig. 1). All three pairs of oligonucleotides produced single, correctly sized PCR products only with human chromosome 19 DNA, indicating that human SYTV is a single-copy gene located on chromosome 19. A human genomic DNA Southern blot (Clontech) probed with SYTV cDNA clone hs10, genomic clone 710S12, or genomic clone 163Pst60 shows banding patterns consistent with a single-copy gene (data not shown).

The 60-Mb human chromosome 19 is physically well characterized, and a high-resolution metric clone map has been constructed (1). This map was used to refine the position of SYTV as follows. DNA probe sequences from positions 479 to 1179 (clone 163Pst60) or 4308 to 5037 (clone 710S12) of the SYTV genomic sequence were hybridized to chromosome 19 cosmid arrays (1). Both sequences hybridized to cosmids lying between FCAR and D19S775 on 19q13.4.

Another synaptotagmin gene, SYTIII, has been mapped to chromosome 19q by somatic cell hybrid analysis and genetically mapped to mouse chromosome 7 (11). Human SYTIII has recently been further localized to a region between FCAR and D19S775 on the chromosome 19 metric map (25). Some of the SYTIII-positive cosmids were also found to be positive for SYTV, indicating that these genes are very closely linked. Another group has also genetically mapped synaptotagmin genes in mouse (13). Their predicted locations for mouse Syt I, II, and III agree with the earlier study (11). Syt IV was localized to mouse chromosome 18. A fifth gene was assigned to mouse chromosome 7, in a region syntenic with human chromosome 11p. This gene, originally referred to as Syt V, has subsequently been designated B/K and is unlikely to be a synaptotagmin as it appears to lack a transmembrane sequence (14). This is the first report of two linked synaptotagmin genes.

The SYTV genomic sequence was compared to the sequence databases using Blastx and Fasta (blast@ncbi.nlm.nih.gov and fasta@ebi.ac.uk). The only regions of SYTV with significant similarity to other known sequences are the exon regions, the *Alu* repeat, and a region in the seventh intron (not including the 26-nt repeats) that is identical to a CpG island clone

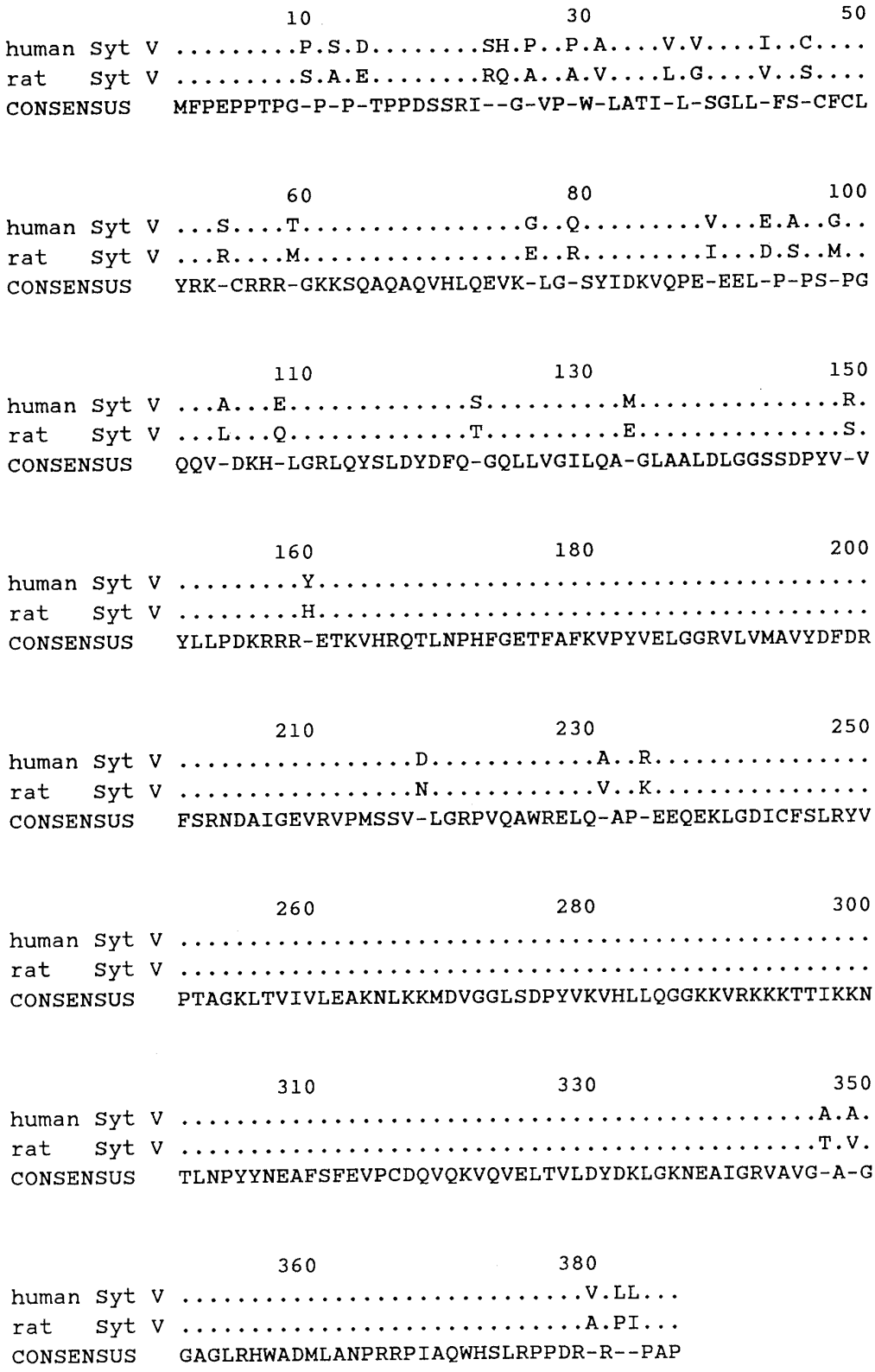

FIG. 2. Sequence comparison of human Syt V and rat Syt V. Identical amino acids, shown as dots, are identified in single-letter code in the consensus. Dashes in the consensus indicate amino acid differences, which are identified above in single-letter code.

(5). Database sequences found to be identical to SYTV and which therefore also map to chromosome 19q13.4 include 26 ESTs and the CpG island clone (Accession Nos. Z62103, H52745, H20299, H21827, H52790, H22498, R46388, H49837, R21342, H49838, H41257, H21826, R87705, H22461, H39018, M78324, H45237,

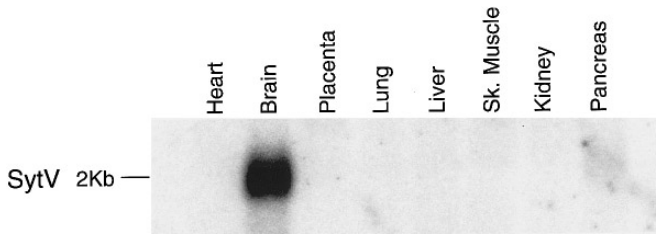

FIG. 3. Northern blot of human mRNA from various tissues, probed with SYTV cDNA clone hs10.

H49950, H39042, H40912, H40953, H48162, D61338, Z19734, H49949, H45200, and H19906).

A small number of sequence disagreements among the fully sequenced cDNA and genomic clones were investigated by sequencing RT-PCR products from human brain cDNA (Clontech). This analysis confirmed the accuracy of the sequence in Fig. 1. It also confirmed that alternative splicing between exons 5 and 6 can lead to the presence (mp2) or absence (hs10) of the glutamine residue at amino acid position 237. Of 40 RT-PCR clones containing the exon 5/6 junction, 33 were found to encode glutamine 237, indicating its prevalent expression. This alternative splicing may be a general phenomenon, as the presence or absence of this glutamine residue was observed in the cDNA sequences of the p65A isoform of the marine ray (30), as well as in rat Syt V (4).

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