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Genetics

Polymorphic human somatostatin gene is located on chromosome 3

(gene mapping/somatic cell hybrids/DNA polymorphism)

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ABSTRACT **Somatostatin is a 14-amino-acid neuropeptide and hormone that inhibits the secretion of several peptide hormones. The human gene for somatostatin *SST* has been cloned, and the sequence has been determined. This clone was used as a probe in chromosome mapping studies to detect the human somatostatin sequence in human–rodent hybrids. Southern blot analysis of 41 hybrids, including some containing translocations of human chromosomes, placed *SST* in the q21→qter region of chromosome 3. Human DNAs from unrelated individuals were screened for restriction fragment polymorphisms detectable by the somatostatin gene probe. Two polymorphisms were found: (*i*) an *Eco*RI variant located at the 3′ end of the gene, found in Caucasian, U.S. Black, and Asian populations with a frequency of approximately 0.10 and (*ii*) a *Bam*HI variant in the intron, which occurs in Caucasians at a frequency of 0.13.**

Somatostatin is a 14-amino-acid hormone and neuropeptide (1, 2) that in vertebrates is found in many regions of the central nervous system and gastrointestinal tract. Somatostatin has several different biological activities: (*i*) it inhibits the release of pituitary, pancreatic, and intestinal hormones; (*ii*) it regulates gastrointestinal function; and (*iii*) it probably is a neurotransmitter (1). A cDNA copy of the human somatostatin mRNA has been isolated, and the sequence has been determined (3). The nucleotide sequence predicted the protein sequence of a 116-amino-acid precursor, the final 14 amino acids of which are somatostatin. The human gene also has been isolated, and its sequence has been determined; it contains a single intervening sequence (unpublished data).

In this study the human somatostatin gene was chromosomally mapped by Southern blot analysis of human–rodent somatic cell hybrids. The gene, located on the end of the long arm of human chromosome 3, has polymorphic sites for *Eco*RI and *Bam*HI segregating in the Caucasian population.

MATERIALS AND METHODS

Cell Lines and Somatic Cell Hybrids. Somatic cell hybrids were constructed by the fusion of human fibroblasts or leukocytes with rodent cell lines possessing selectable markers (4). The hybrid cells were maintained on hypoxanthine/aminopterin/thymidine selection medium (5). Fibroblasts having a normal diploid karyotype were used to construct the WIL (WI38 × LTP), REW (WI38 × RAG), and ICL (GM1006 × LMTK$^-$) hybrid series. Several sets of hybrids were formed from human cell lines containing naturally occurring translocations; these include XER hybrids segregating an X/11 translocation (6); JSR, a 7/9 translocation (7); ATR, a 5/X translocation (8); and NSL, a 17/9 translocation (9). Descriptions of the translocation chromosomes are given in the legend to Table 2. Most relevant to this study are two series of hybrids having translocations with chromosome 3. TSL hybrids, resulting from the fusion of the human fibroblast line GM2808 [46,XX,t(3;17)(p21;p13)] with mouse LMTK$^-$ cells, segregate 3/17 translocation chromosomes. Only those hybrids retaining the 17/3 translocation chromosome (17qter17p13::3p21→3qter) or a normal chromosome 17 having the thymidine kinase gene proliferated on hypoxanthine/aminopterin/thymidine selection medium (10). The second series, XTR hybrid cells, was made from a fusion of RAG mouse cells with the human fibroblast line GM194, which contains an X/3 translocation [46,X,t(X;3)(q28;q21)] (10). The X/3 translocation chromosome (Xpter→Xq28::3q21→3qter) having the human phosphoribosyltransferase gene (*HPRT*) necessary for growth on hypoxanthine/aminopterin/thymidine medium could be selectively eliminated by medium containing 8-azaguanine. Both of the reciprocal translocations—the 3/17 chromosome in the TSL series and the 3/X chromosome in the XTR hybrids—segregated randomly in the hybrids.

Human Chromosome Characterization of Somatic Cell Hybrids. The human chromosomal content of each hybrid clone was determined by either or both of two methods. Human chromosomes could be determined directly by karyotyping and Giemsa/trypsin banding (11) of the hybrid cells. Alternatively, the profile of human genetic markers assigned to specific chromosomes was used as an indicator of the human chromosome composition of a hybrid clone. These genetic markers include human isozymes detected by histochemical and autoradiographic assays and DNA fragments specific for a given chromosome (details of these assays are given in ref. 4).

DNA Isolation and Southern Blot Analysis. DNA was isolated from hybrid cells and their parents as described (12). Each DNA sample (10 μg) was digested with the restriction endonuclease *Eco*RI at 4 units per μg of DNA for 3 hr at 37°C in buffer suggested by the manufacturer. DNA fragments were separated by agarose electrophoresis in a 0.8% gel in a horizontal apparatus and transferred onto nitrocellulose by the method of Southern (13). A 2,667-base-pair (bp) human DNA fragment of known sequences subcloned in pBR322, designated pgHS7-2.7, which includes the somatostatin gene and 1,120 bp of 5′ flanking and 62 bp of 3′ flanking sequence (unpublished data), was used as a probe for somatostatin sequences. The pgHS7-2.7 probe was ^{32}P-labeled by nick translation (14) to a specific activity of $\approx 2 \times 10^8$ cpm/μg. Hybridization of the nick-translated probe to the baked blot was essentially by the method of Wahl as described (15). The blots were hybridized in a formamide solution for 2 days at 42°C and then rinsed briefly in 0.3 M NaCl/0.03 M Na citrate, pH 7/

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Abbreviations: bp, base pair(s); kb, kilobases.

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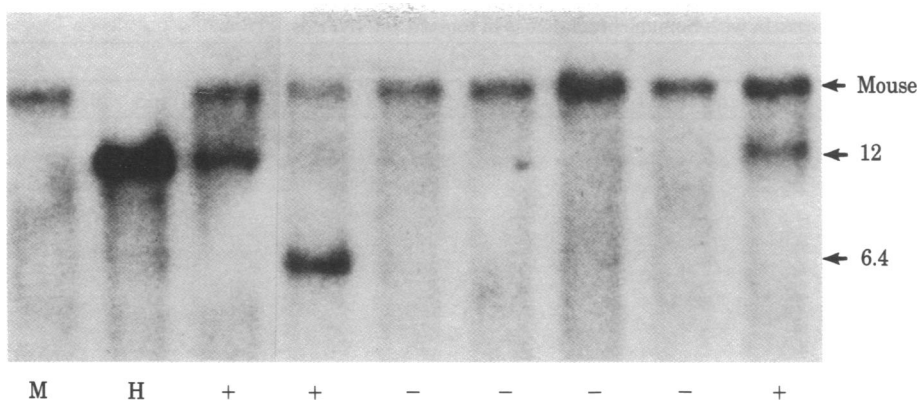

FIG. 1. Hybridization of pgHS7-2.7 to *Eco*RI-digested somatic cell hybrids. The mouse fragment detected (lane M) is easily discernible from the 12-kb human fragment (lane H). Analysis of hybrid fragments in the remaining lanes indicated that not only the 12-kb fragment but also another fragment of 6.4 kb was segregating in the WIL hybrid series.

0.1% $NaDodSO_4$ at room temperature. The blots were washed individually at least twice for 45 min in 500 ml of 0.015 M NaCl/0.0015 Na citrate, pH 7/0.1% $NaDodSO_4$ at 50°C. The dried filters were exposed to XAR x-ray film at −70°C for 1–7 days. The molecular weight standards used were fragments of *Eco*RI- and *Hin*dIII-digested λ phage DNA.

RESULTS

Hybridization of the pgHS7-2.7 Probe with Genomic DNAs. DNAs isolated from mouse, human, and somatic cell hybrid lines were digested with the restriction endonuclease *Eco*RI. The resulting fragments were separated by electrophoresis on 0.8% agarose gels, and the sizes of DNA fragments hybridizing with the somatostatin probe were determined. The human somatostatin gene probe pgHS7-2.7 hybridized to a 6.4- or a 12-kilobase (kb) human *Eco*RI fragment, or to both, and to an approximately 20-kb fragment of mouse DNA (Figs. 1 and 2). As described below, the 6.4-kb human *Eco*RI fragment is a variant generated by an *Eco*RI restriction site polymorphism.

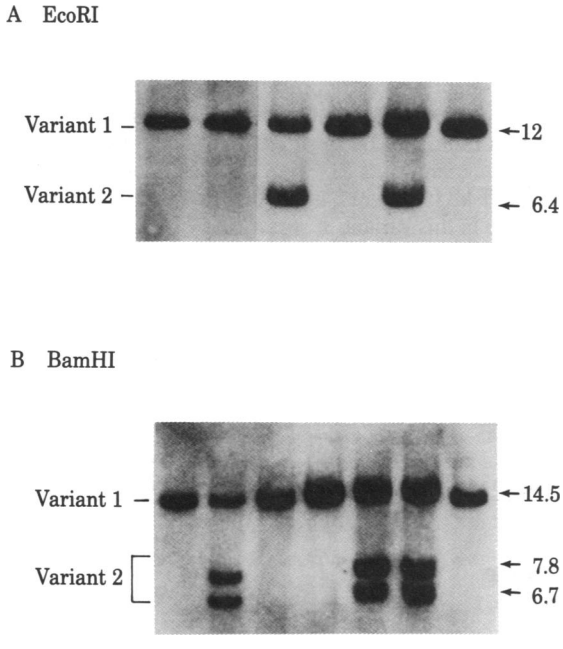

FIG. 2. DNA polymorphisms detected by the pgHS7-2.7 probe. (*A*) The *Eco*RI polymorphic site is located outside the region of homology with the pgHS7-2.7 probe. Allele 1 is the 12 kb fragment, and allele 2 is the 6.4-kb fragment. (*B*) The *Bam*HI polymorphic site is located within the area homologous to the pgHS7-2.7 probe, so that in allele 1, a single 14.5-kb fragment is found, and allele 2 consists of two fragments of 7.8 + 6.7 kb.

Table 1. Segregation of somatostatin with chromosomal markers

Chromosome	Marker*	*SST*/marker +/+	+/−	−/+	−/−	% discordant
1	PEPC, AK2	3	8	2	12	40
2	IDH1, MDH1	4	8	3	13	39
3	ACY1, HS-3†	12	1	0	15	4
4	PEPS	5	8	4	12	41
5	HEXB, 12-65†	6	7	6	9	46
6	ME1, SOD2	5	8	2	13	36
7	GUSB	5	8	3	12	39
8	GSR	7	5	5	10	37
9	AK1, ACO1	0	8	6	8	64
10	GOT1	10	3	6	10	31
11	LDHA, ESA4	8	5	2	12	26
12	LDHB, PEPB	7	6	9	7	52
13	ESD	5	8	6	10	48
14	NP	10	2	7	9	32
15	MPI, PKM2	6	5	4	10	36
16	APRT	5	8	5	11	45
17	GALK	12	1	13	3	48
18	PEPA	10	3	6	10	31
19	GPI	5	8	6	10	48
20	ADA	7	5	9	7	50
21	SOD1	11	2	8	8	34
22	ACO2, MS3-18†	4	9	3	14	43
X	G6PD	8	5	4	12	31

* Genetic marker enzymes assigned to each of the 22 autosomes and the human X chromosome. Marker enzymes are ACO1 (aconitase, soluble), ACO2 (aconitase, mitochondrial), ACY1 (aminoacylase 1), ADA (adenosine deaminase), AK1 (adenylate kinase 1), AK2 (adenylate kinase 2), APRT (adenine phosphoribosyltransferase), ESA4 (esterase A4), ESD (esterase D), GALK (galactokinase), G6PD (glucose-6-phosphate dehydrogenase), GOT1 (glutamic-oxaloacetic transaminase, soluble), GPI (glucose phosphate isomerase), GSR (glutathione reductase), GUSB (β-glucuronidase), HEXB (hexosaminidase B), IDH1 (isocitrate dehydrogenase, soluble), LDHA (lactate dehydrogenase A), LDHB (lactate dehydrogenase B), MDH1 (malate dehydrogenase, soluble), ME1 (malic enzyme, soluble), MPI (mannose phosphate isomerase), NP (nucleoside phosphorylase), PEPA (peptidase A), PEPB (peptidase B), PEPC (peptidase C), PEPS (peptidase S), PKM2 (pyruvate kinase, muscle form), SOD1 (superoxide dismutase, soluble), SOD2 (superoxide dismutase, mitochondrial).

† HS-3, 12-65, and MS3-18 are probes detecting chromosome-specific DNA fragments (unpublished data).

Table 2. Segregation of somatostatin with human chromosomes in somatic cell hybrids

Hybrid cells	*SST**	Chromosomes 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X	Translocations
NSL-7	−	−	−	−	−	−	+	−	−	+	−	−	+	+	+	−	−	+	−	+	−	+	−	−	
NSL-9†	−	−	−	−	−	+	−	−	+	−	+	−	+	+	+	+	+	+	−	−	+	+	+	−	17/9
XER-7‡	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	−	−	+	−	−	+	−	−	11/X
XER-9‡	−	−	+	−	+	−	−	−	+	−	+	−	+	−	+	−	−	+	+	−	−	+	−	−	11/X
XER-11‡	+	+	−	+	+	−	+	+	+	−	+	−	+	−	−	+	+	+	+	−	+	−	+	−	11/X X/11
ATR-13§	+	+	+	+	+	−	+	+	+	−	+	−	+	−	+	+	−	+	+	−	−	−	−	−	5/X
JSR-17S¶	+	+	−	+	−	+	−	−	+	−	+	+	+	+	+	+	+	+	+	−	+	+	+	−	7q⁻
REW8JCSAz3	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	+	−	
WIL-14	+	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	

*Southern blot analyses of hybrid clones and karyotyping by the Giemsa/trypsin banding technique (11) were performed on aliquots of cells from the same passage.
†The 17/9 translocation chromosome is 17qter→17p11::9q12→9qter, and the 9/17 chromosome is 17pter→17p11::9q12→9pter.
‡The 11/X translocation chromosome is Xqter→Xq11::11p11→11qter, and the X/11 chromosome is Xpter→Xq11::11p11→11pter.
§The 5/X translocation is 5pter→5q35::Xq22→Xqter.
¶The 7q⁻ chromosome is 7pter→7q22.

Chromosomal Mapping of the Human Somatostatin Gene. The human *SST* gene was mapped by correlating the presence of specific human chromosomes in human–rodent somatic cell hybrids with human somatostatin gene sequences in the hybrid cell DNA. The human chromosome content of the hybrid cells was determined by karyotyping and Giemsa/trypsin banding and by analysis of human specific gene markers (see Table 1) (4, 11, 16). The human somatostatin gene was found to be present or absent together with the human isozyme of aminoacylase 1, the enzyme marker for human chromosome 3, in an analysis of 29 hybrids (Table 1) (17). The single discordant clone observed was probably due to chromosome breakage. Somatostatin gene sequences also were detected in hybrid DNAs isolated from cells that had been karyotyped at the same cell passage. Only hybrids with human chromosome 3 contained the *SST* gene fragments (Table 2).

Regional Localization of *SST*. The regional localization of the somatostatin gene was determined by analyzing hybrids constructed with human cells containing translocations of chromosome 3. XTR hybrids were made from a fusion of RAG mouse cells with the human fibroblast line GM194, which contains an X/3 translocation [46,X,t(X;3)(q28;q21)] (10). TSL hybrids constructed by the fusion of mouse LMTK⁻ cells with the human line GM2808 [46,XX,t(3;17)(p21;p13)] (10) segregated 3/17 translocation chromosomes (see Table 3). The human somatostatin gene was found only in those hybrids that had retained the distal region of the long arm of chromosome 3 (3q21→3qter)—that is, the Xpter→Xq28::3q21→3qter translocation chromosome in the XTR hybrids and the 3qter→3p21::17p13→17pter translocation chromosome in the TSL hybrids (Table 3). These data assign the somatostatin gene (*SST*) to the q21→qter region of chromosome 3.

DNA Polymorphisms Detected by pgHS7-2.7. The segregation analysis of the human somatostatin gene in somatic cell hybrids indicated that some hybrids had a 12-kb *Eco*RI fragment that contained somatostatin sequences, whereas others segregated a 6.4-kb fragment (Fig. 1). *Eco*RI digestion of the control DNA samples generated a single 12-kb fragment that contained the somatostatin gene. Digestion of DNA from an individual possessing both the 6.4- and 12-kb *Eco*RI fragments with *Bam*HI produced only a single 14.5-kb fragment identical to that generated by *Bam*HI digestion of DNA from the control DNA having only a 12-kb *Eco*RI fragment. Thus, the variant is due to an *Eco*RI restriction site polymorphism and does not indicate another nonallelic somatostatin gene. A survey of DNA samples from 180 individuals represented in Table 4 indicates that this restriction site polymorphism has been observed in Caucasian, Asian, and U.S. Black populations. In this table allele 1 has somatostatin sequences in a 12-kb *Eco*RI fragment, and allele 2 has a 6.4-kb fragment with the somatostatin gene. The allele frequencies are approximately 0.9 for allele 1 and 0.1 for allele 2 in the Caucasian population and in the total population tested. There are insufficient data to evaluate if Asians and U.S. Blacks are significantly different in their allele frequencies. The polymorphic *Eco*RI site is located 3′ to the somatostatin gene sequence (Fig. 3).

In tests of other restriction endonucleases for DNA polymorphisms detectable by the somatostatin probe, a variant was found with *Bam*HI. The most frequent allele was observed as a 14.5-kb fragment (Fig. 2). The variant phenotype consisted of 7.8- and 6.7-kb fragments, indicating that there is an additional *Bam*HI site in this variant. A survey of 67 Caucasian individuals revealed that allele 1 is present in this population at a frequency of 0.87, and allele 2 is at a frequency of 0.13 (where allele 1 is represented by the 14.5-kb fragment, and allele 2 is represented by both the 7.8- and 6.7-kb fragments). The *Bam*HI

Table 3. Regional localization of *SST*

Hybrid cell	Translocation chromosome	Region of chromosome 3 present	*SST*
TSL-2	17/3	3pter→3p21	−
XTR-3BSAgB	3/X	3pter→3q21	−
XTR-22	X/3	3q21→3qter	+

Table 4. DNA polymorphism frequency in the population

Population	Genotype 1,1	1,2	2,2	Frequency of alleles
	*Eco*RI polymorphism			
Caucasian	112	23	3	
U.S. Blacks	20	1	0	
Asians	18	3	0	
Total	150	27	3	1, 0.91 2, 0.09
	*Bam*HI polymorphism			
Caucasian	51	16	0	1, 0.87 2, 0.13

DNA isolated from whole blood samples of unrelated individuals and from cultured lymphocytes and fibroblasts (12) were hybridized to the *SST* probe.

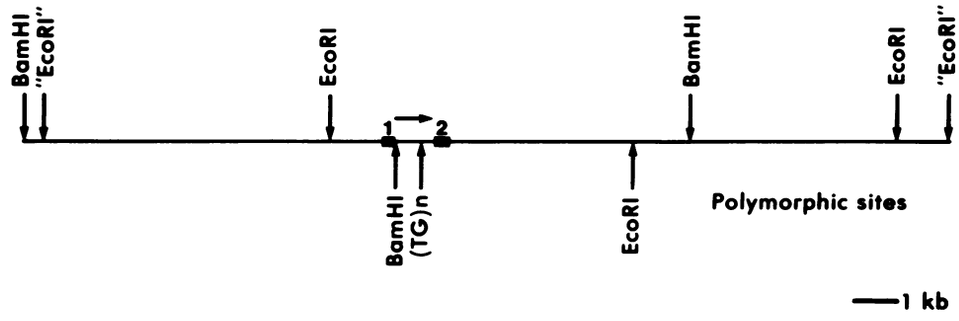

FIG. 3. Linkage map of the human prosomatostatin gene. The two exons coding for somatostatin are indicated by numbers (1 and 2). The location of polymorphic sites are indicated by arrows below the line. The polymorphic *Eco*RI site is located 3′ to the *SST* sequence, whereas the *Bam*HI polymorphic site is in the single intervening sequence. The third polymorphism $(TG)_n$ is a short length variant in the intervening sequence detected by DNA sequence analysis (unpublished data). The *Eco*RI sites in quotation marks indicate the extent of the cloned sequence and are artificial sites generated by the addition of synthetic *Eco*RI linkers. The probe pHS7-2.7 extends from the *Eco*RI site before the gene to a *Hin*dIII site 62 bp after exon 2 (unpublished data).

polymorphism shows Mendelian inheritance in a small nuclear family. A comparison of the DNA sequence of both alleles (unpublished data) indicated that the polymorphic *Bam*HI site is located in the single intervening sequence 31 bp from the exon–intron boundary (Fig. 3). From the limited number of individuals typed for both polymorphisms, it was difficult to determine if linkage disequilibrium exists between these two loci. DNA sequence analyses of the two alleles also revealed a region within the intervening sequence of length polymorphism generated by variation in the tandem repetition of the dinucleotide TG (Fig. 2). There were 24 repetitions in the one allele and 27 in the other.

DISCUSSION

These data indicate that somatostatin is encoded by a single gene on human chromosome 3. In most individuals the entire coding region of the somatostatin precursor is contained on a single 12-kb *Eco*RI fragment that segregates with human chromosome 3 in hybrid cells. Two sets of hybrids (WIL and REW), both made from an individual (WI-38) having the somatostatin gene on 12-kb and 6.4-kb fragments, were found to segregate each band independently in the hybrid cells, indicating that the two fragments are alleles, not linked genes. In addition to this *Eco*RI restriction site polymorphism, a *Bam*HI site polymorphism was also observed. The two polymorphisms do not appear to be in linkage disequilibrium because all four possible combinations of normal and variant sites were observed in the population examined.

Both restriction site polymorphisms detectable by the somatostatin probe are frequent enough to be suitable for gene linkage studies. Although there is no documented association of a somatostatin deficiency or mutant somatostatin protein with any human disease, these DNA polymorphisms will allow us to examine linkage of the somatostatin gene and disease. Because somatostatin inhibits growth hormone and insulin secretion (2), it might be involved in the pathophysiology of some forms of dwarfism or diabetes mellitus. Also, it would be of interest to test the linkage of the somatostatin gene and certain neurological disorders, such as familial Alzheimer disease in which reduced levels of somatostatin-like immunoreactivity are found in the cerebral cortex of affected individuals (18).

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