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SHORT COMMUNICATION

Structure and Chromosomal Localization of the Human Thrombospondin Gene

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Thrombospondin (*THBS1*) is a large modular glycoprotein component of the extracellular matrix and contains a variety of distinct domains, including three repeating subunits (types I, II, and III) that share homology to an assortment of other proteins. Determination of *THBS1* gene structure has revealed that the type I repeat modules are encoded by symmetrical exons and that the heparin-binding domain is encoded by a single exon. To further elucidate the higher level organization of *THBS1*, the gene was localized to the q11–qter region of chromosome 15.

Thrombospondin (*THBS1*) is a homotrimeric glycoprotein with a subunit molecular weight of 140 kDa. As well as being a major component of platelet α-granules involved in mediating the secondary phase of platelet aggregation, *THBS1* is synthesized by fibroblasts and endothelial and smooth muscle cells. Newly synthesized *THBS1* is secreted and incorporated into the extracellular matrix (see Frazier, 1987, for review), where it can serve as an attachment factor for fibroblasts, keratinocytes, squamous carcinoma cells, endothelial cells, and melanoma cells (Varani *et al.*, 1986; Tuszynski *et al.*, 1987; Roberts *et al.*, 1987). Platelet-derived growth factor, a potent mitogen for smooth muscle cells, induces the synthesis of *THBS1* (Majack *et al.*, 1985). Further, epidermal growth factor can exert its mitogenic effect only in the presence of a *THBS1*-enriched matrix, whereas heparin and anti-*THBS1* antibodies that block *THBS1* incorporation into the matrix are antiproliferative (Majack *et al.*, 1986, 1988).

THBS1, a modular glycoprotein, is composed of multiple discrete domains. These domains mediate the binding of *THBS1* to cells and to other components of the extracellular matrix (see Lawler, 1986, for review). The amino terminus of the *THBS1* molecule includes a signal peptide and a 25-kDa heparin-binding domain, the latter containing two clusters of basic amino acids and a single intrachain disulfide bond (Lawler and Hynes, 1986). This domain mediates the incorporation of *THBS1* into the extracellular matrix (McKeown-Longo *et al.*, 1984). Adjacent to the heparin-binding domain is a cysteine-rich 70-kDa domain that binds collagen and laminin and may contribute to the structural integrity of the matrix. By binding both tissue plasminogen activator and plasminogen, the 70-kDa domain increases the catalytic efficiency of plasmin production and may serve as a nidus of extracellular protease activity (Lawler, 1986). The carboxy-terminal domain of *THBS1* includes binding sites for calcium and fibrinogen, and also encodes the Arg–Gly–Asp cell recognition sequence present in other matrix molecules (Lawler, 1986; Lawler and Hynes, 1986; Dixit *et al.*, 1986a). The cDNA sequence for *THBS1* reveals three internal repetitive regions designated types I, II, and III (Lawler and Hynes, 1986; Dixit *et al.*, 1986b; Kobayashi *et al.*, 1986). Sequence motifs similar to the type I repeats are found in complement components (C7, C8α, C8β, C9, and properdin) and in cell surface antigens of *Plasmodium falciparum* (circumsporozite protein and thrombospondin-related anonymous protein) (Goundis and Reid, 1988; Patthy, 1988; Robson *et al.*, 1988; Dame *et al.*, 1984). The type I and II repeats are encompassed by the 70-kDa domain, and the type III repeats are included in the carboxy-terminal domain.

There is some evidence that the evolution of multidomain proteins occurred through exon insertion and exon duplication, each exon encoding a module of protein function (Traut, 1988). Given the numerous sequence homologies that are shared by *THBS1* and other proteins, we have studied its intron/exon organization.

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TABLE 1

Intron–Exon Organization of the Human Thrombospondin Gene

Exon Number	*THBS1* Domain/ Repeat	Exon Size (bp)	Sequence at Intron-Exon Junction: 3' Splice Acceptor	5' Splice Donor	Phase Class	Intron Size (bp)	Location in cDNA
1		95	tgctacag GATCCCTGCTG	ATT CCA G gtgagttt I P E	---	598	46
2	HBD	559	tctaacag AG TCT GGC	AAT TTC CAG gtgaggat N F Q	1-0	268	142
3		75	tgccacag GGG GTG CTG	TCC AGC T gtgagtac S S S	0-1	>528	702
4		199	cttgccag CT ACC AGT	CGC AAA GTG gtcagtgg R K V	1-0	319	778
5	CRD	122	c[redacted]	H C Q	0-0	110	978
6	CRD	93	gcctgcag AAC TCA GTT	TGT TGG C gtaagttt C W P	0-1	>308	1101
7	I	173	c[redacted]	K R F	1-1	>453	1195
8	I	176	g[redacted]	C P I	1-1	486	1369
9	I	173	t[redacted]	P I D	1-1	296	1546
10	II	127	tgtctcag AT GGA TGC	GTT GAT GAG gtaggact V D E	1-0	247	1720
11	II	152	c[redacted]	N K Q	0-0	106	1848
12	II	218	a[redacted]	C K K	0-0	430	2001
13	III	107	t[redacted]	D D R	0-0	468	2220
14	III	159	tcttgcag GAC AAC TGT	GGA GAC G gtaaggtg G D G	0-1	556	2328
15	III	118	ctcttcag GT ATC CTC	CCG GAT CAG gtaggtgg P D Q	1-0	147	2488
16	III	234	tctttcag CTG GAC TCT	TCT GAC G gtgagtca S D G	0-1	>362	2607
17	III	227	g[redacted]	A V G	1-1	183	2842
18		271	tcttccag GT TAT GAT	CCT GGC CAG gtaagaag P G Q	1-0	166	3070
19		97	tccttcag GTG CGC ACC	TTC ATT AG gtcgacat F I R	0-2	>355	3342
20		139	ccctgcag A GTG GTG	TGT AGA G gtaagagc C R D	2-1	101	3440
21		---	tttaacag AT CCC TAA	---------- --------	---	>600	3580

P *

Note. The nucleotide sequence of intron–exon junctions was determined by the dideoxy chain termination method with the use of plasmid clones containing genomic fragments as template and synthetic oligonucleotides as primers. Exon sequences are given in capital letters, intron sequences are given in lowercase letters, and amino acid sequences are given in single letter code. The ochre termination codon is asterisked. The nucleotide numbering referred to under "Location in cDNA" is from Ref. (11). Phase class refers to the position of introns relative to the reading frame. Phase 0 intron, intron lying between two codons; phase 1 intron, intron lying between the first and second nucleotides of a codon; phase 2 intron, intron lying between the second and third nucleotides of a codon. Symmetrical exons—exons that have introns of the same phase class at their 5' and 3' ends—are shaded. *THBS1* domain/repeat refers to the unit of protein function encoded by the particular exon(s). HBD denotes the heparin-binding domain, and CRD denotes the cysteine-rich domain.

In conjunction, we have determined the chromosomal localization of the *THBS1* gene.

A human leukocyte partial *Mbo*I library in the pTCF cosmid vector (kindly provided by Dr. Holers, Washington University) was screened with a previously characterized 1.8-kb *Eco*RI *THBS1* cDNA fragment that encodes the amino terminus of the molecule (Dixit *et al.*, 1986b). The purified cDNA insert was oligolabeled with ^{32}P-labeled deoxynucleotide (Feinberg and Vogelstein, 1983) and used to screen 2×10^5 cosmid

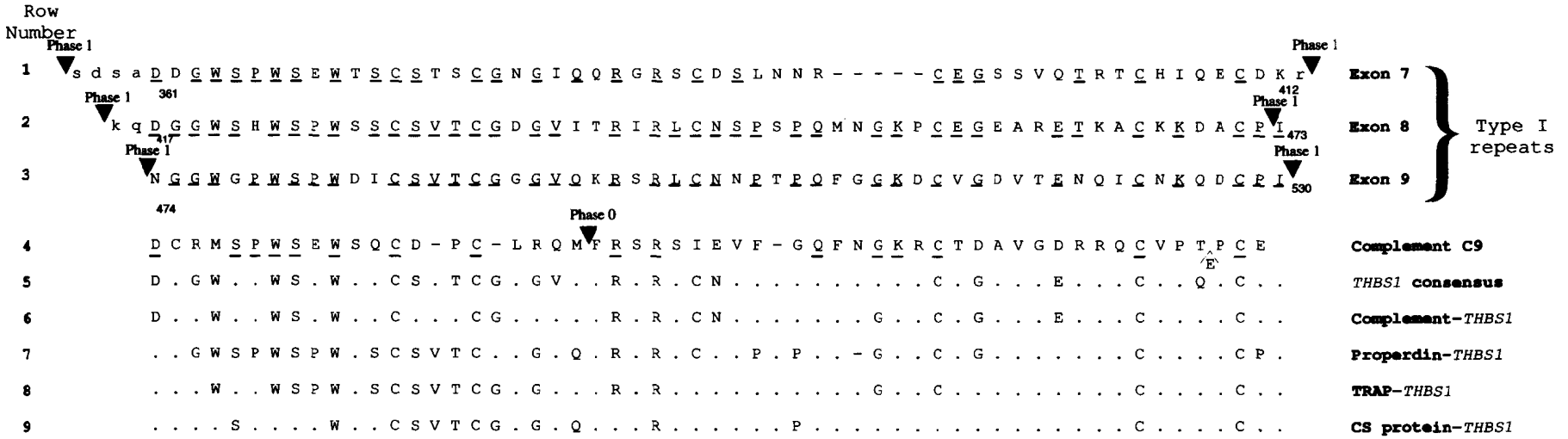

FIG. 1. Homology of *THBS1* type I repeats with sequences in other proteins. Amino acid sequences (single letter code) of the three type I repeats of human *THBS1* are shown in the first three rows, and their positions in the *THBS1* sequence (Lawler and Hynes, 1986) are indicated by subscripts. Positions of introns are marked by closed arrowheads, and amino acid residues that are not part of the type I repeat are indicated by lowercase lettering. The exons encoding the three repeats are identified to the right. Conserved residues in the type I repeats are underlined and dashes indicate gaps. Directly underneath the type I repeats is the exon structure for the relevant portion of complement C9 (16). The fifth row shows the derived consensus sequence of the type I repeat, referred to as *THBS1* consensus. Nonconserved residues are indicated by dots. Also shown by means of consensus sequences are the conserved residues between *THBS1* and the following proteins: (i) complement components C8α, C8β, C9, and *THBS1* (row **6**); (ii) properdin (row **7**); (iii) thrombospondin-related anonymous protein (abbreviated TRAP) of *Plasmodium falciparum* (row **8**); (iv) circumsporozite protein (abbreviated CS) of *P. falciparum* (row **9**).

TABLE 2

Segregation of Human Thrombospondin (*THBS1*) with Human Chromosomes in *Sac*I-Digested Human–Mouse Cell Hybrid DNA

Hybrid	*THBS1*	Human 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
DUM-13	+	+	+	+	−	+	+	+	−	−	+	+	+	−	+	t	+	+	+	+	+	+	+	t	X/15, 15/X
SIR-8	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	
JSR-17S	+	+	+	+	−	+	−	t	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	−	7/9
ATR-13	+	+	+	+	+	+	+	+	+	−	+	−	+	+	+	+	+	+	+	+	−	−	−	t	5/X
REX11 BSAgB	+	−	−	+	−	−	−	−	−	−	+	−	−	−	+	+	−	−	+	−	−	−	−	−	
NSL-16	+	−	−	+	+	+	−	+	+	t	+	−	+	−	+	+	+	+	+	−	+	+	−	−	17/9
WIL-14	+	+	−	+	−	+	−	+	+	−	+	−	+	−	+	+	−	+	−	−	−	−	−	+	
VTL-6	+	−	+	−	−	−	+	+	+	−	+	+	−	−	−	+	−	+	−	+	+	+	+	−	
DUA-6	+	−	+	−	+	+	−	−	−	−	−	−	−	−	−	+	−	−	+	+	−	−	−	+	
XOL-9	+	t	+	+	+	−	+	−	−	−	−	−	+	−	−	+	−	+	+	+	−	+	+	+	X/1
JWR-22H	+	t	t	+	+	−	+	−	+	−	+	+	+	+	+	+	−	+	+	−	+	+	−	−	2/1
JWR-26C	+	t	+	+	+	+	+	+	−	+	+	+	+	−	+	+	+	+	+	−	+	+	−	+	1/2
REW-15	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	−	+	+	+	+	+	+	+	
WIL-2	+	−	−	−	−	−	−	−	+	−	−	−	+	−	−	+	−	+	−	−	−	+	−	+	
VTL-19	+	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	−	+	−	+	−	+	−	+	
DUA-1A	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	t	−	−	−	−	−	−	−	t	X/15
VTL-8	+	−	−	−	−	−	−	−	−	−	−	−	−	+	−	+	−	+	−	−	+	+	+	−	
SIR-11	−	−	−	−	−	−	−	+	−	−	−	−	−	+	−	−	−	−	−	−	−	+	+	+	
JSR-2	−	−	−	+	+	−	−	+	−	−	−	−	−	+	+	−	−	−	−	−	−	−	−	+	
JSR-14	−	−	+	+	+	+	+	−	−	−	−	−	+	+	−	−	−	+	−	−	+	+	−	+	
REX-11BSHF	−	−	−	+	−	−	−	−	−	−	+	−	−	−	+	−	−	−	+	−	−	−	t	t	22/X
XTR-2	−	−	−	t	−	+	−	−	+	−	+	−	+	+	+	−	−	−	+	−	+	+	−	t	3/X
TSL-1	−	−	+	+	+	−	−	−	−	−	+	+	−	+	−	−	+	+	+	−	+	+	−	−	
WIL-1	−	−	−	−	−	−	−	−	+	−	−	−	+	−	+	−	−	+	+	−	−	+	−	+	
WIL-5	−	−	−	−	+	−	−	−	+	−	+	−	−	−	−	−	−	+	+	−	−	+	−	+	
WIL-7	−	−	+	+	−	+	+	−	+	−	+	+	−	+	+	−	−	+	+	−	−	+	−	+	
VTL-7	−	−	−	−	−	−	−	t	−	−	−	+	−	−	+	−	−	+	−	+	+	+	+	−	7q-
W12	−	−	+	−	−	+	−	−	−	−	−	+	−	−	+	−	−	+	−	+	+	+	+	−	11p-
XTR-3BSAGB	−	−	−	t	−	−	−	−	−	+	t	−	+	−	−	−	−	−	−	−	+	+	−	t	3/X 10q-
DUA-3BSAGA	−	−	+	−	−	−	−	+	+	−	−	−	−	+	+	−	−	+	−	−	−	−	−	−	
DUA-5BSAGA	−	−	−	+	−	+	−	−	−	−	−	+	−	−	+	−	−	+	+	−	−	+	−	−	
REW-11	−	−	−	−	+	−	−	+	−	−	−	+	+	+	−	−	+	−	−	−	+	+	+	+	
XOL-6	−	t	−	−	−	+	+	+	−	−	+	+	+	−	+	−	−	+	−	+	+	−	+	t	1/X
XOL-21	−	−	−	+	−	−	−	t	+	+	+	+	+	−	+	−	−	+	+	−	+	−	−	+	ISO7p
DUA-1CSAZB	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	t	−	−	−	−	−	−	−	t	15/X
DUA-1CSAZE	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	t	−	−	−	−	−	−	−	t	15/X
Concordants		24	23	21	22	22	23	19	22	20	22	18	23	17	19	32	23	22	22	24	18	19	20	13	
Discordants		8	12	13	14	14	13	14	14	15	13	18	13	19	17	0	13	14	14	12	18	17	15	14	
% Discordancy		25	34	38	39	39	36	42	39	43	37	50	36	53	47	0	36	39	39	33	50	47	43	52	

Note. This table is compiled from 36 cell hybrids involving 13 unrelated human cell lines and 4 mouse cell lines. The hybrids were characterized by karyotypic analysis and by mapped enzyme markers. The "t" in the table indicates a chromosome translocation for a particular chromosome, but no intact chromosome is present. The DNA probe for *THBS1* was hybridized to Southern blots containing *Sac*I-digested DNA from the human–mouse hybrids listed. The scoring for *THBS1* was determined by the presence (+) or the absence (−) of human bands in the hybrids on the blots. Concordant hybrids have either retained or lost the human bands together with a specific human chromosome. Discordant hybrids either retained the gene, but not a specific chromosome, or the reverse. Percentage discordancy indicates the degree of discordant segregation for a marker and a chromosome. A 0% discordancy is the basis for chromosome assignment.

clones on nitrocellulose membranes by colony hybridization (Maniatis *et al.*, 1982). Hybridization was performed in 5× SSPE (1× SSPE is 0.18 *M* NaCl/1 m*M* EDTA/10 m*M* NaH_2PO_4, pH 7.4), 10× Denhardt's solution (1× Denhardt's is 0.02% polyvinylpyrrolidone/0.02% Ficoll/0.02% bovine serum albumin), 0.1% sodium dodecyl sulfate, and 100 μg/ml sonicated salmon sperm DNA at 68°C for 24 h. The filters were washed five times with 0.1× SSPE, 0.1% sodium dodecyl sulfate at room temperature and were subjected to autoradiography.

A cosmid clone that hybridized with probes from both the extreme 5′ and 3′ ends of a full-length *THBS1* cDNA (thus comprising the entire coding region) was digested with various restriction enzymes, resolved on a 1% agarose gel, blotted onto nitrocellulose membranes, and hybridized to ^{32}P-labeled full-length *THBS1* cDNA. After a washing at appropriate strin-

gency, the filter was autoradiographed. Six hybridizing *Bgl*II fragments representing 14 kb of the 30-kb genomic clone were subcloned into the plasmid vector pGEM7zf(+) (Promega, Madison, WI) by standard techniques (Maniatis *et al.*, 1982). Nucleotide sequence of the CsCl-banded genomic clones was determined using synthetic 20-mer oligonucleotides for double-stranded dideoxynucleotide sequencing. The oligonucleotides were designed at 300-bp intervals along both strands of the known *THBS1* cDNA sequence. Divergence between the genomic sequence and the known cDNA sequence led to the assignment of intron/exon boundaries for the gene. This method defined the exon boundaries for *THBS1*, as shown in Table 1.

The *THBS1* message is encoded by 21 exons, whose average length of 117 bp is comparable to the 140-bp average exon length determined from 80 genes (Traut, 1988). Eight of the 21 exons are symmetrical, having at both ends interrupting codons in the same position within the codon. Patthy (1987) has recently suggested a nomenclature for classifying intronic splice points within a gene, where phase 0 introns are introns that interrupt between codons, and phase 1 and phase 2 introns interrupt the reading frame between the first and second nucleotides of a codon, respectively. Exons are defined by the interrupting position of the introns; e.g., a 0–1 phase exon is preceded by a phase 0 intron and followed by a phase 1 intron. Half of the symmetrical exons in *THBS1* are 0–0 phase class, and half are 1–1 phase class. *THBS1* introns are predominantly phase 0 or phase 1 and are small in size where determined (see Table 1).

The first two exons encompass the signal peptide and the heparin-binding domain. Exon 1 contains the initiator methionine and encodes the signal peptide in addition to the first five amino acids (NRIPE) of the mature *THBS1* protein. The second exon is 559 bp and encodes most of the amino-terminal heparin-binding domain (186 amino acids), including two clusters of basic residues that bind anionic heparin and one of the two cysteines that forms an intrachain disulfide bridge. The large size and modularity of this exon serve as indirect evidence for the evolutionary conservation of *THBS1* heparin-binding domain.

Determination of the intron/exon organization of *THBS1* has led us to believe that the type I repeats found in *THBS1* are susceptible to exon shuffling. Three lines of evidence derived from the exon structure are that (1) the exon boundaries very closely match the defined repeat boundaries for all three repeats (see Fig. 1), thus making them separate units; (2) the exon boundaries for the type I repeats are symmetrical; and (3) these symmetrical boundaries are all of the 1–1 phase class. Symmetrical exons can be inserted, deleted, and duplicated into introns of the same phase class without interruption of the reading frame, allowing them to be candidates for exon shuffling (Patthy, 1988). Further, it has been shown that exons of the 1–1 phase class have participated in exon shuffling during the evolution of proteases, complement, growth factor, and fibronectin genes (Patthy, 1985; Patthy *et al.*, 1984).

Of the other proteins in Fig. 1, only the exon structure for complement C9 has been determined (Marazziti *et al.*, 1988). The sequence in C9 homologous to the *THBS1* type I repeat does not show the same modularity as that in *THBS1* in that the repeat is interrupted by a phase 0 intron and is not delineated by surrounding introns. Thus it is unlikely that these two genes exchanged genetic material through exon shuffling.

The functional significance of the presence of homologous type I modules in other proteins remains unclear. *THBS1* type I repeats constitute the *THBS1* domain which binds laminin, collagen, and plasminogen (Lawler, 1986). It is possible that an altered version of this binding function may be utilized by malaria parasite to bind hepatocytes and red blood cells.

Neither the type II nor the type III repeats are well defined by intron splice points. The type II repeats have six conserved cysteines, have 20% internal amino acid homology, and bear a marginal homology to epidermal growth factor precursor, based mainly on the alignment of the cysteine residues (Lawler and Hynes, 1986). Two of the three type II repeats are encoded by symmetrical exons, the introns occurring near but not at the repeat boundaries. There are seven complete type III repeats in the *THBS1* gene, each highly enriched in aspartate residues, which bind calcium ions to make a calcium-sensitive structure (Lawler and Hynes, 1986; Dixit *et al.*, 1986a). Each repeat contains two clusters of identically spaced aspartate residues which share homology with other calcium-binding proteins (Lawler and Hynes, 1986). Two of the type III-containing exons are symmetrical, but there appears to be no correlation between the defined extent of each repeat and the boundaries of the exons encoding them. The type III repeats may have evolved by a more complex mechanism, such as exon boundary sliding and fusion (Traut, 1988).

The cysteine-rich domain of *THBS1* encodes for the interchain disulfide bridge for the mature trimer protein and is represented by two exons. Human proαI(1) collagen (Chu *et al.*, 1984) and chicken proα1(III) collagen (Yamada *et al.*, 1984) have amino acid homology but little exon structure correspondence to the *THBS1* cysteine-rich domain or between themselves.

To further explore the higher level organization of the *THBS1* gene, its physical location on the chromosomes was determined by gene segregation in somatic cell hybrids. The 36 somatic cell hybrids used in this study have been previously isolated and charac-

terized (Shows *et al.*, 1978, 1982, 1984; Shows, 1983). DNA from human, mouse, and human–mouse hybrids was digested with the restriction enzyme *Sac*I and was blotted onto nitrocellulose membranes (Naylor *et al.*, 1983). The filters were hybridized with a 4.5-kb full-length human *THBS1* oligolabeled cDNA, washed at high stringency, and autoradiographed. In this manner *THBS1* was localized to chromosome 15 (Table 2). The hybrid DUA-1A with the X/15 translocation Xp11→Xter::15q11→qter was positive for the *THBS1* gene, and the hybrid DUA-1CSAZE with the 15/X translocation 15pter→15q11::Xp11→Xpter was negative for the *THBS1* gene, leading to the further localization of *THBS1* to the q11→qter region of chromosome 15.

Chromosome 15 is acrocentric, has a very short p region, and has a relatively high amount of palindromic repeated sequence in the proximal portion of the q region (Donlan *et al.*, 1986). At this time, few pathologic states resulting from chromosome 15 aberrations have been identified (McKusick, 1986).

In summary, the large modular glycoprotein *THBS1* is encoded by at least 21 exons. Modularity of the *THBS1* structure is enforced by the finding that the heparin-binding domain is represented by a single large exon. The exon structure for the type I repeats is optimal for exon shuffling, and this may help explain the existence of type I repeat-like structures in other proteins. Further, *THBS1* has been localized to 15q11–qter and may help serve as a genetic marker in future studies.

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