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Sequence Analysis of the Human Tyrosylprotein Sulfotransferase-2 Gene in Subjects with Chronic Pancreatitis

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Key Words
Chronic pancreatitis · Genetic association study · Tyrosine sulfation · tyrosylprotein sulfotransferase-2 variant · tyrosylprotein sulfotransferase-2 haplotype · PHASE

Abstract
Background/Aims: Human trypsinogens are post-translationally sulfated on Tyr154 by the Golgi resident enzyme tyrosylprotein sulfotransferase-2 (TPST2). Tyrosine sulfation stimulates the autoactivation of human cationic trypsinogen. Because increased trypsinogen autoactivation has been implicated as a pathogenic mechanism in chronic pancreatitis, we hypothesized that genetic variants of *TPST2* might alter the risk for the disease. ***Methods:*** We sequenced the 4 protein-coding exons and the adjacent intronic sequences of *TPST2* in 151 subjects with chronic pancreatitis and in 169 healthy controls. The functional effect of *TPST2* variants on trypsinogen sulfation was analyzed in transfected HEK 293T cells. ***Results:*** We detected 10 common polymorphic variants, including 6 synonymous variants and 4 intronic variants, with similar frequencies in patients and controls. None of the 8 common haplotypes reconstructed from the frequent variants showed an association with chronic pancreatitis. In addition, we identified 5 rare *TPST2* variants, which included 3 synonymous alterations, the c.458G>A (p.R153H) nonsynonymous variant and the c.-9C>T variant in the 5′ untranslated region. The p.R153H variant was found in a family with hereditary pancreatitis; however, it did not segregate with the disease. In functional assays, both the p.R153H and c.-9C>T TPST2 variants catalyzed trypsinogen sulfation as well as wild-type TPST2. ***Conclusion:*** Genetic variants of human *TPST2* exert no influence on the risk of chronic pancreatitis.

Introduction

The imbalance between trypsinogen activation and trypsin inactivation seems to be an important pathogenic factor in the development of chronic pancreatitis. Genetic variants that stimulate the autoactivation of cation-

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ic trypsinogen *(PRSS1*, OMIM 276000) have been found in families with hereditary chronic pancreatitis and in subjects with idiopathic chronic pancreatitis without a family history [1, 2 and references therein]. Triplication and duplication of the trypsinogen locus was identified in a subset of patients with hereditary and idiopathic chronic pancreatitis. This type of genetic alteration is believed to lead to increased trypsinogen expression and activation through a gene dosage effect [3, 4]. Loss of function variants of the pancreatic secretory trypsin inhibitor (*SPINK1*, OMIM 167790) and the trypsin degrading enzyme chymotrypsin C (CTRC, OMIM 601405) are associated with different forms of chronic pancreatitis [5–9]. Thus, not only enhanced trypsin activation, but also impairment of trypsin inhibition or trypsin degradation increases the risk of chronic pancreatitis. In contrast, a degradation-sensitive variant of anionic trypsinogen (*PRSS2*, OMIM 601564) was shown to afford protection against chronic pancreatitis [10–12].

Human trypsinogens are modified post-translationally by the sulfation of Tyr154 [13–15]. Tyrosine sulfation is catalyzed by the enzyme tyrosylprotein sulfotransferase (TPST) in the trans-Golgi network. In humans, 2 TPST isoenzymes TPST1 (OMIM 603125) and TPST2 (OMIM 603126) are expressed in all tissues [16–19]. In cell culture experiments, both TPST enzymes are capable of trypsinogen sulfation [20]. In the human pancreas, TPST2 is expressed at levels 50-fold higher than TPST1, indicating that physiological trypsinogen sulfation is mediated by TPST2 [18, 21, 22]. Notably, TPST2 expression is approximately 10-fold higher in the pancreas than in other human tissues. With respect to trypsinogen function, the only significant consequence of tyrosine sulfation is increased autoactivation of cationic trypsinogen [14, 20]. In contrast, tyrosine sulfation does not affect the autoactivation of anionic trypsinogen. Furthermore, the common p.D153H African polymorphism in anionic trypsinogen abolishes trypsinogen sulfation, suggesting that the modification plays no important functional role in this isoform [20]. Because increased autoactivation of cationic trypsinogen has been linked to the pathogenesis of hereditary pancreatitis [23 and references therein], we hypothesized that altered trypsinogen sulfation due to genetic variants of *TPST2* might modify the risk for chronic pancreatitis. To test this notion, we screened the protein-coding exons of the human *TPST2* gene by direct sequencing in subjects with chronic pancreatitis and healthy controls.

Materials and Methods

Patients

We investigated 151 unrelated patients with chronic pancreatitis (including 73 female and 78 male patients), of which 104 had idiopathic chronic pancreatitis, 40 hereditary chronic pancreatitis and 7 patients alcoholic chronic pancreatitis (median age 33 years, mean age 35.8, range 9–83). All patients were negative for *PRSS1* mutations. The 104 patients with idiopathic chronic pancreatitis were all investigated for *SPINK1* variants; 49 patients were heterozygous and 9 homozygous for p.N34S, and 1 patient carried a c.27delC variant. *CFTR* variants were tested in 66 of the 104 subjects with idiopathic chronic pancreatitis with the following results: 6 × p.F508del, 1 × p.S1235R, 1 × p.R117H, 5 × p.R75Q, 1 × p.R74Q, 5 × p.E528E, 2 × 5T, 1 × p.I507V and 1 × IVS16–2A>G. As healthy controls, 169 subjects were enrolled (131 females, 38 males; median age 47 years, mean age 46.5, range 20–81). This study was approved by the medical ethical review committee of the University of Leipzig. All individuals gave informed consent. The diagnosis of chronic pancreatitis was based on 2 or more of the following findings: presence of a typical history of recurrent pancreatitis, pancreatic calcifications and/or pancreatic ductal irregularities revealed by endoscopic retrograde pancreatography or by magnetic resonance imaging of the pancreas and/or pathological sonographic findings. Hereditary chronic pancreatitis was diagnosed when one 1st-degree relative or 2 or more 2nd-degree relatives suffered from recurrent acute or chronic pancreatitis without any apparent precipitating factors. Idiopathic chronic pancreatitis was diagnosed in the absence of a positive family history or possible precipitating factors, such as alcohol abuse, trauma, medication, infection and metabolic disorders. Alcohol-induced chronic pancreatitis was diagnosed in patients who consumed more than 60 g (females) or 80 g (males) of ethanol per day for more than 2 years.

Sequence Analysis of TPST2

DNA was extracted from peripheral blood leukocytes. We analyzed all 4 protein-coding exons and their flanking intronic sequences in *TPST2* by unidirectional DNA sequencing of PCR amplicons (fig. 1). Sequence variants were confirmed by DNA sequencing of a 2nd independent PCR amplification. The sequences of primers used for PCR amplification and DNA sequencing are given in table 1.

PCR reactions were performed under the following conditions: 0.75 U AmpliTaq Gold polymerase (Applied Biosystems, Foster City, Calif., USA), 450 μmol/l deoxynucleoside triphosphates and 0.3 μmol/l of each primer were used in a total volume of 25 μl. Cycle conditions were as follows: initial denaturation for 6 min at 95°C; 48 cycles of 20-second denaturation at 95°C, 40-second annealing at 62°C and 90-second primer extension at 72°C; and a final extension step for 6 min at 72°C. PCR products were digested with shrimp alkaline phosphatase (USB Europe, Staufen, Germany) and exonuclease I (USB). Cycle sequencing was performed using BigDye terminator mix (Applied Biosystems). The reaction products were purified on a Sephadex G-50 column (GE Healthcare, Piscataway, N.J., USA) or by ethanol precipitation, and were loaded onto an ABI 3100-Avant fluorescence sequencer (Applied Biosystems). Sequencing data were analyzed using ABI DNA sequencing analysis software (Version 1.1.2).

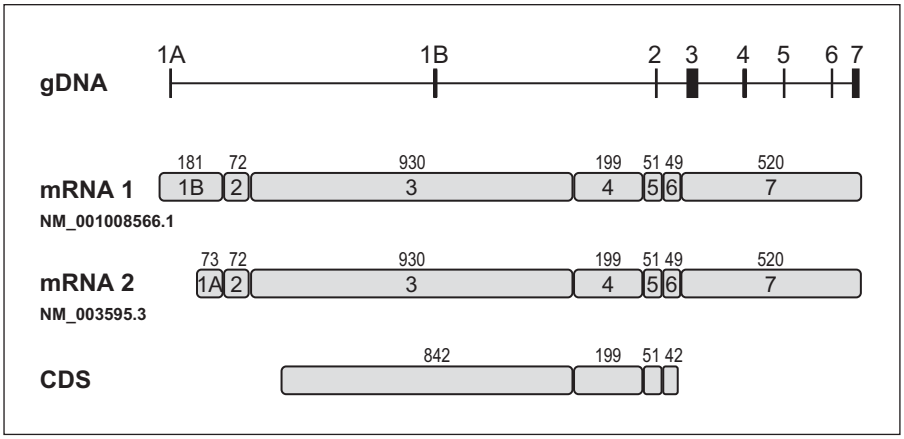


Fig. 1. Organization of the *TPST2* gene and transcripts in humans. The numbers indicate the lengths of the exons in base pairs. NM_001008566.1 and NM_003595.3 are the GenBank accession numbers for the 2 transcript variants. gDNA = Genomic DNA; CDS = protein-coding sequence.

Table 1. Oligonucleotide primers used for PCR amplification and sequencing of the 4 protein-coding exons and the exon/intron junctions in *TPST2*

Exon	Primer name	Primer sequence (5′→3′)
Exon 3 (primer set 1)	1F-T1-PCR	GAAAGGTGCCCTCTGCATTC
	1R-T1-PCR	CCGAGGAAGTCGAGGATGAG
	1F-T1-SEQ	ACTATGCATGTCCCCCTCTCT
Exon 3 (primer set 2)	1F-T2-PCR	GGGACAGCAGGTGCTAGAGT
	1R-T2-PCR	GAAAGGAGAGGAGTAATTGTTGTCA
	1R-T2-SEQ	ATGAAACGGAGGCTCAGAGA
Exon 4	F-PCR	CTGTGCCTGACCTAAACTGTTG
	R-PCR	CTGGCCTGCTGTTAGATGTTAGAG
	F-SEQ	GTACCAATGGTGTATACTGCTAG
Exon 5	F-PCR	CTAATCCCACCTCTTTACTGTAC
	R-PCR	GGAAAACCATCAACCAGAGTGG
	F-SEQ	GCTATGTCCTTGTTTATCTGTG
Exon 6	F-PCR	CTCATAGCAGCCTGTCATATTGC
	R-PCR	CCATCATAGTGGTGCTTCCTGC
	F-SEQ	GCAGCCTGTCATATTGCCCAAG

The annealing temperature was 62°C for all primers listed. F = Forward; R = reverse; PCR = polymerase chain reaction; SEQ = sequencing.

Nucleotide numbering was based on the *TPST2* cDNA sequence (GenBank NM_001008566.1 and NM_003595.3), with the 1st nucleotide of the ATG start codon designated as +1. The mutations are described according to the nomenclature recommended by the Human Genome Variation Society (http://www.hgvs.org/mutnomen). Haplotype reconstruction was performed using PHASE software v2.1 [24, 25]. For the reconstruction of haplotypes, only single nucleotide polymorphisms with a minor allele frequency of ≥5% were considered.

The significance of the differences between variation frequencies in affected individuals and controls was tested by a 2-tailed Fisher's exact test and calculated using GraphPad Prism v4.03. $p < 0.05$ was considered to be of statistical significance.

Plasmid Construction and Mutagenesis

Construction of the pcDNA3.1(-)_PRSS1, pcDNA3.1(–)_PRSS2 and pcDNA3.1(–)_TPST2 plasmids were described previously [26, 20]. Mutation p.R153H was introduced into pcDNA3.1(–)_TPST2 by overlap extension PCR mutagenesis. In the pcDNA3.1(–)_TPST2 expression plasmid, the 5′ untranslated region was only 6 nucleotides long and was altered to generate an optimal Kozak sequence [20]. To analyze the effect of the c.-9C>T variant on the translation of TPST2, we constructed a new TPST2 expression plasmid which contained 29 nucleotides of unadulterated 5′ upstream sequence. This was achieved by amplifying the TPST2 sequence from IMAGE clone No. 4857366 (GenBank BC017509) using the TPST2 *Xho*I ver2 sense primer [5′-CGG GCC CTC GAG

GCC AGG CCT ACC CTG CCT-3′ (where the *Xho*I site is underlined)] and the previously described TPST2 *Bam*HI antisense primer [20]. The PCR product was digested with *Xho*I and *Bam*HI, and cloned into the pcDNA3.1(–) plasmid. Variant c.-9C>T was then introduced into this plasmid by PCR mutagenesis using the following mutagenic primer: 5′-CGG GCC CTC GAG GCC AGG CCT ACC CTG CCT C**T**G GCC CAG-3′ (where the *Xho*I 5′ cloning site is underlined and the altered nucleotide in the c.-9C>T variant is set in bold and underlined).

Functional Analysis of TPST2 Variants

To analyze the activity of TPST2 variants, we used a previously developed method in which HEK 293T cells are cotransfected with a constant amount of pcDNA3.1(–)_PRSS2 or pcDNA3.1(–)_PRSS1 plasmid and increasing amounts of pcDNA3.1(–)_TPST2 plasmid. TPST2 activity is characterized by measuring the level of sulfated trypsinogen in the conditioned medium by Western blotting using an antisulfotyrosine IgG antibody [20, 27, 28]. Cell culture conditions, the transfection protocol and details of Western blotting were described previously [20].

Results

The human *TPST2* gene is located on chromosome 22 and contains 8 exons, including 2 alternatively spliced exons 1 (fig. 1). Only exons 3, 4, 5 and 6 code for protein. Since the majority of known pancreatitis-associated mutations affect the coding region or the exon-intron junctions of the *PRSS1*, *SPINK1* and *CTRC* genes, we focused our investigation on the protein-coding exons and their flanking intronic sequences in *TPST2*.

We sequenced these regions in 151 subjects with chronic pancreatitis and 169 healthy controls, detecting 15 nucleotide variants in the *TPST2* gene including 10 commonly observed polymorphisms (tables 2, 3) and 5 rare mutations (table 4). None of the 10 frequent variants showed a statistically significant distribution difference between patients and controls when allele-frequencies or genotype-frequencies (dominant and recessive model) were compared (tables 2, 3). To determine whether a distinct haplotype might be associated with chronic pancreatitis, haplotypes were reconstructed considering variants with a minor allele frequency of ≥5% using PHASE v2.1. With this method, 22 haplotypes were defined, of which 8 had a frequency of ≥5%. However, the frequencies of the haplotypes were similar in patients and controls (table 5).

The only missense variant found in this study was c.458G>A (p.R153H), which was present in 1 of 151 (0.7%) patients and in 1 of 169 (0.6%) controls in a heterozygous form. The carrier patient was also heterozygous for the p.N34S *SPINK1* variant and had a family history of chronic pancreatitis with an affected father and grandfather.

Table 2. Allelic frequencies of common *TPST2* variants in subjects with chronic pancreatitis and in healthy controls

Variant	Allele	Patients	Controls
c.270C>G (p.=)	C	268/302 (88.7)	306/338 (90.5)
Ala90 rs4822735	G	34/302 (11.3)	32/338 (9.5)
c.276C>T (p.=)	C	273/302 (90.4)	317/338 (93.8)
Pro92 rs17851532	T	29/302 (9.6)	21/338 (6.2)
c.399G>C (p.=)	G	296/302 (98)	333/338 (98.5)
Leu133	C	6/302 (2)	5/338 (1.5)
c.510G>T (p.=)	G	277/302 (91.7)	298/338 (88.2)
Ser170 rs5761587	T	25/302 (8.3)	40/338 (11.8)
c.700C>T (p.=)	C	235/302 (77.8)	268/338 (79.3)
Leu234 rs12169509	T	67/302 (22.2)	70/338 (20.7)
c.831C>T (p.=)	C	272/302 (90.1)	315/338 (93.2)
Val277 rs58274935	T	30/302 (9.9)	23/338 (6.8)
c.1041+104C>T	C	155/302 (51.3)	151/338 (44.7)
rs3752523	T	147/302 (48.7)	187/338 (55.3)
c.1093-108G>A	G	212/302 (70.2)	238/338 (70.4)
rs2283823	A	90/302 (29.8)	100/338 (29.6)
c.1093-84G>A	G	212/302 (70.2)	238/338 (70.4)
rs2283822	A	90/302 (29.8)	100/338 (29.6)
c.*31G>A	G	212/302 (70.2)	238/338 (70.4)
rs2283821	A	90/302 (29.8)	100/338 (29.6)

For the synonymous variants (p.=), the affected codons are indicated. The dbSNP identifiers are also listed. Figures in parentheses are percentages.

The p.R153H *TPST2* variant, however, did not segregate with the disease, as the patient inherited this variant maternally from her unaffected mother and grandmother (fig. 2). Functional assays using HEK 293T cells cotransfected with human anionic or cationic trypsinogen and TPST2 demonstrated that the p.R153H variant catalyzed trypsinogen sulfation as well as wild-type TPST2, indicating that neither TPST2 expression nor activity is affected by the mutation (fig. 3). We conclude that p.R153H is a functionally innocuous *TPST2* variant not associated with chronic pancreatitis.

We also identified a c.-9C>T variant in the 5′ untranslated region of TPST2 in 1 of 151 patients (0.7%). Because of its proximity to the initiator ATG codon, this variant has the potential to alter the translation efficiency of TPST2. We found, however, no difference in the expression of the c.-9C>T variant and wild-type TPST2 in transfected HEK 293T, as judged by the TPST2-mediated

Table 3. Genotype frequencies of common *TPST2* variants in subjects with chronic pancreatitis and in healthy controls

Variant	Genotype	Patients	Controls
c.270C>G	CC	119/151 (78.8)	140/169 (82.8)
	CG	30/151 (19.9)	26/169 (15.4)
	GG	2/151 (1.3)	3/169 (1.8)
c.276C>T	CC	123/151 (81.5)	148/169 (87.6)
	CT	27/151 (17.9)	21/169 (12.4)
	TT	1/151 (0.7)	0/169
c.399G>C	GG	145/151 (96)	164/169 (97)
	GC	6/151 (4)	5/169 (3)
	CC	0/151	0/169
c.510G>T	GG	127/151 (84.1)	135/169 (79.9)
	GT	23/151 (15.2)	28/169 (16.6)
	TT	1/151 (0.7)	6/169 (3.6)
c.700C>T	CC	93/151 (61.6)	107/169 (63.3)
	CT	49/151 (32.5)	54/169 (32)
	TT	9/151 (6)	8/169 (4.7)
c.831C>T	CC	121/151 (80.1)	148/169 (87.6)
	CT	30/151 (19.9)	19/169 (11.2)
	TT	0/151	2/169 (1.2)
c.1041+104C>T	CC	39/151 (25.8)	34/169 (20.1)
	CT	77/151 (51)	83/169 (49.1)
	TT	35/151 (23.2)	52/169 (30.8)
c.1093–108G>A	GG	75/151 (49.7)	87/169 (51.5)
	GA	62/151 (41.1)	64/169 (37.9)
	AA	14/151 (9.3)	18/169 (10.7)
c.1093–84G>A	GG	75/151 (49.7)	87/169 (51.5)
	GA	62/151 (41.1)	64/169 (37.9)
	AA	14/151 (9.3)	18/169 (10.7)
c.*31G>A	GG	75/151 (49.7)	87/169 (51.5)
	GA	62/151 (41.1)	64/169 (37.9)
	AA	14/151 (9.3)	18/169 (10.7)

Figures in parentheses are percentages.

Table 4. Rare *TPST2* variants in subjects with chronic pancreatitis and in healthy controls

Variant	Patients	Controls
c.-9C>T	1/151 (0.7)	0/169 (0)
c.177C>T (p.=) His59	1/151 (0.7)	1/169 (0.6)
c.458G>A (p.R153H)	1/151 (0.7)	1/169 (0.6)
c.822C>T (p.=) Pro274	0/151 (0)	1/169 (0.6)
c.900G>A (p.=) Lys300	1/151 (0.7)	0/169 (0)

For the synonymous variants (p.=), the affected codons are indicated. Figures in parentheses are percentages.

Table 5. Common *TPST2* haplotypes in subjects with chronic pancreatitis and in healthy controls

	Haplotypes	Patients	Controls
1	C-C-G-C-C-C-G-G-G	60/302 (19.9)	60/338 (17.8)
2	G-C-G-C-C-C-G-G-G	28/302 (9.3)	25/338 (7.4)
3	C-C-G-C-C-T-G-G-G	52/302 (17.2)	74/338 (21.9)
4	C-C-G-T-C-T-G-G-G	35/302 (11.6)	46/338 (13.6)
5	C-T-G-T-C-T-G-G-G	23/302 (7.6)	18/338 (5.3)
6	C-C-G-C-C-C-A-A-A	29/302 (9.6)	34/338 (10.1)
7	C-C-G-C-T-C-A-A-A	22/302 (7.3)	18/338 (5.3)
8	C-C-T-C-C-T-A-A-A	23/302 (7.6)	34/338 (10.1)

Haplotypes were reconstructed using PHASE v2.1 considering *TPST2* variants with a minor allele frequency of ≥5%. The variable nucleotides defining the haplotypes are listed according to their order within the haplotypes: c.270C/G, c.276C/T, c.510G/T, c.700C/T, c.831C/T, c.1041+104C/T, c.1093-108G/A, c.1093-84G/A and c.*31G/A. In total 22 haplotypes were defined. The 8 haplotypes listed here occurred with a frequency of ≥5%. Figures in parentheses are percentages.

sulfation of anionic trypsinogen (fig. 4). The results demonstrate that the c.-9C>T variant has no effect on the translation of the TPST2 protein and, therefore, should be classified as a functionally harmless variant.

Discussion

In this study, we tested the hypothesis that changes in tyrosine sulfation of human trypsinogens might alter the risk for chronic pancreatitis. This notion was based on the observation that human trypsinogens undergo post-translational sulfation and this modification leads to increased autoactivation of cationic trypsinogen [14, 20]. Genetic variants of cationic trypsinogen cause hereditary pancreatitis by stimulating trypsinogen autoactivation; therefore, it seemed reasonable to assume that sulfation-stimulated autoactivation might represent a similar risk [23 and references therein]. Conversely, decreased sulfation might be protective against chronic pancreatitis. To determine whether genetic variants of *TPST2* alter the risk of chronic pancreatitis, we sequenced the protein-

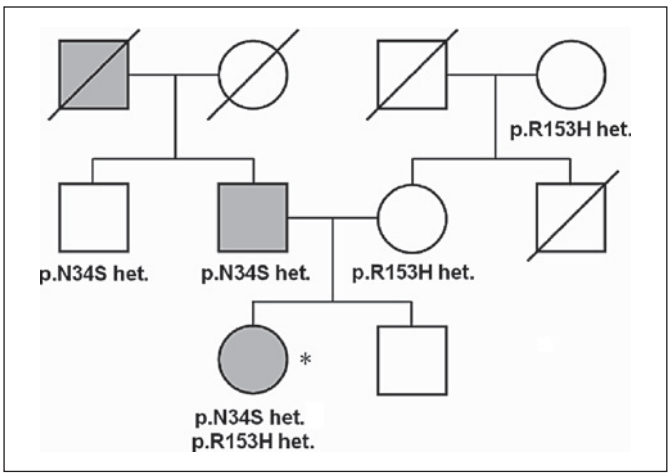


Fig. 2. A hereditary pancreatitis family carrying the *TPST2* p.R153H and the *SPINK1* p.N34S variants. Individuals suffering from chronic pancreatitis are highlighted in gray. Family members tested negative for *PRSS1* (exons 2 and 3) and *CTRC* (exons 2, 3 and 7) variants. The asterisk indicates the index patient. het. = Heterozygous carrier.

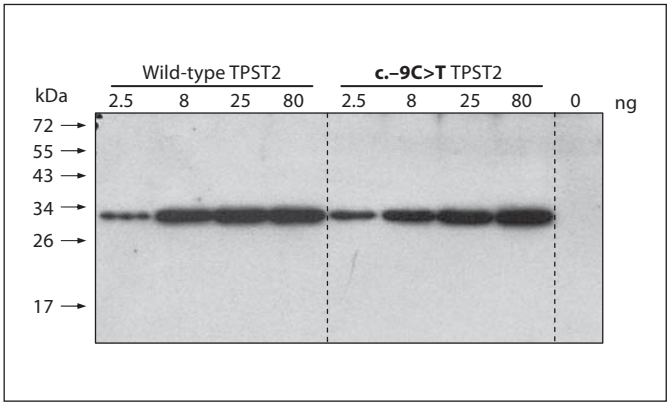
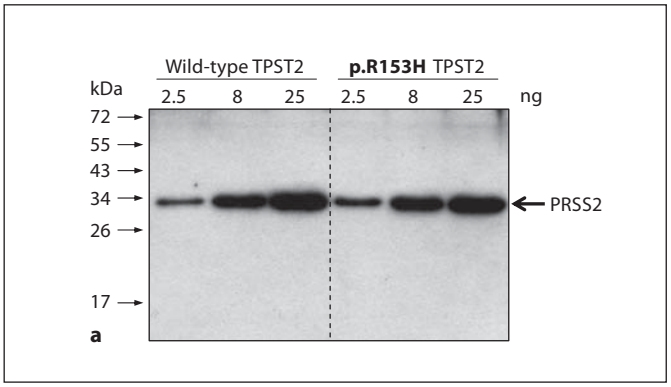


Fig. 4. Expression of TPST2 variant c.-9C>T as assessed by sulfation of human anionic trypsinogen. HEK 293T cells were cotransfected with 2 μg pcDNA3.1(–)_PRSS2 plasmid and increasing amounts (0–80 ng) of wild-type pcDNA3.1(–)_TPST2 plasmid or variant c.-9C>T. Conditioned media were collected after 48 h and sulfated trypsinogen levels were determined by Western blotting as described in figure 3.



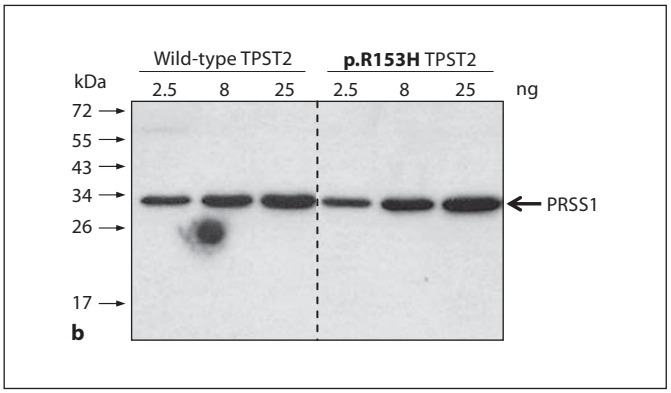


Fig. 3. Sulfation of human anionic trypsinogen (PRSS2) and human cationic trypsinogen (PRSS1) by TPST2 variant p.R153H. HEK 293T cells were cotransfected with 2 μg pcDNA3.1(–)_PRSS2 (**a**) or 2 μg pcDNA3.1(–)_PRSS1 (**b**) plasmid and increasing amounts (0–25 ng) of wild-type pcDNA3.1(–)_TPST2 plasmid or variant p.R153H. Note that for these experiments, the p.K237D/p.N241D PRSS1 variant was used, which is expressed almost 3-fold better than the wild type [see 20]. Conditioned media were collected after 48 h and sulfated trypsinogen levels were determined by Western blotting. Briefly, 50 μl (PRSS2) or 150 μl (PRSS1) aliquots of media were precipitated with 10% trichloroacetic acid, resuspended in reducing Laemmli sample buffer, heat denatured and electrophoresed on 15% SDS-polyacrylamide gels. The proteins were transferred onto an Immobilon-P membrane and the membrane was incubated with antityrosine-sulfate IgG at a dilution of 1:2,000. Horseradish peroxidase conjugated rabbit polyclonal antibody against human IgG was used as secondary antibody at a dilution of 1:10,000. Horseradish peroxidase was detected using the SuperSignal West Pico Chemiluminescent Substrate (Thermo Scientific, Waltham, Mass., USA).

coding exons and their adjacent intronic sequences of *TPST2* in subjects with chronic pancreatitis and healthy control subjects. We identified several variants, but no association with chronic pancreatitis was evident regardless of whether individual variants or haplotypes were considered. Previously, Iida et al. [29] sequenced the *TPST2* gene in 48 healthy Japanese volunteers and described 22 intronic variants and a variant in the 3′ untranslated region. Allele or genotype frequencies were not reported, and there were only 2 variants (c.1041 + 104C>T and c.*31G>A) described in this dataset which were also identified in Germans in our present study, suggesting ethnic differences in *TPST2* variations.

We found a single nonsynonymous variant in the coding region of TPST2, indicating that missense variants in this gene are rare. The p.R153H variant was detected in a healthy control subject and in a family affected with hereditary pancreatitis. However, the variant did not segregate with the disease and functional assays indicated no changes in TPST2 activity. Taken together, the genetic and biochemical data indicate that the p.R153H variant is functionally neutral and does not alter the risk for chronic pancreatitis. The paucity of missense variants in the *TPST2* gene is in line with the high degree of sequence conservation among mammalian TPST2 enzymes. For example, at the amino acid level, the human and mouse enzymes are 94% identical and the human and bovine enzymes are 93% identical. The evolutionary conservation suggests an indispensable physiological function for TPST2. Indeed, selective disruption of the *TPST2* gene in mice resulted in infertility, hypothyroidism and delayed growth [30, 31]. Hypothyroidism was also observed in a dwarf mouse strain which carried the natural p.H266Q mutation in the *TPST2* gene [32].

In a subject with chronic pancreatitis, we identified a c.-9C>T variant that seemed to have the potential to alter the translation efficiency of TPST2 due to its proximity to the initiator ATG codon. Functional analysis, however, demonstrated that expression of wild-type TPST2 and the c.-9C>T variant were comparable, indicating that translation of the TPST2 protein was not affected. Therefore, the c.-9C>T variant is functionally harmless.

In conclusion, the present study found no association between genetic variants of *TPST2* and chronic pancreatitis. The genetic and biochemical data presented here can facilitate further analysis of the *TPST2* gene in a variety of human diseases.

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