PANCREATITIS

Association of cathepsin B gene polymorphisms with tropical calcific pancreatitis

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Background and aims: Tropical calcific pancreatitis (TCP) is a type of chronic pancreatitis unique to countries in the tropics. Mutations in pancreatic secretory trypsin inhibitor (SPINK1) rather than cationic trypsinogen (PRSS1) explain the disease in only 50% of TCP patients. As cathepsin B (CTSB) is known to activate cationic trypsinogen, we attempted to understand the role of CTSB mutations in TCP. Evidence of epistatic interaction was investigated with the previously associated N34S SPINK1 allele, a variant considered to be a modifier rather than a true susceptibility allele.

Subjects and methods: We sequenced the coding region of CTSB gene in 51 TCP patients and 25 controls and further genotyped 89 patients and 130 controls from the same cohort for Leu26Val, C595T, T663C, and Ser53Gly polymorphisms. The positive findings observed in the earlier cohort were re-examined in an ethnically matched replication cohort comprising 166 patients and 175 controls. Appropriate statistical analyses were performed and Bonferroni correction for multiple testing was applied.

Results: We found a statistically significant association of the Val26 allele at Leu26Val polymorphism with an odds ratio (OR) of 2.15 (95% confidence interval (CI) 1.60-2.90 ($p=0.009$)), after Bonferroni correction (corrected p value = 0.025). This significant association of Leu26Val with TCP was replicated in another cohort (OR 2.10 (95% CI 1.56-2.84); $p=0.013$). Val26 allele also showed significantly higher frequency in N34S positive and N34S negative patients than in controls ($p=0.019$ and 0.013, respectively). We also found significant differences in the mutant allele frequencies at Ser53Gly and C595T single nucleotide polymorphisms between N34S positive patients and controls ($p=0.008$ and 0.001, respectively). Although haplotype analysis did not complement the results of allelic association, it did uncover a unique haplotype protective for TCP ($p=0.0035$).

Conclusion: Our study suggests for the first time that CTSB polymorphisms are associated with TCP. As PRSS1 mutations are absent in TCP and the N34S SPINK1 mutation is proposed to play a modifier role, these variants may be critical as a trigger for cationic trypsinogen activation.

Pancreatitis is generally believed to be a disease where the pancreas is injured by enzymes that are normally secreted by acinar cells. Recently, mutations in the genes encoding cationic trypsinogen (PRSS1) and the serine protease inhibitor, Kazal type 1 (SPINK1), were identified as associated with chronic pancreatitis.^{1,2} Tropical calcific pancreatitis (TCP) is a type of chronic pancreatitis unique to developing countries in tropical regions.³ Our previous study showed for the first time that mutations in PRSS1 do not have a role but we did find mutations in the SPINK1 gene in the majority of TCP patients.⁴ Mutated SPINK1 is thought to act by reducing the capacity to block prematurely activated trypsinogen. However, the role of mutated SPINK1 in the presence of a normal autolysis site in cationic trypsinogen is difficult to explain. Intrapaneatic activation of cationic trypsinogen requires a trigger that would cause premature activation of trypsinogen, leading to injury of acinar cells and consequently recurrent attacks of pancreatitis. These triggers could be genetic or environmental factors. Intrapaneatic activation of trypsinogen and presumably other zymogens occurs within acinar cells and is known to be mediated by lysosomal hydrolase cathepsin B (CTSB).^{5,6} CTSB has been shown to activate trypsinogen *in vitro*^{7,8} and *in vivo*^{9,10} in a mouse model of pancreatitis. It is also reported to be redistributed to the zymogen granule enriched subcellular compartment during the initial phase of pancreatitis in animal models.^{11,12} These observations suggest central role for CTSB in the initiation of pancreatitis. However, colocalisation

of lysosomal and digestive proteases may be a physiological event in humans and may not be inherently associated with premature zymogen activation or pancreatitis.¹³ Factors such as pH, stress, and changes in intracellular calcium levels have been shown to affect CTSB induced trypsinogen activation but may not be sufficient as the final explanation.^{14,15} Increased CTSB activity has been detected and proposed to be responsible for invasive properties in head and neck cancers,^{16,17} lung cancers,^{18,19} and in breast carcinoma.²⁰⁻²² Although few reports exist, no study has systematically attempted to associate its activity with polymorphisms in the gene.²³⁻²⁵

The human CTSB gene is 25.6 kb long and has 12 exons, of which three are present in the 5' untranslated region (UTR) that could be alternatively spliced to produce several transcript species.²⁶ We hypothesised that mutations in the CTSB gene may be involved in inappropriate localisation or premature activation of trypsinogen and lead to chronic pancreatitis. The present study screened the CTSB gene and analysed its association in a well characterised cohort of TCP patients and randomly selected controls from Southern India. As N34S SPINK1 mutations account for the majority of these

Abbreviations: CTSB, cathepsin B; Gly, glycine; Leu, leucine; p_{corr} , corrected p value; p_{obs} , observed p value; PRSS1, protease, serine, 1 (trypsin 1); PRSS3, protease, serine, 3 (trypsin 3); Ser, serine; SNP(s), single nucleotide polymorphism(s); SPINK1, serine protease inhibitor, Kazal type I; TCP, tropical calcific pancreatitis; Thr, threonine; UTR, untranslated region; Val, valine; PCR, polymerase chain reaction

patients but its mechanism of action is still not understood, attempts were also made to look for an association, if any, between N34S mutation in SPINK1 gene and CTSB gene polymorphisms. We also performed haplotype analysis to see if a particular CTSB haplotype is associated with the disease. Observations from the first cohort were further verified in another well powered ethnically matched independent case control cohort from the southern region of India. To our knowledge, this is the first extensive study to investigate the role of CTSB variants in any type of pancreatitis.

SUBJECTS AND METHODS

Patients and controls

A total of 140 unrelated patients (97 males and 43 females) were diagnosed with TCP at the Asian Institute of Gastroenterology, Hyderabad, based on the WHO criteria of: (1) recurrent painful abdomen since childhood; (2) large intraductal calculi; (3) evidence of pancreatic calcification on ultrasonography and endoscopic retrograde cholangiopancreatography; (4) absence of any other aetiological factor, such as alcoholism, etc; and (5) diabetes mellitus as defined by the WHO study group E (may or may not be present).⁴ One hundred and fifty five age and sex matched individuals (103 males and 52 females) with an ethnic and geographical background identical to that of the TCP patients were included as controls (cohort I). The control group had no complaints or evidence of pancreatitis on imaging. Both patients and controls completed a structured questionnaire detailing their medical history and underwent similar investigations, including imaging. An additional cohort (cohort II) comprising 166 patients (112 males and 54 females) and 175 controls (124 males and 51 females) of the same ethnic origin (Dravidian) from the Department of Gastroenterology, Medical College, Calicut, in the southern region, was recruited following similar inclusion and exclusion criteria as used for cohort I. Written informed consent was obtained from all patients and controls. The Institutional Ethics Committee of all institutes approved the study following the Indian Council of Medical Research guidelines for research on human subjects.

Genetic analysis

Genomic DNA from a total of 306 TCP patients (209 males and 97 females) and 330 healthy volunteers (227 males and 103 females) were used for this study. As no study has investigated the association of CTSB variations with

pancreatitis, we sequenced the coding region, flanking intron-exon boundary, and the 5'- and 3'-UTR regions of the CTSB gene in 51 patients and 25 controls from cohort I. We designed primers to cover all 12 exons, including the splice site junctions (table 1). Polymerase chain reaction (PCR) products were purified and sequenced individually on both the strands using the Big-dye terminator cycle sequencing ready kit (Applied Biosystems, Foster City, California, USA) on an ABI3730 Genetic Analyzer (Applied Biosystems). The zygosity of the in/del5581-5582 polymorphism was confirmed by SmaI-restriction fragment length polymorphism analysis.²⁵ The remaining patients and controls from cohort I were genotyped for four polymorphisms, namely Leu26Val, C595T, T663C, and Ser53Gly, by sequencing the PCR product spanning exons 3, exon 4, and intron 3 using primers shown in table 1. Subsequently, 166 patients and 175 controls from the replication cohort were also analysed for the same four polymorphisms by sequencing. In the case of unclear sequence data, we repeated direct sequencing under various conditions until the genotype was determined correctly.

Statistical analysis

Allele and genotype frequencies were calculated for each polymorphism (table 2) in both cohorts separately, as well as together (table 3). To analyse deviation from the Hardy-Weinberg equilibrium, observed and expected genotype frequencies were compared using the Markov simulation based goodness of fit test. Pearson's χ^2 test was used to analyse the statistical significance of the difference in allelic distribution of various polymorphisms in patients and controls. Haplotypes were generated using 14 polymorphisms having a minimum allele frequency of 5% with the Expectation-Maximisation algorithm using Arlequin software (version 2.0) and compared between patients and controls and between patients with and without the N34S SPINK1 mutation using a score test with simulated p values from 10^4 replications.²⁷ We applied the Bonferroni correction for multiple testing based on the number of individual sequencing experiments and haplotypes, as applicable. As both cohorts were analysed for four polymorphisms by sequencing specific PCR product using a forward and a reverse primer, a factor of 2 ($n=2$) was used to correct for multiple testing (table 3). Unless indicated specifically, a p value of 0.05 was considered significant in all analyses. This study with random selection of patients and controls was

Table 1 Primer sequences and polymerase chain reaction conditions for the cathepsin B gene

Region†	Name	Sequence (5'-3')	T _{ann} (°C)
Exon 1‡	1F	gcg gcg gaa ggt ggc ggg ag	62*
	1R	cgc gga ctc cca ccc cag cc	
Exon 2‡	2F	gcc tta tgc tcc gcc tct gt	66
	2R	gtg gga gga ggc aag ggg at	
Exon 3 and 4	3F	aga cgg tgc ccc tgt gtg tg	61
	4R	ggc ctt cac tct ccc act tc	
Exon 5	5F	tgt gct cat ttc ctt gtt ag	51
	5R	aaa aga aga ttg cta aga tta t	
Exon 6	6F	gcg tcc cct ggt gtt gag ag	63
	6R	gcc cca ccc cta ccc aca aa	
Exon 7, 8 and 9	7F	tgg tct gga gag ctg gtg gt	64
	8F	tgc ccc agg tct tct ccg tg	
	8R	gcc ctg acc tct tgc ctg ca	
	9R	ccc tct tcc cca gcc cct ca	
Exon 10 and 11	10F	ggg gtg ctg tgg ggc gtg g	61
	11R	ggg gaa gga cgc tct gtg ct	

F, forward; R, reverse; T_{ann}, annealing temperature; *with DMSO.

†As per NCBI sequence Accession No NT_077531 and transcript id = "NM_147782.1".

‡Region from exon 1 to 2 forms part of the 5' untranslated region.

Table 2 Distribution of cathepsin B gene polymorphisms in tropical calcific pancreatitis patients and controls

Region	Polymorphism‡	rs number	AA change‡	Mutant allele frequency¶	
				Patients (n = 51)	Controls (n = 25)
Exon 1*	C14609A†		—	0.02	0.00
Intron 1*	G14520C†		—	0.02	0.00
	G14453A†		—	0.02	0.00
	C14425A	rs1293311	—	0.39	0.42
	T11083C	rs2645415	—	0.08	0.15
Exon 2*	C10927G†		—	0.02	0.00
Exon 3	C76G	rs4292649	Leu26Val	0.46	0.30
Intron 3	A335T	rs1293293	—	0.13	0.20
	G394A†		—	0.02	0.00
	C595T	rs1293292	—	0.12	0.20
	T663C	rs1293291	—	0.51	0.60
Exon 4	A790G†		Ser53Gly	0.09	0.04
Intron 5	C2609T	rs2272766	—	0.41	0.30
Exon 6	A4383C	rs13332	Thr140Thr	0.55	0.60
Intron 6	G4451C†		—	0.09	0.04
	A4735G	rs1736090	—	0.41	0.38
Intron 7	C5516T	rs1692819	—	0.10	0.16
	C5522A	rs2294139	—	0.38	0.30
	5581–5582 (in/del)	rs3215434	—	0.49	0.52
	C5622G†		—	0.40	0.48
Intron 8	G5825A	rs2294138	—	0.40	0.33
Exon 11	C8370G	rs709821	—	0.09	0.15
	A8422G	rs8898	—	0.42	0.37

AA, amino acid; n, number of individuals.

*Region from exon 1 to 2 forms part of the 5' untranslated region.

†Novel polymorphisms.

‡Nomenclature is represented as wild status-position-mutant status.

¶Denotes the mutant allele as per NCBI sequence Accession No NT_077531 and transcript id = "NM_147782.1".

82% powered to detect a relative risk of 1.58 (<http://calculators.stat.ucla.edu/powercalc/>).

RESULTS

Genetic analysis

Analysis in the initial cohort (cohort I)

Sequencing of the CTSB gene in 51 patients and 25 healthy individuals from cohort I identified 23 polymorphisms, of which three were in the coding region and included two non-synonymous mutations (table 2). None of the polymorphisms showed any significant deviation from Hardy-Weinberg equilibrium ($p > 0.05$). Of eight novel SNPs identified in the study, only one, Ser53Gly, was non-synonymous in nature but had comparable frequency in patients and controls. For another reported non-synonymous SNP Leu26Val, the mutant allele Val26 was significantly more prevalent in patients compared with controls (odds ratio (OR) 1.99 (95% confidence interval (CI) 1.48–2.67); $p = 0.029$) but did not reach statistical significance on Bonferroni correction for multiple testing ($p_{\text{corr}} = 0.0035$). A previously reported

intronic polymorphism in/del5581–5582 was also identified but was not statistically significant.²⁵ On analysis of four SNPs—namely, Leu26Val, C595T, T663C, and Ser53Gly—in all the patients and controls in cohort I, the Val26 allele at Leu26Val polymorphism only showed a statistically significant association with an OR of 2.15 (95% CI 1.60–2.90; $p = 0.009$) even after Bonferroni correction (corrected p value (p_{corr}) = 0.025) (table 3). Both heterozygotes and mutant homozygotes were detected in patients as well as controls but no significant differences were noted in the phenotype. Two other polymorphisms, C595T and T663C, did not show any significant difference in allele and genotype frequency between patients and controls.

Analysis in the replication cohort (cohort II)

To confirm the findings of the significant association of CTSB gene polymorphisms with TCP, we investigated four polymorphisms, Leu26Val, C595T, T663C, and Ser53Gly, in the replication cohort comprising ethnically matched patients and controls from the southern region. Interestingly, the

Table 3 Association of cathepsin B gene polymorphisms in tropical calcific pancreatitis patients and controls

SNP†	Cohort I						Cohort II						Total					
	Allele frequency‡		χ^2	OR	95% CI	p Value	Allele frequency‡		χ^2	OR	95% CI	p Value	Allele frequency‡		χ^2	OR	95% CI	p Value
	Patients (n = 140)	Controls (n = 155)					Patients (n = 166)	Controls (n = 175)					Patients (n = 306)	Controls (n = 330)				
Leu26Val*	0.48	0.30	6.81	2.15	1.60–2.90	0.009	0.45	0.28	6.23	2.10	1.56–2.84	0.013	0.46	0.29	6.17	2.09	1.55–2.81	0.013
C595T	0.12	0.20	2.38	0.55	0.37–0.81	0.123	0.10	0.21	4.62	0.41	0.28–0.63	0.032	0.11	0.21	3.72	0.46	0.31–0.70	0.054
T663C	0.52	0.58	0.73	0.78	0.59–1.04	0.394	0.53	0.60	1.00	0.75	0.56–1.00	0.318	0.53	0.59	0.73	0.78	0.59–1.04	0.393
Ser53Gly	0.10	0.04	2.76	2.67	1.45–4.91	0.096	0.09	0.04	2.06	2.37	1.28–4.40	0.152	0.09	0.04	2.06	2.37	1.28–4.40	0.152

n, number of individuals; χ^2 , chi square value; OR, odds ratio; 95% CI, 95% confidence interval; SNP, single nucleotide polymorphism.

†Nomenclature is represented as wild status-position-mutant status.

‡, mutant allele frequency.

The frequency of mutant alleles for each SNP was compared between patients and controls and Pearson's χ^2 and p values were calculated together with OR and 95% CI.*Statistically significant after allowing for multiple post hoc testing ($p_{\text{corr}} = 0.025$, with Bonferroni correction).

Table 4 Distribution of cathepsin B gene polymorphisms in tropical calcific pancreatitis patients based on N34S SPINK1 status

SNP†	Mutant allele frequency			N34S Pos v N34S Neg				N34S Pos v controls				N34S Neg v controls			
	N34S Pos (n = 134)	N34S Neg (n = 172)	Controls (n = 330)	χ^2	OR	95% CI	p Value	χ^2	OR	95% CI	p Value	χ^2	OR	95% CI	p Value
Leu26Val	0.45	0.46	0.29	0.02	0.96	0.72–1.28	0.887	5.49	2.00	1.49–2.70	0.019*	6.17	2.09	1.55–2.81	0.013*
C595T	0.05	0.16	0.21	6.44	0.28	0.16–0.47	0.011*	11.30	0.20	0.12–0.33	0.001*	0.83	0.72	0.50–1.03	0.363
T663C	0.51	0.55	0.59	0.32	0.85	0.64–1.13	0.571	1.29	0.72	0.54–0.96	0.256	0.33	0.85	0.64–1.13	0.568
Ser53Gly	0.15	0.05	0.04	5.56	3.35	1.96–5.74	0.018*	7.04	4.23	2.37–7.58	0.008*	0.12	1.26	0.64–2.51	0.733

N34S Pos, N34S SPINK1 positive; N34S Neg, N34S SPINK1 negative; n, number of individuals; χ^2 , chi square value; OR, odds ratio; 95% CI, 95% confidence interval; SNP, single nucleotide polymorphism.

†Nomenclature is represented as wild status-position-mutant status.

The frequency of mutant allele for each SNP was compared between the three groups and Pearson's χ^2 and p values were calculated together with OR and 95% CI.

*Statistically significant after allowing for multiple post hoc testing ($p_{\text{corr}} = 0.025$, with Bonferroni correction).

analysis showed comparable results and statistical significance for Val26 allele at Leu26Val polymorphism (OR 2.10 (95% CI 1.56–2.84); $p = 0.013$) (table 3). Although the mutant allele at the C595T variant had higher prevalence in the controls than in patients, this finding did not reach statistical significance (observed p value ($p_{\text{obs}} = 0.032$ v $p_{\text{corr}} = 0.025$) while the T663C polymorphism had comparable allele frequency in patients and controls. Observations for these polymorphisms were similar on cumulative analysis of the total cohort of 306 patients and 330 controls, with only the Val26 allele at Leu26Val SNP showing an association with an OR of 2.09 (95% CI 1.55–2.81; $p = 0.013$). The three other polymorphisms had a similar allele frequency and were not statistically significant.

Association of CTSB variants with N34S SPINK1 mutation

Association analysis after dichotomisation of the total cohort based on their N34S SPINK1 mutation status showed interesting results (table 4). The frequency of mutant allele at Leu26Val was similar in N34S negative and positive patients but higher in the N34S SPINK1 positive group with an OR of 2.00 (95% CI 1.49–2.70; $p = 0.019$) and in N34S negative group with an OR of 2.09 (95% CI 1.55–2.81; $p = 0.013$) in comparison with controls. However, no difference in phenotype was observed on comparison of N34S positive and N34S negative patients carrying the Val26 allele (data not presented). This suggests that Val26 at this SNP may act as a susceptibility allele in the pathogenesis of TCP, irrespective of the SPINK1 mutation status. In contrast, Gly53 allele frequency at the Ser53Gly polymorphism was comparable between patients without N34S SPINK1 mutation and controls but much lower than in N34S positive patients. The difference was marginally statistically significant between N34S positive and N34S negative patients (OR 3.35 (95% CI 1.96–5.74); $p = 0.018$) and between N34S positive patients and controls (OR 4.23 (95% CI 2.37–7.58); $p = 0.008$) even after Bonferroni correction ($p_{\text{corr}} = 0.025$). However, Bonferroni correction, while reducing the type I error, also suffers from the drawback of increasing the chances of making a type II error.

Interestingly, at the C595T polymorphism, the prevalence of the T allele was lower in patients carrying the N34S mutation than in controls (OR 0.20 (95% CI 0.12–0.33); $p = 0.001$) whereas it was higher in N34S negative patients compared with those carrying the N34S mutation (OR 0.28 (95% CI 0.16–0.47); $p = 0.011$). It is difficult to surmise any specific role but these observations suggest a protective role for this SNP. T663C variation had comparable frequencies in all groups and was not statistically significant.

Haplotype analysis

Although 28 and 18 haplotypes were identified using 23 polymorphisms in 51 patients and 25 controls, respectively,

only six haplotypes (frequency >2%) were common but none showed a protective or susceptibility role in TCP (data not shown). Certain unique haplotypes were observed in patients but were too infrequent for any further analysis. One haplotype unique to controls (frequency >10%) had the mutant alleles at T663C, Thr140Thr, A4735G, and 5581–5582 in/del positions. However, this finding did not quite reach statistical significance after allowing for multiple post hoc testing ($p_{\text{obs}} = 0.0035$ v $p_{\text{corr}} = 0.0027$, with Bonferroni correction; number of haplotypes, $n = 18$). It was interesting to note that although the controls showed a higher prevalence of mutant alleles at these polymorphisms, no individual association was observed. Thus this haplotype may have a protective role to play in TCP. Analysis of the cohort on the basis of N34S SPINK1 status failed to show any significant difference in haplotypic frequencies.

DISCUSSION

Premature intracellular activation of trypsinogen and other digestive proteases is an early event in the course of pancreatitis and is causally related to the onset of disease. It is already established that several trypsinogen mutations lead to significant change in its activation. Trypsin mediated activation (autoactivation) and cathepsin B induced activation are two enzymatic processes which could confer such a change in intracellular activation of trypsinogen.²⁸ As no trypsinogen mutations have been identified in TCP patients, a pathogenic role for autoactivation is ruled out in them. Additionally, SPINK1 mutations are associated in only ~50% of TCP patients and it is not clear how mutated SPINK1 can cause the disease in the presence of a normal trypsinogen with an intact autolysis site.^{4–29} Hence we hypothesised that CTSB induced trypsinogen activation may play an important role in the pathogenesis of TCP and could be caused by mutations in the CTSB gene. As SPINK1 is proposed to play a modifier role, patients with mutated SPINK1 may still need a trigger for cationic trypsinogen activation and hence CTSB mutations may explain the disease, irrespective of the presence or absence of SPINK1 mutations.

On sequencing of the CTSB gene, only the Leu26Val polymorphism was found to be significantly associated with the disease. A similar strength of association with the Val26 allele in another cohort further indicates its significance in TCP patients. Although two other SNPs, Ser53Gly and C595T, showed differential allele frequencies between patients and controls, they failed to reach statistical significance. However, analysis of TCP patients with and without the N34S SPINK1 mutation produced interesting observations. No CTSB variants were identified exclusively in patients without the N34S SPINK1 mutation. Comparable prevalence of the Val26 allele at the Leu26Val polymorphism in patients with and without the N34S SPINK1 mutation and at a statistically higher frequency than controls suggests that this SNP may play an

independent role as a susceptibility allele in the pathogenesis of TCP, irrespective of SPINK1 mutation status. The novel variant Ser53Gly was more prevalent in patients with mutated SPINK1 compared with controls and patients without N34S SPINK1 mutation. As specific propeptide sequences are known to be essential for the transport of vacuolar proteases, the Leu26Val polymorphism, present in the propeptide region, may affect trafficking of cathepsin B protein.³⁰

A similar mechanism may be operative at the Ser53Gly polymorphism as the hydrophobic amino acid glycine replaces the polar amino acid serine in the propeptide region of CTSB. A lower prevalence of C595T in patients with mutated SPINK1 suggests a protective role for this polymorphism in TCP. C595T being an intronic variation may not play a direct role but could be in linkage disequilibrium with a polymorphism in some other gene in a nearby region. Although allele frequencies for the mutant allele at the 5581–5582 in/del polymorphism were similar in controls and patients, a unique haplotype carrying the mutant allele at this polymorphism was identified exclusively in controls, suggesting a protective role in TCP. This 19 bp insertion/deletion occurs upstream of the putative branch point sequence (5'-gggtgac-3') and thus may have an effect on U₂ small nuclear ribonucleoprotein binding and expression of CTSB.²⁵ The haplotype also contained the mutant alleles at T663C, Thr140Thr, A4735G, but interestingly lacked Leu26Val, C595T, and Ser53Gly mutations. This may suggest that the association of haplotypes might be a better way of identifying an effect of unknown variants.³¹

Cathepsin B is also known to activate mesotrypsinogen preferentially, which in turn is known to degrade SPINK1 and thus lower the physiological levels of the inhibitor.³² Hence it may be speculated that these CTSB variants induce premature activation of trypsinogens, especially cationic trypsinogen, which in the presence of trypsin inhibitor with reduced capacity either due to the N34S SPINK1 mutation or mesotrypsin induced inhibitor degradation leads to recurrent attacks of pancreatitis. It may be interesting to screen TCP patients for mutations in the PRSS3 gene (coding for mesotrypsinogen), which may stabilise mesotrypsin or loss of function mutations that protect against pancreatitis.³² However, based on their location, these changes such as Leu26Val and Ser53Gly appear more likely to affect the transport of cathepsin B and it is still a matter of controversy whether mislocalisation of CTSB in zymogen granules plays a role in activation of cationic trypsinogen. A low level of statistical significance may suggest a minor contribution of these polymorphisms in the causation of disease, which is further strengthened by analogous results in the replication cohort.

Chronic pancreatitis, including TCP, is a complex disease with a possible role for a number of genes together with an interaction with environmental factors.²⁹ As shown by us and other investigators, to date only SPINK1 mutations represent the genetic basis for TCP.^{4–33} Our study suggests the cathepsin B gene as the second candidate gene involved in the pathogenesis of TCP. It also provides evidence of genetic heterogeneity as a number of CTSB variants showed an association in these patients, many of whom also carried the N34S SPINK1 mutation. It will be interesting to investigate the mechanism by which CTSB variants cause the disease as well as their interaction with the N34S SPINK1 mutation. As PRSS1 mutations do not explain the disease in all the patients with different types of chronic pancreatitis, screening such patients for CTSB variants may also shed more light on their significance in the pathogenesis of chronic pancreatitis.

In conclusion, our study shows that polymorphisms in the cathepsin B gene are associated with TCP. Our study suggests

an important role for CTSB polymorphisms in TCP but also advocates emphasis on factors likely to change the pH or alter intracellular calcium levels^{15–34} as trypsinogens and cathepsin B are known to be physiologically present in the secretory vesicle and appear to depend on an opportunity to be in the right environment for CTSB to interact and activate the cationic trypsinogen. As hypothesised in our previous studies,^{29–35} significant association of inhibitor mutations in only ~50% of TCP patients may be suggestive of other genes and/or environments in addition to SPINK1. Our study also suggests that screening for other candidate genes, such as the cystic fibrosis transmembrane regulator, mesotrypsinogen, etc, may be able to explain the disease in the remaining patients.

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EDITOR'S QUIZ: GI SNAPSHOT

Robin Spiller, Editor

Recurrent gastrointestinal haemorrhage from the distal duodenum

Clinical presentation

In October 2004, a 69 year female patient presented with melena and anaemia (haemoglobin level 9 g/dl). The patient had been on warfarin due to construction of an aorto-bifemoral graft because of an aortic aneurysm and an femoropopliteal bypass as a result of a stenosis of the left superficial femoral artery in 1996. Anticoagulation had been monitored regularly and international normalised ratio (INR) values had always been within the desired range of 2.5–3.5. On admission, INR was 3.1. The patient denied complains such as dyspepsia, NSAR intake, irregular bowel movements, or weight loss. Standard oesophagogastroduodenoscopy and colonoscopy revealed no signs of haemorrhage. As blood values remained stable after transfusion, the patient was discharged. In July 2005, the patient presented again with recurrent melena and anaemia (haemoglobin level 8 g/dl), and cardiovascular instability. Immediate enteroscopy with a side view endoscope (Pentax ED3440) revealed the findings shown in fig 1.

Figure 1 Endoscopic finding in the third part of the duodenum far beyond the papilla in the patient with occult gastrointestinal bleeding.

Question

What is the diagnosis?

See page 1331 for answer

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