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A CGC>CAT gene conversion-like event resulting in the R122H mutation in the cationic trypsinogen gene and its implication in the genotyping of pancreatitis

EDITOR—A g.133283G>A (nomenclature in accordance with Rowen *et al*¹) single base change in exon 3 of the cationic trypsinogen gene or T4, resulting in an Arg (CGC) to His (CAC) substitution at amino acid residue 122 (R122H, originally named R117H in the chymotrypsin numbering system; nomenclature discussed in detail in Chen and Ferec²) of the cationic pretrypsinogen, was shown to be associated with hereditary pancreatitis (HP, MIM 167800) in 1996.³ To date, this mutation has been shown to be the most frequent mutation in HP world wide.^{4–9} The occurrence of g.133283G>A could be attributable to a spontaneous deamination of 5-methylcytosine to give thymine in the CpG dinucleotides on the opposite strand.

In an effort to screen for possible cationic trypsinogen gene mutations in sporadic chronic pancreatitis, which was diagnosed on clinical features and pathological sono-

graphic findings, we observed one altered migration pattern different from that of the g.133283G>A transition in exon 3 of the gene from one Belgian patient using a previously established denaturing gradient gel electrophoresis.⁸ Subsequent sequencing analysis showed a novel DNA variant named g.133283–133284GC>AT, which also resulted in an R122H mutation of the protein (fig 1). However, in contrast to the CGC>CAC codon change, CGC>CAT strongly suggests an alternative mutational mechanism, gene conversion.

Gene conversion, the non-reciprocal exchange of genetic information between homologous DNA sequences, has been extensively studied in lower eukaryotes. This mechanism has also been increasingly implicated in human diseases, in which the disease causing mutations identified in the functional genes were believed to be copied from their closely related pseudogenes.^{10–12} In this study, the GC>AT 2 bp mutation in T4 arising as a gene conversion event was supported by the presence of AT at corresponding positions of T6 and T7 among the closely related group I trypsinogen genes (fig 2).¹³ Furthermore, a *Chi*-like sequence (5'-GCTGGAGG-3', complementary strand), which has one mismatch with the consensus *Chi* sequence (5'-GCTGGTGG-3') was found to be located 27 nucleotides downstream from the mutation. *Chi*-like sequences have been reported to stimulate recombination and are frequently observed in the proximity of putatively converted alleles.^{10–14} However, like other gene conversion events, it is difficult to establish the exact boundaries of the conversion tract in our example, which is further complicated by the presence of two possible donor sequences. Nevertheless, we prefer the T7 gene to be the culprit and accordingly this "patchy" microconversion event would involve the exchange of up to 30 nucleotides between the T4 and T7 genes.

The identification of a germline R122H mutation in a sporadic case may represent a de novo mutation in one of the parental alleles, which could be investigated by analysing genomic DNA samples from the non-symptomatic parents. Unfortunately, this was not done owing to the inability to obtain such DNA samples. However, the identification of a clearly pancreatitis causing, germline R122H mutation definitely represents a high risk factor for the carrier's offspring.

This genetic finding also raises practical concerns. Since the g.133283G>A single nucleotide change creates a novel restriction enzyme recognition site for *Alf* III (A▼CRYGT), the specific amplification of exon 3 followed by *Alf* III digestion³ has been widely adopted to

Figure 1 Chromatogram of DNA sequence showing a heterozygous mutation in exon 3 of the cationic trypsinogen gene in one patient with sporadic chronic pancreatitis. Direct sequencing of the PCR product showed a GC>AT 2 bp substitution, which would result in a substitution of Arg (CGC) by His (CAT) at amino acid residue 122 of the cationic pretrypsinogen.

	R122H (CGC>CAT)										Exon 3				
T4	AAC	GCC	CGC	GTG	TCC	ACC	ATC	TCT	CTG	CCC	ACC	GCC	<u>CCT</u>	<u>CCA</u>	<u>GCC</u>
T5	--T	---	-A-	A--	---	---	---	---	--A	---	---	---	---	---	--T
T6	--T	---	AT	---	---	--G	---	---	---	---	--T	---	---	---	--T
T7	--T	---	AT	---	---	---	---	---	---	---	A--	---	---	---	--T
T8	--T	T--	---	---	---	G--	---	---	---	---	--T	---	---	---	--T
T9	--T	---	---	---	---	---	---	---	---	---	---	---	---	---	--T

Figure 2 Partial nucleotide sequence alignment between the cationic trypsinogen gene (T4) and the other group I trypsinogen genes (T5–T9), which share a high sequence homology (~91%). Group II trypsinogen genes, which consist of T1, T2, and T3, were not included because of their significant divergence from group I genes.¹³ Dashes indicate identity with the cationic trypsinogen gene (T4) sequence. Underlined sequence denotes a *Chi*-like sequence (complementary strand) in the neighbourhood of the pathological mutation. The likely donor nucleotides (AT) in T6 and T7 are also indicated by bold characters. GenBank accession numbers used for sequence comparison are U66061 and AF029308.

detect the R122H mutation both in hereditary^{4-6,9} and sporadic^{9,15} cases of pancreatitis. Clearly, this simple method is unable to detect the g.133283-133284GC>AT 2 bp mutation. Thus, the possibility of a gene conversion event should be considered for the genotyping of pancreatitis, particularly when the known mutations are not detectable and linkage to the trypsinogen gene locus cannot be excluded in certain HP families.

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