Mutations in the 5’ upstream region of Chymotrypsinogen C gene are not associated with chronic pancreatitis

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BACKGROUND

Chymotrypsinogen C (*CTRC*) plays a significant role in regulating trypsinogen activation. Early activation of trypsinogen inside the pancreas is a key molecular mechanism in the pathogenesis of pancreatitis that results in self-digestion and local inflammation of the organ. Loss-of-function mutations in the *CTRC* gene encoding Chymotrypsinogen C impair either the catalytic activity or the expression of the enzyme. Impaired expression of *CTRC* might be caused by variants in the 5' upstream region, however, this region of the gene was not investigated yet.

AIM

Our aim was to sequence the 5' upstream region of the CTRC gene in patients and controls in order to identify variants that may predispose to chronic pancreatitis.

METHODS

We selected 125 patients with non-alcoholic (NACP), 168 patients with alcoholic chronic pancreatitis (ACP) and 400 controls (Table 1.) recruited by the Hungarian Pancreatic Study Group (HPSG – https://tm-centre.org). Mutations within the ~1.4 kb CTRC 5’ upstream region were analyzed by Sanger sequencing.

	Controls		NACP patients		ACP patients	
	Male	Female	Male	Female	Male	Female
n =	400		124		168	
	200	200	70	54	148	20
mean age (years)	48.3		57		55	

Table 1. Characteristics of patients with non-alcoholic (NACP), alcoholic (ACP) chronic pancreatitis and controls.

RESULTS

We found 2 common polymorphisms (c.-913A>G and c.-811G>A) and 12 further variants (c.-1331T>A, c.-999G>A, c.-993G>T, c.-755G>A, c.-590G>T, c.-379G>A, c.-314AAAT[5], c.-296T>A, c.-265G>A, c.-92C>T, c.-59C>T, c.-55C>G) in the ~1.4 kb long 5’ upstream region of the CTRC gene. Using the recessive inheritance model the c.-913A>G variant was significantly accumulated in all groups of patients compared to controls (p=0.005–0.03, OR= 1.6-1.7, 95% CI: 1.1-2.7) (Table 2A and 2B). We revealed that haplotypes (Table 3.) carrying the known pathogenic variant c.180C>T were always carried the promoter variant c.-913A>G. In addition, haplotypes containing the c.-913A>G variant without the c.180C>T mutation were not accumulated in patients or in controls (Table 4.).

CONCLUSION

The identified genetic variants in the 1.4 kb long 5’ upstream region of the CTRC gene are not associated with chronic pancreatitis. Accumulation of the c.-913A>G variant in patients can be explained by its linkage with the known pathogenic c.180C>T mutation.

ALCOHOLIC CHRONIC PANCREATITIS

CTRC	Nucleotide change	Genotype	ACP Patients	Controls	OR	*p* Value	95% CI
5’ UTR	c.-913A>G	AA AG GG	45/168 (26.8%) 77/168 (45.8%) 46/168 (27.4%)	94/402 (23.4%) 233/402 (58%) 75/402 (18.7%)	 1.7 1.6	 0.03 0.02	 1.1-2.7 1.1-2.5
5’ UTR	c.-811G>A	GG GA AA	83/168 (49.7%) 68/168 (40.8%) 17/168 (9.5%)	165/402 (41%) 202/402 (50.3%) 35/402 (8.7%)	 0.7 1.2	 0.07 0.6	 0.5-1.0 0.64-2.2
5’ UTR	c.-314AAAT[5]	44 45 55	165/168 (98.2%) 3/168 (1.8%) 0/168 (0%)	388/400 (97%) 12 / 400 (3%) 0 / 400 (0%)	 0.59 2.4	 0.42 0.67	 0.16-2.11 0.05-120.3
Exon3	c.180C>T	CC CT TT	111/168 (66.1%) 53/168 (31.6%) 4/168 (2.3%)	313/390 (80.3%) 76/390 (19.5%) 1/390 (0.2%)	 2.1 9.5	 0.0004 0.045	 1.39-3.1 1.05-85.5

Table 2A. Allele distribution of identified mutations in the *CTRC* promoter region in patients with **alcoholic chronic pancreatitis (ACP)** and in controls.

NON-ALCOHOLIC CHRONIC PANCREATITIS

CTRC	Nucleotide change	Genotype	NACP Patients	Controls	OR	*p* Value	95% CI
5’ UTR	c.-913A>G	AA AG GG	25/125 (20%) 65/125 (52%) 35/125 (28%)	94/402 (23.4%) 233/402 (58%) 75/402 (18.7%)	1.22 1.7	0.43 0.03	0.74-2.0 1.1 – 2.7
5’ UTR	c.-811G>A	GG GA AA	59/125 (47.0%) 49/125 (40.2%) 17/125 (12.8%)	165/402 (41%) 202/402 (50.3%) 35/402 (8.7%)	0.78 1.65	0.22 0.11	0.52-1.17 0.89-3.1
5’ UTR	c.-314AAAT[5]	44 45 55	117/124 (94.4%) 7/124 (5.7%) 0/124 (0%)	388/400 (97%) 12 / 400 (3%) 0 / 400 (0%)	1.94 3.22	0.18 0.56	0.75-5.03 0.06-163
Exon3	c.180C>T	CC CT TT	84/125 (67.2%) 34/125 (27.2%) 7/125 (5.6%)	313/390 (80.3%) 76/390 (19.5%) 1/390 (0.2%)	1.98 23.1	0.003 0.004	1.27-3.1 2.8-189.5

Table 2B. Allele distribution of identified mutations in the *CTRC* promoter region in patients with **non-alcoholic chronic pancreatitis (NACP)** and in controls.

MUTATIONS	HAPLOTYPES 1	2	3	4
c.-1331T>A	T	T	T	T
c.-999G>A	G	G	G	G
c.-993G>T	G	G	G	G
c.-913A>G	A	G	G	G
c.-811G>A	G	A	A	G
c.-755G>A	G	G	G	G
c.-590G>T	G	G	G	G
c.-314AAAT[5]	4	4	4	4
c.-265G>A	G	G	G	G
c.-92C>T	C	C	C	C
c.-59C>T	C	C	C	C
c.180C>T	C	C	C	T
c.493+51C>A	C	C	A	C
c.493+52G>A	G	G	G	A

Table 3. The most frequent haplotypes identified in the CTRC gene.

Haplo-type	NACP patients	Control	OR	p	95% CI
1	102/246 (41.5%)	226/422 (53.6%)	0,61	0,0026	0.45-0.84
2	32/246 (13.0%)	60/422 (14.2%)	0,9	0,66	0.57-1.43
3	47/246 (19.1%)	70/422 (16.6%)	1,19	0,41	0.79-1.79
4	46/246 (18.7%)	40/422 (9.5%)	2,2	0,0007	1.39-3.47

Table 4. Distribution of the most frequent haplotypes in the *CTRC gene* in patients with non-alcoholic chronic pancreatitis (NACP) and in controls.

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