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Common calcium-sensing receptor (*CASR*) gene variants do not modify risk for chronic pancreatitis in a Hungarian cohort

Amanda Takáts[1], **Gergő Berke**[1], **Andrea Szentesi**[1,2,3], **Gyula Farkas Jr**[4], **Ferenc Izbéki**[5], **Bálint Erőss**[1], **László Czakó**[2], **Áron Vincze**[6], **Péter Hegyi**[1,2,3,#], **Miklós Sahin-Tóth**[7,#], **Eszter Hegyi**[1,#,*]

[1]Institute for Translational Medicine, Medical School, University of Pécs, Pécs, Hungary

[2]First Department of Medicine, University of Szeged, Szeged, Hungary

[3]Centre for Translational Medicine, Semmelweis University, Budapest, Hungary

[4]Department of Surgery, University of Szeged, Szeged, Hungary

[5]Szent György University Teaching Hospital of Fejér County, Székesfehérvár, Hungary

[6]Division of Gastroenterology, First Department of Medicine, Medical School, University of Pécs, Pécs, Hungary

[7]Department of Surgery, University of California Los Angeles, Los Angeles, California 90095

Abstract

The calcium-sensing receptor (CASR) is expressed in the pancreas where it might regulate calcium concentrations in pancreatic secretions. Two independent studies reported conflicting results claiming that commonly occurring missense variants of the *CASR* gene are risk factors for chronic pancreatitis (CP). Here, we attempted to replicate the association between *CASR* variants and CP. We analyzed 337 patients and 840 controls from the Hungarian National Pancreas Registry either by direct sequencing of exon 7 and the flanking noncoding regions or by TaqMan SNP genotyping assays. We identified two common missense variants, c.2956G>T (p.A986S), and c.2968A>G (p.R990G), three low-frequency variants, c.3031C>G (p.Q1011E), c.2610G>A (p.E870=) and c.*60T>A, and 8 rare variants including the novel variant c.1895G>A (p.G632D). When allelic or genotype distributions were considered, none of the *CASR* variants associated with CP. Subgroup analysis of nonalcoholic versus alcoholic patients revealed no disease association either. Our results demonstrate that common *CASR* variants do not modify the risk for CP and should not be considered as genetic risk factors in the clinical setting.

[*]Correspondence to Eszter Hegyi, Institute for Translational Medicine, Medical School, University of Pécs, 12 Szigeti street, H-7624 Pécs. Tel: +36 725-36019; eszter.hegyi@aok.pte.hu.

[#]These authors contributed equally.

AUTHOR CONTRIBUTIONS

EH, MST and PH conceived the study. EH directed the study. AT and EH designed the experiments. AT performed the experiments. AT and EH analyzed the data. MST and EH wrote the manuscript; EH prepared the tables; GB prepared the figures. PH planned and organized the collection of all clinical data and biological research samples used in this study. All other co-authors recruited study subjects, collected clinical data and/or provided genomic DNA samples. All authors approved the final manuscript.

CONFLICT OF INTEREST STATEMENT

The authors have declared that no conflict of interest exists.

Keywords

pancreatitis; calcium-sensing receptor; variants; genetic association study

INTRODUCTION

Chronic pancreatitis (CP) is a progressive inflammatory disorder of the pancreas, which often develops in the background of genetic susceptibility and/or chronic alcoholism [1]. Investigations into the genetic underpinning of CP led to the identification of several risk genes/variants that alter intrapancreatic trypsin activation, elicit digestive enzyme misfolding or affect ductal secretions [2–5]. The genes that alter risk in the so-called trypsin-dependent pathological pathway include the serine protease 1 and 2 (*PRSS1, PRSS2*) genes that encode human cationic and anionic trypsinogen, respectively, the serine protease inhibitor Kazal type 1 (*SPINK1*) gene, the chymotrypsinogen C (*CTRC*) gene, and an inversion at the chymotrypsinogen B1-B2 (*CTRB1-CTRB2*) locus. Genetic changes associated with the misfolding-dependent pathological pathway encompass mutations in the carboxypeptidase A1 (*CPA1*) gene, a subset of *PRSS1* variants, and rare mutations and a hybrid allele of the carboxyl-ester lipase (*CEL*) gene. Finally, the ductal pathway of CP risk comprises variants in genes that encode channels predominantly expressed in the pancreatic ductal epithelium such as *CFTR, TRPV6* and *CLDN2*. CP is a multigenic disease and patients may carry multiple genetic alterations that modify risk. However, a genetic basis for CP is not always identified in patients, and the search for yet undiscovered susceptibility genes continues.

The calcium-sensing receptor (*CASR*) gene emerged as a potential candidate for a CP risk gene when Felderbauer et al. described that subjects with familial hypocalciuric hypercalcemia (FHH) caused by heterozygous inactivating *CASR* mutations also developed CP if they carried a heterozygous *SPINK1* p.N34S risk variant [6, 7]. Subsequently, screening of Indian patients with tropical pancreatitis confirmed compound heterozygosity for *SPINK1* p.N34S and *CASR* mutations in a small number of subjects [8, 9]. Finally, a French case report described a patient with recurrent acute pancreatitis and FHH, who carried a heterozygous *CASR* mutation and a heterozygous c.194+2T>C *SPINK1* variant [10]. These anecdotal observations suggested that *CASR* mutations might contribute to CP risk, particularly in carriers of pathogenic *SPINK1* variants.

Two published studies screened larger CP cohorts for *CASR* mutations and identified commonly occurring exon 7 variants as potential risk factors. First, the Whitcomb group reported that *CASR* variant c.2968A>G (p.R990G) increased CP risk about 2-fold and an even more substantial effect was observed in alcoholics [11]. A follow-up study from the Férec group did not confirm this observation but found that the homozygous genotype of the *CASR* variant c.2956G>T (p.A986S) conferred a more than 3-fold increased risk to CP [12]. The authors also observed enrichment of rare variants in their patient cohort relative to controls. Given the discrepant results, additional replication studies are warranted. Here, we investigated the association of *CASR* variants with CP in a Hungarian cohort.

METHODS

Nomenclature.

Nucleotide numbering reflects coding DNA numbering with the first nucleotide of the ATG translation initiation codon designated as +1 in the *CASR* reference sequence (genomic reference: NC_000003.12, Homo sapiens chromosome 3, GRCh38.p13 primary assembly; mRNA reference: NM_000388.4). Amino acids are numbered starting with the initiator methionine of the primary translation product of *CASR*.

Study subjects.

De-identified genomic DNA samples were obtained from the Hungarian National Pancreas Registry (ethical approval: TUKEB 22254–1/2012/EKU, biobanking approval: IF702–19/2012). Subjects were recruited from 11 Hungarian centers between 2012 and 2018, and all gave informed consent according to the ethical guidelines of the Declaration of Helsinki. The discovery cohort analyzed by direct DNA sequencing consisted of 261 patients with CP (106 nonalcoholic and 155 alcoholic cases) and 224 controls. The expanded study cohort analyzed by TaqMan SNP genotyping contained additional 76 CP patients (36 nonalcoholic and 40 alcoholic cases) and 616 controls. In total, 337 unrelated patients with CP (mean age at recruitment 56.4±12 years), including 142 with nonalcoholic CP and 195 with alcoholic CP, and 840 control subjects (mean age at recruitment 39.3±14.6 years) with no pancreatic disease were enrolled. Diagnosis of CP was based on the history of recurrent acute pancreatitis or recurrent abdominal pain typical for CP and/or pathological imaging findings consistent with CP, such as pancreatic calcifications, duct dilatation or irregularities, with or without exocrine pancreatic insufficiency or diabetes. Alcoholic CP was diagnosed when the patient's history included alcohol consumption of more than 80 g/day (men) or 60 g/day (women) for at least two years. Part of this cohort was previously characterized for *SPINK1* variants p.N34S and c.194+2T>C and the common *PRSS1-PRSS2* haplotype [13, 14].

DNA sequencing.

Exon 7 with the flanking intron 6 and 3' UTR regions was amplified using 3 primer pairs; CASR-x7-amp1-FWD 5'-TAT GTA TTC CCA CCA CCA C-3' and CASR-x7-amp1-REV 5'-TGA AGG TGC AGA GGA AAA C-3', CASR-x7-amp2-FWD 5'-GTG TTT GAG GCC AAG ATC C-3' and CASR-x7-amp2-REV 5'-TTG CTC TTG CTG CTG ATG G-3', CASR-x7-amp3-FWD 5'-ACA TCA TTC TCT TCA AGC CAT C-3' and CASR-x7-amp3-REV 5'-AGG AGT CTG GGG CGA TTC-3'. Polymerase chain reaction (PCR) was performed using 0.5 U HotStarTaq DNA Polymerase (Qiagen), 0.2 mM dNTP, 0.5 μM primers, 10x PCR buffer (Qiagen) and 10 to 50 ng of genomic DNA template in a volume of 25 μL. The reaction started with a 15 min initial heat activation at 95°C followed by 35 cycles of 30 sec denaturation at 94°C, 30 sec annealing at 61.1°C (amplicon 1 and 2) or 53.2°C (amplicon 3), and 40 sec (amplicon 1 and 2) or 50 sec (amplicon 3) extension at 72°C; and finished by a final extension for 5 min at 72°C. PCR products were verified by 2% agarose gel electrophoresis. The PCR amplicons (5 μL) were treated with 1 μL FastAP Thermosensitive Alkaline Phosphatase and 0.5 μL Exonuclease I (Thermo Fisher Scientific) for 15 min at 37°C, and the reaction was stopped by heating the samples to 85°C for 15 min. Sanger

sequencing was performed using the forward (amplicon 1 and 3) and reverse (amplicon 2) PCR primers as sequencing primers.

TaqMan SNP genotyping.

TaqMan SNP genotyping assays were used to investigate the p.A986S and p.R990G *CASR* variants in the expanded study cohort (Assay ID: CASR rs1801725_7504853_20 and CASR rs1042636_7504854_20) using a StepOne Real-Time PCR system (Applied Biosystems by Life Technologies). The 20 μL reaction consisted of TaqPath ProAmp Master Mix (2x), TaqMan SNP genotyping assay (20x) and 10–20 ng genomic DNA template. The cycling conditions were as follows: 30 sec holding stage at 60°C followed by a 10 min holding stage at 95°C; 50 cycles of 15 sec denaturation at 92°C and 1 min annealing at 60°C; and a final 30 sec holding stage at 60°C. Allelic discrimination plots were evaluated using the StepOne software. To confirm the results, all homozygous samples and 4–6 heterozygous and wild-type samples from each plate were sequenced.

Statistical analysis.

The significance of the differences in allele frequencies and genotype distribution between cases and controls was assessed by Fisher's exact test using the GraphPad Prism9 software. $P < 0.05$ was considered statistically significant.

RESULTS

DNA sequence analysis of exon 7 of human *CASR* in a discovery cohort.

To investigate whether common *CASR* variants alter risk for CP, we initially sequenced exon 7 and flanking intron 6 and 3' UTR regions of *CASR* in 261 patients with CP (106 nonalcoholic CP and 155 alcoholic CP) and 224 controls from the Hungarian National Pancreas Registry. We identified 2 common missense variants (allele frequency >5%), c.2956G>T (p.A986S) and c.2968A>G (p.R990G) and 3 low-frequency variants (allele frequency 1–5%), which included a missense variant c.3031C>G (p.Q1011E), a synonymous variant c.2610G>A (p.E870=) and a 3' UTR variant c.*60T>A, which was in linkage disequilibrium with p.Q1011E. In addition, we found 8 rare variants (allele frequency <1%), 7 of which were detected in one subject each (Figure 1, Table 1). The rare variants included 3 missense variants; the c.1895G>A (p.G632D) variant was detected in a CP patient, while two previously reported missense variants, c.1775A>G (p.N592S) and c.2405A>G (p.N802S) were present in controls. The novel p.G632D variant was found in a male, nonalcoholic patient who developed CP at the age of 37. He had no history of smoking, and carried no pathogenic *SPINK1* variants. His total serum calcium level was in the normal range (2.39 mmol/L). The serum calcium levels were not available for the carriers of the p.N592S and p.N802S variants.

In silico analysis using the "PredictSNP Consensus classifier for prediction of disease-related mutations" tool classified the p.G632D and p.N802S variants as potentially disease causing and the p.N592S variant as benign.

When allele frequency was considered, distribution of the variants between CP patients and controls showed no significant differences. Genotype distribution of common missense variants p.A986S and p.R990G and the low-frequency variant p.Q1011E was also assessed using dominant and recessive models of inheritance, but no significant differences between CP patients and controls were found (Table 2). In this analysis, a non-significant enrichment of the homozygous p.A986S variant was observed in patients (3.4%) versus controls (0.9%). However, we noticed a deviation from Hardy-Weinberg equilibrium (HWE) in the control population, probably due to the limited sample size.

TaqMan SNP genotyping for the p.A986S and p.R990G variants in an expanded cohort.

Since the homozygous p.A986S variant and the p.R990G variant were previously reported to associate with CP [11, 12], we expanded our study and investigated these two variants using TaqMan SNP genotyping assays in additional 76 CP patients (36 nonalcoholic and 40 alcoholic cases) and 616 controls. Taken the direct sequencing and genotyping results together, allele and genotype frequency of the common p.A986S and p.R990G variants were determined in 337 CP patients (142 nonalcoholic CP and 195 alcoholic CP) and 840 controls. Neither of these variants associated with CP (Table 3). Notably, in the combined results, there was no appreciable enrichment of the homozygous p.A986S variant in patients versus controls (2.7% versus 2.3%, respectively; OR=1.19, 95% CI 0.52–1.92, p=0.68). Subgroup analysis of nonalcoholic CP and alcoholic CP revealed no disease association either (Tables 4–5).

DISCUSSION

The calcium-sensing receptor is a dimeric, G-protein coupled transmembrane receptor that is highly expressed in the parathyroid glands and the kidneys where it regulates systemic calcium homoeostasis [15, 16]. An increase in serum calcium levels activates the receptor, triggering intracellular signaling to inhibit parathyroid hormone (PTH) secretion and calcium resorption. A decrease in serum calcium releases these inhibitions resulting in higher PTH secretion and increased calcium resorption in the kidneys. Similar to PTH regulation in the parathyroid glands, the expression of CASR in the mammary epithelia negatively regulates the secretion of PTH-related peptide, which can mobilize calcium from bones for milk production. Heterozygous inactivating mutations in *CASR* cause FHH, an autosomal dominant disorder characterized by elevated serum calcium and decreased urinary calcium excretion [15, 17]. *CASR* mutations may also cause neonatal severe hyperparathyroidism, typically as a recessive disorder. In contrast, *CASR* mutations are rarely observed in adult-onset hyperparathyroidism and reduced expression of CASR in parathyroid adenomas is the likely explanation for the increased PTH secretion and hypercalcemia in this disease. Finally, activating mutations in *CASR* are associated with autosomal dominant hypocalcemia.

In addition to its systemic, “calcitropic” role, CASR is expressed in several tissues where it contributes to local regulation of various cellular processes. In the rat pancreas, CASR was found in acinar, ductal and islet cells, and activation of the receptor was shown to induce ductal bicarbonate secretion [18]. CASR expression was also documented in all cell types of

the human pancreas, including intrapancreatic nerves and blood vessels [19]. Furthermore, the human pancreatic adenocarcinoma cell line Capan-1 was shown to express functional CASR [19]. Based on these observations, it was suggested that CASR might regulate the calcium concentration of the pancreatic juice by increasing ductal fluid secretion, possibly through activating CFTR [5]. We note, however, that evidence for the exact role(s) of CASR in the pancreas is limited and animal models with pancreas-specific CASR deletion or mutation have been lacking. The strongest indication that CASR mutations may play a role in pancreatitis is the relatively frequent occurrence of pancreatitis in FHH [20]. In a small number of FHH patients, trans-heterozygosity for *SPINK1* and *CASR* mutations was documented [6–10]. However, it seems likely that FHH-associated pancreatitis is due to hypercalcemia rather than the local effects of inactivating CASR mutations in the pancreas. Hypercalcemia is a well-known risk factor for pancreatitis. Hyperparathyroidism and malignancy-associated hypercalcemia are two commonly reported conditions in which pancreatitis frequently occurs in association with elevated serum calcium levels [21–23]. Importantly, experimental studies in rats also demonstrated that hypercalcemia could induce pancreatitis [24–26]. *SPINK1* variants represent an independent risk factor for CP, which often interact with other genetic and environmental risk factors to promote disease onset and progression. Thus, it is not surprising that some FHH patients with pancreatitis might carry *SPINK1* mutations as well.

Considering the role of commonly occurring *CASR* variants in CP risk, human genetic association studies yielded conflicting results (see Introduction). Therefore, in the present study, we examined the contribution of common exon 7 *CASR* variants to CP risk in a Hungarian cohort of nonalcoholic and alcoholic CP cases. A limitation of our analysis was the relatively small size of the patient cohorts. We identified 5 *CASR* variants with a population frequency above 1%, none of which showed an association with CP. No enrichment was observed when allelic or genotype distributions were considered or in a subgroup analysis of nonalcoholic and alcoholic patients. *SPINK1* mutation status was not analyzed, but we note that *SPINK1* variants p.N34S and c.194+2T>C are rare in this Hungarian CP cohort [13]. We conclude that the previously reported associations between common *CASR* variants and CP were likely spurious due to chance and/or multiple testing. This conclusion is in agreement with the reported functional properties of these variants. Thus, in transfected HEK 293 cells variants p.A986S and p.Q1011E behaved exactly as wild-type CASR while variant p.R990G showed slightly enhanced function [27]. The gain-of-function phenotype of variant p.R990G might explain its association with primary hypercalciuria [27] but it seems difficult to reconcile with pancreatitis. Finally, we found a novel missense variant in a CP patient (p.G632D) and two previously reported rare missense variants in controls (p.N592S, p.N802S). Variants p.G632D and p.N802S were predicted to be functionally deleterious. Indeed, variant p.N802S was described as an inactivating mutation associated with FHH [28]. No enrichment of rare missense variants was observed in the patient cohort. However, our analysis was limited to exon 7 and a direct comparison with the relevant results of the Férec group cannot be made [12].

Intracellular calcium signaling plays a critical role in pancreas physiology and aberrant calcium signaling is a hallmark of pancreatitis. Changes in extracellular calcium may have profound effects on calcium signaling and may directly promote activation of digestive

proteases. Besides *CASR*, recent genetic studies focused on other calcium channels and receptors as well [29–32]. While the interesting preliminary findings with *GPRC6A* and *STIM1* await further replication [29, 32], the *TRPV6* gene encoding a constitutive calcium channel was convincingly identified as a high-impact CP risk gene [5, 30, 31]. Loss-of-function mutations in *TRPV6* are strongly associated with CP with a large effect size. TRPV6 is expressed in both acinar and ductal cells and the disease-causing mechanism of *TRPV6* mutations has remained unclear so far. Because higher expression levels were reported in the ductal epithelium, the *TRPV6* gene was tentatively assigned to the ductal pathological pathway of CP risk [5]. One can speculate that TRPV6 might regulate pancreatic juice calcium concentrations in concert with CASR. A recent example for functional interaction between these two molecules was described in the intestinal epithelium, where activation of CASR in the basolateral membrane attenuates TRPV6-dependent intestinal calcium absorption [33]. However, in light of our present data, TRPV6 more likely functions in a manner that is independent of CASR in the pancreas.

In summary, our results demonstrate that common *CASR* variants do not modify the risk for CP and should not be considered as genetic risk factors in the clinical setting.

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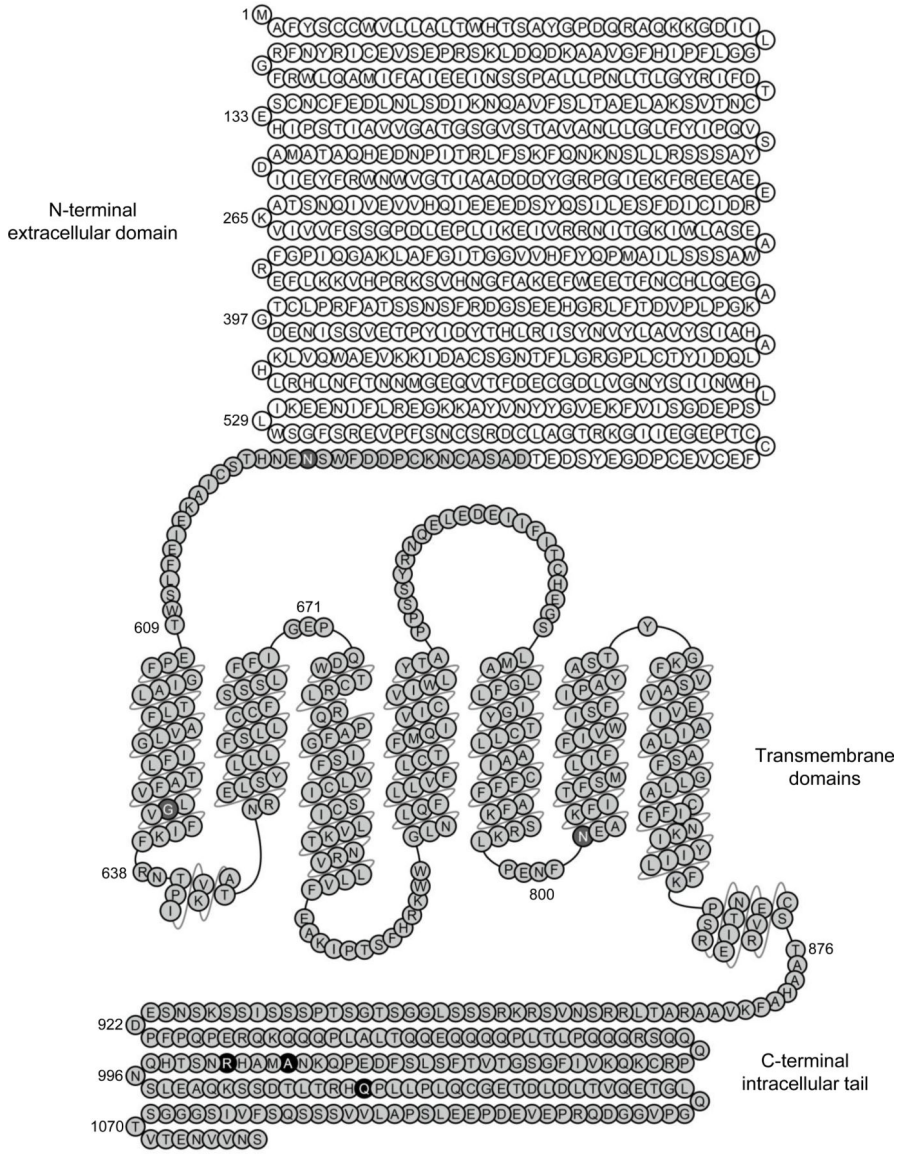

Figure 1.

Snakeplot showing the calcium-sensing receptor (CASR) missense variants found in the Hungarian cohort. The amino acids coded by exon 7 are highlighted with light grey, rare missense variants p.N592S, p.G632D, and p.N802S are shown in dark gray, while common variants p.A986S, and p.R990G, and low-frequency variant p.Q1011E are depicted in black. The snakeplot was generated by GPCRdb [34].

Table 1.

Allele frequency of *CASR* variants in the discovery cohort. OR, odds ratio, CI, confidence interval.

Location	Nucleotide change	Amino-acid change	rs number	Patients	Controls	OR	95% CI	*p*-value
Intron 6	c.1733–9A>G		rs190731787	1/522	0/448			
Exon 7	c.1775A>G	p.N592S	rs117375173	0/522	1/448			
Exon 7	c.1895G>A	p.G632D	-	1/522	0/448			
Exon 7	c.1942C>A	p.R648=	rs104893705	1/522	0/448			
Exon 7	c.2388G>A	p.K796=	rs200701164	0/522	1/448			
Exon 7	c.2405A>G	p.N802S	rs140022350	0/522	1/448			
Exon 7	c.2610G>A	p.E870=	rs143738711	2/522 (0.4%)	5/448 (1.1%)	0.34	0.07–1.58	0.26
Exon 7	c.2838G>A	p.Q946=	rs774889993	2/522 (0.4%)	2/448 (0.5%)			
Exon 7	c.2956G>T	p.A986S	rs1801725	101/522 (19.4%)	83/448 (18.5%)	1.06	0.76–1.45	0.81
Exon 7	c.2968A>G	p.R990G	rs1042636	41/522 (7.9%)	26/448 (5.8%)	1.38	0.84–2.29	0.25
Exon 7	c.2979G>A	p.T993=	rs917914806	1/522	0/448			
Exon 7	c.3031C>G	p.Q1011E	rs1801726	19/522 (3.6%)	20/448 (4.5%)	0.81	0.44–1.57	0.52
3’ UTR	c.*60T>A[a]		rs4677948	19/522 (3.6%)	20/448 (4.5%)	0.81	0.44–1.57	0.52

[a]The reference allele is the minor A allele. The variant is described here with respect to the common T allele.

Table 2.

Genotype distribution of common *CASR* variants in the discovery cohort. Genotypes were analyzed assuming dominant (shown in italics) or recessive models of inheritance. OR, odds ratio, CI, confidence interval, HWE, Hardy-Weinberg equilibrium (*p*-value is shown).

Location	Nucleotide change	Genotype	Patients	Controls	OR	95% CI	*p*-value	HWE
		GG	169/261 (64.8%)	143/224 (63.8%)				
Exon 7	c.2956G>T	GT	83/261 (31.8%)	79/224 (35.3%)	*0.96*	*0.66–1.39*	*0.85*	0.01
		TT	9/261 (3.4%)	2/224 (0.9%)	3.96	1.02–18.43	0.07	
		AA	220/261 (84.3%)	198/224 (88.4%)				
Exon 7	c.2968A>G	AG	41/261 (15.7%)	26/224 (11.6%)	*1.42*	*0.84–2.44*	*0.24*	0.36
		GG	0/261 (0%)	0/224 (0%)				
		CC	242/261 (92.7%)	205/224 (91.5%)				
Exon 7	c.3031C>G	CG	19/261 (7.3%)	18/224 (8%)	*0.85*	*0.44–1.62*	*0.74*	0.39
		GG	0/261 (0%)	1/224 (0.5%)				

Table 3.

Genotype distribution and allele frequency of c.2956G>T (p.A986S) and c.2968A>G (p.R990G) variants in the expanded study population. Genotypes were analyzed assuming dominant (shown in italics) or recessive models of inheritance. OR, odds ratio, CI, confidence interval, HWE, Hardy-Weinberg equilibrium (*p*-value is shown).

Location	Nucleotide change	Genotype	Patients	Controls	OR	95% CI	*p*-value	HWE
Exon 7	c.2956G>T	GG	222/337 (65.9%)	558/840 (66.4%)				
		GT	106/337 (31.4%)	263/840 (31.3%)	*1.03*	*0.79–1.34*	*0.89*	0.06
		TT	9/337 (2.7%)	19/840 (2.3%)	1.19	0.52–1.92	0.68	
		T	124/674 (18.4%)	301/1680 (17.9%)	1.03	0.82–1.30	0.81	
Exon 7	c.2968A>G	AA	289/337 (85.8%)	732/840 (87.2%)				
		AG	48/337 (14.2%)	102/840 (12.1%)	*1.13*	*0.78–1.62*	*0.57*	0.25
		GG	0/337 (0%)	6/840 (0.7%)				
		G	48/674 (7.1%)	114/1680 (6.8%)	1.05	0.75–1.49	0.79	

Table 4.

Genotype distribution and allele frequency of c.2956G>T (p.A986S) and c.2968A>G (p.R990G) variants in patients with nonalcoholic chronic pancreatitis. Genotypes were analyzed assuming dominant (shown in italics) or recessive models of inheritance. OR, odds ratio, CI, confidence interval.

Location	Nucleotide change	Genotype	Patients	Controls	OR	95% CI	*p*-value
Exon 7	c.2956G>T	GG	91/142 (64.1%)	558/840 (66.4%)			
		GT	46/142 (32.4%)	263/840 (31.3%)	*1.11*	*0.76–1.6*	*0.63*
		TT	5/142 (3.5%)	19/840 (2.3%)	1.58	0.63–4.06	0.38
		T	56/284 (19.7%)	301/1680 (17.9%)	1.13	0.82–1.55	0.46
Exon 7	c.2968A>G	AA	125/142 (88%)	732/840 (87.2%)			
		AG	17/142 (12%)	102/840 (12.1%)	*0.92*	*0.53–1.58*	*0.89*
		GG	0/142 (0%)	6/840 (0.7%)			
		G	17/284 (6%)	114/1680 (6.8%)	0.88	0.52–1.47	0.70

Table 5.

Genotype distribution and allele frequency of c.2956G>T (p.A986S) and c.2968A>G (p.R990G) variants in patients with alcoholic chronic pancreatitis. Genotypes were analyzed assuming dominant (shown in italics) or recessive models of inheritance. OR, odds ratio, CI, confidence interval.

Location	Nucleotide change	Genotype	Patients	Controls	OR	95% CI	*p*-value
Exon 7	c.2956G>T	GG	131/195 (67.2%)	558/840 (66.4%)			
		GT	60/195 (30.8%)	263/840 (31.3%)	*0.97*	*0.7–1.35*	*0.87*
		TT	4/195 (2%)	19/840 (2.3%)	0.91	0.33–2.57	>0.99
		T	68/390 (17.4%)	301/1680 (17.9%)	0.97	0.72–1.29	0.88
Exon 7	c.2968A>G	AA	164/195 (84.1%)	732/840 (87.2%)			
		AG	31/195 (15.9%)	102/840 (12.1%)	*1.28*	*0.83–1.97*	*0.29*
		GG	0/195 (0%)	6/840 (0.7%)			
		G	31/390 (8%)	114/1680 (6.8%)	1.19	0.77–1.78	0.44