Online Submissions: wjg.wjgnet.com
wjg@wjgnet.com
doi:10.3748/wjg.14.4486

World J Gastroenterol 2008 July 28; 14(28): 4486-4491
World Journal of Gastroenterology ISSN 1007-9327

RAPID COMMUNICATION

Association between *calcium sensing receptor* gene polymorphisms and chronic pancreatitis in a US population: Role of *serine protease inhibitor Kazal 1type* and alcohol

Venkata Muddana, Janette Lamb, Julia B Greer, Beth Elinoff, Robert H Hawes, Peter B Cotton, Michelle A Anderson, Randall E Brand, Adam Slivka, David C Whitcomb

Venkata Muddana, Janette Lamb, Julia B Greer, Beth Elinoff, Adam Slivka, David C Whitcomb, Division of Gastroenterology, Hepatology and Nutrition, Department of Medicine, University of Pittsburgh Medical Center, Pittsburgh PA 15213, United States
David C Whitcomb, Department of Human Genetics, University of Pittsburgh, Pittsburgh PA 15213, United States
Robert H Hawes, Peter B Cotton, Digestive Disorder Center, Medical University of South Carolina, Charleston SC 29403, United States
Michelle A Anderson, Department of Medicine, University of Michigan Medical Center, Ann Arbor MI 48109, United States
Randall E Brand, Department of medicine, Northwestern University, and Currently at University of Pittsburgh Medical Center, Pittsburgh PA 15213, United States
Supported by NIH R01 DK061451 (DCW) and Andrew and Michelle Aloe
Author contributions: Muddana V: Study design, development of study methodology, data analysis, drafting the original manuscript and reviewing the final manuscript; Lamb J: Method development, data analysis, manuscript editing and review; Greer JB: Data analysis, reviewing, editing and writing of the final manuscript; Elinoff B: Study coordination, clinical data management and analysis, review of final manuscript; Hawes RH: Site PI, subject phenotyping, clinical data collection and analysis, review of final manuscript; Cotton PB: Subject phenotyping, clinical data collection and analysis, review of final manuscript; Anderson MA: Site PI, subject phenotyping, clinical data collection and analysis, review of final manuscript; Brand RE: Site PI, subject phenotyping, clinical data collection and analysis, review of final manuscript; Slivka A: Site PI, subject phenotyping, clinical data collection and analysis, review of final manuscript; Whitcomb DC: Study design, method development, data analysis, manuscript review and editing.
Correspondence to: David C Whitcomb, MD, PhD, Division of Gastroenterology, Hepatology and Nutrition, Department of Medicine, University of Pittsburgh Medical Center, Mezzanine Level 2, C Wing, 200 Lothrop Street, Pittsburgh PA 15213, United States.whitcomb@pitt.edu
Telephone: +1-412-6489604 Fax: +1-412-3837236
Received: April 11, 2008 Revised: July 15, 2008
Accepted: July 22, 2008
Published online: July 28, 2008

Abstract

AIM: To test the hypothesis that calcium sensing receptor (*CASR*) polymorphisms are associated with chronic pancreatitis (CP), and to determine whether *serine protease inhibitor Kazal 1type* (*SPINK1*) N34S or alcohol are necessary co-factors in its etiology.

METHODS: Initially, 115 subjects with pancreatitis and 66 controls were evaluated, of whom 57 patients and 21 controls were predetermined to carry the high-risk *SPINK1* N34S polymorphism. We sequenced *CASR* gene exons 2, 3, 4, 5 and 7, areas containing the majority of reported polymorphisms and novel mutations. Based on the initial results, we added 223 patients and 239 controls to analyze three common nonsynonymous single nucleotide polymorphisms (SNPs) in exon 7 (A986S, R990G, and Q1011E).

RESULTS: The *CASR* exon 7 R990G polymorphism was significantly associated with CP (OR, 2.01; 95% CI, 1.12-3.59; P = 0.015). The association between *CASR* R990G and CP was stronger in subjects who reported moderate or heavy alcohol consumption (OR, 3.12; 95% CI, 1.14-9.13; P = 0.018). There was no association between the various *CASR* genotypes and *SPINK1* N34S in pancreatitis. None of the novel *CASR* polymorphisms reported from Germany and India was detected.

CONCLUSION: Our United States-based study confirmed an association of *CASR* and CP and for the first time demonstrated that *CASR* R990G is a significant risk factor for CP. We also conclude that the risk of CP with *CASR* R990G is increased in subjects with moderate to heavy alcohol consumption.

Key words: Calcium sensing receptor; *Serine protease inhibitor Kazal 1type*; Chronic pancreatitis; Alcohol

Peer reviewers: Asbjørn M Drewes, Professor, Department of Medical Gastroenterology, Center for Visceral Biomechanics and Pain, Aalborg Hospital, Aalborg 9000, Denmark; CS Pitchumoni, Professor, Robert Wood Johnson School of Medicine, Robert Wood Johnson School of Medicine, New Brunswick NJ D8903, United States

Muddana V, Lamb J, Greer JB, Elinoff B, Hawes RH, Cotton PB, Anderson MA, Brand RE, Slivka A, Whitcomb DC. Association between *calcium sensing receptor* gene polymorphisms and chronic pancreatitis in a US population: Role of *serine protease inhibitor Kazal 1type* and alcohol. *World J Gastroenterol* 2008; 14(28): 4486-4491 Available from: URL: http://www.wjgnet.com/1007-9327/14/4486.asp DOI: http://dx.doi.org/10.3748/wjg.14.4486

INTRODUCTION

Chronic pancreatitis (CP) is a debilitating, inflammatory disease of the pancreas, characterized by progressive organ destruction and fibrosis. CP results in profound exocrine and endocrine insufficiency and, in many cases, intractable, chronic pain. As a complex disorder, CP can develop from a variety of etiologies with multiple pathological pathways[1]. For several years, alcohol abuse has been considered the most likely causative agent for CP in Western countries, although etiologies including toxic, metabolic (hypercalcemia, hyperlipidemia), genetic mutations, autoimmune, and duct obstruction, have also been implicated[2,3].

Consistent experimental evidence links elevated acinar cell calcium levels with acute pancreatitis in association with premature trypsinogen activation to trypsin[4]. Recurrent acute pancreatitis (RAP), as illustrated in patients with hereditary and sporadic pancreatitis, can lead to CP[5-7]. Hypercalcemia itself has been associated with the development and complications of CP[2]. Recent studies from Germany and India have reported that novel *calcium sensing receptor (CASR)* gene mutations in combination with the presence of *serine protease inhibitor Kazal 1type (SPINK1)* N34S increased the risk of CP[8-10]. The *SPINK1* N34S "high-risk haplotype" is strongly associated with CP, but only a limited portion of mutation carriers develop CP during their life time, suggesting that additional factors are necessary to develop this complex disorder[11,12].

CASR is a member of the G-protein-coupled receptor (GPCR) superfamily[13]. *CASR* plays an important role in calcium homeostasis, as is reflected in its expression by cells of the parathyroid gland and renal tubules that are involved in calcium metabolism. *CASR* has been identified in both human pancreatic acinar and ductal cells, as well as in various non-exocrine cells[14], although its functional significance in the pancreas has not been determined.

The human *CASR* gene is located on chromosome 3q 13.3-21[15,16]. *CASR* possesses a coding region of 3234 base pairs (bp) which is contained within 6 of the seven exons that make up the gene. One hundred and twelve functional mutations (40 activating and 72 inactivating) have been described in the *CASR* mutation database related to familial hypocalciuric hypercalcemia (FHH), neonatal severe primary hyperparathyroidism (NSHPT), and autosomal dominant hypocalcemia (ADH) families as well as in *de-novo* disease[17]. In addition, single activating or inactivating *CASR* mutations may cause hypercalcemic or hypocalcemic disorders[18,19].

We hypothesized that *CASR* polymorphisms are associated with the development of CP and that *SPINK1* N34S mutations or alcohol may be important co-factors in its etiology. We tested this hypothesis by evaluating subjects with RAP, CP and healthy controls with known *SPINK1* genotypes and alcohol intake for common and novel *CASR* polymorphisms in exons 2, 3, 4, 5 and 7.

MATERIALS AND METHODS

Study population

Subjects were recruited from the North American Pancreatic Study2 (NAPS2). The NAPS2 study is a multicenter, molecular epidemiology study designed to evaluate the genetic and environmental factors predisposing to recurrent acute pancreatitis (RAP) and CP. Detailed description of methods are presented elsewhere[20]. The subjects were stratified into alcohol categories based on self-reported average number of drinks consumed per week during the period of heaviest lifetime drinking. Alcohol categories were defined based on the drinking pattern as: (1) abstainers: no alcohol use or < 20 drinks in lifetime; (2) light drinkers: ⩽ 3 drinks/week; (3) moderate drinkers: 4-7 drinks/week for females; 4-14 drinks/week for males; (4) heavy drinkers; 8-34 drinks/week for females; 15-34 drinks/week for males; (5) very heavy drinkers: ⩾ 35 drinks/week for both males and females. For analysis, alcohol drinking categories were combined into 3 groups based on their risk for causing CP: (1) abstainers and light drinkers were considered very low risk, (2) moderate and heavy drinkers were considered moderate risk, and (3) very heavy drinkers were considered substantial risk[20].

One hundred and fifteen affected individuals and 66 controls were selected initially from four sites of the NAPS2 cohort. These subjects were selected based on the presence or absence of *SPINK1* N34S mutations, of which 57 patients and 21 controls were determined by previous genetic analysis to carry the high-risk *SPINK1* mutation. From the twenty site NAPS2 consortium, 219 affected subjects and 239 controls were later screened for the three common nonsynonymous single nucleotide polymorphisms (SNPs) seen in the coding region of the intracellular *CASR* tail in exon 7 which appeared to be the region of interest. These were A986S (rs # 1801725), R990G (rs # 1042636), and Q1011E (rs # 1801726).

DNA preparation and mutation analysis

Genomic DNA was extracted from whole blood as described[20]. PCR primers were designed for *CASR* gene exons 2, 3, 4, 5 and 7, which contains most of the commonly seen activating and inactivating mutations as well as the novel mutations found in Germany and India (Table 1). Exons 4 and 7 were lengthy and thus were divided into 2 and 4 fragments respectively.

PCR was performed in a total volume of 25 μL; 200 nmol of forward and reverse primer, 200 μmol of dNTP and 1 × PCR Buffer Ⅱ (ABI, CA) with 10 ng of DNA. Amplification settings were 95℃ for 12 min × 1 cycle, 95℃ for 30 s, annealing temperature (Table 1) × 20 s and 72℃ × 20 s for 35 cycles and 72℃ for 2 min × 1 cycle. Annealing temperatures and magnesium concentrations for different primers are shown in Table 1. PCR amplification products were purified with exonuclease Ⅰ (NEB, Beverley, MA) and shrimp alkaline phosphatase (Roche Diagnostics, Indianapolis, IN) according to the manufacturer's recommendations. Cycle sequencing was performed using the ABI Prism Big Dye Terminator Sequencing Kit v3.1 diluted 1:8 (ABI, Foster City, CA) using the appropriate PCR primers. Products from the sequencing reaction were purified by ethanol EDTA precipitation. Sequence products were run on

Table 1 Polymerase chain reaction primer pairs, magnesium concentration and annealing temperatures used for genetic analysis of the *CASR* gene

Scanning region	Forward and reverse primer sequences	$MgCl_2$ (mmol/L)	Annealing temperature (℃)
Exon 2	5′-ACCACCCACATTACAAGTC-3′ 5′-GCTTTTCTCCAACCACTCAG-3′	2.5	55
Exon 3	5′-ATGAAGCCAGAGAGTAGTAAC-3′ 5′-TAAACCGTATGGCTATTGGG-3′	2.5	58
Exon 4a	5′-GCTTTTCCTTACCCTTTCTTTCATC-3′ 5′-ATCACCTCTACCACATGCTG-3′	2	58
Exon 4b	5′-CAGATCTTGAGCCCCTCATC-3′ 5′-GCAGCCCAACTCTGCTTTAT-3′	2	59
Exon 5	5′-TGGGGCTTGTACTCATTCTT-3′ 5′-CTGGTTTTCTGATGGACAGC-3′	1.5	59
Exon 7a	5′-CACACAATAACTCACTCTTCAC-3′ 5′-CAGAGGAAAACCAGCAGGAAC-3′	2.0	61
Exon 7b	5′-AAAACCAACCGTGTCCTCCTG-3′ 5′-ATGGCAATCACCTCTACGGC-3′	1.0	53
Exon 7c	5′-GCTCATCTTCTTCATCGTCTGG-3′ 5′-CGTATCGCTGCTTTTCTGGG-3′	1.0	58
Exon 7d	5′-CCCAGCAAGAGCAGCAG-3′ 5′-ACAACTCTTCAGGGTCCTCC-3′	1.0	58

Table 2 Participant characteristics

Demographic	CP (n = 219)	RAP (n = 115)	Controls (n = 305)
Age, mean (SD)	45.3 (18.1)	46.1 (16.2)	54.7 (14.5)
Race, % White	91	91	94
Sex (M/F)	125/94	49/66	121/184
Alcohol drinking pattern (%)			
Abstainers	45 (22.5)	30 (27)	72 (25)
Light	38 (19)	26 (23)	83 (28)
Moderate	34 (17)	22 (20)	56 (19)
Heavy	39 (19.5)	20 (18)	58 (20)
Very heavy	44 (22)	13 (12)	23 (8)

CP: Chronic pancreatitis; RAP: Recurrent acute pancreatitis; SD: Standard deviation. Abstainers: No alcohol use or < 20 drinks in lifetime; Light: < 3 drinks/week; Moderate: 4-7 drinks/week for females, 4-14 drinks/week for males; Heavy; 8-34 drinks/week for females, 15-34 drinks/week for males; Very heavy: > 35 drinks/week.

an ABI Prism 3730 Genetic Analyzer and sequence data were analyzed using Sequencher 4.7 (Gene Codes Corp., Ann Arbor, MI)[5,21].

Statistical analysis

Genotype frequencies were assessed for Hardy-Weinberg equilibrium. The frequencies of genotypes among cases and controls were compared using Chi square test or the Fisher's exact test when appropriate. Odds ratio (OR) and 95% confidence intervals (95% CI) for genotypes were calculated using an autosomal dominant model. For all statistical comparisons, $P < 0.05$ was considered significant.

RESULTS

Subject demographics and alcohol drinking patterns are given in Table 2. The proportion of subjects reporting a moderate or heavy alcohol drinking pattern was similar between patients and controls. Of the 334 patients with pancreatitis, 219 (66%) had CP and 115 (34%) had RAP.

The initial study consisted of 115 patients (CP = 82 and RAP = 33) and 66 controls, of which 57 patients (CP = 47 and RAP = 10) and 21 controls carried the *SPINK1* N34S high risk haplotype. Of the 58 patients without *SPINK1* N34S, 35 were diagnosed with CP and 23 had RAP.

The genotype frequencies were found to be in Hardy-Weinberg equilibrium. The R990G polymorphism (AGG→ GGG transition) in exon 7 of the *CASR* gene, the G allele was more common among CP patients (n = 35) than controls (n = 45), but only in subjects without *SPINK1* N34S. In comparing CP patients (n = 47) and controls (n = 21) with *SPINK1* N34S, there was a non-significant trend towards an increased occurrence of the G allele in patients (OR, 4.03; 95% CI, 0.48-190.8, P = 0.255). One limitation of this study was the small number of *SPINK1* N34S subjects for comparison; Therefore, caution must be exercised before this association is either accepted or rejected.

From the 112 mutations reported previously, the following three mutations--E191E, Y440C and A746A were each observed once in CP patients with *SPINK1* N34S. Another mutation, P748P, was identified in two CP patients without *SPINK1* N34S. Recently identified novel *CASR* mutations from Germany and India seen in exons 3 (P163R), 4 (L173P, F391F, I425S, D433H), 5 (V477A) and 7 (E870E, R896E)[8-10] were not observed in either patients or controls. Two intronic polymorphisms 493-94 C>T and 493-134 T>C included in exon 4 amplicon occurred with similar frequency in CP patients and controls, both with and without *SPINK1* N34S polymorphisms.

Secondarily, 219 patients (137 CP and 82 RAP) and 239 controls from the NAPS2 study who did not carry *SPINK1* N34S were analyzed to test the association of *CASR* A990G and CP. This ancillary analysis confirmed that the R990G was significantly associated with CP, as shown in Table 3 (OR, 2.01; 95% CI, 1.12-3.59; P = 0.015). The frequencies of R990G among RAP patients and controls, with and without *SPINK1* N34S were similar. There was

Table 3 Genotype analysis of *CASR* R990G polymorphism in patients and controls

	Patients	Controls	P^1	OR (95% CI)
CP patients *vs* controls without *SPINK1* N34S (%)				
AA	140 (82)	255 (90)		
AG	31 (18)	28 (10)		
GG	1 (1)	1 (0.5)		
AA *vs* AG/GG			0.015	2.01 (1.12-3.59)
RAP patients *vs* controls without *SPINK1* N34S (%)				
AA	93 (89)	255 (90)		
AG	10 (9)	28 (10)		
GG	2 (2)	1 (0.5)		
AA *vs* AG/GG			0.712	1.28 (0.67-2.47)
SPINK1 N34S positive CP patients *vs* controls (%)				
AA	39 (83)	20 (95)		
AG	8 (17)	1 (5)		
GG	0	0		
AA *vs* AG/GG			0.255	4.1 (0.48-35.14)

CP: Chronic pancreatitis; RAP: Recurrent acute pancreatitis. *SPINK1*: Serine protease Kazal type 1 gene. [1]Fisher exact test.

Table 4 *CASR* genotype comparison for R990G polymorphism in CP patients and controls with similar alcohol drinking patterns

	Patients	Controls	P^1	OR (95% CI)
A/L Alcohol CP patients *vs* controls (%)				
AA	69 (83)	136 (88)		
AG	13 (16)	18 (11)		
GG	1 (1)	1 (1)		
AA *vs* AG/GG			0.332	1.45 (0.69-3.07)
M/H Alcohol CP patients *vs* controls (%)				
AA	59 (81)	106 (93)		
AG	14 (19)	8 (7)		
GG	0	0		
AA *vs* AG/GG			0.018	3.12 (1.14-9.13)
VH Alcohol CP patients *vs* controls (%)				
AA	37 (84)	21 (91)		
AG	7 (16)	2 (9)		
GG	0	0		
AA *vs* AG/GG			0.708	1.99 (0.38-0.45)

A: Abstainer; L: Light; M: Moderate; H: Heavy; VH: Very heavy. Alcohol categories: Abstainers: No alcohol use or < 20 drinks in lifetime; Light drinkers: < 3 drinks/week; Moderate drinkers: 4-7 drinks/week for females, 4-14 drinks/week for males; Heavy drinkers: 8-34 drinks/week for females, 15-34 drinks/week for males; Very heavy drinkers: > 35 drinks/week. [1]Fisher's exact test.

no difference in A986S and Q1011E polymorphisms among RAP and CP patients, and controls.

To determine if the risk was modified with alcohol use we compared *CASR* R990G genotypes in subjects with moderate and heavy alcohol drinking pattern. CP was strongly associated with the *CASR* R990G in moderate and heavy alcohol drinkers, as is demonstrated in Table 4 (OR, 3.12; 95% CI, 1.14-9.13; $P = 0.018$). No association was observed with this particular polymorphism in abstainers or in subjects with self-reported light or very heavy alcohol drinking patterns.

DISCUSSION

In the past, CP was commonly attributed to heavy alcohol consumption. More recent studies, however, suggest there is also a strong genetic basis for this illness[22]. Growing knowledge of complex gene-environment interactions has provided fundamental insight into the pathophysiological mechanisms that result in fibrotic destruction of the pancreas[11,23-25]. Studies from Germany and India have recently identified 8 novel *CASR* mutations that were associated with *SPINK1* N34S in idiopathic and tropical CP subjects. Our study did not detect these novel *CASR* mutations. However, we were able to demonstrated and verify that *CASR* R990G confers significant risk for developing of CP especially when linked to moderate and heavy alcohol consumption.

Three common nonsynonymous SNPs are located in the region coding the intracellular tail of *CASR*[26] and play an important role in cellular signal transduction that alters serum ionized calcium level[27,28]. Previously, it was reported that individuals carrying the 990 variant G allele may experience very mild decrease in serum ionized calcium levels from 4.92 mg/dL to 4.84 mg/dL[28]. Although serum ionized calcium levels

alter the cytosolic calcium ion concentrations in acinar cells in a concentration-dependent manner, and may alter the risk of acute pancreatitis[29,30], the *CASR* R990G allele associated with increased risk of CP should slightly reduce the risk of acute pancreatitis. Furthermore, the magnitude of change in serum calcium levels due to *CASR* R990G alone is small, and it is difficult to imagine that this small change would, by itself, significantly alter the risk of acute pancreatitis. Indeed, our data suggests that *CASR* R990G is associated with CP rather than RAP. Our speculation is that *CASR* R990G might induce direct changes in the acinar and ductal cells that increase the risk for CP. However, the mechanism remains unknown.

Interestingly, while 55%-80% of pancreatitis cases may be attributed to alcohol abuse, less than 5% of heavy alcohol users develop pancreatitis[31]. Alcohol abuse may not be the sole risk for the development of CP[32]; rather alcoholic CP is likely the result of an interaction of several co-factors[2]. It has been demonstrated that chronic alcohol consumption accelerates fibrosis in response to cerulein-induced CP in rats[33]. Alcohol metabolites in pancreatic acinar cells induce persistent cytosolic Ca^{2+} signals in a concentration-dependent manner and depolarize mitochondria.

The discovery and characterization of a genetic cause of hereditary pancreatitis generated renewed interest in a possible genetic predisposition to alcoholic CP[34]. Several CP-related gene mutations have been described previously with *CFTR*, *PRSS1*, *SPINK1* and others[35]. Our study also demonstrates the association of *CASR* R990G with CP, especially with moderate and heavy alcohol consumption. The presence of *CASR* R990G alone doubled the risk of developing CP, while in those individuals reporting moderate and heavy alcohol consumption, the risk was increased by 3-fold. Our hypothesis for testing *CASR* R990G in subjects with moderate and heavy drinking patterns is that this group represented a "threshold" alcohol pancreatitis risk group in which the addition of another risk factor would increase the overall risk of developing CP. The risk of CP in subjects with *CASR* R990G but with minimal or no alcohol consumption would be lower, while very heavy drinkers would be at high risk, regardless of the *CASR* genotype. Our experimental findings support this hypothesis.

The novel *CASR* gene mutations that were identified in German and Indian populations appeared to be closely associated with the *SPINK1* N34S haplotype. We did not detect these, or other novel *CASR* mutations, and our study was not powered to demonstrate an interaction between *SPINK1* N34S and *CASR* R990G. On the other hand, it was not clear whether or not the German and Indian studies tested for an effect of alcohol in a "threshold" dose range. However, both studies suggest that the overall effect of *CASR* polymorphisms are relatively small, and become clinically significant in the presence of additional risk factors in an additive or multiplicative way. This is consistent with current concepts that CP is a complex syndrome.

The present study confirmed the association of *CASR* genetic variants with CP. Our genotyping results in a US population were different from those reported from Germany and India. *CASR* R990G significantly increased the risk of developing CP and this effect was enhanced in subjects who consumed alcohol in a moderate to heavy dose range. Certain polymorphisms in the *CASR* gene may be considered risk factors for the development of CP, especially within the context of alcohol consumption. The relationship with *SPINK1* mutations warrants further study.

ACKNOWLEDGMENTS

We thank physician members of the NAPS2 Consortium and associates who contributed sample and information for the final data set. These include Christopher Lawrence MD, Joseph Romagnuolo MD, Meredith Korneffel MD, Grace Elta MD, Krik-Gan Wamsteker MD, James Scheiman MD, Peter Banks MD, Michele Bishop MD, John Baillie MD, Paul Jowell MD, Malcom Branch MD, Stuart Sherman MD, Lee McHenry MD, Evan Fogle MD, Nahla Hasabou MD, Michel Goldberg MD, Stephen Amann MD, Nathan Schmulewitz MD, Syed Ahmad MD, Shailendra Chauhan MD, Jim DiSario MD, Frank Burton MD, Timothy Gartner MD, Andres Gelrud MD, Simon Lo MD, Mark DeMeo MD, Sri Komanduri MD, William Steinberg MD, Michael Kochman MD, Gregory Ginsberg MD, Babak Etemad MD, and Christopher Forsmark MD. We also thank Dr. Georgios I Papachristou for helpful comments.

COMMENTS

Background

Chronic pancreatitis is a highly morbid, complex disease whose development depends on the combination of genetic and environmental factors. Elucidating the genetic links to this illness is critical in diagnosis, treatment and risk assessment.

Research frontiers

This study adds another gene to the growing number of genetic and other factors that confers increased risk of chronic pancreatitis. As new factors continue to be identified and confirmed, the emphasis will turn to integrating these risks, using systems approaches, as described in reference #1.

Innovations and breakthroughs

This study is one of the first to consider the complexity of gene-environment and gene-gene interactive paradigms by evaluating alcohol consumption and *serine protease inhibitor Kazal 1type (SPINK1)* N34S variants with *calcium sensing receptor (CASR)* polymorphisms. The confirmation of *CASR* genetic variants as risk factors for chronic pancreatitis strengthens the importance of dysfunctional calcium regulating genes in the etiology of pancreatitis.

Application

With the inclusion of associated *CASR* polymorphisms in comprehensive evaluation of selected patients, we may improve the accuracy of overall pancreatitis risk prediction and may be able to provide a target for preventive approaches and possible treatment options.

Peer review

Our peer reviewers noted this brief manuscript to be well-developed and well-written. They felt that the abstract was clear and the hypothesis being tested and methodology were sound and well presented.

REFERENCES

1 **Aoun E**, Chang CC, Greer JB, Papachristou GI, Barmada MM, Whitcomb DC. Pathways to injury in chronic pancreatitis: decoding the role of the high-risk SPINK1 N34S haplotype

using meta-analysis. *PLoS ONE* 2008; **3**: e2003
2 **Etemad B**, Whitcomb DC. Chronic pancreatitis: diagnosis, classification, and new genetic developments. *Gastroenterology* 2001; **120**: 682-707
3 **Ahmed SA**, Wray C, Rilo HL, Choe KA, Gelrud A, Howington JA, Lowy AM, Matthews JB. Chronic pancreatitis: recent advances and ongoing challenges. *Curr Probl Surg* 2006; **43**: 127-238
4 **Ward JB**, Petersen OH, Jenkins SA, Sutton R. Is an elevated concentration of acinar cytosolic free ionised calcium the trigger for acute pancreatitis? *Lancet* 1995; **346**: 1016-1019
5 **Whitcomb DC**, Gorry MC, Preston RA, Furey W, Sossenheimer MJ, Ulrich CD, Martin SP, Gates LK Jr, Amann ST, Toskes PP, Liddle R, McGrath K, Uomo G, Post JC, Ehrlich GD. Hereditary pancreatitis is caused by a mutation in the cationic trypsinogen gene. *Nat Genet* 1996; **14**: 141-145
6 **Gorry MC**, Gabbaizedeh D, Furey W, Gates LK Jr, Preston RA, Aston CE, Zhang Y, Ulrich C, Ehrlich GD, Whitcomb DC. Mutations in the cationic trypsinogen gene are associated with recurrent acute and chronic pancreatitis. *Gastroenterology* 1997; **113**: 1063-1068
7 **Aoun E**, Slivka A, Papachristou DJ, Gleeson FC, Whitcomb DC, Papachristou GI. Rapid evolution from the first episode of acute pancreatitis to chronic pancreatitis in human subjects. *JOP* 2007; **8**: 573-578
8 **Felderbauer P**, Hoffmann P, Klein W, Bulut K, Ansorge N, Epplen JT, Schmitz F, Schmidt WE. Identification of a novel calcium-sensing receptor gene mutation causing familial hypocalciuric hypercalcemia by single-strand conformation polymorphism analysis. *Exp Clin Endocrinol Diabetes* 2005; **113**: 31-34
9 **Felderbauer P**, Klein W, Bulut K, Ansorge N, Dekomien G, Werner I, Epplen JT, Schmitz F, Schmidt WE. Mutations in the calcium-sensing receptor: a new genetic risk factor for chronic pancreatitis? *Scand J Gastroenterol* 2006; **41**: 343-348
10 **Murugaian EE**, Premkumar RM, Radhakrishnan L, Vallath B. Novel mutations in the calcium sensing receptor gene in tropical chronic pancreatitis in India. *Scand J Gastroenterol* 2008; **43:** 117-121
11 **Muller-Pillasch F**, Menke A, Yamaguchi H, Elsasser HP, Bachem M, Adler G, Gress TM. TGFbeta and the extracellular matrix in pancreatitis. *Hepatogastroenterology* 1999; **46**: 2751-2756
12 **Whitcomb DC**. How to think about SPINK and pancreatitis. *Am J Gastroenterol* 2002; **97**: 1085-1088
13 **Brown EM**, Gamba G, Riccardi D, Lombardi M, Butters R, Kifor O, Sun A, Hediger MA, Lytton J, Hebert SC. Cloning and characterization of an extracellular Ca(2+)-sensing receptor from bovine parathyroid. *Nature* 1993; **366**: 575-580
14 **Bruce JI**, Yang X, Ferguson CJ, Elliott AC, Steward MC, Case RM, Riccardi D. Molecular and functional identification of a Ca2+ (polyvalent cation)-sensing receptor in rat pancreas. *J Biol Chem* 1999; **274**: 20561-20568
15 **Janicic N**, Soliman E, Pausova Z, Seldin MF, Riviere M, Szpirer J, Szpirer C, Hendy GN. Mapping of the calcium-sensing receptor gene (CASR) to human chromosome 3q13.3-21 by fluorescence in situ hybridization, and localization to rat chromosome 11 and mouse chromosome 16. *Mamm Genome* 1995; **6**: 798-801
16 **Aida K**, Koishi S, Tawata M, Onaya T. Molecular cloning of a putative Ca(2+)-sensing receptor cDNA from human kidney. *Biochem Biophys Res Commun* 1995; **214**: 524-529
17 **Pidasheva S**, D'Souza-Li L, Canaff L, Cole DE, Hendy GN. CASRdb: calcium-sensing receptor locus-specific database for mutations causing familial (benign) hypocalciuric hypercalcemia, neonatal severe hyperparathyroidism, and autosomal dominant hypocalcemia. *Hum Mutat* 2004; **24**: 107-111
18 **Brown EM**, Pollak M, Hebert SC. The extracellular calcium-sensing receptor: its role in health and disease. *Annu Rev Med* 1998; **49**: 15-29
19 **Hendy GN**, D'Souza-Li L, Yang B, Canaff L, Cole DE. Mutations of the calcium-sensing receptor (CASR) in familial hypocalciuric hypercalcemia, neonatal severe hyperparathyroidism, and autosomal dominant hypocalcemia. *Hum Mutat* 2000; **16**: 281-296
20 **Whitcomb DC**, Yadav D, Slivka Aea. Multicenter approach to recurrent acute and chronic pancreatitis in the United States: the North American Pancreatitis Study 2 (NAPS2). *Pancreatology* 2008; **8:** 520-531
21 **Pfutzer RH**, Barmada MM, Brunskill AP, Finch R, Hart PS, Neoptolemos J, Furey WF, Whitcomb DC. SPINK1/PSTI polymorphisms act as disease modifiers in familial and idiopathic chronic pancreatitis. *Gastroenterology* 2000; **119**: 615-623
22 **Witt H**. Gene mutations in children with chronic pancreatitis. *Pancreatology* 2001; **1**: 432-438
23 **Van Laethem JL**, Robberecht P, Resibois A, Deviere J. Transforming growth factor beta promotes development of fibrosis after repeated courses of acute pancreatitis in mice. *Gastroenterology* 1996; **110**: 576-582
24 **Bachem MG**, Schneider E, Gross H, Weidenbach H, Schmid RM, Menke A, Siech M, Beger H, Grunert A, Adler G. Identification, culture, and characterization of pancreatic stellate cells in rats and humans. *Gastroenterology* 1998; **115**: 421-432
25 **Apte MV**, Wilson JS. Stellate cell activation in alcoholic pancreatitis. *Pancreas* 2003; **27**: 316-320
26 **Heath H 3rd**, Odelberg S, Jackson CE, Teh BT, Hayward N, Larsson C, Buist NR, Krapcho KJ, Hung BC, Capuano IV, Garrett JE, Leppert MF. Clustered inactivating mutations and benign polymorphisms of the calcium receptor gene in familial benign hypocalciuric hypercalcemia suggest receptor functional domains. *J Clin Endocrinol Metab* 1996; **81**: 1312-1317
27 **Cole DE**, Peltekova VD, Rubin LA, Hawker GA, Vieth R, Liew CC, Hwang DM, Evrovski J, Hendy GN. A986S polymorphism of the calcium-sensing receptor and circulating calcium concentrations. *Lancet* 1999; **353**: 112-115
28 **Scillitani A**, Guarnieri V, De Geronimo S, Muscarella LA, Battista C, D'Agruma L, Bertoldo F, Florio C, Minisola S, Hendy GN, Cole DE. Blood ionized calcium is associated with clustered polymorphisms in the carboxyl-terminal tail of the calcium-sensing receptor. *J Clin Endocrinol Metab* 2004; **89**: 5634-5638
29 **Krims PE**, Pandol SJ. Free cytosolic calcium and secretagogue-stimulated initial pancreatic exocrine secretion. *Pancreas* 1988; **3**: 383-390
30 **Sutton R**, Criddle D, Raraty MG, Tepikin A, Neoptolemos JP, Petersen OH. Signal transduction, calcium and acute pancreatitis. *Pancreatology* 2003; **3**: 497-505
31 **Dani R**, Penna FJ, Nogueira CE. Etiology of chronic calcifying pancreatitis in Brazil: a report of 329 consecutive cases. *Int J Pancreatol* 1986; **1**: 399-406
32 **Lankisch PG**, Lowenfels AB, Maisonneuve P. What is the risk of alcoholic pancreatitis in heavy drinkers? *Pancreas* 2002; **25**: 411-412
33 **Deng X**, Wang L, Elm MS, Gabazadeh D, Diorio GJ, Eagon PK, Whitcomb DC. Chronic alcohol consumption accelerates fibrosis in response to cerulein-induced pancreatitis in rats. *Am J Pathol* 2005; **166**: 93-106
34 **Hanck C**, Schneider A, Whitcomb DC. Genetic polymorphisms in alcoholic pancreatitis. *Best Pract Res Clin Gastroenterol* 2003; **17**: 613-623
35 **Schneider A**, Pfutzer R, Whitcomb DC. Genetics and pancreatic disease. *Curr Opin Gastroenterol* 2002; **18**: 545-551

S- Editor Zhong XY **L- Editor** Anand BS **E- Editor** Lin YP