

Role of the common *PRSS1-PRSS2* haplotype in alcoholic and non-alcoholic chronic pancreatitis: meta- and re-analyses

Anthony F. Herzig¹, Emmanuelle Génin^{1,2}, David N. Cooper³, Emmanuelle Masson^{1,2}, Claude Férec^{1,2}, Jian-Min Chen¹

¹Inserm, Univ Brest, EFS, UMR 1078, GGB, F-29200 Brest, France

²CHRU Brest, Brest, France

³Institute of Medical Genetics, School of Medicine, Cardiff University, Cardiff, United Kingdom

Correspondence: Jian-Min Chen, INSERM UMR1078 – EFS – UBO, 22 avenue Camille Desmoulins, 29238 BREST, France; Tel: +33-2-98018174; Fax: +33-2-98016474; e-mail: jian-min.chen@univ-brest.fr

Abstract

The association between a common *PRSS1-PRSS2* haplotype and alcoholic chronic pancreatitis (ACP), which was revealed by the first genome-wide association study of chronic pancreatitis (CP), has been consistently replicated. However, the association with non-ACP (NACP) has been controversial. Herein, we sought to clarify this basic issue by means of an allele-based meta-analysis of currently available studies. We then used studies informative for genotype distribution to explore the biological mechanisms underlying the association data and to test for gene-environment interaction between the risk haplotype and alcohol consumption by means of a re-analysis. A literature search was conducted to identify eligible studies. Meta-analysis was performed using the Review Manager software. The association between the risk genotypes and NACP or ACP was tested for the best-fitting genetic model. Gene-environment interaction was estimated by both case-only and multinomial approaches. Five and eight studies were employed for the meta-analysis of ACP and NACP findings, respectively. The risk allele was significantly associated with both ACP (pooled OR 1.67, 95% CI 1.56–1.78; $P < 0.00001$) and NACP (pooled OR 1.28, 95% CI 1.17–1.40; $P < 0.00001$). Consistent with a dosage effect of the risk allele on *PRSS1/PRSS2* mRNA expression in human pancreatic tissue, both ACP and NACP association data were best explained by an additive genetic model. Finally, the risk haplotype was found to interact synergistically with ACP.

Keywords: chronic pancreatitis; meta-analysis; *PRSS1*; re-analysis; rs10273639

INTRODUCTION

Chronic pancreatitis (CP) is a chronic inflammatory process of the pancreas that leads to irreversible morphological changes and progressive impairment of both exocrine and endocrine functions (1, 2). CP can be caused by genetic and/or environmental factors (3). In the one extreme, rare gain-of-function missense or copy number variants in the *PRSS1* gene (encoding cationic trypsinogen; MIM# 276000) can cause autosomal dominant hereditary pancreatitis (4, 5). In the other extreme, alcohol abuse is the most frequent environmental factor causing CP worldwide (2, 3).

Eight years ago, the first genome-wide association study of CP was published; a common *PRSS1-PRSS2* haplotype was reported to be associated with both alcoholic CP (ACP) and non-ACP (NACP) (6). *PRSS2* encodes anionic trypsinogen (MIM# 601564), the second major trypsinogen isoform after cationic trypsinogen. This association has a strong biological basis: the risk [C] allele of the lead single nucleotide polymorphism (SNP), rs10273639C/T, appeared to be associated with increased *PRSS1* mRNA expression in pancreatic tissue in a dosage-dependent manner (6). rs10273639C/T is in perfect linkage disequilibrium with rs4726576C/A, which is located 204 bp upstream of the translation initiation codon of the *PRSS1* gene; the C allele of rs4726576C/A increased reporter gene expression as determined by an *in vitro* promoter assay (7). Uncontrolled trypsin activity is central to CP pathogenesis (8).

NOTE: This preprint reports new research that has not been certified by peer review and should not be used to guide clinical practice.

Another interesting finding from the Whitcomb study was that rs10273639C/T was associated with a stronger genetic effect in ACP than in NACP, suggesting that the SNP-conferred risk was amplified by alcohol consumption. Moreover, in a case-only analysis, the SNP seemed to interact with ACP (6). In subsequent studies, the association of the common *PRSS1-PRSS2* haplotype with ACP has been consistently replicated. However, the association with NACP was equivocal, raising the possibility that the common *PRSS1-PRSS2* haplotype modifies CP risk only for ACP but not in NACP (9, 10). To explore this basic question and the underlying biological mechanisms, we firstly performed an allele-based meta-analysis of the reported associations between the common *PRSS1-PRSS2* haplotype and ACP and NACP. We then used informative studies in terms of genotype distribution to perform a new re-analysis with respect to (i) the fit of different genetic models to the association data and (ii) the gene-environment interaction between the common *PRSS1-PRSS2* haplotype and alcohol consumption status in CP.

MATERIALS AND METHODS

Literature search and inclusion criteria

The literature search was performed by keyword search (*PRSS1* and Pancreatitis) via PubMed (as of 13 July 2020), which was complemented by perusal of references citing the Whitcomb and/or Derikx studies (6, 9) via Google Scholar. Only studies that satisfied the following criteria were included for meta-analysis: (i) published in a peer-reviewed journal; (ii) the common *PRSS1-PRSS2* haplotype was analyzed in both ACP and/or NACP patients and controls; and (iii) the tagging SNP of interest was in Hardy–Weinberg equilibrium in the controls.

Data extraction

The protective alleles of the SNPs that tagged the common *PRSS1-PRSS2* haplotype have often been used for statistical analysis in previous publications. To consider the genetic effects of both rare and common *PRSS1* variants in the same framework, herein we used the risk alleles of the tagging SNPs for analysis. To this end, risk allele or genotype distribution data in patients and controls were manually extracted from included studies.

Statistical analysis and meta-analysis with respect to risk allele frequencies in patients and controls

The assessment of significance of the differences between the frequencies of the risk-tagging SNPs in patients and controls in the context of each study was performed by means of χ^2 tests in R (11). A difference was regarded as being statistically significant when the *P* value was ≤ 0.05 . The sample size for case-control association studies was estimated using the Online Sample Size Estimator (12).

Meta-analysis, heterogeneity and sensitivity analyses, and forest plots were all performed using the Review Manager 5.3 software (13). Funnel plots were not performed due to the small number of eligible studies (13, 14). The Mantel-Haenszel fixed effect or random effect model was used to compute pooled odds ratio (OR) in the absence or presence of statistical heterogeneity. Heterogeneity was considered to be significant when the *P* value for the test of heterogeneity was < 0.05 or I^2 was 50% or more (13, 15). The conduct and reporting of the meta-analysis were essentially in accordance with the PRISMA guidelines (16). This meta-analysis was not registered in PROSPERO (17), which does not allow the registration of already finished studies.

Evaluation of the effect of rs10273639 on the expression of *PRSS1* and *PRSS2* in pancreatic tissue

This analysis was performed using the Genotype-Tissue Expression (GTEx) dataset (18).

Re-analysis for association between the common *PRSS1-PRSS2* haplotype and ACP/NACP under different genetic models

Logistic regression models were fitted to examine the association between the risk genotypes and NACP or ACP in the context of single population data (refer to Results). Four models were tested: the

genotypes were coded as either recessive, dominant, additive, or as each having an independent effect (general model) (19). We evaluated the Akaike information criterion (AIC) for each model and tested the change in deviance of the recessive, dominant, and additive models against the general model. Pearson's residuals were calculated to ensure the reasonability of the models fitted. All analyses were carried out using the statistical analysis software R (version 3.6.1) (11).

Re-analysis to test for interaction between the common *PRSS1-PRSS2* haplotype and alcohol consumption status in CP

The studies included in this analysis were those for which we could ascertain individual level genotype data for ACP, NACP and healthy control individuals. We firstly performed case-only analyses before combining results through meta-analysis in order to estimate a pooled OR for the interaction term (also referred to as the synergy index SIM). This analysis was performed using the metagen function from the meta R package (20).

In a second step, we applied the multinomial approach to test for gene-environment interactions as proposed by Kuzma and colleagues (21). ACP and NACP patients were compared using the control individuals as a common baseline through a multinomial logistic regression model (multinom function from the nnet R package) (22). This method of investigating the gene-environment interaction is equivalent to the case-only approach (23); testing the interaction on a multiplicative scale (24). However, unlike the case-only approach, the multinomial model allows us to also include a control group and hence to simultaneously estimate the genetic effects and test for the presence of an interaction term, thereby giving a more complete description.

RESULTS

Included studies for meta-analysis

The keyword search (*PRSS1* and Pancreatitis) via PubMed resulted in 173 entries starting from the pioneering Whitcomb study (6). Of these, 15 publications were identified to report original data on the frequency of the common *PRSS1-PRSS2* haplotype in both pancreatitis patients and controls. Two studies that analyzed patients with a known non-alcohol-related external factor (25, 26) and three studies that analyzed only patients with acute pancreatitis (27-29) were immediately excluded from further consideration. Two additional reports that analyzed chronic pancreatitis (CP) patients were also excluded: one (30) because of a significant overlap of patients with a previous study (9) and the other due to its small sample size (i.e., only 85 CP cases and 78 controls were successfully genotyped) and lack of information on the numbers of CP subtypes among the analyzed patients (31). The remaining eight studies were included in our meta-analysis; five of these reported data on both alcoholic CP (ACP) and non-ACP (NACP) (6, 9, 10, 32, 33) whereas the other three reported data only for NACP (34-36). The search and selection processes of the included studies are outlined in Figure 1. In all included studies, the tagging single nucleotide polymorphism (SNP) of interest was either described in the original publication or verified here to be in Hardy-Weinberg equilibrium in the controls. Additionally, all included studies were evaluated to have a Newcastle-Ottawa Scale (NOS) score (37) of ≥ 7 . Finally, perusal of references citing the Whitcomb study (6) and/or the Derikx study (9) via Google Scholar did not identify additional eligible studies in peer-reviewed journals.

Basic characteristics of the eight included studies are summarized in Table 1. It should be noted that, in the Whitcomb study, the ACP and NACP cohorts actually comprised some patients with recurrent acute pancreatitis; CP and recurrent acute pancreatitis were modeled together based upon the assumption that the two disease states have common susceptibilities (6). As opined by the original authors, this treatment may reduce power relative to that of the analysis of CP alone but is unlikely to have altered the main conclusions of the study.

Allele-based meta-analysis confirmed an association of the common *PRSS1-PRSS2* haplotype with both ACP and NACP

Although the frequencies of the risk-tagging alleles differ significantly (from 22% to 58%) between the American (primarily of European ancestry), European, Japanese, Indian and Hungarian control populations studied, all five ACP studies found a statistically significant association between the common *PRSS1-PRSS2* haplotype and ACP with remarkably comparable ORs (i.e., 1.64 to 1.85; [Table 1](#)). Application of the test for heterogeneity confirmed that the five studies were homogeneous ($\chi^2 = 0.68$, $P = 0.95$; $I^2 = 0\%$). The pooled OR (under a fixed effect model) was 1.67 (95% CI 1.56–1.78; $P < 0.00001$) ([Figure 2](#)).

Of the eight NACP studies, five found a statistically significant association with NACP whereas the other three did not ([Table 1](#)). The test for heterogeneity revealed significant heterogeneity across the studies ($\chi^2 = 15.81$, $P = 0.03$; $I^2 = 56\%$). Sensitivity analysis identified the large European study (9) to be the source of the heterogeneity. Using a random-effect model, the common *PRSS1-PRSS2* haplotype showed significant association with NACP: the pooled OR was 1.28 (95% CI 1.17–1.40; $P < 0.00001$) ([Figure 2](#)).

The three studies that failed to show a significant association with NACP nevertheless all yielded ORs >1 ([Table 1](#)). Notably, the frequency of the tagging rs6666[C] allele among the Hungarian NACP patients was nearly 6% higher than in controls (i.e., 63.1% vs. 57.3%), raising the possibility that the absence of an association ($P = 0.17$) in the corresponding study (10) might have been due to the relatively small sample size (only 103 patients and 296 controls were examined). Assuming (i) the frequencies of the rs6666[C] allele in the Hungarian NACP patients and controls were 63% and 57%, respectively and (ii) a 1:2 ratio of cases to controls, at least 784 patients and 1568 controls would have been required to achieve significance at the 5% level with 80% power.

As for the large European study (9), the risk allele frequency in the NACP patients was 2% higher than in the controls, yielding a P value of 0.085 ([Table 1](#)). This large study used three NACP cohorts, viz. German, French and Dutch. The German cohort was the largest and showed a statistically significant association with NACP (OR = 1.21, $p = 0.0027$; [Table 2](#)).

The third study that failed to achieve a significant association (34) was the smallest of the three Indian studies ([Table 1](#)).

The risk allele number is positively correlated with the pancreatic *PRSS2* mRNA expression level

We evaluated whether the risk [C] allele of rs10273639C/T is associated with increased trypsinogen gene expression in the pancreas in the GTEx dataset. No significant expression quantitative trait loci (eQTLs) for *PRSS1* in the pancreas are currently available in GTEx. However, the genotypes of rs10273639 affected *PRSS2* mRNA expression in the pancreas in a dosage-dependent manner (see [Supplementary Figure S1](#)).

The additive genetic model best fits the associations of the common *PRSS1-PRSS2* haplotype with NACP and ACP

The positive correlation between the risk allele number of rs10273639 and *in vivo* *PRSS1* and *PRSS2* mRNA expression implies that an additive genetic model would best explain the association data. To test this postulate, we firstly examined the three possible genetic models, namely dominant, recessive and additive, in the context of three NACP studies informative for genotype distribution, each from a single population (i.e., the only Japanese study (32), the Paliwal study that analyzed the largest number of Indian patients (35), and the German cohort (the largest of the European cohorts) studied by Derikx et al. (9)). A general model with an independent effect of each genotype was also fitted to aid the evaluation of the three models of interest.

The distribution of the rs10273639 genotypes showed significant differences between patients and controls under all three genetic models ([Table 3](#)). Pearson's residuals (which should not greatly exceed a value of 2) were calculated to establish that all models had provided a reasonable description of the data. By evaluating the Akaike information criterion (AIC), we observed that the best fitting model was invariably the additive model as a lower AIC represents a better model fit. On examining the general models, we observed that the coefficients for the CC genotype were very

close to twice the coefficients of the CT genotype (i.e., 0.39 vs. 0.25 in the German data; 0.87 vs. 0.45 in the Japanese data; and 0.59 vs. 0.34 in the Indian data). This is indicative of an additive dose-dependence relationship between the genotypes and the risk of CP. Further, we performed analyses of variance (Anova) of the recessive, dominant and additive models against the general model. This showed that only the recessive model provided a worse description of the data compared to the general model (P values below 0.05) (Table 3).

We also examined the three possible genetic models in the context of ACP studies. Of the five included ACP studies (Table 1), four used only healthy subjects as controls (6, 10, 32, 33). The Derikx study (9) additionally used German subjects with alcohol dependence (AD) and alcohol-associated liver cirrhosis (ALC) as controls. We therefore limited this analysis exclusively to the German data and tested the fit of the three genetic models to ACP association in the context of three control datasets. Again, the additive model provided the best fit to each dataset as evaluated by the AIC and by Anova tests against the general model. Pearson's residuals did not indicate that the models fitted were unreasonable and in each dataset tested, a clearly significant correlation between genotypes of rs10273639 and the case/control status was observed as evidenced by the significant ORs (see Supplementary Table S1).

A synergistic interaction is evident between the common *PRSS1-PRSS2* haplotype and alcohol consumption status

Using data from ACP and NACP patients from Germany, France, Netherlands, Japan and Hungary (Table 4), we first performed case-only re-analyses of each dataset to estimate the OR of the interaction between alcohol-consumption and copies of the *PRSS1/PRSS2* haplotype. Following the evaluation of different genetic models in NACP and ACP patients, we selected the best-performing additive genetic model for this re-analysis. Subsequently, we performed a meta-analysis of these new results in order to calculate a pooled estimate for the interaction term and to assess the heterogeneity of the studies. Application of the test for heterogeneity confirmed that the five studies were homogeneous ($\chi^2 = 4.78$, $P = 0.31$; $I^2 = 16.4\%$). The pooled OR (under a fixed effect model) of the interaction (or synergy index SIM) was 1.41 (95% CI 1.26–1.58; $P = 2.80 \times 10^{-9}$). The OR being significantly greater than one indicates that the effects between alcohol exposure and the haplotype are more than multiplicative (synergistic effect).

To fully characterize the importance of the interaction, we fitted a multinomial logistic regression model to estimate, within a single framework, the risks associated with the *PRSS1-PRSS2* haplotype in both ACP and NACP cases (Table 5). For this analysis, control groups were required from each population. It should be noted that the purpose of the model is to compare the ACP and NACP against a common baseline and hence no assumption is made about alcohol consumption in the control groups. The multinomial model included co-variables indicating the country- and study-origin of the data, and we again applied the additive genetic model for the genetic effects. The OR for the *PRSS1/PRSS2* haplotype was 1.16 (95% CI 1.07–1.26) in the NACP patients and increased to 1.69 (95% CI 1.54–1.84) in the ACP patients. The difference between these two ORs is statistically significant, as evidenced by the non-overlapping confidence intervals, again signaling the presence of an interaction between the genetic-effect of the haplotype and alcohol consumption. This model allows for a succinct demonstration that alcohol consumption significantly increases the risk associated with the presence of copies of the haplotype on a multiplicative scale across the five populations.

DISCUSSION

We first performed an allele-based meta-analysis of the currently available studies of the association between the common *PRSS1-PRSS2* haplotype and ACP and NACP, demonstrating a significant association in both contexts. Possible causes underlying the previous contentious NACP findings included small sample size, ethnic and population differences in genetic predisposition to CP, and differences in patient selection. For example, using only German samples, Derikx and colleagues

observed a trend towards higher genetic effect sizes in patient groups characterized by a later age of disease onset (9), a possible explanation for the lack of a significant association in the French cohort (see Table 2). More specifically, a significant association was found in the German patients whose age of disease onset was ≥ 20 years but not in those whose age of disease onset was 10-19 years or < 10 years; the age of disease onset was invariably < 20 years in all French patients. Additionally, it should be noted that *PRSS1-PRSS2* rs10273639 was included in a recent meta-analysis, in which ACP and NACP were analyzed together (38).

Having confirmed an association of the common *PRSS1-PRSS2* haplotype with NACP, we further explored the underlying biological basis of this association from two complementary standpoints. As mentioned earlier, the risk [C] allele of rs10273639C/T has been found to be positively associated with *PRSS1* mRNA expression in pancreatic tissue. More specifically, *PRSS1* expression levels were highest in rs10273639[C] homozygotes, intermediate in heterozygotes and lowest in rs10273639[T] homozygotes (6). However, the number of pancreatic tissue samples subjected to quantitative *PRSS1* mRNA expression analysis was limited. We therefore evaluated the effect of rs10273639 genotypes on pancreatic trypsinogen gene expression using the GTEx dataset and found the level of *PRSS2* mRNA expression to correlate directly with the dosage of the risk [C] allele of rs10273639. A role for *PRSS2* in CP is supported by four lines of evidence. Firstly, a loss-of-function *PRSS2* variant is known to be protective against CP (39). Secondly, CP-causing or predisposing trypsinogen copy number variants involve both *PRSS1* and *PRSS2* (5, 40). Thirdly, the *CTRB1-CTRB2* inversion increases CP risk by influencing protective *PRSS2* degradation (30). Finally, transgenic expression of human *PRSS2* exacerbates pancreatitis in mice (41). A dosage effect of the risk allele on *PRSS1/PRSS2* expression in the pancreas implies that the additive gene model represents the best fit to explain the disease association data. Thus, the second approach we adopted was to test this hypothesis by a series of statistical analyses. We demonstrated that the additive genetic model provided the best fit in both the ACP and NACP contexts and the genetic effect of the rs10273639 CC genotype was approximately double that of the CT genotype. Taken together, these findings provide new insights into the pivotal role of increased trypsin in the etiology of CP as well as the genotype-phenotype relationship in ACP/NACP.

Alcohol is an established environmental risk factor for CP (3). However, only 3-5% of heavy drinkers develop the disease (42), suggesting the involvement of additional genetic or environmental factors in the causation of ACP. A higher OR of the common *PRSS1-PRSS2* risk allele associated with ACP than with NACP suggests a gene-environment interaction between the common haplotype and alcohol consumption in CP. By comparing allele distributions at rs10273639 between 447 ACP patients and 1,129 NACP patients, Whitcomb and colleagues (6) suggested the existence of an interaction between the *PRSS1-PRSS2* haplotype and alcohol although they did not attempt to quantify it. Herein, by fitting a similar but more sophisticated model and by combining inference from data of several studies, we confirm that there is indeed a synergistic interaction between the *PRSS1-PRSS2* haplotype and alcohol consumption status i.e. the risk associated with the presence of the *PRSS1-PRSS2* haplotype and exposure to alcohol was significantly greater than the product of the risks associated with each factor singly. Our results are in accordance with those of Whitcomb and colleagues but here, by first determining the correct genetic model (additive), and second by applying multinomial logistic regression, a more complete characterisation of the etiology of CP regarding the *PRSS1-PRSS2* haplotype and alcohol consumption was possible.

This study has several limitations. For example, we could not exclude publication bias in terms of some of the included studies. We did not perform funnel plots to assess this possibility following advice that such a test should be used only when there are at least 10 eligible studies; otherwise the power of the test is too low to distinguish a chance effect from genuine asymmetry (13, 14). Despite lacking detailed records of individual alcohol consumption levels, we have studied the role of alcohol by comparing ACP and NACP cohorts. We can be confident that these two groups will have experienced quite different levels of alcohol consumption, and hence the methods applied here are appropriate for detecting and describing a gene-environment interaction. More detailed data

regarding the environmental exposure variable could enable more precision in the characterization of the interaction. Moreover, there may exist subtle differences between different cohorts in terms of ACP and NACP definitions. Furthermore, none of the included studies employed sex- and age-matched controls. However, we consider it unlikely that either of the abovementioned limitations would affect the main conclusions drawn owing to the mechanistic evidence underpinning the association data.

In conclusion, the results of our allele-based meta-analysis demonstrated significant association of the common *PRSS1-PRSS2* haplotype with both ACP and NACP. In addition, we have provided new information that sheds light on the pivotal role of uncontrolled trypsin in CP. Finally, we have refined the gene-environment interaction between the common *PRSS1-PRSS2* haplotype and alcohol consumption status in the etiology of CP. These findings help to improve our understanding of the complex etiology of CP and strengthen the notion that common risk factors (6, 30, 43) should be considered in risk assessment in the clinical setting. Acute pancreatitis patients carrying the common *PRSS1-PRSS2* haplotype should consider greatly reducing their alcohol consumption or abstaining altogether.

Author contributions: A.F.H. and E.G. designed and performed model testing and gene-environment interaction analysis and wrote the corresponding text. D.N.C. contributed to data interpretation and critically revised the manuscript with important intellectual input. E.M. assisted in performing the literature search and data extraction and contributed to the revision of the manuscript. C.F. contributed to the conception of the study, revised the paper, and provided overall supervision. J.-M.C. conceived the study, performed the literature search, data extraction and meta-analysis, and wrote the paper. All authors have approved the final draft submitted.

Financial support: This work was supported by the Institut National de la Santé et de la Recherche Médicale (INSERM), France. The funding source did not play any roles in the study design, collection, analysis, and interpretation of the data and in the writing of the report.

Potential competing interests: None.

Acknowledgments

We are grateful to the authors who published studies that contributed data for our meta-and re-analyses. Appendix Figure 1 was obtained from the Genotype-Tissue Expression (GTEx) Portal; the GTEx Project was supported by the Common Fund of the Office of the Director of the National Institutes of Health, and by NCI, NHGRI, NHLBI, NIDA, NIMH and NINDS.

Data availability

All data relevant to the study are included in the article or uploaded as supplementary information.

REFERENCES

1. Mayerle J, Sendler M, Hegyi E, et al. Genetics, cell biology, and pathophysiology of pancreatitis. *Gastroenterology* 2019;156(7):1951-68 e1.
2. Kleeff J, Whitcomb DC, Shimosegawa T, et al. Chronic pancreatitis. *Nat Rev Dis Primers* 2017;3:17060.
3. Hegyi P, Parniczky A, Lerch MM, et al. International consensus guidelines for risk factors in chronic pancreatitis. Recommendations from the working group for the international consensus guidelines for chronic pancreatitis in collaboration with the International Association of Pancreatology, the American Pancreatic Association, the Japan Pancreas Society, and European Pancreatic Club. *Pancreatology* 2020;20(4):579-85.
4. Whitcomb DC, Gorrry MC, Preston RA, et al. Hereditary pancreatitis is caused by a mutation in the cationic trypsinogen gene. *Nat Genet* 1996;14(2):141-5.

5. Le Maréchal C, Masson E, Chen JM, et al. Hereditary pancreatitis caused by triplication of the trypsinogen locus. *Nat Genet* 2006;38(12):1372-4.
6. Whitcomb DC, LaRusch J, Krasinskas AM, et al. Common genetic variants in the *CLDN2* and *PRSS1-PRSS2* loci alter risk for alcohol-related and sporadic pancreatitis. *Nat Genet* 2012;44(12):1349-54.
7. Boulling A, Sato M, Masson E, et al. Identification of a functional *PRSS1* promoter variant in linkage disequilibrium with the chronic pancreatitis-protecting rs10273639. *Gut* 2015;64(11):1837-8.
8. Hegyi E, Sahin-Tóth M. Genetic risk in chronic pancreatitis: the trypsin-dependent pathway. *Dig Dis Sci* 2017;62(7):1692-701.
9. Derikx MH, Kovacs P, Scholz M, et al. Polymorphisms at *PRSS1-PRSS2* and *CLDN2-MORC4* loci associate with alcoholic and non-alcoholic chronic pancreatitis in a European replication study. *Gut* 2015;64(9):1426-33.
10. Hegyi E, Toth AZ, Vincze A, et al. Alcohol-dependent effect of *PRSS1-PRSS2* haplotype in chronic pancreatitis. *Gut* 2020;69(9):1-2.
11. R Core Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing V, Austria. <https://www.R-project.org/>. Accessed September 8, 2020.
12. Online Sample Size Estimator (OSSE). <http://osse.bii.a-star.edu.sg/calculation1.php>. Accessed September 4, 2020.
13. Review Manager (RevMan). Version 5.3. Copenhagen: The Nordic Cochrane Centre TCC, 2014. <https://review-manager.software.informer.com/5.3/>. Accessed September 8, 2020.
14. Sterne JA, Sutton AJ, Ioannidis JP, et al. Recommendations for examining and interpreting funnel plot asymmetry in meta-analyses of randomised controlled trials. *BMJ* 2011;343:d4002.
15. Sedgwick P, Marston L. How to read a funnel plot in a meta-analysis. *BMJ* 2015;351:h4718.
16. Moher D, Liberati A, Tetzlaff J, et al. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *PLoS Med* 2009;6(7):e1000097.
17. PROSPERO (International prospective register of systematic reviews). <https://www.crd.york.ac.uk/prospero/>. Accessed October 6, 2020.
18. The Genotype-Tissue Expression (GTEx) dataset. <http://www.gtexportal.org/home/>. Accessed July 16, 2020.
19. Lewis CM. Genetic association studies: design, analysis and interpretation. *Brief Bioinform* 2002;3(2):146-53.
20. Balduzzi S, Rucker G, Schwarzer G. How to perform a meta-analysis with R: a practical tutorial. *Evid Based Ment Health* 2019;22(4):153-60.
21. Kzama R, Babron MC, Genin E. Genetic association and gene-environment interaction: a new method for overcoming the lack of exposure information in controls. *Am J Epidemiol* 2011;173(2):225-35.
22. Venables WN, Ripley BD. *Modern Applied Statistics with S*, Fourth edition. Springer, New York. <http://www.stats.ox.ac.uk/pub/MASS4>. Accessed September 8, 2020.
23. Khoury MJ, Flanders WD. Nontraditional epidemiologic approaches in the analysis of gene-environment interaction: case-control studies with no controls! *Am J Epidemiol* 1996;144(3):207-13.
24. Ottman R. Gene-environment interaction: definitions and study designs. *Prev Med* 1996;25(6):764-70.
25. Angsuwatcharakon P, Sodsai P, Rerknimitr R, et al. The polymorphisms at *PRSS1-PRSS2* and *MORC4* loci and the risk of post-ERCP pancreatitis. *Gastroenterol Res Pract* 2018;2018:1064783.
26. Wolthers BO, Frandsen TL, Patel CJ, et al. Trypsin-encoding *PRSS1-PRSS2* variations influence the risk of asparaginase-associated pancreatitis in children with acute lymphoblastic

- leukemia: a Ponte di Legno toxicity working group report. *Haematologica* 2019;104(3):556-63.
27. Nabi Z, Talukdar R, Venkata R, et al. Genetic evaluation of children with idiopathic recurrent acute pancreatitis. *Dig Dis Sci* 2020.
28. Weiss FU, Hesselbarth N, Parniczky A, et al. Common variants in the *CLDN2-MORC4* and *PRSS1-PRSS2* loci confer susceptibility to acute pancreatitis. *Pancreatology* 2018.
29. Polonikov AV, Samgina TA, Nazarenko PM, et al. Alcohol consumption and cigarette smoking are important modifiers of the association between acute pancreatitis and the *PRSS1-PRSS2* locus in men. *Pancreas* 2017;46(2):230-6.
30. Rosendahl J, Kirsten H, Hegyi E, et al. Genome-wide association study identifies inversion in the *CTRB1-CTRB2* locus to modify risk for alcoholic and non-alcoholic chronic pancreatitis. *Gut* 2018;67(10):1855-63.
31. Chhatrriya B, Paine SK, Das S, et al. Single nucleotide polymorphisms in *PRSS1* and *CASR* genes are associated with chronic pancreatitis in North-Eastern region of India. *J Gastrointest Liver Dis* 2020;29(2):267-8.
32. Masamune A, Nakano E, Hamada S, et al. Common variants at *PRSS1-PRSS2* and *CLDN2-MORC4* loci associate with chronic pancreatitis in Japan. *Gut* 2015;64(8):1345-6.
33. Giri AK, Midha S, Banerjee P, et al. Common variants in *CLDN2* and *MORC4* genes confer disease susceptibility in patients with chronic pancreatitis. *PLoS One* 2016;11(1):e0147345.
34. Avanthi SU, Ravi Kanth VV, Agarwal J, et al. Association of claudin2 and *PRSS1-PRSS2* polymorphisms with idiopathic recurrent acute and chronic pancreatitis: A case-control study from India. *J Gastroenterol Hepatol* 2015;30(12):1796-801.
35. Paliwal S, Bhaskar S, Nageshwar Reddy D, et al. Association analysis of *PRSS1-PRSS2* and *CLDN2-MORC4* variants in nonalcoholic chronic pancreatitis using tropical calcific pancreatitis as model. *Pancreas* 2016;45(8):1153-7.
36. Campa D, Pastore M, Capurso G, et al. Do pancreatic cancer and chronic pancreatitis share the same genetic risk factors? A PANcreatic Disease ReseArch (PANDORA) consortium investigation. *Int J Cancer* 2018;142(2):290-6.
37. Wells GA, Shea B, O'Connell D, et al. The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomised studies in meta-analyses. http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp. Accessed October 6, 2020.
38. Deng Y, Li Z. Effects of *PRSS1-PRSS2* rs10273639, *CLDN2* rs7057398 and *MORC4* rs12688220 polymorphisms on individual susceptibility to pancreatitis: A meta-analysis. *Genomics* 2020;112(1):848-52.
39. Witt H, Sahin-Toth M, Landt O, et al. A degradation-sensitive anionic trypsinogen (*PRSS2*) variant protects against chronic pancreatitis. *Nat Genet* 2006;38(6):668-73.
40. Masson E, Le Maréchal C, Chandak GR, et al. Trypsinogen copy number mutations in patients with idiopathic chronic pancreatitis. *Clin Gastroenterol Hepatol* 2008;6(1):82-8.
41. Wan J, Haddock A, Edenfield B, et al. Transgenic expression of human *PRSS2* exacerbates pancreatitis in mice. *Gut* 2020.
42. Irving HM, Samokhvalov AV, Rehm J. Alcohol as a risk factor for pancreatitis. A systematic review and meta-analysis. *JOP* 2009;10(4):387-92.
43. LaRusch J, Lozano-Leon A, Stello K, et al. The common chymotrypsinogen C (*CTRC*) variant G60G (c.180T) increases risk of chronic pancreatitis but not recurrent acute pancreatitis in a north american population. *Clin Transl Gastroenterol* 2015;6:e68.

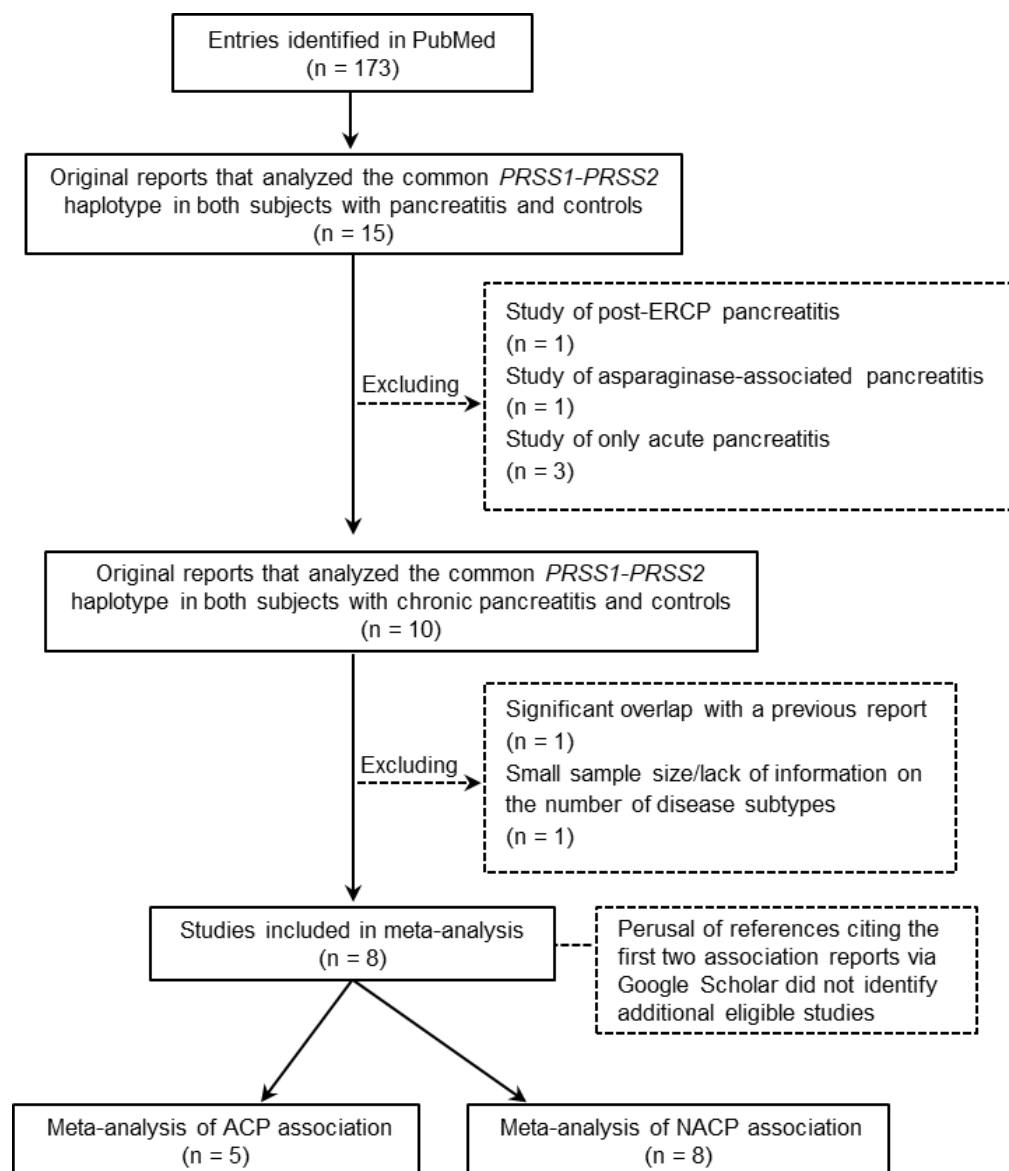


Figure 1. Flow chart of the search and selection process for the studies used for the allele-based meta-analysis. ACP, alcoholic chronic pancreatitis. ERCP, endoscopic retrograde cholangio-pancreatography. NACP, non-alcoholic chronic pancreatitis.

ACP

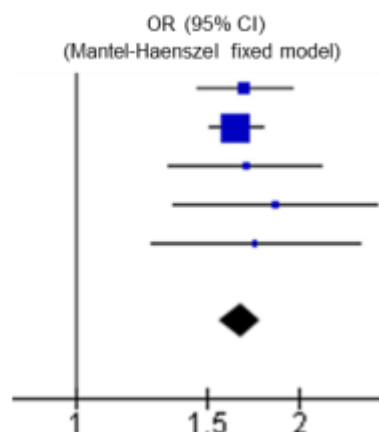
Study	Weight
Whitcomb et al. (2012) ⁶	20.0%
Derikx et al. (2015) ⁹	65.3%
Masamune et al. (2015) ³²	7.0%
Giri et al. (2016) ³³	3.7%
Hegyi et al. (2020) ¹⁰	4.0%

Pooled OR (95% CI): 1.67 (1.56–1.78)

Total events: patients (3610/5556); controls (16512/30316)

Heterogeneity: $Chi^2 = 0.68$, $df = 4$ ($P = 0.95$); $I^2 = 0\%$

Test for overall effect: $Z = 15.52$ ($P < 0.00001$)



NACP

Study	Weight
Whitcomb et al. (2012) ⁶	20.4%
Derikx et al. (2015) ⁹	20.3%
Masamune et al. (2015) ³²	7.8%
Avanthi et al. (2015) ³⁴	4.2%
Giri et al. (2016) ³³	14.2%
Paliwal et al. (2016) ³⁵	13.5%
Campa et al. (2018) ³⁶	13.9%
Hegyi et al. (2020) ¹⁰	5.7%

Pooled OR (95% CI): 1.28 (1.17 – 1.40)

Total events: patients (4341/8094); controls (21471/39936)

Heterogeneity: $Chi^2 = 15.81$, $df = 7$ ($P = 0.03$); $I^2 = 56\%$

Test for overall effect: $Z = 5.52$ ($P < 0.00001$)

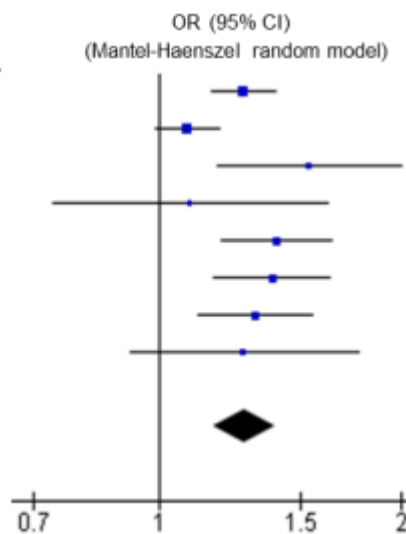


Figure 2. Results of the allele-based meta-analysis of the association between the common *PRSS1-PRSS2* haplotype and ACP or NACP, and the corresponding forest plots. Total events in patients and controls: number of risk alleles/number of total alleles. See Table 1 for the risk allele distribution data, OR (95% CI) and P values in the context of each individual study. ACP, alcoholic chronic pancreatitis. CI, confidence interval. NACP, non-alcoholic chronic pancreatitis. OR, odds ratio.

Table 1. Basic characteristics of the included studies and corresponding risk allele frequencies for the common *PRSSI-PRSS2* haplotype-tagging SNPs in patients and controls

Study	Study population(s)	Risk allele of the tagging SNP	Number of patients/controls	Allele frequency in patients	Allele frequency in controls	OR (95% CI)	P value (χ^2 test)
ACP							
Whitcomb et al. (2012) ⁶	Mainly Americans with European ancestry and a small number of German and British subjects	rs10273639[C]	447 ^a /8029	69.6% (622/894)	57.6% (9249/16058)	1.68 (1.45 – 1.95)	2.03e-12
Derikx et al. (2015) ⁹	European (from 9 countries)	rs10273639[C]	1854/5065	69.0% (2560/3708)	57.6% (5835/10130)	1.64 (1.52 – 1.78)	2.20e-16
Masamune et al. (2015) ³²	Japanese	rs10273639[C]	272/480	32.5% (177/544)	22.2% (213/960)	1.69 (1.34 – 2.14)	1.43e-5
Giri et al. (2016) ³³	Indian	rs2855983[G]	85/1288	48.8% (83/170)	34.0% (876/2576)	1.85 (1.36 – 2.53)	0.00012
Hegyi et al. (2020) ¹⁰	Hungarian	rs6666[C]	120/296	70.0% (168/240)	57.3% (339/592)	1.74 (1.26 – 2.40)	0.00086
NACP							
Whitcomb et al. (2012) ⁶	Mainly Americans with European ancestry and a small number of German and British subjects	rs10273639[C]	1129 ^b /8029	63.4% (1431/2258)	57.6% (9249/16058)	1.27 (1.16 – 1.40)	2.10e-7
Derikx et al. (2015) ⁹	European (from three countries)	rs10273639[C]	1192/4323	59.6% (1421/2384)	57.6% (4981/8646)	1.09 (0.99 – 1.19)	0.085
Masamune et al. (2015) ³²	Japanese	rs10273639[C]	197/480	30.5% (120/394)	22.2% (213/960)	1.54 (1.18 – 2.00)	0.0017
Avanthi et al. (2015) ³⁴	Indian	rs10273639[C]	96/156	30.7% (59/192)	28.9% (90/312)	1.09 (0.74 – 1.62)	0.73
Giri et al. (2016) ³³	Indian	rs2855983[G]	434/1288	42.0% (364/868)	34.0% (876/2576)	1.40 (1.20 – 1.64)	3.07e-5
Paliwal et al. (2016) ³⁵	Indian	rs10273639[C]	551/801	33.8% (372/1102)	26.9% (431/1602)	1.38 (1.17 – 1.64)	0.00015
Campa et al. (2018) ³⁶	European (from 8 countries)	rs10273639[C]	345/4580	64.4% (444/690)	57.8% (5292/9160)	1.32 (1.12 – 1.55)	0.00085
Hegyi et al. (2020) ¹⁰	Hungarian	rs6666[C]	103/296	63.1% (130/206)	57.3% (339/592)	1.28 (0.92 – 1.77)	0.17

^aIncluding 113 patients with recurrent acute pancreatitis.^bIncluding 462 patients with recurrent acute pancreatitis.

Abbreviations: ACP, alcoholic chronic pancreatitis; CI, confidence interval; NACP, non-alcoholic chronic pancreatitis; OR, odds ratio; SNP, single nucleotide polymorphism.

Table 2. Comparison of the rs10273639[C] allele frequencies in the NACP patients and controls in the context of each population studied by Derikx et al. (ref. 9)

Population	Number of patients/controls	All frequency in patients	Allele frequency in controls	OR (95% CI)	<i>P</i> value (χ^2 test)
German	690/2825	62.97% (869/1380)	58.50% (3305/5650)	1.21 (1.07 – 1.36)	0.0027
French	415/1064	54.10% (449/830)	54.61% (1162/2128)	0.98 (0.83 – 1.15)	0.83
Dutch	87/434	59.20% (103/174)	59.22% (514/868)	1.00 (0.72 – 1.39)	1.00

Abbreviations: OR, odds ratio; CI, confidence interval; NACP, non-alcoholic chronic pancreatitis.

Table 3. Test of the fit of different genetic models to the gene association data in three NACP cohorts

NACP cohort	Genotypes of rs10273639			Dominant model			Recessive model			Additive model			General model		
	CC	CT	TT	CC	CT	TT	CC	CT	TT	CC	CT	TT	CC	CT	TT
German ^a				Pearson's residuals			Pearson's residuals			Pearson's residuals			Pearson's residuals		
Patients	274	321	95	1.97			2.31			1.88			1.90		
Controls	985	1335	505	-0.51			-0.43			-0.53			-0.53		
Japanese ^b															
Patients	17	86	94	1.36			1.75			1.12			1.14		
Controls	22	169	289	-0.73			-0.57			-0.89			-0.90		
Indian ^c															
Patients	68	236	247	1.09			1.33			0.97			0.99		
Controls	67	297	437	-0.91			-0.75			-1.03			-1.01		
OR (95% CI); <i>P</i> value	German			1.36 (1.08 – 1.73); 0.010			1.23 (1.04 – 1.46); 0.017			1.20 (1.07 – 1.36); 0.0027			-		
	Japanese			1.66 (1.19 – 2.32); 0.0029			1.97 (1.02 – 3.79); 0.040			1.55 (1.19 – 2.03); 0.0013			-		
	Indian			1.48 (1.19 – 1.84); 0.00044			1.54 (1.08 – 2.20); 0.017			1.36 (1.16 – 1.61); 0.00020			-		
AIC	German			3478.59			3479.89			3476.41			3478.101		
	Japanese			811.68			816.59			810.28			812.27		
	Indian			1819.40			1826.12			1817.92			1819.79		
ANOVA test against General model (<i>P</i> value)	German			0.115			0.052			0.581			-		
	Japanese			0.235			0.0120			0.945			-		
	Indian			0.205			0.00389			0.710			-		

^aUsing NACP data from Derikx et al. (9).^bUsing NACP data from Masamune et al. (32).^cUsing NACP data from Paliwal et al. (35).

Abbreviations: AIC, Akaike information criterion; CI, confidence interval; OR, odds ratio; NACP, non-alcoholic chronic pancreatitis.

Table 4. Estimation of gene-environment interaction between the common *PRSS1-PRSS2* haplotype and alcohol consumption using a case-only analysis

Population ^a	OR (95% CI) ^b	<i>P</i> value
German	1.43 (1.23 – 1.66)	4.14×10^{-6}
French	1.54 (1.11 – 2.15)	0.010
Dutch	1.84 (1.25 – 2.73)	0.0022
Japanese	1.11 (0.83 – 1.49)	0.48
Hungarian	1.34 (0.91 – 1.98)	0.14
Pooled (fixed effect model)	1.41 (1.26 – 1.58)	2.80×10^{-9}

^aOriginal German, French and Dutch data used for analysis were from Derikx et al. (9). Original Japanese and Hungarian data used for analysis were from Masamune et al. (32) and Hegyi et al. (10), respectively.

^bFor the effect of the risk genotypes in the alcoholic chronic pancreatitis group against the non-alcoholic chronic pancreatitis group, equivalent to an estimate of the multiplicative-scale interaction term (or synergy index) between *PRSS1/PRSS2* and alcohol consumption.
Abbreviations: CI, confidence interval; OR, odds ratio.

Table 5. Test of gene-environment interaction between the common *PRSS1-PRSS2* haplotype and alcohol consumption using a multinomial logistic regression model

Groups	Population ^a	Genotypes of the common haplotype ^b			OR (95% CI) ^c
		CC	CT	TT	
Controls	German	985	1335	505	–
	French	316	530	218	
	Dutch	146	222	66	
	Japanese	22	169	289	
	Hungarian	98	143	55	
NACP	German	274	321	95	1.16 (1.07 – 1.26)
	French	130	189	96	
	Dutch	28	47	12	
	Japanese	17	86	94	
	Hungarian	44	42	17	
ACP	German	433	358	73	1.69 (1.54 – 1.84)
	French	36	45	9	
	Dutch	115	102	15	
	Japanese	23	131	118	
	Hungarian	59	50	11	

^aOriginal German, French and Dutch data used for analysis were from Derikx et al. (9). Original Japanese and Hungarian data used for analysis were from Masamune et al. (32) and Hegyi et al. (10), respectively.

^bGenotypes for the German, French, Dutch and Japanese cohorts refer to rs102373639 whilst those for the Hungarian cohort refer to rs6666.

^cFor the effect of the risk genotypes in the ACP or NACP group against the control group.