ORIGINAL ARTICLE

Polymorphisms at *PRSS1–PRSS2* and *CLDN2–MORC4* loci associate with alcoholic and non-alcoholic chronic pancreatitis in a European replication study

Monique H Derikx,[1,*] Peter Kovacs,[2,*] Markus Scholz,[3,4,*] Emmanuelle Masson,[5,6,7,8] Jian-Min Chen,[5,6,7,8] Claudia Ruffert,[9,*] Peter Lichtner,[10,*] Rene H M te Morsche,[1,*] Giulia Martina Cavestro,[11,*] PanEuropean Working group on Alcoholic Chronic Pancreatitis members and collaborators,[†] Claude Férec,[5,6,7,8] Joost P H Drenth,[1,*] Heiko Witt,[12,*] Jonas Rosendahl[9,*]

▶ Additional material is published online only. To view please visit the journal online (http://dx.doi.org/10.1136/gutjnl-2014-307453).

For numbered affiliations see end of article.

Correspondence to
Dr Jonas Rosendahl, Department of Internal Medicine, Neurology and Dermatology, Division of Gastroenterology and Rheumatology, University Clinic of Leipzig, Liebigstraße 20, Leipzig 04103, Germany; jonas.rosendahl@medizin.uni-leipzig.de

*Members of the PanEuropean Working group on Alcoholic Chronic Pancreatitis.

†Names and affiliations of the rest of the co-authors are listed at the end of the manuscript.

MHD, PK, MS, EM, CF, JPHD and HW contributed equally.

Received 11 April 2014
Revised 18 August 2014
Accepted 21 August 2014
Published Online First 24 September 2014

ABSTRACT

Objective Several genetic risk factors have been identified for non-alcoholic chronic pancreatitis (NACP). A genome-wide association study reported an association of chronic pancreatitis (CP) with variants in *PRSS1–PRSS2* (*rs10273639*; near the gene encoding cationic trypsinogen) and *CLDN2–MORC4* loci (*rs7057398* in *RIPPLY1* and *rs12688220* in *MORC4*). We aimed to refine these findings in a large European cohort.

Design We studied 3062 patients with alcohol-related CP (ACP) or NACP and 5107 controls. Also, 1559 German patients with alcohol-associated cirrhosis or alcohol dependence were included for comparison. We performed several meta-analyses to examine genotype–phenotype relationships.

Results Association with ACP was found for *rs10273639* (OR, 0.63; 95% CI 0.55 to 0.72). ACP was also associated with variants *rs7057398* and *rs12688220* in men (OR, 2.26; 95% CI 1.94 to 2.63 and OR, 2.66; 95% CI 2.21 to 3.21, respectively) and in women (OR, 1.57; 95% CI 1.14 to 2.18 and OR 1.71; 95% CI 1.41 to 2.07, respectively). Similar results were obtained when German patients with ACP were compared with those with alcohol-associated cirrhosis or alcohol dependence. In the overall population of patients with NACP, association with *rs10273639* was absent (OR, 0.93; 95% CI 0.79 to 1.01), whereas *rs7057398* of the X chromosomal single nucleotide polymorphisms was associated with NACP in women only (OR, 1.32; 95% CI 1.15 to 1.51).

Conclusions The single-nucleotide polymorphisms *rs10273639* at the *PRSS1–PRSS2* locus and *rs7057398* and *rs12688220* at the *CLDN2–MORC4* locus are associated with CP and strongly associate with ACP, but only *rs7057398* with NACP in female patients.

Significance of this study

What is already known on this subject?

- Genetic associations for non-alcoholic chronic pancreatitis (NACP) in *PRSS1*, *PRSS2*, *CFTR*, *SPINK1*, *CTRC* and *CPA1*, as well as a gene-dosage effect of *PRSS1-PRSS2* locus have been identified.
- Alcohol misuse is the predominant cause of chronic pancreatitis (CP); however, only 3%–5% of alcohol misusers develop the disease. This implicates genetic susceptibility factors, which have not been elucidated so far.
- A recent genome-wide association study (GWAS) reported *PRSS1–PRSS2* and *CLDN2–MORC4* locus variants that affect risk for CP, and the data have not been replicated up to now.

What are the new findings?

- This study in a large European cohort replicates and refines the impact of *PRSS1-PRSS2* and *CLDN2-MORC4* locus variants as susceptibility factors predominantly in ACP.
- Variants at both loci are susceptibility factors for ACP and not alcohol misuse per se according to our comparison with alcohol-dependent and patients with alcoholic liver cirrhosis.
- Risk increase for the X chromosomal *CLDN2-MORC4* locus is comparable in men and women. As such this factor does not explain any sex differences in disease susceptibility.

How might it impact on clinical practice in the foreseeable future?

- The replication and refinement of the recently identified susceptibility variants justifies further studies on their functional properties.
- By the identification of new pathways, new strategies for influencing the clinical picture of NACP and ACP might be developed in the long term.

To cite: Derikx MH, Kovacs P, Scholz M, *et al*. *Gut* 2015;**64**:1426–1433.

INTRODUCTION

The genetic susceptibility to chronic pancreatitis (CP) is best illustrated by the discovery of cationic trypsinogen mutations (*PRSS1* HGNC:9475) in families with autosomal-dominant inherited pancreatitis.[1] There is also strong evidence that genetic variants

contribute to cases of CP without a clear inheritance pattern. Indeed, idiopathic CP (ICP) is associated with genetic alterations in *CFTR* (HGNC:1884), *SPINK1* (HGNC:11244), *PRSS2* (HGNC:9483), *CTRC* (HGNC:2523) and *CPA1* (HGNC:2296).[2–7] The association of genetic variants and disease susceptibility is less clear for alcohol-related CP (ACP). There is a low enrichment of *SPINK1* (p.N34S) and *CTRC* (p.R254W) alleles in ACP populations, and no other consistent genetic risk contributors have been described.[5 8] Similar to ICP, the *PRSS2* p.G191R variant protects against ACP development.[2] All these associations have been discovered through candidate-driven genetic association studies.

A recent paper described a different approach and reported novel risk and protecting loci for CP identified through a genome-wide association study (GWAS). A number of variants in the *PRSS1–PRSS2* but also the *CLDN2–MORC4* locus (Claudin 2; HGNC:2041; RIPPLY1, ripply transcriptional repressor 1, HGNC:25117; MORC4, MORC family CW-type zinc finger 4, HGNC:23485) were captured as risk factors for CP.[9] This study investigated patients with different types of CP as well as recurrent acute pancreatitis (RAP) and stratified individuals into alcohol-related and alcohol-unrelated pancreatitis groups. In a first screening cohort, three single nucleotide polymorphisms (SNPs) *rs10273639* (in the *PRSS1–PRSS2* locus on chromosome 7, in perfect linkage disequilibrium with *rs2011216* in intron 1 and *rs6667* in exon 5 of *PRSS1*), *rs7057398* and *rs12688220* (both in a new locus, *CLDN2–MORC4* on the X chromosome) reached genome-wide significance. After scrutiny, the *PRSS1–PRSS2 rs10273639 T* allele appeared to protect against CP, whereas *RIPPLY1 rs7057398 C* allele and *MORC4 rs12688220 T* allele increased disease susceptibility.[9] There is some biological plausibility for the association with the *PRSS1–PRSS2* locus as it may disturb the balance of pancreatic proteases and antiproteases in favour of the former.[10 11] Claudin 2 represents a tight junction protein involved in low-resistance cation-selective ion and water transport between endothelial cells.[12 13] One might speculate that *CLDN2–MORC4* locus variants lead to miss-localisation of pancreatic CLDN2 that hampers its biological function. However, this speculation warrants further experimental support.

Prior to the design of experimental studies that focus on the biological role of these variants, it is crucial that GWAS results are replicated. This is needed to prove that results are valid and reliable to determine generalisability and to better judge the effect size of the discovered association.[14] We investigated the association of *PRSS1–PRSS2* and *CLDN2–MORC4* locus variants in a large European cohort of ACP and non-alcoholic CP (NACP) to confirm the former finding. In order to assess the effect of alcohol consumption, we further refined our analyses by including cohorts of patients with alcohol-associated cirrhosis (ALC) as well as with alcohol dependence (AD) without hepatic or pancreatic disease.

MATERIALS AND METHODS

Study subjects

The respective medical ethical review committees of all participating centres approved the study protocol and all patients gave written informed consent. The diagnosis of CP was based on two or more of the following findings: (a) presence of a typical history of recurrent pancreatitis or (b) recurrent abdominal pain typical for CP, (c) calcifications and/or (d) pancreatic ductal irregularities revealed by imaging of the pancreas.[15] ACP was diagnosed in patients who had consumed at least 80 g ethanol per day for at least 2 years in men or 60 g per day for women. We labelled patients with NACP in the absence of exogenous factors such as alcohol.

ALC was diagnosed by a history of habitual ethanol intake (see ACP diagnosis above, duration at least 10 years), typical findings in liver biopsy or clinical and laboratory findings indicative for liver disease. Such laboratory and clinical findings included abnormal levels of aminotransferases, gamma glutamyl transpeptidase, coagulation tests, serum albumin concentration, platelet count, complications related to liver cirrhosis such as oesophageal varices, ascites, hepatic encephalopathy and typical liver morphology in imaging studies. Other aetiologies of liver cirrhosis were excluded by standard laboratory tests.

Patients with AD were recruited from psychiatric and addiction medicine departments in different cities across southern and central Germany. AD was diagnosed per *DSM-IV* criteria by consensus of two clinical psychiatrists. All patients were of self-reported German ancestry and did not suffer from CP or ALC.[16]

The study included 1866 patients (male, n=1567) with ACP and 1196 patients (male, n=596) with NACP from different European countries. In addition, we enrolled 5107 controls (male, n=2287), 661 German patients with ALC (male, n=480) and 898 Germans with AD (male, n=797). Characteristics of the patients and controls are summarised in table 1. More details of the controls are summarised in online supplementary table S1.

Genotyping

Details of the methods used for genotyping are summarised in the online supplementary material. As quality controls, 3% of all samples were genotyped in duplicates blinded to the investigator. The concordance rate was >98%. Call rates for *rs10273639*, *rs7057398* and *rs12688220* in the European samples were 99.1% (9641/9730), 99% (9636/9730) and 98.8% (9609/9730), respectively.

Statistical analysis

Quality of SNP genotypes was assessed by study-wise call rate and exact test for Hardy–Weinberg equilibrium in controls (female controls only for the X chromosomal SNPs). We also calculated overall statistics and performed stratified tests of Hardy–Weinberg equilibrium according to Troendle and Yu.[17] According to these measures, genotype qualities were excellent throughout. Study-wise genetic effects were determined by logistic regression analysis assuming an additive model of inheritance. For X chromosomal SNPs, we analysed the subgroups of men and women separately. Following the approach of Loley *et al*,[18] we also determined combined effects by either assuming a model of complete X inactivation (XIA) or no X inactivation (nXIA) at all. The major purpose of our study is to compare allele frequencies of risk variants between different subgroups of patients (ACP, NACP) and controls (healthy, alcohol dependent, patients with cirrhosis). Corresponding contrasts of interest are listed in online supplementary table S2. Study-wise effects were pooled by standard meta-analysis techniques as implemented in the package 'meta' of the statistical software 'R 3.0.1' (ww.r-project.org). Heterogeneity between studies was assessed using Q-statistics. Due to occasionally observed study heterogeneity, we calculated random-effect models throughout. For the purpose of model diagnostics, we analysed and compared likelihoods of XIA, nXIA and sex interaction. In figures 1, 2A, B, 3, 4A, B and 5, we present forest plots of our meta-analysis results as well as other features. Finally, we performed a stratified analysis regarding age of onset in the German population. Forest plots were generated using GraphPad Prism (V.6.0a) (San Diego). p Values <0.05 were considered statistically significant.

Table 1 Characteristics of patients and controls

	ACP				Controls			
Country	Number	Male (%)	Median age (years)	Range (years)	Number	Male (%)	Median age (years)	Range (years)
Germany	871	747 (85.8)	50	20–86	2853	1232 (43.2)	56	18–81
France	90	76 (84.4)	51	30–73	1064	552 (51.9)	n.a.	n.a.
Spain	195	169 (86.7)	50	17–85	46	23 (50)	77	44–91
The Netherlands	237	181 (76.4)	56	33–80	441	166 (37.6)	50	18–99
Hungary	29	24 (82.8)	56	40–80	35	26 (74.3)	58	25–84
Italy	256	212 (82.8)	55	27–88	326	105 (32.2)	36	18–83
Romania	68	60 (88.2)	48	28–78	69	44 (63.8)	60.5	22–88
Poland	85	71 (83.5)	51	28–98	89	41 (46.1)	50	16–91
The UK	35	27 (77.1)	42	17–62	184	98 (53.3)	53	18–104
	NACP				**Controls**			
Country	**Number**	**Male (%)**	**Median age (years)**	**Range (years)**	**Number**	**Male (%)**	**Median age (years)**	**Range (years)**
Germany	694	338 (48.7)	16	0–71	2853*	1,232* (43.2)	56*	18–81*
France	415	210 (50.6)	16	1–20	1064*	552* (51.9)	n.a.*	n.a.*
The Netherlands	87	48 (55.2)	46	7–76	441*	166* (37.6)	50*	18–99*

Median age and range of age are displayed.
*Designates controls that were used for calculations to compare results with patients with alcohol-related CP (ACP) and NACP. In addition, 661 patients with alcohol-associated cirrhosis (480 men; median age 53.5 years; age range 25–80 years) and 898 alcohol-dependent patients (797 men; median age 41 years; age range 18–80 years) from Germany were used for comparison of results with German patients with ACP.
n.a., not available; NACP, non-alcoholic chronic pancreatitis; ACP, alcohol-related chronic pancreatitis.

Online supplementary figures S1a, b and S2a, b display the results of X chromosomal analysis assuming models of complete or no X inactivation.

RESULTS

PRSS1–PRSS2 locus (*rs10273639*)

In meta-analysis, *rs10273639* showed the strongest association with ACP (OR 0.63, 95% CI 0.55 to 0.72, p value 8.5×10^{-11}). No association was observed for NACP (OR 0.93, 95% CI 0.79 to 1.08, p value 0.3). An association was also observed for the comparison between German patients with ACP and patients with ALC (OR 0.58, 95% CI 0.50 to 0.66, p value 2.6×10^{-12}). The association was also found in comparison of German patients with ACP with German patients with AD (OR 0.55, 95% CI 0.47 to 0.63, p value 2.3×10^{-16}). Similar frequencies of the SNP were observed in AD, ALC and healthy controls.

For patients with ACP coming from individual European countries, an association was apparent for Germany, France, the Netherlands, Hungary, Italy, Romania and the UK (Germany OR 0.59, 95% CI 0.52 to 0.66, p value 2.9×10^{-19}; France OR 0.64, 95% CI 0.47 to 0.88, p value 0.007; the Netherlands OR 0.56, 95% CI 0.43 to 0.72, p value 6.3×10^{-6}; Hungary OR 0.43, 95% CI 0.18 to 0.94, p value 0.04; Italy OR 0.77, 95% CI 0.60 to 0.97, p value 0.03; Romania OR 0.41, 95% CI 0.23 to 0.69, p value 0.001; the UK OR 0.53, 95% CI 0.30 to 0.91, p value 0.02). The logistic regression and meta-analysis results of *rs10273639* are summarised in figure 1, while the genotype frequencies for the groups are given in online supplementary tables S3 and S4. The *TT* genotype was underrepresented in all European patients with ACP (all patients 9.5% vs all controls 18.1%, p value 9.6×10^{-33}, except for the samples from Poland (12.9% patients vs 12.4% controls, p value 0.99)). In the NACP cohorts, this underrepresentation was found only in German patients (patients 13.8% vs controls 17.9%, p value 0.01).

RIPPLY1 (*rs7057398*)

In meta-analysis, significant associations were found for *rs7057398* in male patients with ACP (OR 2.26, 95% CI 1.94 to 2.63, p value 5.4×10^{-26}) and in female patients (OR 1.57, 95% CI 1.14 to 2.18, p value 0.007). Upon stratification by countries, we detected a significant association with male patients with ACP originating from Germany, France, Spain, the Netherlands, Italy, Romania and the UK (p values 1.6×10^{-12}, 0.0007, 0.03, 3.0×10^{-5}, 3.1×10^{-5}, 0.002 and 0.02, respectively). We obtained similar results for female patients with ACP from Germany, Poland and the UK (p value 0.004, 0.0005 and 0.04). We then assessed the strength of the association by comparison of the results obtained from patients with ALC and AD. Indeed, *rs7057398* was overrepresented in ACP relative to other alcohol-related disorders. This was especially apparent for the cohort of male German patients with ACP in comparison with ALC (OR 2.32, 95% CI 1.80 to 3.01, p value 1.1×10^{-10}) as well as with AD (OR 2.03, 95% CI 1.64 to 2.51, p value 1.2×10^{-10}). In addition, the SNP is not associated with risk of cirrhosis or AD, neither for men nor for women. Figure 2A, B summarises the results of the meta-analysis of *rs7057398* in patients with ACP. Results of XiA and nXiA are summarised in online supplementary figure S1a, b.

The genotype and allele frequencies of *rs7057398* in patients with ACP are presented in online supplementary tables S5 and S6. The *C* allele was more frequent in male patients with ACP from all European countries investigated (43.8% vs controls 27.5%, p value 10×10^{-25}) and the *C* allele was significantly overrepresented (p value 0.0001) in female patients with ACP (35.2%) compared with controls (27.3%).

We detected a significant association for *rs7057398* with NACP upon logistic regression in female patients (OR 1.30, 95% CI 1.14 to 1.49, p value 1.3×10^{-4}), but not in male patients (figure 3). Estimated genetic effect sizes are always smaller than for ACP. As shown in Supplementary tables S7 and S8, the *C* allele was slightly overrepresented in male patients with NACP (all patients: 32.6% vs 28.3%, p value 0.04; German patients:

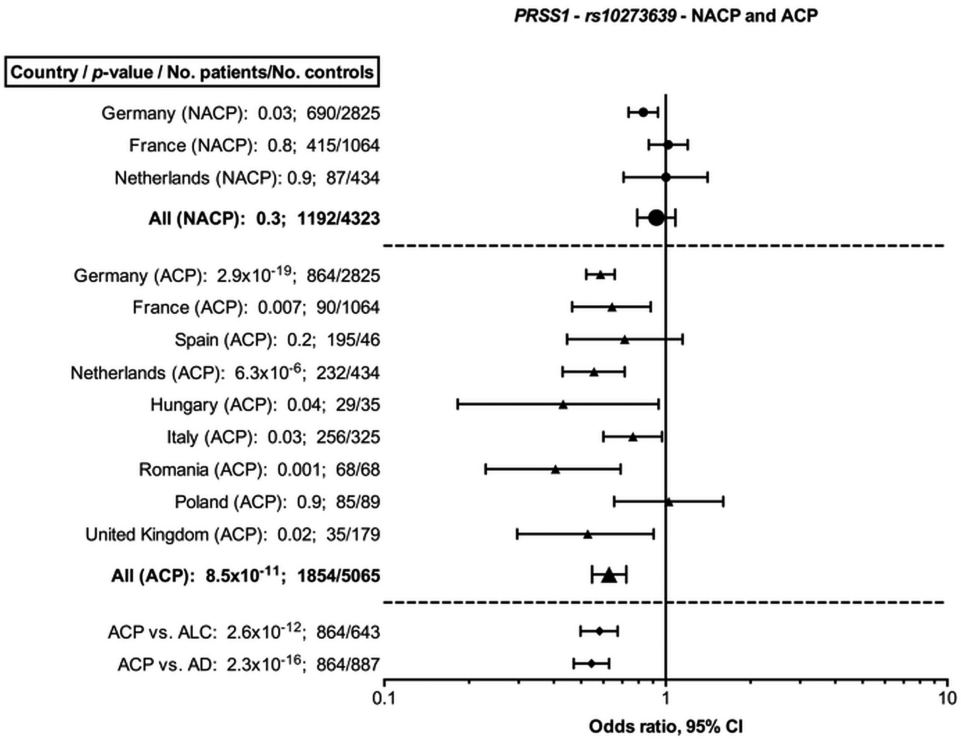


Figure 1 Meta-analysis results for *rs10273639* (*PRSS1–PRSS2*) in patients with non-alcoholic chronic pancreatitis (NACP), alcohol-related chronic pancreatitis (ACP) and comparison of German ACP patients with alcohol-associated cirrhosis (ALC) and with alcohol-dependent (AD) patients. Results are presented in a semi-log scale.

33.3% vs 27.1%, p value 0.03). Subgroup analyses revealed that in German women and in the overall female patients with NACP there was an overrepresentation of *CC* genotype (patients 10.1% vs controls 7.8%, p value 2.6×10^{-4}; patients 10.3% vs controls 8.6%, p value 4.2×10^{-5}).

MORC4 (*rs12688220*)

Similar to the results obtained for *rs7057398* in ACP, *rs12688220* was significantly associated in male (OR 2.66, 95% CI 2.21 to 3.21, p value 1.1×10^{-24}) and female patients (OR 1.71, 95% CI 1.41 to 2.07, p value 3.3×10^{-8}) with ACP (figure 4A, B). The association was also statistically significant in individual male cohorts from Germany, France, Spain, the Netherlands, Italy and Romania (p value 1.2×10^{-16}, 8.4×10^{-6}, 0.02, 2.5×10^{-6}, 5.4×10^{-6} and 0.001, respectively), as well as in the female cohorts from Germany, Poland and the UK (p value 0.0003, 0.003 and 0.03). Results of XiA and nXiA are summarised in online supplementary figure S2a, b.

Online supplementary tables S9 and S10 summarise the genotype and allele distribution of *rs12688220* in ACP. The *T* allele was overrepresented in all European male cohorts (men: all patients 43.9% vs all controls 25.1%, p value 4.6×10^{-33}), while in female ACP cohorts from Germany, Poland and the UK as well as in the overall female group the overrepresentation of the *TT* genotype was statistically significant (women: all patients 10.4% vs all controls 6.7%, p value 2.4×10^{-7}).

In the meta-analysis, we detected no significant association in the overall male and female NACP group (p value 0.2 and 0.1). Again, genetic effect sizes are clearly smaller than for ACP. In single-study analyses, significant differences were found in the German NACP female group (OR 1.41, 95% CI 1.18 to 1.68, p value 0.0002) and in the male NACP groups from Germany (OR 1.34, 95% CI 1.03 to 1.75, p value 0.03) and the Netherlands (OR 2.09, 95% CI 1.05 to 4.12, p value 0.03) (figure 5).

Again, no differences were observed between the three control groups. Genotype and allele distributions of this variant can be found in online supplementary tables S11 and S12.

Additional analyses

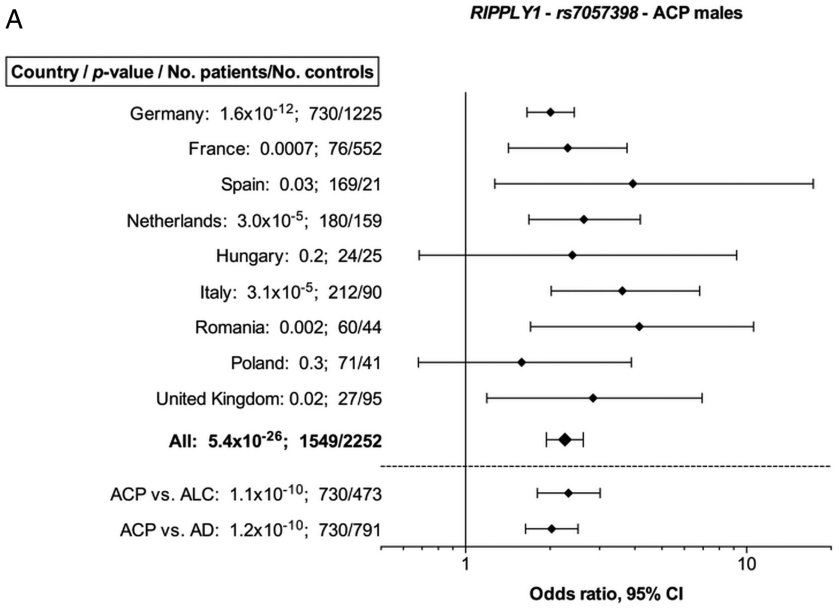
We pooled our cases and control groups in order to compare our results with the analysis published by Whitcomb *et al*. Results are summarised in online supplementary table S13 for all SNPs. Strong associations were observed for all variants, that is, the results of Whitcomb *et al* are clearly replicated.

To analyse whether effect sizes of X chromosomal variants are different between male and female patients, we performed sex-interaction analysis but interaction terms were not significant throughout (results not shown). We also compared the models of XIA and nXIA and observed a non-significant trend that XIA is more likely.

Finally, in order to better understand the lack of associations for NACP, we performed a stratified analysis of the German cohort regarding age of onset. Interestingly, we observed a trend towards higher genetic effect sizes in groups of later age of onset. This could explain, for example, the lack of associations in the French cohort in which the age range is 1–20 years (see online supplementary table S14).

DISCUSSION

This case–control study replicates and refines a robust association between a *PRSS1–PRSS2* locus variant (*rs10273639*) and CP. This is particularly strong in ACP and not apparent in NACP. The effect is independent from alcohol consumption as the



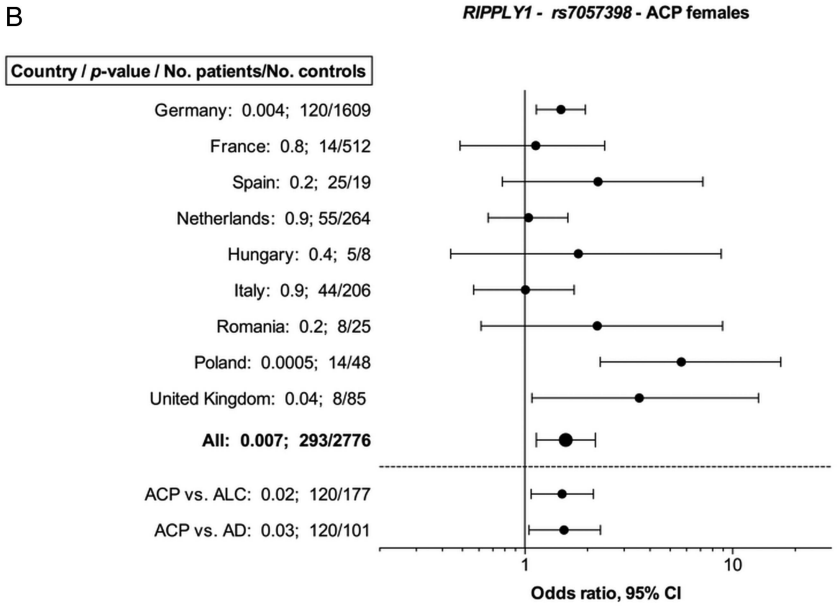


Figure 2 (A and B) Meta-analysis results for *rs7057398* (*RIPPLY1*) in patients with alcohol-related chronic pancreatitis (ACP) and comparison of German ACP patients with alcohol-associated cirrhosis (ALC) and with alcohol-dependent (AD) patients. Results are presented in a semi-log scale.

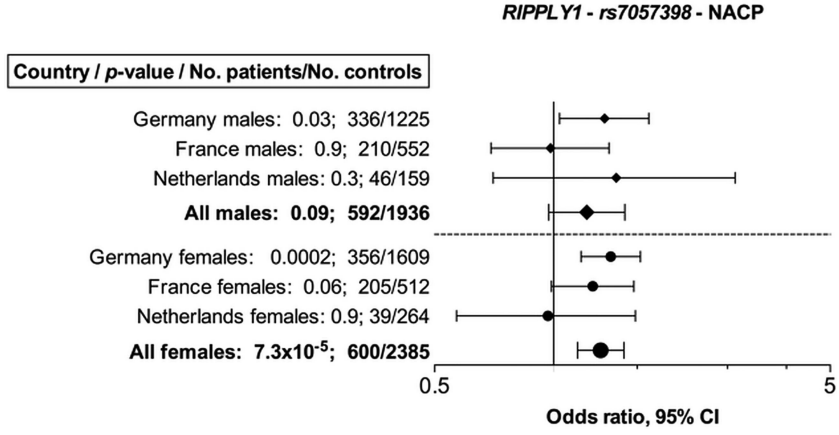


Figure 3 Meta-analysis results for *rs7057398* (*RIPPLY1*) in patients with non-alcoholic chronic pancreatitis (NACP). Results are presented in a semi-log scale. Y-axis intersects x-axis at 1.

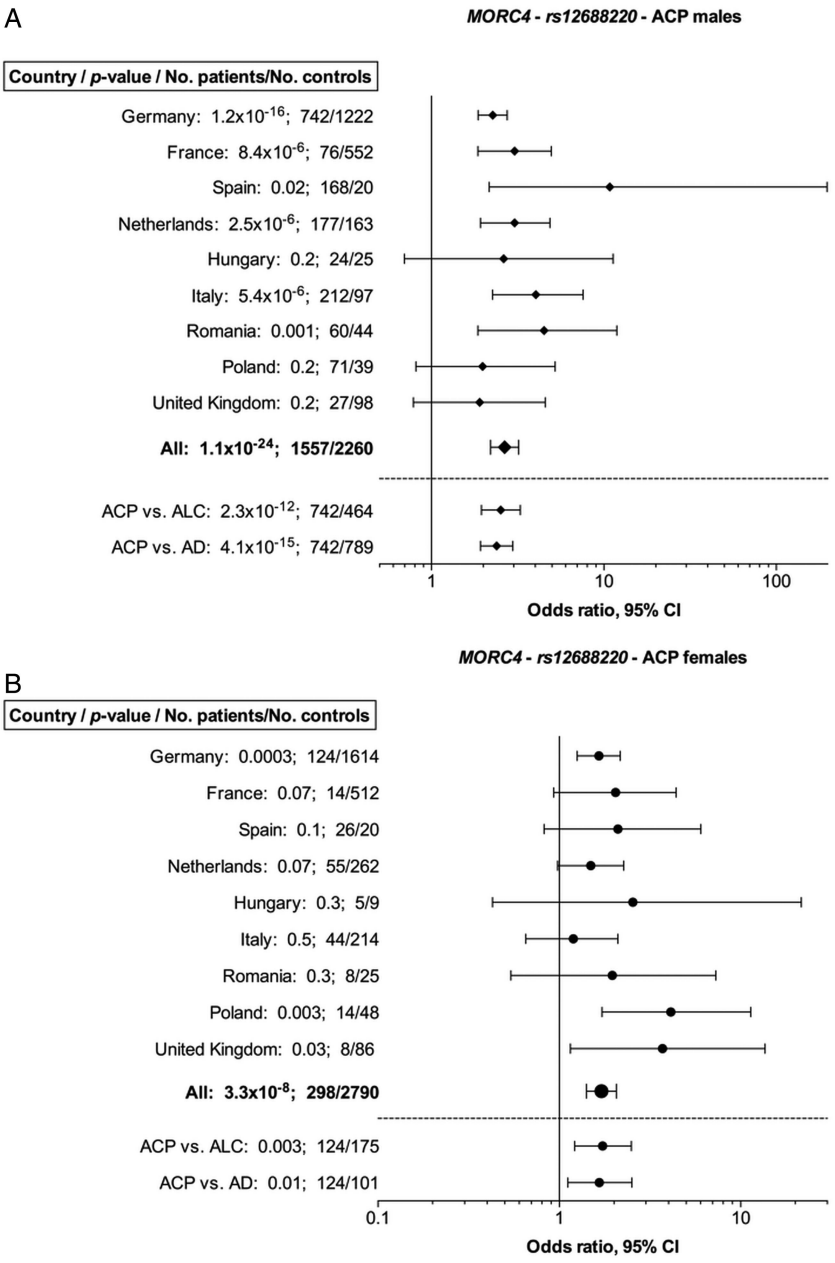


Figure 4 (A and B) Meta-analysis results for *rs12688220* (*MORC4*) in patients with alcohol-related chronic pancreatitis (ACP) and comparison of German ACP patients with alcohol-associated cirrhosis (ALC) and with alcohol-dependent (AD) patients. Results are presented in a semi-log scale.

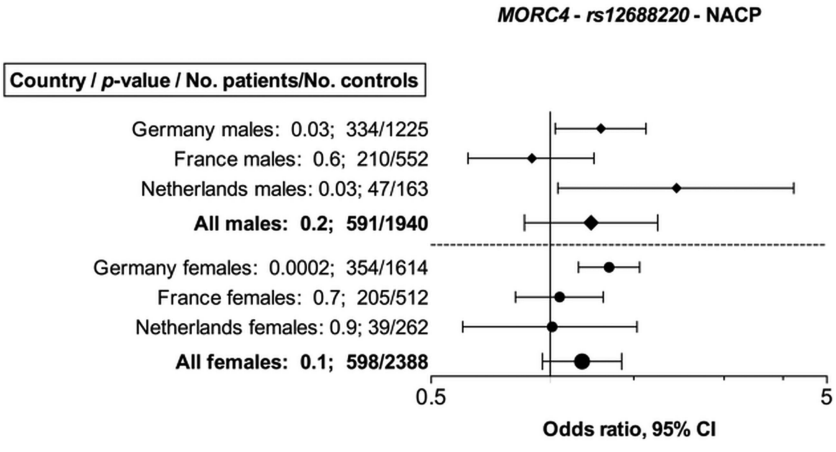


Figure 5 Meta-analysis results for *rs12688220* (*MORC4*) in patients with non-alcoholic chronic pancreatitis (NACP). Results are presented in a semi-log scale. Y-axis intersects x-axis at 1.

difference in allele frequency remained upon comparison with other alcohol-related disorders (ALC and AD). The risk reduction by *rs10273639* was higher in our overall ACP cohort (OR 0.63, CI 0.55 to 0.72) compared with the overall GWAS data (OR 0.73, seOR 0.029), which might be explained by the mixture of different aetiologies of patients with CP and RAP in the recent publication.[9] When using a comparable analysis strategy, similar results were obtained (see online supplementary table S13; OR 0.71, CI 0.61 to 0.84). Thus, the *T* allele confers protection against the development of ACP, but not against NACP. The protective effect of the *T* allele was observed for all single studies except for the samples from Poland. Genetic effect sizes vary between OR=0.41 (Romania) to OR=1.0 (Poland). However, this can be explained by small sample sizes rather than ethnic differences.

What is the biological background of our findings? The *PRSS1–PRSS2* locus SNP *rs10273639* (c.−408T>C) is located 408 nucleotides upstream of the ATG start codon of *PRSS1* and as such might influence PRSS1 expression. Indeed, the SNP seems to correlate with *PRSS1* mRNA levels in 69 pancreas tissue samples pointing towards its role in the regulation of PRSS1 expression.[9] Trypsinogen expression was lowest in *TT* genotypes, which suggests that this genotype might protect against pancreatitis development. However, the normalised gene expression data from pancreatic tissue had high SEs and a p value of 0.01 after removal of two outliers and, therefore, probably warrant further evidence to support this assumption.[9] In addition, SNPs *rs2011216* and *rs6667* were found to be in linkage disequilibrium with *rs10273639* and as such the biological effect might be related to those or even other SNPs.

We obtained similar results for the *CLDN2–MORC4* locus SNPs. We discovered that an association of both the *RIPPLY1* and the *MORC4* SNP with ACP was present in men and in women. Genetic effect sizes in men were somewhat higher than in women (OR=2.66 compared with OR=1.71 for *MORC4* and OR=2.27 compared with OR=1.56 for *RIPPLY1*), but no significant SNP–sex interactions were found. The associations with NACP were weaker throughout and not significant except for the *RIPPLY1* variant in female patients.

In older epidemiological studies, it was shown that women developed ACP at an earlier age and after consumption of a lower total amount of alcohol than men.[19 20] It is a matter of debate whether genetic effects at chromosome X can explain this observation. However, in our study, the genetic effect sizes of men and women were not significantly different for the variants considered. Moreover, by comparing models with and without assuming X inactivation, we did not receive a clear preference towards one of these assumptions. In view of these results, the X chromosomal *CLDN2-MORC4* locus variants do not even partly explain the higher ACP risk in men.

The role of CLDN2/RIPPLY1/MORC4 in pancreatitis is less clear. As a tight junction protein CLDN2 is involved in low-resistance cation-selective ion and water transport between endothelial cells.[12 13] The functional consequence of each investigated SNP is rather unclear so far. The recent paper proposed an atypical localisation of CLDN2 in acinar cells and an increase of CLDN2 expression in one investigated CP pancreas specimen (cDNA expression level) as well as in Western blot analyses from 19 pancreas specimens with different genotypes. Both for *MORC4* and *RIPPLY1* as well as for *TBC1D8B*, another gene within the *CLDN2* locus, the recent paper proposed no relevance for CP development.

For the X chromosomal variants, the effect sizes were smaller in a recently published GWAS.[9] Again, this can be explained by the markedly observed stronger genetic effect sizes of ACP compared with NACP.

The aetiology of AD involves environmental and genetic factors. Its heritability is estimated at ∼50%.[21] Since patients with ACP were compared with controls without defined alcohol consumption in our study as well as in the published GWAS, the described SNPs might represent markers for alcoholism and not for ACP. However, when data of patients with ACP were compared with patients with alcohol-associated liver cirrhosis and alcohol dependence in our study, the association of all investigated SNPs was replicated with similar effect sizes. Therefore, we conclude that the association of the three SNPs is specific for ACP and is unrelated to AD or alcohol-related liver disease.

In summary, our data refine the results of the recently published GWAS. The *PRSS1–PRSS2 rs10273639 T* allele protects against development of ACP but not NACP. The X chromosomal *RIPPLY1* and *MORC4* SNPs showed strong association with ACP. For NACP, the associations are weaker and only significant for the *RIPPLY1* SNP in women. The variants are not associated with the risk of AD or liver cirrhosis. The observed differences in SNP effects between ACP and NACP could be due to interactions of variants with alcohol consumption, which would amplify the risk, or they could result from differences in the pathophysiology of the two forms of CP. These hypotheses warrant future functional investigations.

Author affiliations
[1]Department of Gastroenterology and Hepatology, Radboud UMC, Nijmegen, The Netherlands
[2]Integrated Research and Treatment Centre (IFB) Adiposity Diseases, University of Leipzig, Leipzig, Germany
[3]Institute for Medical Informatics, Statistics and Epidemiology, University of Leipzig, Leipzig, Germany
[4]LIFE- Leipzig Research Center for Civilization Diseases, University of Leipzig, Leipzig, Germany
[5]Institut National de la Santé et de la Recherche Médicale (INSERM), U1078, Brest, France
[6]Etablissement Français du Sang (EFS)—Bretagne, Brest, France
[7]Faculté de Médecine et des Sciences de la Santé, Université de Bretagne Occidentale (UBO), Brest, France
[8]Laboratoire de Génétique Moléculaire et d'Histocompatibilité, Centre Hospitalier Universitaire (CHU) Brest, Hôpital Morvan, Brest, France
[9]Department of Internal Medicine, Neurology and Dermatology, Division of Gastroenterology and Rheumatology, University of Leipzig, Leipzig, Germany
[10]Institute of Human Genetics, Helmholtz Centre Munich, German Research Centre for Environmental Health, Neuherberg, Germany
[11]Unità Operativa di Gastroenterologia ed Endoscopia Digestiva, Università Vita Salute San Raffaele e IRCCS Ospedale San Raffaele, Milan, Italy
[12]Else Kröner-Fresenius-Zentrum für Ernährungsmedizin (EKFZ), Zentralinstitut für Ernährungs- und Lebensmittelforschung (ZIEL) & Paediatric Nutritional Medicine, Technische Universität München (TUM), Munich, Germany

Correction notice The order of the author list in HTML version has been corrected since published Online First. The structure of the author list in PDF version is slightly different from the online version but this difference does not affect the content of this paper.

Acknowledgements The authors thank all individuals who have participated in this study. We also thank Knut Krohn, Kathleen Schön and Birgit Oelzner (IZKF core unit DNA technologies, Leipzig) for excellent technical assistance.

Further authors list Hana Algül,[13] Thomas Berg,[14] Hans Bödeker,[15] Matthias Blüher,[2] Marco J Bruno,[16] Stephan Buch,[17] Peter Bugert,[18] Halina Cichoz-Lach,[19] Andrzej Dabrowski,[20] Antoni Farré,[21] Josef Frank,[22] Anita Gasiorowska,[23] Andrea Geisz,[24] Elisabetta Goni,[11] Johannes Grothaus,[25] Robert Grützmann,[26] Stephan Haas,[27] Jochen Hampe,[17] Claus Hellerbrand,[28] Peter Hegyi,[24] Dominik Huster,[29] Mihai Ioana,[30] Sevastitia Iordache,[31] Grazyna Jurkowska,[20] Volker Keim,[9] Olfert Landt,[32] Milena Di Leo,[11] Markus M Lerch,[33] Philippe Lévy,[34] Matthias J Löhr,[27] Milan Macek,[35] Nuria Malats,[36] Ewa Malecka-Panas,[23] Alberto Mariani,[11] Davide Martorana,[37] Julia Mayerle,[33] Josefina Mora,[38] Joachim Mössner,[9] Sebastian Müller,[39] Johann Ockenga,[40] Jana Paderova,[35] Sergio Pedrazzoli,[41] Stephen P Pereira,[42] Roland Pfützer,[43] Francisco X Real,[44] Vinciane Rebours,[34] Monika Ridinger,[28] Marcella Rietschel,[22]

Kerstin Rohde,[9] Stephan Sack,[45] Adrian Saftoiu,[31] Alexander Schneider,[46] Hans-Ulrich Schulz,[47] Michael Soyka,[48] Peter Simon,[33] James Skipworth,[49] Felix Stickel,[50] Michael Stumvoll,[2, 51] Pier Alberto Testoni,[11] Anke Tönjes,[2, 51] Matthias Treiber,[13] Frank Ulrich Weiss,[33] Jens Werner,[52] Norbert Wodarz[28]

[13]II. Medizinische Klinik, Klinikum rechts der Isar of the Technical University Munich, Munich, Germany
[14]Department of Internal Medicine, Neurology and Dermatology, Clinic of Gastroenterology and Rheumatology, Division of Hepatology, University of Leipzig, Leipzig, Germany
[15]Hospital Freiberg, Academic Hospital of the Technical University Dresden, Clinic for Internal Medicine, Freiberg, Germany
[16]Department of Gastroenterology & Hepatology, Erasmus Medical Centre, University Medical Centre Rotterdam, Rotterdam, The Netherlands
[17]Medical Department I, University Hospital Dresden, TU Dresden, Dresden, Germany
[18]Institute of Transfusion Medicine and Immunology, Medical Faculty of Mannheim University of Heidelberg, German Red Cross Blood Service of Baden-Württemberg-Hessen, Mannheim, Germany
[19]Department of Gastroenterology, Medical University of Lublin, Lublin, Poland
[20]Department of Gastroenterology and Internal Medicine, Medical University Bialystok, Bialystok, Poland
[21]Department of Gastroenterology, Hospital de la Santa Creu i Sant Pau, Barcelona, Spain
[22]Department of Genetic Epidemiology in Psychiatry, Central Institute of Mental Health, University of Heidelberg, Heidelberg, Germany
[23]Department of Digestive Tract Diseases, Medical University of Lodz, Lodz, Poland
[24]First Department of Medicine, University of Szeged, Szeged, Hungary
[25]Department of Medicine I, Altona General Hospital, Hamburg, Germany
[26]Department of General, Thoracic and Vascular Surgery, University Hospital Carl Gustav Carus, Technical University Dresden, Dresden, Germany
[27]Gastrocentrum, Karolinska Institutet CLINTEC, Stockholm, Sweden
[28]Department of Internal Medicine I, University Hospital Regensburg, Regensburg, Germany
[29]Evangelisches Diakonissenkrankenhaus Leipzig, Academic Hospital of the University of Leipzig, Leipzig, Germany
[30]Molecular and Cellular Biology Department, University of Medicine and Pharmacy Craiova, Craiova, Romania
[31]Department of Internal Medicine and Gastroenterology, University of Medicine and Pharmacy, Craiova, Romania
[32]TIB MOLBIOL, Berlin, Germany
[33]Department of Medicine A, University Medicine Greifswald, Greifswald, Germany
[34]Pôle des Maladies de l'Appareil Digestif, Service de Gastroentérologie-Pancréatologie, Hôpital Beaujon, AP-HP, Clichy, France
[35]Department of Biology and Medical Genetics, University Hospital Motol and 2nd Faculty of Medicine, Charles University, Prague, Czech Republic
[36]Grupo de Epidemiología Genética y Molecular Programa de Genética del Cáncer Humano Centro Nacional de Investigaciones Oncológicas (CNIO), Madrid, Spain
[37]Unit of Molecular Genetics, University Hospital of Parma, Parma, Italy
[38]Department of Biochemistry, Hospital de la Santa Creu i Sant Pau, Barcelona, Spain
[39]Department of Internal Medicine, Salem Medical Centre and Centre for Alcohol Research, University of Heidelberg, Heidelberg, Germany
[40]Medical Clinic II, Internal Medicine, Gastroenterology, Endocrinology and Nutritional Medicine, Klinikum Links der Weser, Klinikum Bremen Mitte, Bremen, Germany
[41]IV Surgical Clinic, Department of Medical and Surgical Sciences, University of Padua, Padua, Italy
[42]UCL Institute for Liver and Digesive Health, Division of Medicine, University College London, London, UK
[43]Clinic for Internal Medicine, Hospital Döbeln, Döbeln, Germany
[44]Epithelial Carcinogenesis Group, Molecular Pathology Programme, Centro Nacional de Investigaciones Oncológicas, Madrid, Spain
[45]Clinic for Internal Medicine II, Ev. Klinikum Paul-Gerhardt-Stift, Paul-Gerhardt-Diakonie Hospital and Care GmbH, Lutherstadt Wittenberg, Germany
[46]Department of Gastroenterology, Hepatology, Infectious Diseases, Medical Faculty of Mannheim University of Heidelberg, Mannheim, Germany
[47]Department of Surgery, Otto-von-Guericke University Magdeburg, Magdeburg, Germany
[48]Psychiatric Hospital, University of Munich, Munich, Germany
[49]Department of Surgery and Interventional Science, University College London, London, UK
[50]Department of Visceral Surgery and Medicine, Inselspital Bern, Berne, Switzerland
[51]Department of Internal Medicine, Neurology and Dermatology, Division of Endocrinology, University of Leipzig, Leipzig, Germany
[52]Department of General Surgery, University of Heidelberg, Heidelberg, Germany

Contributors MHD, PK, MS and JR conceived, designed and directed the study. MHD, PK, MS, EM, CR, and JR designed, performed and interpreted genetic analyses with significant contributions from JPHD, CF, and HW. MHD, PK, JR wrote the manuscript with significant contributions from MS, EM, J-MC, CF, JPHD, and HW. OL provided oligonucleotides. All other coauthors recruited study subjects, collected clinical data and provided genomic DNA samples. All authors approved the final manuscript and contributed critical revisions to its intellectual content.

Funding This work was supported by the Deutsche Forschungsgemeinschaft (DFG) grants RO 3929/1-1 & RO 3939/2-1 (to JR), Wi 2036/2-2 & Wi 2036/2-3 (to HW), and SFB 1052 (to MB, MS, AT, PK), by the Boehringer Ingelheim Foundation (to KR), by a grant of the Colora Stiftung gGmbH (to JR), the Else Kröner-Fresenius-Foundation (EKFS) (to HW), by CZ.2.16/3.1.00/24022OPPK and "Conceptual development project of research organization #00064203" (University Hospital Motol, Prague) from the Czech Ministry of Health, Norway Grants PDP3 (to MM), the Institut National de la Santé et de la Recherche Médicale (INSERM; to CF), the Programme Hospitalier de Recherche Clinique (PHRC R 08-04; to CF), the French Association des Pancréatites Chroniques Héréditaires (to CF), the Council of Scientific and Industrial Research (CSIR) (to CF).

Competing interests None.

Ethics approval Ethical Committee of the University Leipzig.

Provenance and peer review Not commissioned; externally peer reviewed.

Data sharing statement All data are included in the manuscript.

REFERENCES

1 Whitcomb DC, Gorry MC, Preston RA, *et al*. Hereditary pancreatitis is caused by a mutation in the cationic trypsinogen gene. *Nat Genet* 1996;14:141–45.
2 Witt H, Luck W, Hennies HC, *et al*. Mutations in the gene encoding the serine protease inhibitor, Kazal type 1 are associated with chronic pancreatitis. *Nat Genet* 2000;25:213–16.
3 Witt H, Sahin-Tóth M, Landt O, *et al*. A degradation-sensitive anionic trypsinogen (*PRSS2*) variant protects against chronic pancreatitis. *Nat Genet* 2006;38:668–73.
4 Le Maréchal C, Masson E, Chen JM, *et al*. Hereditary pancreatitis caused by triplication of the trypsinogen locus. *Nat Genet* 2006;38:1372–74.
5 Rosendahl J, Witt H, Szmola R, *et al*. Chymotrypsin C (*CTRC*) variants that diminish activity or secretion are associated with chronic pancreatitis. *Nat Genet* 2008;40:78–82.
6 Rosendahl J, Landt O, Bernadova J, *et al*. *CFTR*, *SPINK1*, *CTRC* and *PRSS1* variants in chronic pancreatitis: is the role of mutated *CFTR* overestimated? *Gut* 2013;62:582–92.
7 Witt H, Beer S, Rosendahl J, *et al*. Variants in *CPA1* are strongly associated with early-onset chronic pancreatitis. *Nat Genet* 2013;45:1216–20.
8 Witt H, Luck W, Becker M, *et al*. Mutation in the *SPINK1* trypsin inhibitor gene, alcohol use, and chronic pancreatitis. *JAMA* 2001;285:2716–17.
9 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;44:1349–54.
10 Witt H, Apte MV, Keim V, *et al*. Chronic pancreatitis: challenges and advances in pathogenesis, genetics, diagnosis, and therapy. *Gastroenterology* 2007;132:1557–73. Review.
11 Whitcomb DC. Genetic risk factors for pancreatic disorders. *Gastroenterology* 2013;144:1292–302. Review.
12 Amasheh S, Meiri N, Gitter AH, *et al*. Claudin-2 expression induces cation-selective channels in tight junctions of epithelial cells. *J Cell Sci* 2002;115:4969–76.
13 Van Itallie CM, Holmes J, Bridges A, *et al*. The density of small tight junction pores varies among cell types and is increased by expression of claudin-2. *J Cell Sci* 2008;121:298–305.
14 Greene CS, Penrod NM, Williams SM, *et al*. Failure to replicate a genetic association may provide important clues about genetic architecture. *PLoS One* 2009;4:e5639.
15 Rosendahl J, Tönjes A, Schleinitz D, *et al*. A common variant of *PNPLA3* (p.I148M) is not associated with alcoholic chronic pancreatitis. *PLoS One* 2012;7:e29433.
16 Treutlein J, Cichon S, Ridinger M, *et al*. Genome-wide association study of alcohol dependence. *Arch Gen Psychiatry* 2009;66:773–84.
17 Troendle JF, Yu KF. A note on testing the Hardy-Weinberg law across strata. *Ann Hum Genet* 1994;58:397–402.
18 Loley C, König IR, Hothorn L, *et al*. A unifying framework for robust association testing, estimation, and genetic model selection using the generalized linear model. *Eur J Hum Genet* 2013;21:1442–8.
19 Durbec JP, Sarles H. Multicenter survey of the etiology of pancreatic diseases. Relationship between the relative risk of developing chronic pancreatitis and alcohol, protein and lipid consumption. *Digestion* 1978;18:337–50.
20 Masamune A, Kume K, Shimosegawa T. Sex and age differences in alcoholic pancreatitis in Japan: a multicenter nationwide survey. *Pancreas* 2013;42:578–83.
21 Enoch MA, Goldmann D. Problem drinking and alcoholism: diagnosis and treatment. *Am Fam Physician* 2002;65:441–48.

Polymorphisms at *PRSS1*–*PRSS2* and *CLDN2*–*MORC4* loci associate with alcoholic and non-alcoholic chronic pancreatitis in a European replication study

Monique H Derikx, Peter Kovacs, Markus Scholz, Emmanuelle Masson, Jian-Min Chen, Claudia Ruffert, Peter Lichtner, Rene H M te Morsche, Giulia Martina Cavestro, PanEuropean Working group on Alcoholic Chronic Pancreatitis members and collaborators, Claude Férec, Joost P H Drenth, Heiko Witt and Jonas Rosendahl

Gut 2015 64: 1426-1433 originally published online September 24, 2014
doi: 10.1136/gutjnl-2014-307453