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Version of Record: https://www.sciencedirect.com/science/article/pii/S1424390322008225
Manuscript_d34844fb11e677359f1d1b0751d05c85

The *PRSS3P2* and *TRY7* deletion copy number variant modifies risk for chronic pancreatitis

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ABSTRACT

Background: PRSS1 and *PRSS2* constitute the only functional copies of a tandemly-arranged five-trypsinogen-gene cluster (i.e., *PRSS1*, *PRSS3P1*, *PRSS3P2*, *TRY7* and *PRSS2*) on chromosome 7q35. Variants in *PRSS1* and *PRSS2,* including missense and copy number variants (CNVs), have been reported to predispose to or protect against chronic pancreatitis (CP). We wondered whether a common trypsinogen pseudogene deletion CNV (that removes two of the three trypsinogen pseudogenes, *PRSS3P2* and *TRY7*) might be associated with CP causation/predisposition.

Methods: We analyzed the common *PRSS3P2* and *TRY7* deletion CNV in a total of 1536 CP patients and 3506 controls from France, Germany, India and Japan by means of quantitative fluorescent multiplex polymerase chain reaction.

Results: We demonstrated that the deletion CNV variant was associated with a protective effect against CP in the French, German and Japanese cohorts whilst a trend toward the same association was noted in the Indian cohort. Meta-analysis under a dominant model yielded a pooled odds ratio (OR) of 0.68 (95% confidence interval (CI) 0.52-0.89; $p = 0.005$) whereas an allele-based meta-analysis yielded a pooled OR of 0.84 (95% CI 0.77-0.92; $p = 0.0001$). This protective effect is explicable by reference to the recent finding that the still functional *PRSS3P2/TRY7* pseudogene enhancers upregulate pancreatic *PRSS2* expression.

Conclusions: The common *PRSS3P2* and *TRY7* deletion CNV was associated with a reduced risk for CP. This finding provides additional support for the emerging view that dysregulated *PRSS2* expression represents a discrete mechanism underlying CP predisposition or protection.

Keywords: causal variant; case-control study; genetic predisposition to disease; pancreatic tissue; rs10273639

List of abbreviations

CP, chronic pancreatitis

GoF, gain-of-function

CNV, copy number variant

GoP, gain-of-proteotoxicity

LoF, loss-of-function

GWAS, genome-wide association study

SNP, single nucleotide polymorphism

QFM-PCR, quantitative fluorescent multiplex polymerase chain reaction

delCNV, deletion copy number variant

ICP, idiopathic chronic pancreatitis

TCP, tropical chronic pancreatitis

OR, odds ratio

CI, confidence interval

LD, linkage disequilibrium

1. Introduction

PRSS1 (protease, serine, 1; OMIM #276000) encoding cationic trypsinogen, the major isoform of human trypsinogen, was the first gene identified to cause chronic pancreatitis (CP) [1]. Gain-of-function (GoF) missense variants, copy number variants (CNVs) and gain-of-proteotoxicity (GoP) variants in the *PRSS1* gene all cause/predispose to CP whereas loss-of-function (LoF) variants in the gene appear to be protective against CP (see Masson et al. [2] and references therein). Although the *PRSS2* gene (protease, serine, 2; OMIM #601564) encodes the second major isoform of human trypsinogen, anionic trypsinogen, no *PRSS2* GoF or GoP missense variants have so far been reported to cause/predispose to CP [1, 3-5]. This notwithstanding, several lines of evidence suggest that a role for *PRSS2* in CP is wholly plausible. First, a CP-associated missense variant in the *PRSS1* gene, p.Glu79Lys, has been shown by *in vitro* analysis of recombinant wild-type and mutant enzymes to increase transactivation of PRSS2 [6]. Second, a LoF missense variant in the *PRSS2* gene, p.Gly191Arg, has been reported to be protective against CP [5]. Third, the rare examples of GoF trypsinogen duplication and triplication CNVs so far reported in the literature have invariably involved not only *PRSS1* but also *PRSS2* [7-10]. Fourth, our recent survey [11] of the Genotype-Tissue Expression (GTEx) database (https://www.gtexportal.org/home/) revealed that the CP-risk (C) allele of rs10273639 (located 408-bp upstream of the translation initiation codon of *PRSS1*), discovered through genome-wide association studies (GWAS) on CP [12], was associated with increased expression of *PRSS2* (but not *PRSS1*) in pancreatic tissue. Fifth, the European GWAS-identified common inversion at the *CTRB1-CTRB2* (chymotrypsin B1 and B2; OMIM #118890 and #619620) locus modifies CP risk by reversing the expression ratio of these isoforms in favor of CTRB2 [13, 14] whilst carriers of the inversion allele appeared to be protected against CP via their increased capacity for CTRB2-mediated PRSS2 degradation [13, 15]. Finally, transgenic expression of wild-type *PRSS2* was found to exacerbate the clinical severity of caerulein-induced pancreatitis [16] and induced spontaneous pancreatitis only when co-expressed with p.Arg122His-carrying *PRSS1* [17] in mice (note that p.Arg122His was the first mutation identified to cause CP in humans [1]).

PRSS1 and *PRSS2* constitute the only functional gene copies in the tandemly-arranged five-trypsinogen-gene cluster, which is intercalated with the T-cell receptor locus on chromosome 7q35 [18]. These five tandemly-arranged trypsinogen genes, in centromeric to telomeric order, are termed *PRSS1*, *PRSS3P1*, *PRSS3P2*, *TRY7* and *PRSS2* (Figure 1a). All five copies are ~10-kb in length and exhibit 90% nucleotide sequence similarity between them [18]. Here it should be noted that these five genes were previously known as T4 to T8 or TRY4 to TRY8 (N.B. *TRY7* has retained its symbol) [18, 19]. T4 to T8 were annotated relative to T1 to T3 (now known as

PRSS58, *TRY2P* and *PRSS3P3*; Figure 1b), a second trypsinogen gene cluster located on chromosome 7q35 [18]. The T1-T3 gene cluster is located ~490-kb centromeric to the T4-T8 gene cluster (Figure 1c). The two gene clusters have evolved largely as separate gene families since duplication [18].

Using dense single nucleotide polymorphism (SNP) genotype data from 269 HapMap population samples [20], McCarroll et al. reported a common deletion CNV (delCNV) involving *PRSS3P2* (TRY6) in 2006 [21]. The frequencies of this deletion allele in the CEU (European ancestry), JCH (Japanese and Chinese ancestry) and YRI (Yoruba ancestry) populations were 41%, 74% and 12% respectively. During analysis of the rare GoF trypsinogen duplication and triplication CNVs by means of quantitative fluorescent multiplex polymerase chain reaction (QFM-PCR), we found that this common deletion also encompasses *TRY7* [8]. We wondered whether this delCNV might be associated with CP causation/predisposition. To explore this postulate, we genotyped the delCNV in four cohorts from four distinct populations. We now provide genetic evidence that the deletion allele of the *PRSS3P2* and *TRY7* delCNV is protective against CP. This is consistent with the recent finding that this deletion allele removes the still functional *PRSS3P2/TRY7* pseudogene enhancers, which serve to upregulate *PRSS2* expression in the pancreas [22]. Two recent studies have refined the structure of the *PRSS3P2* and *TRY7* delCNV [23, 24]; the chromosome 7 GRCh38 assembly corresponds to the deletion allele at the *PRSS3P2* and *TRY7* loci whereas the GRCh38 alternative contig, KI270803.1, contains both these genes (Figure 1a).

2. Materials and Methods

2.1. Subjects

A total of 1536 CP patients and 3506 healthy controls participated in this study (Table 1). The French, German and Japanese patients had a diagnosis of idiopathic CP (ICP) whereas the Indian patients had a diagnosis of tropical CP (TCP). The terms ICP and TCP were defined as in our previous publications [25, 26]. None of the participating patients had been found to carry *PRSS1* p.Arg122His [1] or p.Asn29Ile [27], the two most frequently reported CP-causing variants worldwide. Moreover, none of the French patients had been found to harbor the rare GoF trypsinogen duplication or triplication CNV [7, 8].

This study was approved by the respective Institutional Ethics Committees of the Brest (France), Munich (Germany), Hyderabad (India), and Sendai (Japan) groups. Informed consent was obtained from all participants (or parents/guardians when the participants were under the age of 18).

2.2. QFM-PCR analysis

To detect the *PRSS3P2* and *TRY7* delCNV by means of QFM-PCR, we designed primers to amplify exonic sequences from *PRSS1*, *PRSS3P2*, *TRY7* and *PRSS2* as well as from two control genes, viz. *MGAM* (maltase-glucoamylase) on chromosome 7 and *DSC2* (desmocollin 2) on chromosome 18. One of each primer pair was 5'-labeled with 6-HEX fluorochrome. Primer sequences and lengths for the six amplicons are provided in Supplementary Table 1.

QFM-PCR was performed in the Brest genetics laboratory, using the Qiagen® multiplex PCR Kit (Qiagen, Courtaboeuf, France) according to the manufacturer's instructions. PCR was performed in a 12 µL mixture containing 100 ng genomic DNA (2 µL of a DNA solution with a concentration of 50 ng/µL), 5 µL primer mix (see Supplementary Table 1 for end primer concentrations) and 5 µL Qiagen® multiplex PCR Master Mix. The PCR program comprised an initial denaturation at 95°C for 15 min, followed by 25 cycles of denaturation at 95°C for 30 sec, annealing at 58°C for 60 sec and extension at 72°C for 90 sec, and a final extension at 72°C for 45 min. Amplified DNA fragments were separated on an ABI Prism 3130 sequencer (Applied Biosystems, Foster City, CA) and the data were analyzed with Genemapper v4.0 (Applied Biosystems).

2.3. Statistical analysis

Departure from Hardy-Weinberg equilibrium was tested for among controls in each population using a publicly available web-based tool at https://ihg.helmholtz-muenchen.de/cgi-bin/hw/hwa1.pl. The genotype and allele distributions of the *PRSS3P2* and *TRY7* delCNV were described in cases and controls and compared using the χ^2 test. The strength and precision of the associations were estimated by odds ratios (OR) with their 95% confidence intervals (CI). Differences were regarded as being statistically significant when the *p* values were ≤ 0.05.

A meta-analysis of genetic effects was performed using the Review Manager 5.3 software [28]. In the absence of heterogeneity (i.e., the *p* value for the test of heterogeneity was >0.05 or I^2 was <50%), the pooled OR was computed using the Mantel–Haenszel fixed-effect model; otherwise, the Mantel–Haenszel random-effect model was employed [28, 29]. Funnel plots were not performed due to the number of cohorts (n = 4) included for meta-analysis being <10 [28, 30].

3. Results

We have routinely used QFM-PCR to detect CNVs in several pancreatitis-related genes (e.g., refs. [8, 31-33]). Herein, we describe a QFM-PCR method for genotyping the *PRSS3P2* and *TRY7* delCNV. QFM-PCR

electropherograms of a *PRSS3P2* and *TRY7* delCNV heterozygote and a deletion homozygote are depicted in Figure 2. The QFM-PCR method employed in this study was also capable of detecting the rare *PRSS1* and *PRSS2* duplication and triplication CNVs (allele frequency of the former was 0.00004610 although the latter was absent by reference to gnomAD SVs v2.1 [34]). No such variants were found in the analyzed German, Indian and Japanese subjects primarily owing to a common founder effect [8, 35].

Genotype distributions of the *PRSS3P2* and *TRY7* delCNV in the four studied cohorts and the OR (95% CI) and *p* values from three different association tests are provided in Table 2. The allele frequency distributions of the delCNV in the four cohorts (patients and controls) and their corresponding OR (95% CI) and *p* values are provided in Table 3. The delCNV variant was noted to be in Hardy–Weinberg equilibrium among the controls across all four populations (French, $p = 0.0766$; German, $p = 0.0768$; Japanese, $p = 0.1491$; Indian, $p = 0.9912$).

Genotype and allele frequencies of the *PRSS3P2* and *TRY7* delCNV differed significantly between normal populations (Tables 2 and 3). In terms of allele frequency, the largest difference was between the German and Japanese controls (42.0% *vs*. 74.8%), which is consistent with the abovementioned McCarroll report [21].

Despite the observed inter-population differences, a statistically significant protective effect of the *PRSS3P2* and *TRY7* delCNV in relation to CP was consistently observed in the French, German and Japanese cohorts, with respect to three of the four association tests, namely, dw *vs*. ww, (dd + dw) *vs*. ww (see Table 2 for genotype definitions), and allele frequency distributions between patients and controls (Table 3). Same association trends but without statistical significance were found for the Indian cohort which comprised TCP patients (Tables 2 and 3).

We further performed a meta-analysis combining data from all four cohorts. Under the dominant model (i.e., (dd + dw) *vs*. ww), the *PRSS3P2* and *TRY7* delCNV was significantly associated with a protective effect against CP (pooled OR 0.68, 95% CI 0.52-0.89; $p = 0.005$) (Figure 3a). In the context of allele-based meta-analysis, the *PRSS3P2* and *TRY7* delCNV was also significantly associated with a protective effect against CP (pooled OR 0.84, 95% CI 0.77-0.92; $p = 0.0001$) (Figure 4a). Exclusion of the Indian TCP cohort from the meta-analysis yielded a slightly smaller pooled OR but slightly larger *p* values in both contexts (Figure 3b; Figure 4b).

4. Discussion

Employing QFM-PCR, we genotyped the common *PRSS3P2* and *TRY7* CNV in three ICP cohorts and one TCP cohort. The deletion CNV variant was significantly associated with a protective effect against ICP in the French, German and Japanese cohorts whilst a trend toward the same association was noted in the Indian TCP

cohort. Here it is pertinent to mention that ICP and TCP exhibit overlapping clinical features; however, genetic studies in Indian CP patients have invariably generated different results as compared to those from other ethnicities (e.g., refs. [26, 36-39]) owing to the unique genetic architecture and environmental risk exposures underlying TCP in the Indian population [40].

Irrespective of the inclusion or exclusion of the TCP cohort, two types (allele-based and dominant model-based) of meta-analysis showed that the common *PRSS3P2* and *TRY7* CNV was associated with a reduced risk for CP. What is the biological mechanism underlying this association? A recent preprint may provide one possible answer [22]. Having explored existing publicly available databases (including particularly multiomic single-cell sequencing data of Assay from Transposase-Accessible Chromatin with high-throughput sequencing (ATAC-seq) and RNA sequencing (RNA-seq)) by means of state-of-the-art bioinformatics analyses, Lou and colleagues convincingly demonstrated that the *PRSS3P2* and *TRY7* delCNV removes a region that serves to maintain *PRSS2* expression. Specifically, the still functional *PRSS3P2* and *TRY7* pseudogene enhancers were shown to exert an influence on *PRSS2* expression in the pancreas, thereby contributing to a ~15% higher *PRSS2* expression level than the haplotype harboring the *PRSS3P2/TRY7* deletion. This finding may shed new light upon the biological mechanism underlying the first GWAS-identified, rs10273639-tagged *PRSS1-PRSS2* risk haplotype [11, 12]: Lou and colleagues showed that the wild-type *PRSS1-PRSS3P1-PRSS3P2-TRY7-PRSS2* haplotype (i.e., without the *PRSS3P2* and *TRY7* deletion) is in complete linkage disequilibrium (LD) with rs2855983, a lead SNP associated with CP in a European GWAS [13]. Moreover, in the European GWAS study, rs2855983 was reported to be in LD with rs10273639 [12]. Thus, the first GWAS-identified CP-risk (C) allele of rs10273639 is in high LD with the wild-type *PRSS1-PRSS3P1-PRSS3P2-TRY7-PRSS2* haplotype, which is associated with markedly higher *PRSS2* expression than the deletion *PRSS1-PRSS3P1- PRSS2* haplotype. This notwithstanding, a minor contribution from rs4726576, which is located 204-bp upstream of the translation initiation codon of *PRSS1* and is in high LD with rs10273639 [41], to a higher *PRSS1* expression level of the wild-type *PRSS1-PRSS3P1-PRSS3P2-TRY7-PRSS2* haplotype cannot be excluded for several reasons. Firstly, analysis of 69 pancreas tissue samples by means of TaqMan-based RT-PCR assays showed that the risk (C) allele of rs10273639 is associated with increased *PRSS1* mRNA expression [12]. Very recently, RNA sequencing using pancreatic samples from 12 individuals heterozygous for *PRSS1* p.Asn246= (rs6667, which is in high LD with rs10273639 [42] and has been experimentally shown to be associated with CP [43]) showed that the high-risk (C) allele was associated with 5% higher *PRSS1* expression than the low-risk (T) allele (52.5%±4.0% *vs*. 47.5%±4.0%; Wilcoxon signed ranked text, p=0.021) [44]. On the other hand, the high-risk (C) allele of

rs4726576 was found to increase gene expression in a reporter gene assay [41]. *In silico* analysis has also predicted that rs4726576 has the highest regulatory potential among linked SNPs within the rs10273639-tagged haplotype [10]. Finally, it may be relevant that the rs10273639-tagged haplotype was recently noted to alter T cell receptor beta repertoire more strongly than *PRSS1* expression [44]. However, it remains unknown whether the altered cell receptor beta repertoire is the cause of the increased CP risk or is instead secondary to pancreatic inflammation.

Lou and colleagues also showed that the CP-protective LoF missense variant in the *PRSS2* gene, p.Gly191Arg [5], was exclusively located on the wild-type *PRSS1-PRSS3P1-PRSS3P2-TRY7-PRSS2* trypsinogen haplotype [22]. Thus, the *PRSS2* p.Gly191Arg variant (allele frequency 0.01453 in combined gnomAD populations [34]) and the *PRSS3P2* and *TRY7* delCNV (allele frequency 0.42-0.75 in this study) represent independent protective factors against CP, both of which lead to a functional loss of *PRSS2*.

As mentioned earlier, the delCNV variant was shown to be in Hardy–Weinberg equilibrium across all four populations. However, the *p* values from the French ($p = 0.0766$) and German ($p = 0.0768$) populations were markedly different from those obtained from the Japanese ($p = 0.1491$) and Indian ($p = 0.9912$) populations. This is potentially explicable in terms of the significant allele frequency difference manifested by the delCNV variant in Europeans (allele frequency ~40%) and East and South Asians (allele frequency ~70%; Table 3), which may in turn be determined by a difference in the degree of positive selection exerted on these populations. In the Lou study, positive selection was demonstrated to start earlier in, and to have had a much stronger effect on, East Asians as compared to Europeans, whilst selection might still be ongoing in Eurasian populations [22]. Unfortunately, such studies are lacking in South Asians (Indians) but such a possibility has been previously proposed [36]. The remarkable *p* value difference observed with the Hardy–Weinberg equilibrium test in the two European countries is not inconsistent with ongoing weak selection acting on the delCNV allele in European populations.

This study has various limitations. First and foremost, the sample size of each individual patient cohort was relatively small. Second, the study would have been enhanced had an alcoholic CP cohort been analyzed. In this regard, two points are pertinent to mention. On the one hand, there is good evidence for an interaction between alcohol intake and the rs10273639-tagged haplotype in the etiology of CP [12, 42, 43, 45, 46]. On the other hand, although alcohol abuse significantly increases the concentration of all three trypsinogen isoforms (i.e., cationic, anionic and mesotrypsinogen) in pure pancreatic juice of chronic alcoholics, most of this increase results from an approximately five-fold increase of PRSS2 [47].

In summary, we have shown that the deletion allele of the *PRSS3P2* and *TRY7* loci is associated with a reduced risk of CP, although with heterogeneity in the effect size across the studied populations. Our finding provides further evidence to support the emerging view that increased expression of *PRSS2* is an independent mechanism underlying pancreatitis risk [10, 16, 22]. The increased expression of *PRSS2* may account not only for the increased risk of CP conferred by the wild-type *PRSS1-PRSS3P1-PRSS3P2-TRY7-PRSS2* haplotype but also for the increased risk of cystic fibrosis-related diabetes and decreased risk for cystic fibrosis-related meconium ileus associated with this haplotype [24, 48].

Financial support

This research was funded by the Institut National de la Santé et de la Recherche Médicale (INSERM), the Association des Pancréatites Chroniques Héréditaires and the Association Gaétan Saleün, France; Else Kröner-Fresenius-Foundation (EKFS) 2017_A108 – EKFZ-Witt (to H.W.) and DFG WI 2036/2-3 (to H.W.); and CSIR-Centre for Cellular and Molecular Biology (CSIR-CCMB), Ministry of Science and Technology, Govt. of India.

Author contributions

E.M., C.F. and J.M.C. conceived the study.

E.M. performed QFM-PCR analysis.

E.M., V.S., K.R. and J.M.C. performed genetic data collection and statistical analysis.

E.M., M.E., S.P., K. Kume, A.M., G.R.C., H.W., C.F. and J.M.C. analyzed and interpreted the data.

J.M.C. drafted and revised the manuscript with substantial assistance from E.M., M.E., S.P., K. Kume, D.N.C., A.M., G.R.C. and H.W.

All other co-authors recruited study subjects and/or provided genomic DNA samples. All authors approved the final manuscript and contributed critical revisions to its intellectual content.

Acknowledgment

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Declaration of competing interest

The authors are unaware of any conflict of interest.

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FIGURE LEGENDS

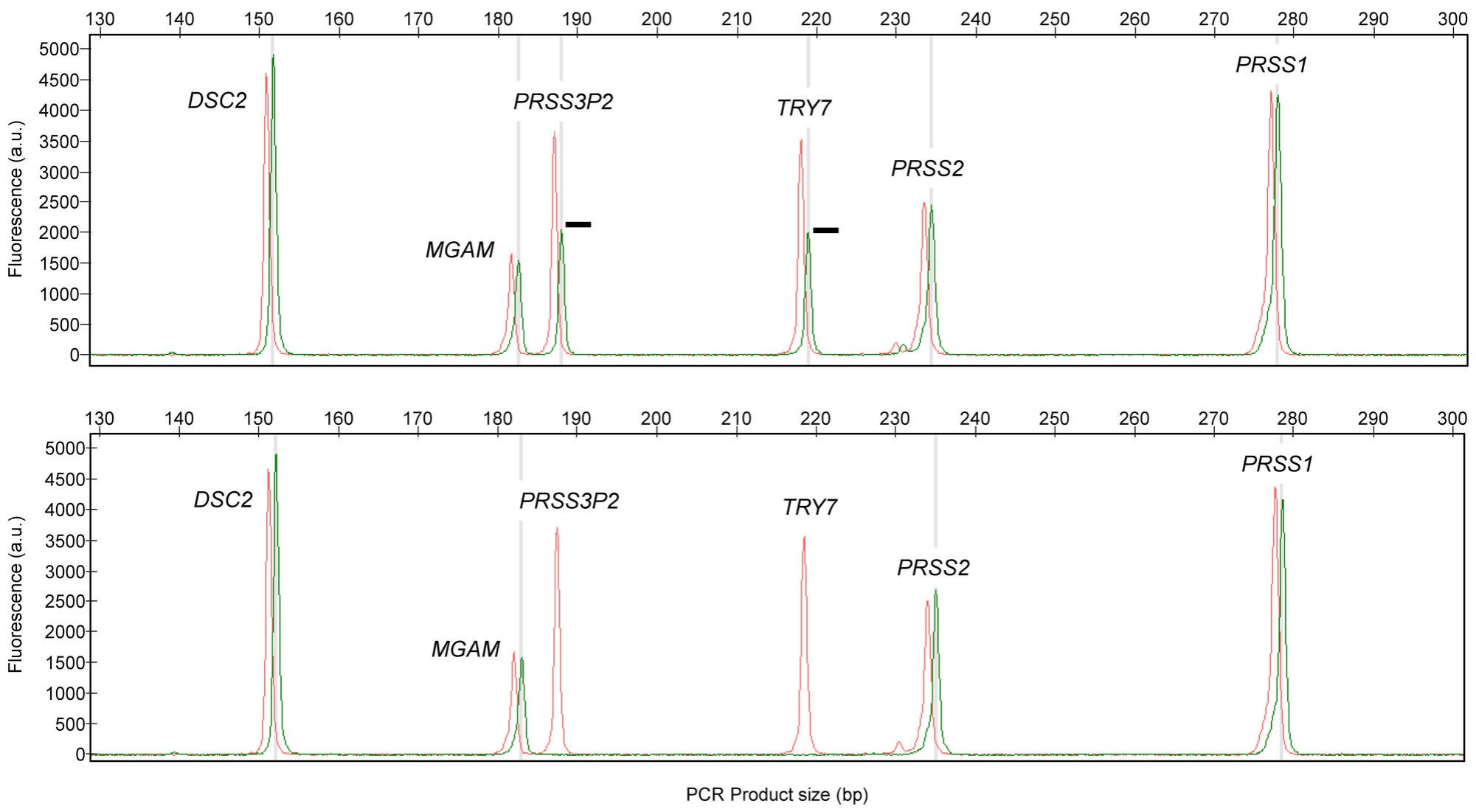
Figure 1. The two trypsinogen gene clusters on human chromosome 7q35. (**a**) The tandemly-arranged five-trypsinogen-gene cluster comprising *PRSS1*, *PRSS3P1*, *PRSS3P2*, *TRY7* and *PRSS2*. Gene organization is in accordance with the GRCh38 alternative contig, KI270803.1. The GRCh38 assembly, which does not include *PRSS3P2* and *TRY7*, corresponds to the deletion allele of the *PRSS3P2* and *TRY7* loci discussed in the present study. Older, now discontinued, symbols for the five trypsinogen genes are also provided in the Figure. (**b**) The tandemly-arranged three-trypsinogen-gene cluster comprising *PRSS58*, *TRY2P* and *PRSS3P3.* Reference genome assembly used was GRCh38. Older symbols for the three genes are also provided in the Figure. (**c**) Relative locations of the two trypsinogen gene clusters (indicated by arrows) in the context of the GRCh38 alternative contig, KI270803.1.

Figure 2. QFM-PCR electropherograms of a *PRSS3P2* and *TRY7* delCNV heterozygote (upper panel) and a deletion homozygote (lower panel). In each panel, the QFM-PCR electropherogram of the subject under study (green) was superimposed upon that of a normal control (red).

Figure 3. Meta-analysis of the association between the *PRSS3P2* and *TRY7* delCNV and CP under a dominant model (i.e., (dd + dw) *vs.* ww), with (**a**) or without (**b**) the inclusion of the Indian TCP cohort. See Table 2 for the genotype distribution data in the four cohorts as well as for the genotype definitions.

Figure 4. Allele-based meta-analysis of the association between the *PRSS3P2* and *TRY7* delCNV and CP, with (**a**) or without (**b**) the inclusion of the Indian TCP cohort. See Table 3 for the allele distribution data in the four cohorts.


a
chr7_KI270803v1_alt:734,167-894,566
740,000 745,000 750,000 755,000 760,000 765,000 770,000 775,000 780,000 785,000 790,000 795,000 800,000 805,000 810,000
Reference Assembl
Reference Assembly A
GENCODE
TRBV29-1
PRSS1
PRSS1
PRSS1
PRSS3P1
PRSS3P2
TRY7
PRSS2
TRBD1
TRBJ1-1
TRBJ1-2
Older names
T4
TRY4
T5
TRY5
T6
TRY6
T7
TRY7
T8
TRY8
b
chr7:142,204,653-142,393,985
142,250,000 142,260,000 142,270,000 142,280,000 142,290,000 142,300,000
Reference Assembly Fix Patc
GENCODE V41 (11 it
PRSS58
PRSS58
ENSG00000241881
TRBV2
RefSeq genes f
Gene Expression in 54 tissues from GTEx RNA-seq
U66059.58
PRSS58
TRBV1
TRY2P
TRBV2
PRSS3P3
Older names
T1
TRY1
T2
TRY2
T3
TRY3
c
chr7_KI270803v1_alt:1-1,111,570
200,000 250,000 300,000 350,000 400,000 450,000 500,000 550,000 600,000 650,000 700,000 750,000 800,000
Reference Assembly Fix Patch Sequence Alignments
Reference Assembly Alternate Haplotype Sequence Alignments
GENCODE V41 (62 items filtered out)
PRSS58
PRSS58
TRBV2
ENSG00000282264
TRBV4-1
TRBV5-1
TRBV7-3
TRBV4-2
TRBV3-1
TRBV6-1
TRBV7-2
TRBV6-4
TRBV5-3
TRBV9
TRBV10-1
TRBV10-2
TRBV6-5
TRBV7-4
TRBV5-4
TRBV6-6
TRBV6-7
TRBV5-5
TRBV6-8
TRBV7-7
TRBV5-7
TRBV10-3
TRBV12-4
TRBV7-9
TRBV13
TRBV11-3
TRBV12-3
TRBV10-3
TRBV12-4
TRBV14
TRBV15
TRBV18
TRBV19
TRBV16
TRBV20-1
TRBV23-1
TRBV24-1
TRBV25-1
TRBV27
TRBV28
TRBV29-1
PRSS1
PRSS1
PRSS1
PRSS2
TRBD1
TRBJ1-1
TRBJ1-2
TRBJ1-3
TRBJ1-4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4500
4000
3500
3000
2500
2000
1500
1000
500
0
Fluorescence (a.u.)
DSC2
MGAM
PRSS3P2
TRY7
PRSS2
PRSS1
PCR Product size (bp)

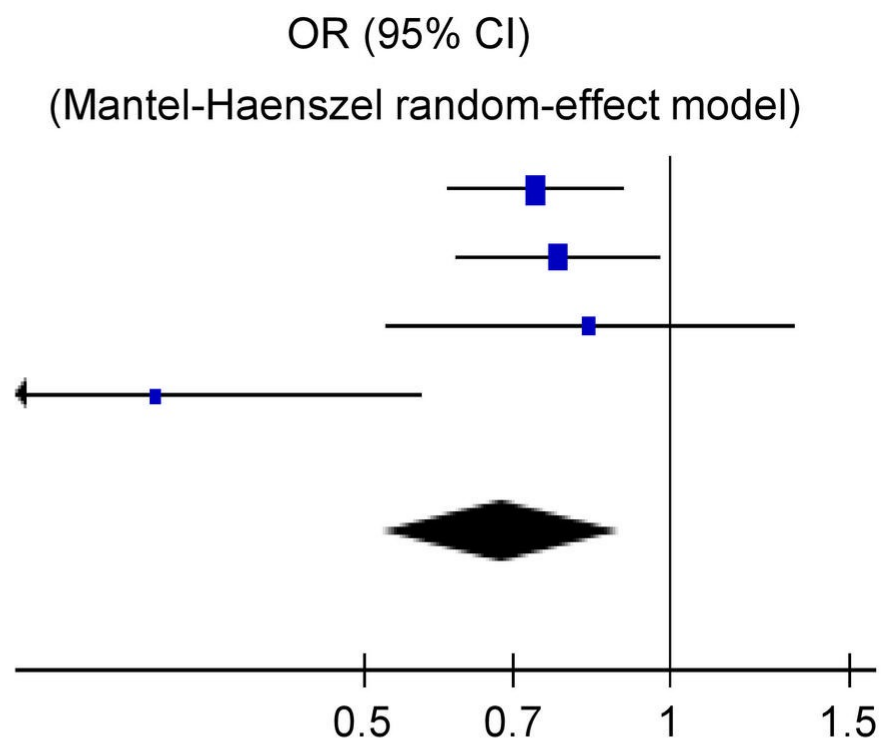
a

Cohort	Weight
French ICP	34.6%
German ICP	32.7%
Indian TCP	19.0%
Japanese ICP	13.7%

Pooled OR (95% CI): 0.68 (0.52-0.89)

Heterogeneity: *Chi*² = 8.41, df = 3 (*p* = 0.04); I² = 64%

Test for overall effect: Z = 2.79 (*p* = 0.005)



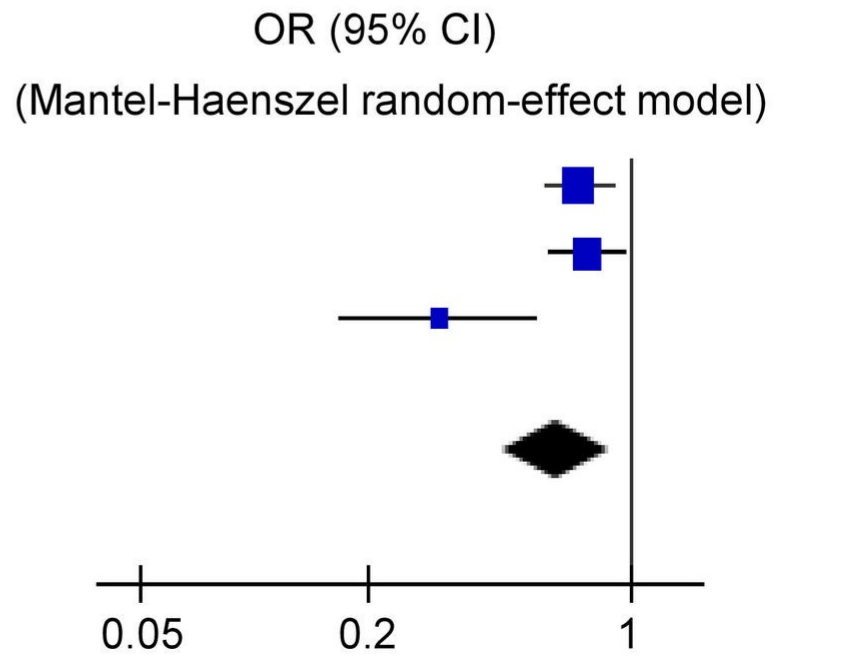
b

Cohort	Weight
French ICP	41.3%
German ICP	39.6%
Japanese ICP	19.1%

Pooled OR (95% CI): 0.64 (0.46-0.89)

Heterogeneity: *Chi*² = 8.02, df = 2 (*p* = 0.02); I² = 75%

Test for overall effect: Z = 2.62 (*p* = 0.009)



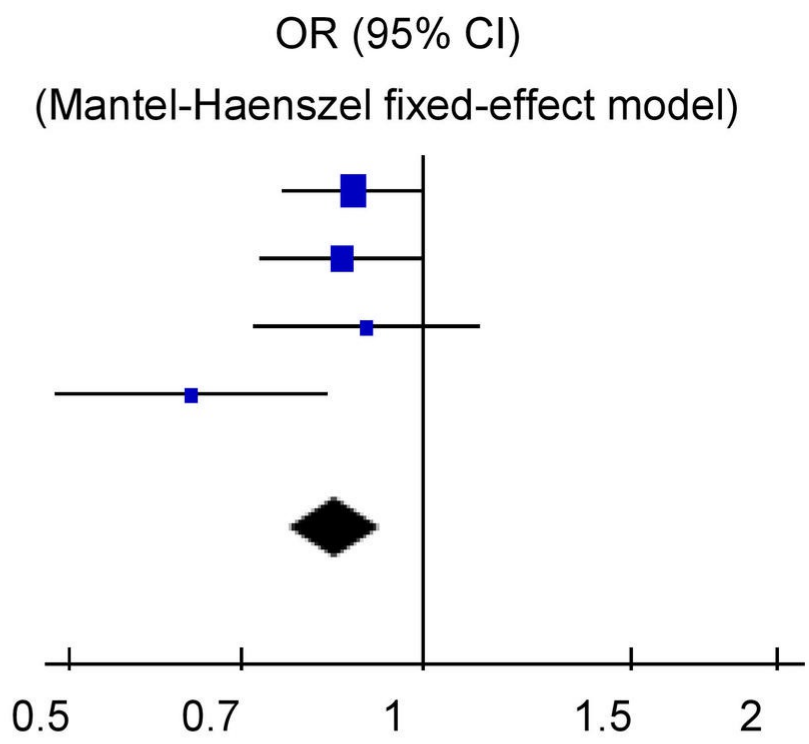
a

Cohort	Weight
French ICP	41.5%
German ICP	30.8%
Indian TCP	15.4%
Japanese ICP	12.3%

Pooled OR (95% CI): 0.84 (0.77-0.92)

Heterogeneity: Chi^2 = 5.05, df = 3 (p = 0.17); I^2 = 41%

Test for overall effect: Z = 3.86 (p = 0.0001)



b

Cohort	Weight
French ICP	41.1%
German ICP	37.0%
Japanese ICP	21.9%

Pooled OR (95% CI): 0.81 (0.69-0.94)

Heterogeneity: Chi^2 = 4.46, df = 2 (p = 0.10); I^2 = 57%

Test for overall effect: Z = 2.69 (p = 0.007)

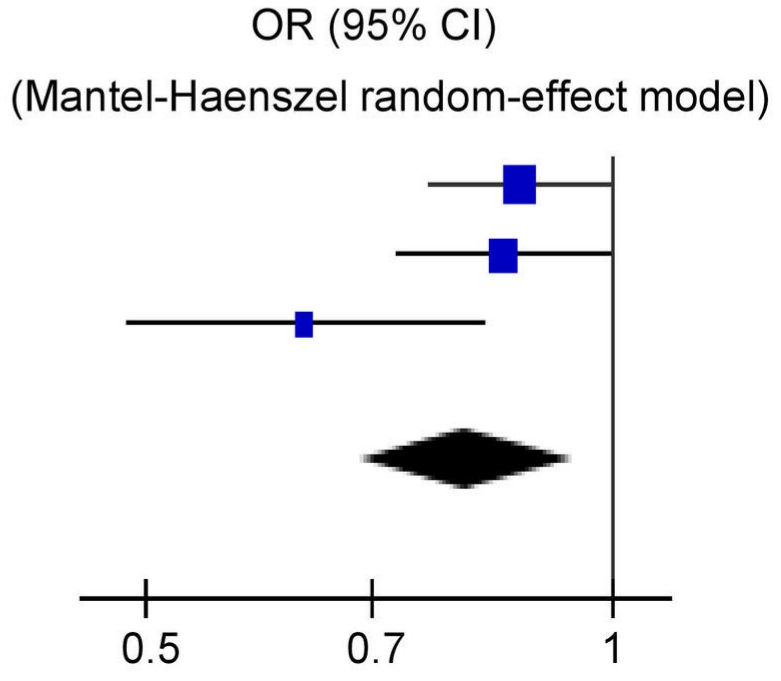


Table 1

CP patients and healthy controls analyzed in this study.

Cohort	Number of patients (number of males; age range)	Number of healthy controls (number of males; age range)
French	588 (332; 3-88)	1611 (922; 24-52)
German	484 (223; 0-67)	977 (376; 18-61)
Indian	278 (190; 6-73)	499 (261; 15-75)
Japanese	186 (99; 4-78)	419 (237; 21-61)

Table 2

Genotype distribution of the *PRSS3P2* and *TRY7* delCNV in CP patients and controls.

Cohort	Genotype[a]	Patients n (%)	Controls n (%)	Association test	OR (95% CI)	*p* value
French	dd	112 (19.0%)	306 (19.0%)	dd *vs*. ww	0.82 (0.62-1.07)	0.1417
	dw	264 (44.9%)	832 (51.6%)	dw *vs*. ww	0.71 (0.57-0.88)	**0.0015**
	ww	212 (36.1%)	473 (29.4%)	(dd + dw) *vs*. ww	0.74 (0.60-0.90)	**0.0027**
German	dd	70 (14.5%)	159 (16.3%)	dd *vs*. ww	0.75 (0.54-1.07)	0.0974
	dw	230 (47.5%)	503 (51.5%)	dw *vs*. ww	0.78 (0.62-1.00)	**0.0450**
	ww	184 (38.0%)	315 (32.2%)	(dd + dw) *vs*. ww	0.78 (0.62-0.97)	**0.0285**
Indian	dd	119 (42.8%)	229 (45.9%)	dd *vs*. ww	0.79 (0.49-1.29)	0.3534
	dw	125 (45.0%)	218 (43.7%)	dw *vs*. ww	0.88 (0.54-1.42)	0.5955
	ww	34 (12.2%)	52 (10.4%)	(dd + dw) *vs*. ww	0.83 (0.53-1.32)	0.4410
Japanese	dd	84 (45.2%)	229 (54.7%)	dd *vs*. ww	0.29 (0.15-0.53)	**<0.0001**
	dw	75 (40.3%)	169 (40.3%)	dw *vs*. ww	0.35 (0.18-0.65)	**0.0007**
	ww	27 (14.5%)	21 (5.0%)	(dd + dw) *vs*. ww	0.31 (0.17-0.57)	**<0.0001**

[a]Genotype definitions: dd, deletion homozygote; dw, deletion heterozygote; ww, wild-type homozygote.

Table 3

Allele frequency distribution of the *PRSS3P2* and *TRY7* delCNV in CP patients and controls.

Cohort	Allele	Patients n (%)	Controls n (%)	OR (95% CI)	*p* value
French	Deletion Wild-type	488 (41.5%) 688 (58.5%)	1444 (44.8%) 1778 (55.2%)	0.87 (0.76-1.00)	**0.0496**
German	Deletion Wild-type	370 (38.2%) 598 (61.8%)	821 (42.0%) 1133 (58.0%)	0.85 (0.73-1.00)	**0.0495**
Indian	Deletion Wild-type	363 (65.3%) 193 (34.7%)	676 (67.7%) 322 (32.3%)	0.90 (0.72-1.12)	0.3258
Japanese	Deletion Wild-type	243 (65.3%) 129 (34.7%)	627 (74.8%) 211 (25.2%)	0.63 (0.49-0.83)	**0.0007**