Classification of *PRSS1* variants responsible for chronic pancreatitis: an expert perspective from the Franco-Chinese GREPAN Study Group

Short title: Classification of *PRSS1* variants

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ABSTRACT

Background: PRSS1 was the first reported chronic pancreatitis (CP) gene. The existence of both gain-of-function (GoF) and gain-of-proteotoxicity (GoP) pathological *PRSS1* variants, together with the fact that *PRSS1* variants have been identified in CP subtypes spanning the range from monogenic to multifactorial, has made the classification of *PRSS1* variants very challenging.

Methods: All currently reported *PRSS1* variants (derived from two databases) were manually reviewed with respect to their clinical genetics, functional analysis and population allele frequency. They were classified by variant type and pathological mechanism within the framework of our recently proposed ACMG/AMP guidelines-based seven-category system.

Results: The total number of distinct germline *PRSS1* variants included for analysis was 90, comprising 3 copy number variants (CNVs), 12 5' and 3' variants, 18 intronic variants, 4 nonsense variants, 1 frameshift deletion variant, 4 synonymous variants, 1 in-frame duplication, 3 gene conversions and 44 missense variants. Based upon a combination of clinical genetic and functional analysis, population data and *in silico* analysis, we classified 26 variants (all 3 CNVs, the in-frame duplication, all 3 gene conversions and 19 missense) as "pathogenic", 2 variants (missense) as "likely pathogenic", 5 variants (four missense and one promoter) as "predisposing", 7 variants (all missense) as "unknown significance", 3 variants (all missense) as "likely benign", and all remaining 50 variants as "benign".

Conclusions: We describe an expert classification of the 90 *PRSS1* variants reported to date. The results have immediate implications for reclassifying many ClinVar-registered *PRSS1* variants as well as providing optimal guidelines/standards for reporting *PRSS1* variants.

Keywords: Chronic pancreatitis; Genetic predisposition to disease; *PRSS1* gene; Trypsinogen/trypsin; Variant classification

List of abbreviations

ACMG/AMP, the American College of Medical Genetics and Genomics/Association for Molecular Pathology

ACP, alcoholic chronic pancreatitis

AIP, autoimmune pancreatitis

AP, acute pancreatitis

CFTR, cystic fibrosis transmembrane conductance regulator

CI, confidence interval

CP, chronic pancreatitis

CNV, copy number variant

CTRC, chymotrypsin C

ER, endoplasmic reticulum

FCP, familial chronic pancreatitis

gnomAD, the Genome Aggregation Database

GoF, gain-of-function

GoP, gain-of-proteotoxicity

gpAF, global population allele frequency

GREPAN, Genetic REsearch on PANcreatitis

GWAS, genome-wide association study

HGMD, Human Gene Mutation Database

hspAF, highest subpopulation allele frequency

ICP, idiopathic chronic pancreatitis

8.3KJPN, 8.3 K Japanese population reference panel

KRGDB, Korean Reference Genome Database

LD, linkage disequilibrium

LoF, loss-of-function

NA, not available

OR, odds ratio

RAP, recurrent acute pancreatitis

1. Introduction

Chronic pancreatitis (CP) is a chronic inflammatory process leading to progressive morphological and functional changes of the pancreas [1, 2]. It has a prevalence of 36-125 per 100,000 individuals [3]. The process of CP is thought to be irreversible once initiated [4, 5] and there is currently no cure for the disease. Therefore, determining the genetic basis of CP holds out promise for developing new options in disease prevention and treatment. In 1996, a missense variant, p.Arg122His, in the *PRSS1* gene (encoding cationic trypsinogen) was identified as a cause of an inherited form of CP, namely autosomal dominant hereditary CP (HCP) [6]. This marked the beginning of a new era in CP research. To date, more than 10 CP-related gene loci have been reported (for references, see [7]). Moreover, studies of *PRSS1* variants led to the recognition of two distinct pathological pathways in the etiology of CP, namely the trypsin-dependent pathway [8] and the misfolding-dependent pathway [9]. *PRSS1* variants belonging to the former pathway include missense variants that increase trypsinogen (auto)activation and/or trypsin stability as well as copy number and regulatory variants that increase *PRSS1* dosage; these variants have been collectively termed gain-of-function (GoF) variants [10]. *PRSS1* variants belonging to the latter pathway include only missense variants that could induce the formation of misfolded proteins that would in turn elicit endoplasmic reticulum (ER) stress; these variants have been termed gain-of-proteotoxicity (GoP) variants [10]. The existence of both GoF and GoP pathologically relevant *PRSS1* variants, together with the fact that *PRSS1* variants have been identified in CP subtypes spanning monogenic to multifactorial, complicate the classification and interpretation of *PRSS1* variants [11].

The American College of Medical Genetics and Genomics/Association for Molecular Pathology (ACMG/AMP)-recommended five-category scheme (i.e., “pathogenic”, “likely pathogenic”, “uncertain significance”, “likely benign” and “benign”) for classifying variants in Mendelian disease genes [12] has been widely used in the human genetics field. However, a serious drawback of this five-category scheme is that it cannot deal properly with variants that fall somewhere between “pathogenic” and “benign”. Employing CP as a disease model, and focusing on the four most studied CP-related genes (i.e., *PRSS1*, *CFTR* (encoding cystic fibrosis transmembrane conductance regulator) [13, 14], *SPINK1* (encoding pancreatic secretory trypsin inhibitor) [15] and *CTRC* (encoding chymotrypsin C [16, 17]), we have recently proposed a seven-category system (i.e., “pathogenic”, “likely pathogenic”, “predisposing”, “likely predisposing”, “unknown significance”, “likely benign” and “benign”) for classifying variants in any disease-causing gene [10]. In a preprint, we have provided evidence to support our contention that the newly added “*predisposing*” variant classificatory category

is an appropriate repository for the many intermediate variants that fall somewhere between “pathogenic” and “benign” [18].

As far as *PRSS1* is concerned, our previous study employed many of the experimentally demonstrated GoF and GoP variants to establish proof of concept for our seven-category variant classification system [10]. Prior to our own study, two papers were directly relevant to the classification of *PRSS1* variants. In 2014, Németh and Sahin-Tóth provided an early classification of the then published *PRSS1* variants [19]. Their classifications relied strongly upon functional analysis data. For example, all variants that had not been functionally analyzed (apart from nonsense and canonical GT-AG splice site variants) were classified as being of “unknown significance”. Moreover, all missense variants that were experimentally shown to be compatible with a GoF or GoP mechanism were classified as “pathogenic”. The second paper did not include 5' and 3' variants or intronic variants (except for those occurring within the GT-AG canonical splice sites) and designated specific terms for classifying pathologically relevant *PRSS1* variants (e.g., “pathogenic variants with established risk to be disease-causing”) [16]. Herein, we describe a classification of all currently known *PRSS1* variants for CP within the framework of the ACMG/AMP guidelines-based seven-category variant classification system; an expert perspective from the Franco-Chinese GREPAN (Genetic REsearch on PANcreatitis) Study Group.

2. Methods

2.1. The Franco-Chinese GREPAN Study Group

The Franco-Chinese GREPAN Study Group is composed of clinicians, geneticists, bioinformaticists and basic researchers from various regions of France and China. One of its tasks is to provide expert classifications of genetic variants reported in CP-related genes. In classifying the *PRSS1* variants, the Study Group attempted to adhere to the ACMG/AMP guidelines wherever possible while making amendments and additions when required.

2.2. *PRSS1* variants

PRSS1 variants were derived from a combination of data derived from the Genetic Risk Factors in Chronic Pancreatitis Database (https://pancreasgenetics.org/; accessed 08/31/2022) [19] (N.B. *PRSS1* variant data have been removed from the database since November 2022) and the Human Gene Mutation Database (HGMD; https://www.hgmd.cf.ac.uk. Accessed 02/22/2023) [20]. Original reports describing the registered *PRSS1* variants were manually reviewed with respect to clinical genetic findings and data from functional analyses (see

below). Inquiries were made to the original authors only in cases of uncertainty about the described sequence changes or variant nomenclature. Three variants were excluded from further consideration: one somatic variant and two variants obtained via personal communications. Cross-reference examination and keyword search ("*PRSS1*" plus "variant" or "mutation") in PubMed did not identify additional variants beyond those registered in the two Databases (Figure 1).

Variant nomenclature employed here was in accordance with Human Genome Variation Society recommendations [21]. Nomenclature at the DNA level was in relation to the hg19 chromosome 7 sequence whilst the nomenclature at the coding DNA reference followed NM_002769.5.

2.3. Approaches to, and principles for, variant classification

Functionality, a prerequisite for pathological relevance, is closely bound up with the type of variant or the variant's location within the genomic sequence of the gene in question. This is of particular importance for classifying *PRSS1* variants because both GoF and GoP variants are of pathological relevance whereas loss-of-function (LoF) variants are benign in relation to CP [10]. We therefore classified them by variant type and pathological mechanism within the framework of our recently proposed ACMG/AMP guidelines-based seven-category framework (Figure 1). Here, it is worth emphasizing three points. First, we previously proposed an allele frequency threshold, 0.001 as an aid to distinguish *PRSS1* "pathogenic" variants from "predisposing" variants with respect to CP [10]. Thus, any pathologically relevant *PRSS1* variant having an allele frequency of ≥0.001 in the general population would be considered to be too common to cause CP; rather, it would be regarded as predisposing to CP. This proposition, which was based on the allele frequency cutoff recommended for filtering dominant Mendelian disease-causing variants [22], and validated against most of the experimentally confirmed *PRSS1* GoF and GoP variants [10], will be adopted in the current study. Second, we previously proposed to classify LoF *PRSS1* variants (e.g., nonsense and canonical GT-AG splice site variants) as "benign" whilst specifying their *protective* nature in parentheses after the primary variant classificatory category [10]. Herein, we will classify all predicted and experimentally demonstrated LoF variants as "benign" whilst using the complementary term *protective* only for those variants causing a complete or almost complete LoF of the affected allele. Third, *PRSS1* variants were not only reported in subjects with CP but also in subjects with other diseases (see subsection 2.4.1). Here we included all known *PRSS1* variants in our classification. However, for those variants that were reported only in the context of non-CP diseases, their classification was carried out solely in relation to their pathological relevance to CP rather than to the non-CP diseases.

2.4. Factors taken into consideration for variant classification

2.4.1. Clinical genetic data

Clinical genetic data refer to whether or not the variant in question has been found in subjects with CP or other diseases, or in controls. Data from some original reports were reinterpreted according to our working definitions of HCP, familial CP (FCP) and idiopathic CP (ICP) (for disease subtype definitions, see Masson et al. [15] and references therein). For frequently reported variants, usually only the first three and/or representative publications were cited. Definitions of a variant as common (allele frequency of ≥0.05), low frequency (≥0.005 to <0.05), rare (≥0.001 to <0.005) or very rare (<0.001) are in accordance with Manolio et al. [23].

PRSS1 variants have been confirmed to play a pathological role (either causative or predisposing) in not only HCP, FCP and ICP but also in alcoholic CP (ACP) (e.g., [24, 25]). *PRSS1* variants may also play a role in autoimmune pancreatitis (AIP) (e.g., [26]) or asparaginase-associated pancreatitis [27] but definitive conclusions cannot be drawn at this stage owing to the limited data available and/or lack of replication. Here, clinical genetic findings made in these two rather specific manifestations of pancreatitis will not be considered as being informative with regard to the pathological role of *PRSS1* variants in the etiology of CP.

Whilst GoF and GoP *PRSS1* variants predispose to CP, CP itself increases the risk of pancreatic cancer [28]. However, to date, a direct causal link between GoF and GoP *PRSS1* variants and an increased risk of pancreatic cancer has not been established, let alone for cancers affecting other organs/tissues. Thus, germline *PRSS1* variants found in individuals with any type of cancer will not be interpolated to those variants found in individuals with CP.

PRSS1 variants have sometimes been identified in patients with acute pancreatitis (AP), particularly recurrent AP (RAP) without known etiological factors. Given that 10% of subjects with a first attack of AP and 36% of subjects with RAP would progress to CP [29], such findings will be considered here as clinical genetic evidence pertinent to the pathological role of *PRSS1* variants in the etiology of CP.

2.4.2. Functional analysis data

Functional analysis data (with respect to the functional effect of the variant in question) refer to laboratory findings obtained from either biochemical analysis, cell transfection experiments, transgenic mouse studies or analyses performed using patient-derived material.

2.4.3. Population allele frequency data

Global population allele frequency (gpAF) and highest subpopulation allele frequency (hspAF) of studied variants were obtained from the Genome Aggregation Database (gnomAD) [30, 31].

2.4.4. In silico analyses and evidence-based conjecture

The linkage disequilibrium (LD) between two variants was evaluated by means of the LDpair Tool available on the LD link website [32]. The regulatory potential of 5' and 3' variants was evaluated in terms of their RegulomeDB probability scores [33, 34], a model that integrated functional genomics features along with continuous values such as ChIP-seq signals, DNase-seq signals, information content change, and DeepSEA scores. The score ranges from 0 to 1. The higher the score, the more likely the variant is to be a functional regulatory variant [35]. SpliceAI [36, 37], the currently most accurate tool for predicting splicing variants (e.g., [38, 39]), was used to classify intronic, nonsense and synonymous variants in terms of their contribution to aberrant splicing. Evidence-based conjecture was employed for classifying variants without supporting functional data whenever appropriate.

3. Results

A total of 90 distinct germline *PRSS1* variants, all reported in peer-reviewed papers, were derived from the two Databases that contain *PRSS1* variant data (Figure 1). Mindful of the existence of two discrete pathological mechanisms underlying CP-related *PRSS1* variants, these were divided into five subcategories for classificatory purposes. Before going into the details, we reiterate that some *PRSS1* variants have so far only been reported in subjects with diseases other than CP; these variants were classified with respect to their relevance to CP rather than the non-CP diseases.

3.1. Classification of gain of trypsinogen CNVs

Consistent with previous publications [10, 19], all three gain of trypsinogen CNVs were classified as "pathogenic" because (i) they have been identified in multiple ICP patients and/or HCP or FCP families; (ii) they are absent or extremely rare in the general population; and (iii) their presumed dosage effect on the etiology of CP was confirmed by transgenic mouse studies (Table 1).

3.2. Classification of 5' and 3' variants

For ease of discussion, the 12 5' and 3' variants will be divided into common and uncommon variants.

3.2.1. Common variants

All six common variants are in high LD, as indicated by the R^2 values in Table 2. The first reported variant, c.-408T>C (rs10273639), will henceforth be used as a tag for this common haplotype. c.-408T>C has a global population allele frequency (gpAF) of 0.5189 in gnomAD (v2.1.1) [31]. A previous meta-analysis showed that c.-408T>C had a pooled odds ratio (OR) of 1.28 (95% confidence interval (CI) 1.17-1.40; $p < 0.00001$) for ICP [40].

c.-204A>C (rs4726576) has been shown, both *in silico* and *in vitro*, to be a functional regulatory variant [41, 42]. It has previously been classified as "predisposing" in the context of CP [10]. Of the remaining five variants, only c.-408T>C was subjected to a promoter reporter gene assay, and was shown to have no effect on gene expression [42].

Herein, we assessed the regulatory potential of the six common variants in terms of the RegulomeDB probability score [33, 34]. As shown in Table 2, the experimentally demonstrated functional c.-204A>C variant has a RegulomeDB probability score of 0.75713 whereas the other five variants have scores of <0.14. We further correlated the six common variants with DNase I-accessible DNA regions in pancreatic tissue using data available from the NIH Roadmap Epigenomics Mapping Consortium website [43]. Only c.-204A>C was found to be located within a significant DNaseI-accessible DNA region within the *PRSS1* locus (Supplementary Figure 1). Additionally, c.-204A>C occurred within a MAF transcription factor binding site motif, according to RegulomDB [34] (Supplementary Figure 2a). Although MAF protein is highly expressed in the pancreas [44], to date, no studies have investigated its role in the exocrine pancreas. MAF proteins can act as transcriptional activators or repressors depending upon the target genes [45].

The above cross-variant comparisons have demonstrated a clear difference between c.-204A>C and the other five common variants in terms of their potential regulatory features. This has served to strengthen the previous classification of c.-204A>C as "predisposing" while suggesting that the other five variants could be classified as "benign". It should however be emphasized that c.-204A>C may not be the sole functional variant underlying the increased risk of the c.-408T>C-tagged haplotype. This risk haplotype has been recently shown to contain the *PRSS3P2* and *TRY7* pseudogenes whereas the alternative haplotype has lost the two pseudogenes [46, 47]; this alternative haplotype was reported to be associated with a protective effect against CP [48]. Interestingly, the risk haplotype appears to contain still functional *PRSS3P2/TRY7* pseudogene enhancers that serve to upregulate pancreatic *PRSS2* expression [49], which is consistent with the increasingly appreciated role of *PRSS2* in

pancreatitis (see Masson et al. [48] and references therein). It is plausible that the risk conferred by the c.-408T>C-tagged haplotype is attributable to two non-mutually exclusive mechanisms.

3.2.2. Uncommon 5' variants

Except for c.-338T>G, which was identified in 3 of 65 Chinese patients with pancreatic cancer but without pancreatitis [50], all six uncommon 5' variants have been reported only once, either in CP patients or controls (Table 3). Herein, it should be noted that whereas c.-338T>G is very rare in combined gnomAD populations, it occurs as a low frequency variant in the Japanese and Korean populations according to their respective population-specific databases (i.e., 0.01468 in accordance with the 8.3 K Japanese population reference panel (8.3KJPN) project and 0.0109 in accordance with the Korean Reference Genome Database (KRGDB); Table 3).

Four variants (c.-184G>A, c.-173C>T, c.-147C>T and c.-30_-28delTCC) were subjected to a promoter reporter gene assay but only c.-147C>T exhibited a significant (and negative) effect on gene expression [51]. Notably, c.-147C>T was identified as affecting a binding site for ATF4 [51]; ATF4 is highly expressed in the pancreas [44] and knockout of ATF4 has been reported to cause pancreatic deficiency in mice [52].

We further performed a cross-variant comparison in terms of regulatory potential. All six uncommon 5' variants are located within the significant DNase I-accessible DNA region that contains the common c.-204A>C variant (refer to Supplementary Figure 1a). Presumably, the high RegulomeDB probability score for the functional c.-147C>T variant, 0.98, would be largely attributable to its location within this significant DNase I-accessible DNA region and its disruption of the binding site for transcription factor ATF4 that has an experimentally confirmed role in the exocrine pancreas (Supplementary Figure 2b). By contrast, the identical and lower RegulomeDB probability score, 0.60906, for the other five variants would be accounted for by their location within the same significant DNase I-accessible DNA region even though they do not affect any known transcription factor binding sites (Table 3).

Taken together, all six uncommon 5' variants could be classified as "benign" (Table 3).

3.3. Classification of intronic variants

3.3.1. Two canonical splice site variants

Neither of the two canonical splice site variants, c.40+1G>A and c.200+1G>A, was found in subjects with pancreatitis (Table 4). Both of them represent predicted LoF (pLoF) variants in accordance with [30]. Herein, we confirmed their LoF nature by means of SpliceAI [36, 37]. c.40+1G>A was predicted to disrupt the splice donor

site of intron 1 whilst activating a nearby downstream cryptic splice donor site; the mutant transcript, which would be predicted to encode a product comprising only 19 amino acids (Supplementary Figure 3), is likely to be subject to significant degradation by nonsense-mediated mRNA decay [53]. c.200+1G>A was predicted to disrupt the splice donor site of intron 2 whilst activating an upstream cryptic splice donor site within exon 2; this would be predicted to lead to the loss of the terminal 33 bp of exon 2, which would in turn lead to the translation of a protein with an internal deletion of 11 amino acids; the mutant protein cannot be functional owing to the loss of histidine 63, one of trypsin's catalytic triad residues (Supplementary Figure 4).

Consistent with our previous publication [10], these two pLoF variants were classified as "benign (protective)".

3.3.2. A pentanucleotide deletion variant with accompanying *in vivo* functional data

c.200+64_68delCCCAG was detected in a Chinese AIP family but did not segregate with the disease [all three patients (one homozygote) and 3 of 7 genetically analyzed healthy members carried the variant]; it was also detected in a Chinese idiopathic AIP patient but was absent from 520 unrelated healthy controls; RNA analysis using patient blood cells revealed a mutant transcript harboring an in-frame deletion of the first 141 nucleotides of exon 2 [54]. As illustrated in Supplementary Figure 5, the mutant transcript would encode a protein lacking amino acids 14-60 in the context of pretrypsinogen and the first 37 amino acids in the context of trypsin. Such a significantly truncated trypsin at the N-terminal end is unlikely to function properly. Indeed, *in vitro* biochemical characterization demonstrated that the mutant protein had lower trypsin activity than the wild-type protein with or without enterokinase treatment [54]. These, together with the fact that *PRSS1* has no confirmed role in AIP, would suggest that the detection of c.200+64_68delCCCAG in AIP was most probably a chance finding. Consequently, we think that it is reasonable to classify c.200+64_68delCCCAG as "benign" in the context of CP.

3.3.3. The remaining intronic variants

Two of the remaining 15 entries are worthy of mention. The first is c.41-49C>T, which was identified in 2 patients (father and son) of an FCP family. However, c.41-49C>T has a rather high allele frequency in the general population (i.e., gpAF, 0.001122; hspAF, 0.005499 (Table 4)). Applying our previously proposed allele frequency threshold of 0.001 [10] suggests that it is unlikely to be a "pathogenic" variant. The second is

c.454+172C>T, which was found in a HCP family. However, it was co-inherited with *PRSS1* p.Asn29Ile, the second most frequent *PRSS1* variant causing HCP.

Importantly, none of these 15 variants were predicted by SpliceAI to affect splicing, supporting their "benign" nature from a mechanistic viewpoint.

3.4. Classification of nonsense, frameshift deletion and synonymous variants

In common with the two canonical splice site LoF variants (Table 4), none of the five pLoF variants including four nonsense (p.Y37*, p.W57*, p.Q86*and p.C160*) and one frameshift deletion (p.P164Lfs*3), were found in patients with CP (Supplementary Table 1). They are unequivocal LoF variants and were thus classified as "benign (protective)".

Two of the four silent variants, c.486C>T (p.D162=) and c.738C>T (p.N246=), are in high LD with the previously discussed c.-408T>C variant. The other two variants, c.273C>A (p.A91=) and c.417C>T (p.C139=), are rare in the general population. However, one was identified in a healthy subject whereas the other was identified in a patient with a disease (i.e., AIP) that was not considered relevant to *PRSS1* variants (Supplementary Table 1). Additionally, the four silent variants were not predicted to affect splicing by SpliceAI. Taken together, all four silent variants could be reasonably classified as "benign" despite not being functionally analyzed.

3.5. Classification of missense, small in-frame duplication and gene conversion variants

3.5.1. Variants with supporting functional analysis

Thirty-six of the 48 missense, small in-frame duplication and gene conversion variants have been functionally analyzed, mainly by the Sahin-Tóth group. A functional test is generally regarded as the gold standard for classifying any variant. Therefore, it is relatively straightforward to classify these 36 variants on the basis of their supporting functional data.

All 17 experimentally demonstrated GoF variants had a hspAF of <0.001. Of these variants, 7 affected the activation peptide sequence (i.e., the first 7 variants in Table 5), 3 affected Asn29, 4 affected Arg122, p.Val39Ala segregated perfectly with the disease in a large HCP family (i.e., the variant was identified in all eight analyzed patients but not in any of the four healthy family members analyzed [55]) whilst p.E190K was identified in an 11-year-old girl with ICP. All these 16 variants were characterized by a GoF effect on PRSS1 itself. The remaining p.Glu79Lys variant, which was identified in multiple ICP and FCP patients in four

independent studies (see Table 5), may be regarded as an outlier with respect to the GoF mechanism. The p.Glu79Lys-cationic trypsin was shown to transactivate PRSS2 (encoding anionic trypsinogen, the second major trypsinogen isoform after PRSS1) more efficiently than the wild-type [56]; a finding compatible with the growing view that increased PRSS2 expression acts as an independent GoF mechanism underlying CP [48]. In short, the clinical genetic, functional and population data concurred, supporting a "pathogenic" classification for all 17 variants.

Of the 10 experimentally demonstrated GoP variants, 6 were characterized by a severe secretion effect and all these 6 variants had a hspAF of <0.001. Of the 6 variants associated with a severe secretion effect, 3 were further analyzed with respect to ER stress in transfected cells; all three were found to exhibit increased ER stress markers. Of these 3 variants, two had been identified in HCP families (Table 5). By contrast, none of the four variants characterized by a moderate secretion effect were found in HCP families. Most importantly, one of these variants, p.Gly208Ala, had an allele frequency of 0.009514 in East Asians; based upon data from a large Chinese cohort study [57], p.Gly208Ala was calculated to have an OR of 4.72 for ICP (95% CI 2.88-7.72, $p = 9.2 \times 10^{-11}$) [58]. As a matter of fact, it was these two latter observations that prompted our proposal of the seven-category variant classification system [10]. Now that p.Gly208Ala is unequivocally categorized as a "predisposing" variant, it would appear reasonable to use "moderate secretion defect" and "severe secretion effect" as additional and functional criteria for classifying these GoF variants. Thus, the six variants with a severe secretion effect were classified as "pathogenic" whereas the four variants with a moderate secretion effect were classified as "predisposing" (Table 6).

All nine variants characterized by no effect or LoF were classified as "benign" (Table 5).

3.5.2. Variants without supporting functional data

The 12 missense variants lacking supporting functional data are summarized in Table 6. All were reported in single studies and were almost invariably found in single subjects. Moreover, seven of these variants were either found in controls or individuals with a disease that was not considered to be relevant to a pathological role for *PRSS1* variants in CP. Nonetheless, just as a rare benign variant may be found in a CP patient by chance (see Table 5), a rare pathological variant may also be found in a non-CP patient or control by chance for many reasons (e.g., asymptomatic disease, late disease onset, low penetrance, *de novo*). It should also be emphasized that *in silico* prediction tools tend to have relatively poor overall performance in relation to missense variants [59]. Their utility would be even more limited for the prediction of functional consequences of *PRSS1* variants

owing to the existence of two possible pathological mechanisms. GoF variants are inherently refractory to prediction [60] and we are not aware of any *in silico* tools that can distinguish GoP variants from LoF variants. Therefore, to be on the safe side, and following in the footsteps of Németh and Sahin-Tóth [19], all 12 missense variants could conservatively be classed as being of "unknown significance". We nevertheless attempted to improve upon the classification of five of these variants using evidence-based conjecture.

p.Val39Glu was found in a patient with RAP and is absent from gnomAD (Table 6). p.Val39Ala, which affected the same amino acid site as p.Val39Glu, was a "pathogenic" GoF variant (see Table 5). p.Val39Ala is thought to modify PRSS1 structure in such a way as to reduce trypsinogen cleavage at Arg122 by trypsin and at Leu81 by CTRC as well as reducing trypsin degradation by CTRC [61]. It is possible that p.Val39Glu (as well as p.V39G that was identified serendipitously in a pediatric soft tissue sarcoma survivor; Table 6) has a similar effect to p.Val39Ala. Taken together, we are minded to classify both p.Val39Glu and p.V39G as "likely pathogenic".

p.Gln98Arg was identified in a single patient with pancreatitis and has an extremely low allele frequency in gnomAD (Table 6). Interestingly, p.Gln98Lys was a "benign" variant (see Table 5). Given that arginine and lysine have remarkably similar physicochemical properties (i.e., both are polar and positively charged) [62], we suggest classifying p.Gln98Arg as "likely benign".

p.Leu104Val was identified in two patients and in two healthy members of a family with solid pseudopapillary tumors and is absent in gnomAD (Table 6). p.Leu104Pro was a pathogenic GoP variant (see Table 5). Since leucine is markedly different from proline but very similar to valine in terms of its physicochemical properties [62], we would classify p.Leu104Val as "likely benign"

p.Val123Leu was found in a subject without any overt pancreatic disease and is very rare in gnomAD (Table 6). p.Val123Met was a "benign" variant (see Table 5) and leucine is a conservative substitution for methionine [63], suggesting that p.Val123Leu is a "likely benign" variant.

4. Discussion

The Franco-Chinese GREPAN Study Group comprises clinicians, geneticists and basic researchers with diverse and complementary expertise in CP. In this study, the Group provides an expert classification of the currently reported 90 *PRSS1* variants with respect to their clinical relevance in relation to CP. Based upon the combined consideration of clinical genetic, functional analysis, population data and *in silico* analysis and evidence-based conjecture when appropriate, we classified 26 variants as "pathogenic", 2 variants as "likely

pathogenic", 5 variants as "predisposing", 7 variants as of "unknown significance", 3 variants as "likely benign", and 47 variants as "benign". Apart from its completeness in terms of variants included, this study is characterized by two features. First, it employed *in silico* tools to aid the classification of 5' and 3', nonsense, silent and intronic variants but not missense variants. This decision was made on the basis of balancing the biological plausibility of a given type of variant being pathologically relevant to CP or not with the reliability (and feasibility) of the *in silico* prediction tools on the other. For example, SpliceAI is a highly accurate tool for predicting the potential effects of single nucleotide substitutions or small indel variants on splicing. Therefore, a negative prediction for any silent or intronic *PRSS1* variant strongly supports a "benign" classification. Even in the case of a positive prediction (i.e., predicted to affect splicing), from a mechanistic viewpoint, the aberrantly spliced transcript would most likely lead to a LoF rather than a GoF or GoP. In this regard, it is worth mentioning that the vast majority of the silent and intronic *PRSS1* variants registered by ClinVar are classified as "uncertain significance", "conflicting interpretations of pathogenicity" or "likely benign" [64]. In line with our above reasoning, most, if not all, of these ClinVar-registered silent and intronic *PRSS1* variants should be reclassified as "benign" with respect to CP. By contrast, the existence of two types of *PRSS1* pathologically relevant missense variants, GoF and GoP, made *in silico* predictions undesirable because these predictions could be potentially misleading. Therefore, one may have to rely heavily on functional analysis to classify clinically relevant *PRSS1* missense variants. Second, having compared the GoP variants with respect to their clinical genetic, functional analysis and population data, we proposed to classify the variants with a "moderate secretion defect" as "predisposing" and the variants with a "severe secretion defect" as "pathogenic". This functional phenotype-based classification criterion, together with our previously proposed allele frequency threshold, promises to improve our understanding of the complexity underlying disease expression and the genotype-phenotype relationship in CP.

Variant classification is an important, complex and evolving issue in the field of human genetics, and this is reflected in the constantly refined variant classification standards/guidelines and the ever increasing number of reports of reclassified variants (for examples in both contexts, see [18]). In this vein, our proposed allele frequency threshold and functional phenotype-based classification criterion for distinguishing *PRSS1* "pathogenic" variants from "predisposing" variants (as well as our proposed classifications for some rare *PRSS1* missense variants) may need to be refined as more data become available. Indeed, separating "predisposing" variants from "pathogenic" variants is a difficult task, a process that would require us to make semi-arbitrary but nevertheless operational thresholds. As we noted previously, all such decisions "need to be made on a gene-by-

gene basis and would require close collaboration between researchers and clinicians with specific expertise in the diseases/genes in question" [19].

In summary, this study provides a systematic classification of the so far reported 90 *PRSS1* variants for CP within the framework of the ACMG/AMP guidelines-based seven-category variant classification system. We believe that our study will have immediate implications for interpreting ClinVar-registered *PRSS1* variants and should serve to provide optimal guidelines/standards for reporting *PRSS1* variants in the future.

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Author contributions

E.M. and W.B.Z. contributed to the study design, data collection, performed variant classification and contributed to manuscript writing. N.P. collected data and performed variant classification. V.R. and E.G. analyzed data and critically revised the manuscript with important intellectual input. H.W, J.H.L. and Y.C.W. contributed to data collection and variant classification. Z.S.L. contributed to study conception and critically revised the manuscript with important intellectual input. D.N.C. contributed to the study design and variant classification and led manuscript revision. C.F. contributed to study conception, supervised the study and critically revised the manuscript with important intellectual input. Z.L. contributed to study conception, led the Chinese group and contributed to variant classification. J.M.C. conceived and coordinated the study, collected data, performed variant classification and drafted the manuscript. All authors approved the final manuscript submitted.

Declaration of competing interest

The authors are unaware of any conflict of interest.

Appendix A. Supplementary data

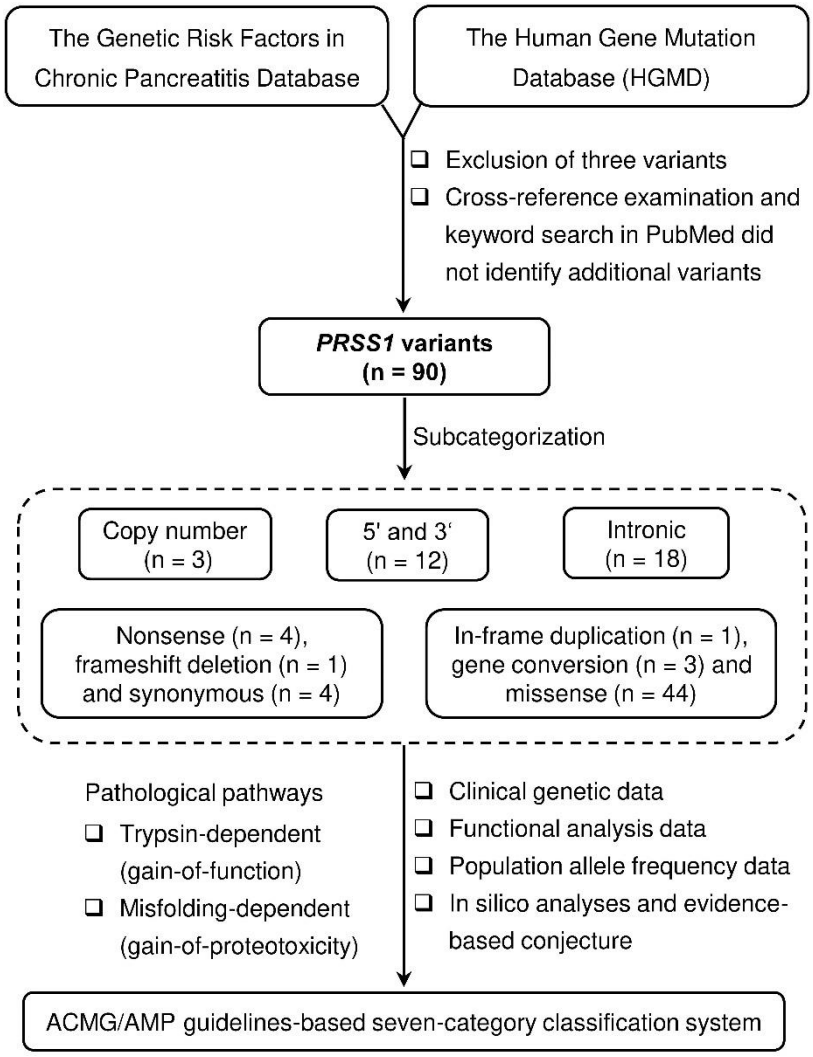

Figure 1. Variant collection and classification procedures.

Table 1. Classification of gain of trypsinogen CNVs

Variant	**Clinical genetic data**	**Functional analytic data**	**gpAF (hspAF)[a]**	**Classification (for CP)**
Triplication	Causes HCP in multiple families; has also been identified in cases with FCP and ICP [41, 65-67]	GoF (three mouse transgenic studies [68-70] were retrospectively identified to be informative in relation to the pathogenic mechanism underlying the trypsinogen gene dosage effect in pancreatitis [41])	NA	Pathogenic
Duplication	Detected in multiple ICP patients and one patient with FCP [41, 66, 67]	Same as above	0.00004610 (0.0001049, African/African American)	Pathogenic
Double GoF hybrid variant	Identified in 1 HCP family with 6 patients across 3 generations [71]	GoF (gene dosage plus the effect of p.Asn29Ile) [71])	NA	Pathogenic

[a]In accordance with gnomAD SVs v2.1 [31]. NA, not available.

Table 2. Classification of common *PRSS1* 5' and 3' variants

Location	Variant[a]			R^2 (with respect to rs10273639)[b]	Clinical genetic data	Functional data	RegulomeDB score[c]	Classification (for CP)
	c.nomenclature (NM_002769.5)	g.nomenclature (chr7, hg19)	rs number					
5'	c.-408T>C	g.142456928T>C	rs10273639		Association with CP discovered by means of GWAS [24]; replicated in multiple subsequent studies (pooled odds ratio for ICP, 1.28 [40], which was calculated here to correspond to an increase in absolute risk from 0.0084% to 0.011%)	Not functional by means of a promoter reporter gene assay [42]; the risk haplotype is associated with slightly increased *PRSS1* and *PRSS2* mRNA expression in pancreatic tissue in a dosage-dependent manner [24, 40]; the risk haplotype contains still functional *PRSS3P2/TRY7* pseudogene enhancers that upregulate pancreatic *PRSS2* expression [48, 49]	0.13454	Benign
5'	c.-204A>C	g.142457132A>C	rs4726576	0.9365	Found to be in complete LD with c.-408T>C by resequencing the promoter region of *PRSS1* in French Caucasian individuals [42]	GoF (increased gene expression by means of a promoter reporter gene assay [42])	0.75713	Predisposing
5'	c.-1809A>G	g.142455527A>G	rs3757378	0.7111	Found by direct sequencing of 2.1 kb *PRSS1* 5' region; linked with c.-408T>C and c.-204A>C but not associated with tropical calcific pancreatitis [72]	No data available	0.00347	Benign
5'	c.-1798T>C	g.142455538T>C	rs3757377	0.7158	Same as c.-1809A>G	No data available	0.13454	Benign
5'	c.-1383A>G	g.142455953A>G	rs9969188	0.9568	Same as c.-1809A>G	No data available	0.0	Benign

3'	c.*12,596G>A	g.142473466G>A	rs13228878	0.6593	Identified by GWAS; linked with c.-408T>C and associated with asparaginase-associated pancreatitis [27]	No data available	0.09659	Benign

[a]Variants are listed in the order of publication year.
[b]Data were obtained using the LDproxy Tool available on the LD link website [32] in the context of all populations.
[c]Data were obtained from the RegulomeDB website [34].

Table 3. Classification of uncommon *PRSS1* 5' variants

Variant			Clinical genetic data	Functional data	gpAF (hspAF)[a]	RegulomeDB[b]		Classification (for CP)
c.nomenclature (NM_002769.5)	**g.nomenclature (chr7, hg19)**	**rs number**				**Score**	**Motif**	
c.-338T>G	g.142456998T>G	rs184553357	Identified in 3 of 65 Chinese patients with pancreatic cancer (but without pancreatitis) [50]	No data available	0.00006389 (0.001284, East Asian) [N.B. allele frequency in the Japanese population, 0.01468 (8.3KJPN project) [73]; allele frequency in the Korean population, 0.0109 (KRGDB project) [74]]	0.60906	No	Benign
c.-184G>A	g.142457152G>A	rs139432246	Found in 1 of 242 French ICP patients (not in 384 controls) by resequencing [51]	No effect on reporter gene expression [51]	0.005121 (0.01849, African/African American)	0.60906	No	Benign
c.-173C>T	g.142457163C>T	rs572772014	Found in 1 of 384 French controls (not in 242 ICP patients) by resequencing [51]	No effect on reporter gene expression [51]	0.00003225 (0.00006529, non-Finnish European)	0.60906	No	Benign
c.-147C>T	g.142457189C>T	rs754367025	Found in 1 of 384 French controls (not in 242 ICP patients) by resequencing [51]	Significantly reduced reporter gene expression (a reduction of 86% of normal) by reducing the affinity of an ATF4 transcription factor binding site [51]	Absent	0.98[c]	ATF4[c]	Benign
c.-36G>A	g.142457300G>A	rs377134514	Found in 1 of 236 healthy Greek subjects [75]	No data available	0.00002475 (0.00005014, East Asian)	0.60906	No	Benign
c.-30_-28delTCC	g.142457306_142457308del	rs1287463139	Found in a single CP patient [76]	Mild effect on reporter gene expression (a	0.0004914 (0.004245, African/African American)	0.60906[d]	No[d]	Benign

				reduction of 24% of normal) [51]				

[a]In accordance with gnomAD v2.1.1 [31].
[b]Data were obtained from the RegulomeDB website [34].
[c]Used chr7:142457189-142457190 for querying RegulomeDB [34].
[d]Used chr7:142457306-142457307 for querying RegulomeDB [34].

Table 4. Classification of *PRSS1* intronic variants

Intron	Variant			Clinical genetic data	Functional data	gpAF (hspAF)[a]	Classification (for CP)
	c.nomenclature (NM_002769.5)	g.nomenclature (chr7, hg19)	rs number				
1	c.40+1G>A	g.142457376G>A	rs149125789	Identified in a patient with benign pancreatic hyperenzymemia but without pancreatitis [77]	No data available	0.00006010 (0.0003204, African/African American)	Benign (protective)
1	c.40+40delC	g.142457415del	rs779579792	Identified in 1 of 381 patients with pancreatitis; no controls were analyzed [78]	No data available	0.0002134 (0.0005651, Latino/Admixed American)	Benign
1	c.41-49C>T	g.142458357C>T	rs190942214	Detected in 2 patients (father and son) from a FCP family; no controls were analyzed [79]	No data available	0.001122 (0.005499, Ashkenazi Jewish)	Benign
2	c.200+1G>A	g.142458566G>A	rs143909348	Identified in 1 of 55 alcoholics without pancreatitis [80]	No data available	0.004579 (0.0287 African/African American)	Benign (protective)
2	c.200+64_68delCCCAG[b]	g.142458629_142458633del	rs377590054	Detected in all three patients (one homozygote) and in 3 of 7 healthy members of a Chinese AIP family; detected also in a Chinese idiopathic AIP patient; absent in 520 unrelated healthy controls [54]	In-frame deletion of the first 141 nucleotides of exon 2 (RNA analysis using patient's blood cells); *in vitro* biochemical characterization demonstrated that the mutant protein had lower trypsin activity than the wild-type protein [54]	0.00001422 (0.0002009, East Asian)	Benign
2	c.201-99G>C	g.142459526G>C	rs530407004	Identified in 1 of 50 patients with familial intestinal gastric cancer and 1 of 107 subjects from the normal Italian Tuscany population [81]	No data available	0.0007646 (0.001297, European (non-Finnish))	Benign
3	c.454+10A>C[c]	g.142459888A>C	Not available	Identified in 5 of 253 Han Chinese patients	No data available	NA	Benign

				with pancreatitis (comprising 22 with alcohol-related diseases, 16 with idiopathic disease, 30 cases with hyperlipidemia, 35 with the hereditary form from six families, and 150 with gallbladder disease) [82]			
3	c.454+36T>C[d]	g.142459914T>C	rs761438114	Identified in 2 of 65 Chinese patients with pancreatic cancer [83]	No data available	0.0001275 (0.001655, East Asian)	Benign
3	c.454+75A>G	g.142459953A>G	rs1376416883	Identified in 27 of 253 Chinese patients with pancreatitis (comprising 22 with alcohol-related diseases, 16 with idiopathic disease, 30 cases with hyperlipidemia, 35 with the hereditary form from six families, and 150 with gallbladder disease) and in 4 of 320 controls [82]	No data available	NA	Benign
3	c.454+127A>T[e]	g.142460005A>T	rs376116875	Detected in 1 of 29 Chinese patients with pancreatitis; this patient also carried c.454+157C>G (incorrectly named c.454+157G>C) [83]. Whether the two variants were in *cis* or in *trans* is unknown	No data available	NA	Benign
3	c.454+157C>G[f]	g.142460035C>G	rs371236770	Detected in 2 of 156 Chinese patients with pancreatic cancer [84]	No data available	NA	Benign

3	c.454+157C>A[g]	g.142460035C>A	Not available	Detected in 1 Chinese patient with CP [85]	No data available	NA	Benign
3	c.454+172C>T	g.142460050C>T	rs878977606	Co-inherited with *PRSS1* p.Asn29Ile in four patients from a HCP family [86]	No data available	NA	Benign
3	c.455-192T>A[g]	g.142460090T>A	Not available	Detected in 1 Chinese patient with CP [85]	No data available	NA	
4	c.592-79G>A[h]	g.142460640G>A	rs531271210	Detected in 2 (one Italian male and one Turkish female) of 109 patients with ICP [87]	No data available	NA	Benign
4	c.592-78G>A[h]	g.142460641G>A	rs1337286040	Detected in 2 (one Italian male and one Turkish female) of 109 patients with ICP [87]	No data available	NA	
4	c.592-24C>T	g.142460695C>T	rs192452846	Detected in 1 of 381 patients with pancreatitis; no controls were analyzed [78]	No data available	0.003316 (0.005373, non-Finnish European)	Benign
4	c.592[-11C>T;-8C>T][i]	g.[142460708C>T;142460711C>T]	rs183791770; rs200381474	Detected in 1 of 381 patients with pancreatitis; no controls were analyzed [78]	No data available	0.003305 (0.01476, South Asian)	Benign

[a]In accordance with gnomAD v2.1.1 [31].
[b]Incorrectly named as c.200+56_60delCCCAG in the original report [54].
[c]Incorrectly named as c.454+10T>G in the original report [82].
[d]Incorrectly named as c.454+36A>G in the original report [83].
[e]Incorrectly named as c.454+127T>A in the original report [83].
[f]Incorrectly named as c.454+157G>C in the original report [84].
[g]These variants were incorrectly described as exon 3 variants in the original report and were identified in the same patient [85]. Whether the two variants were in *cis* or in *trans* is unknown.
[h]These two variants were detected together. They are likely in *cis* given their adjacent positions.
[i]The two variants were annotated here to be in *cis* since they are in complete LD (R^2 = 1.0) using the LDpair Tool [32] in the context of all populations.

Table 5. Classification of functionally analyzed *PRSS1* missense variants, small in-frame duplication and gene conversion variants

Exon	Variant		Clinical genetic data[a]		Functional consequence	gpAF (hspAF)[d]	Classification (for CP)
	c.nomenclature (NM_002769.5)	Amino acid change	Number of HCP families (family description) reported[b]	Other[c]			
GoF							
2	c.47C>T	p.A16V	2 (4 patients across 2 generations; 3 patients across 2 generations) [88]	Also reported in patients with FCP or ICP (e.g., [89-91]. It is the third most commonly detected rare *PRSS1* variant in CP [92]	Increased activation [61]	NA [92]	Pathogenic
2	c.49C>A	p.P17T		Arose *de novo* in a Hungarian CP patient [93]	Increased activation [93]	NA	Pathogenic
2	c.56A>C	p.D19A		Identified in a French ICP patient [94]	Increased autoactivation [94]	NA	Pathogenic
2	c.62A>C	p.D21A	1 (5 patients across 3 generations) [95]		Increased activation [96]	NA	Pathogenic
2	c.65A>G	p.D22G		Identified in two patients of a FCP family [97]	Increased autoactivation [94]	NA	Pathogenic
2	c.68A>G	p.K23R		Reported in a dozen of CP patients (e.g., [76, 98-100])	Increased autoactivation [94, 101]	NA	Pathogenic
2	c.63_71dup	p.K23_I24insIDK	1 (3 patients across 2 generations) [102]		Increased activation [102]	NA	Pathogenic
2	c.86A>T	p.N29I	The second most frequent variant causing HCP; in the first report, one family had 19 patients across 7 generations [103]	Also frequently reported in patients with ICP (e.g., [57, 104, 105]	Increased activation and stability [61]	NA	Pathogenic
2	c.[86A>T;161A>G] (gene conversion)	p.[Asn29Ile; Asn54Ser]		Identified in a patient with ICP [106]	Increased autoactivation (solely due to p.Asn29Ile) [106]	NA	Pathogenic
2	c.86A>C	p.N29T	1 (8 patients across 3 generations) [107]	Also reported in patients with FCP, ICP or RAP [108, 109]	Increased activation and stability [61]	NA	Pathogenic
2	c.116T>C	p.V39A	1 (9 patients across 3 generations) [55]		Increased stability [61]	NA	Pathogenic

3	c.235G>A	p.E79K		Identified in a French ICP patient [110]; two German FCP patients (brothers), a French FCP patient and a Belgian ICP patient [56]; a Brazilian ACP patient [111]; two Polish FCP patients and one Polish ICP patient [112]	Increased transactivation of PRSS2 [56]; there is growing evidence that increased expression of PRSS2 is an independent mechanism underlying CP [48]	0.00003579 (0.0003076, African/African American)	Pathogenic
3	c.364C>T	p.R122C		Identified in cases with ICP worldwide (e.g., [57, 105, 113]) as well as in FCP families [108, 114]	Increased autoactivation and stability [61, 114]	0.00001988 (0.00003517, European (non-Finnish))	Pathogenic
3	c.365G>A	p.R122H	The most frequent variant causing HCP; in the discovery report, one family had 20 patients across 4 generations [6]	Also frequently reported in ICP patients (e.g., [57, 104, 105]	Increased autoactivation and stability [61, 115]	0.00001062 (0.00002327, European (non-Finnish))	Pathogenic
3	c.365_366GC>AT (gene conversion)	p.R122H	1 (4 patients across 4 generations) [116]	Identified in a Belgian patient with ICP [117]	Increased autoactivation and stability [61, 115]	NA	Pathogenic
3	c.[343T>A;347G>C;365_366delinsAT] (gene conversion)	p.[S115T;R116P;R122H]		Identified in a 20-year-old German male with RAP; *de novo* occurrence in a 7-year-old Polish girl with CP [118]	A combination of increased trypsinogen activation (attributable to p.Arg122His) and secretion (attributable to p.Arg116Pro) [118]	NA	Pathogenic
4	c.568G>A	p.E190K		Identified in an 11-year-old girl with ICP [119]	Increased autoactivation [119]	NA	Pathogenic
GoP							
3	c.276G>T	p.K92N		Identified in a Caucasian ICP patient [110] and a Chinese AIP patient [26]	Moderate secretion defect [120]	NA	Predisposing
3	c.298G>C	p.D100H		Identified in 1/109 Caucasian patients with ICP [87]	Severe secretion defect [120]	NA	Pathogenic

3	c.311T>C	p.L104P	2 (both having 3 patients across 3 generations) [91, 121];	Also identified in a Chinese patient with ICP [122]	Severe secretion defect and elevation of ER stress marker [123]	NA	Pathogenic
3	c.346C>T	p.R116C	2 (3 patients across 2 generations [124]; 3 patients across 3 generations [125])	Also reported in patients with ICP (e.g. [57, 87, 126]), AIP [26] or pancreatic cancer [127]	Severe secretion defect and elevation of ER stress marker [125]	0. 00007072 (0.0007018, East Asian)	Pathogenic
3	c.371C>T	p.S124F		Identified in 1 of 660 German CP patients [105]	Moderate secretion effect [120]	0.000003977 (0.00005437, East Asian)	Predisposing
3	c.380C>G	p.S127C		Identified in an ICP patient and his unaffected mother [128]	Severe secretion defect and elevation of ER stress marker [128]	NA	Pathogenic
3	c.415T>A	p.C139S		Identified in a 7-year-old white girl with CP [78] and a Chinese patient with AIP [26]	Severe secretion defect [125]	NA	Pathogenic
3	c.416G>T	p.C139F		Identified in a German patient with RAP and her unaffected mother [91] and a Chinese patient with AIP [26]	Severe secretion defect [120]	NA	Pathogenic
4	c.508A>G	p.K170E		Identified in a patient with CP [129]	Moderate secretion effect [120]	NA	Predisposing
5	c.623G>C	p.G208A		Found in a 12-year-old Asian boy with pancreatitis (homozygote), who also carried *CFTR* p.Phe508del and p.Gln1352His [78]; Identified in 1 of 18 Korean children with RAP [109]; Identified in 8 of 232 Japanese ACP patients, 9 of 198 Japanese ICP patients and 1 of 411 controls [130]; Identified in 89 (2 homozygotes) of 1061	Moderate secretion defect [120]	0.0007141 (0.009873, East Asian)	Predisposing

				Chinese ICP patients and 22 of 1196 controls [57]; Based upon data from ref. [57], the allele-based OR of p.Gly208Ala for ICP was calculated to be 4.72 (95% CI 2.88-7.72, $p = 9.2 \times 10^{-11}$) [58]			
No effect or loss-of-function							
2	c.107C>G	p.P36R		Identified in two patients with ICP [110, 120]	Loss-of-function (degradation by CTRC) [120]	0.00009908 (0.001353, East Asian)	Benign
3	c.241C>A	p.L81M		Not segregated with CP in a Chinese AIP family (i.e., the variant was identified in the index patient with CP (12 y, female), her grandmother with CP and her healthy aunt but not in her grandfather with CP) [131]	Biochemical analysis of recombined trypsinogen expressed in *E. coli* did not find differences in autoactivation or trypsin activity between the mutant and wild-type molecules [131]. Cleavage at Leu81 by CTRC is required for CTRC-dependent degradation of PRSS1 [61, 132]. Methionine is one of the conservative substitutions for leucine [63] and is one of the preferential cleavage sites of CTRC [133]	NA	Benign
3	c.248G>A	p.G83E		Identified in 1 patient with ICP [110]	Loss-of-function (degradation by CTRC) [120]	NA	Benign
3	c.263T>A	p.I88N		Identified in 1 patient with CP [78]	Loss-of-function (degradation by CTRC) [120]	0.000007953 (0.00004619, European (Finnish))	Benign
3	c.292C>A	p.Q98K		Identified in 1 patient with CP [129]	Functionally neutral [120]	0.0001494 (0.0005880, Ashkenazi Jewish)	Benign

3	c.361G>A	p.A121T		Identified in a Chinese patient with FCP (the index patient and his unaffected son carried the variant; the deceased index patient's father was reported to have CP) [134] and a German patient with ICP [135]	Functionally neutral by biochemical analysis; and no secretion defect [136]	0.00001995 (0.00006172, African/African American)	Benign
3	c.367G>A	p.V123M		Identified in 1 patient with ICP [110]	Loss-of-function (degradation by CTRC) [120]	0. 00003183 (0.0001003, East Asian)	Benign
3	c.410C>T	p.T137M		Identified in an 13-year-old Asian girl with AP [78]; 1/129 Chinese patients with ICP [122]; and a Chinese patient with pancreatic cancer (who also carried c.403A>G (p.Thr135Ala)) [84]	Functionally neutral [120]	0.0006792 (0.009425, East Asian)	Benign
4	c.541A>G	p.S181G		Identified in 6-year-old Italian boy with RAP; the patient also carried *CFTR* p.Phe508del; the two variants were present in the clinically normal mother [137]	Functionally neutral [120]	0.00006362 (0.0002613, South Asian)	Benign

[a]Data from some original reports were reinterpreted according to our working definitions of HCP, FCP and ICP.
[b]Most of these data were taken from Masson et al. [10].
[c]For frequently reported variants, usually only the first three and/or representative publications were cited.
[d]In accordance with gnomAD v2.1.1 [31].

Table 6. Classification of *PRSS1* missense variants without supporting functional data

Exon	Variant		Clinical genetic data	gpAF (hspAF)[a]	Classification
	c.nomenclature (NM_002769.5)	Amino acid change			
2	c.116T>A	p.V39E	Identified in 1 patient with RAP [138]	NA	Likely pathogenic
2	c.116T>G	p.V39G	Identified in a pediatric soft tissue sarcoma survivor [139]	NA	Likely pathogenic
2	c.125A>G	p.N42S	Identified in 1 patient with RAP [138]	NA	Unknown significance
2	c.200C>T	p.S67F	Reported by a study of 10,389 cases from 33 cancer types [140]	0.00001593 (0.00002892, Latino/Admixed American)	Unknown significance
3	c.293A>G	p.Q98R	Identified in 1 patient with pancreatitis [99]	0.000003976 (0.000008790, European (non-Finnish))	Likely benign
3	c.296A>G	p.Y99C	Identified in a patient with pancreatic ductal adenocarcinoma [141]	0.000007953 (0.00001758, European (non-Finnish)	Unknown significance
3	c.310C>G	p.L104V	Identified in two patients and two healthy members of a family with solid pseudopapillary tumors [142]	NA	Likely benign
3	c.367G>T	p.V123L	Found in 1/1000 German subjects without any pancreatic disease [120]	0.00003579 (0.00007033, European (non-Finnish))	Likely benign
3	c.403A>G	p.T135A	Found in a Chinese patient with pancreatic cancer who also carried c.410C>T (p.Thr137Met; see Table 6); whether the two variants are in *cis* or in *trans* remains unknown [84]	0.000007141 (0.00002828, Latino/Admixed American)	Unknown significance
3	c.443C>T	p.A148V	Identified in a patient with benign pancreatic hyperenzymemia [77]	0.0003605 (0.002564, African/African American)	Unknown significance
4	c.487G>A	p.A163T	Identified in an ICP patient [143]	0.00004242 (0. 0002257, Latino/Admixed American)	Unknown significance
4	c.544A>T	p.N182Y	Identified in 1 of 1061 Chinese patients with ICP [57]	NA	Unknown significance

[a]In accordance with gnomAD v2.1.1 [31].

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