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## **Clinical interpretation of *PRSS1* variants in patients with pancreatitis**

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**Summary** Since the description of the *PRSS1* gene encoding the cationic trypsinogen as being involved in dominant hereditary pancreatitis, more than 50 *PRSS1* variants have been reported. Among the *PRSS1* variants that have been classified as pathogenic, some have a high penetrance and others have a low penetrance. Assessing the clinical relevance of *PRSS1* variants is often complicated in the absence of functional evidence and interpretation of rare variants is not very easy in clinical practice. The aim of this study was to review the different variants identified in the *PRSS1* gene and to classify them according to their degree of deleterious effect. This classification was based on the results of several *in vitro* experiments and on population data, in comparing the allelic frequency of each variant in patients with pancreatitis and in unaffected individuals. This review should help geneticists and clinicians in charge of patient's care and genetic counseling to interpret molecular results.

#### **KEYWORDS**

Pancreatitis;*PRSS1*; penetrance; variant classification



## Introduction

Hereditary pancreatitis (HP) is a rare autosomal condition characterized by recurrent attacks of acute pancreatitis (AP) and progressing to the development of chronic pancreatitis (CP) over a variable period of time [1]. The genetic basis of CP has been extensively characterized since 1996 when the *PRSS1* (protease, serine, 1) gene encoding the cationic trypsinogen was reported to be associated with dominant HP independently by two research groups [2-3]. *PRSS1* is considered as the most frequent gene involved in autosomal dominant HP [4-6]. This gene has been extensively analyzed in different cohorts of patients with familial, hereditary and sporadic pancreatitis in absence or presence of environmental factors such as alcohol and it was suggested that *PRSS1* variants are risk factors for CP through recurrent acute pancreatitis (RAP) [7-11]. It was also suggested that autoimmune pancreatitis may be related to *PRSS1* variants [12]. However, a recent large meta-analysis has shown that one of the most frequent *PRSS1* variant, p.R112H, was strongly associated with an increased risk of hereditary CP but not with sporadic CP, either alcoholic or idiopathic [13]. Since 1996, many *PRSS1* variants have been reported. Among the *PRSS1* variants that have been classified as pathogenic, some were shown to be associated with a high penetrance and others with a low penetrance [14-16]. However, assessing the clinical relevance of rare or private *PRSS1* variants is often complicated in the absence of functional evidence [17]. Several reviews on *PRSS1* variants have been published during the last decade, and the Genetic Risk Factors in Chronic Pancreatitis Database (<http://pancreasgenetics.org/index.php>) has been developed and may be used as the primary data source [18-19].

Pathogenic *PRSS1* variants exert their effect via a so-called trypsin-dependent pathological pathway and/or a misfolding dependent pathway, which involves increased autoactivation of mutant cationic trypsinogens resulting in elevated intrapancreatic trypsin activity, due to a gain of function of the mutant proteins. This inappropriate autoactivation in the pancreas is considered to cause pancreatitis [20].

The aim of this study was to review *PRSS1* variants and to classify them according to their degree of deleterious effect. This classification was based upon: 1) description in clinical reports including databases and publications; 2) results of *in vitro* experiments and functional data; 3) variant population frequency, in comparing the allelic frequency of each variant in patients with pancreatitis and in unaffected individuals (gnomAD database, <https://gnomad.broadinstitute.org/>). *In silico* prediction of the impact of each variant on the messenger RNA splicing and the protein structure was analyzed but not used for the classification. This review should be of interest to geneticists in charge



of genetic counseling and to pediatricians, gastroenterologists and pancreatic surgeons in charge of monitoring patients with pancreatitis.

## Methods

The study was designed as a systematic review of the published literature related to the involvement of the *PRSS1* gene in pancreatitis (including idiopathic chronic pancreatitis and recurrent acute pancreatitis) and the impact of *PRSS1* variants on PRSS1 function in experimental models. We performed an online search of PubMed to identify all experimental and molecular studies related to *PRSS1* in pancreatitis. We performed a systematic search of original publications according to the following criteria for inclusion: English language articles published between 01/01/1996 and 31/12/2019; molecular analysis of the *PRSS1* gene in patients with pancreatitis; experimental studies analyzing the impact of the *PRSS1* variants on PRSS1 function. The keywords *PRSS1* or mutation/variant were combined with pancreatitis. The filter “human” was applied. We also used data from the database “Genetic risk factors in chronic pancreatitis” (<http://www.pancreasgenetics.org/>) [19].

Among the different *PRSS1* gene molecular studies, we selected data from 18 reports on *PRSS1* variants in different populations (Europe, Asia, India, Africa, Central America, South America). To avoid duplicate results, we chose to select only one publication per scientific team. The nomenclature for the description of sequence variants followed HGVS recommendations. The allelic prevalence (p) of each variant in the general population was taken from gnomAD (overall population) (<https://gnomad.broadinstitute.org/>). gnomAD v2.1.1 collects exome and genome data from 125,748 and 15,708 unrelated individuals, respectively, who were enrolled in various disease-specific and population genetic studies. These allelic prevalences were compared with those observed in CP patients, taken from 18 cohorts as described above. For each variant, the Odds-Ratio (OR) was estimated as described above ([www.medcalc.org/calc/odds\\_ratio.php/](http://www.medcalc.org/calc/odds_ratio.php/)) and its 95% confidence interval (CI) was determined. The exact binomial test was used to compare the observed genotype frequency in the pancreatitis cohorts with the observed frequency in gnomAD.

In 2015, the American College of Medical Genetics and Genomics (ACMG) and the Association for Molecular Pathology (AMP) recommended the classification of variants into five categories [21]. According to this system, a variant can be classified as: pathogenic; likely pathogenic; uncertain significance; likely benign; and benign. However, unlike in Mendelian diseases, this classification is most complicated to use in complex and/or multifactorial disorders, such as idiopathic pancreatitis. For common diseases, the terms “pathogenic”, and “likely pathogenic” are



not appropriate in this context even when an association is statistically valid. A suggested interim solution was to report these variants as “risk alleles” and can be express as “established risk allele”, “likely risk allele”, or “uncertain risk allele”. Following the publication of updated guidelines for the interpretation of sequence variants and for classifying them into five categories [21-22], comprehensive tools combining criteria based on population, computational, functional and segregation data have been developed to facilitate their interpretation. Here, based on results of functional studies, clinical observations in unrelated patients, variant frequencies in relevant control populations, we classified all variants in four categories: i) pathogenic variants with established risk to be disease-causing (extremely rare or absent in the general population and gain of function (or highly suspected gain of function) in a well-validated functional assay); ii) pathogenic variant with established risk to be disease-contributing in association with other genetic and/or environmental factors (rare but with similar frequency in the general population or specific ethnic population and with functional gain in a well-validated functional assay); iii) variants of uncertain significance (rare or absent in the general population with no available functional analysis); iv) benign variants (similar or higher frequency in the general population than in CP patients and/or no functional gain in a well-validated functional assay).

## Results

The serine protease 1 (*PRSS1*) gene in humans is located on chromosome 7q35, within the T cell receptor beta locus. Besides *PRSS1*, the locus also contains five trypsinogen pseudogenes (such as *PRSS3P2*), and *PRSS2*, that encodes anionic trypsinogen. The *PRSS3* gene coding for mesotrypsinogen is found on chromosome 9p13 (<https://genome.ucsc.edu/>). *PRSS1* consists of 5 exons (NM\_002769.2) encoding a TRY-1 protein of 247 amino-acids (aa). This protein is cleaved after Arg-122 into two alpha-trypsin chain 1 (aa 24-122) and 2 (123-247). Tyr aa at position 154 is modified by sulfation. Different disulfide bonds have been identified (Cys30-Cys160; Cys48-Cys64; Cys139-Cys206; Cys139-Cys206; Cys171-Cys185; Cys196-Cys220).

To date, only 56 different *PRSS1* variants have been identified in patients with hereditary, familial or sporadic chronic pancreatitis. Three kinds of variants have been identified: copy number variants (CNV), gene conversion and point variants.

### ***Copy number variants***

Heterozygous triplication and duplication of an approximately 605-kb segment containing the trypsinogen genes on chromosome 7q35 was initially found in French patients with hereditary and sporadic idiopathic chronic pancreatitis [23-24]. Since this first description, other triplication and



duplication have been identified (Table 1) [10, 25-28]. Such copy number variants should result in higher trypsinogen expression through a gene-dosage effect, although this has not been demonstrated directly. Higher trypsinogen concentrations, in turn, would increase the likelihood of autoactivation and development of intrapancreatic trypsin activity. Up to now, their penetrance was not estimated [28].

### ***Gene Conversions within Trypsinogen Genes***

Human trypsinogen genes (*PRSS1*, *PRSS2* and *PRSS3*) exhibit a high level of sequence identity, which may facilitate gene conversion events [23]. This gene conversion mechanism between two functional paralogous trypsinogen genes lead to introduce point variants which cause genetically determined chronic pancreatitis. In fact, gene conversion was suggested as a mechanism for the evolutionary origin of the most common hereditary pancreatitis-associated variants. A gene conversion event was identified in a 6-yr-old German girl with sporadic chronic pancreatitis, which replaced exon 2 in *PRSS1* with that from *PRSS2* (Table 1) resulting in the *PRSS1* variants p.N29I and p.N54S [29]. Since exon 1 codes for part of the signal peptide which is removed in the endoplasmic reticulum, only changes in exon 2 affect the mature trypsinogen protein. The amino acids encoded by exon 2 are nearly identical between the two isoforms, with the exception of Ile29 and Ser54 found in anionic trypsinogen (*PRSS2*). Whereas p.N54S is of uncertain significance, p.N29I is considered disease-causing [31]. A gene duplication event in a French family with hereditary pancreatitis resulted in a similar hybrid gene, containing exons 1–2 from *PRSS2* and exons 3–4–5 from *PRSS1* [29]. More recently, a gene conversion event between exon 3 of the *PRSS1* gene and the *PRSS3P2* pseudogene, resulting in three amino-acid alterations (p.S115T, p.R116P, p.R122H), was reported in two unrelated individuals [31]. This rare conversion was *de novo* in one case and was inherited from the father in the other case but no other case was described in the family.

### ***Single nucleotide variants***

Up to now, 54 *PRSS1* point variations located in exonic and exon-intron boundaries have been described in patients with pancreatitis (after exclusion of four polymorphisms with a population frequency >5%, *i.e.* c.486C>T, p.D162=; c.738C>T, p.N246=; and c.-204C>A, c.-408C>T). Three variants were loss-of-function variants (and expected to be not involved in pancreatitis), one was an in-frame insertion, three were synonymous variants (p.A91=; p.C139=; and a new one p.R68=) and 47 were missense variants (Tables 1-4). The majority of these missense variants are located in three clusters within the trypsinogen sequence: in the TAP (trypsinogen activation peptide, aa 16-to-23, 7 variants), in the N-terminal part of trypsin (between aa 29 and aa39, 6 variants) or in the longest peptide segment not stabilized by disulfide bonds between Glu79 and Cys139 (22 variants). Only 12 variants



were identified in the C-terminal part of the protein (from aa 140 to aa 247). In the gnomAD database, apart the common polymorphisms, these *PRSS1* variants are extremely rare in the general population. 29 (29/54, 54%) of these variants have not been identified in more than 250.000 alleles (Tables 1-4). Only 6 variants were identified in more than 50 healthy individuals among more than 250 000 alleles (p.A16V, c.200+1G>A, p.T137M, p.C139=, p.A148V, and p.G208A).

We classified all *PRSS1* variants in four categories: pathogenic variants with established risk to be disease-causing, pathogenic variant with established risk to be disease-contributing in association with other genetic and/or environmental factors, variants of uncertain significance and benign variants.

### ***Pathogenic variants with established risk to be disease-causing***

The variants that were highly suspected to cause a gain of function of the mutant TRY-1 proteins were considered disease-causing. These variants are described in Table 1. We included in this category: 1- a duplication and a triplication of the *PRSS1* gene, which probably lead to an excess of autoactivation of the protein and which have never been reported in the general population [28]; 2- gene conversions within trypsinogen genes that probably increase autoactivation [31]; and 3- rare or ultra-rare (not identified in databases) missense variants that stimulate TRY-1 autoactivation directly or indirectly. Increased trypsin stability and/or increased TRY-1 autoactivation was demonstrated for 13 *PRSS1* variants i.e. p.R122H, p.N29I, p.N29T, p.D19A, p.D21A, p.D22G, p.K23R, p.K23\_I24insIDK, p.V39A, p.D100H, p.E190K, p.P17T, and p.R122C [24, 31, 31-34]. Moreover, moderate to severe reduction in secretion of trypsinogen was noted for several *PRSS1* variants (p.D100H, p.C139F, p.K92N, p.R116C, p.S124F, and p.G208A) [34-36]. Variants with reduced secretion are likely pathogenic due to mutation-induced misfolding and consequent endoplasmic reticulum stress. Finally, misfolding-dependent pathogenic pathway with intracellular retention and degradation, inducing endoplasmic reticulum (ER) stress that may be responsible for pancreas injury was observed with several variants such as p.K92N, p.L104P, p.R116C, p.C139F, p.C139S, and p.G208A [36-37].

Except for five variants, all were not described in controls and in gnomAD database from the general population (Table 1). p.R122C, p.S124F, p.R116C, p.R122H and p.G208A were found in 5/251452 (0.002%), 1/251458 (3.97e-4 %), 20/282814 (0.007%), 3/282442 (0.001%) and in 202/282874 (0.07%) alleles from “normal” individuals, respectively. 13 and 6 of the missense variants (Table 1) were predicted to have an impact on the protein structure or function using the SIFT and Polyphen-2 prediction softwares, respectively, but these bioinformatic tools are more suitable for predicting loss of function than gain-of-function effects.



Based on these experimental and epidemiological data, two CNVs (duplication and triplication of 605 kb on chromosome 7q35 increasing the copy number of *PRSS1*), two hybrids (hybrid with *PRSS2* exons 1 and 2 and *PRSS1* exons 3 to 5; hybrid with *PRSS1* and *PRSS3P2*), the in-frame insertion (c.63\_71dup; p.K23\_I24insIDK) and 19 very rare missense variants were classified as pathogenic variants with established risk to be disease-causing.

Interestingly, the two most prevalent *PRSS1* variants p.R122H and p.N29I are classified in this category and are found in more than 80% of HP worldwide. These variants were almost always identified in the heterozygous state. However, penetrance of each *PRSS1* variant in HP families is incomplete. This penetrance varies according to the variant and according to the families, which suggests the contribution of other factors.

Although p.A16V was found to increase autoactivation *in vitro* and that it was involved previously in familial and sporadic cases, we chose not to classify it in this category yet, due to its notable frequency in the general population (1728/261532 (0.66%)).

**Table 1:** Pathogenic gain-of-function *PRSS1* variants with established risk to be disease-causing. NI: not identified; NE: not estimated; GOF: Gain-Of-Function

Nucleotide change	Amino acid change	Allele frequency general population gnomAD	Allele frequency Pancreatitis patients (n=3033 from 18 reports)	Odds Ratio Ratio patients carrier/ population carrier	95% IC (%)	Significance (binomial test)	Functional effect	SIFT	POLYPHEN-2	References	Described in the Genetic Risk Factors Database
<i>PRSS1</i> duplication		NI	4	NE			NE			25,69	Yes
<i>PRSS1</i> triplication		NI	9	NE			NE			23,27,69	Yes
c.49C>A	p.P17T	NI		NE			GOF	Deleterious	Benign	19,38	Yes
c.56A>C	p.D19A	NI		NE			GOF	Tolerated	Benign	32	Yes
c.62A>C	p.D21A	NI	2	NE			GOF	Deleterious	Probably damaging	53	Yes
c.65A>G	p.D22G	NI		NE			GOF	Deleterious	Probably damaging	32,7	Yes
c.68A>G	p.K23R	NI	9	NE			GOF	Deleterious	Possibly damaging	32,5	Yes
c.63_71dup	p.K23_I24insIDK	NI		NE			GOF			25	Yes
c.86A>T	p.N29I	NI	45	NE			GOF	Deleterious	Benign	27,57	Yes
c.86A>C	p.N29T	NI	2	NE			GOF	Tolerated	Benign	34	Yes
c.116T>C	p.Y39A	NI		NE			GOF	Deleterious	Possibly damaging	71	Yes
<i>PRSS1-PRSS2</i> hybrid	p.N29I + p.N54S (large conversion)	NI		NE			GOF			72	Yes
<i>PRSS1-PRSS3P2</i> hybrid	p.S115T + p.R116P + p.R122H	NI		NE			GOF			31	Yes
c.276G>T	p.K92N	NI		NE			GOF	Deleterious	Benign	34,68	Yes
c.298G>C	p.D100H	NI		NE			GOF	Deleterious	Benign	34	Yes
c.311T>C	p.L104P	NI		NE				Deleterious	Benign	37	Yes
c.346G>T	p.R116C	20/282814	14	29,72	15.00-58.91	p<0.0001	GOF	Tolerated	Benign	18,32	Yes
c.365G>A; c.365_366GC>AT	p.R122H	3/282442	136	2008	639.5-6309.6	p<0.0001	GOF	Tolerated	Benign	18,27,52,53	Yes
c.364C>T	p.R122C	5/251452	11	83.51	28.99-240.49	p<0.0001	GOF	Tolerated	Probably damaging	5,27,57	Yes
c.371C>T	p.S124F	1/251458	1	378.47	23.66-6052.38	p<0.0001	GOF	Deleterious	Probably damaging	31	Yes
c.415T>A	p.C139S	NI		NE			GOF	Deleterious	Benign	47	Yes
c.416G>T	p.C139F	NI		NE			GOF	Deleterious	Probably damaging	34,73	Yes
c.568G>A	p.E190K	NI		NE			GOF	Tolerated	Benign	35	No
c.623G>C	p.G208A	202/282874	75	16.14	12.35-21.09	p<0.0001	GOF	Deleterious	Probably damaging	44,57,76	Yes

### ***Pathogenic variant with established risk to be disease-contributing in association with other genetic and/or environmental factors***

Taking into account the small impact *in vitro* of the p.A16V variant on autoactivation [33], this disease-contributing variant may cause the disease in association with other genetic and/or environmental factors (Table 2). The presence or absence of other predisposing factors may explain



the low odds-ratio observed with this variant and the highly variable and family dependent penetrance [39]. The estimated OR was 0.47 for p.A16V. Interestingly, the frequency of the p.A16V variant varied significantly between populations (817/18906 (4.32%) in the African population, and 567/120490 (0.47%) in the European (non Finnish) population). It is important to note that the frequency of this variant found in gnomAD may not be used for accurate calculation, and may explain the low OR observed with this variant. The variable penetrance suggested the impact of common variants/polymorphisms in modifier genes and/or of the shared environment. Indeed, the effect of gain-of-function variant in *PRSS1* may be accentuated by loss-of-function in other known genes involved in idiopathic pancreatitis such as pancreatic secretory trypsin inhibitor (*SPINK1*), chymotrypsin C (*CTRC*), calcium-sensing receptor (*CASR*) and *CFTR* [40-41; 57-58].

**Table 2:** *PRSS1* pathogenic variant with established risk to be disease-contributing in association with other genetic and/or environmental factors and *PRSS1* variants of unknown significance. Note that p.A16V is relatively frequent in Africa (817/1728 mutated cases). NI: not identified; NE: not estimated; ND: not determined; GOF: Gain-Of-Function; Odds-Ratio [https://www.medcalc.org/calc/odds\\_ratio.php](https://www.medcalc.org/calc/odds_ratio.php)\*, new variants reported in this study

<i>PRSS1</i> Gene	Nucleotide change	Amino acid change	Allele frequency General population gnomAD	Allele frequency Pancreatitis patients (n=3833 from 18 reports)	Odds Ratio Ratio patients carrier/ population carrier	95% IC (%)	Significance (binomial test)	Functional effect	SIFT	POLYPHEN-2	References	Described in the Genetic Risk Factors Database
exon 2	c.47C>T	p.A16V	1728/261532	19	0.42	0.27-0.67	p=0.0003	Small effect	Tolerated	Benign	39, 72, 75	Yes
exon 2	c.97A>G	p.N33D	NI		NE			ND	Tolerated	Benign	This study*	No
exon 2	c.116T>A	p.V39E	NI	1	NE			ND	Deleterious	Probably damaging	11	Yes
exon 2	c.125A>G	p.N42S	NI	1	NE			ND	Deleterious	Possibly damaging	11	Yes
	c.204C>T	p.R68-	NI		NE			ND	NE	NE	This study*	No
exon 3	c.235G>A	p.E79K	9/251490	3	12.62	3.41-46.6	p=0.0001	No effect	Tolerated	Benign	68,77	Yes
exon 3	c.241C>A	p.L81M	NI		NE			ND	Tolerated	Benign	12	Yes
exon 3	c.273C>A	p.A91-	NI		NE			ND	NE	NE	12	Yes
exon 3	c.293A>G	p.Q98R	1/251496	1	37.85	2.36-605.3	p=0.0102	ND	Tolerated	Benign	55	Yes
exon 3	c.303G>C	p.R101S	NI		NE			ND	Tolerated	Benign	This study*	No
exon 3	c.310C>G	p.L104Y	NI		NE			ND	Deleterious	Benign	76	Yes
exon 3	c.403A>G	p.T135A	2/280096		NE			ND	Tolerated	Benign	19	Yes
exon 3	c.417C>T	p.C139-	108/282894		NE			ND	NE	NE	19	Yes
exon 3	c.443C>T	p.A148V	98/271810		NE			ND	Tolerated	Benign	19	Yes
exon 4	c.486T>G	p.D162N	NI		NE			ND	Tolerated	Benign	77	Yes
exon 4	c.487G>A	p.A163T	12/282894		NE			ND	Deleterious	Benign	78	Yes
exon 4	c.508A>G	p.K170E	NI		NE			Small GOF	Tolerated	Benign	19,34	Yes
exon 4	c.544A>T	p.N182Y	NI	1	NE			ND	Tolerated	Probably damaging	57	No
exon 4	c.557T>C	p.Y186A	NI		NE			ND	Tolerated	Benign	This study*	No
exon 4	c.571G>A	p.G191R	36/281544		NE			ND	Deleterious	Probably damaging	79	Yes
exon 4	c.689C>T	p.T230I	NI		NE			ND	Deleterious	Probably damaging	This study*	No
exon 4	c.721A>G	p.N241D	1/251200		37.80			ND	Tolerated	Benign	This study*	No

### Benign variants

In this category, we included all variants that cause no functional gain of the mutant proteins *in vitro* and/or that were found in CP patients and in controls at a similar frequency. These variants are described in Tables 3 and 4. We included in this category 1- missense variants such as p.P36R, p.G83E, p.I88N, p.Q98K, p.V123M, p.T137M, and p.S181G [34], and 2- variants observed more frequently in the general population than in patients with pancreatitis (p.A121T, p.V123L) [17,33]



(Table 4). Three loss-of-function variants (non-sense or splicing variants) are probably protective against pancreatitis (Table 3)[38]. It has also been shown that promoter variants (not included in our tables) such as c.-147C>T may confer protection against CP by reducing the affinity of an ATF4 transcription factor binding site [42]. Another promoter variant, c.-204C>T (rs4726576), which is in linkage disequilibrium with c.-408C>T (rs10273639), was also suggested to have a protective effect [43]. In total, twelve exonic and intronic splice variants were considered as benign.

**Table 3:** Loss-of-function *PRSS1* variants playing a potential protective effect against pancreatitis. Note that c.401+1G>A and c.200+1G>A are more frequent in Africa (8/17 cases and 541/1228, respectively).

<i>PRSS1</i> Gene	Nucleotide change	Amino acid change	gnomAD	Clinical significance	References	Described in the Genetic Risk Factors Database
intron 1	c.40+1G>A		17/282846	Protective	38	Yes
exon 2	c.111C>A	p.Y37X	NA	Protective	38	Yes
intron 2	c.200+1G>A		1128/246316	Protective	38	Yes

**Table 4:** *PRSS1* variants commonly found in subjects with pancreatitis and healthy controls alike. The table does not show two frequent (>5%) polymorphisms: c.486C>G, p.D162=, and c.738C>T, p.N246=. NI: not identified; NE: not estimated; ND: not determined ; GOF: Gain-OF-Function

<i>PRSS1</i> Gene	Nucleotide change	Amino acid change	Allele frequency General population gnomAD	Allele frequency Pancreatitis patients (n=3033 from 18 reports)	Functional studies	SIFT	POLYPHEN-2	References	Described in the Genetic Risk Factors Database
exon 2	c.107C>G	p.P36R	28/282592	1	No GOF	Deleterious	Probably Damaging	34,68	Yes
exon 3	c.248G>A	p.G83E	NI	0	No GOF	Deleterious	Probably damaging	34,68	Yes
exon 3	c.263T>A	p.I88N	2/251490	1	No GOF	Deleterious	Probably damaging	34, 47	Yes
exon 3	c.292C>A	p.Q98K	4/274382	0	No GOF	Tolerated	Benign	34	Yes
exon 3	c.361G>A	p.A121T	5/250602	0	No GOF	Tolerated	Benign	17	Yes
exon 3	c.367G>A	p.V123M	9/282790	0	No GOF	Deleterious	Probably damaging	34,68	Yes
exon 3	c.367G>T	p.V123L	10/282790	0	ND	Tolerated	Possibly damaging	68	Yes
exon 3	c.410C>T	p.T137M	192/282690	2	No GOF	Deleterious	Probably damaging	34,47	Yes
exon 4	c.541A>G	p.S181G	16/251490	0	No GOF	Tolerated	Benign	34	Yes

### Variants of uncertain significance

In absence of convergent functional and epidemiological data, and to classify the other promoter, synonymous and missense variants, we determined the frequency of each variant in the gnomAD database, and compared the variant frequency with the frequency of each variant in cohorts of patients with idiopathic CP, IPA, RAP and HP from different studies. We used data from 18 patient cohorts from different countries [7,8, 11, 27, 44-57] including 3323 patients with idiopathic pancreatitis.



In this category, we found 18 missense variants and three synonymous variants. 13 of these variants were not described in the gnomAD database. Six were considered as ultra-rare (less than 50 cases in gnomAD) in the general population (Table 2). Only two were considered as rare: p.C139= (108/282894) and p.A148V (98/271810). Among the 18 missense variants, only 6 were found as deleterious using *in silico* algorithms from SIFT, only five of them being predicted to be possibly or probably damaging by Poly-phen-2 (Table 2). As previously observed, these prediction algorithms do not seem pertinent for gain-of-function variants. Using data from 18 reports (including 3323 patients with pancreatitis), we determined, if possible, the OR for several variants identified in these studies. The estimated OR were : p.E79K OR=12.62, p.Q98R OR=37.85, and p.N241D OR=37.80 (Table 2). Interestingly, the p.C139= variant was found in 96/19952 (0.48%) in the East Asian population but only in 1/24934 (0.004%) and 1/129074 (7.74e-4 %) alleles in African or European populations, respectively. Finally, the p.E79K variant, known to be associated with decreased autoactivation [58], was identified in 5/16256 (0.03%) alleles from African population but in only 1/113764 (8.79e-4 %) and 1/18390 (0.005%) from European and East Asian populations, respectively. These data are extremely important especially when meta-analyses are performed. Up to now, in absence of functional analysis, these rare *PRSS1* variants were classified as variants of uncertain significance.

## Discussion

*PRSS1* genetic testing can determine if a person, especially in familial forms of pancreatitis, has or has not inherited a *PRSS1* variant that confers a high risk of pancreatitis. This genetic test result has various impacts depending on the clinical situation, whether a variant is found in a patient with symptoms suggestive of pancreatitis, in an asymptomatic individual from the family, or as an unsolicited finding during whole exome or genome sequencing in a family investigated for another genetic disease.

Although this test cannot determine if an asymptomatic individual will develop a disease, or predict the age of disease onset and disease severity, this molecular analysis has several important interests: i) evaluation of parents of the patient to evaluate if one of them has escaped a previous diagnosis because of a milder presentation; ii) evaluation of the risk in the sibs of a patient. It has been demonstrated that early-onset pancreatitis is strongly associated with *PRSS1* variants [52-54], although all sibs who inherit the *PRSS1* variant may not have pancreatitis because of incomplete penetrance; iii) recommendations to prevent recurrent manifestations, including low-fat diet, multiple small meals and avoidance of alcohol, tobacco, dehydration, physical and emotional stresses; iv) medical surveillance, as having a *PRSS1* variant is associated with a fast progression from acute pancreatitis and chronic pancreatitis from the first attack of pancreatitis [59]. It has been



confirmed that the progression to CP is faster in children with pathogenic *PRSS1* variants than in children without *PRSS1* variants [60]; v) surveillance for pancreatic cancer. Several reports suggested that *PRSS1* variants were associated with autosomal dominant pancreatitis and increased cancer risk [61-62]. It has been shown experimentally that migration and invasion were increased in p.R116C-bearing PANC-1 cells compared with wild-type counterparts. This led to suggest that this *PRSS1* variant plays a role in pancreatic carcinogenesis [63]. However, a German study showed that *PRSS1* variants do not seem to significantly increase the risk for pancreatic adenocarcinoma [64]. This report was recently confirmed by a study which showed that CP and pancreatic ductal adenocarcinoma probably do not share genetic susceptibility at least in terms of high frequency variants [65]. Patients with HP have an absolute increased risk of pancreatic adenocarcinoma as compared to the general population, especially in smokers, but there is no correlation with the type of *PRSS1* variant [66]. On the contrary, the c.-409C>T variant was revealed to be protective factor against pancreatic cancer in the Han Chinese population [67]; vii) information on reproductive options, as prenatal diagnosis or preimplantation genetic diagnosis in familial forms with very severe chronic pancreatitis and may be discussed.

**Table 5:** Genetic and environmental recommendations according to the class of *PRSS1* variants

Variant Class	<i>PRSS1</i> analysis in affected individuals	<i>PRSS1</i> analysis in unaffected related individuals	Other pancreatitis-related genes testing	Dietetic recommendations	Surveillance to pancreatic cancer
<i>Pathogenic PRSS1 variants with established risk to be disease-causing</i>	yes	yes	yes	yes	yes
<i>Benign variants</i>	no	no	yes	yes	no
<i>Pathogenic variant with established risk to be disease-contributing in association with other genetic and/or environmental factors</i>	yes	yes	yes	yes	no
<i>Variants of uncertain significance</i>	yes	no	yes	yes	no

In conclusion, when a pathogenic variant with established risk to be disease-causing (from Table 1) is identified in a patient with pancreatitis, a familial analysis of the affected or unaffected parents and first-degree relatives should be offered during a genetic counseling interview. When a pathogenic variant characterized by a low penetrance or of uncertain significance (from Table 2) is identified, the search for other genetic and environmental factors must be performed. The identification of other additional genetic factors makes the variant very likely involved and a familial analysis of affected or unaffected parents and first-degree relatives should be proposed. If no other predisposing factor is identified, only a study of symptomatic relatives can be considered. Finally, in the presence of a benign variant (from Tables 3 and 4), no molecular study in the family should be proposed. All these recommendations are summarized in table 5. They are also of utmost importance in the era of increasing requests for genetic testing in the general population by using wide genome analysis, with a high risk of interpretation errors by using current bioinformatics tools and metapredictors.



**Disclosure of interest**

The authors declare that they have no competing interest.



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#### **Legends of tables S:**



Table S1: Cohorts of patients with pancreatitis included in this report. TCP, tropical chronic pancreatitis, ACP alcoholic chronic pancreatitis, ICP idiopathic chronic pancreatitis, RAP recurrent acute pancreatitis, IAP idiopathic acute pancreatitis.