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Emmanuelle Masson, Wen-Bin Zou, Claudia Ruffert, Vanessa Holste ...+16 more authors

Institutions: French Institute of Health and Medical Research, Second Military Medical University, Martin Luther University of Halle-Wittenberg, Technische Universität München ...+3 more institutions

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1 **Genetic analysis of the *STIM1* gene in chronic pancreatitis**

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3 Emmanuelle Masson,^{1,2,†} Wen-Bin Zou,^{3,4,†} Claudia Ruffert,⁵ Vanessa Holste,⁶ Patrick Michl,⁵
4 Joachim Mössner,⁷ Maren Ewers,⁶ Helmut Laumen,⁶ Hao Wu,^{3,4} Dai-Zhan Zhou,⁸ Zhao-Shen Li,^{3,4}
5 Dong Yu,⁹ Arnaud Boulling,¹ Cédric Le Maréchal,^{1,2} David N. Cooper,¹⁰ Jian-Min Chen,¹
6 Heiko Witt,⁶ Jonas Rosendahl,⁵ Zhuan Liao,^{3,4,*} Claude Férec^{1,3,*}

7

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

9 ²CHU Brest, Service de Génétique, Brest, France

10 ³Department of Gastroenterology, Changhai Hospital, the Second Military Medical University,
11 Shanghai, China

12 ⁴Shanghai Institute of Pancreatic Diseases, Shanghai, China

13 ⁵Department of Internal Medicine I, Martin Luther University, Halle, Germany

14 ⁶Paediatric Nutritional Medicine, Else Kröner-Fresenius Zentrum für Ernährungsmedizin (EKfZ),
15 Technische Universität München (TUM), Freising, Germany

16 ⁷Department of Internal Medicine, Neurology and Dermatology, Division of Gastroenterology,
17 University of Leipzig, Leipzig, Germany

18 ⁸Key Laboratory of Developmental Genetics and Neuropsychiatric Diseases (Ministry of Education),
19 Bio-X Institutes, Shanghai Jiao Tong University, Shanghai, China

20 ⁹Center of Translational Medicine, the Second Military Medical University, Shanghai, China

21 ¹⁰Institute of Medical Genetics, School of Medicine, Cardiff University, Cardiff, United Kingdom

22

23 †Emmanuelle Masson and Wen-Bin Zou share co-first authorship.

24

25 ***Correspondence to:**

26 **Claude Férec**, INSERM U1078 and UBO, 22 avenue Camille Desmoulins, 29200 Brest, France.

27 email: claud.ferec@univ-brest.fr.

28 **Zhuan Liao**, Department of Gastroenterology, Changhai Hospital, The Second Military Medical

29 University, 168 Changhai Road, Shanghai 200433, China. e-mail: liao zhuan@smmu.edu.cn.

30 **ABSTRACT**

31 Chronic pancreatitis is a complex disease that involves many factors, both genetic and
32 environmental. Over the past two decades, molecular genetic analysis of five genes that are highly
33 expressed in human pancreatic acinar cells, namely *PRSS1*, *PRSS2*, *SPINK1*, *CTRC* and
34 *CTRB1/CTRB2*, has established that a trypsin-dependent pathway plays a key role in the etiology
35 of chronic pancreatitis. Since Ca^{2+} deregulation can lead to intracellular trypsin activation in
36 experimental acute pancreatitis, we analyzed *STIM1* (encoding stromal interaction molecule-1, the
37 main regulator of Ca^{2+} homeostasis in pancreatic acinar cells) as a candidate modifier gene in
38 French, German and Chinese patients with chronic pancreatitis. The French and German subjects
39 were analyzed by Sanger sequencing whereas the Chinese subjects were analyzed by targeted
40 next-generation sequencing confirmed by Sanger sequencing. A total of 37 rare coding variants (35
41 missense and 2 nonsense) were identified, which were enriched in patients as compared with
42 controls [2.28% (47/2,057) vs. 0.99% (33/3,322); odds ratio = 2.33, $P = 0.0001$]. This is the first
43 large case-control study to demonstrate a putative association of rare *STIM1* coding variants with
44 chronic pancreatitis. Functional analysis will be required to clarify whether or not the rare *STIM1*
45 variants detected predispose to pancreatitis.

46 47 **INTRODUCTION**

48 Chronic pancreatitis is a complex disease that is defined as “a pathologic fibro-inflammatory
49 syndrome of the pancreas in individuals with genetic, environmental and/or other risk factors who
50 develop persistent pathologic responses to parenchymal injury or stress” (Whitcomb et al., 2016).
51 Over the past two decades, molecular genetic analysis of five genes that are highly expressed in
52 human pancreatic acinar cells, namely *PRSS1* encoding cationic trypsinogen (Le Maréchal et al.,
53 2006; Whitcomb et al., 1996), *PRSS2* encoding anionic trypsinogen (Witt et al., 2006), *SPINK1*
54 encoding pancreatic secretory trypsin inhibitor (Witt et al., 2000), *CTRC* encoding chymotrypsin C
55 (Masson et al., 2008; Rosendahl et al., 2008) and *CTRB1-CTRB2* encoding chymotrypsin B1 and
56 B2 (Rosendahl et al., 2018), has established a trypsin-dependent pathway in the etiology of chronic
57 pancreatitis (Hegyí and Sahin-Toth, 2017).

58 The majority of patients with chronic pancreatitis had prior clinically recognized acute
59 pancreatitis (LaRusch et al., 2015), an acute inflammatory disease of the pancreas postulated to be
60 an autodigestive disease triggered by prematurely activated trypsin within the pancreas (Chiari,
61 1896). The association of gain-of-function *PRSS1* variants with both recurrent acute pancreatitis
62 and chronic pancreatitis (Gorry et al., 1997; Whitcomb et al., 1996) not only provided support for
63 Chiari’s original hypothesis (Chiari, 1896) but has also contributed to the Sentinel Acute
64 Pancreatitis Event model for the development of chronic pancreatitis (Whitcomb, 1999).

65 Importantly, successive developments of spontaneous acute pancreatitis and chronic pancreatitis
66 have recently been observed in genetically modified mice that carried a heterozygous p.Asp23Ala
67 mutation within the activation peptide of the mouse cationic trypsinogen (Prss1) T7 isoform (the
68 p.Asp23Ala mutant autoactivates to trypsin 50-fold faster than wild-type) (Geisz and Sahin-Tóth,
69 2018).

70 The above notwithstanding, our understanding of the early events leading to pancreatitis is still
71 rather limited. In this regard, prolonged and global Ca²⁺ elevation (elicited by bile, alcohol
72 metabolites and other causes) has been described to result in trypsin activation, vacuolization and
73 necrosis of the pancreatic acinar cells in experimental acute pancreatitis (review in (Li et al., 2014));
74 and stromal interaction molecule-1 (STIM1) is a key regulator for Ca²⁺ homeostasis in both non-
75 excitable and excitable cells (Yuan et al., 2009). These findings suggest that variants in the *STIM1*
76 gene may contribute to the early steps of pancreatitis by disturbing Ca²⁺ homeostasis within the
77 pancreatic tissue.

78 Variants in the *STIM1* gene have been previously associated a number of diseases such as
79 immunodeficiency and autoimmunity (Picard et al., 2009; Shaw et al., 2013), a novel syndrome of
80 amelogenesis imperfecta and hypohidrosis (Parry et al., 2016), tubular-aggregate myopathy (Bohm
81 et al., 2013; Nesin et al., 2014; Noury et al., 2017), or Stormorken syndrome (Misceo et al., 2014;
82 Morin et al., 2014). Also, tubular aggregate myopathy and Stormorken syndrome patients carrying
83 *STIM1* variants additionally manifested psychiatric disorders (Harris et al., 2017). Moreover, Sofia
84 and colleagues have recently analyzed the *STIM1* gene (included within a panel of 70 genes
85 related to six different pancreatic pathways) in 80 patients with idiopathic chronic pancreatitis (ICP)
86 and found three missense mutations [i.e., c.1310G>A (p.Cys437Tyr), c.1589G>A (p.Arg530His),
87 and c.2246G>A (p.Arg749His)] in different patients (Sofia et al., 2016). In addition to the relatively
88 small number of patients analyzed, this study was limited by the lack of data from a corresponding
89 control population. Herein, we report our findings from a comprehensive variant analysis of the
90 *STIM1* gene in three ICP cohorts.

91

92 **PATIENTS AND METHODS**

93 **Patients**

94 This study included 436 French, 517 German and 1,104 Chinese patients with ICP (i.e., absence of
95 both a positive family history and any of the following external precipitating factors, namely alcohol
96 abuse, post-traumatic, hypercalcemic, hyperlipidemic and autoimmune) and corresponding healthy
97 controls. The diagnosis of chronic pancreatitis was made as previously described (Witt et al., 2013;
98 Zou et al., 2016). Informed consent was obtained from each patient and the study was approved by
99 the respective ethics committees.

100 **Variant screening**

101 The French and German subjects were analyzed by Sanger sequencing; three multiplex PCRs
102 were designed to amplify the entire coding sequence and flanking intronic sequences of the *STIM1*
103 gene (see [additional file, Figures. S1 and S2](#)). The Chinese subjects were analyzed by targeted
104 next-generation sequencing followed by Sanger sequencing confirmation, essentially as previously
105 described (Wu et al., 2017; Zou et al., 2018), the primer sequences are provided in [Additional file,](#)
106 [Figure S3](#).

108 **Variant nomenclature and reference sequences**

109 Variant nomenclature followed Human Genome Variation Society recommendations
110 (<http://www.hgvs.org/mutnomen/recs.html>) (den Dunnen et al., 2016). GenBank accession number
111 NM_003156.3 was used as the *STIM1* mRNA reference sequence. *STIM1* genomic sequence was
112 obtained from human GRCh38/hg38 (<https://genome.ucsc.edu/>).

114 **Pathogenicity prediction**

115 This was performed using the Combined Annotation-Dependent Depletion (CADD) method (Kircher
116 et al., 2014) available at <https://cadd.gs.washington.edu/>.

118 **Statistical analyses**

119 The assessment of statistical significance of the differences between the carrier frequencies of the
120 *STIM1* variants in patients and controls was performed by the 2x2 contingency table available at
121 <http://vassarstats.net/odds2x2.html>. The difference was considered as being statistically significant
122 when the *P* value was ≤ 0.05 .

124 **RESULTS AND DISCUSSION**

125 Given the importance of Ca²⁺ signaling for the regulation of pancreatic zymogen activation and the
126 key role of STIM1 in Ca²⁺ homeostasis, we analyzed the *STIM1* gene as a candidate modifier gene
127 for chronic pancreatitis. Employing Sanger sequencing, we first analyzed the entire coding
128 sequence (2,058 bp; NM_003156.3) and exon/intron boundaries of the 12-exon *STIM1* gene in 436
129 French ICP patients and 1,005 controls, and then repeated this analysis with 517 German ICP
130 patients and 1,121 controls. Our subsequent analysis was limited to coding sequence variants that
131 resulted in amino acid changes and intronic variants that affected canonical donor/acceptor splice
132 sites. Eight such variants were identified in the French cohort and ten in the German cohort; all
133 these variants were single nucleotide substitutions and all were predicted to result in missense
134 substitutions ([Additional file, Tables S1 and S2](#)). Since all detected variants were rare variants

135 (defined as having a minor allele frequency of <0.5% in the control population as previously
136 described (Manolio et al., 2009; Tennessen et al., 2012), we first performed aggregate association
137 analysis in the context of each cohort. A significant enrichment of rare variants in patients as
138 compared to controls was noted in the French cohort (odds ratio (OR) = 4.04, $P = 0.002$) but not in
139 the German cohort (OR = 1.64, $P = 0.26$) (Table 1).

140 We also analyzed the *STIM1* gene in 1,104 Chinese ICP patients and 1,196 controls by means
141 of targeted sequencing followed by Sanger sequencing validation. A total of 24 rare variants were
142 identified (Additional file, Table S3), which when taken together were significantly overrepresented
143 in patients as compared to controls (OR = 2.03, $P = 0.03$; Table 1). A Breslow-Day test for
144 homogeneity of the ORs (<https://www.prostatservices.com/>) between the French, German and
145 Chinese cohorts showed no significant difference ($P = 0.14$). We therefore combined data from
146 these three cohorts (Table 2), the carrier frequency of the aggregated rare variants being
147 significantly higher in patients than in controls (OR = 2.33, $P = 0.0001$; Table 1).

148 Our comprehensive analysis of the *STIM1* gene in three ICP cohorts identified a significant
149 enrichment of rare coding *STIM1* variants in patients as compared to controls by means of
150 aggregate association analysis (Table 1). Notably, none of the identified 37 rare *STIM1* variants
151 correspond to those previously reported to cause or predispose to other diseases (Lacruz and
152 Feske, 2015), potentially strengthening the notion of the tissue-specific effects of different *STIM1*
153 variants. However, pathogenicity prediction by means of the CADD method yielded similar findings
154 among the three groups of variants namely, (i) variants found in only patients, (ii) variants found in
155 both patients and controls and (iii) variants found in only controls (Table 2).

156 In summary, this is the first large case-control study to demonstrate a putative association of
157 rare *STIM1* coding variants with chronic pancreatitis. Functional analysis will be required to clarify
158 whether or not rare coding *STIM1* variants predispose to pancreatitis.

159

160 **Disclosure statement**

161 The authors declare no conflict of interest.

162

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303 **Table 1.** Prevalence of *STIM1* variants in ICP patients versus controls in the French, German and
304 Chinese Populations

Population	Cases	Controls	Odds ratio	95% confidence interval	P value
	+/n (%)	+/n (%)			
French	12/436 (2.75)	7/1,005 (0.70)	4.04	1.58-10.32	0.002
German	9/517 (1.74)	12/1,121 (1.07)	1.64	0.69-3.91	0.26
Chinese	26/1,104 (2.36)	14/1,196 (1.17)	2.03	1.06-3.92	0.03
All three combined	47/2,057 (2.28)	33/3,322 (0.99)	2.33	1.49-3.65	0.0001

305

Table 2. *STIM1* variants in the combined French, German and Chinese cohorts

Exon	Nucleotide change	Amino acid change	Patients (<i>n</i> = 2,057)		Controls (<i>n</i> = 3,322)		rs number	Allele frequency in gnomAD	CADD score
			+ (%)	Population(s)	+ (%)	Population(s)			
Variants detected in patients only									
1	c.91G>C	p.Ala31Pro	1 (0.05)	F	0 (0)		rs368091975	1.19e-5	16.10
1	c.107C>T	p.Ser36Leu	1 (0.05)	G	0 (0)		rs200907515	3.18e-5	13.72
1	c.112G>C	p.Ala38Pro	1 (0.05)	F	0 (0)		rs774499633	No	14.95
1	c.113C>T	p.Ala38Val	1 (0.05)	C	0 (0)		No	No	13.93
4	c.454G>A	p.Glu152Lys ^a	3 (0.15)	C (1), F (2)	0 (0)		rs143916878	1.16e-4	23.2
6	c.747G>C	p.Glu249Asp	1 (0.05)	C	0 (0)		No	No	18.57
9	c.1231A>G	p.Thr411Ala	1 (0.09)	C	0 (0)		No	No	18.54
11	c.1498C>T	p.Arg500Trp	1 (0.05)	C	0 (0)		rs772902514	1.59e-5	33
12	c.1562C>T	p.Ser521Leu	2 (0.10)	C (1), G (1)	0 (0)		rs745539009	1.59e-5	24.5
12	c.1595G>A	p.Arg532His	1 (0.05)	C	0 (0)		rs771442242	7.96e-6	26.5
12	c.1615C>T	p.Gln539Ter	1 (0.05)	C	0 (0)		No	No	41
12	c.1668C>G	p.Ser556Arg	5 (0.24)	C	0 (0)		rs201543900	4.24e-5	22.6
12	c.1801C>T	p.Pro601Ser	1 (0.05)	G	0 (0)		rs200960094	3.98e-5	16.68
12	c.1808C>T	p.Ala603Val	1 (0.05)	C	0 (0)		rs749622475	1.19e-5	19.32
12	c.1843C>T	p.Arg615Cys	3 (0.15)	C	0 (0)		rs560566339	1.19e-5	28.5
12	c.2012G>A	p.Arg671Gln	1 (0.05)	C	0 (0)		rs779204802	8.04e-6	24.3
Variants detected in patients and controls									
4	c.458C>T	p.Thr153Ile	3 (0.15)	F (1), G (2)	1 (0.03)	F	rs144602692	1.94e-4	23.8

11	c.1511C>T	p.Thr504Met	2 (0.10)	C	1 (0.03)	C	rs146873551	8.28e-4	20.8
12	c.1571C>T	p.Ser524Phe	5 (0.24)	F (2), G (3)	8 (0.24)	F (4), G (4)	rs141215990	1.78e-3	29.1
12	c.1589G>A	p.Arg530His	6 (0.29)	C	3 (0.09)	C	rs746517083	3.18e-5	24.5
12	c.1612C>T	p.Pro538Ser	4 (0.19)	F	1 (0.03)	F	rs35960304	5.95e-3	20.2
12	c.1636G>A	p.Glu546Lys	2 (0.10)	F (1), G (1)	2 (0.06)	G	rs371443357	3.54e-5	23.9
Variants detected in controls only									
4	c.408G>C	p.Glu136Asp	0 (0)		1 (0.03)	C	rs200648767	1.77e-4	13.79
4	c.472C>G	p.Gln158Glu	0 (0)		1 (0.03)	C	No	No	21.3
5	c.530C>T	p.Thr177Ile	0 (0)		1 (0.03)	C	rs761973338	3.18e-5	24.6
7	c.826G>C	p.Glu276Gln	0 (0)		1 (0.03)	C	No	No	23.0
8	c.1010C>T	p.Ser337Phe	0 (0)		1 (0.03)	G	No	No	27.6
11	c.1499G>A	p.Arg500Gln	0 (0)		1 (0.03)	C	rs760242778	7.97e-6	29.4
11	c.1505G>A	p.Arg502His	0 (0)		1 (0.03)	C	rs555016539	1.19e-5	27.3
12	c.1583G>A	p.Ser528Asn	0 (0)		1 (0.03)	C	rs200078549	2.39e-5	24.0
12	c.1601C>A	p.Ala534Asp	0 (0)		1 (0.03)	F	No	No	14.12
12	c.1624C>T	p.Arg542Cys	0 (0)		1 (0.03)	C	rs370846246	3.58e-5	28.3
12	c.1673G>A	p.Arg558Gln	0 (0)		1 (0.03)	C	rs199503470	1.59e-5	24.4
12	c.1681G>A	p.Glu561Lys	0 (0)		1 (0.03)	G	rs200557274	1.99e-5	31
12	c.1928G>A	p.Arg643His	0 (0)		3 (0.09)	G	rs140080199	7.57e-4	31
12	c.1960G>A	p.Ala654Thr	0 (0)		1 (0.03)	G	rs201466902	1.41e-4	21.8
12	c.2053A>T	p.Lys685Ter	0 (0)		1 (0.03)	C	No	No	42
Total			47 (2.28)		33 (0.99)				

All variants were found in the heterozygous state. C, Chinese. F, French. G, Germany.