https://doi.org/10.1016/j.hpb.2020.06.002 HPB

ORIGINAL ARTICLE

A high prevalence of genetic polymorphisms in idiopathic and alcohol-associated chronic pancreatitis patients in Ireland

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Abstract

Background: Individual genetic architecture is considered central to susceptibility and progression of disease in chronic pancreatitis. The study aimed to evaluate the presence of common pancreatic gene mutations in a defined cohort of idiopathic and alcohol-induced chronic pancreatitis patients in Ireland.

Methods: The study comprised patients with idiopathic and alcohol-induced chronic pancreatitis and historic controls. Variants in the cystic fibrosis transmembrane conductance regulator (CFTR) gene, cationic trypsinogen (PRSS1) gene and serine protease inhibitor kazal type-1 (SPINK1) gene, were assessed by Taqman© genotyping assay.

Results: Of n = 126 patients and n = 167 controls, mutations were detected in 23 (20%) and in 10 (6%) respectively ($P < 0.001$). The majority of mutations found were in the SPINK1 gene variant N34S (13%) which increased disease risk almost six-fold (OR 5.9). Neither CFTR severe mutation (F508del) ($P = 0.649$) nor mild variant (R117H) ($P = 0.327$) were over-represented amongst patients compared to control subjects. PRSS1 variants were not detected in either patient or control subjects.

Conclusion: There was a significant prevalence of chronic pancreatitis-associated gene mutations in this well-phenotyped cohort. In patients with alcohol-related or idiopathic chronic pancreatitis, the possibility of genetic mutations in the SPINK 1 gene should be considered as a contributing aetiology factor.

Received 26 April 2020; accepted 4 June 2020

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Introduction

Chronic pancreatitis is a progressive inflammatory disease associated with destruction of the pancreatic parenchyma, which is replaced by fibrotic tissue eventually leading to impairment of exocrine and endocrine functions.[1–3] Previously, it was believed that alcohol was the most important factor driving chronic pancreatitis development. While the role of alcohol appears to be a significant etiological factor in predisposed patients,[4] there are high numbers of patients in whom the cause remains unclear, despite sophisticated imaging and biochemical investigations. The pathogenesis of chronic pancreatitis is now considered a complex, multifactorial process. There is strong evidence that individual genetic susceptibility contributes significantly to disease development,[2,6,7] in tandem with exposure to environmental factors such as smoking, excessive alcohol intake, hypertriglyceridemia, among others. Together these result in organ injury, dysfunction or modify the nature of severity of disease process.[5]

This study was presented at the HNiC as a podium presentation (plenary section) at the European & African Hepato-Biliary Assocation (E-AHPBA) meeting, 2019, Amsterdam.

The diagnosis of chronic pancreatitis is difficult especially early in the disease process, and is often only reached years after the initial pancreatitis attack when end-stage features become evident. Knowledge and understanding of individual genetic susceptibility and etiological pathways could allow improved and more timely diagnosis as well as highlighting more effective therapeutic approaches.

The important contribution of common gene polymorphisms to disease development has been documented in a variety of cohorts.[8–13] The principal susceptibility genes currently known are cationic trypsinogen (PRSS1) gene, serine protease inhibitor kazal type 1 (SPINK1) gene, and cystic fibrosis transmembrane conductance regulator (CFTR) gene. These three genes are mechanistically linked to the control of trypsin activity within the pancreas.[5] SPINK1 is a specific trypsin inhibitor, and CFTR is a ductal anion channel responsible for generation of fluid secretion which flushes prematurely activated trypsin in the ducts out of the pancreas. PRSS1 gene mutations are thought to prevent the inactivation of trypsin within the pancreas, which leads to pancreatic autodigestion and subsequent pancreatitis. The management of disease and prevention of recurrence would benefit from specific knowledge of a given individual patient's etiology. Whilst several studies have examined the association of major pancreatic gene mutations in chronic pancreatitis patients, no cohorts have been examined in Ireland, and only a small number assessed in the UK.[14–17] Carriage of CFTR gene mutations is known to be enriched within the general population of Ireland, contributing to one of the highest observed rates of cystic fibrosis worldwide.[18] Studies have described increased frequency of CFTR mutations (commonly F508del) amongst chronic pancreatitis patients and thus the possibility that F508del is a significant hazard for the development of chronic pancreatitis in Ireland is an open question.[9,11,19] More broadly, it is possible that frequencies of disease associated variants may differ between populations and understanding this may improve local diagnosis and clinical decision making. The causative role of pancreatitis-related gene mutations may be of particular relevance amongst idiopathic patients, or amongst those with documented alcohol-related chronic pancreatitis but with apparently modest alcohol consumption. Currently, genetic testing is not part of standard diagnostic work-up, besides the examination of PRSS1 mutations in hereditary pancreatitis patients. By clarifying the nature of pancreatitis susceptibility genes in chronic pancreatitis, protocols for assessments and management of patients may be formulated to optimise the diagnosis of patients, allowing targeted etiology-based patient care. The aim of the current study was to evaluate the presence of common pancreatic gene variants in an Irish cohort which have been reported in previous studies of chronic pancreatitis cohorts worldwide. Specifically; the presence of SPINK1 variant N34S, CFTR variant F508del, and PRSS1 variants R122H, N29I, A16V were examined in patients with the diagnosis of idiopathic or alcohol-induced pancreatitis.

Methods

This study had a prospective controlled-cohort design. Patients with diagnoses of idiopathic and alcohol-induced chronic pancreatitis were identified from specialist pancreatitis clinics in the Centre for Pancreatico-Biliary Diseases, Tallaght University Hospital Dublin and St. Vincent's University Hospital Dublin (both are university-affiliated, tertiary pancreatic referral centers). The study had full ethical approval from the Joint Tallaght University Hospital/St James's Hospital Joint Research Ethics Committee (JREC), and St Vincent's University Hospital Ethics and Medical Research Committee. Diagnosis of chronic pancreatitis was based on at least two of the following criteria: patient history (abdominal pain typical of pancreatitis), functional deficits (exocrine/endocrine impairment) and/or findings of radiologic/endoscopic studies (computed tomography/endoscopic ultrasonography). Aetiology was determined from clinical history or investigations performed at the time of admission. Idiopathic aetiology was diagnosed in the absence of other known clinical causes. The aetiology of alcohol-induced chronic pancreatitis was assessed based on a self-reported chronic alcohol intake which typically exceeded the recommended intakes according to Irish government guidelines (17 and 11 standard drinks for men and women per week, respectively), and in the absence of other clinical causes. All abdominal imaging and endoscopic examinations were reviewed to evaluate pancreatic morphology. The medical records of all patients were reviewed for demographic and clinical data.

Patient recruitment

Irish Caucasian patients with a diagnosis of idiopathic or alcohol-induced chronic pancreatitis were identified as eligible for study inclusion through discussion with the medical team, nurse specialists, and through attendance at specialist pancreatic clinics. Patients were excluded if they had a diagnosis of cystic fibrosis, if they were pregnant, less than 18 years of age, or if they were unable to provide informed written consent. The recruitment period ran between November 2017 and March 2018. All patients were offered genetic counselling prior to inclusion in the study. Patients were made aware that the presence of a mutation may not predict the course of the disease, nor would it affect clinical management. All patients underwent genetic testing for the specified mutations of SPINK1, PRSS1, and CFTR. Patients' medical records were evaluated, and the following indices were recorded: personal or family history of pancreatitis, smoking status (self-reported current-, past- or never-smoker), history of alcohol consumption (self-reported current-, past- or never-drinker; from medical notes and patient interview), history of acute pancreatitis, age of onset of chronic pancreatitis, diagnosis,

Please cite this article as: Ní Chonchubhair HM et al., A high prevalence of genetic polymorphisms in idiopathic and alcohol-associated chronic pancreatitis patients in Ireland, HPB, https://doi.org/10.1016/j.hpb.2020.06.002

medical and surgical history, and prescribed medication. Patients were recorded as having pancreatic exocrine insufficiency (PEI) if there was a faecal elastase-1 level <200 μg/g stool,[20] and/or if the patients were clinically deemed by the pancreatic team to have PEI based on symptomology. Patients were recorded as having diabetes if they had a prior recorded diagnosis of diabetes (defined as fasting plasma venous glucose >7.0 mmol/L (126 mg/dl), or a HbA1c of >48 mmol/mol (6.5%).[21]

Control recruitment

Control subjects were obtained from an anonymous, national control DNA database which is held in the Trinity Translational Medicine Institute (TTMI), St James's Hospital, Dublin. This control database included 2047 healthy persons, without known disease, who were representative of the general population. The historical database was populated by persons attending the Irish Blood Transfusion Service for blood donation, during which DNA was extracted following informed consent. Controls for this study were selected using a randomised block sampling method. In total, 167 consecutive controls were randomly selected from the database and included in the current analysis. Whilst the age and gender of controls were known, no other clinical or socio-economic characteristics were available.

A *post hoc* phase two to this study was conducted to more accurately assess allele frequency of SPINK N34S in the general population. Therefore, an additional n = 341 controls, obtained from TTMI's control database, were analysed bringing the control group to a total of n = 508.

DNA extraction

DNA was extracted manually from whole blood samples at the TTMI Biobank, using an automated Gentra extraction facility. DNA was re-suspended in TE buffer pH8.0, and stored at −20 °C or −80 °C.

Genotyping method

Genotyping of patients and controls was performed using TaqMan (ThermoFisher Scientific) assay for CFTR mutations R117H (rs78655421), F508del (rs113993960); SPINK1 mutation N34S (rs17107315); and PRSS1 mutations A16V (rs202003805), N29I (rs111033566) and R122H (rs111033565). 20ul reactions were prepared using 10 ng of genomic DNA, TaqMan Genotyping Master Mix (ThermoFisher Scientific, United Kingdom) and purified DNase free water. Each plate contained one template negative control. Thermocycling was performed on a 7500 Real Time PCR System (Applied Biosystems) in a two-step PCR protocol, following recommended settings by ThermoFisher Scientific. Results were analysed using Applied Biosystem's 7500 Software (V2.0.6). Results were checked for clear cluster separation and genotype calling was performed manually. Any results which failed to cluster were re-analysed on a separate plate together with one previously genotyped sample and one template negative control.

Statistical analysis

Statistical analysis was conducted using SPSS Version 22 (SPSS, Chicago, IL, USA, 2015). Data were compared by means of Fisher's exact test, and Chi square test for categorical data. Continuous variables were analysed using Student's T test or Mann-Whitney U for independent samples. A P-value of <0.05 was considered statistically significant.

Results

Patient and control characteristics

The CONSORT diagram of the recruitment process is shown in Fig. 1. A total of 126 patients were evaluated for the presence of PRSS1, SPINK1 and CFTR mutations and the clinical characteristics are detailed on Table 1. Mutations were more common in patients (23/126, 20%) than in controls (10/167, 6%) (P < 0.001) (Table 2).

Breakdown of mutational analysis is shown in Table 2. Of the 16 patients with chronic pancreatitis, nine with alcohol-induced disease and seven with idiopathic disease were heterozygotes for the SPINK1 N34S mutation. One patient was heterozygous for both SPINK1 N34S and CFTR F508del.

Table 3 details the characteristics of patients with one or more positive mutation (n = 25) compared to patients negative for mutations.

Discussion

This study aimed to determine if pathogenic genetic variants of the SPINK1, CFTR or PRSS1 gene were present in idiopathic and alcohol-associated chronic pancreatitis patients in Ireland. Twenty percent of the chronic pancreatitis patient cohort had disease-associated mutations in one or more of these genes. The combined burden of mutations in these genes increases chronic pancreatitis risk more than three-fold. Importantly, the N34S allele which is an established disease modifier conferred an almost six-fold increased risk of chronic pancreatitis when compared to healthy controls. Previous studies have shown that mutated N34S occurs in ~1% of the general population and increases pancreatitis risk almost 20-fold.[22,23] Notably, patients with idiopathic and alcohol-associated chronic pancreatitis were recruited and this therefore shows that patients with either of these aetiologies may have a genetic component to their disease. In a US study comparing patients of European ancestry to those of African ancestry, pathogenic gene variants (including SPINK1, PRSS1, CFTR and CTRC) were significantly more common among European ancestry patients.[24] Together, these data bolster information on the pattern of genetic susceptibility worldwide.

Variable rates of PRSS1 gene mutations have been reported in chronic pancreatitis patients of idiopathic etiology (0–21%).[14,25–28] Similar to the current findings, several studies internationally have reported that PRSS1 mutations were rare or absent in patients with chronic pancreatitis,[28–33] although others

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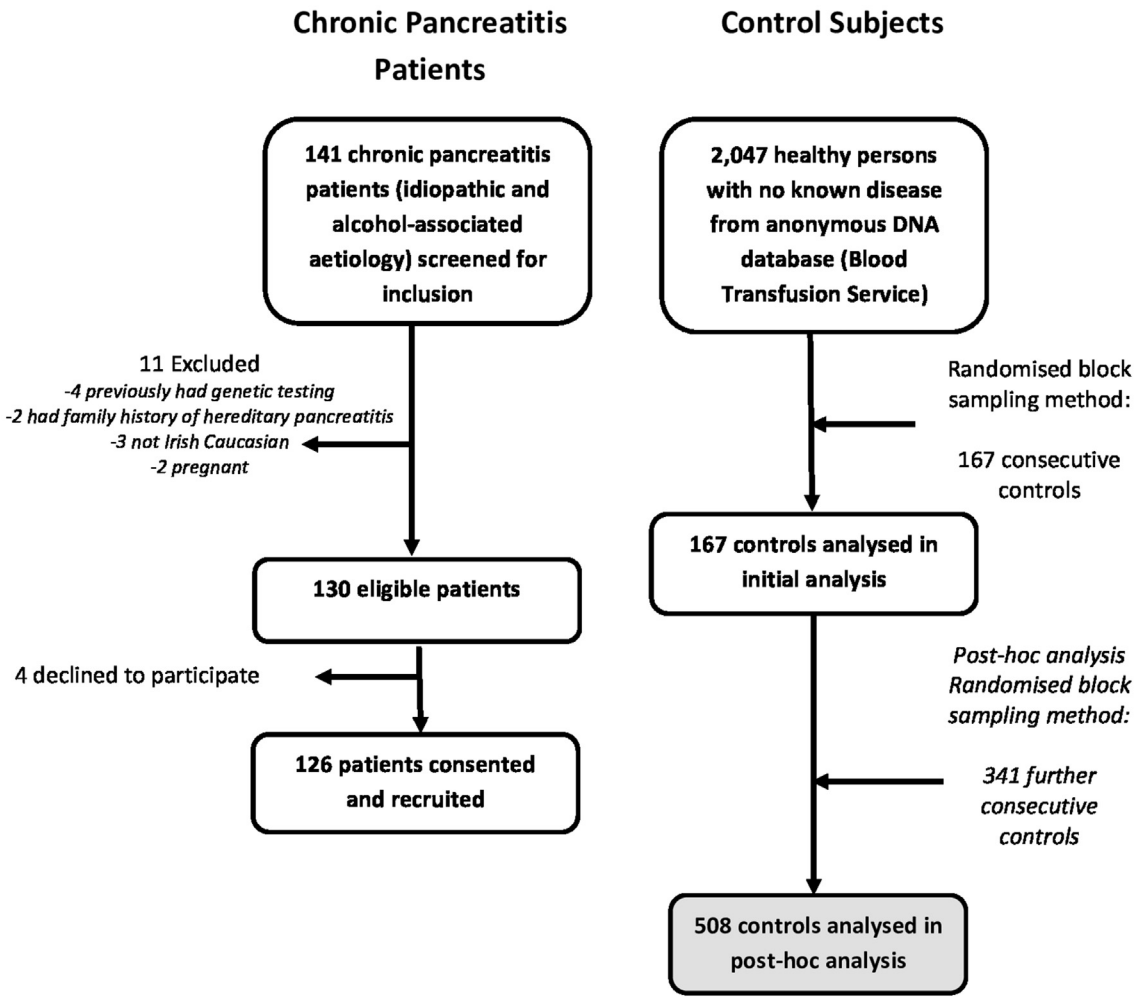

Figure 1 CONSORT diagram detailing study recruitment

reported PRSS1 mutations at frequencies of 1.5–33% amongst alcohol-related pancreatitis patients.[27,34]

SPINK1 mutations are found in 1–3% of the general population,[24] and idiopathic chronic pancreatitis is strongly associated with the common SPINK1 high-risk genotype N34S.[35] There may be a small effect of N34S in alcohol-related chronic pancreatitis patients, suggesting that alcohol's primary effects are driven through a non-trypsin related pathway.[35] N34S was associated with a 9-fold increased risk of disease in European chronic pancreatitis patients,[38] with the frequency of N34S more than twice as high in idiopathic compared to alcohol-related patients.

This study examined the prevalence of the most commonly seen CFTR (severe and mild) mutations. Results demonstrated that neither heterozygous F508del of R117H were higher amongst patients with chronic pancreatitis relative to controls, similar to previous findings.[39,40] Therefore, while carriage rates of mild and severe CFTR mutations are relatively high amongst the Irish general population, this did not significantly contribute as a risk factor for chronic pancreatitis in the absence of cystic fibrosis.[18] While F508del of R117H are relatively common CFTR mutations more than 1600 mutations and 200 polymorphisms have been reported, most of which are rare and of unknown significance in consideration of the potential effects on pancreatic duct cells and phenotypes.[8] This study examined only two common CFTR mutations and rare mutations were not investigated. However, due to the number and lack of functional attribution of these variants, it would be difficult to ascribe causality to these variants in the absence of a large study cohort. Compound heterozygosity in CFTR does not appear to further increase CP disease risk over that observed in regular heterozygotic forms.[39,41]

When to test for genetic mutations in chronic pancreatitis, and in whom, is controversial.[42,43] Current recommendations regarding genetic testing do not identify all possible gene mutations or mutation combinations, and therefore, may have limited predictive value. Furthermore, there is little evidence to suggest a genetic basis for disease prognosis and most patients

Table 1 Characteristics of patients and controls who were tested for pancreatic gene mutations

	Patients (n = 126)	Controls (n = 167)	P-value
Demographics			
Age in years, mean (SD)	55 (12.2)	40.9 (14.1)	< **0.001**
Male, (%)	68	46	< **0.001**
Etiology and Presentation			
Alcohol-related etiology	69 (54.8)	N/A	–
Idiopathic etiology	57 (45.2)	N/A	–
PEI present	92 (73)	Unknown	–
DM present	51 (40.5)	Unknown	–
Smoking			
Current	62 (49.2)	Unknown	–
Past	30 (23.8)	Unknown	–
Never	34 (27)	Unknown	–
Alcohol Use			
Current	60 (47.6)	Unknown	–
Past	55 (43.7)	Unknown	–
Never	11 (8.7)	Unknown	

Values are given as number (percentage) unless otherwise indicated. Student's t-test used for statistical analysis of continuous data and χ^2 test for categorical data. Significant results are highlighted in bold.
SD standard deviation, PEI pancreatic exocrine insufficiency, DM diabetes mellitus.

Table 2 Distribution of SPINK1, CFTR, and PRSS1[a] mutations in chronic pancreatitis patients and controls

Gene Variant	Patients (n = 126)	Controls (n = 167)	P-value	OR	OR 95% CI
Any mutation	23 (20)	10 (6)	**0.001**	3.05	1.4–6.6
SPINK1					
N34S	16 (13)	4 (2)	**0.001**[d]	5.9	1.9–18.1
N34S (*post-hoc*)[b]	16 (13)	12 (2)[c]	< **0.001**	6.0	2.7–12.9
CFTR					
F508del	5 (4)	5 (3)	0.65[d]	1.3	0.4–4.7
R117H	3 (2)	1 (1)	0.33[d]	4.0	0.4–40

Values are given as number (percentage) unless otherwise indicated. Statistical analysis performed by χ^2 test, significant results are highlighted in bold. One patient was heterozygous for N34S and F508del.
OR odds ratio, CI confidence interval, SPINK1 serine protease inhibitor kazal type-1, CFTR cystic fibrosis transmembrane conductance regulator, PRSS1 cationic trypsinogen.
[a] PRSS1 analysis: Neither patients nor controls were positive for disease mutations at any of the R122H, A16V and N29I sites.
[b] Post-hoc analyses of an additional 341 control subjects for SPINK1 N34S identified a further 8 carriers of the variant.
[c] Including originally tested control subjects, n = 508 for this group.
[d] Fisher's exact test.

Table 3 A comparison of clinical and demographic characteristics between patients with one or more identified mutations in SPINK1 or CFTR and patients with no mutations detected

	One or more mutation (n = 25)	No mutations detected (n = 101)	P-value
Demographics			
Age at assessment in years, mean (SD)	53 (10.9)	56 (12.5)	0.29
Age at diagnosis in years, mean (SD)	43 (11.1)	47.5 (12.8)	0.075
Male (n)	20	65	0.135
Etiology and Presentation			
Alcohol-related etiology	15	54	0.55
Idiopathic etiology	10	47	0.55
Family history of chronic pancreatitis	1	2	0.49[a]
PEI present	15	77	0.13
DM present	10	41	0.96
Smoking and alcohol			
Current smoker	13	51	0.9
Current alcohol drinker	24	90	0.29

Values are given as number, unless otherwise indicated.
Statistical analysis of categorical data was performed by χ^2 test. Continuous data were interrogated by Student's t-test or Mann Whitney U test.
SD standard deviation, PEI pancreatic exocrine insufficiency, DM diabetes mellitus.
[a] Fisher's exact test used.

with established mutations never develop chronic pancreatitis.[42] Recent European guidelines have recommended that those with a family history of pancreatitis,[20,43,44] an unknown aetiology,[43] and recurrent disease[43] should be tested for genetic mutations, but that routine genetic testing cannot be recommended for those with alcohol-associated chronic pancreatitis.[20] However, these data suggest that even those with alcohol-associated chronic pancreatitis may have underlying genetic aetiologies.

The study had several limitations. Rather than conducting a comprehensive genetic analysis requiring targeted sequencing of the genes, selected genes were evaluated based on the most common variants previously linked to chronic pancreatitis, a similar approach to that of a recent US study.[24] Therefore, the true morbidity associated with these genes is likely to be underestimated and the diagnostic yield reported of almost 20% of patients with chronic pancreatitis is likely to be represent the lower boundary of affected patients in this cohort. The controls for this study were selected from a National control DNA biobank of healthy blood donors with a National geographic distribution, but otherwise unselected. The random selection from this control group had more females and were younger than the patient group however this is not anticipated to affect allele frequency estimation. Despite these limitations, this study

included well-phenotyped idiopathic and alcohol-associated patients and the risk factors for the development of chronic pancreatitis were well defined. The variants employed in the mutational screening panel were selected according to published reports, and genetic variants of unknown clinical significance were excluded.

Chronic pancreatitis is a disease of complex aetiology, comprising environmental and genetic factors which likely coalesce to confer increased disease risk. An accurate classification of the disease is required to define which patients carry genetic susceptibility in association with morphological conditions and exogenous factors and are at risk of developing acute, recurrent and chronic pancreatitis. While significantly more pathogenic variants were found in patients than in controls, there is insufficient evidence to support blanket genetic screening at this point. Those with severe acute pancreatitis or recurrent acute pancreatitis may benefit from genetic testing, particularly in the absence of a convincing history of excess alcohol consumption.

Acknowledgements

Thank you to the staff of the specialist chronic pancreatitis clinics at Tallaght University Hospital Dublin and St. Vincent's University Hospital Dublin for their assistance with patient recruitment. Thank you to the staff at the Trinity Translational Medicine Institute, St James Hospital, Trinity College Dublin for their assistance regarding genetic testing and analysis. Thank you to Mylan EPD, United Kingdom for an unrestricted educational grant which supported the PhD studies of the primary author (HNíC).

Funding sources

This work was supported by Mylan EPD, United Kingdom (an unrestricted education grant to KCC and SND, supporting the PhD studies of HNiC).

Conflicts of interest

None declared.

References

1. Steer ML, Waxman I, Freedman S. (1995) Chronic pancreatitis. *N Engl J Med* 332:1482–1490.
2. Witt H, Apte MV, Keim V, Wilson JS. (2007) Chronic pancreatitis: challenges and advances in pathogenesis, genetics, diagnosis, and therapy. *Gastroenterology* 132:1557–1573.
3. Braganza JM, Lee SH, McCloy RF, McMahon MJ. (2011) Chronic pancreatitis. *Lancet* 377:1184–1197.
4. Apte MV, Haber PS, Norton ID, Wilson JS. (1998) Alcohol and the pancreas. *Addiction Biol* 3:137–150.
5. Whitcomb DC. (2013) Genetic risk factors for pancreatic disorders. *Gastroenterology* 144:1292–1302.
6. Whitcomb DC. (2000) Genetic predispositions to acute and chronic pancreatitis. *Med Clin North Am* 84:531–547.
7. Zator Z, Whitcomb DC. (2017) Insights into the genetic risk factors for the development of pancreatic disease. *Ther Adv Gastroenterol* 10: 323–336.
8. Schneider A, Larusch J, Sun X, Aloe A, Lamb J, Hawes R. (2011) Combined bicarbonate conductance-impairing variants in CFTR and SPINK1 variants are associated with chronic pancreatitis in patients without cystic fibrosis. *Gastroenterology* 140:162–171.
9. LaRusch J, Jung J, General IJ, Lewis MD, Park HW, Brand RE *et al*. (2014) Mechanisms of CFTR functional variants that impair regulated bicarbonate permeation and increase risk for pancreatitis but not for cystic fibrosis [published correction appears in PLoS Genet. 2014 Oct;10(10):e1004778]. *PLoS Genet* 10e1004376.
10. Cohn JA, Friedman KJ, Noone PG, Knowles MR, Silverman LM, Jowell PS. (1998) Relation between mutations of the cystic fibrosis gene and idiopathic pancreatitis. *N Engl J Med* 339:653–658.
11. Sharer N, Schwarz M, Malone G, Howarth A, Painter J, Super M *et al*. (1998) Mutations of the cystic fibrosis gene in patients with chronic pancreatitis. *N Engl J Med* 339:645–652.
12. Masson E, Chen JM, Audrézet MP, Cooper DN, Férec C. (2013) A conservative assessment of the major genetic causes of idiopathic chronic pancreatitis: data from a comprehensive analysis of PRSS1, SPINK1, CTRC and CFTR genes in 253 young French patients. *PloS One* 8e73522.
13. Rosendahl J, Landt O, Bernadova J, Kovacs P, Teich N, Bodeker H *et al*. (2013) CFTR, SPINK1, CTRC and PRSS1 variants in chronic pancreatitis: is the role of mutated CFTR overestimated? *Gut* 62:582–592.
14. O'Reilly DA, Yang BM, Creighton JE, Demaine AG, Kingsnorth AN. (2001) Mutations of the cationic trypsinogen gene in hereditary and non-hereditary pancreatitis. *Digestion* 64:54–60.
15. Threadgold J, Greenhalf W, Ellis I, Howes N, Lerch MM, Simon P *et al*. (2002) The N34S mutation of SPINK1 (PSTI) is associated with a familial pattern of idiopathic chronic pancreatitis but does not cause the disease. *Gut* 50:675.
16. Creighton JE, Lyall R, Wilson DI, Curtis A, Charnley RM. (2000) Mutations of the cationic trypsinogen gene in patients with hereditary pancreatitis. *Br J Surg* 87:170–175.
17. Cohn JA, Neoptolemos JP, Feng J, Yan J, Jiang Z, Greenhalf W *et al*. (2005) Increased risk of idiopathic chronic pancreatitis in cystic fibrosis carriers. *Hum Mutat* 26:303–307.
18. Farrell PM. (2008) The prevalence of cystic fibrosis in the European Union. *J Cyst Fibros* 7:450–453.
19. LaRusch J, Whitcomb DC. (2011) Genetics of pancreatitis. *Curr Opin Gastroenterol* 27:467–474.
20. Löhr JM, Dominguez-Munoz E, Rosendahl J, Besselink M, Mayerle J, Lerch MM *et al*. (2017) United European Gastroenterology evidence-based guidelines for the diagnosis and therapy of chronic pancreatitis (HaPanEU). *Unit Eur Gastroenterol J* 5:153-199.
21. Classification and diagnosis of diabetes. American diabetes association. *Diabetes Care* 38(Supplement 1), (Jan 2015):S8–S16.
22. Noone PG, Zhou Z, Silverman LM, Jowell PS, Knowles MR, Cohn JA. (2001) Cystic fibrosis gene mutations and pancreatitis risk: relation to epithelial ion transport and trypsin inhibitor gene mutations. *Gastroenterology* 121:1310–1319.
23. Pfützer RH, Barmada MM, Brunskill AP, Finch R, Hart PS, Neoptolemos J *et al*. (2000) SPINK1/PSTI polymorphisms act as disease modifiers in familial and idiopathic chronic pancreatitis. *Gastroenterology* 119:615–623.
24. Phillips AE, LaRusch J, Greer P, Abberbock J, Alkaade S, Amann ST *et al*. (2018) Known genetic susceptibility factors for chronic pancreatitis in patients of European ancestry are rare in patients of African ancestry. *Pancreatology* 18:528–535.
25. Creighton J, Lyall R, Wilson DI, Curtis A, Charnley R. (1999) Mutations of the cationic trypsinogen gene in patients with chronic pancreatitis. *Lancet* 354:42–43.

26. Ockenga J, Stuhrmann M, Ballmann M, Teich N, Dork T, Manns MP. (2000) Mutations of the cystic fibrosis gene, but not cationic trypsinogen gene, are associated with recurrent or chronic idiopathic pancreatitis. *Am J Gastroenterol* 95:2061–2067.

27. Ockenga J, Stuhrmann M, Ballmann M, Teich N, Dork T, Manns MP *et al*. (2011) The prevalence of cationic trypsinogen (PRSS1) and serine protease inhibitor, Kazal type 1 (SPINK1) gene mutations in Polish patients with alcoholic and idiopathic chronic pancreatitis. *Dig Dis Sci* 56: 894–901.

28. Witt H, Luck W, Becker M. (1999) A signal peptide cleavage site mutation in the cationic trypsinogen gene is strongly associated with chronic pancreatitis. *Gastroenterology* 117:7–10.

29. Hu C, Wen L, Deng L, Zhang C, Lugea A, Su HY *et al*. (2017) The differential role of human cationic trypsinogen (PRSS1) p.R122H mutation in hereditary and nonhereditary chronic pancreatitis: a systematic review and meta-analysis. *Gastroenterol Res Pract* 2017:9505460.

30. Teich N, Mossner J, Keim V. (1999) Screening for mutations of the cationic trypsinogen gene: are they of relevance in chronic alcoholic pancreatitis? *Gut* 44:413–416.

31. Monaghan KG, Jackson CE, KuKuruga DL, Feldman GL. (2000) Mutation analysis of the cystic fibrosis and cationic trypsinogen genes in patients with alcohol-related pancreatitis. *Am J Med Genet* 94: 120–124.

32. Chandak GR, Idris MM, Reddy DN, Mani KR, Bhaskar S, Rao GV, Singh L. (2004) Absence of PRSS1 mutations and association of SPINK1 trypsin inhibitor mutations in hereditary and non-hereditary chronic pancreatitis. *Gut* 53:723–728.

33. Drenth JP, te Morsche R, Jansen JB. (2002) Mutations in serine protease inhibitor Kazal type 1 are strongly associated with chronic pancreatitis. *Gut* 50:687–692.

34. Bernardino AL, Guarita DR, Mott CB, Pedroso MR, Machado MC, Laudanna AA. (2003) CFTR, PRSS1 and SPINK1 mutations in the development of pancreatitis in Brazilian patients. *JOP* 4:169–177.

35. Aoun E, Chang CC, Greer JB, Papachristou GI, Barmada MM, Whitcomb DC. (2008) Pathways to injury in chronic pancreatitis: decoding the role of the high-risk SPINK1 N34S haplotype using meta-analysis. *PloS One* 3:e2003.

38. Di Leo M, Bianco M, Zuppardo RA, Guslandi M, Calabrese F, Mannucci A *et al*. (2017) Meta-analysis of the impact of SPINK1 p.N34S gene variation in Caucasic patients with chronic pancreatitis. *Update Dig Liver Dis : Off J Ital Soc Gastroenterol Ital Assoc Stud Liver* 49: 847–853.

39. Cavestro GM, Zuppardo RA, Bertolini S, Sereni G, Frulloni L, Okolicsanyi S *et al*. (2010) Connections between genetics and clinical data: role of MCP-1, CFTR, and SPINK-1 in the setting of acute, acute recurrent, and chronic pancreatitis. *Am J Gastroenterol* 105:199–206.

40. Hegyi P, Wilschanski M, Muallem S, Lukacs GL, Sahin-Tóth M, Uc A. (2016) CFTR: a new horizon in the pathomechanism and treatment of pancreatitis. *Rev Physiol Biochem Pharmacol* 170:37–66.

41. Pelletier AL, Bienvenu T, Rebours V, O'Toole D, Hentic O, Maire F *et al*. (2010) CFTR gene mutation in patients with apparently idiopathic pancreatitis: lack of phenotype-genotype correlation. *Pancreatology* 10: 158–164.

42. Cohn JA. (2003) Motion–genetic testing is useful in the diagnosis of nonhereditary pancreatic conditions: arguments against the motion. *Can J Gastroenterol* 17:53–55.

43. Martinez J, Abad-Gonzalez A, Aparicio JR *et al*. (2013) [Recommendations of the Spanish Pancreatic Club on the diagnosis and treatment of chronic pancreatitis: part 1 (diagnosis)]. *Gastroenterol Hepatol* 36: 326–339.

44. Mayerle J, Hoffmeister A, Werner J *et al*. (2013) Chronic pancreatitis–definition, etiology, investigation and treatment. *Dtsch Arzteblatt Int* 110:387–393.