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ORIGINAL ARTICLE

Keratin 8 sequence variants in patients with pancreatitis and pancreatic cancer

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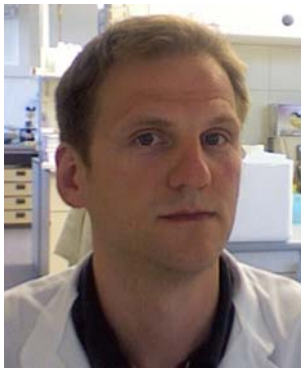
Abstract Keratin 8 (KRT8) is one of the major intermediate filament proteins expressed in single-layered epithelia of the gastrointestinal tract. Transgenic mice over-expressing human *KRT8* display pancreatic mononuclear infiltration, interstitial fibrosis and dysplasia of acinar cells resulting in exocrine pancreatic insufficiency. These experimental data are in accordance with a recent report describing an association between *KRT8* variations and chronic pancreatitis. This prompted us to investigate *KRT8* polymorphisms in patients with pancreatic disorders. The *KRT8* Y54H and G62C polymorphisms were assessed in a cohort of patients with acute and chronic pancreatitis of various aetiologies or pancreatic cancer originating from Austria (n=16), the Czech Republic (n=90), Germany (n=1698), Great Britain (n=36), India (n=60), Italy (n=143), the Netherlands (n=128), Romania (n=3), Spain (n=133), and Switzerland (n=129). We also studied 4,234 control subjects from these countries and 1,492 control subjects originating from Benin, Cameroon, Ethiopia, Ecuador, and Turkey. Polymorphisms were analysed by melting curve analysis with fluorescence resonance energy transfer probes. The frequency of G62C did not differ between patients with acute or chronic pancreatitis, pancreatic adenocarcinoma and control individuals. The frequency of G62C varied in European



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populations from 0.4 to 3.8%, showing a northwest to southeast decline. The Y54H alteration was not detected in any of the 2,436 patients. Only 3/4,580 (0.07%) European, Turkish and Indian control subjects were heterozygous for Y54H in contrast to 34/951 (3.6%) control subjects of African descent. Our data suggest that the *KRT8* alterations, Y54H and G62C, do not predispose patients to the development of pancreatitis or pancreatic cancer.

Keywords Keratin 8 · Acute pancreatitis · Chronic pancreatitis · Pancreatic carcinoma

Introduction

Chronic pancreatitis (CP) is a continuing or relapsing inflammatory process leading to irreversible morphological changes and in advanced stage to exocrine insufficiency, endocrine insufficiency, or both [1]. Ethanol abuse, trauma, anatomic anomalies, and metabolic disorders have been related to the development of CP. In approximately 20 to 30% of all patients a conspicuous etiological factor is absent. These patients are classified to have idiopathic disease or, if they present with a family history for the disease, as hereditary pancreatitis.

Mutations in the genes encoding cationic trypsinogen (*PRSS1*) and the serine protease inhibitor, Kazal type 1 (*SPINK1*) have been associated with hereditary and idiopathic CP [2–5]. It can be hypothesised that these mutations lead to an imbalance of proteases and their inhibitors within the pancreatic parenchyma. Additionally, mutations in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene are significantly enriched in patients with idiopathic CP [6–8]. The majority of patients with so-called hereditary or idiopathic CP, however, do not carry a mutation in either of the above-mentioned genes, indicating that defects in additional genes contribute to the pathogenesis.

The cytoskeleton comprises three filamentous systems: intermediate filaments, actin-containing microfilaments and microtubules. These cytoskeletal proteins function as regulators of cell division, stress response and maintenance of mechanical cellular integrity. Keratin proteins are made up of two types: acidic (type I) and basic (type II), and determine the composition of intermediate filaments. As a rule, keratins are synthesised in pairs so that complementary members of the two families are expressed in every cell. In epithelial cells, type I keratins such as keratin 18 (KRT18) and type II keratins such as keratin 8 (KRT8) polymerise to form the intermediate filaments. KRT18 and KRT8 represent the major keratins expressed in single-layered epithelia of the gastrointestinal tract including the liver and the pancreas. In addition, keratins 7 and 19 are expressed in ducts and islets of the human pancreas, but not in the acinar compartment [9, 10].

The function of KRT8 as a regulator of cell stress and cell division suggests that altered KRT8 might contribute to pancreatic inflammation and/or neoplastic transformation. Transgenic mice over-expressing human *KRT8* have a reduction in the size of the pancreas and develop severe exocrine pancreatic insufficiency characterised by focal inflammation, fibrosis, and substitution of exocrine by adipose tissue. Moreover, other anomalies found in these mice include loss of acinar architecture, dysplasia and increased cell proliferation, all characteristics of early neoplastic stages [11].

Two *KRT8* variations, a tyrosine to histidine exchange at codon 54 (Y54H) and a glycine to cysteine exchange at codon 62 (G62C), have been linked to cryptogenic liver cirrhosis as well as other liver diseases [12, 13]. These two variants were labelled Y53H and G61C in the publications of Ku et al. and the difference in nomenclature arises because our codon numbering starts at the ATG initiation codon.

More recently, an association between CP and *KRT8* variations has been described. In this study, the G62C variant was found in six out of 67 CP patients (8.9%), but in none of 100 control subjects ($P<0.003$) [14]. These data suggest that *KRT8* variations may contribute to the risk of developing pancreatitis and possibly to the risk of developing pancreatic cancer. Thus, we investigated *KRT8* variations in a large cohort of pancreatitis and pancreatic cancer patients as well as control subjects of different ethnic background.

Study subjects and methods

Study subjects

The study was approved by the local medical ethical review committee of the Charité. All patients gave their informed consent for genetic analysis. The study included 2,436 patients originating from Austria (n=16), the Czech Republic (n=90), Germany (n=1698), Great Britain (n=36), India (n=60), Italy (n=143), the Netherlands (n=128), Romania (n=3), Spain (n=133) and Switzerland (n=129) (Table 1).

The alcoholic chronic pancreatitis (ACP) group consisted of 508 patients. We also enrolled in our study 1,114 patients with non-alcoholic CP (idiopathic or hereditary CP) and 60 patients with tropical calcific pancreatitis, an idiopathic chronic pancreatitis prevalent in tropical countries (Table 1).

The 256 German ACP patients were recruited in the university hospitals in Berlin in 2003 (n=38), Leipzig, Saxony in 2000 (n=30) and in Magdeburg, Saxony-Anhalt

Table 1 Characteristics of patients and control subjects

Population	ACP			ICP/HP			PC			Controls	
	n	Gender Female/male	Age (years): Mean±SD	*n*	Gender Female/male	Age (years): Mean±SD	*n*	Gender Female/male	Age (years): Mean±SD	*n*	Gender Female/male
Austria	–	–	–	16	8/8	18.3±13.9	–	–	–	405	163/242
Czech Republic	35	7/28	51.2±7.5	55	21/34	29.5±18.4	–	–	–	486	205/281
Germany	256	33/223	45.1±9.5	789	399/390	30.3±19.0	382	166/216	61.7±10.3	1,532	914/618
Great Britain	25	7/18	50.1±7.8	11	8/3	52.0±15.9	–	–	–	200	72/128
Italy	–	–	–	143	54/89	38.8±15.9	–	–	–	254	120/134
The Netherlands	71	25/46	49.7±9.0	57	33/24	42.1±17.7	–	–	–	262	105/157
Romania	–	–	–	3	0/3	45.0±7.3	–	–	–	150	75/75
Spain	62	3/59	47.9±12.9	5	3/2	68.8±15.2	66	28/38	69.7±11.8	539	138/401
Switzerland	59	3/56	57.0±8.4	35	14/21	42.8±16.1	35	16/19	63.4±10.0	339	116/223
India *	–	–	–	60	21/39	30.3±10.5	–	–	–	67	Unknown

ACP Alcoholic chronic pancreatitis, *ICP/HP* idiopathic/hereditary chronic pancreatitis, *PC* pancreatic adenocarcinoma

between 1996 and 2003 (*n*=188). The 789 German non-alcoholic CP patients originated from Berlin (*n*=262; collected between 1998 and 2003), Leipzig, Saxony (*n*=202; between 1997 and 2003), Magdeburg, Saxony-Anhalt (*n*=118; between 1996 and 2003), and Ulm, Baden-Wuerttemberg (*n*=207; between 1998 and 2001). Because the German contributing university hospitals represent tertiary care centres specialised for pancreatic diseases, most of the patients, especially of the non-alcoholic CP cases, were referred to these centres from virtually all parts of Germany.

The clinical diagnosis of CP was based on two or more of the following criteria: presence of a typical history of recurrent pancreatitis, radiological findings such as pancreatic calcifications and/or pancreatic ductal irregularities revealed by endoscopic retrograde pancreaticography or magnetic resonance imaging of the pancreas and/or pathological sonographic findings. Hereditary CP was diagnosed when two first-degree relatives or three or more second-degree relatives suffered from recurrent acute or chronic pancreatitis without any apparent precipitating factor. Alcohol-induced CP was diagnosed in patients who consumed more than 60 g (women) or 80 g (men) of ethanol per day for more than 2 years. Patients were classified as having idiopathic CP when precipitating factors such as alcohol abuse, trauma, medication, infection, metabolic disorders and/or a positive family history were absent.

The acute pancreatitis (AP) patients group consisted of 192 German patients (72 women, 120 men; mean age±SD: 53.1±17.2) of alcoholic (*n*=51), biliary (*n*=78), idiopathic (*n*=33) or miscellaneous origin (*n*=30) (postoperative [*n*=13], trauma [*n*=5], ERCP-induced [*n*=6], hyperlipoproteinaemia [*n*=4], hyperparathyroidism [*n*=1], drug-induced [*n*=1]). All AP patients were recruited at the university hospital Magdeburg, Saxony-Anhalt, between 1996 and 2003. Acute pancreatitis was defined as acute abdominal pain with a typical clinical picture (severe abdominal pain/vomiting) and a serum amylase or lipase concentration of at least three times above the upper limit of normal and typical findings on imaging studies. Severity of AP was classified following the Atlanta symposium in 1992 [15]. In accordance with this classification system, an attack was classified as mild (grade 1) if associated with minimal organ dysfunction and an uneventful recovery. An attack was classified as severe if associated with local complications (grade 2) or systemic complications (grade 3). Twenty-nine out of 192 (15%) patients showed mild (grade 1), 103/192 (54%) grade 2, and 60/192 (31%) grade 3 AP. Because the university hospital in Magdeburg represents a tertiary care centre for pancreatic surgery, the AP patients were selected for severe pancreatitis.

We also included 483 patients with pancreatic ductal adenocarcinoma (PC) of German [*n*=382; all recruited between 1996 and 2003 in the university hospitals in Berlin (*n*=89), Leipzig, Saxony (*n*=12) and Magdeburg, Saxony-Anhalt (*n*=281)], Swiss (*n*=35) and Spanish (*n*=66) origin in this study (Table 1). The diagnosis of pancreatic adenocarcinoma was either confirmed histologically or classified as exocrine pancreas cancer reviewed by a panel of experts [16].

Additionally, 79 German patients with other types of pancreatic neoplasms were enrolled in the study (15 serous cystadenoma, two mucinous cystadenoma, ten neuroendocrine carcinoma, 24 carcinoma of papilla Vateri, 12 adenoma of papilla Vateri, five intraductal papillary

mucinous carcinoma, six insulinoma, one pancreatic lymphangioma, two cystic adenocarcinoma, two solid-pseudopapillary tumour). The patients were recruited in the university hospital of Magdeburg, Saxony-Anhalt, between 1996 and 2003. Fifty patients were women and 29 men; the mean age±SD was 59.7±12.2.

Altogether 5,726 control subjects were recruited from each of the above investigated populations, as well as from five additional countries (Tables 2 and 3).

The 1,532 German control subjects consisted of 143 blood donors originating from Leipzig, Saxony, recruited in 2000 (n=50) and Münster, Westphalia, recruited in 2001 (n=93); 220 medical students and staff recruited in Berlin between 1996 and 1998 (n=149) and in Magdeburg, Saxony-Anhalt between 1998 and 2003 (n=71); 975 parents of healthy newborns recruited between 1990 and 1992 in Berlin (n=393), Düsseldorf, North Rhine-Westphalia (n=62), Freiburg, Baden-Wuerttemberg (n=216), Mainz, Rhineland-Palatinate (n=143), and Munich, Bavaria (n=161); 164 healthy individuals recruited in Berlin for the Berlin Aging Study (BASE) between 1993 and 1995, and 30 individuals with alcoholism without pancreatic diseases recruited in Magdeburg (Saxony-Anhalt) in 2000; 560 of these control subjects were analysed in a previous study [17].

The other controls originated from Austria (n=405; all recruited for genetic population studies), the Czech Republic [n=486; 235 healthy newborns (cord blood), 251 healthy sperm and oocyte donors], Great Britain (n=200; all recruited for genetic population studies), India (n=67; recruited for genetic association studies), Italy (n=254; individuals presenting for paternity testing), the Netherlands (n=262; recruited for genetic association studies by advertisement in a newspaper), Romania (n=150; all hospital-based controls admitted for non-pancreatic diseases), Spain (n=539; recruited for genetic association or population studies), and Switzerland (n=339; all blood donors), and Turkey and Northern Cyprus (n=346; students and medical staff).

To determine the frequency of *KRT8* variants in non-Caucasians, we studied 951 individuals of African descent originating from Benin (n=185), Cameroon (n=384), Ethiopia (n=153), and Ecuador (n=229) and 195 Native Americans originating from Ecuador (Table 3). All non-Caucasian samples were recruited for genetic population studies between 1986 and 1990 (samples originating from Cameroon) and 1990–1998 (samples originating from Benin, Ethiopia and Ecuador).

PCR

Genomic DNA was extracted from peripheral blood leukocytes according to standard protocols. Primers flanking exon 1 of the *KRT8* gene and fluorescence resonance energy transfer (FRET) probes were synthesised based on the published nucleotide sequence (GenBank #M34482). Primer sequences used for PCR were: 5′-CGCTCCTTCTAG GATCTCCG-3′ and 5′-GGCACAGTCAGCCACGCAG-3′. We performed PCR using 0.75 U AmpliTaq Gold (Applied Biosystems), 400 μM dNTPs, 1.5 mM $MgCl_2$ and 0.1 μM of each primer in a final volume of 25 μl. The reaction mix was denatured at 95°C for 12 min followed by 48 cycles of denaturation at 95°C for 20 s, annealing at 56°C for 40 s, elongation at 72°C for 90 s, and a final extension step for 2 min at 72°C in a thermocycler.

Table 2 Frequency of G62C and Y54H in patients and controls of different European and Western Asian populations

	G62C						Y54H
Country	ACP	ICP/HP	AP	PC 1	PC 2	Controls	Control[a]
Austria		0/16 (0.0)				9/405 (2.2)	0/405 (0.0)
Czech Republic	0/35 (0.0)	2/55 (3.6)				4/486 (0.8)	1/486 (0.2)
Germany	2/256 (0.8)	12/789 (1.5)	0/192 (0.0)	11/382 (2.9)	2/79 (2.5)	23/1532 (1.5)	1/1532 (0.1)
Great Britain	0/25 (0.0)	0/11 (0.0)				7/200 (3.5)	0/200 (0.0)
Italy		1/143 (0.7)				2/254 (0.8)	1/254 (0.4)
Netherlands	1/71 (1.4)	1/57 (1.8)				10/262 (3.8)	0/262 (0.0)
Romania		0/3 (0.0)				1/150 (0.7)	0/150 (0.0)
Spain	0/62 (0.0)	0/5 (0.0)		1/66 (1.5)		2/539 (0.4)	0/539 (0.0)
Switzerland	0/59 (0.0)	1/35 (2.9)		1/35 (2.9)		5/339 (1.5)	0/339 (0.0)
Turkey						2/346 (0.6)	0/346 (0.0)
India		0/60 (0.0)				0/67 (0.0)	0/67 (0.0)

ACP Alcoholic Chronic Pancreatitis, *ICP/HP* Idiopathic Chronic Pancreatitis/Hereditary Chronic Pancreatitis, *AP* Acute Pancreatitis, *PC* Pancreatic Cancer, *PC 1* adenocarcinoma, *PC 2* rare types of pancreatic cancer

[a] The Y54H variation was not detected in any patient

Table 3 Distribution of the G62C and Y54H variants in African and Ecuadorian populations

Country	*n*	Gender	Variation	
		Female/male	G62C	Y54H
Benin	185	58/127	0/185 (0.0)	6/185 (3.2)
Cameroon	384	283/101	0/384 (0.0)	10/384 (2.6)
Ethiopia	153	78/75	2/153 (1.3)	1/153 (0.7)
Ecuador				
African Ecuadorians	229	108/121	0/229 (0.0)	17/229 (7.4)
Native Americans	195	104/91	0/195 (0.0)	0/195 (0.0)

Melting curve analysis

The detection of the mutant alleles was carried out by melting curve analysis with FRET probes in the Light-Cycler instrument (Roche Diagnostics). The probes were designed complementary to the mutant allele of both codons. For detection of the Y54H alteration the sequence of the sensor-fluoresceine-labelled probe was 5′-CCCCAC CATGGCCGCC-FL and that of the anchor LC Red 705 labelled probe was 5′-LC 705-CCCAGGCCACCGCG AAAGTTGC-ph. For identification of G62C the sensor probe 5′-LC 640-GTGATGCATCCCATGCCGCT-ph and the anchor probe 5′-TCAGCAGGCTCTGGTTGACCGTA ACTGC-FL were used. All FRET probes were designed and synthesised by TIB MOLBIOL, Berlin, Germany. We numbered the mutations according to the recommendations of the Nomenclature Working Group for human gene mutations [18].

Statistical analysis

Statistical analysis was carried out using Chi-square test and Fisher's exact test. *P*-values less than 0.05 were considered to be statistically significant. The SPSS software version 11.0 for Windows (Chicago, USA) was used to perform statistical analyses.

Results

The *KRT8* genotyping results are presented in Tables 2 and 3. No statistical differences in the distribution of the *KRT8* genotypes among the different patient and control groups were observed.

G62C variation

The frequency of the G62C variation varied in control subjects of European populations from 0.4 to 3.8% and displayed a northwest to southeast decline (Fig. 1).

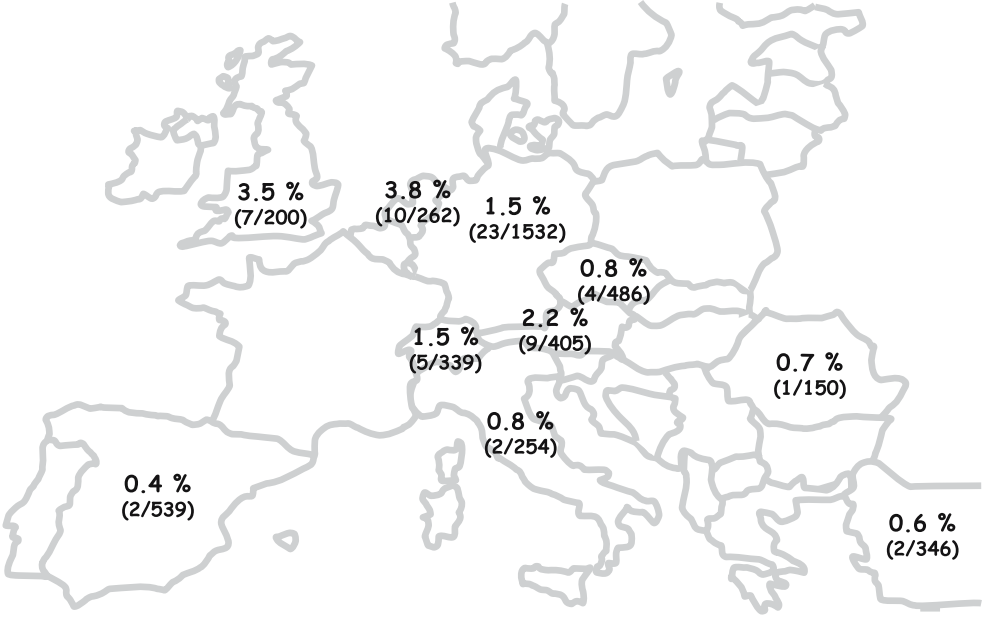

Fig. 1 Geographical distribution of the G62C *KRT8* variant in Europe showing a northwest to southeast decline in the different control populations. Absolute numbers of mutated and analysed subjects are given in parentheses

In alcoholic CP, we found the G62C alteration in 2/256 German (0.8%) and in 1/71 (1.4%) Dutch patients, but in none of the 181 patients originating from the Czech Republic, Great Britain, Spain or Switzerland. The frequency of G62C in alcoholic CP was lower than in the corresponding controls of all studied six populations (Table 2).

The distribution of the G62C alteration did also not differ significantly between patients with idiopathic, hereditary or tropical CP and controls from the respective country.

The G62C variant was detected in 11/382 German (2.9%), in 1/66 (1.5%) Spanish, and in 1/35 (2.9%) Swiss patients with pancreatic adenocarcinoma. None of these G62C frequencies was statistically different from the respective control cohort in each country.

None of the 192 German patients suffering from acute pancreatitis and only 2/79 (2.5%) German patients with rare types of pancreatic cancer (a patient with pancreatic lymphangioma and a patient with a serous cystadenoma) were found to carry a G62C alteration.

Moreover, G62C can be detected in Europeans and Western Asians of various ethnicity, whereas is it rare in individuals of African origin (100/6889 Europeans and Western Asians vs 2/951 individuals of African origin; P=0.00035).

Y54H variation

The Y54H alteration was not detected in any of the 2,436 European or Indian patients with pancreatic diseases. Only three out of 4,580 control subjects (0.07%) originating from Europe, Turkey or India were heterozygous for Y54H. One heterozygous control originated from the Czech Republic, one from Germany and one from Italy (Table 2). None of these three subjects showed evidence for an African descent. In contrast, we found a considerable higher prevalence of Y54H in control subjects originating from Africa (3/7016 non-Africans vs 34/951 individuals of African origin; $P<10^{-10}$). We also detected Y54H frequently in African Americans, but not in Native Americans from Ecuador (Table 3).

No patient or control subject was homozygous or compound heterozygous for these two variations.

Discussion

This study, which examined more than 2,400 patients with pancreatic diseases and almost 5,700 control subjects, fails to establish an association between *KRT8* alterations and acute or chronic pancreatitis of various aetiologies or pancreatic cancer. This contrasts with another study that suggested that the *KRT8* G62C variation is overrepresented in CP [14]. The discrepancies between our data and the previous report might be explained by the differences in the number of patients and controls investigated. Cavestro et al. investigated only 67 patients and 100 control subjects. It is likely that this study is under-powered and that the positive results are due to a type II error. The different results highlight the importance of investigating adequately large patient and control cohorts in case of genetic association studies.

The Italian population we investigated differs from the cohort studied by Cavestro et al. with respect to the aetiology of CP (Table 4). Whereas Cavestro et al. included patients from northern Italy with CP of various aetiology, we analysed northern Italian patients with ICP/HP only. Noteworthy is also the high frequency of autoimmune pancreatitis (9%) in the previous report. Direct comparison of both studies is hampered by the fact that 5/67 (7.5%) of the CP patients published earlier were classified as having *CFTR*–related disease [14]. It is unclear whether these patients would be classified as having idiopathic or alcoholic CP without knowledge of their *CFTR* status. Unfortunately, we did not investigate *CFTR* in our study population. Furthermore, a clear information about the definitions of the subgroups was not provided: neither the term “obstructive” pancreatitis nor the amount of alcohol consumption per day in the ACP group was specified. With the exception of one patient suffering most probably from alcoholic CP, it is impossible to draw any conclusion about the aetiology of CP in the *KRT8*-mutated patients described by Cavestro et al., which makes a comparison even more difficult.

A recent study also investigated the *KRT8* Y54H and G62C variants in pancreatitis patients originating from the United States and detected G62C in 5/198 (2.5%) unrelated ICP/HP patients, in none of the 61 ACP patients, and in 2/271 (0.7%) control subjects [19]. Patients with a family history of CP who did not carry a *PRSS1* mutation, showed with 3.8% actually a higher G62C frequency. However, due

Table 4 Aetiology of chronic pancreatitis in the population investigated by Cavestro et al. and in the current study

	Cavestro et al.	Italy	Germany
ACP	43.2%	0%	24.7%
ICP/HP	29.9%	100%	75.3%
obstructive CP	10.4%	0%	0%
autoimmune CP	9.0%	0%	0%
CFTR-associated CP	7.5%	NA	NA

ACP Alcoholic chronic pancreatitis
ICP/HP Idiopathic chronic or hereditary pancreatitis

to the low sample size in the patient groups, these differences did not reach significance (P=0.12) [19].

We detected Y54H only in three of 6,889 European and Western Asian and in none of the 127 Indian individuals, but in 34/951 (3.6%) control subjects of African descent. In the population studied by Ku et al., three out of five patients and the single positive control subject carrying the Y54H alteration were interestingly all African Americans [13]. These observations may imply that Y54H represents a frequent variation among individuals of African origin, and may suggest a founder effect. Indeed, this founder effect became particularly obvious in our population from Ecuador where 7.4% of those individuals originating from Africa carried the Y54H variation, whereas this alteration was not found in subjects descendent from Native inhabitants of Ecuador.

The relatively small number of patients in the groups other than those from Germany or Italy rises the question of sufficient study power in these cohorts. However, it is rather unlikely that a study with a larger sample size might yield different results. We do not believe that the same genetic variant, which can be found in all European populations, represents a risk factor for pancreatitis in one European ethnicity but not in another one.

We investigated two relatively frequent *KRT8* variations and the absence of an association in our study population does not completely rule out an involvement of KRT8 in pancreatic disorders. Keratins fulfil a cytoprotective function and the fact that KRT8 and KRT18 are the only cytokeratins expressed in the acinar compartment of the pancreas would suggest an organ specific protective role [10, 20]. Additionally, functional studies on mutated KRT8 indicated a loss of function of these alterations. In transfection experiments, Ku et al. showed that NIH-3T3 cells transfected with the G62C- or Y54H-mutated KRT8 were less resistant to several cell-stress inducing agents known to cause reorganisation of keratins compared to those expressing the wild type KRT8 [12]. However, it is not clear if these in vitro observations also match the in vivo situation or if they are only applicable to the given experimental settings.

Indeed, keratin null animal models tell a different story. *Krt8* null mice showed, depending on the genetic background, mid-gestational lethality with intrahepatic bleeding or colorectal hyperplasia and inflammation [21, 22]. Despite the fact that pancreatic acinar cells and hepatocytes of these mice lack keratin cytoplasmic proteins, pancreata of *Krt8* null mice are equally susceptible to injury as wild-type mice when tested current models to induce pancreatitis [23]. The chief experimental evidence in favour of a role of KRT8 stems from data elicited from *KRT8* transgenic mice [11]. However, it is unclear whether these findings are directly related to KRT8 function or whether they are indirectly related to variables such as high expression levels of the transgene protein and/or differences in human vs mouse KRT8 structure. In support of the hypothesis that over-expression of keratins may cause detrimental effects on pancreatic cells is the observation that mice with a transgene construct containing *KRT1* under the insulin promoter develop diabetes [24].

Although we investigated two of the most frequent genetic variations of *KRT8* in our study, we cannot exclude that further *KRT8*mutations or *KRT18* mutations might be involved in the pathogenesis of pancreatic diseases.

In this study, we did not examine rigorously our patients for the prevalence of liver disease. Several studies investigated *KRT8* variations in various liver diseases, which led to controversial results [13, 25, 26]. One American study [13] described an association of *KRT8*polymorphisms and liver cirrhosis of various origin. However, two European studies, one of them using a much larger sample size, failed to confirm an association [25, 26]. Taking into account that the association between *KRT8* variants and liver disease was not confirmed in these two studies together with the fact that idiopathic CP is not associated with liver disease and that most individuals with alcohol abuse develop either pancreatic or liver disease but rarely both, we do not believe that a concomitant liver disease biased our study results.

In summary, our data indicate that carriers of the two *KRT8* alterations, Y54H and G62C, are not at increased risk for developing pancreatitis or pancreatic cancer. Furthermore, G62C can be detected in healthy Europeans of various ethnicity, but is rare in individuals of African origin. In contrast, Y54H occurs mainly among people of African descent. Both sequence variations may, therefore, rather represent non-pathogenic genetic alterations than disease-causing mutations.

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