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Original Article

Analysis of *GPRC6A* variants in different pancreatitis etiologies

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ABSTRACT

Background: The G-protein-coupled receptor Class C Group 6 Member A (GPRC6A) is activated by multiple ligands and is important for the regulation of calcium homeostasis. Extracellular calcium is capable to increase NLRP3 inflammasome activity of the innate immune system and deletion of this proinflammatory pathway mitigated pancreatitis severity *in vivo*. As such this pathway and the GPRC6A receptor is a reasonable candidate gene for pancreatitis. Here we investigated the prevalence of sequence variants in the *GPRC6A* locus in different pancreatitis aetiologies.
Methods: We selected 6 tagging SNPs with the SNPinfo LD TAG SNP Selection tool and the functional relevant SNP *rs6907580* for genotyping. Cohorts from Germany, further European countries and China with up to 1,124 patients and 1,999 controls were screened for single SNPs with melting curve analysis.

Abbreviations: ACP, alcoholic chronic pancreatitis; AP, acute pancreatitis; CASR, Calcium-sensing receptor; CI, confidence interval; CP, chronic pancreatitis; GPRC6A, G-protein-coupled receptor Class C Group 6 Member A; HWE, Hardy-Weinberg-disequilibrium; NACP, non-alcoholic chronic pancreatitis; NLRP3, NLR Family Pyrin Domain Containing 3; OR, odds ratio; PCR, polymerase chain reaction; SNP, single nucleotide polymorphism.

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Results: We identified an association of *rs1606365(G)* with alcoholic chronic pancreatitis in a German (odds ratio (OR) 0.76, 95% confidence interval (CI) 0.65–0.89, $p = 8 \times 10^{-5}$) and a Chinese cohort (OR 0.78, 95% CI 0.64–0.96, $p = 0.02$). However, this association was not replicated in a combined cohort of European patients (OR 1.18, 95% CI 0.99–1.41, $p = 0.07$). Finally, no association was found with acute and non-alcoholic chronic pancreatitis.
Conclusions: Our results support a potential role of calcium sensing receptors and inflammasome activation in alcoholic chronic pancreatitis development. As the functional consequence of the associated variant is unclear, further investigations might elucidate the relevant mechanisms.

Introduction

Inflammatory pancreatic diseases are one of the leading causes for hospital admissions of gastroenterological diseases [1]. In patients with acute (AP) and chronic pancreatitis (CP) alcohol abuse is the predominant aetiological factor [2]. In addition, several genetic associations with CP have been identified, which indicated the complex pathophysiology of the disease. As summarized recently, most of the associated genes induced pancreatitis within pancreatic acinar cells by a trypsin-dependent (e.g. *PRSS1*, *SPINK1*, *CTRC*, *CTRB1-CTRB2*) or misfolding-dependent pathway (e.g. *CPA1*, *CEL*) [3–10].

On the other hand, pancreatitis risk genes such as *CFTR* [11,12], *CASR* [13], *CLDN2* [14,15] or *TRPV6* [16] could not be assigned to any of the previously mentioned mechanisms and some of these proteins are expressed in ductal and not acinar cells [17]. Furthermore, some of these associations highlight the importance of calcium homeostasis in pancreatitis, as variants in *TRPV6* and *CASR* have been associated with pancreatitis. Generally calcium ions (Ca^{2+}) are crucial for the secretory function of the pancreas and the pathological release of Ca^{2+} is derived from the endoplasmatic reticulum (ER) most likely [18]. Mechanistically, it has been demonstrated that cytosolic Ca^{2+} is responsible for premature trypsinogen activation, vacuolization and acinar cell death [19]. In addition, free extracellular Ca^{2+} acts as damage associated molecular pattern (DAMP) via the G-protein-coupled receptor Class C Group 6 Member A (*GPRC6A*) and Calcium-sensing receptor (*CASR*). Thereby, the NLRP3-inflammasome is activated and pro-inflammatory cytokines such as IL1β in murine monocytes/macrophages are secreted [20]. In a caerulein-induced acute pancreatitis model the genetic deletion of the *NLRP3* gene as well as the deletion of mediators of sterile inflammation like *CASP1, TLR9, ASC, P2RX7* ameliorated the phenotype with reduction of oedema and inflammation [21]. In addition, a recent study showed a protective role of the genetic *NLRP3 knockout* in a severe acute pancreatitis model with duct-ligation [22]. Otherwise, allosteric modulators of CASR and GPRC6a affected on the death of isolated acini that was induced by basic amino acids *in vitro* [23]. Taken together these data indicated the importance of this pathway for pancreatitis development and implied the need for further investigations.

As part of this pathway GPRC6A is activated by multiple ligands (Osteocalcin, Testosterone, basic amino acids) and various cations (Ca^{2+}, Mg^{2+}, Zn^{2+}, or Al^{3+}) [24]. Moreover, GPRC6A impacts on complex endocrine networks and metabolic processes including glucose metabolism and progression of prostate cancer [25]. Thus far, the influence of GPRC6A on AP and CP has not been elucidated. Here we investigated whether single nucleotide polymorphisms of the *GPRC6A* locus are associative in AP, non-alcoholic CP (NACP) and alcoholic CP (ACP) in large European and Chinese cohorts.

Material and methods

Patients and controls

The medical ethical review committees of the Martin-Luther-University of Halle-Wittenberg (Medical ethical committee, University Halle-Wittenberg, Medical Faculty, Bearbeitungsnummer 2015-106, date: January 22, 2016, title: “Erforschung molekulargenetischer Ursachen von Pankreaserkrankungen”) and all participating study centres approved this study. All patients and blood donors gave written informed consent. AP was defined as in our recent publication [15]. The CP study cohorts comprised patients with a history of recurrent AP or recurrent or persisting abdominal pain typical for CP, pancreatic calcifications and/or pancreatic ductal irregularities indicated by computed tomography imaging, magnetic resonance imaging, endoscopic retrograde pancreaticography or (endo)sonography of the pancreas and/or the diagnosis of exocrine pancreatic insufficiency [25]. ACP was diagnosed when alcohol consumption was >80 g per day for males or >60 g per day for females for more than 2 years. Patients without known precipitating factors were classified as NACP. The characteristics of the different cohorts of patients and controls are summarized in Table 1.

Selection of tagging SNPs in the GPRC6A locus

We selected six tagging SNPs using the SNPinfo LD TAG SNP Selection tool (linkage disequilibrium (LD) map Supplementary Fig. 1) to cover the whole *GPRC6A* locus. Tagging SNPs were determined with an LD threshold (R^2) of 0.8, a minimum of 5 valid genotypes to calculate LD in populations with European ancestry (CEU) and we extended the region of interest by 5.000 bp in the 5′-region and the 3′-region.

Additionally the SNP *rs6907580* encoding a stop codon (p.Arg57*) was analysed [26]. The functionally relevant SNP *rs2274911* [27–30] was tagged by *rs1512655*. A description of the cohorts analysed for the distinct SNPs is depicted in Fig. 1.

DNA extraction and SNP genotyping

DNA was isolated from EDTA blood using a commercial system (QIAamp Blood DNA Mini Kit; Qiagen, Hilden, Germany). Polymerase chain reaction (PCR) was performed with the following cycle conditions in a thermal cycler (initial denaturation at 95 °C for 5 min, followed by 45 cycles of 20 s denaturation at 95 °C, 40 s annealing (Supplementary Table 1), 90 s primer extension at 72 °C followed by final extension for 5 min at 72 °C). PCR was conducted using OneTaq® 2X Master Mix (NEB) with 200 μM dNTPs, 1.8 mM $MgCl_2$ and 0.1 μM forward primer as well as 0.1 μM reverse primer in a total volume of 25 μl. For SNP *rs7766085* (0.2 μM forward

Table 1
Description of the cohorts analysed.

Cohort type	No.	Age (mean)	Age (median)	Age range	Male sex
Screening cohorts					
Controls[a]	336	63.9	63.5	60–70	50.0%
AP[a]	59	47.3	48	17–79	30.5%
ACP[a]	152	49.3	50	21–79	88.8%
NACP[a]	166	40.6	42	7–77	56.6%
Controls[b]	258	37.1	37	18–65	61.2%
AP[b]	175	56.0	58	19–93	43.6%
Extended cohort from Germany					
Controls	1,999	63.8	63	60–70	50.5%
ACP	1,124	49.5	49	21–85	86.6%
European replication cohort (Italy, France, Poland, Romania, Hungary, The Netherlands)					
Controls	1,671	53.0	52	18–79	55.6%
ACP	780	41.0	40	20–98	81.6%
Controls China	504	41.0	41	18–62	66.9%
ACP China	303	50.4	51	9–80	98.3%
NACP China	363	43.6	45	5–91	64.5%

[a] Cohorts from Germany.
[b] Cohorts from Hungary; age in years. Abbreviations: AP = acute pancreatitis, ACP = alcoholic chronic pancreatitis, NACP = non alcoholic chronic pancreatitis, No. = number.

primer) and *rs1398404* (0.2 μM reverse primer) asymmetric PCRs were performed.

Primers and probes (Supplementary Table 1) were synthesized by TIB Molbiol (Berlin, Germany). Genotyping was performed using the LightCycler480® system (Roche Diagnostics). For genotyping we used the PCR products from standard PCR (see above) with 50 nM (final) of probe oligomers followed by melting curve analysis with the following protocol: 95 °C for 60 s, 40 °C for 60 s, continuous increase to 70 °C with a ramp rate of 0.19 °C/s.

Call rates for all SNPs were >95%. For quality control 1.8% of all samples were genotyped in duplicates blinded to the investigator. Resulting concordance rate was 99%.

Statistics

P-values were computed using GraphPad Prism 5 and IBM SPSS Statistics 25. The significance of differences of genotype frequencies between patients and healthy controls and all other models (recessive, dominant, allele frequencies) were calculated with Chi-square test and two-tailed Fisher's Exact test, respectively. A p-value of less than 7×10^{-3} was considered to be significant in the screening cohorts (significance level after Bonferroni correction accounting for seven tests). In case of a significant or nominal significant association of the polymorphisms in the initial screening cohort of ACP patients and controls from Germany, an extended sample of German patients was analysed. Other European cohorts and a Chinese replication cohort were screened for replication. The quality of SNP genotypes was checked by study-wise call rate and test for Hardy-Weinberg equilibrium (HWE) in patients and controls.

Results

No association with AP or NACP

In the screening cohort of AP patients from Germany and Hungary we did not identify any significant genetic association (Supplementary Table 2). Furthermore, we did not find any differences in the genotype distribution in the German NACP screening cohort compared to the controls (Supplementary Table 3) or in

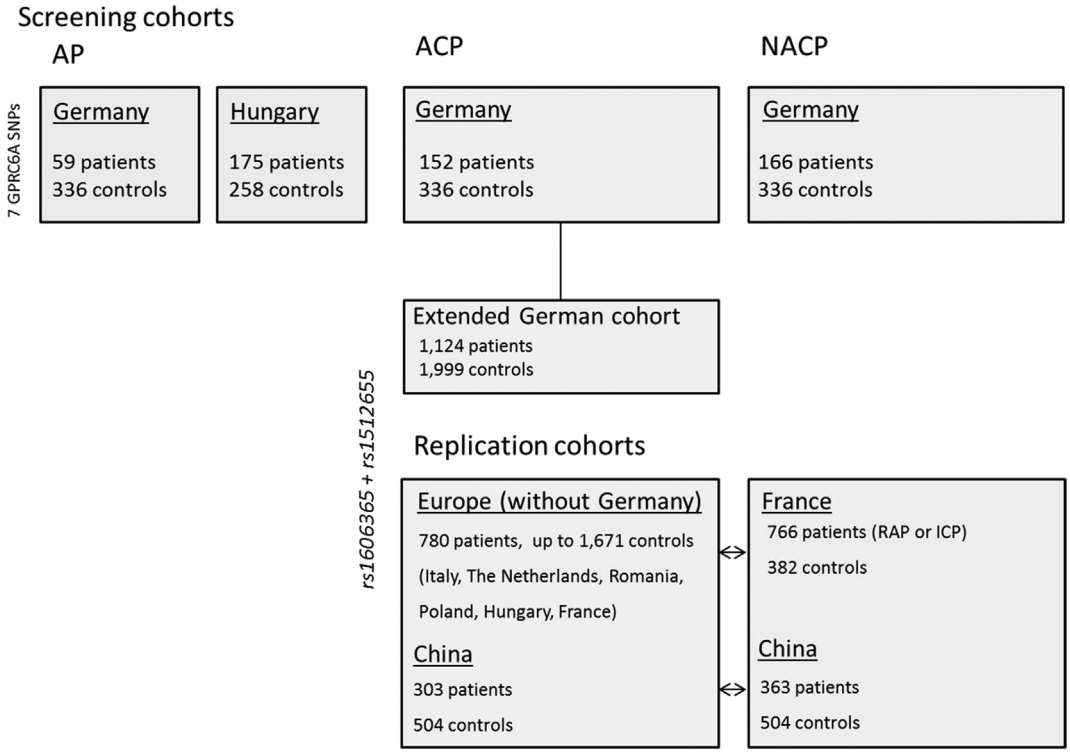

Fig. 1. Flowchart of samples included in this study.
Extended German cohort = Screening cohort + additional patients and controls. Abbreviations: AP = acute pancreatitis, ACP = alcoholic chronic pancreatitis, NACP = non alcoholic chronic pancreatitis.

further models (allele, recessive or dominant) of our analysis. No deviation of the Hardy-Weinberg equilibrium (HWE) was found for all SNPs.

SNPs rs1606365 and rs1512655 as potential risk factors in ACP

In the screening cohort the tagging SNPs *rs1606365* and *rs1512655* displayed a nominal or borderline significant difference in genotype distribution in ACP patients compared to healthy controls before Bonferroni correction (Table 2 and Supplementary Table 4, p = 0.057 and p = 0.049, respectively). Therefore we extended the German ACP cohort for both SNPs (n = 1,124) as well as the control cohort (n = 1,999). Here, the significant deviation of genotype distribution for *rs1606365* was confirmed (Supplementary Table 5, p = 0.001). This association was additionally demonstrated in the other statistical models (C/G: p = 0.0008, OR 0.765, 95% CI 0.653–0.894; dominant model CC/CG + GG: p = 0.0003, OR 0.724, 95% CI 0.610–0.861) (Table 3). However, in the summarized European populations (without Germany) we were not able to confirm this association. For further validation we screened a Chinese cohort and confirmed the association (C/G: p = 0.019; OR 0.782; 95% CI 0.637–0.961) (Table 3 and Supplementary Table 5). In addition, we investigated the two SNPs in NACP in additional cohorts from France and China. Here, again no associations were seen for both SNPs in NACP.

The different models comprise (order from top to bottom), allele frequencies, the recessive and the dominant model for computations. The number of patients and the genotype distribution of each variant are summarized in Supplementary Table 4. For all calculations the Fisher's exact test was used. Abbreviations: OR = odds ratio, 95% CI = 95% confidence interval.

The different models comprise (order from top to bottom), allele frequencies, the recessive and the dominant model for computations. The number of patients and the genotype distribution of each variant are summarized in Supplementary Table 5. For all calculations the Fisher's exact test was used. Abbreviations: OR = odds ratio, 95% CI = 95% confidence interval.

Discussion

Several studies demonstrated the importance of the widely expressed GPRC6A receptor in distinct diseases and inflammatory processes [24]. On macrophages, the receptor can bind several ligands and free extracellular Ca^{2+} that act as DAMP to amplify proinflammatory signalling by inflammasome activation [20]. Deletion of the inflammasome response *in vivo* ameliorated pancreatitis severity and highlighted the potential importance of this pathway and the GPRC6A receptor for inflammatory pancreatic diseases [21,22].

In the present study, we investigated *GPRC6A* SNPs in pancreatitis and identified a putative association of *rs1606365* with ACP in German and Chinese patients, whereas no association was found in AP or in NACP. Our analysis revealed that the *G* allele of this intronic variant reduces ACP risk (OR 0.765, 95% CI 0.653–0.894), but this association was not replicated in cohorts from all over Europe. One of the reasons for this observation might be the smaller sample size of the individual European cohorts that ranged from 55 to 291 patients resulting in an insufficient power in the cohorts derived from different countries (<0.80). Otherwise, as in the overall European cohort no association was found there might be differences in genotype frequencies of this variant throughout Europe as also demonstrated in the Chinese cohort. We observed different allele frequencies for *rs1606365* in the European and Chinese cohorts, which might be explained by the evolutionary background of these populations. However, we are not capable to fully clarify this issue with our data.

Interestingly, the *rs1606365 G* allele has recently been described to increase risk of aggressiveness in prostate cancer in Northern Chinese men indicating a functional relevance [28]. This observation, however, requires replication and finally functional studies are warranted to understand the disease causing mechanisms in prostate cancer and additionally in ACP. So far there are no data of the clinical significance of *rs1606365* available in public databases.

In addition, the SNP *rs1512655* was associated with ACP in our extended German cohort, but this finding was not confirmed in the European or the Chinese cohort. Here, we observed a similar trend

Table 2
Data of the analysed SNPs in the German alcoholic chronic pancreatitis screening cohort and controls calculated with different genetic models.

SNP/Genetic model for calculation		*p*-value	*OR*	*95% CI*
rs6919622 (T)	C/T	0.44	1.122	0.835–1.507
	CC + CT/TT	0.61	1.215	0.649–2.307
	CC/CT + TT	0.55	0.880	0.598–1.300
rs1606365 (G)	C/G	**0.03**	0.644	0.431–0.961
	CC + CG/GG	–	–	–
	CC/CG + GG	**0.05**	0.641	0.414–0.993
rs1512655 (A)	A/G	0.64	1.081	0.797–1.467
	AA + AG/GG	0.62	0.902	0.613–1.329
	AA/AG + GG	**0.05**	2.011	1.038–3.897
rs6907580 (A)	G/A	0.67	1.116	0.638–1.926
	GG + GA/AA	–	–	–
	GG/GA + AA	0.66	1.157	0.670–2.025
rs7766085 (C)	C/G	1.00	0.995	0.705–1.406
	CC + CG/GG	1.00	0.978	0.653–1.466
	CC/CG + GG	0.80	1.103	0.406–2.999
rs1398404 (C)	A/C	0.57	0.923	0.697–1.433
	AA + AC/CC	0.80	0.917	0.561–1.502
	AA/AC + CC	0.59	0.881	0.584–1.342
rs11153632 (G)	A/G	0.47	0.727	0.337–1.508
	AA + AG/GG	–	–	–
	AA/AG + GG	0.46	0.718	0.326–1.534

Table 3
Data of the SNPs *rs1606365* and *rs1512655* in the extended German, the independent European and the Chinese ACP replication cohorts calculated with different genetic models.

SNP/Genetic model for calculation		*p*-value	*OR*	*95% CI*
rs1606365 (G)				
Germany (extended)	C/G	**0.0008**	0.765	0.653–0.894
	CC + CG/GG	0.89	0.911	0.509–1.596
	CC/CG + GG	**0.0003**	0.724	0.610–0.861
Europe (without Germany)	C/G	0.07	1.182	0.991–1.409
	CC + CG/GG	0.44	1.268	0.691–2.302
	CC/CG + GG	0.07	1.203	0.989–1.467
China	C/G	**0.019**	0.782	0.637–0.961
	CC + CG/GG	0.21	1.281	0.867–1.890
	CC/CG + GG	**0.012**	0.679	0.505–0.916
rs1512655 (A)				
Germany (extended)	A/G	**0.02**	1.152	1.026–1.294
	AA + AG/GG	0.17	1.110	0.957–1.287
	AA/AG + GG	**0.004**	1.482	1.142–1.922
Europe (without Germany)	A/G	0.42	1.060	0.925–1.217
	AA + AG/GG	0.76	1.029	0.867–1.221
	AA/AG + GG	0.16	1.269	0.914–1.765
China	A/G	0.35	1.102	0.901–1.347
	AA + AG/GG	0.42	1.148	0.839–1.571
	AA/AG + GG	0.54	1.124	0.805–1.571

for the association in the patients from The Netherlands only ($p = 0.07$). The variant *rs1512655* is in perfect linkage disequilibrium with *rs2274911* (p.Pro91Ser) located in the so called Venus flytrap of the protein and this variant was associated with increased insulin resistance [30], as a risk factor of prostate cancer [28] and with testis failure [29]. Again, the functional consequences of this SNP in human disorders remain unclear and indicate the need of further studies. Finally, to exclude an association of the two SNPs with NACP we screened cohorts from China and France and again found no association for both SNPs (Data not shown).

A limitation of our study is the small sample size in the individual European ACP replication cohorts. Here, a significant association in distinct cohorts may have been missed, whereas the power of the combined analysis of all European cohorts that yielded a negative result seems reliable. As we have gathered the thus far largest European ACP cohort, we are not able to extend our analysis.

In conclusion, we have demonstrated association of the intronic variant *rs1606365* with ACP in German patients. Although we replicated the finding in an independent Chinese cohort we cannot present data on the functional consequences of the associated variant. As GPRC6A is involved in several processes it remains unclear whether the association truly is pancreatitis related and as such warrants further replication.

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Author contributions

T.K. and J.R. conceived, designed and directed the study.

T.K., C.R., E.M., J.M.C., W.-B.Z., S.-J.D., and Z.L. performed genotyping.

T.K. and J.R. drafted and revised the manuscript.

T.K. and C.R. designed, performed and interpreted genetic analyses.

All other co-authors recruited study subjects, collected clinical data and/or provided genomic DNA samples. All authors approved the final manuscript and contributed critical revisions to its intellectual content.

Declaration of competing interest

The authors report no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.pan.2020.08.001.

References

[1] Yadav D, Lowenfels AB. The epidemiology of pancreatitis and pancreatic cancer. Gastroenterology 2013;144(6):1252–61. https://doi.org/10.1053/j.gastro.2013.01.068.
[2] Herreros-Villanueva M, Hijona E, Bañales JM, Cosme A, Bujanda L. Alcohol consumption on pancreatic diseases. World J Gastroenterol 2013;19(5):638–47.
[3] Hegyi E, Sahin-Tóth M. Genetic risk in chronic pancreatitis: the trypsin-

dependent pathway. Dig Dis Sci 2017;62(7):1692–701.
[4] Whitcomb DC, Gorry MC, Preston RA, Furey W, Sossenheimer MJ, Ulrich CD, et al. Hereditary pancreatitis is caused by a mutation in the cationic trypsinogen gene. Nat Genet 1996;14(2):141–5.
[5] Witt H, Luck W, Hennies HC, Classen M, Kage A, Lass U, et al. Mutations in the gene encoding the serine protease inhibitor, Kazal type 1 are associated with chronic pancreatitis. Nat Genet 2000;25(2):213–6.
[6] Rosendahl J, Witt H, Szmola R, Bhatia E, Ozsvári B, Landt O, et al. Chymotrypsin C (CTRC) variants that diminish activity or secretion are associated with chronic pancreatitis. Nat Genet 2008;40(1):78–82.
[7] Rosendahl J, Kirsten H, Hegyi E, Kovacs P, Weiss FU, Laumen H, et al. Genome-wide association study identifies inversion in the CTRB1-CTRB2 locus to modify risk for alcoholic and non-alcoholic chronic pancreatitis. Gut 2018;67(10):1855–63.
[8] Sahin-Tóth M. Genetic risk in chronic pancreatitis: the misfolding-dependent pathway. Curr Opin Gastroenterol 2017;33(5):390–5.
[9] Witt H, Beer S, Rosendahl J, Chen J-M, Chandak GR, Masamune A, et al. Variants in CPA1 are strongly associated with early onset chronic pancreatitis. Nat Genet 2013;45(10):1216–20.
[10] Fjeld K, Weiss FU, Lasher D, Rosendahl J, Chen J-M, Johansson BB, et al. A recombined allele of the lipase gene CEL and its pseudogene CELP confers susceptibility to chronic pancreatitis. Nat Genet 2015;47(5):518–22.
[11] Cohn JA, Friedman KJ, Noone PG, Knowles MR, Silverman LM, Jowell PS. Relation between mutations of the cystic fibrosis gene and idiopathic pancreatitis. N Engl J Med 1998;339(10):653–8.
[12] Sharer N, Schwarz M, Malone G, Howarth A, Painter J, Super M, et al. Mutations of the cystic fibrosis gene in patients with chronic pancreatitis. N Engl J Med 1998;339(10):645–52.
[13] Felderbauer P, Hoffmann P, Einwächter H, Bulut K, Ansorge N, Schmitz F, et al. A novel mutation of the calcium sensing receptor gene is associated with chronic pancreatitis in a family with heterozygous SPINK1 mutations. BMC Gastroenterol 2003 Nov 29;3:34.
[14] Whitcomb DC, LaRusch J, Krasinskas AM, Klei L, Smith JP, Brand RE, et al. Common genetic variants in the CLDN2 and PRSS1-PRSS2 loci alter risk for alcohol-related and sporadic pancreatitis. Nat Genet 2012;44(12):1349–54.
[15] Derikx MH, Kovacs P, Scholz M, Masson E, Chen J-M, Ruffert C, et al. Polymorphisms at PRSS1-PRSS2 and CLDN2-MORC4 loci associate with alcoholic and non-alcoholic chronic pancreatitis in a European replication study. Gut 2015;64(9):1426–33.
[16] Masamune A, Kotani H, Sörgel FL, Chen J-M, Hamada S, Sakaguchi R, et al. Variants that affect function of calcium channel TRPV6 are associated with early-onset chronic pancreatitis. Gastroenterology 2020;158(6):1626–1641.e8. https://doi.org/10.1053/j.gastro.2020.01.005. Epub 2020 Jan 10.
[17] Mayerle J, Sendler M, Hegyi E, Beyer G, Lerch MM, Sahin-Tóth M. Genetics, cell biology, and pathophysiology of pancreatitis. Gastroenterology 2019;156(7).1951-1968.e1.
[18] Gerasimenko JV, Gerasimenko OV, Petersen OH. The role of Ca^{2+} in the pathophysiology of pancreatitis. J Physiol (Lond) 2014;592(2):269–80.
[19] Li J, Zhou R, Zhang J, Li Z-F. Calcium signaling of pancreatic acinar cells in the pathogenesis of pancreatitis. World J Gastroenterol 2014;20(43):16146–52.
[20] Rossol M, Pierer M, Raulien N, Quandt D, Meusch U, Rothe K, et al. Extracellular Ca^{2+} is a danger signal activating the NLRP3 inflammasome through G protein-coupled calcium sensing receptors. Nat Commun 2012;3:1329.
[21] Sendler M, van den Brandt C, Glaubitz J, Wilden A, Golchert J, Weiss1 FU, et al. NLRP3 inflammasome regulates development of systemic inflammatory response and compensatory anti-inflammatory response syndromes in mice with acute pancreatitis. Gastroenterology 2020;158:253–69.
[22] Zhang X, Jin T, Shi N, Yao L, Yang X, Han C, et al. Mechanisms of pancreatic injury induced by basic amino acids differ between L-Arginine, L-Ornithine, and L-Histidine. Front Physiol 1922;9.
[23] Hoque R, Sohail M, Malik A, Sarwar S, Luo Y, Shah A, et al. TLR9 and the NLRP3 inflammasome link acinar cell death with inflammation in acute pancreatitis. Gastroenterology 2011 Jul;141(1):358–69.
[24] Pi M, Nishimoto SK, Quarles LD. GPRC6A: Jack of all metabolism (or master of none). Mol Metab 2017;6(2):185–93.
[25] Löhr JM, Dominguez-Munoz E, Rosendahl J, Besselink M, Mayerle J, Lerch MM, et al. United European Gastroenterology evidence-based guidelines for the diagnosis and therapy of chronic pancreatitis (HaPanEU). United European Gastroenterol J 2017;5(2):153–99.
[26] Jørgensen S, Have CT, Underwood CR, Johansen LD, Wellendorph P, Gjesing AP, et al. Genetic variations in the human G protein-coupled receptor Class C, Group 6, member A (GPRC6A) control cell surface expression and function. J Biol Chem 2017;292(4):1524–34.
[27] Hazelett DJ, Rhie SK, Gaddis M, Yan C, Lakeland DL, Coetzee SG, et al. Comprehensive functional annotation of 77 prostate cancer risk loci. PLoS Genet 2014;10(1):e1004102.
[28] Liu M, Zhao Y-Y, Yang F, Wang J-Y, Shi X-H, Zhu X-Q, et al. Evidence for a role of GPRC6A in prostate cancer metastasis based on case-control and in vitro analyses. Eur Rev Med Pharmacol Sci 2016;20(11):2235–48.
[29] de Toni L, Di Nisio A, Speltra E, Rocca MS, Ghezzi M, Zuccarello D, et al. Polymorphism rs2274911 of GPRC6A as a novel risk factor for testis failure. J Clin Endocrinol Metab 2016;101(3):953–61.
[30] Di Nisio A, Rocca MS, Fadini GP, de Toni L, Marcuzzo G, Marescotti MC, et al. The rs2274911 polymorphism in GPRC6A gene is associated with insulin resistance in normal weight and obese subjects. Clin Endocrinol 2017;86(2):185–91.