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*HLA-DRB1*16* and *-DQB1*05* alleles are strongly associated with autoimmune pancreatitis in a cohort of hundred patients

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ABSTRACT

Background/objectives: Autoimmune diseases are often associated with human leukocyte antigen (HLA) haplotypes, indicating that changes in major histocompatibility complex (MHC)-dependent self-peptide or antigen presentation contribute to autoimmunity. In our study, we aimed to investigate HLA alleles in a large European cohort of autoimmune pancreatitis (AIP) patients.
Methods: Hundred patients with AIP, diagnosed and classified according to the International Consensus Diagnostic Criteria (ICDC), were prospectively enrolled in the study. Forty-four patients with chronic pancreatitis (CP) and 254 healthy subjects served as control groups. DNA was isolated from blood samples and two-digit HLA typing was performed with sequence-specific primer (SSP-) PCR. HLA allele association strength to AIP was calculated as odds ratio.
Results: We uncovered a strong enrichment of *HLA-DQB1* homozygosity in type 1 and type 2 AIP patients. Moreover, a significantly increased incidence of the *HLA-DRB1*16* and *HLA-DQB1*05* alleles and a concomitant lack of the *HLA-DRB1*13* allele was detected in AIP type 1 and type 2 patients. In contrast, the *HLA-DQB1*02* allele was underrepresented in the 'not otherwise specified' (NOS) AIP subtype. We detected no significant difference in the *HLA-DRB3*, *HLA-DRB4* and *HLA-DRB5* allele frequency in our cohort.
Conclusions: Although AIP type 1 and type 2 are characterized by distinct histopathological characteristics, both subtypes are associated with the same HLA alleles, indicating that the disease might rely on similar immunogenic mechanisms. However, AIP NOS represented another subclass of AIP.
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1. Introduction

Autoimmune pancreatitis (AIP) is a relatively rare and distinct form of chronic pancreatitis and comprises approximately 5% of all chronic pancreatitis (CP) cases [1,2]. The overall prevalence rate of AIP increased in the last years in the Japanese population with a current incidence of around 10 per 1,000,000 population [3,4]. Histopathological features of AIP are a dense lymphoplasmacytic

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infiltrate and fibrosis of the pancreas. Moreover, AIP patients respond dramatically to corticosteroid therapy (5). Based on the presence or absence of IgG4+ plasma cells in the inflamed pancreatic tissue, AIP is sub-classified in AIP type 1 and AIP type 2, respectively. In the histological analysis, AIP type 1 shows high $IgG4^{+}$ lymphoplasmacytic infiltrates, a storiform fibrosis and obliterative phlebitis. In contrast, AIP type 2 is described as an idiopathic duct-centric pancreatitis (IDCP) with granulocytic epithelial lesions (GEL) and very low to absent IgG4+ cell infiltrations [5–7]. Thus, AIP type 1 is the pancreatic manifestation of the IgG4-related syndrome, characterized by elevated serum IgG4 levels and other organ involvements (OOI). In case of ambiguous clinical features with inconclusive histology, AIP can also be classified as 'not otherwise specified' [8]. To facilitate the diagnosis of AIP, international consensus diagnostic criteria (ICDC) have been developed in 2011 [8]. Notably, imaging studies have shown that AIP patients exhibit a diffuse or focal parenchymal enlargement, whereby the presence of a capsule-like rim favors AIP diagnosis over pancreatic cancer [9–11]. Besides IgG4, blood serum studies of AIP patients have detected autoantibodies such as those against carbonic anhydrase [12,13], lactoferrin [14], pancreatic secretory trypsin inhibitor [15] and PBP peptide [16]. However, none of them has provided satisfactory specificity and sensitivity for diagnosis.

Although the trigger of AIP remains poorly understood, genetic correlation studies have shown that genetic predispositions facilitate AIP development. Interestingly, human leukocyte antigen (HLA) gene variants, coding for the major histocompatibility complex (MHC), are linked to many autoimmune diseases (ADs) [17]. The HLA system is responsible for the presentation of intracellular and extracellular peptides to immune cells and the regulation of innate and adaptive immune responses [18]. Particularly, HLA class II molecules are highly associated with an increased susceptibility towards different ADs and an association between *HLA-DRB1* and ADs has been reported in several studies [19–22]. The products of the HLA class II genes *-DR, -DP, and -DQ* are expressed on B-lymphocytes, antigen-presenting cells and activated T-lymphocytes, which are important mediators in the immune response [23]. Kawa S. et al. recognized for the first time in 2002 a potential genetic predisposition in AIP patients with specific HLA genotypes [24]. The authors detected that *HLA-DRB1*0405* and *-DQB1*0401* serotypes were significantly associated with AIP in the Japanese population. Due to a linkage disequilibrium of *HLA-DRB1*0405* with *-DQB1*0401*, a majority of the analyzed AIP patients carried both alleles and formed a 2-loci haplotype [24]. Addressing other gene variants, the group analyzed single nucleotide polymorphisms (SNPs) in the *TNF* and *NFKBIL1*, which were, however, not associated with an increased risk for AIP in an HLA-independent analysis [25]. Nevertheless, various SNPs in non-HLA genes, such as *CTLA4* [26,27], *FCRL3* [28] and *PRSS1* [29] have been identified in several studies showing a higher frequency in AIP patients in different Asian population studies. Interestingly, the 2-loci *DRB1*0405-DQB1*0401* haplotype, which was identified in the Japanese AIP cohort [24], showed no significant association with AIP in a Korean population cohort [30]. In our study, we aim at identifying, if HLA class II alleles are associated with an increased susceptibility for AIP in a Caucasian cohort.

2. Methods

2.1. Patient recruitment and ethics

Between 2010 and 2019, 100 AIP patients and 254 healthy controls from four referral centers (University of Verona, Italy; University of Parma, Italy; University Medicine Greifswald, Germany and Ludwig Maximilians University Hospital, Germany) were prospectively enrolled. All AIP patients were diagnosed according to the ICDC and stratified in AIP type 1, AIP type 2 and AIP "not otherwise specified" (NOS). Briefly, in the ICDC the diagnosis of AIP is categorized into a definitive or probable type 1 or type 2 AIP, respectively, which is based on a combination of features containing pancreas imaging and histology, serology, other organ involvement (OOI), and response to steroid therapy. In some cases, the classification into a subtype may not be possible and AIP is considered as AIP NOS [8]. Clinical data, such as age, sex, age of onset, serum IgG4 levels, other ADs in medical history, inflammatory bowel diseases (IBD) as co-morbidity and country of origin were recorded. The study was performed according with "The Code of ethics of the World Medical Association" (Declaration of Helsinki). All participants provided written informed consent for the experimentation with human subjects, including the blood sample collections and the genetic testing. At all four participating centers, the local ethics review boards approved the study (IRB number: 2010–01, University of Parma).

2.2. Blood sampling and HLA typing

We collected EDTA blood samples (5 ml, stored at −80 °C before DNA isolation) from 100 patients with AIP and from 254 blood donors as healthy controls. Genomic DNA was extracted and purified from whole blood using DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. Isolated DNA was stored at −20 °C until further use. Low-resolution (two-digit) DNA typing of class II *HLA-DRB1, -DRB3, -DRB4, -DRB5* and *-DQB1* alleles was performed using Micro SSP™ Generic Trays (SSPML02, Micro SSP Generic HLA Class I and II DNA Typing Tray ABDRDQ) with accompanied separation of PCR products on a 2% agarose gel or using One Lambda rSSO (Sequence Specific Oligonucleotide) typing kit; both from the company One Lambda, Canoga Park, CA, USA according to the manufacturer's protocol. After an HLA locus specific PCR amplification with labelled primers, the amplification product was hybridized to bead fixed oligonucleotides; every bead of a distinct color is assigned to an oligonucleotide of defined sequence. All reaction and washing steps were performed following the instructions of the manufacturer. A LABScan3D system, based on Luminex® xMAP® technology served as detection platform and used Luminex® xPONENT® software for data acquisition. xPONENT® raw data were imported in the One Lambda HLA Fusion software for calculating HLA typing.

2.3. Statistical analysis

Overall, our study cohort (398 subjects) consisted of 254 healthy controls (Co), 44 CP patients, 53 AIP type 1, 23 AIP type 2 and 24 AIP NOS patients. Corresponding HLA class II genotypes from 44 CP patients from a previous published cohort [31] were included in our study analyses. Considering an effect size of 1.5 (power of 0.8, alpha 0.05), the power calculation reveals a number of 93 total samples to include in the study. We included 100 AIP patients. The allele frequency calculation and the statistical analyses were performed with R (version 3.6.3, https://www.R-project.org/) and RStudio (Version 1.3.959, https://github.com/rstudio/rstudio). The characteristics of the study population are listed in Supplementary Table 1. The group variables gender, country and age were assessed with Chi-square statistics with Yates continuity correction and Student's t-test, respectively. Contingencies tables were generated for the determined allele frequency and allele zygosity. The odds ratio with a 95% confidence interval was calculated in a univariate analysis in comparison to the controls with Chi-square statistics with Yates continuity correction. A two-sided p-value of < 0.05 was considered significant.

3. Results

3.1. Study population characteristics and different HLA allele distribution

A total of 398 subjects (males m, n = 198, 49.7%) originating from Italy (n = 324, 81.4%) and Germany (n = 74, 18.6%) were analyzed. The study population consists of 100 AIP patients, 53 patients diagnosed with AIP type 1, 23 patients with AIP type 2 and 24 patients with AIP NOS. In addition, we included 44 CP patients from a previous study [31] and 254 healthy controls (Supplementary Table 1). We observed an overrepresentation of males in the CP (m = 34, 77.3%) and in the AIP type 2 (m = 14, 60.9%) cohort, which included also younger patients with a mean age of 34.7 ± 13.7 (SD) years. Higher IgG4 serum levels with a mean of 628.3 ± 578.3 (SD) mg/dL were identified in AIP type 1 patients. Intestinal bowel disease (IBD) was present in 18 AIP patients. Five patients (9.4%) with AIP type 1 had an ulcerative colitis. From 13 (56.5%) AIP type 2 patients, 11 had an ulcerative colitis and two patients had Crohn's disease (Supplementary Table 1). We performed allele typing for *HLA-DRB1* and *HLA-DQB1* and determined *HLA-DRB3*, *HLA-DRB4* and *HLA-DRB5* incidence in AIP patients and healthy controls. In seven AIP type 1, three AIP type 2 and two AIP NOS cases, *HLA-DQB1* allele typing results were not clearly depicted and excluded from our analyses. HLA analyses from the CP cohort was already available [31]. Independent from the disease groups, we identified that the Italian population carries the *HLA-DRB1*04* (p = 0.0002), *-DRB1*09* (p = 0.0236) and *-DRB1*15* (p = 0.0185) alleles less frequent (Supplementary Fig. 1A). Moreover, we found significant differences in the incidence of *HLA-DQB1*01* (p = 0.0285), *-DQB1*03* (p = 0.0006) and *-DQB1*07* (p = 0.0121) between the Italian and German cohort (Supplementary Fig. 1B), whereas the *HLA-DRB3*, *HLA-DRB4* and *HLA-DRB5* gene loci were equally distributed (Supplementary Fig. 1C). After clarifying country-specific variations in the HLA allele distribution, we aimed at identifying HLA genotypes associated with an increased susceptibility for AIP.

3.2. HLA-DQB1 homozygosity is strongly associated with AIP disease

We assessed whether *HLA-DRB1* and *HLA-DQB1* allele variants occur in a heterozygous or homozygous allele distribution in the controls, CP and AIP patients. We detected that most of the study subjects were heterozygous for their *HLA-DRB1* and *HLA-DQB1* alleles (Fig. 1A). Interestingly, we uncovered that homozygous genotypes of *HLA-DRB1* and *HLA-DQB1* alleles can be found more often in AIP patients, whereby particularly homozygous *HLA-DQB1* allele carriers are strongly enriched in the AIP cohort with a odds ratio (OR) of 3.02, 95% confidence interval (CI) 1.82–5.03, p < 0.001 (Fig. 1A, Supplementary Table 2). From the data, we concluded that individuals, which are homozygous for *HLA-DQB1* allele variants have an increased susceptibility to develop AIP.

3.3. Association of HLA-DRB1 and -DQB1 allele frequencies with AIP susceptibility

We performed a univariate analysis of the *HLA-DRB1* (Table 1) and *-DQB1* (Table 2) allele distribution in AIP patients in comparison to healthy controls. We detected that *HLA-DRB1*16* (OR 2.49, 95% CI 1.29–5.19, p = 0.02) and *HLA-DRB1*11* (OR = 1.53, 95%CI 1.02–2.26, p = 0.04) alleles are positively correlated with AIP. However, a negative correlation was uncovered for carrying the *HLA-DRB1*13* allele (OR = 0.51, 95%CI 0.29–0.86, p = 0.02) (Table 1). Focusing on *-DQB1* alleles, a strong positive association with AIP was identified for *HLA-DQB1*05* (OR = 2.16, 95%CI 1.43–3.25, p < 0.001) and *-DQB1*06* (OR = 1.57, 95%CI 1.05–2.33, p = 0.03) in comparison to healthy controls. In contrast, *HLA-DQB1*02* (OR = 0.59, 95%CI 0.36–0.93, p = 0.03) showed a reduced allele frequency and it was negatively associated with AIP. The overall less frequent *HLA-DQB1* gene variants, such as *HLA-DQB1*01* or *HLA-DQB1*07*, were not detected in the AIP patients' cohort, which makes it difficult to evaluate their significant negative association with AIP (Table 2). Notably, the identified AIP-associated alleles

Table 1
Distribution of *HLA-DRB1** alleles.

allele	Co n = 508	CP n = 88	AIP n = 200	AIP OR (95% CI)#	p-value
01	48 (9.4)	6 (6.8)	25 (12.5)	1.37 (0.81–2.28)	0.29
03	61 (12.0)	15 (17.0)	17 (8.5)	0.69 (0.38–1.18)	0.23
04	46 (9.1)	13 (14.8)	19 (9.5)	1.06 (0.59–1.83)	0.97
07	66 (13.0)	8 (9.1)	16 (8.0)	0.59 (0.32–1.02)	0.08
08	16 (3.1)	0	2 (1.0)	0.33 (0.05–1.19)	0.17
09	3 (0.6)	0	1 (0.5)	0.92 (0.03–7.99)	1.00
10	5 (1.0)	0	2 (1.0)	1.06 (0.14–5.20)	1.00
11	89 (17.5)	17 (19.3)	49 (24.5)	**1.53 (1.02–2.26)**	***0.04***
12	7 (1.4)	2 (2.3)	3 (1.5)	1.12 (0.23–4.19)	1.00
13	83 (16.3)	12 (13.6)	18 (9.0)	**0.51 (0.29–0.86)**	***0.02***
14	25 (4.9)	5 (5.7)	6 (3.0)	0.61 (0.22–1.42)	0.36
15	43 (8.5)	5 (5.7)	27 (13.5)	1.69 (1.00–2.81)	0.06
16	16 (3.1)	5 (5.7)	15 (7.5)	**2.49 (1.19–5.19)**	***0.02***

Total number and percentage in parentheses of *HLA-DRB1** alleles. Co: healthy controls, CP: chronic pancreatitis, AIP: autoimmune pancreatitis, OR: odds ratio, CI: confidence interval, # in respect to Co.

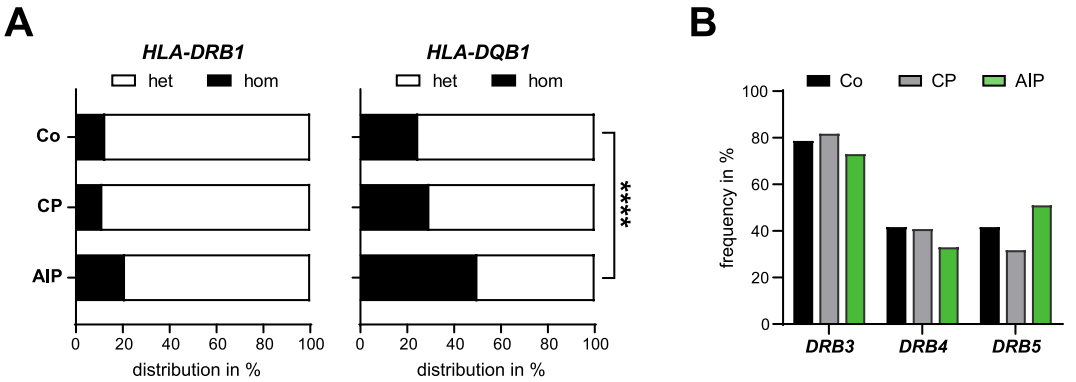


Fig. 1. *HLA-DQB1* homozygosity is strongly associated with AIP disease. (A) Co: healthy controls, CP: chronic pancreatitis, AIP: autoimmune pancreatitis. Distribution of homozygous and heterozygous *HLA-DRB1* and *-DQB1* alleles in Co, CP and AIP patients in percent. *HLA-DQB1* alleles were not determined in 12 AIP patients. Numbers are listed in Supplementary Table 2. ****p < 0.0001. (B) Frequency of the *HLA-DRB3*, *-DRB4*, *-DRB5* genes in percent in Co, CP and AIP patients.

Table 2
Distribution of *HLA-DQB1** alleles.

allele	Co n = 508	CP n = 88	AIP n = 176	AIP OR (95% CI)#	p-value
01	37 (7.3)	0	0	**0.08 (0.00–0.38)**	***< 0.01***
02	116 (22.8)	20 (22.7)	26 (14.8)	**0.59 (0.36–0.92)**	***0.03***
03	111 (21.9)	35 (39.8)	50 (28.4)	1.42 (0.96–2.09)	0.10
04	14 (2.8)	0	3 (1.7)	0.64 (0.14–2.00)	0.62
05	77 (15.2)	17 (19.3)	49 (27.8)	**2.16 (1.43–3.25)**	***< 0.001***
06	98 (19.3)	16 (18.2)	48 (27.3)	**1.57 (1.05–2.33)**	***0.03***
07	44 (8.7)	0	0	**0.07 (0.00–0.31)**	***< 0.001***
08	8 (1.6)	0	0	0.40 (0.02–2.24)	0.53
09	3 (0.6)	0	0	1.04 (0.04–9.03)	1.00

Total number and percentage in parentheses of *HLA-DQB1** alleles. Co: healthy controls, CP: chronic pancreatitis, AIP: autoimmune pancreatitis, OR: odds ratio, CI: confidence interval, # in respect to Co. *HLA-DQB1* alleles were not determined in 12 AIP patients.

were country-independent (Supplementary Fig. 1A and B). Analyzing *HLA-DRB3*, *-DRB4* and *-DRB5* allele frequency, we detected no differences between control, CP or AIP subjects (Fig. 1B). Altogether, our findings revealed that an increased *HLA-DRB1*16* and *-DQB1*05* allele frequency is strongly associated with AIP disease, whereas a reduced *HLA-DQB1*02* allele frequency might have a protective effect. Interestingly, the *HLA-DRB1*16* allele was linked to *HLA-DQB1*05* in almost all control, CP and AIP cases, suggesting a linkage disequilibrium of these two alleles.

3.4. Association of HLA-DRB1 and -DQB1 allele frequencies with AIP subtypes

AIP type 1 and AIP type 2 patients showed a strong enrichment of homozygous *HLA-DQB1* gene variants (AIP type 1: OR = 3.59, 95% CI 1.88–6.93, $p < 0.001$; AIP type 2: OR = 3.67, 95%CI 1.44–9.63, $p < 0.01$). In contrast, AIP NOS patients revealed a similar *HLA-DQB1* zygosity pattern as controls and CP patients (Fig. 2A, Supplementary Table 3). The HLA alleles *HLA-DRB3*, *-DRB4* and *-DRB5* were equally distributed in our subgroup analysis (Fig. 2B). Notably, we uncovered that solely patients with AIP type 1 and AIP type 2 had a higher allele frequency of *HLA-DRB1*16* (AIP type 1: OR = 3.21, 95%CI 1.36–7.25, $p < 0.01$; AIP type 2: OR = 3.81, 95%CI 1.17–10.41, $p = 0.03$) (Table 3) and the linked *HLA-DQB1*05* allele (AIP type 1: OR = 2.33, 95%CI 1.38–3.85, $p < 0.01$; AIP type 2: OR = 2.41, 95%CI 1.13–4.86, $p = 0.03$) (Table 4). In addition, both AIP subtypes showed a pronounced reduction in the *HLA-DRB1*13* allele frequency, although the negative association was only significant for AIP type 1 ($p = 0.03$) (Table 3). Interestingly, a loss of the *HLA-DQB1*02* allele (OR = 0.26, 95%CI 0.06–0.73, $p = 0.02$) was only present in the AIP NOS group, indicating a possible protective effect (Table 4). The significant negative association of *HLA-DQB1*01* and *-DQB1*07* to AIP type 1 is again of unclear biological relevance, since the less common alleles are completely absent in the whole AIP cohort (Table 4). In summary, the *HLA-DRB1*16-HLA-DQB1*05* linkage disequilibrium was highly frequent in AIP type 1 and AIP type 2 disease, whereas the protective *HLA-DRB1*13* allele was much less frequent. Thus, we concluded that individuals with these allele frequencies have an increased susceptibility to develop AIP type 1 or type 2 disease. Moreover, whether a reduction of the HLA-*DQB1*02* allele frequency protects from AIP NOS needs to be investigated in a larger cohort.

4. Discussion

The enrichment of specific HLA gene variants in ADs is a known phenomenon. The gene loci of the HLA system code for MHC molecules, which represent an enormous range of self and non-self peptides to the immune system [32]. The MHC class II-presented antigens are recognized by $CD4^+$ T-cells [33]. Under physiological conditions, only T-lymphocytes with a low or intermediate avidity to self-peptides are released into the circulation. In contrast, autoimmune diseases reveal an inefficient inactivation of autoreactive T-cells, which trigger the autoimmune response due to increased reactivity to self-antigens [34].

The pathogenesis of AIP subtypes and the involvement of genetic factors are not well characterized, although a genetic predisposition for *DRB1*0405* and *DQB1*0401* HLA alleles was described in a Japanese AIP cohort [24]. Thus, we examined HLA class II allele frequencies in healthy controls, CP and in 100 AIP patients from Italy and Germany. It is, so far, the largest European AIP cohort undergoing genomic HLA typing. Interestingly, we observed that a homozygous presence of *HLA-DQB1* gene variants was highly associated with AIP type 1 and type 2 disease, whereas CP or AIP NOS did not show a correlation to allele zygosity. *HLA-DQB1* homozygosity was also detected in chronic lymphocytic leukemia [35] and in celiac disease [36]. A low somatic *HLA-DQB1* diversity might impair the inactivation of T-cells with higher avidity to self-peptides, which triggers autoimmunity.

Reduced heterozygosity of HLA class II genes was also found in patients with ulcerative colitis (UC), an IBD that is along with Crohn's disease (CD) associated with both AIP subtypes, but with a higher frequency in AIP type 2 [8,37]. For the allele *DRB1*0103*, a strong genetic association with UC and CD was found in European populations, while risk alleles of the *DRB1*15* group were common

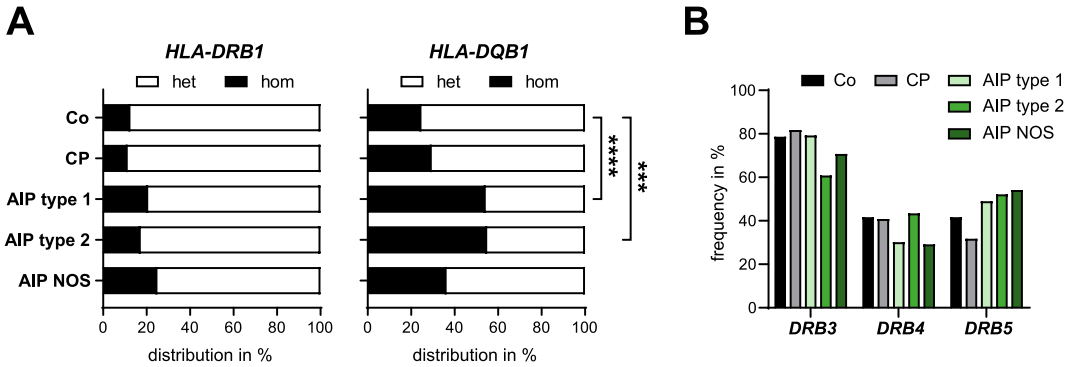


Fig. 2. Enrichment of homozygous *HLA-DQB1* alleles occurs mostly in AIP type 1 and type 2. Co: healthy controls, CP: chronic pancreatitis, AIP: autoimmune pancreatitis, NOS: not otherwise specified. A) Distribution of homozygous and heterozygous *HLA-DRB1* and *-DQB1* alleles in Co, CP and AIP type 1, AIP type 2 and AIP NOS patients in percent. *HLA-DQB1* alleles were not determined in 12 AIP patients. Numbers are listed in Supplementary Table 3. ***$p < 0.001$, ****$p < 0.0001$. (B) Frequency of the *HLA-DRB3*, *-DRB4*, *-DRB5* genes in percent in Co, CP and AIP type 1, AIP type 2 and AIP NOS patients.

Table 3
Distribution of *HLA-DRB1** alleles in AIP subtypes.

allele	Co	CP	AIP type1	AIP type2	AIP NOS	AIP type 1		AIP type 2		AIP NOS	
	n = 508	n = 88	n = 106	n = 46	n = 48	OR (95% CI)[#]	p-value	OR (95% CI)[#]	p-value	OR (95% CI)[#]	p-value
01	48 (9.4)	6 (6.8)	12 (11.3)	5 (10.9)	8 (16.7)	1.23 (0.60–2.35)	0.68	1.20 (0.39–2.94)	0.96	1.94 (0.80–4.21)	0.18
03	61 (12.0)	15 (17.0)	15 (14.2)	1 (2.2)	1 (2.1)	1.22 (0.64–2.19)	0.65	0.19 (0.01–0.87)	0.07	0.18 (0.01–0.83)	0.06
04	46 (9.1)	13 (14.8)	9 (8.5)	6 (13.0)	4 (8.3)	0.94 (0.42–1.91)	1.00	1.54 (0.55–3.59)	0.53	0.94 (0.27–2.47)	1.00
07	66 (13.0)	8 (9.1)	9 (8.5)	4 (8.7)	3 (6.3)	0.63 (0.28–1.25)	0.26	0.66 (0.19–1.71)	0.54	0.47 (0.11–1.33)	0.26
08	16 (3.1)	0	0	0	2 (4.2)	0.33 (0.01–1.64)	0.34	0.76 (0.03–3.85)	1.00	1.42 (0.20–5.27)	1.00
09	3 (0.6)	0	1 (0.9)	0	0	1.74 (0.06–15.17)	1.00	3.96 (0.14–34.89)	0.77	3.80 (0.13–33.42)	0.79
10	5 (1.0)	0	0	2 (4.3)	0	1.05 (0.04–6.94)	1.00	4.74 (0.60–23.80)	0.21	2.32 (0.09–15.49)	1.00
11	89 (17.5)	17 (19.3)	26 (24.5)	12 (26.1)	11 (22.9)	1.53 (0.92–2.50)	0.12	1.67 (0.80–3.29)	0.21	1.41 (0.66–2.80)	0.46
12	7 (1.4)	2 (2.3)	1 (0.9)	1 (2.2)	1 (2.1)	0.76 (0.03–4.48)	1.00	1.78 (0.07–10.60)	1.00	1.70 (0.07–10.13)	1.00
13	83 (16.3)	12 (13.6)	8 (7.5)	2 (4.3)	8 (16.7)	***0.43 (0.18–0.86)***	***0.03***	0.25 (0.04–0.83)	0.05	1.04 (0.43–2.20)	1.00
14	25 (4.9)	5 (5.7)	1 (0.9)	2 (4.3)	3 (6.3)	0.21 (0.01–1.00)	0.11	0.94 (0.14–3.32)	1.00	1.34 (0.30–4.06)	0.95
15	43 (8.5)	5 (5.7)	14 (13.2)	6 (13.0)	7 (14.6)	1.66 (0.84–3.09)	0.18	1.65 (0.59–3.88)	0.44	1.87 (0.73–4.22)	0.25
16	16 (3.1)	5 (5.7)	10 (9.4)	5 (10.9)	0	***3.21 (1.36–7.25)***	< ***0.01***	***3.81 (1.17–10.41)***	***0.03***	0.73 (0.03–3.69)	1.00

Total number and percentage in parentheses of *HLA-DRB1** alleles in AIP subtypes. Co: healthy controls, CP: chronic pancreatitis, AIP: autoimmune pancreatitis, NOS: not otherwise specified, OR: odds ratio, CI: confidence interval, # in respect to Co.

Table 4
Distribution of *HLA-DQB1** alleles in AIP subtypes.

allele	Co	CP	AIP type1	AIP type2	AIP NOS	AIP type 1		AIP type 2		AIP NOS	
	n = 508	n = 88	n = 92	n = 40	n = 44	OR (95% CI)[#]	p-value	OR (95% CI)[#]	p-value	OR (95% CI)[#]	p-value
01	37 (7.3)	0	0	0	0	**0.16 (0.01–0.74)**	***0.04***	0.36 (0.02–1.73)	0.39	0.33 (0.01–1.57)	0.33
02	116 (22.8)	20 (22.7)	19 (20.7)	4 (10.0)	3 (6.8)	0.88 (0.50–1.50)	0.74	0.39 (0.11–1.00)	0.09	**0.26 (0.06–0.73)**	***0.02***
03	111 (21.9)	35 (39.8)	25 (27.2)	10 (25.0)	15 (34.1)	1.34 (0.79–2.20)	0.32	1.20 (0.54–2.47)	0.79	1.86 (0.94–3.55)	0.10
04	14 (2.8)	0	1 (1.1)	0	2 (4.5)	0.44 (0.02–2.23)	0.56	1.0 (0.04–5.19)	1.00	1.78 (0.25–6.76)	0.83
05	77 (15.2)	17 (19.3)	27 (29.3)	12 (30.0)	10 (22.7)	**2.33 (1.38–3.85)**	< ***0.01***	**2.41 (1.13–4.86)**	***0.03***	1.66 (0.75–3.40)	0.27
06	98 (19.3)	16 (18.2)	20 (21.7)	14 (35.0)	14 (31.8)	1.17 (0.66–1.98)	0.69	**2.26 (1.10–4.44)**	***0.03***	1.97 (0.97–3.79)	0.07
07	44 (8.7)	0	0	0	0	**0.13 (0.01–0.60)**	***0.02***	0.30 (0.01–1.43)	0.27	0.27 (0.01–1.29)	0.22
08	8 (1.6)	0	0	0	0	0.76 (0.03–4.32)	1.00	1.75 (0.07–10.11)	1.00	1.59 (0.06–9.17)	1.00
09	3 (0.6)	0	0	0	0	1.99 (0.07–17.32)	1.00	4.55 (0.16–40.21)	0.70	4.14 (0.14–36.50)	0.75

Total number and percentage in parentheses of *HLA-DQB1** alleles in AIP subtypes. Co: healthy controls, CP: chronic pancreatitis, AIP: autoimmune pancreatitis, OR: odds ratio, NOS: not otherwise specified, CI: confidence interval, # in respect to Co. *HLA-DQB1* alleles were not determined in 12 AIP patients.

in UC in all ethnic groups [37,38]. In our selected cohort of 100 AIP patients, IBD was present in 18 patients, but no significant association with the described risk alleles was found because of the small numbers (data not shown). In our study, we uncovered for the first time a significant enrichment of the *HLA-DRB1*16* and *HLA-DQB1*05* alleles in AIP type 1 and type 2 patients, whereas CP and AIP NOS patients did not show an association to this linkage disequilibrium. An interesting aspect was observed in a recent meta-analysis, showing an association of the *HLA-DRB1*1602* allele with autoimmune diseases that were primarily triggered by autoantibodies [39]. Various studies have identified different autoantibodies in AIP patients [40], although no correlation to specific AIP subtypes was performed, so far. The enrichment of *HLA-DRB1*16* and *HLA-DQB1*05* haplotypes in AIP type 1 and type 2 patients might lead to an increased number of autoreactive T-cells or the production of autoantibodies through an overrepresentation of self-peptides, although this needs to be examined in further studies. In our analysis, we also detected a reduced abundance of the *HLA-DRB1*13* allele in AIP type 1 and type 2 patients, for which a protective effect was described in other autoimmune diseases [41].

Similar to the study of Park et al. investigating HLA genotypes in a Korean AIP cohort, we could not confirm the *DRB1*0405* and *DQB1*0401* gene association that was identified in the Japanese AIP cohort [29], indicating a region-specific association of this genetic predisposition [42,43]. Although some autoimmune diseases were associated with *HLA-DRB3*, *-DRB4* or *-DRB5* alleles [44], our data revealed no correlation of these alleles with AIP. This is in line with the study of Buechter et al., which demonstrated a significant enrichment of the class I *HLA-B8* allele frequency in AIP type 1 patients, but no further association to *HLA-DRB3* and *HLA-DRB4* class II alleles [45].

In conclusion, our data revealed that AIP type 1 and type 2 are associated with the same genetic predispostions, which indicates that the onset of the disease might rely on similar immunogenic mechanisms, although the two subtypes differ in histological features. Furthermore, we suppose that AIP NOS evolves from different pathomechanisms or represents another type of chronic pancreatitis, since patients with AIP NOS revealed merely an underrepresentation of the *HLA-DQB1*02* allele. Our study gives important insights into the different subtypes and immunogenetic heterogeneity of the AIP disease.

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b) Specific author contributions: EG, IR, AA, GB, GDM were involved in the patient enrollment, acquisition of the data, analysis and interpretation of the data and drafting of the manuscript. UMM performed statistical analysis. IR, FUW, UMM, FB, ML served as expert geneticists and immunologists. RAZ, MDL, TK, AD provided healthy control samples for the analysis. GMC, US, MML, LF and JM provided relevant intellectual input. EG, IR, GMC, LF and JM: Study concept and design, drafting of the manuscript, study supervision. All authors read and approved the final manuscript.

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Appendix A. Supplementary data

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