Published in final edited form as:

Gastroenterology. 2015 May ; 148(5): 924–927.e2. doi:10.1053/j.gastro.2015.01.041.

Association Between HLA Haplotypes and Increased Serum Levels of IgG4 in Patients with Primary Sclerosing Cholangitis

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Abstract

Increased serum levels of immunoglobulin (Ig)G4 have been reported in 9%–15% of patients with primary sclerosing cholangitis (PSC); it is not clear whether this increase contributes to pathogenesis. We performed genetic analyses of the HLA complex in patients with PSC from

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Disclosures: No relevant conflict of interests.

Author contributions: NLB: analysis and interpretation of data, drafting of the manuscript, critical revision of the manuscript for important intellectual content; OK: acquisition of data, analysis and interpretation of data, critical revision of the manuscript for important intellectual content; BDJ: acquisition of data, critical revision of the manuscript for important intellectual content; MBdV: acquisition of data, critical revision of the manuscript for important intellectual content; BL: acquisition of data, critical revision of the manuscript for important intellectual content; KNL: acquisition of data, critical revision of the manuscript for important intellectual content; KMB: acquisition of data, critical revision of the manuscript for important intellectual content; THK: analysis and interpretation of data, critical revision of the manuscript for important intellectual content; JRH: acquisition of data, analysis and interpretation of data, drafting of the manuscript, critical revision of the manuscript for important intellectual content.

Norway, Sweden, and the United States. We found an association between levels of IgG4 above the upper reference limit and specific HLA haplotypes. These patients had a significantly lower frequency of the strongest PSC risk factor, HLA-B* 08, than patients without increased IgG4, and significantly higher frequencies of HLA-B* 07 and DRB1*15. HLA genotype might therefore affect the serum concentration IgG4, and increased IgG4 might be a marker of a distinct phenotype of PSC.

Keywords

genetic association study; immunogenetics; IgG4-associated cholangitis; immune response

The clinical phenotype of primary sclerosing cholangitis (PSC) is heterogeneous. Several comorbidities are found only in subgroups of the patients, *e.g.* inflammatory bowel disease (IBD) (up to 80%), other autoimmune disorders (approximately 25%) and features of autoimmune hepatitis (approximately 10%)¹. There is also reason to believe that the nature of the bile duct affection varies, *e.g.* small-duct affection only is found in approximately 10% of the patients¹. The finding of elevated serum concentration of immunoglobulin G4 (IgG4) in a proportion of PSC patients was first reported in 2006². Later studies have corroborated this observation, yielding frequencies of 9–15%^{2–4}. Elevated IgG4 in PSC seems to be a marker of a more severe disease course². How or whether it may relate to IgG4-associated cholangitis (IAC), occurring in the context of systemic inflammatory IgG4-related disease⁵, is obscure. In contrast to PSC, IAC responds to immunosuppression, but to which extent that also pertains to PSC patients with elevated IgG4 is undetermined⁶.

Recently it was shown that the IgG4-producing B cells in IAC exhibit a large degree of clonality⁷, suggesting the presence of specific antigenic triggers. There is also considerable evidence to support an autoimmune component to the pathogenesis in PSC¹, but how this relates to high IgG4 concentrations observed in a fraction of patients is unknown. The strongest genetic risk factors in PSC are encoded within the human leukocyte antigen (HLA) complex on chromosome 6p21⁸. Due to genetic properties of the HLA complex (strong linkage disequilibrium) and the presence of multiple, independent association signals, it has proven exceedingly difficult to determine the biologically relevant gene variants^{8,9}. We hypothesized that elevated IgG4 concentrations serve as a marker for a pathogenetically distinct group of PSC patients, and therefore aimed to explore the clinical features and HLA background of this group.

We determined IgG4 in 263 Norwegian PSC patients (Supplementary Table 1 and Supplementary Material and Methods). Several IgG4 assays with different upper reference levels (URLs) exist. In this study, elevated serum IgG4 concentration was defined as above either: i) 1.35g/L (suggested threshold for IAC⁴ and similar to the 1.4g/L URL used in PSC by Mendes *et al.*²) or ii) 2.01g/L (URL of the assay employed in the present study). According to these thresholds, elevated IgG4 was found in 47 (18%) and 23 (9%) of the patients, respectively (Supplementary Table 1).

In line with previous observations², elevated IgG4, irrespective of cut-off, was associated with more advanced disease stage, as evaluated by liver biochemistry and revised Mayo risk

score (Supplementary Table 1). Considering elevated IgG4 as a phenotype, there was a significant association between elevated IgG4 (cut-off at 1.35) and reduced transplant-free survival from time of PSC diagnosis ($P=0.05$, Supplementary Figure 1).

To test the hypothesis of a genetic basis for elevated IgG4 in PSC, we compared PSC patients with high and low IgG4, and both these groups with healthy controls ($n=368$) for *HLA-B* and *HLA-DRB1*, focusing on previously identified PSC associated alleles (Table 1 and Supplementary Tables 2 and 3). Considering $\text{IgG4} > 1.35$ as cut-off, the strongest genetic risk factor in PSC⁸, the *HLA-B*08* allele, was less prevalent in patients with high than low IgG4 (29% vs. 42%, $P=0.02$, Supplementary Table 2). When considering URL ($\text{IgG4} > 2.01$) as cutoff, a significantly reduced *HLA-B*08* frequency was still observed in the high IgG4 group, with the additional observations that *HLA-B*07* and *DRB1*15* were significantly more prevalent in PSC patients with high than low IgG4 (Table 1).

To validate these findings, we included PSC patients from Sweden ($n=68$) and the USA ($n=90$), focusing on high IgG4 using the cut-off $\text{IgG4} > \text{URL}$, as other IgG4 assays were applied (Supplementary Material and Methods). Using imputed HLA data⁸, the significantly lower frequency of *HLA-B*08* and the higher frequencies of *HLA-B*07* and *DRB1*15* in PSC patients with high IgG4 were confirmed in the combined Swedish-USA panel (Table 2). A meta-analysis of all patients yielded P -values of 0.004, 0.005 and 0.002 for the differences observed for *HLA-B*07*, *B*08* and *DRB1*15*, respectively (Table 2). When comparing PSC patients with healthy controls in the Norwegian panel, *HLA-DRB1*15* was only associated with PSC patients with $\text{IgG4} > 2.01$ (odds ratio, 2.0; 95% confidence interval 1.0–3.9; $P=0.05$; Table 1). This observation was also replicated in the combined Swedish-USA panel (odds ratio, 3.1; 95% confidence interval, 1.5–6.6; $P=0.003$; Supplementary Table 4).

Studies on the genetics of systemic IgG4-related disease are very limited. An association with the *HLA-DRB1*0405-DQB1*0801* haplotype has been observed in a Japanese population of IgG4-associated autoimmune pancreatitis¹⁰. This haplotype is rarely observed in the Norwegian population, meaning results cannot be compared. *HLA-B*08* is part of the *HLA-B*08-DRB1*03* haplotype, which has been associated with multiple autoimmune diseases¹¹. The increased frequency of *HLA-B*08* in the low IgG4 group parallels our observation of higher *HLA-B*08* frequency in large than small duct PSC¹², suggesting that exclusion of patients exhibiting extreme phenotypes leaves a genetically more homogeneous PSC population.

The increased frequencies of both *HLA-B*07* and *DRB1*15* (serotype DR2) observed in patients with $\text{IgG4} > \text{URL}$ may be explained by the typical co-occurrence of these two alleles on the same conserved haplotype (AH7.1). An association of *HLA-DR2* with PSC was first described in 1991, in a study where clinical differences also suggested that *HLA-DR2* and *DR3* could represent different etiological subsets of PSC¹³. Association studies for *HLA-DR2/DRB1*15* in PSC have yielded different results. Except for one study in Italian PSC¹⁴, significant associations have only been detected when patients carrying *HLA-DRB1*03* (serotype DR3) and *DRB1*13:01* (serotype DR6) are excluded from the analysis^{9,15}. Our

study is the first to explain the presence of this secondary association with DR2 in PSC by stratification of the patient population according to serum IgG4 concentrations.

While the power of the study was limited since the high IgG4 groups are small, the independent replication of the main findings represents a strength of the study. The study was specifically assessing differences in established PSC HLA risk factors between patients with high and low IgG4. Due to multiple testing concerns and low effect size of non-HLA associations in PSC⁸, associations outside the HLA complex warrant further investigations in accordingly sized study panels. Furthermore, since most of the patients in the present study were included prior to the awareness of the IAC entity, we lack available data to evaluate the presence of pancreatic disease and systemic IgG4-related disease, as relevant investigations were not performed in this period. In the present study, the similar prevalence of high IgG4 (9–10%) and HLA associations in patient panels analyzed with different assays but using URL as cut-off, suggest that categorizing patients according to URL rather than an absolute IgG4 threshold value is beneficial. However, in the Norwegian panel, a more advanced disease stage and a different HLA association was observed already in patients with IgG4 above a lower cut-off than URL, meaning that IgG4 concentrations below URL could be clinically relevant.

In conclusion, we report for the first time that PSC patients with elevated serum IgG4 concentrations show distinct HLA associations, suggesting that the IgG4 concentrations are in part genetically determined, and that phenotypic heterogeneity may contribute to the complexity of the HLA associations observed in PSC. The possibility that elevated IgG4 is a marker for a distinct PSC entity should be explored in studies of pathophysiology or therapeutic trials targeting this sub-population.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

We would like to thank all study participants for contributing to this study. Prof. Benedicte A. Lie and the Norwegian Bone Marrow Donor Registry at Oslo University Hospital are acknowledged for generously contributing Norwegian control data. Trine Folseraas and Jimmy Liu are acknowledged for help with the use of imputed HLA data from the ImmunoChip study.

Grant support: The study was funded by the Norwegian PSC Research Center. Konstantinos N. Lazaridis is funded by NIH (DK 084960).

Abbreviations

HLA	human leukocyte antigen
IAC	IgG4 associated cholangitis
IBD	inflammatory bowel disease
IgG4	immunoglobulin G4
PSC	primary sclerosing cholangitis

URL upper reference limit

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HLA associations in Norwegian PSC patients stratified according to IgG4 concentrations using upper reference limit (IgG4>2.01) as cut-off

Table 1

HLA allele ^a	Allele frequency, n (%)			High IgG4 vs. Low IgG4		Low IgG4 vs. Ctr		High IgG4 vs. Ctr	
	Ctr (2n=736)	Low IgG4 (2n=480)	High IgG4 (2n=46)	OR (95% CI)	P ^b	OR (95% CI)	P	OR (95% CI)	P ^b
B*07	118 (16)	63 (13)	11 (24)	2.1 (1.1–4.3)	0.04	0.8 (0.6–1.1)	0.15	1.7 (0.8–3.3)	0.17
B*08	94 (13)	196 (41)	12 (26)	0.5 (0.3–1.0)	0.05	4.7 (3.5–6.2)	<0.001	2.4 (1.2–4.8)	0.01
DRB1*03	106 (14)	191 (40)	16 (35)	0.8 (0.4–1.5)	0.48	3.9 (3.0–5.2)	<0.001	3.2 (1.7–6.0)	<0.001
DRB1*04	157 (21)	36 (8)	4 (9)	1.3 (0.5–3.4)	0.77	0.3 (0.2–0.4)	<0.001	0.4 (0.2–1.0)	0.04
DRB1*07	55 (7)	17 (4)	2 (4)	1.5 (0.4–5.2)	0.68	0.5 (0.3–0.8)	0.005	0.7 (0.2–2.3)	0.57
DRB1*11	43 (6)	10 (2)	2 (4)	2.5 (0.7–9.3)	0.29	0.4 (0.2–0.7)	0.002	0.9 (0.3–3.0)	0.67
DRB1*13:01	47 (6)	65 (14)	6 (13)	1.0 (0.4–2.3)	0.91	2.3 (1.6–3.4)	<0.001	2.3 (1.0–5.4)	0.12
DRB1*15	112 (15)	69 (14)	12 (26)	2.1 (1.1–4.2)	0.04	0.9 (0.7–1.3)	0.70	2.0 (1.0–3.9)	0.05

^a Only previously PSC associated HLA alleles shown, for full listing see Supplementary Tables 2 and 3;

^b Marked in **bold** are alleles significantly different between high and low IgG4, as well as alleles that are associated with high but not low IgG4 when compared with healthy controls. n: number of patients; Ctr: Healthy controls; OR: odds ratio.

Table 2

HLA allele frequencies and replication association analyses in PSC patients from Sweden and USA comparing individuals with high and low IgG4

	Sweden		USA		Sweden + US		Norway + Sweden + USA ^a	
	Allele frequency, n (%)		Allele frequency, n (%)					
	Low IgG4 2n=124	High IgG4 ^c 2n=12	Low IgG4 2n=162	High IgG4 ^c 2n=18	OR _{CMH}	P _{CMH} ^d	OR _{CMH}	P _{CMH} ^d
HLA allele ^b								
B*07	18 (15)	4 (33)	26 (16)	5 (28)	2.1 (0.7–5.9)	0.05	2.2 (1.3–2.8)	0.004
B*08	45 (36)	2 (17)	48 (29)	2 (11)	0.4 (0.1–1.3)	0.04	0.4 (0.2–0.8)	0.005
DRB1*15	28 (23)	4 (33)	27 (16)	7 (39)	3.2 (1.2–8.6)	0.03	2.2 (1.3–2.8)	0.002

^a Meta-analysis across both the Sweden+USA replication panel and the original Norwegian panel (Table 1).

^b Only alleles significantly different between high and low IgG4 PSC (upper reference limit as cut-off) was analysed in the replication panel.

^c High IgG4 was defined as above upper reference limit.

^d No significant heterogeneity of the odds ratios (Breslow-Day test, P>0.05 for all three alleles); n: number of patients; OR: odds ratio (95% confidence interval), low IgG4 defined as control population; CMH: Cochran-Mantel-Haenszel test.