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ARTICLE

HLA variants associated with azathioprine-induced pancreatitis in patients with Crohn's disease

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Abstract

The immunosuppressant drug azathioprine is associated with a 4% risk of acute pancreatitis in patients with inflammatory bowel disease (IBD). Studies have demonstrated an increased risk in carriers of HLA-DQA1*02:01 and HLA-DRB1*07:01. We investigated whether these human leukocyte antigen (HLA) types were associated with azathioprine-induced pancreatitis also in Swedish patients with IBD, and whether the type of disease affected the association. Nineteen individuals with IBD who developed acute pancreatitis after initiation of azathioprine were genotyped and compared with a population control cohort ($n = 4891$) and a control group matched for disease ($n = 81$). HLA-DQA1*02:01 and HLA-DRB1*07:01 were in full linkage disequilibrium, and were significantly associated with acute pancreatitis both when cases were compared with population controls (OR 3.97 [95% CI 1.57–9.97], $p = 0.0035$) and matched controls (OR 3.55 [95% CI 1.23–10.98], $p = 0.0275$). In a disease-specific analysis, the correlation was positive in patients with Crohn's disease versus matched controls (OR 9.27 [95% CI 1.86–46.19], $p = 0.0066$), but not in those with ulcerative colitis versus matched controls (OR 0.69 [95% CI 0.07–6.74], $p = 0.749$). In patients with Crohn's disease, we estimated the conditional risk of carriers of HLA-DQA1*02:01-HLA-DRB1*07:01 to 7.3%, and the conditional risk of a non-carrier to 2.2%. We conclude that HLA-DQA1*02:01-HLA-DRB1*07:01 is a marker for increased risk of acute pancreatitis in individuals of Swedish genetic origin, treated with azathioprine for Crohn's disease.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

HLA-DQA1*02:01 and HLA-DRB1*07:01 have been shown to increase the risk of acute pancreatitis in individuals with inflammatory bowel disease (IBD) treated with azathioprine.

WHAT QUESTION DID THIS STUDY ADDRESS?

It is unknown whether this risk also applies to patients of Swedish origin and if the risk differs depending on type of IBD.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

We show that HLA-DQA1*02:01 and HLA-DRB1*07:01 are risk markers for azathioprine-induced acute pancreatitis in patients of Swedish origin. We propose that this risk could be restricted to those with Crohn's disease, where the estimated risk equals 7.3% for carriers and 2.2% for non-carriers.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

As the HLA-DQA1*02:01-HLA-DRB1*07:01 haplotype has a frequency of ~7% in the Swedish population, preemptive HLA-typing could be useful for the selection of patients with Crohn's disease that need intensified monitoring or for choice of a different therapy.

INTRODUCTION

The thiopurine azathioprine and its metabolite mercaptopurine are immunosuppressant medications used for the treatment of inflammatory bowel diseases (IBDs) such as Crohn's disease and ulcerative colitis, autoimmune disorders such as rheumatoid arthritis, vasculitis, and autoimmune hepatitis, and to prevent transplant rejection.[1] Around 17% of patients taking these medications develop adverse drug reactions (ADRs) that necessitate drug withdrawal.[2] Acute pancreatitis is a well-recognized dose-independent and potentially life-threatening ADR that occurs in 4% of Crohn's patients taking azathioprine.[3] The pathogenesis of thiopurine-associated pancreatitis is not fully understood, but a genetic predisposition has been proposed.

In a genome-wide association study (GWAS) on 172 cases and 2035 IBD controls of predominantly European genetic ancestry, the strongest association with azathioprine-induced pancreatitis was with a single nucleotide polymorphism (SNP) in the class II HLA region, rs2647087 (odds ratio [OR] = 2.59).[4] An association was also found with the human leukocyte antigen (HLA) alleles HLA-DQA1*02:01 (OR = 2.54) and HLA-DRB1*07:01 (OR = 2.55) that are partially tagged by rs2647087 ($R^2 = 0.49$). The risk of developing pancreatitis in rs2647087C homozygotes was calculated at about 17%, and the risk in heterozygotes at 9%.[4] The association with rs2647087 was replicated in a Canadian study on IBD patients: 13 cases of azathioprine-induced pancreatitis (85% Crohn's disease) and 360 controls (65% Crohn's disease).[5] Genetic ancestry was not reported.

In this study, we aimed to investigate whether the association between the HLA region and azathioprine-induced pancreatitis is valid also in a population of Swedish genetic ancestry. In addition, we aimed to investigate whether there is a difference in risk between patients with Crohn's disease and ulcerative colitis.

METHODS

Ethical statement

The study was approved by the regional ethical review boards in Uppsala and Stockholm (2010/231 in Uppsala; 2007/644-31 and 2011/463-32 in Stockholm). Written informed consent was obtained from all participants.

Sample description

The patients were recruited within the larger SWEDEGENE project, which aims to identify genetic markers for ADRs, as described.[6] The basis for case recruitment was through nationwide spontaneous ADR reports sent from health care professionals to the Swedish Medical Products Agency (MPA) between January 1990 and February 2017. To be included, cases were required to meet the following criteria:

a. A diagnosis of Crohn's disease or ulcerative colitis,
b. Onset of abdominal pain after initiation of therapy with azathioprine,
c. An increase in P-amylase or P-lipase above the upper level of normal,
d. Resolution after azathioprine withdrawal,
e. No other identifiable cause for pancreatitis, namely, gallstones, alcohol abuse, hypertriglyceridemia, infections (e.g., mumps, coxsackievirus, hepatitis B, cytomegalovirus [CMV], varicella-zoster, herpes simplex virus [HSV]), recently performed endoscopic retrograde cholangiopancreatography (ERCP), ischemia, trauma, or concomitant treatment with other drugs known to be associated with pancreatitis as defined by Badalov et al.,[7]
f. A Naranjo score indicating at least a possible association with azathioprine exposure,[8]

g. Age at least 18 years at the time of recruitment,
h. Able to provide informed consent.

A total of 21 reports of azathioprine-associated pancreatitis were retrieved from the MPA. We collected clinical data (demographics, medical history, drug treatment history, laboratory data, and country of birth of parents as proxy for genetic ancestry) through interviews using a standardized questionnaire, and by obtaining and reviewing medical records. Each case was adjudicated by two specialists in clinical pharmacology and drug safety. Based on this evaluation, two cases were excluded for the following reasons: no exact information on the increase in P-amylase and mismatching disease (juvenile arthritis as opposed to IBD). Unrelated individuals from the Swedish Twin Registry, which contains genome-wide data for about 10,000 individuals of predominantly Swedish genetic ancestry that were born between 1911 and 1958, were used as controls.[9] To investigate any possible association between findings and the underlying IBD type, we selected a subgroup of controls from the Swedish Twin Registry with a matching diagnosis of IBD ($n = 81$). The diagnoses were obtained by linking the Swedish Twin Registry to the National Disease Register operated by the Swedish National Board of Health and Social Welfare. Finally, we explored whether the underlying type of IBD (i.e., Crohn's disease or ulcerative colitis) affected the risk estimates.

Genome-wide array data

DNA was extracted from peripheral venous blood. The cases were genotyped with the Illumina Infinium OmniExpress 8v1-3_A 1M or Illumina GSAMD-24v3 650K array. The controls were genotyped with the Illumina HumanOmniExpress 700K array and consisted of 4891 individuals, where 81 had matching diseases (Table 1). Genotype calls were generated using the GenomeStudio software from Illumina and the Genome Reference Consortium human assembly GRCh37.

Genotyping quality control (QC) and data management were performed using PLINK v1.9.[10] QC included gender checks, exclusion of markers with call rate <0.98, Hardy–Weinberg $p < 5 \times 10^{-8}$ and minor allele frequency (MAF) <0.005 followed by exclusion of individuals with call rate <0.98. Imputation was performed using the Sanger imputation server[11] separately on the different batches. The pipeline performed prephasing using Eagle2 (v2.0.5)[11,12] and Positional Burrows–Wheeler Transform imputation,[13] with the haplotype reference consortium panel as reference (v1.1) that is predominantly of European genetic ancestry.[11] Post-imputation variants were filtered out if impute2 quality metric was <0.7 or MAF <0.0001 and converted to hard calls using PLINK. Using principal component analysis (PCA) as implemented in PLINK, the cases and controls were checked for stratification (Figure S1) between the different batches and stratification between cases and controls (Figure S2). PCA was performed on the merged genotyped material, which consisted of approximately 160,000 markers in common across panels. No pronounced genetic outliers were seen.

The variant rs2647087 was not present in our imputed dataset, and we therefore selected a variant in strong linkage disequilibrium (LD), rs2647085 (LD: 0.98 r^2, 0.99 D′ vs. rs2647087),[14] for the analysis.

HLA allele imputation

HLA allele imputation to first and second field resolution of 180 classical HLA alleles, amino acid residues, and individual SNPs was performed on the merged imputed datasets using the software SNP2HLA with the T1DGC European HLA reference panel of 5225 individuals.[13,15] The Beagle R2 imputation quality was 0.959 and 0.950 for HLA-DRB1*07:01 and HLA-DQA1*02:01, respectively.

Statistical analysis

The variant rs2647085 was encoded to zero or one, indicating carrier and wild-type, and was tested for association in a dominant model. HLA-DQA1*02:01 and HLA-DRB1*07:01 were encoded the same way.

HLA-DQA1*02:01 and HLA-DRB1*07:01 were in full LD both in cases and controls matched for disease. Furthermore, the SNP rs2647085 and the HLA haplotype were strongly correlated ($r^2 = 0.71$). We therefore set the significance level to 0.05. Comparison of proportions of rs2647085 and the HLA haplotype with the outcome pancreatitis was performed using logistic regression and expressed as ORs with 95% confidence intervals (CIs). These calculations were done using the *lrm* function from the *rms* R package (R version 4.1.0, rms version 6.2-0).[16] All regressions were calculated with four PCs included as covariates to control for genetic population structure.

We investigated the influence of the underlying type of IBD by including interaction between IBD type and genetic variant in the model. Effects per IBD type were estimated using contrasts from the interaction model, as implemented in the *contrast* function in the *rms* R package. These calculations were restricted to controls with matching disease, of whom six individuals with a diagnosis of both Crohn's disease and ulcerative colitis were excluded, resulting in a total number of 75 controls.

TABLE 1 Characteristics of included cases and controls matched for disease

Characteristic	Cases ($n = 19$)	Controls ($n = 81$)
Sex (n male/female)	10/9	42/39
Mean age[a] (years), [range]	43 [12–69]	51 [15–77]
Time to onset[b] (days), [range]	54.4 [7–365]	N/A
Median daily dose of azathioprine (mg/day), [range]	100 [50–200]	N/A
Disease[c]		
Crohn's disease (n)	11	39
Ulcerative colitis (n)	8	48
Mean maximum P-pancreas specific amylase[d] (μkat/L), [range]	4.0 [1.3–8.9]	N/A
Mean maximum P-amylase (μkat/L), [range][e]	21.2 [3.9–44]	N/A
Mean alcohol consumption at time of onset (units/week), [range]	1.8 [0–7]	N/A
Naranjo score		
9 points (Definite), n	2	N/A
7 points (Probable), n	16	N/A
4 points (Possible), n	1	N/A

Abbreviations: N/A, not applicable; P, plasma.

Note: All cases had laboratory measurements of P-pancreas specific amylase or P-amylase, except one patient who had a P-lipase of 40 μkat/L (reference 0.4–5.0).

[a]Age for cases is at time of onset and at first recorded diagnosis of Crohn's disease or ulcerative colitis for controls.

[b]Missing data for 1 patient.

[c]A total of 6 controls had a diagnosis of both Crohn's disease and ulcerative colitis.

[d]Reference interval 0.15–1.1 μkat/L for adults and 0.10–0.60 μkat/L for one 12-year-old patient. Data are for 14 patients.

[e]Reference interval 0.4–2.0 μkat/L. Data are for 4 patients who did not have measurements of P-pancreas-specific amylase.

RESULTS

Characteristics of the 19 cases and the 81 matched controls are shown in Table 1. All cases were of Swedish origin, except for one who had mixed Swedish and Spanish ancestry. All had been treated with azathioprine for Crohn's disease or ulcerative colitis, and had developed abdominal pain after initiation of azathioprine therapy, with laboratory data in agreement with a diagnosis of acute pancreatitis. In all cases, symptoms abated after azathioprine was withdrawn. Two cases exhibited positive rechallenge after switching to therapy with the thiopurine mercaptopurine. No patient had a history of alcohol abuse or another suspected differential diagnosis. Zygosity frequencies among cases and controls are shown in Table 2.

When cases were compared with all 4891 controls, statistically significant associations between pancreatitis and rs2647085 (OR = 3.00 [95% CI 1.91–7.55], $p = 0.0197$) and the HLA-DQA1*02:01-HLA-DRB1*07:01 haplotype (OR = 3.966. [95% CI 1.57–9.97], $p = 0.0035$) were detected (Table 3). When restricting controls to those matched for underlying disease, the association between pancreatitis and HLA-DQA1*02:01-HLA-DRB1*07:01 (OR = 3.55 [95% CI 1.15–10.98], $p = 0.0275$) remained, as well as the association with rs2647085 (OR = 3.67 [95% CI 1.23–11.08], $p = 0.0201$; Table 3).

IBD type specific analysis

For HLA-DQA1*02:01-HLA-DRB1*07:01 and IBD type the interaction was marginally significant ($p = 0.066$), but not for rs2647085 and IBD type ($p = 0.287$; Table S1). Estimated effects per IBD type showed the largest effect in Crohn's patients with OR = 9.27 (95% CI 1.86–46.19, $p = 0.0066$, Figure 1) for HLA-DQA1*02:01-HLA-DRB1*07:01, and OR = 6.61 (95% CI 1.24–35.08, $p = 0.0266$, Figure 1) for the SNP rs2647085. No associations between pancreatitis and HLA-DQA1*02:01-HLA-DRB1*07:01 or rs2647085 were observed for ulcerative colitis (OR = 0.69 [95% CI 0.07–6.74, $p = 0.7489$] and OR = 1.89 [95% CI 0.37–9.70, $p = 0.4447$], respectively; Figure 1).

Frequencies of carriers of the haplotype HLA-DQA1*02:01-HLA-DRB1*07:01 and rs2647085 are shown in Table 4.

TABLE 2 Zygosity frequencies

	Wild-type *n* (%)	Heterozygosity *n* (%)	Homozygosity *n* (%)
HLA-DQA1*02:01-HLA-DRB1*07:01			
Cases ($n = 19$)	11 (57.89)	8 (42.11)	0
Matched controls ($n = 81$)	67 (82.71)	13 (16.04)	1 (1.23)
All controls ($n = 4891$)	4150 (84.84)	704 (14.39)	37 (0.76)
rs2647085			
Cases ($n = 19$)	11 (57.89)	8 (42.11)	0
Matched controls ($n = 81$)	55 (67.90)	21 (25.93)	5 (6.17)
All controls ($n = 4891$)	3425 (70.03)	1331 (27.21)	135 (2.76)

Note: Zygosity frequencies in cases of azathioprine-associated pancreatitis, controls matched for disease, and all controls.

TABLE 3 Odds ratios for the risk of azathioprine-induced pancreatitis

Target	OR	OR CI 95%	*P* value	β_0 estimate	Cases(*n*)	Controls(*n*)
rs2647085	3.0008	[1.91–7.55]	0.0197	−6.00	19	4891
HLA-DQA1*02:01-HLA-DRB1*07:01	3.966	[1.57–9.97]	0.0035	−5.89	19	4981
rs2647085	3.67	[1.23–11.07]	0.0201	−1.86	19	81
HLA-DQA1*02:01-HLA-DRB1*07:01	3.55	[1.15–10.98]	0.0275	−1.65	19	81

Note: Analysis was performed using logistic regression and expressed as odds ratios with 95% confidence intervals. Principal component one to four was included as covariates.

Cases of azathioprine-induced pancreatitis were compared with all population controls ($n = 4891$) and controls matched for disease ($n = 81$).

Abbreviations: CI, confidence interval; OR, odds ratio.

Probability of pancreatitis in patients carrying HLA-DRB1*07:01 with Crohn's disease

The probability of developing pancreatitis from azathioprine was calculated with the assumption that 4% of Crohn's patients develop pancreatitis.[3,17]

A previous study estimated that the 35% of patients with Crohn's in the UK are carriers of HLA-DRB1*07:01.[18] By assuming a similar frequency among patients in Sweden, we can estimate the probability of pancreatitis conditioned on carrying HLA-DQA1*02:01-HLA-DRB1*07:01. This was calculated to 7.3% among carriers, while the probability of a non-carrier developing was estimated to be 2.2% (Supplement S1).

DISCUSSION

Previous studies in predominantly European patients have shown an association between the risk of azathioprine-induced pancreatitis and the class II HLA haplotype HLA-DQA1*02:01-HLA-DRB1*07:01 as well as with SNPs in high LD with this haplotype.[4,5]

All cases in our study had IBD, which is also associated with specific HLA class II types. The inheritance pattern for IBD is, however, complicated and differs between genetic ancestry and diseases. In individuals of European and American genetic ancestry, the most replicated association between HLA class II and ulcerative colitis is with the rare HLA-DRB1*01:03 allele.[19] One of the most consistent associations between HLA class II and Crohn's disease in Europeans is with the common HLA-DRB1*07 allele.[18,19] By using control subjects matched for IBD type and studying interaction terms, we can control for confounding by indication, which occurs when a risk factor for disease that promotes exposure to the drug in question is erroneously detected as a risk factor for the ADR.[18-20]

In the present study, we showed that the haplotype HLA-DQA1*02:01-HLA-DRB1*07:01 and rs2647085 are predictive of azathioprine-associated pancreatitis in a Swedish population.

Interestingly, our results suggest that the genetic association with pancreatitis is markedly different depending

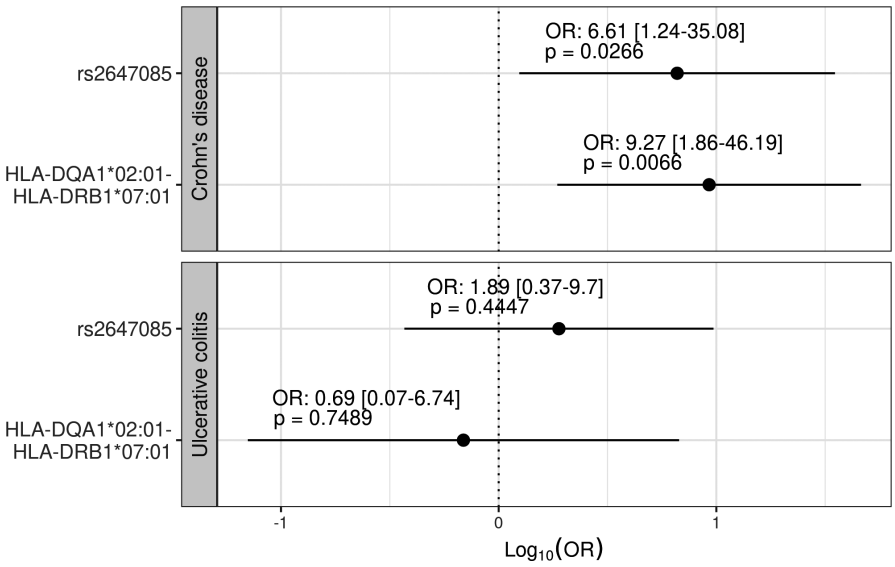

FIGURE 1 Odds ratio for azathioprine-induced pancreatitis from inflammatory bowel disease (IBD) type specific contrast tests. The term being contrasted is the genetic factor, while the IBD subtype is being held constant for each type. Each test was performed for both HLA-DQA1*02:01-HLA-DRB1*07:01 and rs2647085 as genetic factor. Odds ratio (OR), *p* value and 95% confidence interval (CI) can be found above each line

TABLE 4 Number of carriers of genetic variants among cases and controls divided by type of inflammatory bowel disease

Inflammatory bowel disease	HLA-DQA1*02:01-HLA-DRB1*07:01 carriers	rs2647085 carriers
Crohn's disease	Cases: 7/11	Cases: 8/11
	Controls: 6/33	Controls: 12/33
Ulcerative colitis	Cases: 1/8	Cases: 3/8
	Controls: 7/42	Controls: 11/42

on type of IBD, in that patients with Crohn's disease exhibit a strong association with both HLA-DQA1*02:01-HLA-DRB1*07:01 and rs2647085, while no correlation was observed in cases with ulcerative colitis. These findings are contrary to the study by Heap et al.[4] who did not observe a difference in risk between patients with Crohn's disease and ulcerative colitis. Due to the low number of cases in our study, and the increase in sample size needed for interaction studies, the results should be interpreted with caution.[21] Furthermore, since we do not know if our disease-matched controls had been exposed to azathioprine, any estimates should be considered conservative. A possibility for a disease-specific risk is supported by the epidemiological finding that azathioprine-induced pancreatitis appears to be more common in patients with Crohn's disease than in patients with other diseases for which it is prescribed,[2,22-24] although this has been disputed.[25,26] As mentioned above, the HLA-DRB1*07 allele is also overrepresented in European patients with Crohn's disease.[18,19] The mechanism through which these genetic markers increases the risk of pancreatitis in azathioprine-treated patients with Crohn's disease is not known, and it cannot be excluded that genetic variation other than HLA plays a role. However, a growing number of immune-mediated drug reactions are associated with specific HLA alleles.[27] In general the mechanisms underlying these associations are unknown, but for some medications, mechanistic studies have been performed. Abacavir and carbamazepine that are associated with severe hypersensitivity, and carbamazepine in patients carrying certain MHC class I HLA-B types (HLA-B*57:01 and HLA-B*15:02, respectively) have been shown to interact with these HLA-B types.[28] The binding of these medications to the respective HLA- B types changes the shape and chemistry of the antigen-binding cleft, thereby altering the repertoire of endogenous peptides that it can bind to. In this way, new endogenous peptides act as antigens, thereby inducing the activation of T-cells and causing an immune response manifesting as hypersensitivity or Stevens–Johnson syndrome in certain patients.

HLA-DQA1 and HLA-DRB1 belong to the MHC class II,[29] and whether a similar mechanism is applicable also to HLA-D type II is currently not known. HLA class I genes are ubiquitously expressed in cells, while expression of class II genes is mainly restricted to thymic epithelial cells, B-cells, macrophages, and dendritic cells.[29] Expression of HLA-DQA1 and HLA-DRB1 in pancreatic cell lines is limited,[30] but extensive expression of HLA-DQA1 and HLA-DRB1 has been demonstrated in both monocytes and macrophages within pancreatic tissue (http://www.proteinatlas.org).[31]

In our study, patients with Crohn's disease carrying HLA-DQA1*02:01-HLA-DRB1*07:01 had an estimated 3.3-fold increased risk of experiencing pancreatitis. This

risk is calculated under the assumption that the carrier frequency of patients in Sweden is the same as in the UK; however, the carrier frequency was estimated to be 28% in the British population for HLA-DRB1*07:01 but only 14% in the Swedish population.[17,32] Due to this difference, the risk of developing pancreatitis among Swedish patients with HLA-DRB1*07:01 might be underestimated. Therefore, preemptive HLA-typing could be useful for the selection of patients who need intensified monitoring or choice of a different therapy. In conclusion, we have confirmed the association between the HLA-DQA1*02:01-HLA-DRB1*07:01 haplotype and azathioprine-associated pancreatitis in Swedish patients with Crohn's disease.

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CONFLICT OF INTEREST

The authors declared no competing interests for this work.

AUTHOR CONTRIBUTIONS

J.Å., I.B., N.E., P.H., and M.W. wrote the manuscript. P.H., M.W., and P.K.E.M. designed the research. P.H. and I.B. performed the research. J.Å. and N.E. analyzed the data.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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