


ORIGINAL ARTICLE

Two tagging single-nucleotide polymorphisms to capture *HLA-DRB1*07:01-DQA1*02:01-DQB1*02:02* haplotype associated with asparaginase hypersensitivity

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Aims: Asparaginase (ASP) hypersensitivity is a well-known challenge in the treatment of lymphoblastic malignancies. In terms of cost considerations, the cheap native *Escherichia coli* ASP, the most immunogenic form of this medication, is used in the first line in middle-income countries. Previously, the role of the *HLA-DRB1*07:01-DQA1*02:01-DQB1*02:02* haplotype had been established to associate with *E. coli* ASP hypersensitivity. We investigated a possible cost-effective genetic testing method to identify patients harbouring the risk HLA haplotype in order to pave the way for safer ASP treatment.

Methods: In 241 patients with previously determined *HLA-DRB1*07:01-DQA1*02:01-DQB1*02:02* haplotype and known ASP hypersensitivity status, 4 candidate HLA-tagging single-nucleotide polymorphisms (SNPs) were measured, and the performance of the different sets of these tag SNPs was evaluated.

Results: We identified a combination of 2 SNPs – rs28383172 and rs7775228 – as a tag for *HLA-DRB1*07:01-DQA1*02:01-DQB1*02:02* haplotype with sensitivity and specificity values >95%. In line with previous findings, we found complete concordance between *HLA-DRB1*07:01* and rs28383172. With bioinformatics methods, the results were also confirmed in the 1000 Genomes dataset in different ethnic groups.

Conclusion: Rs28383172 and rs7775228 are suitable for identifying *HLA-DRB1*07:01-DQA1*02:01-DQB1*02:02* carriers. Compared to the rest of the population, patients with hypersensitivity-prone genotype would benefit more from the administration of less immunogenic PEGylated ASP before the hypersensitivity evolves, incurring minimal extra cost.

KEYWORDS

acute lymphoblastic leukaemia, allergy, asparaginase hypersensitivity, *HLA-DRB1*07:01*, screening test, tagging SNPs

Csaba Szalai and Dániel J. Erdélyi contributed equally to this work and are considered as joined last authors.

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1 | INTRODUCTION

Due to the high survival rate of paediatric acute lymphoblastic leukaemia (ALL), research has now come to focus on reducing the toxic effects of chemotherapy. Asparaginase (ASP), responsible for the asparagine depletion of the extracellular fluid compartment, is a key component of the therapy. However, unpredictable immunological reactions to this enzyme with bacterial origin are major challenges in clinical practice. ASP hypersensitivity may manifest even as an anaphylactic reaction that can be life-threatening. Besides, the allergic reaction is associated with the development of anti-ASP antibodies, which may have long-term adverse consequences. These antibodies can neutralise ASP, resulting in a suboptimal treatment response through reduced drug exposure and subsequent recurrence of the disease.^{1,2}

According to the current ALL IC-BFM 2009 treatment protocol in Hungary, paediatric patients with ALL receive *Escherichia coli*-derived ASP as a first-line treatment. While in higher-income countries the less immunogenic, but more expensive PEGylated (PEG) ASP preparation is the first-line drug, the cheaper *E. coli* ASP is the first choice in lower-income countries. The most common reason for the discontinuation of ASP therapy is hypersensitivity, with an incidence rate of up to 45% for native *E. coli* ASP. However, hypersensitivity is less likely to develop (in 3 to 24% of patients) against the PEGylated form of the drug.³

First, Fernandez *et al.* published that the *HLA-DRB1*07:01* allele predisposes to *E. coli* ASP hypersensitivity in paediatric ALL patients.⁴ Later, in a subsequent genome-wide association study by the same group, a single-nucleotide polymorphism (SNP) linked to *HLA-DRB1*07:01* also acted as a risk allele for ASP hypersensitivity in patients of diverse ancestry.⁵ In 2017, we published our results confirming the role of the *DRB1*07:01* allele in the development of ASP hypersensitivity in Hungarian patients using a next-generation sequencing method; and we also showed with bioinformatics tools that this association was restricted to the extended *HLA-DRB1*07:01-DQA1*02:01-DQB1*02:02* haplotype.⁶ The *HLA-DRB1*07:01* allele only increased the risk of hypersensitivity if it occurred in a haplotype with *HLA-DQB1*02:02*, while the *HLA-DRB1*07:01-HLA-DQB1*03:03* haplotype were not associated with an increased risk. The *HLA-DQA1*02:01* allele was in complete linkage disequilibrium with the *HLA-DRB1*07:01* allele.⁶ Recently, the implication of *HLA-DRB1*07:01*, *DQA1*02:01* and *DQB1*02:02* alleles in ASP hypersensitivity was confirmed by Gagné *et al.*⁷

Current diagnostic HLA genotyping methods are time-consuming and expensive, requiring specially equipped laboratories. In addition, the cost and time required for these laboratory-based tests are further increased implicitly by sample collection, transport and reporting, limiting the utility of HLA genotyping in precision medicine.⁸ Alternative testing methods have been explored to overcome these barriers and enable the application of HLA testing at the point of care. One of these possible methods is to determine tagging SNPs.⁹

The *HLA-DRB1*07:01* is one of the pharmacogenomic biomarkers in drug labelling approved by the US Food and Drug Administration.¹⁰

What is already known about this subject

- *HLA-DRB1*07:01-DQA1*02:01-DQB1*02:02* haplotype is associated with *Escherichia coli* asparaginase hypersensitivity.
- Patients with hypersensitivity-prone genotype would benefit more from the administration of less immunogenic PEGylated asparaginase.
- Cost-effective, less laborious testing of the risk haplotype is needed to enable application at the point of care.

What this study adds

- The combination of rs28383172 and rs7775228 is widely suitable for tagging *HLA-DRB1*07:01-DQA1*02:01-DQB1*02:02* carrier status.

In a recent work of Erlichster *et al.* previously described tagging SNPs were re-evaluated, and a novel rs28383172 was identified as a potential cross-ethnicity tagging SNP for *HLA-DRB1*07:01* allele using bioinformatics analyses on the 1000 Genomes dataset.⁸ Previously, Monsuur *et al.* identified 6 tagging SNPs to predict risk-conferring HLA variants, including 3 SNPs for *DQA1*02:01/DQB1*02:02* (*DQ2.2*) haplotype in coeliac disease.¹¹ Later, Koskinen *et al.* validated their method in Finnish, Hungarian and Italian populations.¹²

Based on these, we selected a total of 4 tagging SNPs: rs28383172, rs2395182, rs4713586 and rs7775228 (Table 1) for capturing the *HLA-DRB1*07:01-DQA1*02:01-DQB1*02:02* haplotype. Our results revealed that the most efficient haplotype tagging could be achieved by using 2 SNP tags. In addition, we evaluated the clinical performance of this screening method for *E. coli* ASP hypersensitivity in a retrospective manner.

2 | METHODS

In a previous study ALL patients with known ASP hypersensitivity status ($n = 359$) were typed at high-resolution level for *HLA-DRB1* (exon 2) and *HLA-DQB1* (exons 2 and 3), in the Beijing Genomics Institute using an NGS-based genotyping method that applies a massively parallel paired-end sequencing on Illumina MiSeq, effective variant phasing and haploid sequence assembling pipeline.⁶ According to the results we found that the *HLA-DRB1*07:01-DQA1*02:01-DQB1*02:02* haplotype was associated with *E. coli* ASP hypersensitivity, and suggested it as a possible screening marker for this phenomenon. A case was regarded as having *E. coli* ASP hypersensitivity when signs of allergic reactions or anaphylactic reactions National Cancer Institute Common Toxicity Criteria grade 1 and above were noted, as described in detail earlier. In this cohort of

TABLE 1 The 4 single-nucleotide polymorphisms reported to tag the risk HLA allele and haplotype

Rs number	Allele call		Method	Base pair position ^b	Tagged HLA type	Positive predicting allele	Negative predicting allele	Reference
	FAM	VIC (HEX ^a)						
rs28383172	G	A	TaqMan	chr6:32598202	HLA-DRB1*07:01	G		8
rs2395182	G	T	KASP	chr6:32445540	DQ2.2	T		11,12
rs4713586	C	T	KASP	chr6:32691805	DQ2.2		C	11,12
rs7775228	C	T	KASP	chr6:32690302	DQ2.2	C		11,12

^aIn KASP chemistry, one of the allele-specific primers is labelled with HEX dye, the excitation and emission values of which are very close to VIC.

^bAccording to GRCh38.p12.

patients, the overall *E. coli* ASP hypersensitivity rate was 39%.^{6,13} A subset of patients from the original study ($n = 241$) with known HLA-DRB1*07:01-DQA1*02:01-DQB1*02:02 haplotype and available DNA samples was chosen as reference population for evaluating the SNP tagging method. Characteristics of the patients in the original cohort and in the successfully SNP-genotyped subpopulation ($n = 233$) are presented in Table 2.

Written informed consent was obtained from the study participants or from the next of kin, caretakers or guardians on the behalf of the minors/children participants involved in the study. The study was conducted according to the principles expressed in the Declaration of Helsinki and was approved by the Hungarian Scientific and Research Ethics Committee of the Medical Research Council (ETT TUKEB; Case No.:8-374/2009-1018Eku 914/PI/08).

TABLE 2 Characteristics of the patients in the original cohort and the single-nucleotide polymorphism (SNP)-genotyped subpopulation

	Original cohort $n = 359$	SNP-genotyped subpopulation $n = 233$
Sex (%)		
Male	200 (55.7)	134 (57.5)
Female	159 (44.3)	99 (42.5)
Age at diagnosis (y)		
Median	4.8	4.6
Range	1-18	1-18
Lower quartile	3.0	3.0
Upper quartile	8.2	8.5
Risk category (%)		
Low risk	102 (28.4)	60 (25.8)
Medium risk	213 (59.3)	143 (61.4)
High risk	44 (12.3)	30 (12.9)
Immunophenotype (%)		
Pre-B ALL	287 (79.9)	180 (77.3)
T-ALL	46 (12.8)	34 (14.6)
Unknown	26 (7.2)	19 (8.2)

ALL: acute lymphoblastic leukaemia

The rs28383172 was determined using a Custom TaqMan SNP Genotyping Assay (4331349, ANNKYYJ; Applied Biosystems, Foster City, CA, USA). Since we did not have any sample from a patient homozygous for HLA-DRB1*07:01-DQA1*02:01-DQB1*02:02 haplotype, a DNA sample obtained from the MOU human cell line (88052050, IHW Number: 9050; Human Leukocyte Antigen Typed Collection maintained by the European Collection of Cell Cultures) representing HLA-DRB1*0701, HLA-DQA1*0201 and HLA-DQB1*0202 in homozygous form was used as a positive control. The rs2395182, rs4713586 and rs7775228 were determined using KASPar-on-Demand prevalidated assays (Aliquot IDs: 0303639501, 0303736579 and 0303639507, respectively; LGC Genomics, Berlin, Germany). In all cases, genotyping was carried out using 7900HT Fast Real-Time PCR System (Applied Biosystems, Foster City, CA, USA).

Statistical analyses of sensitivity, specificity, predictive values and Cohen- κ values were performed using VassarStats (<http://vassarstats.net/>).¹⁴ Venn diagrams were created by using Displayr online software (<https://www.displayr.com/>). To compare sensitivities, specificities and predictive values of the screening tests, McNemar χ^2 test and the method of Moskowitz and Pepe were applied using R statistical software (3.6.3) and the DTComPair package.¹⁵⁻¹⁷ The performance of the different combinations of the reported SNPs for tagging HLA-DRB1*07:01-DQB1*02:02 haplotype was assessed separately for each of the 1000 Genomes super populations (AFR, AMR, EAS, EUR and SAS). Haplotype estimation was performed using the PHASE software (v.2.1.1) based on the reported HLA allele data.¹⁸

3 | RESULTS

In the present study we utilized results from a previous investigation, namely, the sequencing-based genotyping results of exon 2 in the HLA-DRB1 and exons 2 and 3 in the HLA-DQB1 genes of 359 ALL children with known ASP hypersensitivity status.⁶ As a follow-up cohort, a subset of these patients ($n = 241$) was enrolled. They were chosen based on the availability of their DNA samples. In the follow-up cohort, we performed the genotyping of the 4 selected SNPs (Table 1) and investigated their potentials as predictors for HLA-DRB1*07:01-DQA1*02:01-DQB1*02:02 carrier status (SNP-genotyped subpopulation). Using the Custom TaqMan SNP Genotyping Assay the success

rate of rs28383172 genotyping was 98%. Two patients had to be excluded due to the poor quality of their DNA samples; therefore, out of 241, a total of 233 patients were finally included in the analysis (Table 2). From the genotyping using KASPar-on-Demand prevalidated assays, genotypes were available for all 3 SNPs (rs2395182, rs4713586 and rs7775228) in all 233 patients. Based on the genotyping results, linkage disequilibrium (LD) coefficients were calculated between the 4 SNPs in our population and based on the 1000 Genomes dataset in several super populations. As can be seen in Figures S1 and S2 there was LD between the investigated SNPs in all populations and the LD coefficients in the Hungarian population were most similar to those of in the EUR super population.

Based on the literature, the genotypes determined for each SNP were classified as positive and negative predictors of HLA type separately (Supporting Information Table S1). We then evaluated the results as follows: (i) the association of rs28383172 AG or GG genotypes and *HLA-DRB1*07:01* carrier status; (ii) the association of rs28383172 AG or GG genotypes and *HLA-DRB1*07:01-DQA1*02:01-DQB1*02:02* haplotype carrier status; (iii) the association of rs2395182 TT or TG and rs4713586 TT and rs7775228 TC or CC genotypes and *HLA-DRB1*07:01-DQA1*02:01-DQB1*02:02* haplotype carrier status; (iv) patients meeting both the second and the third conditions were checked for *HLA-DRB1*07:01-DQA1*02:01-DQB1*02:02* haplotype carrier status; and (v) patients with rs28383172 AG or GG and rs7775228 TC or CC genotypes were examined for *HLA-DRB1*07:01-DQA1*02:01-DQB1*02:02* haplotype carrier status (Supporting Information Table S2). The fifth algorithm was raised as a possible approach in data evaluation, during which we examined the contribution of each SNP in different combinations to positive prediction (Figure 1).

The performance of each test can be seen in Table 3. Carrying the G allele of rs28383172 was in perfect concordance with the *HLA-DRB1*07:01* allele carrier status. Two-thirds of the patients harbouring *HLA-DRB1*07:01* allele were *HLA-DRB1*07:01-DQA1*02:01-DQB1*02:02* haplotype carriers. The rest of the patients did not carry the *HLA-DQB1*02:02* allele. Thus, algorithms II–V were designed to identify the same group of patients. The last of these, i.e. the 2-SNP approach, seems to be the most suitable for clinical

utilization with sensitivity, specificity, positive and negative predictive values all greater than 95%.

To confirm our results in other populations, the performance of the different combinations of the tag SNPs was also assessed in the 1000 Genomes dataset. The results obtained for algorithm I were the same as those described by Erlichster et al.⁸ For algorithm V, we found 100% sensitivity and >95% specificity in 3 out of 5 super populations, in AMR, EAS and EUR (Figure S3). The SNP combinations used for *HLA-DRB1*07:01-DQA1*02:01-DQB1*02:02* haplotype-tagging in algorithms III and IV did not achieve the performance required for validation in any super population.

We performed a pairwise comparison of the performance of algorithms II–V on both the Hungarian and the 1000 Genomes data. In the Hungarian SNP-genotyped subpopulation, specificity and the positive predictive value of algorithms III, IV and V were significantly higher compared to algorithm II, and, similarly, the specificity and the positive predictive value of algorithm IV were significantly higher compared to algorithm III, while the results of further comparisons were not found to be significant (Supporting Information Table S3). However, in the EUR super population, sensitivity and the negative predictive value of algorithm V, i.e. the 2-SNP tagging, were significantly higher compared to algorithms III and IV. Besides, specificity and the positive predictive value of algorithm V were significantly higher compared to algorithm II. Overall, in the different comparisons on the 1000 Genomes dataset, the values of the different parameters of algorithm V were, in almost all cases, better than the values of algorithms II, III and IV (Figure S3).

In the SNP-genotyped subpopulation, we evaluated the performance of the *HLA-DRB1*07:01-DQA1*02:01-DQB1*02:02* and its surrogate markers as screening tests for *E. coli* ASP hypersensitivity (Table 4). The test identified a small proportion of affected patients (sensitivity value of 23.4%), but with good specificity (88.5%) and its positive and negative predictive values were 57.9 and 63.1%, respectively. We note that the performance measures of the *HLA-DRB1*07:01-DQA1*02:01-DQB1*02:02* haplotype and of its best tagger algorithm (V) were identical meanwhile, the latter is not a perfect predictor for the haplotype (i.e. the sensitivity and specificity of algorithm V to tag the haplotype were <100%). The reason for this

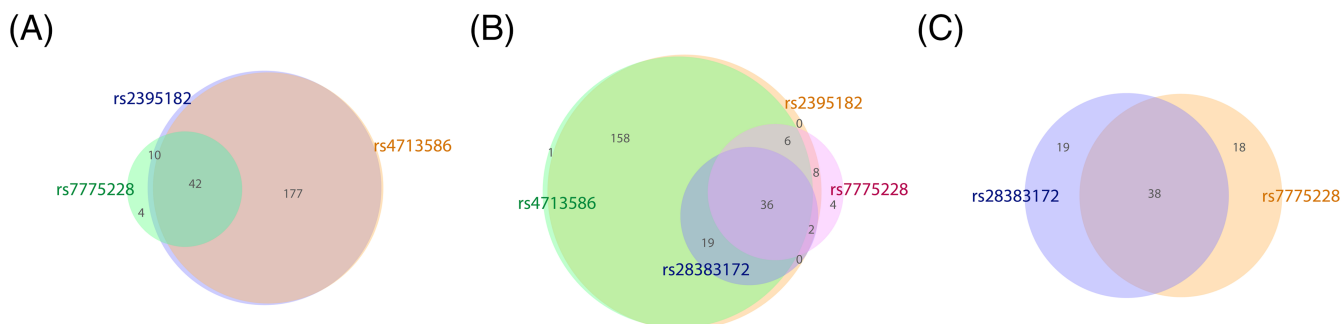


FIGURE 1 Contributions of each single-nucleotide polymorphism (SNP) to positive prediction for *HLA-DRB1*07:01-DQA1*02:01-DQB1*02:02* haplotype carrier status in the SNP-genotyped subpopulation. Venn diagrams for 3 different testing algorithms: application of (A) previously reported markers, rs2395182, rs4713586 and rs7775228; (B) combination of rs28383172, rs2395182, rs4713586 and rs7775228; and (C) 2 SNPs, rs28383172 and rs7775228

TABLE 3 Performance of the different single-nucleotide polymorphism sets in predicting the risk HLA allele and haplotype in the single-nucleotide polymorphism-genotyped subpopulation ($n = 233$)

Test	Marker	HLA genotype	Prevalence (HLA genotype)	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	Unweighted κ (95% CI)
I	rs28383172 (AG or GG)	HLA-DRB1*07:01	24.5%v (19.2–30.6)	100.0% (92.1–100.0)	100.0% (97.3–100.0)	100.0% (92.1–100.0)	100.0% (97.3–100.0)	1.00 (1.00–1.00)
II	rs28383172 (AG or GG)	HLA-DRB1*07:01-DQA1*02:01-DQB1*02:02	16.3% (11.9–21.8)	100.0% (88.6–100.0)	90.3% (85.0–93.9)	66.7% (52.8–78.2)	100.0% (97.3–100.0)	0.75 (0.64–0.86)
III	rs2395182 (TT or TG) AND rs4713586 (TT) AND rs7775228 (TC or CC)	HLA-DRB1*07:01-DQA1*02:01-DQB1*02:02	16.3% (11.9–21.8)	94.7% (80.9–99.1)	96.9% (93.1–98.7)	85.7% (70.8–94.1)	99.0% (95.9–99.8)	0.88 (0.80–0.96)
IV	rs28383172 (AG or GG) AND rs2395182 (TT or TG) AND rs4713586 (TT) AND rs7775228 (TC or CC)	HLA-DRB1*07:01-DQA1*02:01-DQB1*02:02	16.3% (11.9–21.8)	94.7% (80.9–99.1)	100.0% (97.6–100.0)	100.0% (88.0–100.0)	99.0% (96.0–99.8)	0.97 (0.92–1.00)
V	rs28383172 (AG or GG) AND rs7775228 (TC or CC)	HLA-DRB1*07:01-DQA1*02:01-DQB1*02:02	16.3% (11.9–21.8)	97.4% (84.6–99.9)	99.5% (96.7–99.9)	97.4% (84.6–99.9)	99.5% (96.7–100.0)	0.97 (0.93–1.00)

CI: confidence interval; PPV: positive predictive value—percentage of the patients carrying the given HLA genotype or haplotype among patients carrying the marker genotypes; NPV: negative predictive value—percentage of the patients without the given HLA genotype or haplotype among patients not carrying the marker genotypes

seemingly contradictory result is that there were 2 patients for whom the tagger algorithm V missed the true haplotype carrier status (one in both directions), but neither had *E. coli* ASP hypersensitivity, and therefore they did not change the proportion of allergic patients.

4 | DISCUSSION

The aim of the present study was to investigate 4 SNPs as possible tags for the *HLA-DRB1*07:01-DQA1*02:01-DQB1*02:02* haplotype found to be associated previously with ASP hypersensitivity and to provide experimental evidence for their possible clinical application. For patients with increased risk, the less immunogenic (but more expensive) PEG ASP could be administered as a first-line treatment instead of *E. coli* ASP. PEG ASP does not carry unnecessary additional risks, rather just benefits for the patients. Compared to the rest of the population, patients with hypersensitivity-prone genotype would benefit more from the administration of PEG ASP before the hypersensitivity evolves. The lower the proportion of false negative cases is (i.e. the higher the negative predictive value), the higher patient safety is. However, from a cost-effectiveness point of view, the specificity of the test should be high so that the additional cost of treatment modification is kept to a minimum with maximum efficacy. The genotype-based drug choice would require very little extra cost compared to a strategy with PEG ASP therapy as frontline treatment for all. It must be added that although the PEG ASP therapy is associated with significantly lower prevalence of hypersensitivity, within the PEG ASP treated group, the *HLA-DRB1*07:01* allele was also shown to be associated with increased risk of hypersensitivity.⁴

We identified 2 SNPs, rs28383172 and rs7775228, as most suitable tags for *HLA-DRB1*07:01-DQA1*02:01-DQB1*02:02* haplotype in 233 Hungarian patients with paediatric ALL. The role of rs28383172 as a cross-ethnicity tagging SNP for *HLA-DRB1*07:01* was predicted previously by Erlichster *et al.* based on analyses on the 1000 Genomes dataset.⁸ In other studies, rs28724121 and rs17885382 SNPs were also used to tag *HLA-DRB1*07:01* allele.^{5,7} Our measurements in the follow-up cohort with known *HLA-DRB1* genotypes revealed complete concordance between rs28383172 and *HLA-DRB1*07:01* allele. Thus, we confirmed that rs28383172 is an ideal and sufficient surrogate marker for *HLA-DRB1*07:01* carrier status. The chosen custom TaqMan genotyping method is considered the gold standard for genotyping with fluorescence-based homogeneous assays. Functionally tested TaqMan genotyping assay for rs7775228 is also available.

Rs7775228 is one of the previously identified tagging SNPs for DQ2.2 haplotype.^{11,12} This SNP has been found to be predictive for the haplotype in several European populations, but its applicability in a non-European population has not yet been elucidated. In combination with rs28383172, we verified their applicability as *HLA-DRB1*07:01-DQB1*02:02* haplotype tags in AMR, EAS and EUR super populations using bioinformatics analyses on the 1000 Genomes dataset. Haplotype phasing could not be performed for 3 loci in the absence of *HLA-DQA1* data; however, considering the

TABLE 4 Performance of the risk HLA haplotype and the different combinations of its tagging single-nucleotide polymorphisms for predicting *E. coli* ASP hypersensitivity in the single-nucleotide polymorphism-genotyped subpopulation

		<i>E. coli</i> ASP hypersensitivity			Performance	Value (CI 95%)
		Yes	No	Total	Sensitivity	
HLA-DRB1*07:01-DQA1*02:01-DQB1*02:02	Carrier	22	16	38	Specificity	23.4% (15.5–33.5)
	Non-carrier	72	123	195	PPV	88.5% (81.7–93.1)
	Total	94	139	233	NPV	57.9% (40.9–73.3)
						63.1% (55.9–69.8)
rs28383172	Carrier	31	26	57	Specificity	33.0% (23.8–43.6)
	Non-carrier	63	113	176	PPV	81.3% (73.6–87.2)
	Total	94	139	233	NPV	54.4% (40.7–67.4)
						64.2% (56.6–71.2)
rs2395182 AND rs7775228 AND rs4713586	Carrier	21	21	42	Specificity	22.3% (14.7–32.3)
	Non-carrier	73	118	191	PPV	84.9% (77.6–90.2)
	Total	94	139	233	NPV	50.0% (34.4–65.6)
						61.8% (54.5–68.6)
rs28383172 AND rs2395182 AND rs7775228 AND rs4713586	Carrier	21	15	36	Specificity	22.3% (14.7–32.3)
	Non-carrier	73	124	197	PPV	89.2% (82.5–93.6)
	Total	94	139	233	NPV	58.3% (40.9–74.0)
						62.9% (55.8–69.6)
rs28383172 AND rs7775228	Carrier	22	16	38	Specificity	23.4% (15.5–33.5)
	Non-carrier	72	123	195	PPV	88.5% (81.7–93.1)
	Total	94	139	233	NPV	57.9% (40.9–73.3)
						63.1% (55.9–69.8)

ASP, asparaginase; CI: confidence interval; PPV: positive predictive value—percentage of the patients with *E. coli* ASP hypersensitivity among carriers of the risk HLA allele and haplotype; NPV: negative predictive value—percentage of the patients with no *E. coli* ASP hypersensitivity among noncarriers of the risk HLA allele and haplotype

global frequency of *HLA-DQA1* alleles associated with the *HLA-DRB1*07:01-DQB1*02:02* haplotype, *HLA-DQA1*02:01* is found in this haplotype block in approximately 95% of the cases. Based on these, we concluded that the combination of rs7775228 and rs28383172 was widely suitable for tagging *DRB1*07:01-DQA1*02:01-DQB1*02:02* haplotype carrier status.

The role of *HLA-DRB1*07:01* has been described in connection to drugs other than ASP. *HLA-DRB1*07:01*, as a part of the *DRB1*07:01-DQA1*02:01* haplotype, is one of the Food and Drug Administration-annotated pharmacogenomic biomarkers based on its association with lapatinib-induced hepatotoxicity.¹⁹ The same haplotype was also implicated in thiopurine-induced pancreatitis among patients with inflammatory bowel disease.²⁰ Earlier, at lower resolution, the *DRB1*07-DQA1*02* haplotype has been described as being associated with drug-induced liver-injury resulting from anticoagulant ximelagatran.²¹

The limitations of this study are its retrospective design and the lack of ASP activity monitoring of the cohort. Because of the latter, a small proportion of patients categorized as hypersensitive may have only had non-immunological infusion reactions.^{22,23} We do not think, however, that this issue would substantially undermine our results. The evidence for the association of HLA-genotype and ASP hypersensitivity is robust, similarly described by 3 separate research groups. If cases of infusion reactions could be

taken away, we would expect that the predictive value of genotypes may be even better.

In conclusion, we confirmed the previous findings experimentally that rs28383172 is an efficient tagging SNP for *HLA-DRB1*07:01*. In addition, we demonstrated that in combination with rs7775228 these 2 SNPs are suitable for identifying *HLA-DRB1*07:01-DQA1*02:01-DQB1*02:02* haplotype carrier status, proposing a clinical use algorithm for ASP hypersensitivity screening. A genotype-based drug choice would require very little extra cost compared to a strategy with PEG ASP therapy as front-line treatment for all. To confirm our hypothesis, prospective clinical studies are required.

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COMPETING INTERESTS

The authors declare that they have no conflict of interest.

CONTRIBUTORS

N.K. and D.J.E. conceived the original idea. N.K. carried out the experiments. Á.F.S. and E.R.K. contributed to sample collection and preparation; A.G. performed bioinformatics analyses; J.M., R.S. and K.H. organized clinical data collection, and all these 6 coauthors contributed to the interpretation of the results. G.T.K and C.S. supervised the project. N.K. wrote the manuscript with support from D.J.E and C.S. All authors provided critical feedback and helped shape the research, analysis and manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

PRINCIPAL INVESTIGATOR

Due to the nature of the study, there is no Principal Investigator.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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