

# Allopurinol hepatotoxicity is associated with human leukocyte antigen Class I alleles

Robert J. Fontana<sup>1</sup>  | Yi-Ju Li<sup>2</sup> | Elizabeth Phillips<sup>3</sup> | Naba Saeed<sup>4</sup> |  
 Huiman Barnhart<sup>2</sup> | David Kleiner<sup>5</sup>  | Jay Hoofnagle<sup>6</sup> |  
 the Drug Induced Liver Injury Network

<sup>1</sup>Division of Gastroenterology and Hepatology, University of Michigan, Ann Arbor, MI, USA

<sup>2</sup>Department of Biostatistics and Bioinformatics, Duke University Medical Center, Durham, NC, USA

<sup>3</sup>Vanderbilt University Medical Center, Nashville, TN, USA

<sup>4</sup>Department of Internal Medicine, University of Kentucky, Louisville, KY, USA

<sup>5</sup>Laboratory of Pathology, National Cancer Institute, National Institutes of Health (NIH), Bethesda, MD, USA

<sup>6</sup>NIH, Liver Disease Research Branch, Division of Digestive Diseases and Nutrition, National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, MD, USA

## Correspondence

Robert J. Fontana, MD, 3912 Taubman Center, Ann Arbor, MI 48103, USA.  
 Email: rfontana@med.umich.edu

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## Abstract

**Background/Aims:** Allopurinol can cause HLA class I-associated life-threatening severe skin reactions. However, HLA risk and association with clinical features in allopurinol hepatotoxicity are unknown.

**Methods:** Eleven of 17 patients with suspected allopurinol hepatotoxicity enrolled into the Drug-Induced Liver Injury Network were adjudicated as definite, highly likely, or probable. High-resolution HLA sequencing was undertaken in cases and compared with population and other DILI controls.

**Result:** Median age was 60 years, 54% were male, and 63% African-American, 27% Caucasian, and 9% Hispanic. Patients presented at a median of 52 days after starting allopurinol, all were hospitalized and six were jaundiced. The median peak ALT, alkaline phosphatase, and total bilirubin were 525 U/L, 521 U/L, and 7.8 mg/dl, respectively, with a median R ratio of 2.7 at onset. During follow-up, nine patients were treated with corticosteroids including five of the six with suspected DRESS. Three patients died including two from liver failure at 38 and 45 days after onset, and the remaining eight recovered. Three HLA alleles were found to be overrepresented in allopurinol cases, particularly in African Americans: HLA-B\*58:01, which has been previously linked to severe skin reactions, and HLA-B\*53:01 and HLA-A\*34:02, all of which are more frequently found in African Americans than European Americans or Latinos.

**Conclusions:** Allopurinol hepatotoxicity is associated with systemic hypersensitivity, a short latency to onset, African-American race and three HLA risk alleles, HLA-B\*58:01, HLA-B\*53:01, and HLA-A\*34:02-58:01 testing may help confirm a diagnosis of hepatotoxicity in allopurinol-treated patients.

**Abbreviations:** AF, allele frequency; Alk P, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CF, carriage frequency; CMV, cytomegalovirus; DILI, drug-induced liver injury; DILIN, drug-induced liver injury network; DRESS, drug reaction and eosinophilia and systemic symptoms; EBV, Epstein-Barr virus; GFR, glomerular filtration rate; GWA, genome-wide association; HLA, human leukocyte antigen; INR, international normalized ratio; SCAR, severe cutaneous adverse reaction; SJS, Stevens-Johnson syndrome; SNP, single nucleotide polymorphism; TEN, toxic epidermal necrolysis; ULN, upper limit of normal.

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## KEYWORDS

genetic polymorphisms, gout, human leukocyte antigen, hyperuricaemia, liver injury

## 1 | INTRODUCTION

Allopurinol is a xanthine oxidase inhibitor that is widely used to treat gout.<sup>1</sup> Chronic therapy with allopurinol is associated with minor serum aminotransferase elevations in 2%-6% of treated patients, which usually resolve spontaneously or with drug discontinuation.<sup>2,3</sup> Allopurinol is also a rare but well known cause of potentially severe acute liver injury that frequently presents with prominent hypersensitivity features such as those seen with drug reaction with eosinophilia and systemic symptoms (DRESS). This includes fever, widespread rash, eosinophilia, atypical lymphocytosis, lymphadenopathy, and other organ involvement with a typical latency of 2-6 weeks.<sup>2-4</sup>

Allopurinol hypersensitivity syndrome has a high case fatality rate, either from acute liver failure or complications of other allergic manifestations such as the Stevens-Johnson syndrome and toxic epidermal necrolysis (SJS/TEN), vasculitis, pancreatitis, and renal dysfunction. African-American and Asian race as well as preexisting renal disease appear to be particular risk factors for severe cutaneous adverse drugs reactions (SCAR) such as SJS/TEN and DRESS associated with allopurinol.<sup>2-4</sup> The mechanism of allopurinol hepatotoxicity is believed to be a hypersensitivity reaction, and many cases resemble those of anticonvulsant hypersensitivity. Recently, several cases have been associated with reactivation of human herpesvirus-6, Epstein-Barr virus (EBV), or cytomegalovirus (CMV) infections.<sup>4-7</sup> Allopurinol SCAR have been closely linked to HLA-B\*58:01 particularly in Asian populations and also to a lesser extent in Caucasians where HLA-B\*58:01 appears to account for approximately 60% of allopurinol associated SCAR.<sup>4-7</sup>

The Drug-Induced Liver Injury Network (DILIN) has been conducting studies of the presenting features, risk factors, and outcomes of patients with drug-induced liver injury (DILI) since 2003.<sup>8</sup> In addition, mechanistic studies exploring the potential genetic susceptibility in DILI patients have been undertaken using genome-wide association (GWA) studies and targeted human leukocyte antigen (HLA) genotyping. In the current study, we describe the presenting features, clinical outcomes, and liver histopathology in a well-characterized cohort of consecutive patients with suspected allopurinol hepatotoxicity. We also compared HLA alleles associated with allopurinol DILI cases with those associated with drug-induced DILI cases in DILIN and population controls.

## 2 | METHODS

### 2.1 | DILIN prospective study

Patients with liver injury were enrolled into the ongoing DILIN prospective, observational registry study.<sup>8</sup> Liver injury onset was

### Lay summary

Allopurinol is commonly used to treat gout but can be associated with potentially severe skin and liver reactions in a minority of patients. The majority of the 11 patients with allopurinol-related liver injury in the current study had evidence of a hypersensitivity reaction and jaundice when they presented. During follow-up, nine patients were treated with corticosteroids and three died of their illness. Going forward, testing for the three human leukocyte antigen Class I genes identified in this study, HLA-B\*58:01, HLA-B\*53:01, and HLA-A\*34:02, may help doctors more rapidly diagnose and treat future patients.

defined as the first date after a subject met one of the predefined laboratory criteria of either a serum aspartate aminotransferase (AST) or alanine aminotransferase (ALT) level that exceeded five times the upper limit of normal (ULN) (or five times pretreatment baseline if baseline abnormal), a serum alkaline phosphatase (ALP) that exceeded two times the ULN (or two times pretreatment baseline if baseline abnormal), a total bilirubin of 2.5 mg/dl or above, or an international normalized ratio (INR) greater than 1.5 on two consecutive blood draws. All study participants provided written informed consent and were enrolled within 6 months of DILI onset.

A detailed medical history was obtained at the baseline study visit, and additional laboratory and radiological testing were performed to more fully characterize the DILI event and exclude competing etiologies via testing for hepatitis A, B, C, E, HIV, anti-nuclear antibody (ANA) and smooth muscle antibody (SMA) titers, CMV, and EBV infection at the local laboratory. Cases without available hepatitis E virus testing or without HCV RNA results were tested centrally using stored serum samples. Enrolled patients were seen for a follow-up study visit 6 months after initial enrollment, and those with evidence of chronic injury were asked to return for further study visits up to 48 months.<sup>8</sup> Chronic DILI was defined as any patient having a persistently elevated serum AST, ALT, or ALP level, histological evidence of liver injury, or clinical evidence of portal hypertension at 6 months after DILI onset.<sup>8</sup>

The severity of the DILI episode was categorized on a 5-point scale from mild (1), moderate (2), moderate-hospitalized (3), severe (4), and fatal (5), where a fatal score was assigned only if the patient died or had liver transplant due to DILI within 6 months of onset.<sup>8</sup>

While self-reported races and ethnicity were available, we used genome-wide single nucleotide polymorphism (SNP) genotypes to infer individual's ethnicity. Genome-wide SNP data were generated for samples in DILIN over time by different Illumina platform

including Infinium HumanCoreExome Bead Chip 1Mduo array, Multi-Ethnic Genotyping Array (MEGA), and Expanded MEGA (MEGAEX). For each genotyping platform, we computed principal components (PCs) for the combined SNP data of DILIN and all racial groups of the 1000 genomes project and then designated individual genetic ethnicity based on the PC clusters and their boundaries as described previously.<sup>9</sup> If a subject's race could not be inferred by PC criteria due to the uncertainty of cluster assignment or lack of genome-wide SNP data, self-reported race was used.

## 2.2 | Liver histopathology

Available liver biopsies were reviewed by a single expert liver histopathologist (DEK). All samples were scored for multiple histological features as well as an overall pattern of liver injury.<sup>10</sup>

## 2.3 | Causality assessment

The causal relationship between the liver injury episode and allopurinol use was evaluated in a standardized fashion by the DILIN causality committee. A DILIN expert opinion causality score ranging from 1 (Definite >95% likelihood), 2 (Highly Likely 75%-95% likelihood), 3 (Probable 50%-74% likelihood), 4 (Possible 25%-49% likelihood), to 5 (Unlikely <25% likelihood) was assigned by consensus agreement of committee members for all of the retrospective and prospective DILIN cases. In subjects with two or more implicated drugs, an overall causality score was assigned to the case and then an individual causality score for each drug was assigned. In this study, cases with a causality score of 1, 2, or 3 were referred to as high confidence DILI cases and included in the analysis. The previously published RegiSCAR Score with a range of 0 to 6 points was used to identify patients with DRESS.<sup>11</sup>

## 2.4 | HLA sequencing

HLA deep sequencing for the HLA Class I and II genes was performed for DILIN cases at the Vanderbilt Immunogenomics, Microbial Genetics and Single Cell Technologies core (IMGSCCT), a laboratory accredited by the American Society for Histocompatibility and Immunogenetics (ASHI). Specific HLA loci were PCR amplified using sample-specific MID-tagged primers that amplify polymorphic exons from Class I (A, B, C Exons 2 and 3) and Class II (DQ, Exons 2 and 3; DRB and DPB1, Exon 1) MHC genes. MID-tagged primers were optimized to minimize allele dropouts and primer bias. Amplified DNA products from unique MID-tagged products (up to 48 MIDs) were pooled in equimolar ratios for library preparation. Libraries were quantified using the KAPA library quantitation kit (Kapa Biosystems) and high sensitivity D1000 screen tape on an Agilent 2200 TapeStation (Agilent) for concentration and size distribution. Normalized libraries were sequenced on the Illumina MiSeq platform

using the MiSeq V3 600-cycle kit (2X300bp reads). Sequences were separated by MID tags and alleles called using an in-house accredited HLA allele caller software pipeline that minimizes the influence of sequencing errors. Alleles were called using the IMGT HLA allele sequence database release v3270 (March 24, 2017), IIID HLA analysis suite release v3.11, and IIID allele caller release v2.7 HLA analysis reporting software that performs comprehensive allele balance and contamination checks on the final dataset.

## 2.5 | Controls

Two control groups were used in the HLA analysis. One is the population controls using the GWAS dataset of PAGE: The Charles Bronfman Institute for Personalized Medicine BioMe, from NCBI dbGAP. (phs000925.v1.p1), hereafter, referred to as BioMe. The BioMe GWAS dataset consists of 8780 subjects with a diverse racial background, and SNP genotyping was based on Illumina MEGA platform. Similarly, genome-wide SNP data were used to infer individual race as described above. Of 8780 BioMe subjects, 5816 were African Americans. The 4-digit HLA alleles in Class I and Class II genes were imputed by GWAS SNPs for each racial group, respectively, using the HLA genotype imputation with attributable bagging (HiBAG) program.<sup>12</sup> The second control group is referred to as disease control group, which consisted of all other DILI cases not caused by allopurinol (other drugs or herbal and dietary supplements) from the DILIN cohort. This included 1512 DILI cases, of which 177 were African Americans. The comparison of allopurinol cases to nonallopurinol DILI cases in DILIN allows us to assess if the HLA risk alleles identified are more specific for allopurinol DILI.

## 2.6 | Statistical analysis

Descriptive statistics were computed for demographic and patient characteristics by frequency (percentage) for categorical variables and mean, standard deviation (SD), median, and the range for continuous variables. Although 10 of 11 allopurinol cases have genome-wide genotype data available, there was inadequate statistical power to perform GWAS in this small dataset. Alternatively, we checked if any of the allopurinol DILI cases carried the minor allele of rs2476601 in PTPN22, the GWAS signal published previously for DILI.<sup>13</sup> Because the distribution of HLA alleles often differs by racial and ethnicity groups, we focused on the HLA analysis in African-American subset as seven of 11 allopurinol DILI cases are African Americans. However, considering the limited number of allopurinol cases, we also followed up the top interesting HLA alleles using all cases in the dataset. Fisher exact tests were performed to test the allele frequency (AF) difference between allopurinol cases and the two control groups for each HLA allele. In addition, the population AF of each HLA allele in African Americans were obtained from BeTheMatch (<https://bioinformatics.bethematchclinical.org/>) and Allele Frequency Net Database (AFND, <http://www.allelefrequencys.net/>) to serve as the reference. Due to

the small number of cases, candidate HLA alleles were not selected using the conventional criteria for false discovery rate that corrects for multiple testing. Instead, we focused more on the differences in AF between groups. Therefore, we determined the candidate HLA alleles for allopurinol-induced DILI if the HLA allele met the following two criteria: (1) the absolute difference of AF in cases and population AF is >15%; (2) *P* value < .05 from the Fisher exact test for the comparison of allopurinol cases to BioMe controls.

For the HLA genes that harbour the candidate HLA alleles we identified above, we followed up with allelic cluster and haplotype analysis. First, the NetMHCpan-2.8 algorithm in MHCcluster<sup>14</sup> was used to identify clusters of HLA alleles in the gene of interest based on their predicted peptide-binding specificity. We then tested the association between each allelic cluster and allopurinol DILI. That is, HLA alleles in the same cluster were coded as the same marker. We then tested each cluster frequency difference between cases and controls by the Fisher exact test. Second, haplotype association was tested for two HLA gene combinations for the genes of interest using the score statistics in the haplo.stats program.<sup>15</sup> Clusters or haplotypes meeting *P* < .05 were considered significant.

### 3 | RESULTS

#### 3.1 | Patient population

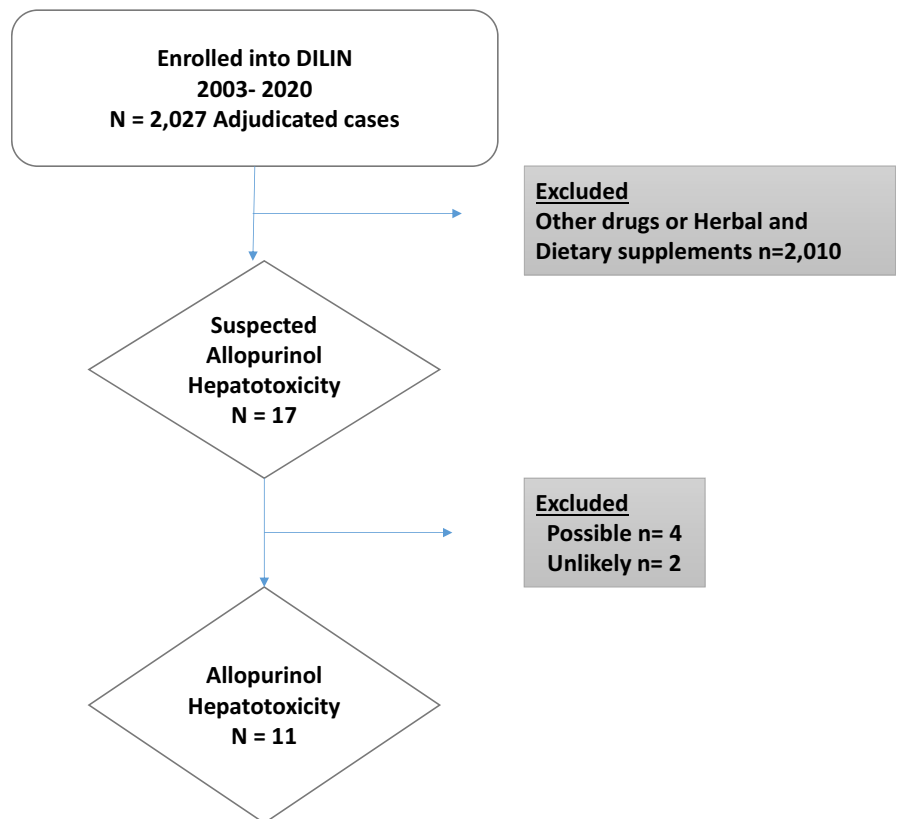
Between January 2003 and May 2020, 2220 cases were enrolled in the DILIN Prospective Study of which 2027 had undergone

6 months of follow up and formal adjudication for causality. In 17 cases, allopurinol was potentially implicated, but only 11 cases were judged to be definite (*n* = 4), highly likely (*n* = 4), or probably (*n* = 3) related to allopurinol. In the other six cases, allopurinol was considered only possible (*n* = 4) or unlikely (*n* = 2) the cause; these cases were not included in the analysis (Figure 1).

The 11 confirmed cases of allopurinol hepatotoxicity included six men and five women, with a median age of 60 years (range: 27-74 years) and a median BMI of 33.7 kg/m<sup>2</sup> (range: 24.1 to 42.1 kg/m<sup>2</sup>). Self-reported race was consistent with the genetically inferred race which was seven African Americans, three European Americans, and one White Hispanic (Table 1).

The median time from drug start to onset of liver injury was 52 days with a range of 12-89 days. The daily dose of allopurinol prescribed ranged from 100 to 600 mg/day, with a median dose of 200 mg/day. Other prescription drugs were implicated in four cases, but none were considered probable. Hypersensitivity features were frequent with rash observed in 91%, fever in 45%, and eosinophilia in 45% of patients. Medical comorbidities included type 2 diabetes in 46% and occasional alcohol use 18%. The median estimated glomerular filtration rate (GFR) at the time allopurinol was started was 70 ml/min and was below 50 ml/min in three patients (range 10.2-168.6).

At onset, all 11 patients were symptomatic and required hospitalization, and six were jaundiced. The median initial ALT was 362 U/L (range 84-716), Alk P 252 U/L (range 150-1176), total bilirubin 1.4 mg/dl (0.5-15.4), and R ratio 2.7 (0.4-13.8). The median peak bilirubin was 7.8 mg/dl, but in only three patients did the INR rise above 1.5. Only three (27%) patients had a low titer anti-nuclear antibody,



**FIGURE 1** Overview of study population. Amongst the 2027 adjudicated DILIN cases, there were 17 cases of suspected allopurinol hepatotoxicity. Eleven were categorized as definite/ highly likely/ probable allopurinol hepatotoxicity while 6 cases were attributed to other medications or disease processes

**TABLE 1** Clinical features in 11 allopurinol hepatotoxicity patients

Clinical feature	Allopurinol cases N = 11	African American N = 7	White or Caucasian N = 4
Age (yrs)	60 (27-74)	67 (42-74)	38 (27-52)
Male sex	6 (54%)	2 (29%)	4 (100%)
Hispanic	1 (9%)	0 (0%)	1 (25%)
BMI (Kg/m <sup>2</sup> )	33.7 (24.1-42.1)	34.6 (24.1-40.7)	30.4 (26.4-42.1)
Diabetes	5 (46%)	3 (43%)	2 (50%)
Time to onset (days)	52 ( 12-89)	52 (12-89)	49 (37-69)
Daily allopurinol dose (mg)	200 (100-600)	175 (100, 600)	300 (100, 300)
GFR (mg/dl)	70 (10.2-168.6)	36 (10.2, 116.4)	124 (70, 169)
Blood test results at DILI onset			
AST (U/L)	226 (105, 591)	180 (105, 485)	368 (168, 591)
ALT (U/L)	362 (84, 716)	331 (84, 651)	532 (135, 716)
Alk P (U/L)	252 (150, 1176)	220 (150, 829)	466 (252, 1176)
Total bilirubin (mg/dl)	1.4 (0.5, 15.4)	1.4 (0.5, 15.4)	1.5 (0.5, 3.9)
INR	1.1 (1.0, 1.4)	1.1 (1.0, 1.4)	1.1 (1.1, 1.1)
R ratio	2.7 (0.4, 13.8)	4.0 (0.4, 13.8)	2.2 (1.5, 3.3)
Peak Blood test results			
AST (U/L)	412 (122, 1174)	212 (122, 731)	622 (310, 1174)
ALT (U/L)	525 (173, 3388)	381 (173, 2075)	767 (522, 3388)
Alk P (U/L)	521 (157, 1415)	288 (157, 1105)	1242 (252, 1415)
Total bilirubin (mg/dl)	7.8 (1.0, 50.3)	7.8 (1.0, 45.7)	9.4 (2.4, 50.3)
INR	1.4 (1.1, 7.3)	1.4 (1.1, 2.2)	1.2 (1.1, 7.3)
Corticosteroids given	9 (82%)	5 (71%)	4 (100%)
DRESS	6 (54%)	4 (57%)	2 (50%)
Death within 6 months	3 (27%)	3 (43%)	0 (0%)
DILIN Severity score			
Mild	3 (28%)	2 (29%)	1 (25%)
Moderate	0 (0%)	0 (0%)	0 (0%)
Moderate -hospitalization	4 (36%)	3 (43%)	1 (25%)
Severe	2 (18%)	0 (0%)	2 (50%)
Fatal	2 (18%)	2 (29%)	0 (0%)

Note: Data reported as Median (range) or N (%)

Abbreviation: GFR, glomerular filtration rate obtained prior to treatment.

and none had detectable smooth muscle antibody. The course of liver injury varied, with five patients presenting first with rash and fever and later developing liver biochemical abnormalities after allopurinol had been discontinued.

### 3.2 | Clinical outcomes

During follow-up, 9 patients were treated with corticosteroids and 7 of these patients experienced clinical and biochemical improvement. Two corticosteroid-treated patients had progressive liver injury and died from liver failure at 38 and 45 days after presentation. Of the remaining two patients who did not receive corticosteroids, one

died 6 months after presentation from multiorgan failure and one improved spontaneously. No patient had evidence of chronic liver injury at 6 month follow-up. Severity scores ranked three patients as mild (1+: anicteric), four as moderate (3+: jaundiced and hospitalized), two as severe (4+: evidence of hepatic failure), and two as fatal (5+: death within 6 months of onset).

### 3.3 | DRESS

Although 10 patients had rash and seven had a fever, six were judged to have DRESS syndrome (cases 1,3,5,7,9,10) as judged by a RegiSCAR score  $\geq 4$ . RegiSCAR scores were 6 or more indicative of

definite DRESS in three patients and 4 or 5 indicative of probable DRESS in three patients (Table S1). Two patients underwent skin biopsy, which contributed to the diagnosis (Cases 3 and 7). No patient had the diagnosis of Stevens–Johnson syndrome or toxic epidermal necrolysis. Testing for human herpes virus-6 was done in one patient and was negative. One patient (Case 9) had CMV-DNA detectable by PCR at 9 days after DILI onset, but a liver biopsy on Day 8 shows no evidence of viral inclusions or changes typical of CMV hepatitis.

### 3.4 | Liver histopathology

Seven patients underwent liver biopsy at a median of 8 days after DILI onset (Table 2). A review of six available biopsies demonstrated three patterns of injury that had been previously established with allopurinol DILI: granulomatous hepatitis in one patient, acute hepatitis with necrosis in one patient, and cholestasis with mild inflammation in three patients. The sixth patient showed cholestatic injury with mild duct paucity superimposed on underlying chronic hepatitis C. One biopsy was no longer available for review but was reported to show granulomatous hepatitis. Three of the six available biopsies for review showed increased numbers of eosinophils in portal areas. Two cases showed evidence of ductopenia.

### 3.5 | Genetic and HLA associations

All 10 allopurinol DILI cases carried GG genotype at SNP rs2476601 in the PTPN22 gene, where G is the major allele, which is not surprising due to the low frequency of the minor allele, the A nucleotide (AF =0.1 for European Americans and 0.015 for African Americans from *gnomAD* (<https://gnomad.broadinstitute.org/>)).

HLA typing demonstrated an excess distribution of three class I HLA types: HLA-A\*34:02, HLA-B\*53:01, and HLA-B\*58:01 with allele frequencies that were twofold to 18-fold higher than both population and disease controls (Table 3, upper row; Table S2). HLA-B\*53:01 was present in five whereas HLA-A\*34:02 and HLA-B\*58:01 were present in four of the 11 patients. Thus, all except two subjects had at least one of the three candidate risk alleles (Table 4). These three alleles are rare in European-American and US Hispanic populations (AF <0.01 for all three alleles in both populations) and are several-fold more frequent among African Americans (AF 0.034, 0.118, and 0.038). Nevertheless, the limitation of the analysis to the seven African American patients with allopurinol hepatotoxicity still revealed a significantly higher frequency of the three alleles than among racially matched population and DILIN disease controls (Table 3, lower row). All six cases of DRESS syndrome had one of the three alleles and four had HLA-B\*58:01. The two subjects without even one of these alleles were both European Americans without DRESS in whom the causality score was only probable (Table 4).

Analysis of functional clustering of HLA class I alleles based on their predicted binding specificity (Figure S1) showed significant

TABLE 2 Liver histopathology findings\*

CASE	Days from onset	R ratio/ Bilirubin at biopsy	Total inflammation score	Granulomas	Eosinophils	Cholestasis	Ductopenia	Pattern of Injury
CASE 2	5	3.3/2.0	14	+	+	-	None	Granulomatous hepatitis
CASE 4	6	1.3/12.8	9	-	+	+	Mild	Chronic cholestatic hepatitis on Chronic hepatitis C
CASE 6	4	4.5/5.3	11	-	+	-	None	Acute hepatitis
CASE 7	29	3.9/1.0	4	-	-	+	None	Cholestatic hepatitis
CASE 9	8	1.6/15.4	3	-	+	+	None	Cholestatic hepatitis
CASE 11	9	1.5/0.5	4	-	-	+	Marked	Cholestatic hepatitis

\*Case 1 was not available for central review. By description it showed granulomatous hepatitis and extramedullary hematopoiesis.

**TABLE 3** Summary of HLA Alleles with Potential Association with Allopurinol Hepatotoxicity

Cases & controls:		Allopurinol cases		BioMe controls		DILIN controls		
All subjects	Sample sizes	n = 11		n = 8,780		n = 1,512		
	HLA allele	AF	count	AF	P value: Cases vs BioMe AF	AF	P value: Cases vs DILIN AF	
	A*34:02	0.182	4	0.023	.001	0.010	8.22E-05	
	B*53:01	0.227	5	0.085	.035	0.026	.0002	
African Americans	Sample size	n = 7		n = 5,816		n = 177		
	HLA allele	AF	count	AF	P value: Cases vs BioMe AF	AF	P value: Cases vs DILIN AF	
	A*34:02	0.214	3	0.033	.010	0.057	.050	
	B*53:01	0.357	5	0.120	.020	0.184	.155	
		B*58:01	0.214	3	0.046	.024	0.020	.005

Note: p-values were from Fisher exact tests to compare allopurinol Cases to BioMe controls and to DILIN controls

**TABLE 4** HLA Genotyping results for HLA-A and HLA-B

Case #	HLA-A		HLA-B		Race or ethnicity	DRESS	Causality score
	Allele 1	Allele 2	Allele 1	Allele 2			
Case 1	A*02:01	A*02:01	B*39:06	B*44:03	European American	No	3
Case 2	A*02:01	<b>A*34:02</b>	B*35:01	B*51:01	Hispanic	Yes	1
Case 3	A*02:02	A*33:03	<b>B*53:01</b>	<b>B*58:01</b>	African American	Yes	1
Case 4	A*33:03	A*74:01	B*35:01	<b>B*53:01</b>	African American	No	2
Case 5	A*03:01	<b>A*34:02</b>	<b>B*53:01</b>	B*58:02	African American	Yes	2
Case 6	<b>A*34:02</b>	A*68:02	B*15:10	B*41:02	African American	No	2
Case 7	A*30:02	A*68:02	B*07:02	<b>B*58:01</b>	African American	Yes	1
Case 8	A*01:01	A*02:05	B*13:02	<b>B*58:01</b>	European American	Yes	1
Case 9	A*02:01	A*26:01	<b>B*53:01</b>	<b>B*58:01</b>	African American	Yes	3
Case 10	A*01:01	<b>A*34:02</b>	B*37:01	<b>B*53:01</b>	African American	Yes	2
Case 11	A*02:01	A*02:01	B*08:01	B*56:01	European American	No	3

Note: The candidate alleles identified are highlighted in bold.

clustering of HLA-B alleles based on shared peptide binding specificities. HLA-B\*56:01, B\*51:01, B\*35:01, and B\*53:01 formed the first large cluster, and HLA-B\*53:01 and B\*58:01 were in the second cluster. However, because there were no instances of HLA-B\*56:01 and HLA-B\*51:01 in allopurinol cases, the first cluster analysis was limited to HLA-B\*35:01 and HLA-B\*53:01. The HLA-B\*35:01 and HLA-B\*53:01 cluster did not show significant association with allopurinol DILI in African Americans when compared with either BioMe controls (cluster frequency: 0.43 vs. 0.21,  $P = .051$ ) or DILIN controls (cluster frequency: 0.43 vs. 0.25,  $P = .128$ ). On the other hand, the HLA-B\*53:01 and HLA-B\*58:01 cluster was significantly associated with allopurinol DILI in comparison with either BioMe controls (cluster frequency: 0.57 vs. 0.17,  $P = 6.67 \times 10^{-4}$ ) or DILIN controls (cluster frequency: 0.57 vs. 0.20,  $P = .004$ ) in African-American subset and remained significant when all subjects were included (Table 5).

Haplotype analysis was performed for HLA-A\*34:02 with either HLA-B\*53:01 or HLA-B\*58:01 in the African-American subset. Haplotype HLA-A\*34:02-HLA-B\*53:01 showed higher frequency in allopurinol cases (0.14) than in both BioMe population controls (0.005,  $P = 8.79 \times 10^{-15}$ ) and DILIN controls (0.012,  $P = 3.01 \times 10^{-16}$ ) suggesting linkage disequilibrium between the two class I alleles. However, in contrast, the HLA-A\*34:02-HLA-B\*58:01 haplotype was not found in the 11 allopurinol cases and was in lower frequency (0.0004) in BioMe population controls, which implies that HLA-A\*34:02 is not in strong linkage disequilibrium with HLA-B\*58:01 and that the association of allopurinol liver injury with HLA-A\*34:02 and HLA-B\*58:01 is likely independent of each other.

Of note, none of the five liver injury cases with exposure to allopurinol that were excluded from this case series because of a low causality score for allopurinol carried HLA-B\*58:01, HLA-B\*53:01, or HLA-A\*34:02 (Table S3).

**TABLE 5** Allelic cluster association between allopurinol cases and controls from BioMe cohort, and between allopurinol cases and disease controls from DILIN

Analysis subset	Clusters	Cases AF	BioME controls AF	p value: Cases vs. BioMe AF	DILIN Controls AF	P values: Case vs. DILIN AF
African Americans	B*35:01,B*53:01	0.43	0.21	0.051	0.25	0.128
	B*53:01,B*58:01	0.57	0.17	6.67x10 <sup>-4</sup>	0.20	0.004
All subjects	B*35:01,B*53:01	0.32	0.17	0.085	0.10	0.004
	B*53:01,B*58:01	0.41	0.12	0.0007	0.04	1.54x10 <sup>-7</sup>

Note: P-values were from Fisher exact test by comparing the allele counts in allopurinol cases to controls from BioMe, and to DILIN cases due to other drugs or herbal (DILIN controls) for all alleles listed in the cluster.

## 4 | DISCUSSION

Allopurinol is a xanthine oxidase inhibitor that is commonly used to treat and prevent gout and hyperuricaemia. In calendar year 2017, over 14 million prescriptions of allopurinol were dispensed in the United States.<sup>16</sup> In the current study, we report 11 cases of well-documented acute liver injury associated with allopurinol use that were enrolled into the DILIN Prospective Study over a 16-year period. All of the cases developed evidence of liver injury within the first 3 months of treatment and were severe with all 11 patients requiring hospitalization and the majority developing jaundice. Similar to previous reports showing overrepresentation of allopurinol associated SJS/TEN in African Americans and Asians, there appeared to be an overrepresentation of African Americans compared with the overall DILIN cohort (60% vs 13%).<sup>20</sup> However, the frequency of alcohol use, diabetes, and concomitant medications as well as patient age and gender were similar to other cases enrolled into DILIN (data not shown).

In comparison with prior studies, most patients with allopurinol-induced liver injury had a mixed or cholestatic profile at presentation.<sup>4-7</sup> This difference may have been due to the inclusion of an older group of patients. However, most of the patients had prominent hypersensitivity features at presentation with rash noted in 10 cases, fever in five, and eosinophilia in five. Interestingly, four of the six patients who presented with prominent features of a DRESS reaction had initially normal liver biochemistries, and the liver enzymes increased only after their hospital admission (Case nos. 1, 3, 5, and 7). Subsequently, two of these delayed onset cases were confirmed to be DRESS via skin biopsy. (Cases 7 and 3). The peak bilirubin in three of the four cases was less than 2.0 mg/dl. One patient (Case 1) had a history of multiple congenital anomalies and hypogammaglobulinaemia as well suggestive of an immunodeficiency syndrome. In addition to a delayed onset of his granulomatous hepatitis, he also subsequently developed evidence of glomerulonephritis. In addition to these four, two other cases (Cases 9 and 10) had DRESS with high RegiSCAR scores and received corticosteroid therapy. Ultimately, nine patients in all were treated with corticosteroids. Three patients died 38, 45, and 182 days after DILI onset, and in two cases, death was considered to be liver related.

Liver histopathology demonstrated a spectrum of abnormalities (Table 2). One patient had a picture of granulomatous hepatitis (Case

1) in the setting of extra medullary haematopoiesis, whereas three patients showed cholestasis with mild inflammation. Two patients had evidence of ductopenia despite the fact that the biopsies were only obtained 6 and 9 days after DILI onset. These histopathological features are compatible with prior reports that report three patterns of injury that are typical for allopurinol: granulomatous hepatitis (particularly with fibrin ring granulomas), cholestatic hepatitis, and acute hepatitis with necrosis<sup>17</sup>

HLA analysis revealed an established association between HLA-B\*58:01 and allopurinol hypersensitivity skin reactions.<sup>18</sup> In addition, potential new associations are demonstrated for allopurinol DILI with HLA-B\*53:01 and HLA-A\*34:02 based on their higher frequencies in the cases than BioMe controls in African Americans. Although we did not have a large enough sample size to assess their effect in other racial groups, the only Hispanic patient carried both HLA-A\*34:02 and HLA-B\*53:01, and one of three European-American patients carried HLA-B\*58:01. The results of our haplotype analysis implied that the effect of HLA-B\*53:01 on DILI is likely due to being on the same haplotype with HLA-A\*34:02, but the effect of HLA-B\*58:01 is likely independent from HLA-A\*34:02. Although we cannot rule out other ethnicities, our data support the potential role of HLA-B\*58:01 as a risk factor for allopurinol DILI in African Americans.

In the comparison with other nonallopurinol DILIN cases (disease controls), the HLA-A\*34:02 and HLA-B\*58:01 showed significantly higher AF in allopurinol DILI cases amongst African Americans. In particular, the AF for HLA-B\*58:01 was 10-fold higher in allopurinol DILI cases than in nonallopurinol DILI cases. This is an encouraging observation as these two HLA alleles may specifically increase the risk of allopurinol-induced liver injury. However, it should be noted that in our comparison with the nonallopurinol DILI cases in DILIN ( $N = 1512$  in Table 3), the AF may be biased due to the different proportion of European Americans between allopurinol cases and DILIN disease controls (27% vs. 70%). This could explain the reason why more significant results were found in the full dataset than in the smaller African-American subset (eg, HLA-B\*34:02:  $P = 8.22 \times 10^{-5}$  vs. 0.05).

Prior studies have demonstrated a strong association of HLA-B\*58:01 and allopurinol associated SJS/TEN in both Asians and Caucasians with an odds ratio of 80 to 100.<sup>18,19</sup> Furthermore, epidemiological evidence suggests that African Americans and Asians



are more likely to be hospitalized for allopurinol-associated SCAR.<sup>20</sup> The pathogenesis of allopurinol-induced SCAR such as SJS/TEN and DRESS are consistent with a delayed immune-mediated reaction. Due to its high positive predictive value, pretreatment genotyping for HLA-B\*58:01 has been introduced in some Southeast Asian countries in an effort to avoid the use of the drug in these high-risk patients and has proven to be effective and reduce the incidence of severe skin reactions in some Asian countries.<sup>21</sup> However, up to 45% of patients of European ancestry with allopurinol-associated SJS/TENS do not carry HLA-B\*58:01.<sup>3</sup> Our current data now demonstrate a promising association between the HLA-B\*58:01 allele and allopurinol hepatotoxicity in African Americans. In terms of the suspected mechanism, allopurinol is metabolized to its active metabolite, oxypurinol, which is then cleared by the kidney. As a result, it is recommended that the dose of allopurinol be adjusted in patients with renal impairment.<sup>22</sup> We note that several of the patients in our study had impaired renal function and received high daily doses of allopurinol (Table 1 and Table S1). A recent study demonstrated that oxypurinol can activate HLA-B\*58:01-restricted CD8+ T cells.<sup>23</sup>

Limitations of our study include the relatively small number of allopurinol DILI cases available for genetic testing. For this reason, our selection of top HLA alleles was based on the objective criteria of AF difference and nominal significance threshold without considering the correction of multiple testing. However, the association signals were supported in the subset of African-American cases. Furthermore, we did not have access to an independent validation cohort to confirm our genetic associations. However, identification of a replication cohort may prove difficult because allopurinol accounted for only one of 133 cases of acute liver failure attributed to drugs in the Acute Liver Failure Study Group Registry<sup>24</sup> and for only one of 661 cases of acute liver failure due to drugs reported to the US liver transplant registry.<sup>25</sup> Furthermore, no cases of allopurinol hepatotoxicity were reported in two large European DILI registries.<sup>26,27</sup> However, our findings are in line with prior studies demonstrating a strong association between HLA-B\*58:01 and allopurinol associated SCAR.<sup>4-7</sup>

In summary, allopurinol hepatotoxicity is characterized by a short latency with prominent features of systemic hypersensitivity and a mixed or cholestatic serum enzyme profile at presentation. The majority of patients were treated with corticosteroids, which appeared to speed up the recovery from the symptoms and signs of hypersensitivity but which had unclear effects on the course of the liver injury as two of the nine treated subjects died. These results also demonstrate that African Americans who are at a higher risk of severe cutaneous adverse reactions from allopurinol are similarly at higher risk of liver injury from this agent. This overrepresentation may be due to the higher frequency of HLA risk alleles among African Americans compared with European Americans as well as to an increased frequency of comorbidities such as chronic kidney disease. Interestingly, African Americans do not appear to be at higher risk of gout, despite the overrepresentation of hypertension and chronic kidney disease.<sup>20</sup> Because allopurinol was recently recommended as the first-line agent for patients with hyperuricaemia

and gout including those with renal impairment, additional cases of allopurinol DILI will likely be seen.<sup>28</sup>

Overall, 50% of subjects who had a RegiSCAR score of 4 or more for DRESS in this cohort carried HLA-B\*58:01 and two thirds were African American. The strong genetic association with HLA-B\*58:01 in both African Americans and the overall population strongly implicated HLA-B\*58:01 in the pathogenesis of allopurinol DILI. Currently, HLA-B\*58:01 has a positive predictive value of 1% to 3% for allopurinol SCAR, which given the overall prevalence of disease would be expected to be lower for allopurinol DILI. This suggests that using HLA-B\*58:01 testing in isolation, more than 1000 allopurinol-treated patients would need to be tested to prevent one case of either SCAR or DILI. Furthermore the negative predictive value of HLA-B\*58:01 is not 100%, meaning that cases of DILI will arise that do not carry HLA-B\*58:01. Febuxostat is an alternative to allopurinol; however, it is more expensive and carries a black box warning for increased risk of all cause and cardiac associated death.<sup>28</sup> Therefore, in the current environment, allopurinol will likely to continue to be used as a first-line agent for gout and hyperuricaemia. From our data, testing for HLA-B\*58:01 and potentially other class I risk alleles may be of value in assessing allopurinol-treated patients with unexplained liver injury.

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#### CONFLICTS OF INTEREST

Dr Fontana has received research support from Gilead, BMS, and Abbvie and consulted for Sanofi. Dr Phillips has no disclosures relevant to this manuscript. Within the past 3 years, she has served as

a consultant to Biocryst Pharma and Janssen Pharmaceuticals; she serves as Section Editor for Drug Allergy for UpToDate. She is co-director of IIID Pty Ltd that holds a patent for a method for identification and determination of hypersensitivity to abacavir, and she holds a patent for detection of human leukocyte antigen A\*32:01 in connection with diagnosing vancomycin associated drug reaction with eosinophilia and systemic symptoms without any financial remuneration. Dr Barnhart, Dr Hoofnagle, Dr Kleiner, Dr Li, and Dr Saeed have no reported conflicts.

## ETHICS APPROVAL STATEMENT

The study protocol and consent forms were reviewed and approved by local institutional review boards and a central data safety and monitoring board. All study participants provided written informed consent.

## ORCID

Robert J. Fontana  <https://orcid.org/0000-0001-9161-5892>

David Kleiner  <https://orcid.org/0000-0003-3442-4453>

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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