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*HLA-B*53:01* is a significant risk factor for liver injury due to phenytoin and other anti-epileptic drugs in African Americans

Paola Nicoletti[1], **Andrew Dellinger**[2], **Yi-Ju Li**[2], **Huiman Barnhart**[3], **Elizabeth Phillips**[4], **Naga Chalasani**[5] **Drug Induced Liver Injury Network (DILIN) investigators**

[1]Icahn School of Medicine at Mount Sinai, New York, NY, USA

[2]Duke University School of Medicine, Durham, NC, USA

[3]Duke Clinical Research Institute, Duke University, Durham, NC, USA

[4]Vanderbilt University, Nashville, TN, USA

[5]Indiana University Health & Indiana University School of Medicine, Indianapolis, IN, USA

Abstract

Objective: To investigate HLA alleles associated with anti-epileptics (AEDs) liver injury in African Americans (AA).

Methods: 21 AA with AED DILI, 176 AA with DILI due to non-AEDs, and 5816 AA population controls were included.

Results: *HLA-B*53:01* was significantly associated with aromatic AED-DILI (OR: 4.52, 95% CI: 2.42-8.44, P = $1.46x10^{-5}$). Phenytoin DILI showed the strongest association with *HLA-B*53:01* (OR: 9.17; 95% CI: 3.61 - 23.28, P = $1.1x10^{-5}$). The *HLA-B*53:01* allele was carried by 8 out of 9 AA phenytoin-DILI cases.

Conclusion: *HLA-B*53:01* is a significant risk factor for liver injury due to anti-epileptics, particularly phenytoin, in African Americans.

Keywords

DILI; HLA; *HLA-B*53:01* ; DRESS

Correspondence should be addressed to Naga Chalasani, MD, Indiana University School of Medicine at nchalasa@iu.edu.
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Introduction

Anti-epileptic drugs (AEDs) are an important cause for idiosyncratic drug induced liver injury (DILI) in African Americans (AA). Phenytoin is the third most common cause for DILI in African Americans, behind trimethoprim-sulfamethoxazole (TMP-SMZ) and isoniazid.[1] AEDs, including phenytoin and carbamazepine can cause severe cutaneous adverse drug reactions and drug reaction with eosinophilia and systemic symptoms (DRESS), which are strongly associated with *HLA-B*15:02* and *HLA-A*31:01* in European and Asian populations.[2] Recent studies identified HLA alleles as risk factors for DILI due to agents such as allopurinol, TMP-SMZ and green tea extract.[3,4,5] As HLA risk alleles for AED-DILI have not yet been identified, we investigated their role among AA in whom there is heightened burden of AED-DILI.

Methods

We analyzed 21 AA patients with AED-DILI and 176 AA patients with non-AED prescription DILI (DILI controls) enrolled into DILIN studies. DILI cases with definite, highly likely, or probable causality were included. [3, 4, 6] DRESS was assessed in AED-DILI cases by RegiSCAR scoring system as previously described.[7] Given the low DILI incidence rate in the general population, we constructed an AA control group (N = 5816) from the PAGE BioME biobank cohort (phs000925.v1.p1). HLA class I and II alleles were determined by direct sequencing (Illumina MiSeq) in DILI cases and were imputed from genotype data in controls as previously described.[4]

Genetic ancestry was inferred by EIGENSTRAT as previously described.[4] A case/control association test was conducted for each allele using a Fisher Exact test or Firth logistic regression (≥ 40 cases) adjusting for population structure using PC 1 and 2. [4, 6] The false discovery rate (FDR) was computed to correct for multiple testing.[4] Alleles with qvalue < 0.10 were considered as significant. See supplementary material for additional information on methods.

Results

Selected characteristics of 21 AA with AED DILI and 176 AA DILI controls are shown in Table S1. Of 21 AA with AED-DILI, 17 were due to aromatic AEDs (phenytoin 9, carbamazepine 3, lamotrigine 4, and ethosuximide 1) and 4 were due to non-aromatic AEDs (topiramate 2, valproate 1, and pregabalin 1).

*HLA-B*53:01* was significantly associated with AED-DILI (OR: 4.52, 95% CI: 2.42-8.44, P = 1.4×10^{-5}, FDR = 0.001). *HLA-B*53:01* allele frequency was 3 times higher in AED-DILI cases than in controls (0.38 vs 0.12). Phenytoin DILI showed the strongest association with *HLA-B*53:01* (OR: 9.17; 95% CI: 3.61 - 23.28, P = 1.1×10^{-5}, FDR = 0.0006, Table 1). The *HLA-B*53:01* allele was carried by 8 out of 9 African Americans with phenytoin DILI (22% were homozygous). All *HLA-B*53:01* carriers with phenytoin DILI developed DRESS and 87% had the *HLA-B*53:01*/*HLA-C*04:01* haplotype (P = 4.0×10^{-5}). *HLA-B*53:01* was not carried by any of 11 European American patients with phenytoin-DILI. A

second allele, *HLA-A*03:01* was also independently associated with phenytoin-DILI (OR = 9.31; 95% CI: 3.12-26.73, P = 0.0002, Table S2).

*HLA-B*53:01* was also carried by AA patients with DILI due to carbamazepine, pregabalin, topiramate and lamotrigine, suggesting that *HLA-B*53:01* could be a shared genetic risk variant across different AED drugs for liver injury among AA. *HLA-B*53:01* allele frequencies for phenytoin DILI, non-phenytoin AED DILI, and AA population controls were 0.56 (Carriage rare [CR] 0.89), 0.25 (CR 0.33), and 0.12 (CR 0.22), respectively. 67% of *HLA-B*53:01* carriers with AED DILI developed DRESS and 62% had the *HLA-B*53:01*/*HLA-C*04:01* haplotype ($P = 3.0x10^{-4}$). *HLA-B*53:01* carriers were older and had more severe DILI than non-carriers (Table S3).

Interestingly, *HLA-B*53:01* was significantly associated with DILI due to any causal agent in the entire cohort of 197 African American patients with DILI due to prescription agents (OR: 1.66, 95% CI: 1.3, 2.15, P = 0.0003, Table 2). Among 176 African Americans with non-AED DILI, *HLA-B*53*:01 allele frequency was significantly higher compared to controls (AF = 0.16 vs 0.12, OR: 1.4, 95% CI: 1.04, 1.87, P = 0.03). In this group, *HLA-B*53:01* was enriched in allopurinol (independently from *HLA-B*58:01*) isoniazid, and TMP-SMZ DILI cases.

Among the 17 AA with DILI due to aromatic-AEDs, *HLA-B*53:01* was still the most significant associated allele (Table 1), with 90% of the carriers developing DRESS. African Americans with DILI due to aromatic-AEDs also showed a higher frequency of *HLA-A*32:01* compared to controls (0.09 vs 0.01; P = 0.009). *HLA-A*32:01* was strongly associated with lamotrigine DILI in African Americans (0.25 vs 0.01; P = 0.004, FDR = 0.07 Table 1). For comparison, *HLA-A*32:01* showed a higher frequency in 9 European Americans (0.17 vs 0.03, P = 0.02) and in 2 Hispanic lamotrigine DILI patients (0.50 vs 0.04, P = 0.008) in DILIN studies than in population controls.

The performance of *HLA-B*53:01* for predicting liver injury from phenytoin or any AED, and *HLA-A*32:01* for predicting liver injury from lamotrigine among AA are shown in Table S4. They showed very high specificity and NPV albeit low sensitivity and PPV. *HLA B*53:01* showed excellent sensitivity (89%), specificity (100%), and NPV (100%) but a PPV of only 0.006 for predicting phenytoin DILI.

Discussion

Our results suggest *HLA-B*53:01* as a significant risk factor for liver injury due to phenytoin and other AEDs in AA. *HLA-B*53:01* has previously been associated with raltegravir-induced DRESS in AA and Hispanic patients with HIV.[8] AA with allopurinol DILI were also enriched for the allele, and 75% of carriers developed DRESS.[3] Similarly, the majority of AA patients with AED-DILI with *HLA B*53:01* developed DRESS. Interestingly, DILI due to TMP-SMZ,[4] and possibly allopurinol[3], is also associated with *HLA-B*35:01* among AA. *HLA-B*53:01* and *HLA-B*35:01* differ in a few amino acids showing analogous binding site affinities;[8] consequently they may elicit a similar

T-mediated hypersensitivity. Our study adds to growing literature on the importance of HLA B*53 in human disease.[9-11]

Given the high NPV of *HLA-B*53:01* and *HLA-A*32:01* and the serious consequences of AED DILI and DRESS among AA, addition of these HLA alleles might improve the risk stratification at a US population level of commercially available panels, which currently include only *HLA-A*31:01* and *HLA-B*15:02*.[12] Limitations of our study include its small sample size and lack of AED-exposed controls without DILI. Future studies should be designed to overcome these limitations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Data sharing statement:

Research Data for this article where pertinent are included in the supplemental material. Patient level data are available in a deidentified fashion from the NIDDK repository in accordance with NIH data sharing policies.

Abbreviations:

DILI	drug induced liver injury
DILIN	Drug Induced Liver Injury Network
DRESS	Drug reaction with eosinophilia and systemic symptoms
ALT	serum alanine aminotransferase
AST	serum aspartate aminotransferase
Alk P	serum alkaline phosphatase

T Bili	serum total bilirubin
HLA	human leukocyte antigen
SNP	single nucleotide polymorphism
OR	odds ratio
CI	confidence interval
AF	allele frequency
CR	carriage frequency
PC	principal component
FDR	False discovery rate

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Table 1

Association of AED-DILI with HLA alleles across drug classes in African American DILI patients

Drug	Allele	OR (95%CI)	P	FDR	AF cases	AF controls	CF cases	% Carriers with DRESS
Phenytoin (N=9)	***B*53:01***	**9.17 (3.61-23.28)**	**$1.1x10^{-5}$**	**0.0006**	**0.56**	**0.12**	**0.89**	**100%**
	*A*03:01*	6.12 (2.29-16.34)	0.002	0.04	0.33	0.08	0.44	100%
Lamotrigine (N=4)	*A*32:01*	26.97 (5.40-134.8)	0.004	0.07	0.25	0.01	0.50	100%
All AEDs (N = 21)	***B*53:01***	**4.52 (2.42-8.44)**	**$1.46x10^{-5}$**	**0.001**	**0.38**	**0.12**	**0.57**	**75%**
	*A*32:01*	6.22 (1.90-20.38)	0.01	0.28	0.07	0.01	0.14	67%
Aromatic AED (N =17)	***B*53:01***	**4.54 (2.27-9.09)**	**$8.7x10^{-5}$**	**0.006**	**0.38**	**0.12**	**0.59**	**90%**
	*A*32:01*	7.83 (2.37-25.91)	0.009	0.14	0.09	0.01	0.18	67%

Abbreviations: AA: African Americans; AED: Anti-epileptic drugs; OR: Odds ratios ; 95% CI: 95% confidence intervals (95%CI); P= p-values; AF = Allele Frequency; FDR = False Discovery Rate; DRESS: Drug reaction with eosinophilia and systemic symptoms.

Table 2

Association of *HLA-B*53:01* with DILI across multiple drug groups in African American patients

Drug group	n	Case AF¶	OR (95% CI)	P
All DILI	197	0.18	1.66 (1.27,2.15)	0.0003 *
Non-AED-DILI	176	0.16	1.40 (1.04,1.87)	0.03
Non-AED, non-allopurinol DILI	169	0.15	1.32 (0.96,1.77)	0.09
Non-phenytoin, non-allopurinol DILI	181	0.15	1.39 (1.03,1.84)	0.03
Phenytoin DILI	9	0.56	9.17 (3.61-23.28)	1.1x10−5 **
Allopurinol DILI	7	0.36	4.08 (1.36,12.18)	0.02
Isoniazid DILI	21	0.24	2.29 (1.13,4.68)	0.03
TMP-SMZ DILI	14	0.18	1.60 (0.61,4.20)	0.37
Methyldopa DILI	7	0.21	2.00 (0.56,7.18)	0.23

¶HLA-B*53:01 allele frequency in AA population controls is 0.12; *FDR= 0.004; **FDR<0.001

Abbreviations: AA: African Americans; AED: Anti-epileptic drugs; Odds ratios (OR), 95% confidence intervals (95%CI) and p-values (P). AF = Allele Frequency; FDR = False Discovery Rate; AF not-AED DILI = Allele is computed on the entire DILIN DILI cases across non-AED drugs for the appropriate ethnic group; CF = Carriage Frequency; TMP-SMZ: Trimethoprim-sulfamethoxazole.

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