Ahmad Jawad (Orcid ID: 0000-0003-1384-2349)
Fontana Robert (Orcid ID: 0000-0001-9161-5892)
Kleiner David (Orcid ID: 0000-0003-3442-4453)

Liver Injury due to Intravenous Methylprednisolone in the Drug-Induced Liver Injury Network

Short Title: Methylprednisolone Liver Injury

Authors: Jawad Ahmad M.D.[1], Yi-Ju Li[2], Elizabeth Phillips[3], Andrew Dellinger[2], Paul H. Hayashi[4], Naga Chalasani[5], Robert J. Fontana[6], David E. Kleiner[7], Huiman X. Barnhart[8], Jay H. Hoofnagle[9] for the Drug-Induced Liver Injury Network.

From: [1]Recanati-Miller Transplantation Institute, Icahn School of Medicine at Mount Sinai, New York; [2]Department of Biostatistics and Bioinformatics, Duke School of Medicine, Durham, NC; [3]Vanderbilt University Medical Center, Nashville, TN, [4]Food and Drug Administration; [5]Indiana University; [6]Division of Gastroenterology and Hepatology, University of Michigan, Ann Arbor, MI; [7]Laboratory of Pathology, Intramural Division, National Cancer Institute, National Institutes of Health (NIH), Bethesda, MD; [8]Duke Clinical Research Institute, Durham, NC; [9]Liver Disease Research Branch, Division of Digestive Diseases and Nutrition, National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), NIH, Bethesda, MD

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Correspondence and reprint requests to:

Jawad Ahmad, M.D.

Professor of Medicine

Division of Liver Diseases

Icahn School of Medicine at Mount Sinai, New York, NY 10029

Phone: 212 241 8035

Fax: 212 731 7340

E-mail: jawad.ahmad@mountsinai.org

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DILIN prospective study was approved by the Institutional Review Boards at each clinical site and data-coordinating center and by a central Data Safety and Monitoring Board appointed by the NIDDK. All enrolled subjects provided written informed consent.

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Abbreviations

AF Allele frequency

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AIH	Autoimmune hepatitis
ALT	Alanine aminotransferase
Alk P	Alkaline phosphatase
CF	Carrier frequency
DILI	Drug induced liver injury
DILIN	Drug Induced Liver Injury Network
HiC	High confidence
iv	Intravenous
MP	Methylprednisolone
MS	Multiple Sclerosis
NIH	National Institutes of Health
NIDDK	National Institute of Diabetes and Digestive and Kidney Diseases

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Abstract

Background and Aims: Short courses of intravenous (iv) methylprednisolone (MP) can cause drug induced liver injury (DILI). The aim of this study was to assess the clinical features and HLA associations of MP-related DILI enrolled in the US DILI Network (DILIN).

Methods: DILIN cases with MP as a suspected drug were reviewed. DILIN causality scoring was assigned on a 5-point scale (definite, highly likely, probable, possible, unlikely). All cases with MP causality scores of definite, highly likely or probable were analyzed. HLA data from direct sequencing were analyzed.

Results: Eleven cases of definite, highly likely, or probable MP DILI were identified. The median age was 48 years; 73% were female; median latency to onset was 30 days; 55% were jaundiced; and all had hepatocellular injury with one patient requiring transplantation. Nine of the 11cases were in patients with multiple sclerosis (MS). Liver biopsies in 7 cases revealed mild acute hepatitis with/without cholestasis. HLA data demonstrated that *HLA-DRB1*15:01*, the primary HLA class II allele associated with MS was over-represented. *HLA-DQB1*06:02-HLA-DQA1*01:02* which is haplotypic with the *HLA-DRB1*15* haplotype was more common in the MP DILI cases compared to other DILI controls (p=0.03) and to DILI controls exposed to MP (p=0.04).

Conclusion: MP DILI is characterized by hepatocellular injury, short latency and generally rapid recovery. There was no independent HLA haplotype associated with MP DILI.

Keywords: Drug induced liver injury; methylprednisolone; multiple sclerosis; HLA genotype associations.

Lay Summary: Intravenous methylprednisolone (MP) can cause liver injury, particularly when used in patients with multiple sclerosis (MS). The injury can be severe with more than half the patients developing jaundice. There were no genetic risk factors associated with MP use beyond the known genetic risk factors for MS.

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Introduction

Intravenous (iv) methylprednisolone (MP) is a potent anti-inflammatory agent used to treat a variety of immune-mediated diseases including multiple sclerosis (MS) and Graves ophthalmopathy. It has a rapid onset of action and is almost completely excreted within 12 hours (1). Typically, iv MP is administered 0.5-1 gram daily for 2-3 days until the patient's condition stabilizes and then continued at a lower dose or converted to oral prednisolone or prednisone. Lower iv daily doses of 40-80 mg are routinely used in patients with inflammatory bowel disease and asthma requiring hospitalization.

In patients with multiple sclerosis (MS), national guidelines recommend iv MP 1 gram daily for 3 to 5 days for patients with a relapse or exacerbation of disease in whom oral corticosteroids have failed or hospitalization is required (2). Idiosyncratic liver injury following MP administration has been reported with a similar presentation as autoimmune hepatitis (AIH)- a disease with known underlying HLA associations and which is also routinely treated with corticosteroids (3).

The Drug-Induced Liver Injury Network (DILIN) is a National Institutes of Health (NIH)-funded multicenter observational cohort study that has been enrolling patients with suspected DILI in the United States since 2004 (4,5). Because DILI is a diagnosis of exclusion, patients who have a temporal exposure to a drug or herbal and dietary supplement (HDS) undergo a thorough evaluation with laboratory data and imaging. Causality assessment is performed by expert review of each case after exclusion of competing causes and 6 months of follow-up. As the literature on DILI has advanced over the last 2 decades, the clinical phenotype of liver injury attributed to iv MP

particularly in patients with MS or Graves' ophthalmopathy has been reported (3, 6-11). The primary objective of this present study was to identify the clinical, histological, and genetic characteristics of cases of DILI attributed to iv MP enrolled in DILIN from 2004 to 2022. We also reviewed the available literature on other cases of iv MP hepatotoxicity to provide summary data and guidance to practicing clinicians.

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Methods

A detailed description of the DILIN study design has been previously published (4). The DILIN prospective study was approved by the Institutional Review Boards at each clinical site and data-coordinating center and by a central Data Safety and Monitoring Board appointed by the NIDDK. All enrolled subjects provided written informed consent. All authors had access to the study data, reviewed, and approved the final manuscript.

Briefly, patients with suspected DILI are eligible who meet predefined laboratory criteria including elevations in aspartate aminotransferase (AST), alanine aminotransferase (ALT), or alkaline phosphatase (Alk P) levels with or without jaundice or coagulopathy. Liver biopsy, though not required for enrollment, is centrally reviewed by an expert pathologist whenever available. Prospective subjects are enrolled within 6 months of onset of injury and followed up at 6 months after enrollment. Patients with persistent liver injury at 6 months are considered to have chronicity and are asked to return annually for up to 4 years. Formal causality adjudication is undertaken at least 6 months after enrollment using expert opinion by 3 hepatologists independently (12). The 3 reviewers come to a consensus opinion score which is voted upon by the full Causality Committee in cases of disagreement. The DILIN expert system employs a 5-point likelihood score: 1 (definite: ≥ 95% likelihood), 2 (highly likely: 75%-94% likelihood), 3 (probable: 50%-74% likelihood), 4 (possible: 25%-49% likelihood) or 5 (unlikely: <25% likelihood). Scores of 1, 2 and 3 are considered high confidence (HiC) cases, while scores of 4 and 5 are considered possibly or unlikely DILI and an alternative diagnosis is determined. In cases in which more than one agent is

implicated, an overall causality score is assigned, and separate causality scores are assigned to each suspected drug, one being assigned the primary cause. No more than 3 suspect drugs are allowed to be listed as possibly implicated, but all drugs taken within 2 months of onset were recorded as concomitant medications.

The current study focusing upon MP involved two stages. Initially patients enrolled into the DILIN where the liver injury was deemed at least possibly related to MP (i.e. scores 1-4) were reassessed. In the second stage, all cases in which MP was listed as a concomitant drug (i.e. not originally implicated as causal) were reassessed by two investigators independently (JA, JHH). All cases that the two investigators considered as "possibly" related to MP were then re-adjudicated by the full Causality Committee.

HLA sequencing was performed on all DILIN cases using the Illumina MiSeq platform at the Vanderbilt University Medical Center as previously described (13). Since many of the HiC MP DILI cases occurred in MS patients, the HLA associations may have been related to MS rather than a risk of DILI related to MP. Hence, to compare HLA allelic associations with the HiC MP DILI cases we looked at 3 control groups. Firstly, HiC cases of DILI attributed to another medication but who had been exposed to MP within the previous 2 months and adjudicated as unlikely to be MP DILI (MP exposure controls). Secondly, patients with MS who developed HiC DILI attributed to another medication other than MP (MS disease controls). Thirdly, HiC cases of DILI attributed to other conventional medications (excluding those due to HDS) who were not exposed to MP and did not have MS (DILI controls).

Statistical Analysis:

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Summary statistics were computed for all MP implicated cases and DILI cases not caused by MP occurring in patients with MS (the MS controls). Due to the limited number of MP cases, direct allele frequency (AF) difference between MP cases and each comparison group was tested by Fisher's exact tests. As most MP cases also had MS, the HLA allele associations of HiC DILI cases with MS were compared to those associated with other DILI cases without MP and MS. Multivariable logistic regression was performed to test HLA allelic association with MS DILI after adjusting for age and the first principal component (PC) for the potential impact from population stratification. PCs were derived from the genome-wide genotype data in the DILIN cohort as previously described (13). To correct for multiple testing, the Qvalue, one of the false discovery rate (FDR) methods, was computed for each HLA allele using the QVALUE program (14). Haplotypes for the combination of HLA alleles of interest were explored by the Cobygram software, and haplotype frequency was compared between the HiC MS DILI group and the control groups. Finally, the three previously reported genetic variants related to DILI in DILIN, rs2476601 in PTPN22 (15), the GG genotype of rs1363907 in ERAP2 (16), and rs61745685 in ERAP1 (17) were assessed for association with MP DILI.

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Results

Of the 2493 patients enrolled in the DILIN Prospective Study between 2004 and 2022, 11 were adjudicated as HiC MP-DILI cases (Table 1, Supplementary Table 1), 10 were possible MP-DILI, and 64 were exposed to MP but considered unlikely to be MP-DILI (Figure 1). Among the 11 HiC cases, 2 were definite, 4 highly likely, and 5 probable. Two HiC DILI cases initially had MP listed as a concomitant medication that upon re-review and re-adjudication was attributed to MP. All 11 had received iv MP at a dose of at least 1 gram daily, nine for 3 to 5 days, one for 11 days and one intermittently over 150 days. The median age was 48 years, 8 (73%) were female, 8 European American, 2 African American, and one Asian. Nine patients were being treated for MS, one for a spinal cord contusion and one for an ulcerative colitis flare. The median latency (from drug start to onset of DILI) was 30 days (range 4 to 150 days). All patients had hepatocellular injury with usually rapid improvement. A 49-year-old female patient, however, without known underlying liver disease, developed severe hepatocellular injury (ALT 1874 U/L) with jaundice (total bilirubin 32.4 mg/dL) and synthetic dysfunction (INR 3.5) several weeks after receiving 3 days of iv MP 1g for her MS flare. She did not improve, was listed as a status 1, and underwent successful liver transplantation with the explanted liver showing portal inflammation with increased plasma cells, bridging necrosis and parenychmal collapse. Autoantibodies were noted in a minority of patients. Liver biopsies in 7 patients showed mild acute hepatitis with lobular and interface necroinflammation. Three cases had features of autoimmune hepatitis (AIH) with mild to moderate interface hepatitis. Three HiC cases were treated

for the liver injury with tapering doses of oral prednisone with normalization of liver tests within 5 weeks. One patient was also treated with ongoing azathioprine.

Of the 10 cases that were considered possibly related to MP, demographic data were similar to the HiC MP cases (Supplementary Table 2). The dose of MP was unavailable in most cases, and 4 patients were being treated for MS. Five cases were adjudicated as HiC DILI but were attributed to another agent rather than MP. All 10 cases had a hepatocellular injury and 3 had jaundice. Eight patients recovered while 2 had residual evidence of liver injury at six months.

In the entire DILIN cohort of 2439 patients, 35 occurred in MS patients. Excluding 9 cases attributed to MP and 5 cases that were judged to be only possibly or unlikely DILI, 21 were adjudicated as having HiC DILI due to other drugs (MS disease controls) (Supplementary Table 3). Of the 21, 13 were attributed to drugs being used to treat MS, including 11 due to interferon beta, one to dimethyl fumarate, and one to natalizumab. In the 8 remaining MS patients, the injury was attributed to nitrofurantoin (N=2) and one each from amoxicillin/clavulanate, oxaprozin, ribociclib, carbamazepine, atorvastatin, and nivolumab.

HLA data were available in most cases and controls (Table 2, Supplementary Table 4). Three class II alleles; *HLA-DQB1*06:02*, *HLA*DQA1*01:02*, and *HLA-DRB1*15:01*, were significantly more frequent in HiC MP DILI cases than in MP exposure and DILI controls. These three alleles are associated with MS (18). They have also been associated with protection from type I diabetes although the greatest effect comes from HLA-DQB1*06:02 (19). Regarding MP DILI, among these three HLA alleles, *HLA-DQB1*06:02* was most significant with an allele frequency (AF) of 0.40

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(carrier frequency, CF =70%), which is higher than estimated from USA population databases, 0.12 for European, 0.20 for African, and 0.04 for Asian-Americans (allellefrequencies.net). *HLA*DQA1*01:02* ranked as the second most significant association with MP DILI with an AF of 0.45 (and CF of 80%), higher than the frequencies in US population databases (0.23 in European Americans). The third strongest HLA genotype association with the MP cases was *HLA-DRB1*15:01*, a well-recognized HLA class II allele associated with MS. This allele was found in 5 of the 10 MP cases, all of whom were heterozygotes (AF=0.25) and had MS. A similar AF was also observed in MS disease (non-MP DILI) controls (AF=0.24). In contrast, lower AF of this allele was observed in the DILI controls (AF=0.13) and MP exposure controls (AF=0.11), which are a similar rate to those in the US Non-Hispanic White population (AF=0.11).

These three class II alleles identified for MP DILI also form a haplotype called "DR15 haplotype" (*DRB1*15:01-DQA1*01:02-DQB1*06:02*), which is a known primary HLA haplotype risk factor for MS (18). The visualization of the DR15 haplotype is depicted in Figure 2, which shows the difference in haplotype frequency between MP DILI cases and two non-MS comparison groups. The DR15 haplotype was present in half of MP DILI cases (one case being homozygous), leading to a higher haplotype frequency as compared to DILI controls (Table 2). In contrast, there was no difference in the haplotype frequency between the MP cases and the MS disease controls (haplotype frequency: 0.30 vs. 0.24, p=0.75).

The two HLA alleles, *HLA-DRB*03:01* and *DRB*04:01*, known to be associated with spontaneous AIH were not overrepresented in HiC MP cases and MS disease

controls as compared to other DILI controls (e.g. *DRB1*03:01* AF= 0.05 vs. 0.12; *DRB1*04:01* AF= 0 and 0.05) (Table 2). The frequencies of these alleles in the MS disease controls (0.12 for DRB1*03:01 and 0.05 for DRB1*04:01) were similar to those in Non-Hispanic White control populations (*DRB1*03:01* AF= 0.12 and *DRB1*04:01* AF= 0.09: from allellefrequencies.net). Finally, the target variants in PTPN22, ERAP1, and ERAP2 were not associated with MP DILI when compared to DILI controls and MP exposure controls. However, the ERAP1 rs62376414 showed nominal significant higher AF in HiC MP cases than in MS disease controls (AF: 0.20 vs. 0.02, p=0.03) (Table 2).

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Discussion

Idiosyncratic DILI from iv MP has been increasingly reported over the last 20 years (3,6-11) typically occurring in patients with MS or Graves' disease who frequently receive 0.5-1 gram daily for 3 to 5 days or more. Of the 11 HiC DILI cases due to MP seen in DILIN, 9 occurred in MS patients but none were associated with Graves' disease. The clinical presentation was very consistent, with a rapid onset of severe acute hepatocellular injury with or without jaundice arising within a few days to weeks after iv MP treatment for a flare of MS. Autoimmune serologies were positive in a minority of patients and the liver histology demonstrated varying degrees of an acute hepatitis, with features of AIH in some patients. This association seems paradoxical as corticosteroids are used to treat de novo AIH and drug-induced AIH. However, an immune mediated hyper-reactivity via rebound or reconstitution after MP is stopped or tapered may be occurring in susceptible patients (11). One case developed acute hepatic failure requiring liver transplantation while almost all the remaining cases had self-limited hepatitis without the need for long-term immunosuppressive therapy.

Prospective data in MS patients treated with pulsed iv MP suggests that liver injury is not infrequent. Amongst 175 MS patients given iv MP at a dose of 1g daily for 5 days, 15 (8.6%) patients developed liver injury, 4 with jaundice. Case adjudication suggested MP-DILI in 3 patients and de novo AIH in another 3 (3). The current data from DILIN did not suggest co-incidental AIH as HLA alleles of AIH were not over-represented although the sample size was small.

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Recent studies with liver biopsy have also suggested MP liver injury is due to idiosyncratic DILI rather than sporadic AIH, particularly because the injury was self-limited and spontaneously resolved without need for long-term immunosuppression (6, 7). In the French pharmacovigilance database, 97 MP-DILI cases were analyzed with similar findings as the current study with a mild to moderate hepatocellular injury with quick resolution (7). Patients with MS were again over-represented, but there were also patients on MP for other autoimmune diseases and 20% developed DILI from oral corticosteroids. In the current study, 10 patients recovered completely but one patient needed liver transplantation. Interestingly this patient had an episode of acute hepatitis 12 years prior, not recognized as DILI but treated with oral corticosteroids (without treatment in the interim), suggesting that the current episode was an inadvertent rechallenge, a phenomenon seen in 10 of 13 MP-DILI patients in the French pharmacovigilance database (7). Advanced fibrosis over several years is also possible with repeated exposure to iv MP as noted in an MS patient that improved after withdrawal of corticosteroids (20).

All the HiC MP cases with MS had been exposed to doses of iv MP of at least 1g per infusion), a similar dose to that used in Graves' disease. A study of more than 1000 consecutive patients with Graves' ophthalmopathy treated with similar doses of iv corticosteroids noted a significantly increased risk of liver injury in older patients (>53 years) and in doses >0.8 g per infusion (8). Similarly, various iv MP regimens caused a dose-dependent effect on ALT levels in 49 patients with Graves' orbitopathy (9). By using a lower dose of iv corticosteroids and adding oral prednisone to prevent a

rebound AIH in patients with elevated ALT, a lower risk of severe liver injury was noted (10).

The mechanism of liver injury associated with iv MP is unclear as is the predilection to affect patients with MS. Although development of MS is strongly associated with EBV infection (21), there was no clinical evidence of adenopathy or rash in our patients suggestive of EBV hepatitis. Furthermore, testing for EBV infection was negative and the clinical presentation and pathological findings are more like AIH. Seven of the 11 HiC patients had liver biopsies and histologic features of AIH were seen in 3 patients with 3 patients treated with tapering oral prednisone for the liver injury, one of whom was continued on azathioprine. Similar features were seen in a recent report of 8 patients (most with MS) who developed DILI after MP administration where liver biopsies all showed some degree of interface hepatitis (22). In contrast to the hepatotoxicity of high dose iv amiodarone that has been attributed to the vehicle additive of polysorbate, it is unlikely that iv MP hepatotoxicity is mediated by a vehicle additive since the buffers of sodium succinate and sodium phosphate and sucrose are endogenous molecular species (23).

HLA alleles play a significant role in the susceptibility of immune-mediated adverse drug reactions (24). Multiple HLA associations with DILI have been reported such as HLA-A*33:01 with terbinafine (25), HLA-B*35:02 with minocycline (26), HLA-DRB1*15:01 with amoxicillin clavulanic acid (27), and DRB1*16:01/DQB1*05:02 with flupirtine-DILI among others (28). Some HLA alleles are associated with the risk of DILI across several drugs, particularly HLA-B*35:01, seen with DILI due to trimethoprim-sulfamethoxazole (13), green tea extract (29), Polygonum multiflorum (30), and Garcinia

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cambogia (31). It should be remembered that many of these studies had a limited number of cases so designating specific HLA polymorphisms as risk alleles for DILI based solely on allele frequency comparisons would require further studies and a validation cohort. The carrier frequency of HLA alleles in the general population is very variable and the incidence of DILI, even with well recognized hepatotoxic drugs, is too low to recommend testing for HLA alleles before prescribing a medication. However, testing for a drug-specific HLA risk allele may help in establishing a diagnosis of DILI (32)

The HLA analysis identified three HLA alleles, *HLA-DQB1*06:02*, *HLA-DQA1*01:02*, and *DRB1*15:01* as well as the DR15 haplotype formed by these three alleles as potential susceptibility HLA alleles for MP-DILI. The *DRB1*15:01* allele and DR15 are known risk HLA allele and haplotypes for MS in patients of European descent (33). As 9 of 10 MP DILI cases with HLA data had MS, the HLA alleles and DR15 haplotype identified were more likely due to the underlying MS rather than an association with MP-DILI. These data highlight the potential for confounding of interpretation of genetic data for risk of DILI by the underlying disease being treated.

The represented SNPs in PTPN22, ERAP1, and ERAP2 previously identified for other drug specific DILI in DILIN showed no association with MP DILI. However, the SNP rs62376414 in ERAP1 showed more prevalence in the 10 MP cases than the 21 MS disease controls (AF: 0.20 vs. 0.02), which may be an indicator to differentiate MP effect from MS effects in those with DILI. However, the number of cases was small and further studies are needed to validate this finding.

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A comparison of the current study to other selected series of iv MP liver injury is shown in Table 3, highlighting very similar clinical features and its predominance in MS patients. Although other authors suggest this could be AIH, the data in the current study demonstrated a low incidence of autoantibodies and lack of known HLA alleles associated with AIH.

There are some limitations to this study. Although the data was collected prospectively, recognition of drugs associated with DILI has changed over time and accurate documentation of MP usage as an implicated agent may have been lacking. Eight of the 11 HiC MP cases were enrolled in the last 10 years, compared to only 3 in the first 10 years of DILIN. Non-Hispanic White participants are over-represented in DILIN and recent studies have demonstrated that there are significant racial differences in the risk of MS, partially due to conserved extended haplotypes (34); and DILI due to some classes of drugs such as anti-epileptics (35).

In conclusion, MP DILI is characterized by hepatocellular injury with or without jaundice, short latency, and usually rapid recovery, although fatal MP DILI can rarely occur. In DILIN, MP DILI was seen largely in MS patients and the HLA haplotypes associated with an increased risk of MS likely represented an association with the treated patient population rather than a distinct DILI risk. The observation that nearly all cases in this series and those reported in the literature arose after administration of 0.8 to 1 gram per day for at least 3 to 5 days suggests that clinicians using these drugs should be aware for this potential adverse event and have a low threshold for monitoring liver tests in patients who develop new symptoms after iv infusion.

Table 1: Clinical characteristics of 11 high confidence methylprednisolone DILI cases in the DILIN prospective cohort from 2004-2022.

Characteristic	Median or Number	Range or %
Age (years)	48	18-69
Female sex (N)	8	73%
Non-Hispanic White (N)	8	73%
BMI (kg/m^2)	26.8	21.4-49.3
Multiple Sclerosis (N)	9	82%
Symptoms (N)	6	55%
Rash (N)	1	11%
Eosinophilia >500/microL (N)	1	11%
Hospitalized (N)	10	91%
Duration of treatment (days)	5	3-150
Latency to DILI onset (days)	30	4-150
Initial *R* value	16.5	5.0-35.6
Initial ALT (U/L) Peak ALT (U/L)	643 1168	228-2669 228-2669
Initial total bilirubin (mg/dL) Peak total bilirubin (mg/dL)	1.5 3.2	0.3-31.4 0.4-31.4
Peak bilirubin ≥ 2.5 mg/dL	6	55%
Liver biopsy (N)	7	64%
ANA positive (N) SMA positive (N)	4 2	36% 18%
Ig >1500 mg/dL (N=7)	4	57%

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Days to >50% decrease from peak ALT	19	3-36
Days to >50% decrease from peak total bilirubin	18	14-33
Days to recovery (N=10)	65	30-186
Oral corticosteroid therapy for injury (N)	3	27%
Recovered (N) Chronic liver injury (N) Fatal/transplant (N)	10 0 1	91% 0% 9%

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Table 2: Results of HLA alleles and DR15 haplotype association with MP DILI with comparison to other DILI cases without MP or MS and unlikely MP DILI cases.

	MP DILI Cases (n=10)		**Non-MP, non-MS DILI Controls (n=1489)**			**MP Exposure Controls (n=61)**			**MS Disease Controls (n=21)**		
Allele/Haplotype	**CF**	**AF**	**CF**	**AF**	**Fisher P**	**CF**	**AF**	**Fisher P**	**CF**	**AF**	**Fisher P**
DQB1*06:02	0.70	0.40	0.27	0.15	0.01	0.21	0.13	0.01	0.52	0.29	0.40
DQA1*01:02	0.80	0.45	0.37	0.22	0.02	0.36	0.20	0.02	0.52	0.29	0.26
DRB1*15:01	0.50	0.30	0.24	0.13	0.04	0.21	0.11	0.04	0.48	0.24	0.75
DR15 haplotype	0.50	0.30	0.22	0.12	0.03	0.21	0.11	0.04	0.48	0.24	0.75
AIH associated HLA risk alleles											
DRB1*03:01	0.1	0.05	0.21	0.12	0.72	0.28	0.14	0.47	0.19	0.12	0.66
DRB1*04:01*	0	0	0.11	0.06	---	0.05	0.02	---	0.10	0.05	---
Other DILI risk alleles											
PTPN22: rs2476601	0.20	0.10	0.19	0.10	>0.99	0.15	0.07	0.65	0.24	0.12	>0.99
ERAP1: rs62376414	0.30	0.20	0.17	0.09	0.10	0.15	0.07	0.09	0.05	0.02	0.03
ERAP2**:** rs1363907 **	0.40	---	0.39	--	> 0.99	0.31	---	0.72	0.33	--	> 0.99

*Because DRB1*04:01 was not present in our MP DILI cases, association tests were not performed.
** GG genotype

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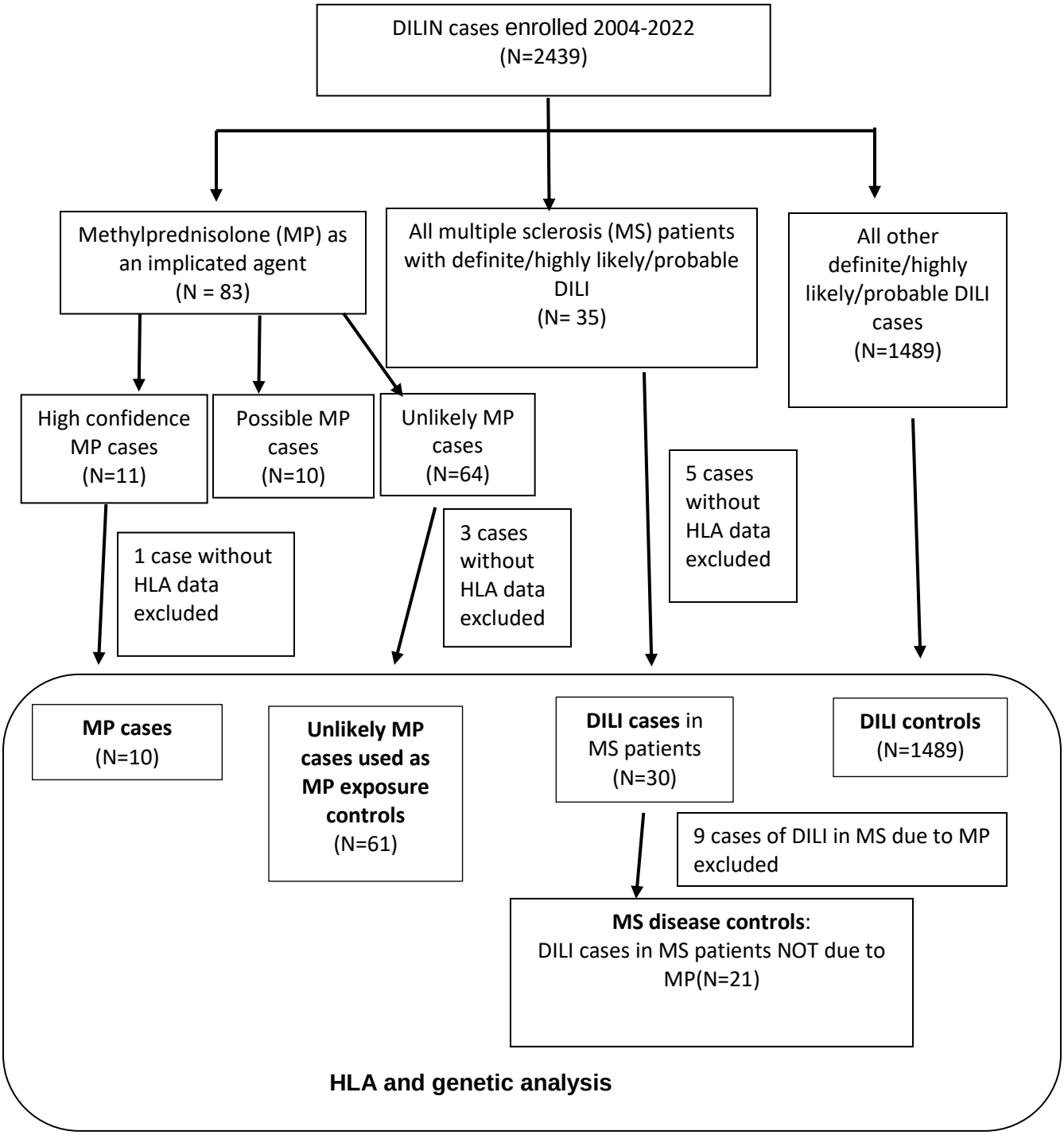
Table 3: Selected published series of iv MP associated liver injury.

	Current study 2023 *	Nociti 2018	Kimura 2020	Allgeier 2022	Cottin 2020	Sisti 2015
DILI cases	11	6	8	13	77	14
Median age/years (range)	48 (18-69)	28 (19-59)	37 (28-49)	39 (20-74)	45.7	55 (30-78)
Female	73%	83%	100%	92%	59%	79%
MS Graves Other diseases	82% 18%	100%	100%	92% 8%	27% 73%	 100%
Median peak ALT/U/L (range)	1168 (228-2669)	931 (778-2956)	912 (170-3720)	1431 (336-2667)	NA (79% hepatocellular injury)	NA (ALT >300)
Median peak bilirubin/mg/dL (range)	3.2 (0.4-31.4)	1.5 (1.3-10.8)	1.15 (0.4-21.6)	10 (0.8-29.7)	NA	NA
Jaundice	55%	17%	25%	62%	34%	NA
ANA and/or SMA positive	45%	33%	0%	92%	NA	NA
Liver biopsy	67%	83%	88%	77%	17%	NA
% Fatal/ liver txp	9%	Not reported	Not reported	Not reported	Not reported	Not reported

* Only study with genetic analyses reported

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Figure 1: Consort diagram of patients included in the study are shown.

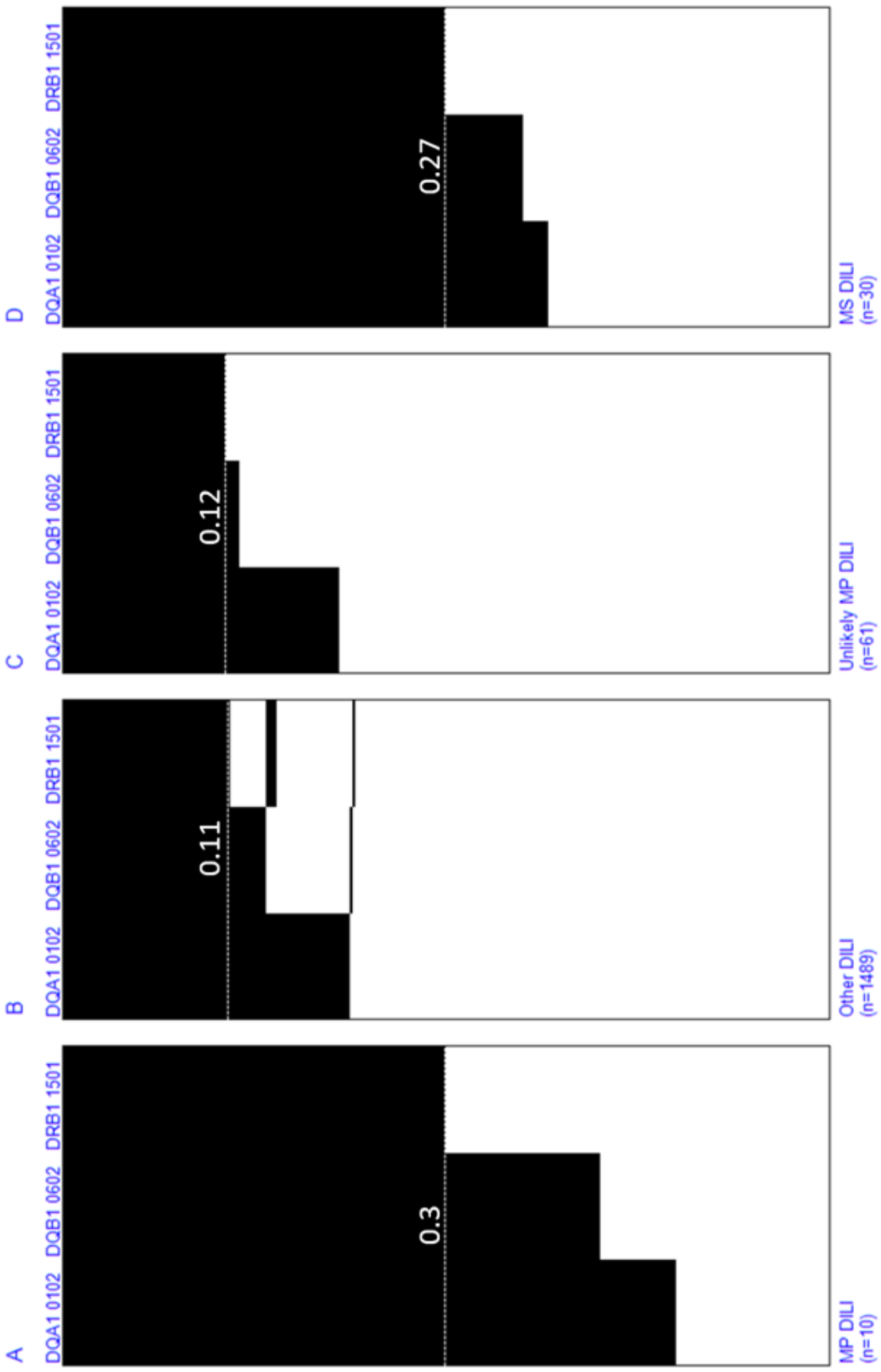


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Figure 2: Frequencies of HLA haplotypes derived from different combinations of DRB1*15:01, DQA1*01:02, DQB1*06:02 alleles in MP DILI cases and the 3 control groups: DILI, MP exposure and MS disease control groups. The black color indicates the present of the allele. The vertical axis indicates the frequency of each allele or haplotype. The dashed line annotates DR15 (DRB1*15:01-DQA1*01:02-DQB1*06:02) haplotype with its haplotype frequency showing above. The DR15 haplotype frequency is similar in MP DILI cases and MS disease control groups but lower in the DILI controls and MP exposure controls.

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Supplementary table 1: Clinical characteristics of 11 high confidence methylprednisolone DILI cases in the DILIN prospective cohort from 2004-2022.

Patient	Age /years	Sex	Indication	Latency /days	Peak bilirubin mg/dL	Peak ALT U/L	Peak INR	AI markers	Liver biopsy
1	69	F	Spinal contusion	6	2	463	1	neg	NA
2	49	F	MS	150	13.2	1168	1.2	ANA 1:160	Piecemeal necrosis with collapse
3	51	M	MS	41	1	228	ND	neg	NA
4	32	F	MS	9	0.5	1653	1.1	ANA 1:640 IGG 1773	Mild hepatitis, No AI features
5	48	F	MS	36	8.9	1189	1.2	ANA 1:320	NA
6	47	F	MS	26	3.7	1694	1.3	IGG 1710	AI hepatitis type
7	18	M	MS	14	3.2	916	1	neg	Acute hepatitis
8	48	F	MS	44	2.2	921	0.9	neg	Mmild to moderate interface hepatitis with portal and periportal fibrosis (grade 1-2, stage 1-2)
9	48	M	UC	4	0.4	606	1	neg	NA
10	66	F	MS	30	31	2669	2.1	ANA 1:160 SMA 27 IGG 1866	Submassive necrosis
11	49	F	MS	38	31.4	1874	5.3	SMA 21 IGG 2271	Portal inflammation, increased plasma cells, bridging necrosis, parenychmal collapse

Abbreviations F- female, M- male, MS- multiple sclerosis, UC- ulcerative colitis, AI- autoimmune, ANA- anti-nuclear antibody (normal <1:40), IGG immunoglobulin G (normal <1600 mg/dL), SMA smooth muscle antibody (normal <19 U/L), NA- not available.

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Supplementary table 2: Clinical characteristics of 10 possible methylprednisolone DILI patients in the DILIN prospective cohort from 2004-2022.

Characteristic	Median or Number	Range or %
Age /years	43	36-81
Female sex (N)	8	80%
BMI (kg/m^2)	26.6	21-39.5
Overall Causality (N)		
1,2,3	5	50%
4,5	5	50%
Multiple Sclerosis (N)	4	40%
Symptoms (N)	3	30%
Hospitalized N)	6	60%
Duration of treatment /days	21.5	4-210
Latency to DILI onset /days	35.5	3-300
Initial R value	24.5	6.7-63.7
Initial ALT /U/L	1118	282-1731
Peak ALT /U/L	1184	282-2700
Initial total bilirubin /mg/dL	1.4	0.6-13.9
Peak total bilirubin /mg/dL	1.7	0.6-24.9
Jaundice (N)	3	30%
Days to >50% decrease in peak ALT	13	3-32
Days to >50% decrease from peak total bilirubin (N=3)	29	12-32
Days to recovery (N=7)	68	38-160
Recovered (N)	8	80%
Chronic DILI (N)	2	20%

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Fatal/transplant (N)	0	0%
Liver biopsy (N)	5	50%
ANA positive (N)	3/9	33%
SMA positive (N)	2/9	22%

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Supplementary table 3: Clinical characteristics of 21 patients with Multiple Sclerosis with suspected DILI in the DILIN prospective cohort from 2004-2022 and the 11 high confidence MP DILI cases.

	MS DILI (N=21)		HiC MP DILI (N=11)	
Characteristic	Median or Number	Range or %	Median or Number	Range or %
Age /years	55	32-71	48	18-69
Female sex (N)	17	81%	8	73%
BMI (kg/m^2)	26.7	17.1-47.8	26.8	21.4-49.3
Implicated drug used to treat MS (N)	13	62%	9	82%
Symptoms (N)	11	52%	6	55%
Hospitalized (N)	13	62%	10	91%
Latency to DILI onset /days	108	6-1728	30	4-150
Pattern of liver injury (N=23) Hepatocellular Cholestatic Mixed	 13 5 3	 62% 24% 14%	 11	 100%
Initial R value	8.9	0.5-35.4	16.5	5.0-35.6
Initial ALT /U/L Peak ALT /U/L	513 713	168-2417 168-2417	643 1168	228-2669 228-2669
Initial ALP /U/L Peak ALP /U/L	224 304	74-857 83-1378	111 194	70-246 70-246
Initial total bilirubin /mg/dL Peak total bilirubin /mg/dL	1.6 6.7	0.3-11.5 0.4-29.8	1.5 3.2	0.3-31.4 0.4-31.4
Jaundice (N)	13	62%	6	55%

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Days to >50% decrease in peak ALT (N=18)	14	3-39	19	3-36
Days to >50% decrease in peak total bilirubin (N=11)	15	5-49	18	14-33
Days to recovery (N=12)	99	34-225	65	30-186
Recovered (N)	16	76%	10	91%
Chronic DILI (N)	2	10%	0	0%
Fatal/transplant (N)	3	14%	1	9%
Liver biopsy (N)	11	52%	7	64%
ANA positive (N)	6	29%	4	36%
SMA positive (N)	5	24%	2	18%

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Supplementary Table 4: Clinical and HLA results for the three class II alleles in the 11 high confidence MP DILI cases

Sex	Race	Causality Score	RECAM score	MS	LATENCY	PEAK ALT	PEAK AKP	PEAK TB	Severity	Outcome	DQA1_1	DQA1_2	DQB1_1	DQB1_2	DRB1_1	DRB1_2
F	White	2	3	No	6	463	217	2	Mild	Alive	NA	NA	NA	NA	NA	NA
F	White	2	1	Yes	150	1168	137	13.2	Moderate-hospitalized	Alive	01:02	02:01	02:02	06:02	07:01	16:01
M	Asian	2	7	No	4	606	103	0.5	Mild	Alive	01:03	03:01	03:03	06:01	04:10	15:02
M	White	3	6	Yes	41	228	79	1.8	Mild	Alive	01:02	01:02	06:02	06:02	15:01	15:01
F	Black	2	4	Yes	9	1653	233	0.5	Mild	Alive	01:02	03:01	02:02	06:02	11:01	15:03
F	White	2	6	Yes	44	921	119	2.2	Mild	Alive	01:02	05:01	02:01	06:02	03:01	15:01
F	White	2	13	Yes	36	1189	194	8.9	Moderate-hospitalized	Alive	01:02	03:01	03:02	06:02	04:04	15:01
F	White	3	4	Yes	26	1694	246	8.2	Moderate-hospitalized	Alive	01:02	03:01	03:02	06:02	04:05	15:01
F	White	1	6	Yes	30	2669	210	31	Severe	Alive	01:03	02:01	03:03	06:03	07:01	13:01
M	White	1	10	Yes	14	916	139	3.2	Moderate-hospitalized	Alive	01:01	01:02	05:01	06:02	01:02	15:01
F	Black	2	3	Yes	38	1874	239	32.4	Fatal	Transplant; Alive	01:01	01:02	05:01	06:09	01:01	13:02

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