nature genetics

LETTERS

https://doi.org/10.1038/s41588-018-0168-y

Common variation near *IRF6* is associated with IFN-β-induced liver injury in multiple sclerosis

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Multiple sclerosis (MS) is a disease of the central nervous system treated with disease-modifying therapies, including the biologic, interferon-β (IFN-β). Up to 60% of IFN-β-exposed MS patients develop abnormal biochemical liver test results[1,2], and 1 in 50 experiences drug-induced liver injury[3]. Since genomic variation contributes to other forms of drug-induced liver injury[4,5], we aimed to identify biomarkers of IFN-β-induced liver injury using a two-stage genome-wide association study. The rs2205986 variant, previously linked to differential expression of *IRF6*, surpassed genome-wide significance in the combined two-stage analysis ($P = 2.3 \times 10^{-8}$, odds ratio = 8.3, 95% confidence interval = 3.6–19.2). Analysis of an independent cohort of IFN-β-treated MS patients identified via electronic medical records showed that rs2205986 was also associated with increased peak levels of aspartate aminotransferase ($P = 7.6 \times 10^{-5}$) and alkaline phosphatase ($P = 4.9 \times 10^{-4}$). We show that these findings may be applicable to predicting IFN-β-induced liver injury, offering insight into its safer use.

While the therapeutic options for multiple sclerosis (MS) are expanding, interferon-βs (IFN-βs) remain the most widely used disease-modifying therapy. Liver injury secondary to IFN-β has potentially serious sequelae, yet there are no means of predicting this adverse reaction. In the USA, drug-induced liver injury (DILI) is the leading cause of acute liver failure[6] and the most common reason for drug withdrawal from the market[7]. Genome-wide association studies (GWAS) have successfully discovered variants of large effect sizes associated with DILI due to non-biologics using relatively small but rigorously phenotyped cohorts[4,8]. However, studies identifying variants associated with DILI from biologics, including IFN-β, have not been reported.

Patients who exhibited normal baseline biochemical liver test results before IFN-β exposure were included in this study (see Methods). Cases met a published DILI definition[9], and controls were exposed to IFN-β for ≥2 years, with all biochemical liver test results within the normal range. We recruited 170 patients from Canadian-based MS clinics for stage 1 analyses employing genome-wide genotyping. Upon exclusion of samples failing quality control or those of non-European genetic ancestry (Supplementary Fig. 1), 151 samples (38 cases, 113 controls) were subject to whole-genome and human leukocyte antigen (*HLA*)-allele imputation. Variants reaching $P < 1.0 \times 10^{-6}$ in stage 1 were tested in stage 2. The clinical and demographic characteristics of stage 1 participants were similar between cases and controls, apart from the controls being more likely to have a relapsing–remitting MS course ($P = 0.035$; Supplementary Table 1).

Genome-wide analysis identified three regions associated with IFN-β-induced liver injury, after adjusting for MS disease course (Table 1, Supplementary Tables 2 and 3, and Supplementary Fig. 2a). The strongest association was located on chromosome 1q32.2

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Table 1 | Pharmacogenomic association analyses for rs2205986 and IFN-β-induced liver injury in multiple sclerosis patients enrolled from Canada (stage 1) and the USA or Sweden (stage 2)

Genetic variant information		Population					Logistic regression (additive)[a]		
Variant	Annotation	Study stage	Cases (*n*)	Controls (*n*)	MAF cases	MAF controls	*P* value	OR	95% CI
rs22059861:210,116,112[b]	*IRF6* eQTL/*SYT14* intronic	Stage 1	38	113	0.24	0.04	1.9×10^{-7}	8.5	3.5–20.4
		Stage 2	18	13	0.17	0.00	4.3×10^{-3}	–	–
		Combined (stages 1+2)	56	126	0.21	0.03	2.3×10^{-8}	8.3	3.6–19.2
		BioVU 'mild DILI'[c]	9	78	0.22	0.05	0.048	4.3	1.02–17.8

[a]Logistic regression was performed in stages 1 (adjusted for MS disease course) and 2 (adjusted for age). [b]GRCh37 assembly position (chromosome:base pair), HGVS notation: NC_000001.10:g.210116112G>A. [c]Defined as twice the upper limit of normal for either ALT or AST.

(rs2205986[G>A], $P=1.9\times10^{-7}$, odds ratio (OR) = 8.5, 95% confidence interval (CI) = 3.5–20.4; Table 1). This variant also surpassed our screening threshold unadjusted for covariates ($P=3.1\times10^{-7}$) and when adjusted for the first 5 principal components and MS disease course ($P=2.6\times10^{-7}$). Analyses of the *HLA* region did not identify any association with IFN-β-induced liver injury, including *HLA* variants previously associated with DILI caused by other medications (Supplementary Table 4).

The prioritized genomic regions were subsequently tested in stage 2 using a separate cohort of MS subjects from the USA and Sweden (18 cases, 13 controls of European ancestry). Cases were significantly older ($P=0.026$), but were similar to controls across other characteristics (Supplementary Table 1). Of the variants tested in stage 2, only the 1q32.2 region (rs2205986) was associated with IFN-β-induced liver injury ($P=0.004$; Table 1 and Supplementary Table 3). This variant was only observed in cases (Supplementary Table 2), and the overall effect (combined stages 1 and 2) surpassed genome-wide significance ($P=2.3\times10^{-8}$, OR = 8.3).

Next, we evaluated array-genotyped MS patients receiving IFN-β (identified via electronic medical records (EMRs) in the Vanderbilt University Medical Center repository (BioVU)) to assess the influence of the rs2205986 DILI-risk variant on peak biochemical liver test results during IFN-β treatment. Of the 4 liver test results analyzed, rs2205986 was significantly associated with increased aspartate aminotransferase (AST; $P=7.6\times10^{-5}$) and alkaline phosphatase (ALP; $P=4.9\times10^{-4}$) levels (Table 2 and Supplementary Fig. 3). On average, each rs2205986 G allele contributed to an increase of 52.3 units l^{-1} ALP and 29.4 units l^{-1} AST.

Of note, the one BioVU patient homozygous for the rs2205986 G risk-allele presented with elevated peak alanine aminotransferase (ALT), AST and ALP levels (Table 2), potentially indicating a marked increase in risk associated with this rare genotype. We performed a complementary analysis excluding this sample, which showed a significant association with AST ($P=0.017$) but no association for the remaining liver test results. Furthermore, since IFN-β-induced liver injury typically presents with a hepatocellular pattern, we also performed an exploratory case-control analysis of 'mild DILI' (ALT or AST >2× the upper limit of normal) in the BioVU cohort. These analyses also detected evidence for an association with this phenotype ($P=0.048$; OR = 4.3, 95% CI = 1.02–17.8; Table 1), indicating that the biomarker may also be useful in identifying milder forms of liver injury.

We also examined the frequency of the top stage 1 regions in a cohort of 1,319 disease-matched population controls that were unscreened for biochemical liver test abnormalities. This confirmed a higher frequency of rs2205986 in cases (minor allele frequency (MAF) = 21.4%) compared with MS population controls (MAF = 9.4%). Although these analyses were no longer genome-wide significant ($P=3.0\times10^{-4}$), the use of population controls is best suited to adverse drug reactions with prevalence rates of <1%[10]. In contrast, 2% of IFN-β-treated MS patients developed DILI, and up to 60% exhibited abnormal biochemical liver test results in a population-based cohort study[3]. Since the BioVU biochemical liver test analyses indicated that rs2205986 is associated with elevated peak liver test results, the depletion in MAF observed in drug-exposed and screened controls may have been caused by the removal of carriers with abnormal biochemical liver test results during the stringent selection process of controls.

Inspection of the 1q32.2 region showed that only rs2205986 surpassed $P<1.0\times10^{-6}$, while 30 variants within a 266-kilobase (kb) linkage disequilibrium block displayed $P<5.0\times10^{-5}$ (Fig. 1). Upon adjusting for rs2205986, no variants were independently associated with DILI ($P>0.05$; Supplementary Table 5). rs2205986 is an intronic variant in synaptotagmin-14 (*SYT14*); however, this marker is approximately 4.5 kb from the nearest canonical exon and is not predicted to alter *SYT14* transcription, splicing, or expression. Notably, in silico annotation using the Genotype-Tissue Expression Project data showed that rs2205986 is an expression quantitative trait locus (eQTL) for the interferon regulatory factor-6 gene (*IRF6*; $P_{\text{multi-tissue}}=5.89\times10^{-17}$)[11], located 137 kb upstream from rs2205986 (Fig. 1).

Interferon regulatory factors (IRFs) are a family of IFN transcription factors that synchronize the type I IFN pathway[12]. Many of the nine known IRFs are associated with promoting liver damage in another model of liver cell death: hepatic ischemia and reperfusion injury[13]. Furthermore, recent gene expression studies have identified *IRF6* as an IFN-β drug response biomarker[14], while *IRF3* has been implicated in tolvaptan-induced liver injury[15]. IRF6 promotes apoptosis following brain injury[16], and previous case reports of MS patients experiencing IFN-β-induced liver injury have shown hepatocyte apoptosis[17], suggesting that rs2205986-induced alterations in IRF6 expression may promote apoptosis in the presence of IFN-β. The rs2205986-*IRF6* eQTL was not significant in liver tissue; however, the effect of this variant on gene expression may be amplified in the presence of IFN-β, or the variant may exert its influence via the blood (Supplementary Fig. 4). Future studies should therefore investigate the influence of this eQTL on IFN-β-induced hepatic expression.

The *HLA* region has been shown to confer risk to DILI caused by certain drugs[4,5]; however, no *HLA* alleles or variants outside the *HLA* region that have previously been associated with DILI (for example, glutathione *S*-transferase and ATP-binding cassette transporter genes)[18,19] reached the screening threshold. As previous pharmacogenomic studies of DILI investigated non-biologics, these results might be expected given the differences in the metabolism of biologics compared with small-molecule drugs[19], and the limited evidence surrounding IFN-β metabolism[20].

We also incorporated rs2205986 into a predictive model for DILI. Including rs2205986 significantly improved the prediction of

Table 2 | Association analysis of the rs2205986 genotype on peak biochemical liver test results during IFN-β treatment in MS patients from the VUMC repository

Biochemical liver test	Measurement	β coefficient[a]	*P* value[a]	First recorded value (mean)	Highest recorded value (mean)	Mean highest value by genotype (GG/GA/AA)
ALP	Units l^{-1}	52.28	4.9×10^{-4}	79	101	255/140/94
	Upper limit of normal	0.35	5.0×10^{-4}	NA	NA	NA
AST	Units l^{-1}	29.39	7.6×10^{-5}	29	42	147/59/38
	Upper limit of normal	0.73	7.6×10^{-5}	NA	NA	NA
ALT	Units l^{-1}	5.29	0.54	34	47	112/43/47
	Upper limit of normal	0.10	0.54	NA	NA	NA
TBIL	mg dl^{-1}	0.00	0.98	0.49	0.77	1.00/0.59/0.77
	Upper limit of normal	0.00	0.98	NA	NA	NA

[a]Linear regressions using the additive genetic model for highest values, adjusted for age at biochemical liver test date, sex, and the first two principal components in 87 MS patients exposed to interferon-β. NA, not applicable.

liver injury over clinical factors alone ($P=0.0039$; Supplementary Fig. 5). rs2205986 had a specificity of 93.7% (95% CI = 87.9–97.2) and a sensitivity of 41.1% (95% CI = 28.1–55.0). Notably, the only patient requiring a liver transplant was an rs2205986 carrier. The negative and positive predictive values of rs2205986 were 98.7 and 12.2%, respectively, and the number of individuals needed to screen for rs2205986 to prevent 1 case was 117. These metrics are similar to those reported for the testing of *HLA-B*1502* and carbamazepine-induced Stevens–Johnson syndrome[21]. Pharmacogenomic testing for *HLA-B*1502* before carbamazepine use is recommended by the Food and Drug Administration for certain ancestries, highlighting the importance of the current findings. Future studies could consider incorporating additional variables, such as the absolute baseline liver biochemistry values, into predictive models to further improve prediction.

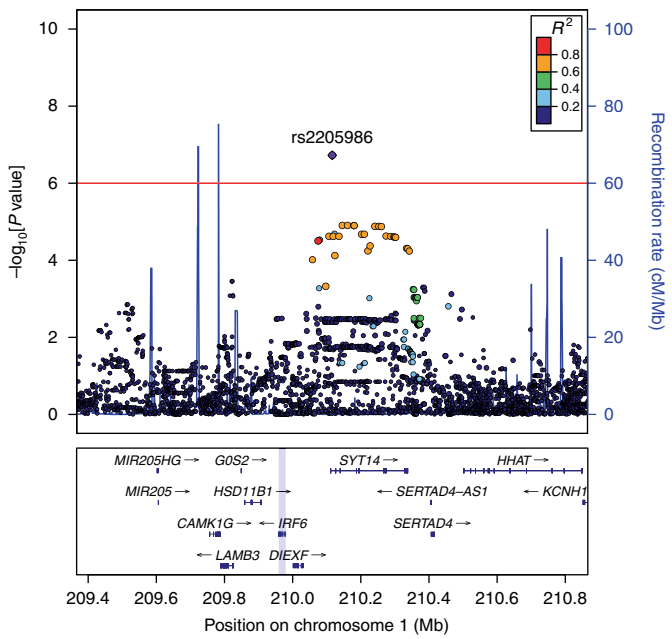

Fig. 1 | Regional association plot of chromosome 1q32.2, demonstrating a pharmacogenomic association between rs2205986 and IFN-β-induced liver injury. *IRF6* is located ~132 kb upstream of *SYT14* (light blue). Association results (primary *y* axis) are shown for genetic variants, along with recombination rates (secondary *y* axis) for a 1.5-megabase region on chromosome 1. Each circle represents the $-\log_{10}$[*P* value] from the logistic regression analysis, adjusted for MS disease course in the stage 1 case-control cohort ($n=151$). Genetic variants are colored according to their pairwise correlation (R^2) with rs2205986 (purple circle) using linkage disequilibrium data from the 1000 Genomes Project (European population). The red horizontal line indicates $P=1.0\times10^{-6}$. Three genes (*MIR4260*, *TRAF3IP3*, and *C1orf74*) were omitted from the figure due to space requirements.

To our knowledge, few others have investigated the genetic architecture of adverse drug reactions from an MS therapy. These analyses were restricted to European genetic ancestry patients to minimize population stratification. However, since MS is known to be most prevalent in those with northern European ancestry[22], these results are expected to be applicable to the majority of people with MS. Furthermore, objectively defining DILI[9] and applying stringent inclusion criteria for the controls in this study enhanced the statistical power[10]. Nonetheless, sample size remains a limitation, and as a consequence, we were only able to identify one pharmacogenomic predictor of IFN-β-induced liver injury. Future studies of larger cohorts might improve the ability to detect additional variants of smaller effect.

In conclusion, we have identified an association between an IRF-related eQTL and IFN-β-induced liver injury. These findings have important implications for the development of strategies to reduce the occurrence of IFN-β-induced liver injury in MS patients. Pharmacogenomic testing for this variant before IFN-β therapy, rather than only monitoring liver enzymes during treatment, may prevent DILI in at-risk patients. Prevention of DILI in rs2205986 carriers could then be achieved by either considering alternative therapies or increased monitoring of liver injury. In addition, our findings set the stage for functional assessments of *IRF6*, rs2205986, and IFN-β treatment to provide a mechanistic understanding of this pharmacogenomic association that can be specifically targeted to prevent DILI.

URLs

1000 Genomes database, http://www.1000genomes.org; PLINK, https://www.cog-genomics.org/plink2; R for statistical computing 3.2.3, http://www.r-project.org/; Wellcome Trust Case Control Consortium, www.wtccc.org.uk; GTEx Portal, www.gtexportal.org

Methods

Methods, including statements of data availability and any associated accession codes and references, are available at https://doi.org/10.1038/s41588-018-0168-y.

Received: 2 December 2016; Accepted: 31 May 2018;
Published online: 16 July 2018

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Acknowledgements

We gratefully acknowledge the participation of all patients and families who took part in this study across Canada, Europe, and the USA. This work would not have been possible without their generous participation. We also acknowledge the contributions of the Canadian Pharmacogenomics Network for Drug Safety Consortium (C. Hildebrand, A. Borrie, T. Wong, T. Bendyshe-Walton, N. Massah, F. Miao, M. Higginson, M. Staub, K. Shaw, B. Malkin, and J. Stortz), Winnipeg Health Sciences Centre MS Clinic (N. Hall and B. Stranger), London Health Sciences Centre MS Clinic (H. Rosehart), Dalhousie MS Clinic (K. Stadnyk, K. Sabourin, and B. DeCoste), and Centre Hospitalier de L'Université de Montréal (É. Roger). We thank all the MS clinic neurologists who contributed to the study through patient examination and data collection. This study makes use of data generated by the Wellcome Trust Case Control Consortium. A full list of the investigators who contributed to the generation of the data is available from www.wtccc.org.uk. Funding for the original Wellcome Trust Case Control Consortium project was provided by the following Wellcome Trust awards: 076113, 085475 and 090355. This investigation was supported by a pilot grant from the British Columbia Clinical Genomics Network (to H.T. (the principal investigator)). Doctoral studentships provided additional funding for K.K. from the Canadian Institutes of Health Research (CIHR), CIHR Drug Safety and Effectiveness Cross-Disciplinary Training Program (DSECT), MS Society of Canada, University of British Columbia, and Canadian Pharmacogenomics Network for Drug Safety. G.E.B.W. received fellowships from the CIHR and CIHR DSECT programs. B.I.D. received stipends from the British Columbia Children's Hospital Research Institute, CIHR DSECT, CIHR, and the Michael Smith Foundation for Health Research during the period of this study. The Drug-Induced Liver Injury Network is supported by the National Institute of Diabetes and Digestive and Kidney Diseases of the National Institutes of Health (NIH) as part of the Cooperative Agreements (U01s) project under grants U01-DK065176 (Duke), U01-DK065201 (UNC), U01-DK065184 (Michigan), U01-DK065211 (Indiana), U01DK065193 (UConn), U01-DK065238 (UCSF/CPMC), U01-DK083023 (UTSW), U01-DK083027 (TJH/UPenn), U01-DK082992 (Mayo), U01-DK083020 (USC), and U01-DK100928 (Icahn). Additional funding was provided by CTSA grants UL1 RR025761 (Indiana), UL1 RR025747 (UNC), and UL1 UL1 RR024986 (UMich), and the Intramural Research Program of the National Cancer Institute. Vanderbilt University Medical Center BioVU is supported by institutional funding and Vanderbilt CTSA grant ULTR000445 from NCATS/NIH. Genome-wide genotyping was funded by P50GM115305 from NIGMS. H.T. and B.C.C. had full access to all the data in the study, and take full responsibility for the integrity of the data and the accuracy of the data analysis. The study funders had no role in the study design, data collection, data analysis, data interpretation, or writing of the report.

Author contributions

K.K. performed the baseline epidemiological analyses of the clinical characteristics and genetic association analyses, compiled the corresponding sections, performed literature reviews, and wrote the first draft of the manuscript. G.E.B.W. performed whole-genome imputation association analyses and population control analyses, and compiled the corresponding sections. B.I.D. performed the *HLA* imputation and related association analyses, and compiled the corresponding sections. M.F.D., J.C.D., G.E.B.W., and B.C.C. performed the analysis and interpretation of the BioVU cohorts. K.K., G.E.B.W., B.I.D., F.A., A.P.B., E.K., and C.J.D.R. provided further data interpretation. C.J.D.R., B.C.C., and H.T. were responsible for study design and interpretation of the data. K.K., E.M.Y., A.T., R.A.M., M.K., T.L.C., P.D., N.C., M.W., P.H., Z.X., and P.L.D.J. were responsible for patient recruitment. All authors contributed to drafting and revising the work for important intellectual content, and made substantial contributions to the concept and design of the study and analysis and interpretation of the data.

Competing interests

K.K. has a consulting agreement with Emerald Lake Safety. E.M.Y. is an investigator of clinical trials sponsored by Gilead, Merck, Vertex, Hoffman LaRoche, Abbvie, Janssen, Boeringher Ingelheim, Intercept, and Genfit. He has also received honoraria for continuing medical education lectures from Merck Canada and Gilead Canada, spoken at Advisory Board Meetings of Boeringher Ingelheim Canada, Hoffman LaRoche Canada, Abbvie Canada, and Celgene Canada (for which he received an honorarium), and is a member of the Gilead Canada compassionate release program adjudication committee, for which he received an honorarium. A.T. has received grant support from Hoffman la Roche, Sanofi Genzyme, Chugai, Novartis, and Biogen, as well as performing consultancy work for Biogen, EMD Serono, Hoffman la Roche, Sanofi Genzyme, and Teva Neuroscience. R.A.M. has conducted clinical trials for Sanofi-Aventis, and receives research funding from the CIHR, National MS Society, MS Society of Canada, Research Manitoba, Consortium of MS Centers, Crohn's and Colitis Canada, and Waugh Family Chair in Multiple Sclerosis. In the past two years, M.K. or M.K.'s institution (London Health Sciences Centre and Department of Neurological Sciences, Western University) has received research support or grants from CIHR. T.L.C. was a principal investigator for a research study for Biogen Idec Canada, has received honoraria for lectures from EMD Merck Serono Canada, Biogen Idec Canada, Teva Canada Innovation, and Genzyme, has participated in or spoken at Advisory Board Meetings of EMD Merck Serono, Teva Canada Innovation, Biogen Idec Canada, Genzyme, and Novartis (for which an honorarium and travel support were received in exchange for attendance). P.D. has received support as a consultant, membership on advisory councils, and grants from the pharmaceutical industry, and is funded by the MS Society of Canada and CIHR. N.C. has consulting agreements with Abbvie, Lilly, DS Biopharma (Afimmune), Tobira (Allergan), NuSirt, Celgene, Axovant, Shire, and Madrigal, and has received research grants from Cumberland, Galectin, and Intercept. M.W. and P.H. receive funding from the Swedish Research Council (Medicine 521-2011-2440 and 521-2014-3370) and Clinical Research Support (ALF) at Uppsala University. M.W. has also received grants from the Swedish Heart and Lung Foundation (20120557 and 20140291). Z.X. was a recipient of the Clinician Scientist Development Award from the National Multiple Sclerosis Society and American Academy of Neurology, and is supported by NINDS NIH K08-NS079493 and NINDS R01 NS098023. P.L.D.J. has received research funding from Sanofi/Genzyme and Biogen, and has consulted for Teva Pharmaceuticals and Sanofi/Genzyme. C.J.D.R. receives funding support from the CIHR, Canadian Foundation for Innovation, Canadian Hearing Foundation, BC Children's Hospital Foundation, Child & Family Research Institute, Canadian Gene Cure Foundation, Teva Pharmaceutical Industries, Genome BC, and CIHR Drug Safety and Effectiveness Network. H.T. is the Canada Research Chair for Neuroepidemiology and Multiple Sclerosis, and currently receives research support from the National MS Society, CIHR, MS Society of Canada, and MS Scientific Research Foundation. In the past five years, H.T. has received support from the Multiple Sclerosis Society of Canada (Don Paty Career Development Award),

Michael Smith Foundation for Health Research (Scholar Award), and UK MS Trust. H.T. has received speaker honoraria and/or travel expenses to attend conferences from the Consortium of MS Centres (2013), National MS Society (2014 and 2016), ECTRIMS (2013–2018), Biogen Idec (2014), and American Academy of Neurology (2013–2016). All speaker honoraria were either declined or donated to an MS charity or unrestricted grant for use by H.T.'s research group. B.C.C. currently receives research funding from the CIHR, Genome Canada, Genome British Columbia, and British Columbia Children's Hospital Research Institute (Vancouver, Canada), and has previously received matching funds support for Genome Canada funding from Pfizer Canada (unrestricted).

Additional information

Supplementary information is available for this paper at https://doi.org/10.1038/s41588-018-0168-y.

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Methods

Study participants. Subjects were eligible for inclusion in our study if they had either relapsing–remitting or secondary-progressive definite MS (based on Poser or McDonald criteria)[23,24], documented exposure to an IFN-β product (subcutaneous IFN-β-1b (250 μg every other day), subcutaneous IFN-β-1a (22 μg or 44 μg 3× weekly) or intramuscular IFN-β-1a (30 μg weekly)) and had a normal baseline liver enzyme test. At least one ALT test result was required for baseline assessment. Cases met at least one of the following criteria[9]: (1) ALT or AST ≥5× the upper limit of normal; (2) ALT ≥3× the upper limit of normal, with simultaneous elevation of bilirubin >2× the upper limit of normal; or (3) ALP >2× the upper limit of normal. Controls were exposed to IFN-β for at least two years with all biochemical liver test results within normal limits based on the normal ranges for the site-specific laboratory.

As the first 15 months of IFN-β exposure are considered the greatest risk period for developing de novo ALT elevations[2], and all cases developed DILI within 700 days of beginning IFN-β[3], 2 years of IFN-β exposure was required to determine whether a participant was truly a control. As expected due to the study design, stage 1 controls were exposed to IFN-β for a significantly longer duration (median: 82 months, interquartile range: 51–110.5 months) than cases (median: 4 months; interquartile range: 2.5–27.5 months; $P = 6.0 \times 10^{-15}$). Moreover, all biochemical liver test results for controls had to be within the normal reporting range, which further limited the size of our control sample given that 30–60% of MS patients exposed to IFN-β will experience de novo liver enzyme elevations[1,2]. Although limiting our sample size for IFN-β-exposed controls, these stringent inclusion criteria increased the confidence in the clinical phenotype, enhancing our power to detect genetic variants of clinical relevance.

Participants included in stage 2 were recruited from three sites: a USA-based clinic (Partners HealthCare MS Clinic, Boston, MA) and two national adverse drug reaction surveillance networks located in the USA (the Drug-Induced Liver Injury Network[25,26]) and Sweden (SWEDEGENE; http://www.swedegene.se/). Inclusion criteria for patients recruited from the Drug-Induced Liver Injury Network matched those from other centers except that two consecutive elevations of the same magnitude described above for ALT, AST, or ALP were required[26]. The relevant research ethics board of each participating institution approved the study, and all participants provided written informed consent. This study complies with all relevant ethical regulations.

Clinical characterization. Each patient's medical record was reviewed before genotyping to capture demographic and clinical information and a comprehensive characterization of the adverse drug reaction, including drug exposure information and biochemical liver test results. The following information was collected from medical charts for all patients: demographics (sex, date of birth, and self-reported ancestry), body mass index, MS disease characteristics (that is, the MS disease course at IFN-β initiation (relapsing–remitting or secondary-progressive)), medications (IFN-β product (dose, route of administration, and start and stop dates) and concurrent medication usage (generic name, route of administration, dose, frequency, and start and stop dates, where possible)), and biochemical liver test results (date of test, test result (and value, if abnormal), and reporting laboratory upper limit of normal, if abnormal).

Genotyping, quality control, and imputation. Genome-wide genotyping was performed for the stage 1 participants ($n = 170$) using the Illumina MEGA array (1,705,969 genetic variants) followed by stringent sample and variant quality control methods. The stage 2 cohort was genotyped either in the same manner ($n = 10$) or as part of a genome-wide analysis of MS disease risk, as described previously[27] ($n = 24$). For the participants genotyped as part of the MS disease risk study, access to imputed genotype data allowed for the extraction of the variants of interest where necessary.

The following combination of thresholds for quality control metrics were implemented using either QCTOOL (version 2; http://www.well.ox.ac.uk/~gav/qctool_v2/), GTOOL (version 0.7.5; http://www.well.ox.ac.uk/~cfreeman/software/gwas/gtool.html) or PLINK (version 1.90)[28]. Genetic variants with a low call rate (<95%), a MAF of <1% in both cases and controls, and those deviating from Hardy–Weinberg equilibrium genotype distributions ($P < 1.0 \times 10^{-6}$ in controls) were excluded. No samples were related (using an identity-by-descent estimation metric ≤0.15). Three patient samples in the stage 1 cohort were excluded due to a low sample call rate. Non-autosomal markers were excluded from the analyses.

Genotype Harmonizer (1.4.15)[29] was used to ensure that variants were on the correct strand to facilitate imputation. Phasing was performed with SHAPEIT (version 2), followed by whole-genome imputation using IMPUTE2 (version 2.3.2)[29] and the Phase 3 1000 Genomes Project reference panel[30]. Markers with imputation information metrics ≥0.5 were included in the subsequent analyses. Imputation of classical *HLA* alleles and *HLA* region variants was performed with SNP2HLA (version 1.0.2)[31] using the stage 1 cohort genotype data and the Type 1 Diabetes Genetics Consortium reference panel. *HLA* alleles and related variants with imputation scores of $R^2 \leq 0.5$ and a call rate <0.85 were excluded from the subsequent analyses.

Genotyping calls of genome-wide significant variants (rs2205986) were validated in the stage 1 cohort patients using TaqMan genotyping assays (TaqMan Genotyper Software; Thermo Fisher Scientific) and exhibited 100% concordance with the array genotype (Supplementary Fig. 6).

GWAS stage 1 and stage 2 statistical analyses. Categorical variables (sex, MS disease course (relapsing–remitting or secondary-progressive), IFN-β product, liver injury pattern (hepatocellular, cholestatic, or mixed), and concomitant hepatotoxic medication use) were summarized by frequency (%), with age at IFN-β initiation and body mass index (continuous variables) summarized using the median (interquartile range) or mean (s.d.). Clinical and demographic factors were compared between cases and controls using the appropriate parametric (Pearson's chi-squared test or Student's *t*-test) or non-parametric tests (Fisher's exact test or Mann-Whitney *U*-test), and associations with $P < 0.05$ were considered significant (all *P* values were two-tailed).

Genetic ancestry was confirmed using principal components analysis (EIGENSTRAT method)[32], which was subsequently compared with self-reported ethnicity, with patients excluded based on non-European ancestry. To minimize the potential confounding effects of population stratification, a total of 16 (stage 1) and 3 (stage 2) samples were removed from the analyses owing to non-European ancestry (Supplementary Fig. 1). The first 10 principal components were re-calculated within the individuals who were of European genetic ancestry in stage 1 ($n = 151$), with no significant difference between cases and controls (Student's *t*-test, $P > 0.1$). Additionally, a genomic inflation factor of 1.06 indicates that the stage 1 participants ($n = 151$) used for genome-wide discovery were not notably influenced by population stratification (Supplementary Fig. 2b).

The association for each genomic marker passing quality control assessment with case or control status was tested using logistic regression in an additive model (adjusted for relevant clinical and demographic factors), with findings expressed as ORs with 95% CIs. A screening threshold ($P < 1.0 \times 10^{-6}$) was applied to the stage 1 cohort to prioritize variants for subsequent stage 2 analyses, where $P < 0.05$ was considered significant. Associations reaching the standard genome-wide significance threshold ($P < 5.0 \times 10^{-8}$) across the combined cohort (that is, stages 1 and 2) were considered to be statistically significant. For the *HLA* region, an *HLA*-wide significance threshold of $P < 2.3 \times 10^{-4}$ was set to account for Bonferroni correction for the 219 *HLA* alleles present in the cohort. $P < 0.05$ was considered significant for the replication of previously reported associations with *HLA* alleles and DILI.

Genome-wide association analyses were performed with SNPTEST (version 2; https://mathgen.stats.ox.ac.uk/genetics_software/snptest/snptest.html), and other statistical analyses were performed using Golden Helix SVS (version 8.4; Bozeman, MT, USA), IBM SPSS (version 22.0; Mississauga, Canada), or R for Statistical Computing (version 3.2.3). Plots (Manhattan plot, regional association plot, and the receiver operating characteristic (ROC) curve) were generated using LocusZoom[33], R for Statistical Computing, or Golden Helix SVS.

BioVU EMR analyses. *BioVU population.* A total of 279 MS patient samples previously genotyped at Vanderbilt University Medical Center (VUMC) were accessed. The samples were part of BioVU—a de-identified collection of DNA samples extracted from discarded blood and linked to de-identified EMRs[34]. All samples were identified as being from an individual with MS by previously published algorithms[35]. The EMRs were evaluated manually to identify dates of IFN-β treatment. Biochemical liver test results (ALT, AST, ALP, and total bilirubin) were extracted from structured fields of the EMRs during IFN-β treatment. A total of 87 unique MS patients were exposed to IFN-β, had a sample and available biochemical liver test results during IFN-β treatment, and were used in subsequent analyses. The highest value for each biochemical liver test result was identified independent of the other biochemical liver test results. For patients with more than one IFN-β treatment period, only the treatment period with the highest overall value was analyzed. Additionally, IFN-β-induced liver injury often presents with an hepatocellular pattern[3]. An exploratory analysis of 'mild DILI' was performed where cases had either ALT or AST levels >2× the upper limit of normal.

BioVU genotyping, quality control, and statistical analyses. Samples were genotyped on the Illumina MEGA^{EX} array at VUMC. Quality control was performed by BioVU as previously described[36], and array genotype data for rs2205986 were extracted. Relationship status was evaluated using PLINK[28] and revealed no related individuals (identity by descent: ≤0.15). Principal components were determined by multidimensional scaling in PLINK[28]. One patient sample was excluded from the ALP analyses due to being an outlier (>3 s.d. above the mean). BioVU association analyses were performed using PLINK[28]. The rs2205986 genotype was analyzed using an additive genetic model by linear regression for association with the highest values for each of the four biochemical liver test results during IFN-β treatment, while logistic regression was employed in the case-control association analyses. BioVU linear regression analyses were adjusted for age at biochemical liver testing, sex, and the first two principal components.

Disease-matched population control analyses. Genotype data (Illumina Human670-QuadCustom v1) for disease-matched population controls were obtained from the MS Wellcome Trust Case Control Consortium 2 cohort

(EGAD00000000120)[37] after approval from the relevant data access committees. We included MS patients recruited in North America and determined to be of northern European genetic ancestry (Supplementary Fig. 7), leaving 1,319 patients for these analyses. Quality control, strand alignment, and whole-genome imputation were performed as described above.

Predictive test analyses. Genomic markers of statistical significance were evaluated for specificity, sensitivity, negative predictive value, positive predictive value, and the number needed to screen within the combined patient cohort. Negative predictive value, positive predictive value, and the number needed to screen were calculated using sensitivity, specificity, and the population incidence of IFN-β-induced liver injury (2%)[3]. Post-test probabilities to estimate the proportion of patients testing positive for the variant who will develop DILI were assessed using likelihood ratios and pre- and post-test odds (http://www.cebm.net/likelihood-ratios/). ROC curves, the corresponding area under the curve estimates, and 95% CIs were generated for two predictive models of IFN-β-induced liver injury. The clinical model included age, IFN-β product, and sex (selected a priori based on previous DILI literature reporting significantly associated factors)[38], and a separate model incorporated these same variables in addition to any significantly associated genomic variants. The ROC curves of these two prediction models were compared using DeLong's test[39,40].

Reporting Summary. Further information on experimental design is available in the Nature Research Reporting Summary linked to this article.

Data availability. Data referenced in this study for the population control analysis are available from the Wellcome Trust Case Control Consortium 2 cohort under accession code EGAD00000000120 (data access committee approval is required). All remaining data are not publicly available owing to them containing information that could compromise research participant privacy or consent. Explicit consent to deposit data in public databases or to share them outside the specific use of this research study was not obtained from the patients.

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☐	☒	Clearly defined error bars *State explicitly what error bars represent (e.g. SD, SE, CI)*

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Software and code

Policy information about availability of computer code

Data collection	No software was used for data collection.
Data analysis	Analyses were performed with SNPTEST (version 2, https://mathgen.stats.ox.ac.uk/genetics_software/snptest/snptest.html), QCTOOL (version 2, http://www.well.ox.ac.uk/~gav/qctool_v2/), GTOOL (version 0.7.5, http://www.well.ox.ac.uk/~cfreeman/software/gwas/gtool.html), PLINK (version 1.90), Genotype Harmonizer (version 1.4.15), SHAPEIT (version 2), IMPUTE2 (version 2.3.2), SNP2HLA (version 1.0.2), Golden Helix SVS (version 8.4, Bozeman, USA), IBM SPSS (version 22.0, Mississauga, Canada), and R for Statistical Computing (version 3.2.3).

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- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

Data referenced in this study for the population control analysis are available from the Wellcome Trust Case Control Consortium 2 cohort under the following accession code: EGAD00000000120 (data access committee approval is required). All remaining data are not publicly available due to them containing information that could compromise research participant privacy or consent. Explicit consent to deposit data in public databases or to be shared outside the specific use of this research study was not obtained from the patients.

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Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	Our study was designed and powered (>85%) to identify associations with large effect sizes (per-allele OR ≥ 8.0) at a minor allele frequency of ≥ 0.10. Sample size estimation was performed using Quanto (v.1.2.4)
Data exclusions	Samples were excluded from analyses if they failed quality control or if they were of non-European genetic ancestry.
Replication	The experimental findings were reliably reproduced in two independent cohorts of interferon-beta treated multiple sclerosis patients.
Randomization	Samples were not randomly allocated into experimental groups, however all analyses were adjusted for any relevant covariates. More specifically the following adjustments were made: Genome-wide association analyses for the Stage 1 cohort (Table 1) was adjusted for MS disease course because controls were more likely to have a relapsing-remitting MS course than cases. The genome-wide association analyses in Stage 1 were also adjusted for the first five principal components and MS disease course. Genomic association analyses for the Stage 2 cohort were adjusted for age given that cases were significantly older than controls. We also adjusted the genomic association analyses of any additional variants that were within a 266-kb linkage disequilibrium block of the top variant (rs2205986) and were P<5.0x10-5 for the presence of rs2205986. BioVU linear regression analyses were adjusted for age at biochemical liver testing, sex, and the first two principal components.
Blinding	Not applicable. Investigators were not blinded to group allocation.

Reporting for specific materials, systems and methods

Materials & experimental systems

n/a	Involved in the study
☒	☐ Unique biological materials
☒	☐ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology
☒	☐ Animals and other organisms
☐	☒ Human research participants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Human research participants

Policy information about studies involving human research participants

Population characteristics	The characteristics of the sample population utilized in the Stage 1 and 2 aspects of this study were typical of a interferon-beta

Population characteristics	exposed MS population. The clinical and demographic characteristics of participants were similar between cases and controls across the study. There were minor differences in age and multiple sclerosis disease course between cases and controls in Stage 1 and 2 analyses, however the relevant analyses were adjusted for these potential confounders.
Recruitment	Subjects were recruited if they had either relapsing-remitting or secondary-progressive definite MS (based on Poser or McDonald criteria), documented exposure to an IFN-β product and had a normal baseline liver enzyme test. At least one alanine aminotransferase test result was required for baseline assessment. We recruited patients from Canadian-based MS clinics for stage one analyses. Participants included in stage two were recruited from three sites: a USA-based clinic (Partners HealthCare MS Clinic, Boston, USA) and two national adverse drug reaction surveillance networks, situated in the USA (the Drug-Induced Liver Injury Network) and Sweden (SWEDEGENE, http://www.swedegene.se/). Inclusion criteria for patients recruited from the Drug-Induced Liver Injury Network matched that from other centers except that two consecutive elevations of the same magnitude described above of ALT, AST or ALP were required. For the BioVU population, MS patient samples that were previously genotyped at Vanderbilt University Medical Center (VUMC), USA were accessed. The samples are part of BioVU, a de-identified collection of DNA samples extracted from discarded blood and linked to de-identified electronic medical records, which were evaluated manually to identify dates of IFN-β treatment and biochemical liver test results. For the disease-matched population control analyses, we obtained genotype data for MS disease-matched population control from the MS Wellcome Trust Case Control Consortium 2 cohort after approval by the relevant data access committees. We included MS patients recruited in North America and determined to be of Northern European genetic ancestry.