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A genome-wide study identifies HLA alleles associated with lumiracoxib-related liver injury

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Lumiracoxib is a selective cyclooxygenase-2 inhibitor developed for the symptomatic treatment of osteoarthritis and acute pain[1]. Concerns over hepatotoxicity have contributed to the withdrawal or non-approval of lumiracoxib in most major drug markets worldwide. We performed a case-control genome-wide association study on 41 lumiracoxib-treated patients with liver injury (cases) and 176 matched lumiracoxib-treated patients without liver injury (controls). Several SNPs from the MHC class II region showed strong evidence of association (the top SNP was rs9270986 with $P = 2.8 \times 10^{-10}$). These findings were replicated in an independent set of 98 lumiracoxib-treated cases and 405 matched lumiracoxib-treated controls (top SNP rs3129900, $P = 4.4 \times 10^{-12}$). Fine mapping identified a strong association to a common HLA haplotype (*HLA-DRB1*1501-HLA-DQB1*0602-HLA-DRB5*0101-HLA-DQA1*0102*, most significant allele $P = 6.8 \times 10^{-25}$, allelic odds ratio = 5.0, 95% CI 3.6–7.0). These results offer the potential to improve the safety profile of lumiracoxib by identifying individuals at elevated risk for liver injury and excluding them from lumiracoxib treatment.

Idiosyncratic drug-induced liver injury (DILI) is a major safety concern and has been a common cause for the marketing withdrawal of a wide range of drugs[2–4]. Most drugs causing severe idiosyncratic liver injury show no signs of hepatotoxicity in preclinical animal models and have very low rates of hepatotoxicity in humans; these drugs have an estimated incidence of liver transplantation or death in humans of ≤1 per 10,000 individuals taking the drug[5]. Due to the unpredictable and rare nature of these events, it is often not until the post-marketing stage, during which a large number of patients are exposed to the drug, that a drug's propensity for idiosyncratic liver injury is revealed; these events usually result in the marketing withdrawal of the drug. The discovery of genetic markers able to identify individuals at risk for developing DILI could potentially make otherwise safe and efficacious drugs available for use.

Lumiracoxib (Prexige/Joicela), a selective cyclooxygenase-2 (COX-2) inhibitor, has been shown to be efficacious in the symptomatic treatment of osteoarthritis and acute pain[1]. The risk of developing cardiovascular events, a major safety concern with selective COX-2 inhibitors, has been shown for lumiracoxib to be similar to nonsteroidal anti-inflammatory drugs[6,7]. However, concern over hepatotoxicity has led to market withdrawal or nonapproval of lumiracoxib in most drug markets worldwide. To identify genetic markers predictive of lumiracoxib-related hepatotoxicity, a retrospective pharmacogenetic case-control study was conducted using DNA samples collected from the Therapeutic Arthritis Research and Gastrointestinal Event Trial (TARGET)[6,8], an international, randomized 52-week gastrointestinal clinical safety study of more than 18,000 patients with osteoarthritis. TARGET consisted of two identically designed substudies, one comparing patients taking 400 mg of lumiracoxib once daily to those taking 500 mg of naproxen twice daily, and the other comparing patients taking 400 mg of lumiracoxib once daily to those taking 800 mg of ibuprofen three times daily.

An exploratory case-control genome-wide association study was conducted using 41 lumiracoxib-treated patients with peak liver enzyme (alanine aminotransferase (ALT) and/or aspartate aminotransferase (AST)) elevations exceeding five times the upper limit of normal (>5 times ULN) and 176 lumiracoxib-treated controls (predominantly having <1.2 times ULN). Controls were matched to cases at a ratio of approximately 4:1 based on race, sex, age, study and, where possible, country. The resulting genomic inflation factor was 1.025, indicating that population stratification was well controlled. This analysis identified a large number of SNPs from chromosome 6 (**Fig. 1**) showing evidence of association, with rs9270986 as the most significant SNP ($P = 2.8 \times 10^{-10}$; **Table 1**). Most of these significant SNPs are located in the extended MHC region. A total of seven SNPs, all mapping to the MHC class II region (6p21.32), remained significant after genome-wide multiple-testing correction, with rs9270986 yielding the most significant study-wide finding (permutation test $P = 0.0075$). A comparison of the observed and expected P value distributions showed an excess of small P values, with most of the deviation from expectation occurring in the MHC region (**Supplementary Fig. 1**).

To provide independent replication of these results, the seven SNPs that attained study-wide significance were evaluated in an independent set of lumiracoxib-treated patients in the TARGET study consisting of 98 cases with >3 times ULN ALT and/or AST and 405 controls matched to the cases as described above. All of these SNPs

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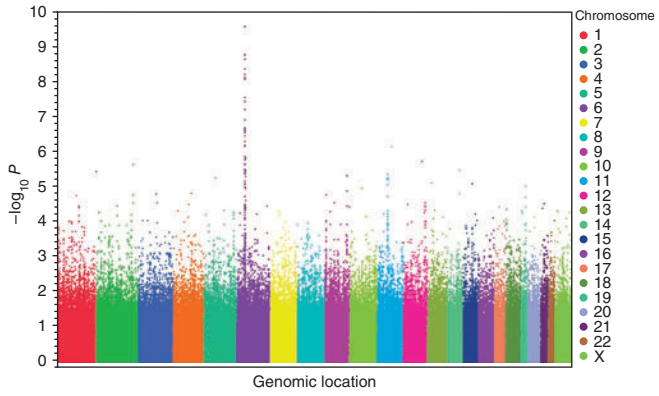

Figure 1 Genome-wide association results for all SNPs (n = 682,386) included in the analysis ($-\log_{10} P$ values graphed by genomic location).

were found to be associated with lumiracoxib-related liver enzyme elevation (**Table 1**), with rs3129900 being the most significant SNP ($P = 4.4 \times 10^{-12}$).

To further refine this association, a fine-mapping study was undertaken by directly genotyping genes within the MHC class II region. Genotyping was performed for *HLA-DRB1*, *HLA-DRB3-5*, *HLA-DQA1* and *HLA-DQB1* on all patients included in the genome-wide screen and the replication study. A total of 137 cases with >3 times ULN ALT and/or AST and 577 matched controls were successfully genotyped and used for a case-control analysis. Four alleles yielded highly significant associations (**Table 2**), with *HLA-DRB1*1501* being the most significant allele ($P = 6.8 \times 10^{-25}$, allelic OR = 5.0, 95% CI 3.6–7.0). These alleles lie on a common haplotype (*HLA-DRB1*1501-HLA-DQB1*0602-HLA-DRB5*0101-HLA-DQA1*0102*) that has been associated with multiple sclerosis[9].

Although the HLA analysis was predominantly comprised of patients of European descent (self-identified 'Caucasians') (comprising 84.7% of the study cohort), it also included a small number of self-reported Hispanics (13.9% of the cohort) (**Supplementary Table 1**). Consistent with the overall analysis, *HLA-DRB1*1501* was the most significantly associated allele within the Hispanic subgroup ($P = 0.0007$, allelic OR = 6.0, 95% CI 2.1–16.9; **Supplementary Note** and **Supplementary Fig. 2**).

Using data from the TARGET study, we evaluated the potential for each of these HLA alleles to function as a predictive marker using the predictive parameters of sensitivity, specificity, positive and negative predictive values (PPV and NPV), and odds ratio (OR) for developing >3 times ULN ALT and/or AST (**Table 2**). Patients carrying at least one copy of the risk allele were considered to have the risk marker, as little difference in risk or magnitude of liver enzyme elevation was observed between homozygotes and heterozygotes. PPV and NPV were extrapolated to the full TARGET lumiracoxib-treated population by assuming the same carrier frequency among non-genotyped cases as among genotyped cases, and the same assumption was made for controls. Because the utility of predictive safety markers is to exclude patients who are at risk for developing adverse events, NPV and sensitivity are the parameters of greatest importance in analyses of this type. Although multiple HLA alleles demonstrated a highly significant association (P values ranging from 1.2×10^{-18} to 6.8×10^{-25}; **Table 2**), *HLA-DQA1*0102* had the best results for these parameters, with 99.0% NPV and 73.6% sensitivity. Hence, although the incidence of ALT and/or AST elevation to >3 times ULN was 2.5% among all lumiracoxib recipients in TARGET, it is extrapolated that this number would decrease to 1.0% among non-carriers of *HLA-DQA1*0102*. Multiallele markers, including *HLA-DQA1*0102* and other alleles on the haplotype, did not improve NPV because nearly all chromosomes carrying the other alleles also carried *HLA-DQA1*0102*.

The low PPVs of the alleles on the haplotype (ranging from 5.8% to 8.0%; **Table 2**) are due to the relatively high frequency of the allele in the study population (~20% to 30% of controls carry the allele) as compared to the 2.5% incidence of actual ALT and/or AST elevations (defined as >3 times ULN). These results show that the presence of the risk allele is not sufficient to trigger hepatotoxicity in lumiracoxib-treated patients. The process underlying the development of hepatotoxicity is likely combinatorial, with other factors, such as environment, diet, comorbid diseases, co-medications and possibly other genetic factors, likely contributing as well. A follow-up genome-wide analysis failed to identify other SNPs associated with liver enzyme elevation independently of the identified HLA alleles (**Supplementary Table 2**).

The sensitivity of the *HLA-DQA1*0102* allele as a predictor of lumiracoxib-related liver enzyme elevation was found to increase as more severe elevation thresholds were examined (**Table 3**). The sensitivity was 73.6% for patients with elevations to >3 times ULN but improved to 84.1% for those with >5 times ULN, 91.2% for >8 times ULN and, ultimately, reached 100% for patients with >20 times ULN (all patients (eight in total) with >20 times ULN carried the allele). The most severe type of hepatotoxicity examined was that commonly termed 'Hy's law', defined as drug-related ALT and/or AST >3 times ULN and serum bilirubin >2 times ULN. Patients meeting these criteria have a greater risk of developing severe hepatotoxicity compared to those with elevations in transaminase enzymes only[10]. All three lumiracoxib-treated patients meeting Hy's law criteria in this study for whom DNA was available were found to be heterozygous for the allele. Moreover, cases (defined as having >3 times

Table 1 Significant findings identified in the genome-wide analysis from the MHC region on chromosome 6

		Genome scan		Replication study		All samples			
SNP ID	Position	P value	Allelic OR (95% CI)	P value	Allelic OR (95% CI)	P value	Allelic OR (95% CI)	MAF cases (%)	MAF controls (%)
rs9270986	32,682,038	2.8×10^{-10}	5.3 (3.0–9.2)	1.0×10^{-9}	3.0 (2.1–4.4)	3.6×10^{-18}	3.8 (2.8–5.2)	37.3	13.8
rs3129900	32,413,957	1.8×10^{-9}	4.7 (2.7–8.2)	4.4×10^{-12}	3.4 (2.3–4.8)	8.3×10^{-18}	3.5 (2.6–4.8)	38.3	14.7
rs3132943	32,416,443	1.9×10^{-9}	4.5 (2.6–7.7)	4.4×10^{-7}	2.4 (1.7–3.4)	5.4×10^{-14}	2.9 (2.2–3.9)	43.6	21.0
rs3129934	32,444,165	2.5×10^{-9}	4.7 (2.7–8.1)	4.9×10^{-11}	3.2 (2.2–4.7)	6.1×10^{-16}	3.4 (2.5–4.6)	36.8	14.6
rs3135365	32,497,233	4.5×10^{-9}	4.4 (2.6–7.6)	6.3×10^{-10}	2.9 (2.1–4.1)	6.0×10^{-17}	3.4 (2.5–4.7)	42.2	18.0
rs3129932	32,444,105	6.5×10^{-9}	4.3 (2.5–7.4)	2.5×10^{-7}	2.5 (1.7–3.5)	7.0×10^{-14}	2.9 (2.2–3.9)	43.3	20.9
rs910049	32,423,705	6.6×10^{-9}	4.3 (2.5–7.4)	2.6×10^{-7}	2.5 (1.7–3.5)	6.9×10^{-14}	2.9 (2.2–3.9)	44.7	22.0

The genome scan was performed on 41 lumiracoxib-treated cases with peak ALT and/or AST elevation to >5 times ULN and 176 matched lumiracoxib-treated controls. The replication study was performed on an independent set of 98 lumiracoxib-treated cases with peak ALT and/or AST elevation to >3 times ULN and 405 matched lumiracoxib-treated controls. Study-wide significance was assessed by permutation test. MAF, minor allele frequency.

Table 2 Most significant HLA alleles associated with elevated liver enzymes and their predictive parameters

Gene and allele	*P* value	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	OR (95% CI)	Allele frequency case, control (%)
*HLA-DRB1*1501*	6.8×10^{-25}	64.2	80.8	8.0	98.9	7.5 (5.0–11.3)	35.4, 10.5
*HLA-DQB1*0602*	1.1×10^{-22}	62.0	80.8	7.7	98.8	6.9 (4.6–10.3)	34.3, 10.5
*HLA-DRB5*0101*	1.6×10^{-20}	64.2	80.1	7.7	98.9	7.2 (4.8–10.8)	32.1, 10.0
*HLA-DQA1*0102*	1.2×10^{-18}	73.6	69.2	5.8	99.0	6.3 (4.1–9.6)	42.7, 17.4

Elevated liver enzymes defined here as ALT and/or AST of >3 times ULN; n=137 cases and n=577 controls. PPV, positive predictive value; NPV, negative predictive value; OR, odds ratio. For the calculations of sensitivity, specificity, PPV, NPV and OR, patients carrying at least one copy of the risk allele were considered to have the risk marker. The sensitivity is the frequency among cases of carrying at least one copy of the risk allele. The specificity is equivalent to 100% minus the frequency among controls of carrying at least one copy of the risk allele.

ULN ALT and/or AST) who carry the allele had higher mean peak ALT and/or AST values than cases who are non-carriers (8.7 times ULN compared to 5.1 times ULN, $P = 0.019$; **Supplementary Fig. 3**). Taken together, these results indicate that, among patients with liver enzyme elevations, carriers of the *HLA-DQA1*0102* allele are more likely to experience severe hepatotoxicity than non-carriers. This may reflect a difference in the hepatotoxicity mechanism between *HLA-DQA1*0102* carriers and non-carriers, or it may suggest that many of the less severe elevations were caused by sporadic, non–drug related factors that would skew the marker sensitivity toward lower values for lower thresholds.

Further comparison of *HLA-DQA1*0102* carriers and non-carriers among lumiracoxib-treated patients with ALT and/or AST elevations to >3 times ULN revealed a difference with respect to the type of liver injury. A total of 76.5% of the *HLA-DQA1*0102* carriers had hepatocellular injury compared to only 45.9% of non-carriers ($P = 0.014$) (**Supplementary Table 3**), again suggesting that the underlying mechanisms of hepatotoxicity may differ by *HLA-DQA1*0102* carrier status.

To further examine the performance characteristics of the *HLA-DQA1*0102* marker, all remaining TARGET lumiracoxib-treated patients with DNA available who had given informed consent were genotyped to determine presence or absence of the *HLA-DQA1*0102* allele. A total of 4,518 patients were genotyped. The predictive parameters were recalculated for this sample set at various severity thresholds (**Supplementary Table 4**). Kaplan-Meier estimates of the cumulative incidence of liver enzyme elevations were obtained for *HLA-DQA1*0102* carriers and non-carriers and were compared to estimates for all lumiracoxib-treated, ibuprofen-treated and naproxen-treated patients (**Fig. 2**). Estimates were obtained for five time intervals (days 1–49, 50–105, 106–196, 197–287 and >287) to enable a comparison of time patterns of incidence between these populations. The *HLA-DQA1*0102* carriers treated with lumiracoxib showed a dramatic increase in incidence of liver toxicity between days 106 and 196 in the populations with >3 and >5 times ULN, suggesting that HLA-driven liver toxicity does not typically manifest clinically until after several weeks of exposure to lumiracoxib. These findings are in contrast to those found in the non-carriers treated with lumiracoxib, who had a consistent and more gradual slope for cumulative incidence of liver enzyme elevation throughout the study. These differing incidence patterns also support the hypothesis that *HLA-DQA1*0102* carriers and non-carriers have different underlying mechanisms of hepatotoxicity. It is also noteworthy that the lumiracoxib-treated non-carriers have an incidence pattern remarkably similar to that of ibuprofen-treated patients, particularly in those with elevations to >5 times ULN ALT and/or AST (**Fig. 2** and **Supplementary Fig. 4**).

The mapping of the genetic association to the MHC class II region suggests a possible immunological mechanism for lumiracoxib-related hepatotoxicity. Metabolism of lumiracoxib may produce reactive metabolites that lead to the formation of immunogenic adducts. These adducts could be recognized and presented at the cell surface by specific HLA complexes, triggering a T-cell–mediated immune response (hapten hypothesis)[11]. Alternatively, lumiracoxib may directly interact with the HLA complex (pharmacological interaction hypothesis)[12], again triggering an immune reaction.

Table 3 Sensitivity of the *HLA-DQA1*0102* allele as a function of increasing ALT and/or AST threshold

ALT and/or AST elevation threshold	Sensitivity (%)	Number of cases with at least one copy of the *HLA-DQA1*0102* allele	Total number of genotyped cases
>3× ULN	73.6	103	140
>5× ULN	84.1	53	63
>8× ULN	91.2	31	34
>10× ULN	92.0	23	25
>15× ULN	94.1	16	17
>20× ULN	100.0	8	8

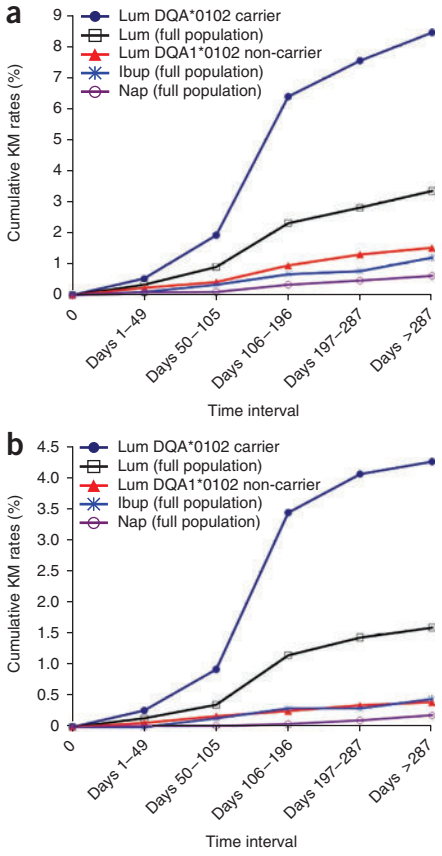

Figure 2 Cumulative Kaplan-Meier incidence estimates (shown as percentages) for liver enzyme elevations in the TARGET study comparing *HLA-DQA1*0102* carrier status and comparator arms. (Lum, lumiracoxib 400 mg once a day; ibup, ibuprofen 800 mg three times a day; nap, naproxen 500 mg twice a day.) (**a**) Elevations to >3 times ULN ALT and/or AST. (**b**) Elevations to >5 times ULN ALT and/or AST. See **Supplementary Table 6** for number of subjects at risk for each time interval.

In vitro and *in vivo* studies will be required to further elucidate the mechanisms underlying lumiracoxib-related hepatotoxicity.

The findings of this study are consistent with several studies linking the MHC region to the development of drug-related serious adverse events. The *HLA-B*5701* allele has previously been strongly associated with hypersensitivity reactions in patients treated with abacavir[13–15] and with DILI in patients treated with flucloxacillin[16]. An association has been established between the *HLA-B*1502* allele and toxic epidermal necrolysis and Stevens-Johnson syndrome in patients of Asian descent treated with carbamazepine[17]. The *HLA-DRB1*07* and *DQA1*02* alleles have been associated with elevated ALT levels in patients treated with ximelagatran[18]. Also, an association has been identified between the haplotype *HLA-DRB1*1501-HLA-DQB1*0602-HLA-DRB5*0101* and amoxicillin-clavulanate–induced hepatotoxicity[19,20]. Notably, this is the same haplotype identified in the present study of lumiracoxib-induced hepatotoxicity, marking, to our knowledge, the first time that the same allele or haplotype has been associated with hepatotoxicity for two different drugs. Lumiracoxib, however, shares no obvious structural similarity with either amoxicillin or clavulanate. Moreover, amoxicillin-clavulanate liver injury is usually mixed or cholestatic[19] and occurs an average of 25 d after first exposure, whereas lumiracoxib-related liver injury is predominantly hepatocellular and typically occurs after 106–196 d of exposure. The reasons for these differences are not clear. The causal alleles for these associations, although present on the same haplotype, may reside within different genes. Alternatively, underlying differences in drug metabolism or other factors may contribute to the different DILI phenotypes observed.

The results presented here provide strong evidence that the *HLA-DQA1*0102* allele would have clinical utility as a screening marker to exclude carriers from lumiracoxib treatment. Because 33.6% of the genotyped patients in TARGET carry this allele (**Supplementary Table 5**), but only 5.6% of the allele carriers would be expected to develop ALT and/or AST elevations if treated with lumiracoxib (**Supplementary Table 4**), the use of this allele for exclusion would result in substantial overexclusion. Nevertheless, it would result in a substantial reduction in risk for those patients eligible to receive lumiracoxib.

The study described here offers clear and strong evidence for an association between polymorphisms in the MHC class II region and lumiracoxib-induced hepatotoxicity. In addition to providing insight into the mechanisms of lumiracoxib-related hepatotoxicity, these findings offer the potential to improve the safety profile of lumiracoxib and move the field of personalized medicine forward with one of the first marketed DILI safety markers.

METHODS

Methods and any associated references are available in the online version of the paper at http://www.nature.com/naturegenetics/.

Note: Supplementary information is available on the Nature Genetics website.

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AUTHOR CONTRIBUTIONS

The genome-wide association study was designed by C.A.P., S.L., J.M. and J.B.S. with analysis and interpretation performed by C.A.P., S.L., J.B.S., J.M. and F.Y. The HLA fine mapping study was designed by C.A.P., J.B.S. and S.L., and analysis and interpretation was performed by C.A.P., J.B.S., S.L., J.M., L.K. and T.M.W. Genotyping was supervised and performed by E.L. and X.Z. C.A.P., S.L. and J.B.S. wrote the first draft of the manuscript, and all other authors contributed to and approved the final draft.

COMPETING FINANCIAL INTERESTS

The authors declare competing financial interests: details accompany the full-text HTML version of the paper at http://www.nature.com/naturegenetics/.

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ONLINE METHODS

Study population. TARGET was an international, multicenter, randomized, double-blind, double-dummy, 52-week gastrointestinal clinical safety study of patients with osteoarthritis[6,8]. For logistical reasons, TARGET was conducted in two identical sub-studies: CCOX189 0117 (with comparator naproxen) and CCOX189A2332 (with comparator ibuprofen). A total of 18,244 subjects received treatment (9,117 with 400 mg of lumiracoxib once a day, 4,730 with 500 mg of naproxen twice a day and 4,397 with 800 mg of ibuprofen three times a day). Participation in the pharmacogenetic component was optional. DNA was extracted from blood from 10,057 patients, all of which gave informed written consent. Liver enzyme elevation cases were adjudicated by an independent liver safety committee. All instances of lumiracoxib-associated liver enzyme elevations in the TARGET study were shown to be reversible after stopping lumiracoxib treatment. Demographics of the subjects included in the pharmacogenetic analysis are provided in **Supplementary Table 1**.

Genotyping. Genomic DNA was extracted from the subject's blood by Covance using the Autopure System (Qiagen). Whole-genome amplification was performed on all samples used for the genome-wide association studies and initial HLA genotyping using the illustra GenomiPhi V2 DNA Amplification Kit (GE Healthcare). Genomic DNA was used in the replication study and in subsequent HLA genotyping.

Genotyping for the genome-wide screens was performed using the Affymetrix Genome-Wide Human SNP Array 6.0. Initial quality control screening for call rate and called gender was performed in Genotyping Console 2.0 (Affymetrix). Chips with an initial call rate >84% were genotyped with the Birdseed algorithm in apt-probeset-genotype (Affymetrix). Samples whose inferred gender failed to match the clinical database were excluded. The following inclusion criteria were applied: minimum 97% call rate for SNPs, minimum 90% call rate for cases, minimum Hardy-Weinberg *P* value in controls of 5×10^{-8}, and minimum 2% minor allele frequency. Following quality control, a total of 682,386 SNPs met inclusion criteria.

Genotyping of patient polymorphisms in the replication study was performed using TaqMan Assays-by-Design and Assays-on-Demand on a 7900HT Fast Real Time PCR system according to the manufacturer's instructions (Applied Biosystems). Genotyping for the HLA allele identification study was performed by IMGM Laboratories GmbH.

HLA-DQA1 and *HLA-DQB1* genotyping was performed using LABType SSO DQA1/DQB1Typing test kit (One Lambda) coupled with the Luminex xMAP technology (Luminex) according to the manufacturer's instructions. Ambiguities in the results were then resolved using the Olerup SSP DQA1 and SSP DQB1 03,04,05,06 kits from Genovision (Qiagen). The Genovision Helmberg SCORE software was used to assign allele designations. *HLA-DRB1* genotyping was performed using the LABType SSO DRB1 High Definition Typing Test. *HLA-DRB3, HLA-DRB4* and *HLA-DRB5* genotyping was performed using the LABType SSO DRB3, 4, 5 Typing test. Ambiguities in the results were then resolved using Olerup SSP kits DRB3*, B4*, B5*. In rare instances, additional ambiguities were resolved using IMGM in-house sequence-based-typing test. The assignment of HLA alleles was based on the comparison of the test reaction pattern to patterns associated with HLA allele definitions in the IMGT/HLA database (see URLs).

For the last stage of the analysis, the *HLA-DQA1*0102* allele was genotyped in house by direct sequencing of exon 2 of *HLA-DQA1* using the dideoxy termination method (ABI BigDye v1.1) on an ABI 3730X DNA analyzer (Applied Biosystems). *HLA-DQA1* genotypes were called by analyzing sequence traces from both sequencing primers using the software SBTengine (Genome Diagnostics).

Statistical analysis. The case-control analysis was comprised of three stages: an initial genome-wide screen, replication of the top findings in an independent set of cases and controls, and a fine-mapping study of nearby HLA genes. All association tests were two-tailed, single-point tests for an additive allelic effect using the Cochran-Mantel-Haenszel test for 2-by-2-by-K stratified tables (allele by case or control status for K strata, with each stratum consisting of one case and approximately four matched controls). Study-wide significance was assessed by permutation test. All tests were performed using PLINK[21].

In the first stage, an exploratory case-control genome-wide screen was performed on an initial set of lumiracoxib-treated patients including 41 cases with peak ALT and/or AST level >5 times ULN and 176 controls who never experienced either ALT or AST elevations >3 times ULN. Based on self-classification, the cases consisted of 37 Caucasians (90.2%), 3 Hispanics (7.3%) and 1 patient classified as 'other' (2.4%). Hence, to control for population stratification, controls were matched to cases at a ratio of approximately 4:1 on the basis of self-reported race and, where possible, country, in addition to gender, age (matched as ±2 years of age from each other, where possible) and clinical study (CCOX189 0117 or CCOX1892332). Controls were selected to have peak ALT and/or AST <1.2 times ULN where possible; otherwise, they were selected to have the lowest peak ALT and/or AST among available matching candidates.

As shown by the quantile-quantile plot excluding the MHC region (**Supplementary Fig. 1**) and the genomic inflation factor (equaling 1.025), population stratification was reasonably well controlled in this analysis. Moreover, inspection of the scatter plot of the first two principal components (**Supplementary Fig. 5**) shows no evidence that the cases and controls included in this analysis had differing genetic backgrounds, whether among all patients, Caucasians only or Hispanics only (**Supplementary Fig. 5**). Hence, although *HLA-DQA1*0102* allele frequencies vary across races (**Supplementary Table 5**), these results indicate that any bias due to population stratification was very small and did not have substantial influence on the identification of the association between this allele and risk of liver enzyme elevation.

In the second stage, a replication analysis was performed in which those SNPs attaining study-wide significance in the first stage were tested in an independent set of lumiracoxib-treated patients, including 98 cases with peak ALT and/or AST >3 times ULN and 405 matched controls. Matching was again performed at a ratio of approximately 4:1 using the above criteria.

In the third stage, HLA genotypes were obtained for the genes neighboring the most strongly associated SNPs. These genes are in the MHC class II region and include *HLA-DRB1*, *HLA-DRB3-5*, *HLA-DQA1* and *HLA-DQB1*. HLA genotyping was performed for all cases and controls included in the first two stages. For each four-digit allele at each HLA gene, cases were compared to controls with respect to frequency of chromosomes carrying the given allele.

Sensitivity, specificity, PPV and NPV, and OR were calculated for each of the most strongly associated alleles based on data from the lumiracoxib-treated patients in the TARGET study. For these calculations, patients carrying at least one copy of the risk allele were considered to have the risk marker. Sensitivity was calculated as the ratio of the number of cases with at least one copy of the risk allele to the total number of cases, and specificity was calculated as the ratio of the number of controls with no copies of the risk allele to the total number of controls. The OR was calculated as the ratio of the odds of being a case conditional on carrying at least one copy of the risk allele to the odds of being a case conditional on carrying no copies of the risk allele. PPV and NPV were extrapolated to the full TARGET population by assuming the same carrier frequency among nongenotyped cases as among genotyped cases, and this same assumption was made for controls. From the resulting extrapolated 2×2 table, the PPV was calculated as the ratio of the number of cases with at least one copy of the risk allele to the total number of patients with at least one copy of the risk allele, and the NPV was calculated as the ratio of the number of controls with no copies of the risk allele to the total number of patients with no copies of the risk allele.

The *HLA-DQA1*0102* allele was genotyped in all remaining lumiracoxib-treated patients in TARGET for whom DNA was available and who had given informed written consent. A total of 4,518 patients were successfully genotyped, including those genotyped previously. Among the newly genotyped patients were three additional cases (defined as having >3 times ULN ALT and/or AST) who had not been successfully genotyped in the earlier stages, bringing the number of genotyped cases to 140. All subsequent analyses were based on this population. Sensitivity, specificity, PPV, NPV and OR were recalculated as above. Kaplan-Meier cumulative incidence estimates of ALT and/or AST elevations were obtained for *HLA-DQA1*0102* carriers and non-carriers, as well as for all lumiracoxib-treated, ibuprofen-treated and naproxen-treated patients.

Tests on patients with ALT and/or AST >3 times ULN to detect differences between *HLA-DQA1*0102* carriers and non-carriers in mean peak ALT and/or AST level (log-transformed) and frequency of hepatocellular injury were performed by linear and logistic regression, respectively, with race as a covariate.

URL. IMGT/HLA database, http://www.ebi.ac.uk/imgt/hla/.

21. Purcell, S. *et al.* PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am. J. Hum. Genet.* **81**, 559–575 (2007).

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