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ORIGINAL ARTICLE

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ABSTRACT

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BACKGROUND

Carbamazepine causes various forms of hypersensitivity reactions, ranging from maculopapular exanthema to severe blistering reactions. The HLA-B*1502 allele has been shown to be strongly correlated with carbamazepine-induced Stevens–Johnson syndrome and toxic epidermal necrolysis (SJS–TEN) in the Han Chinese and other Asian populations but not in European populations.

METHODS

We performed a genomewide association study of samples obtained from 22 subjects with carbamazepine-induced hypersensitivity syndrome, 43 subjects with carbamazepine-induced maculopapular exanthema, and 3987 control subjects, all of European descent. We tested for an association between disease and HLA alleles through proxy single-nucleotide polymorphisms and imputation, confirming associations by high-resolution sequence-based HLA typing. We replicated the associations in samples from 145 subjects with carbamazepine-induced hypersensitivity reactions.

RESULTS

The HLA-A*3101 allele, which has a prevalence of 2 to 5% in Northern European populations, was significantly associated with the hypersensitivity syndrome ($P=3.5\times10^{-8}$). An independent genomewide association study of samples from subjects with maculopapular exanthema also showed an association with the HLA-A*3101 allele ($P=1.1\times10^{-6}$). Follow-up genotyping confirmed the variant as a risk factor for the hypersensitivity syndrome (odds ratio, 12.41; 95% confidence interval [CI], 1.27 to 121.03), maculopapular exanthema (odds ratio, 8.33; 95% CI, 3.59 to 19.36), and SJS–TEN (odds ratio, 25.93; 95% CI, 4.93 to 116.18).

CONCLUSIONS

The presence of the HLA-A*3101 allele was associated with carbamazepine-induced hypersensitivity reactions among subjects of Northern European ancestry. The presence of the allele increased the risk from 5.0% to 26.0%, whereas its absence reduced the risk from 5.0% to 3.8%. (Funded by the U.K. Department of Health and others.)

CARBAMAZEPINE IS ONE OF THE MOST commonly prescribed drugs for the treatment of epilepsy, as well as trigeminal neuralgia and bipolar disorder. A minority of treated persons have hypersensitivity reactions that vary in prevalence and severity,[1] with some forms associated with substantial morbidity and mortality. The mildest form, maculopapular exanthema, occurs in 5 to 10% of treated persons of European ancestry and resolves spontaneously after drug discontinuation. More severe reactions, such as the hypersensitivity syndrome, are associated with mortality of up to 10%[2] and include symptoms such as rash, fever, eosinophilia, hepatitis, and nephritis. The most severe reactions, such as the Stevens–Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN), are characterized by a blistering rash affecting a variable percentage of the body-surface area. The rate of death increases with the degree of epidermal detachment. TEN is the rarest of these phenotypes and is associated with mortality of up to 30%. According to the labeling of carbamazepine, as mandated by the Food and Drug Administration (FDA), the estimated incidence of SJS–TEN is 1 to 6 cases in 10,000 persons of European ancestry who are exposed to the drug.

Genomewide approaches are increasingly used to identify genetic predisposing factors for drug-induced hypersensitivity reactions and drug-induced liver injury.[3] For example, the HLA-B*1502 allele has been identified as a clinically important predictor of SJS–TEN in Asians of Han Chinese descent who are candidates for treatment with carbamazepine.[4] This finding led the FDA to require a warning label for carbamazepine to indicate the need for genotyping for HLA-B*1502 before the drug is prescribed. This change seems warranted, given the prospective study described in this issue of the *Journal*,[5] which shows that withholding carbamazepine from subjects of Han Chinese ancestry who carry the HLA-B*1502 allele substantially decreased the rate of the development of SJS–TEN. The value of HLA-B*1502 in predicting the risk of SJS–TEN has been shown in other Asian populations, including those of Thailand, Malaysia, and India.[4,6-8] This effect is specific for SJS–TEN, as compared with the full spectrum of carbamazepine-related hypersensitivity reactions, which led to the suggestion that different phenotypes of carbamaze-pine-induced hypersensitivity may have distinct genetic predictors. Furthermore, HLA-B*1502 is rare (with a prevalence of less than 2%) in populations of European descent, in which carbamazepine-induced SJS–TEN occurs at a lower incidence than in Asian populations.[9-12] Using a candidate-gene approach, previous studies have shown weak or moderate signals of association between maculopapular exanthema or the DRESS (drug reaction with eosinophilia and systemic symptoms) syndrome and single-nucleotide polymorphisms (SNPs) in the major histocompatibility complex (MHC) region,[13-15] and these associations have not been consistently replicated.[13,14]

We present data from two independent studies that suggest the relevance of an HLA variant to the clinical spectrum of carbamazepine-related hypersensitivity reactions, including maculopapular exanthema, the hypersensitivity syndrome, and SJS–TEN.

METHODS

CASE SUBJECTS

Recruitment

We recruited case subjects at centers collaborating with the University of Liverpool and Walton Centre for Neurology (both hereinafter referred to as "the Liverpool collaborators") or at centers affiliated with the EPIGEN consortium (for details, see the Supplementary Appendix, available with the full text of this article at NEJM.org). All case subjects and control subjects were of European ancestry as determined by either self-report or genetic-marker analysis.[13] Many of these subjects have been described previously in the context of hypersensitivity reactions.[9,13,16]

Hypersensitivity Syndrome

The group with the hypersensitivity syndrome consisted of 26 subjects: 23 who were recruited through the Liverpool collaborators and 3 who were recruited through the EPIGEN consortium. The hypersensitivity syndrome was defined as the presence of rash or liver involvement within 3 months after the initiation of carbamazepine treatment, accompanied by a minimum of two of the following manifestations: a prolonged recovery phase, despite withdrawal of the drug; fever; involvement of other internal organs (liver, kidney, lung, central nervous system, heart, muscle, thy-

roid, or lymphoid system); or the presence of he-
matologic abnormalities, such as eosinophilia
and atypical lymphocytosis.[17] We considered the
DRESS syndrome to be the same as the hyper-
sensitivity syndrome.

Acute Generalized Exanthematous Pustulosis

We recruited one subject with acute generalized
exanthematous pustulosis (AGEP), who had a
typical pustular reaction with high fever,[18] from
a collaborating clinic in Switzerland. We then
analyzed the data from this subject together with
data from the subjects with the hypersensitivity
syndrome.

Maculopapular Exanthema

The group with maculopapular exanthema con-
sisted of 106 subjects: 57 who were recruited
through the Liverpool collaborators and 49 who
were recruited through the EPIGEN consortium.
Maculopapular exanthema was defined as rash
without systemic symptoms that required the dis-
continuation of carbamazepine within 3 months
after the initiation of drug therapy.

SJS–TEN

The SJS–TEN group consisted of 12 subjects: 8 who
were recruited through the Liverpool collaborators
and 4 who were recruited through the EPIGEN
consortium. The diagnostic criteria suggested by
the RegiSCAR Project (a research network for se-
vere cutaneous adverse drug reactions)[11,19] were
used for this group (see the Supplementary Ap-
pendix).

CONTROL SUBJECTS

Population Controls

Genotype data from the Wellcome Trust Case
Control Consortium (WTCCC) (www.wtccc.org
.uk) were used as a population control group. The
Liverpool collaborators used data from a genome-
wide association study of samples from 2691
subjects in the U.K. National Blood Services Col-
lection. EPIGEN used samples from a homoge-
neous subgroup of 1296 subjects in the 1958 Brit-
ish Birth Cohort that were selected by principal
component analysis. We did not screen either set
of population controls for carbamazepine-related
adverse drug reactions. (For details about our
access to these data, see the Supplementary Ap-
pendix.)

Clinical Controls

The clinical control group consisted of 257 sub-
jects with epilepsy who had been taking carba-
mazepine for at least 3 months with no clinical
or biochemical signs of hypersensitivity. Of these
subjects, 44 were recruited by the Liverpool col-
laborators and 213 were recruited from clinics
affiliated with EPIGEN.

GENOMEWIDE GENOTYPING

Genomewide analysis of samples that were ini-
tially available from the 22 Liverpool subjects
with carbamazepine-induced hypersensitivity syn-
drome was performed with the use of the Illu-
mina Infinium 1.2M chip at the Wellcome Trust
Sanger Institute. Samples from 43 case subjects
and 200 control subjects from the EPIGEN con-
sortium were genotyped with the use of the Illu-
mina 610K Quad platform at the Duke University
Center for Human Genome Variation. (Details
regarding quality controls and principal-compo-
nents analysis are provided in the Supplementary
Appendix.)

IMPUTATION OF HLA ALLELES

We based the imputation of classic HLA alleles in
samples from the 22 Liverpool subjects with the
hypersensitivity syndrome and control subjects
from the U.K. National Blood Service on a dense
reference panel of SNP data and four-digit HLA
types in 2767 unrelated subjects of European de-
scent, according to a method described recently.[20]
We imputed classic HLA alleles in the samples
from the EPIGEN consortium, using MACH 1.0 on
the Web site of the Center for Statistical Genetics
(www.sph.umich.edu/csg/abecasis/MACH).[21] The
1458 subjects in the HLA-typed 1958 British Birth
Cohort constituted the reference population. (See
the Supplementary Appendix for further details.)
We used different approaches to impute the HLA
alleles in the Liverpool and EPIGEN subjects,
because the two studies were originally inde-
pendent.

HLA-A HIGH-RESOLUTION GENOTYPING

To confirm the accuracy of imputation, high-
resolution, sequence-based HLA-A typing of sam-
ples from 22 subjects with the hypersensitivity
syndrome and from a subgroup of 44 clinical
control subjects (i.e., subjects who did not have
adverse reactions to carbamazepine) was per-

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formed by Histogenetics. We determined the HLA-A alleles in samples from 49 subjects with maculopapular exanthema and a subgroup of 213 clinical control subjects from the EPIGEN consortium, using sequence-specific polymerase-chain-reaction assay primers (details available on request). Direct genotyping showed 100% concordance with the imputed alleles.

HLA-A*3101 PROXY SNP GENOTYPING

Genotyping of the rs1061235 SNP in samples from all Liverpool subjects (including case subjects with maculopapular exanthema, the hypersensitivity syndrome, or SJS–TEN and clinical control subjects) was performed by KBioscience. This allele has previously been shown to be in complete linkage disequilibrium ($r^2=1$) with the HLA-A*3101 allele in populations of European descent.[22]

STATISTICAL ANALYSIS

Statistical analyses were performed with the use of PLINK (version 1.05),[23] RevMan (version 5) (http://ims.cochrane.org/revman), and Haploview[24] software packages. For the genomewide association studies, both the Liverpool collaborators and the EPIGEN consortium used logistic regression with an additive model of inheritance. They adjusted for population stratification by including significant principal components as covariates in the logistic-regression model and used a P value of less than 5.0×10^{-8} to indicate genomewide significance. Pooled analysis of the different groups of subjects was performed to estimate odds ratios and confidence intervals from summary data with the use of a random-effects model. Between-study heterogeneity was tested by calculating I^2 and Tau.^{2} The z-score directionality or estimated risk was checked for consistency with observed data.

RESULTS

ASSOCIATION WITH THE HYPERSENSITIVITY SYNDROME

We first conducted an association analysis involving 1.2M SNPs in samples from the 22 Liverpool subjects with carbamazepine-induced hypersensitivity syndrome and from 2691 healthy controls from the U.K. National Blood Services Collection. (The full set of results can be obtained from the European Genotype Archive, access num-

ber EGAS00000000037.) We identified a strong signal in the MHC region on chromosome 6, with several SNPs around HLA-A reaching genomewide significance ($P=3.5\times10^{-8}$) (Fig. 1A and 1B). This variant was seen in 40.0% of case subjects but in only 4.9% of control subjects. The top hit (rs1061235) had previously been shown to be a proxy for the HLA-A*3101 allele in persons of European descent.[22] Therefore, we focused on this locus as a predictor of response through high-resolution, sequence-based HLA-A typing, which confirmed the absolute correlation ($r^2=1$) between rs1061235 and HLA-A*3101 that had been observed previously.[22] No other classic HLA-A alleles reached nominal significance (Table 1 in the Supplementary Appendix).

During the course of this study, samples from 4 additional subjects with the hypersensitivity syndrome and from 1 additional subject with AGEP became available from the Liverpool and EPIGEN centers. Of these 5 subjects, 2 (including the single subject with AGEP) tested positive for HLA-A*3101. A comparison of all 27 subjects with the 257 control subjects without adverse drug reactions produced an odds ratio of 12.41 (95% confidence interval [CI], 1.27 to 121.03; P=0.03) (Fig. 2).

ASSOCIATION WITH MACULOPAPULAR EXANTHEMA

EPIGEN investigators performed a genomewide association study of samples from 43 subjects with maculopapular exanthema and 1296 healthy control subjects from the 1958 British Birth Cohort. (The full set of results can be obtained from dbGAP, access number EGAS00000000036.) HLA-A*3101 was the most strongly associated allele ($P=1.11\times10^{-6}$) and was observed in 27% of case subjects and 4% of control subjects, although the effect was not significant genomewide (Fig. 3 in the Supplementary Appendix). However, we had access to another 63 case subjects with maculopapular exanthema and 257 control subjects without adverse drug reactions. Our combined analysis of all 106 subjects with maculopapular exanthema and the 257 clinical control subjects showed a significant allelic association with HLA-A*3101 ($P=8.0\times10^{-7}$), generating an odds ratio of 8.33 (95% CI, 3.59 to 19.36) (Fig. 2). Although the analyses of samples from subjects with maculopapular exanthema and those with the hypersensitivity syndrome were carried out in the Nether-

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P Value ($-\log_{10}$)

7

6

0

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 22
                                                                    21

Chromosome

B SNPs in the HLA-A Region

P Value ($-\log_{10}$)

Recombination Rate (cM/Mb)

HLA-A*3101

LD
Typed
◆ 0.80 to 1.00
◆ 0.50 to <0.80
◆ 0.20 to <0.50
◇ <0.20
Imputed
❘ 0.80 to 1.00
❘ 0.50 to <0.80
❘ 0.20 to <0.50
❘ <0.20

HLA-A

29.7   30.0   30.3
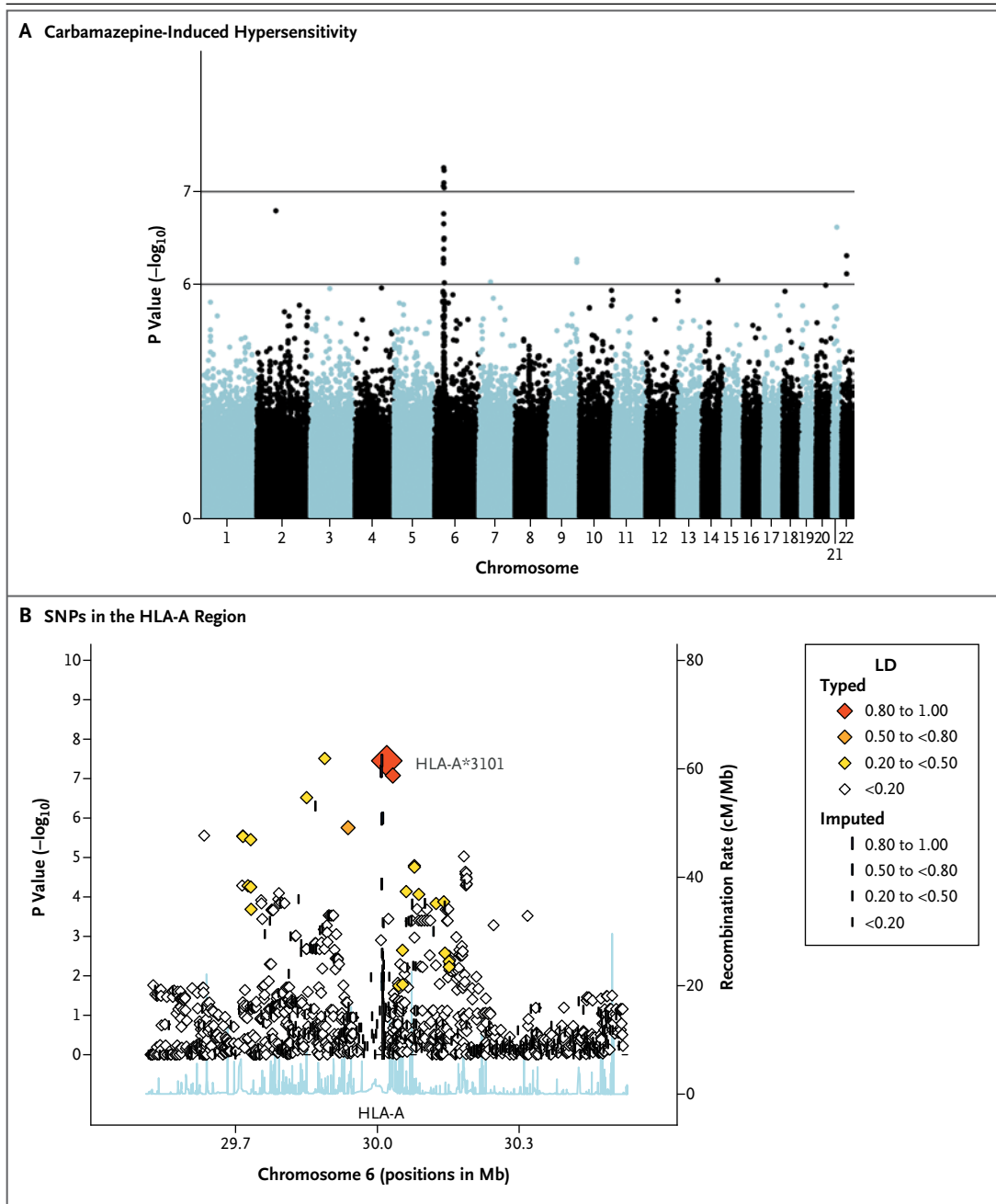
Chromosome 6 (positions in Mb)

Figure 1. Results of a Genomewide Association Study of Samples from Case Subjects with Carbamazepine-Induced Hypersensitivity Syndrome and Control Subjects.

Panel A shows a Manhattan plot for logistic regression with a strong signal in the HLA-A region on chromosome 6. Each dot represents a P value for the comparison of 22 case subjects with the hypersensitivity syndrome and 2691 healthy control subjects from the Wellcome Trust Case Control Consortium. Panel B shows P values for single-nucleotide polymorphisms (SNPs) in the HLA-A region. There is a strong association between carbamazepine-induced hypersensitivity syndrome and the HLA-A*3101 allele, with identification of the allele in 40.0% of case subjects and only 4.9% of control subjects. The x axis shows the SNP position on chromosome 6 (National Center for Biotechnology Information build 36). The left y axis shows the negative $\log_{10}$ of P values for the comparison between case subjects and control subjects, as calculated with logistic regression. The right y axis shows the recombination rate on chromosome 6 between 29 and 31 Mb. The diamonds show the degree of linkage disequilibrium (LD) in the samples.

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lands and Liverpool, respectively, the effect size of HLA-A*3101 on risk was consistent across the EPIGEN and U.K. groups.

ASSOCIATION WITH SJS–TEN

On the basis of our hypothesis that the HLA-A*3101 allele is associated with carbamazepine-induced SJS–TEN, we genotyped HLA-A*3101 in a group of 12 subjects with SJS–TEN who were recruited from both the Liverpool and EPIGEN-affiliated centers. Of these 12 subjects, 5 (42%) carried the allele, as compared with 10 (4%) of the 257 clinical control subjects (odds ratio, 25.93; 95% CI, 4.93 to 116.18; P=8.0×10⁻⁵) (Fig. 2).

A pooled analysis of all 145 subjects with carbamazepine-induced hypersensitivity and 257 control subjects without adverse drug reactions showed a strong association between hypersensitivity and the HLA-A*3101 allele (odds ratio, 9.12; 95% CI, 4.03 to 20.65; P=1.0×10⁻⁷). On the basis of this pooled collection, we calculated that the presence of HLA-A*3101 had a sensitivity of 26% and a specificity of 96% as a predictor of carbamazepine-associated hypersensitivity. Given that carbamazepine hypersensitivity has a prevalence of 5% (1:20), application of the test criteria from our pooled analysis would increase the post-test probability to 26% (approximately 1:4) on the basis of the positive likelihood ratio of 6.74 (95% CI, 3.51 to 13.00). Since the negative likelihood ratio was 0.77 (95% CI, 0.67 to 0.84), a negative HLA-A*3101 test would reduce the probability of hypersensitivity from 5.0% to 3.8% (1:26 ratio).

DISCUSSION

We found that a variation in the presence of the HLA-A*3101 allele is an important predictor of the full spectrum of carbamazepine-induced hypersensitivity reactions in persons of European descent. Such reactions range from the relatively mild but nevertheless troublesome maculopapular exanthema to more severe reactions, such as the hypersensitivity syndrome and SJS–TEN. Although the presence of the HLA-A*3101 allele is neither necessary nor sufficient for the development of hypersensitivity to carbamazepine, it is associated with a significantly increased risk.

Our data add to growing evidence of the role of different HLA alleles in predisposing patients to immune-mediated adverse reactions, such as drug-induced hypersensitivity[4,25-27] and liver injury.[3,28] The most prominent example thus far is the strong predictive value of the HLA-B*5701 allele for hypersensitivity to the drug abacavir, used for the treatment of human immunodeficiency virus infection.[25,26,29] FDA guidelines now recommend HLA-B*5701 testing in advance of abacavir prescription. Testing results in a reduction in the incidence of abacavir hypersensitivity[27,30] and is cost-effective.[29,31]

Our study now provides strong evidence suggesting that HLA-A*3101 is a predictor of carbamazepine hypersensitivity in Europeans. First, despite the small number of subjects who were included in the analysis, this signal had genome-wide significance. Second, the signal was observed across independent groups of case subjects with different phenotypes of carbamazepine hypersensitivity, with the use of independent sets of control subjects. Third, HLA-A*3101 has previously been shown to be associated with multiple carbamazepine-hypersensitivity phenotypes, including SJS–TEN in Japanese subjects[15,32] and with carbamazepine-induced maculopapular exanthema in subjects of Han Chinese ancestry.[14] The one subject in our study who had AGEP, a reaction characterized by sterile pustules, neutrophilic inflammation, and high levels of interleukin-8 in skin,[33] also was found to carry the HLA-A*3101 allele. The prevalence of this allele is 2 to 5% in Northern European populations, 2% in Han Chinese populations, and 9% in Japanese populations.[32,34,35] The finding that HLA-A*3101 is associated with carbamazepine-induced hypersensitivity in populations of different ancestries parallels the finding of HLA-B*5701-associated abacavir hypersensitivity.[30,36]

HLA-B*1502, a predictor of carbamazepine hypersensitivity reactions in Asian populations, seems to be phenotype-specific in that it predicts the development of SJS–TEN but not the development of the hypersensitivity syndrome or maculopapular exanthema.[14] This finding has led to the suggestion that the pathogenesis of carbamazepine-induced SJS–TEN is distinct from that of the hypersensitivity syndrome. This hypothesis is based on the fact that class I alleles are usually associated with specific CD8+ cytotoxic T-cell reactions, which is consistent with the known pathologic features of SJS–TEN.[37] How-
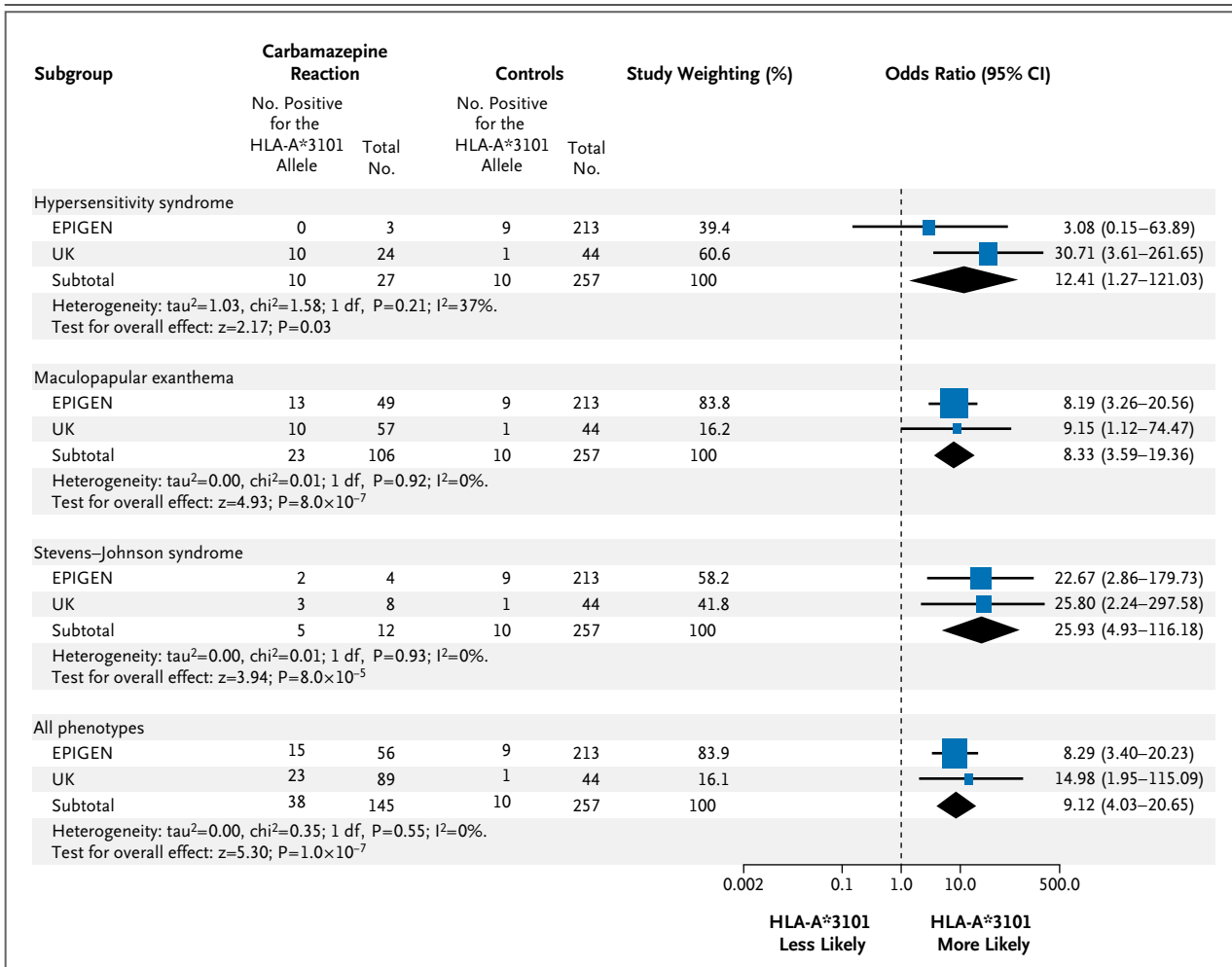
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Subgroup	Carbamazepine Reaction		Controls		Study Weighting (%)	Odds Ratio (95% CI)
	No. Positive for the HLA-A*3101 Allele	Total No.	No. Positive for the HLA-A*3101 Allele	Total No.		
Hypersensitivity syndrome						
EPIGEN	0	3	9	213	39.4	3.08 (0.15–63.89)
UK	10	24	1	44	60.6	30.71 (3.61–261.65)
Subtotal	10	27	10	257	100	12.41 (1.27–121.03)
Heterogeneity: tau²=1.03, chi²=1.58; 1 df, P=0.21; I²=37%. Test for overall effect: z=2.17; P=0.03						
Maculopapular exanthema						
EPIGEN	13	49	9	213	83.8	8.19 (3.26–20.56)
UK	10	57	1	44	16.2	9.15 (1.12–74.47)
Subtotal	23	106	10	257	100	8.33 (3.59–19.36)
Heterogeneity: tau²=0.00, chi²=0.01; 1 df, P=0.92; I²=0%. Test for overall effect: z=4.93; P=8.0×10⁻⁷						
Stevens–Johnson syndrome						
EPIGEN	2	4	9	213	58.2	22.67 (2.86–179.73)
UK	3	8	1	44	41.8	25.80 (2.24–297.58)
Subtotal	5	12	10	257	100	25.93 (4.93–116.18)
Heterogeneity: tau²=0.00, chi²=0.01; 1 df, P=0.93; I²=0%. Test for overall effect: z=3.94; P=8.0×10⁻⁵						
All phenotypes						
EPIGEN	15	56	9	213	83.9	8.29 (3.40–20.23)
UK	23	89	1	44	16.1	14.98 (1.95–115.09)
Subtotal	38	145	10	257	100	9.12 (4.03–20.65)
Heterogeneity: tau²=0.00, chi²=0.35; 1 df, P=0.55; I²=0%. Test for overall effect: z=5.30; P=1.0×10⁻⁷						

Axis: 0.002 0.1 1.0 10.0 500.0

HLA-A*3101 Less Likely — HLA-A*3101 More Likely

Figure 2. Distribution of the HLA-A*3101 Allele across the Spectrum of Clinical Phenotypes of Case Subjects with Carbamazepine-Induced Hypersensitivity and Control Subjects without Adverse Reactions to Carbamazepine.

Subjects were recruited at centers collaborating with the University of Liverpool and Walton Centre for Neurology (UK) or centers affiliated with the EPIGEN consortium. The sample obtained from one patient with acute generalized exanthematous pustulosis (AGEP) was analyzed with the samples for the group of case subjects with the hypersensitivity syndrome. Since there were no observations of the HLA-A*3101 allele in the three case subjects with the hypersensitivity syndrome in the EPIGEN cohort, 0.5 was added to each value in a two-by-two contingency table to estimate an odds ratio. One patient with the Stevens–Johnson syndrome and toxic epidermal necrolysis (SJS–TEN) was of mixed European and Thai ancestry. Study weighting (indicated by different sizes of squares) refers to the proportion of subjects who were recruited from each study cohort. Diamonds indicate pooled odds ratios. The horizontal lines indicate 95% confidence intervals. The abbreviation df denotes degrees of freedom, and I² is the percentage of total variation that is due to heterogeneity rather than chance.

ever, our data suggest that the class I allele HLA-A*3101 may be important for an increased range of phenotypes, including maculopapular exanthema and the hypersensitivity syndrome, at least in European populations. Persons with carbamazepine-induced hypersensitivity syndrome have been shown to have several types of drug-specific T cells in peripheral blood, including CD4+, CD8+, and CD4−CD8+ cells.[38] Although there is

no obvious relationship between HLA-A*3101 and HLA-B*1502, reports have indicated a possible overlap in peptide-binding specificity.[39] Given the degree of linkage disequilibrium across the extended MHC region, we cannot be certain that either HLA-B*1502 or HLA-A*3101 is causal.

We used samples from healthy volunteers in the WTCCC as control subjects for both genomewide association studies. Given that the

hypersensitivity syndrome occurs in 1 in 5000 to 1 in 10,000 persons, it is unlikely that more than one or two population control subjects would be at risk for hypersensitivity with exposure to carbamazepine. The larger WTCCC collections therefore afforded increased power over that provided by comparison of case subjects with the clinical control subjects. Nevertheless, in analyses involving the 257 clinical control subjects, the association with HLA-A*3101 remained strong for both the hypersensitivity syndrome (odds ratio, 12.41; 95% CI, 1.27 to 121.03) and SJS (odds ratio, 25.93; 95% CI, 4.93 to 116.18), although the effect size was smaller with maculopapular exanthema (odds ratio, 8.33; 95% CI, 3.59 to 19.36).

Additional studies in populations of European descent and other ancestry are required to further characterize this association. The International Serious Adverse Event Consortium is coordinating a global effort to recruit subjects with drug-induced severe adverse events (www.saeconsortium.org). Efforts such as this will facilitate the analysis of larger groups of subjects, which may well uncover other genetic variants associated with a smaller effect size or population-specific variants. Additional work is also required to determine whether the effect of the HLA-A*3101 allele is specific to carbamazepine or whether it also applies to other drugs. A prospective study that is based on the results we present here would facilitate the routine clinical use of HLA-A*3101 testing in carbamazepine treatment. In this issue of the *Journal*, a report describes how prospective HLA-B*1502 typing can prevent carbamazepine-induced SJS–TEN in Han Chinese populations.[5]

We propose that HLA-A*3101 is clinically relevant as a marker to predict hypersensitivity reactions. On the assumption that the prevalence of carbamazepine-induced hypersensitivity is 5.0%, the presence of the HLA-A*3101 allele increases the risk of hypersensitivity to 26.0%, whereas its absence reduces the risk to 3.8%. Among Japanese patients, the estimated reduction is from 2.9% to 1.1%. The sensitivity and specificity values estimated here for Europeans (26% and 96%) and by others for Japanese populations (61% and 88%)[32] vary. Among a number of possible reasons for these differences are differences in study size, in the frequency of HLA-A*3101 (and potential interacting variation), and in the effect size of the allele across the two populations. Nonetheless, these numbers are similar to those underlying the test criteria calculated for the presence of the HLA-B*5701 allele and abacavir hypersensitivity (sensitivity, 45.5%; specificity, 97.6%; and a risk reduction from 7.8% to 2.7%).

On the basis of our data, we have calculated that 83 patients would need to be screened to prevent one case of carbamazepine hypersensitivity; the number in the Japanese population is 56. However, it is important to note that this calculation is based on a conservative estimate of the prevalence of carbamazepine hypersensitivity (5%). The prevalence of carbamazepine-induced hypersensitivity that was determined in the context of the Standard and New Antiepileptic Drugs (SANAD) trial (Current Controlled Trials number, ISRCTN38354748) (also involving subjects of European descent) was 10%.[40] On the basis of this prevalence, the number of persons who would need to be screened to prevent one instance of carbamazepine-induced hypersensitivity is 39. We therefore suggest that consideration be given to adding the association with HLA-A*3101 to the drug label for carbamazepine.

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APPENDIX

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Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: McCormack M, Alfirevic A, Bourgeois S, et al. HLA-A*3101 and carbamazepine-induced hypersensitivity reactions in Europeans. N Engl J Med 2011;364:1134-43.

Supplementary Appendix

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Methods

Recruiting centres

University of Liverpool collaborators included the UK national prospective SANAD study, the EUDRAGENE study and Universitätsspital Bern (Switzerland). The EPIGEN Consortium includes Duke University (North Carolina), University College London (UK), Université Libre de Bruxelles (Brussels, Belgium) and the Royal College of Surgeons and Beaumont Hospital in Dublin, Ireland.

Definition of Stevens Johnson Syndrome

SJS was defined as skin detachment 1-10% (SJS), 10-30% (overlap syndrome) and >30% (TEN), the presence of atypical target lesions predominately on the trunk and face, severe, often haemorrhagic, erosions of mucous membranes. Other manifestations indicating systemic involvement included fever, liver chemistry elevations, intestinal and pulmonary manifestations, presence of lymphopenia).

Data access for healthy controls

Individual-level genotype data (Illumina 1.2M (custom chip) were accessed for both sets of healthy controls by application to the Wellcome Trust Case Control Consortium Data Access Committee (https://www.wtccc.org.uk/ccc1/access_to_data_samples.shtml).

Details on cleaning of Illumina data

Illumina 1.2M on 22 HSS cases: The Illumina gender markers were utilized to compare clinical data with the DNA sample gender. Hardy-Weinberg equilibrium was estimated and SNPs which failed (p< 0.0001) in control population were excluded from further analyses. In addition, SNPs with MAF <1% and SNPs with genotype success rate < 97% were excluded. Final Manhattan plot cleansing step was based on low intensities and regional LD estimates. Accurate annotation of the variant names and strand orientation by explicit specification of the version of the human genome assembly and of dbSNP ensured compatibility of different datasets for comparison in pooled analysis of case-control studies.

Illumina 610k on 49 EPIGEN **MPE cases**: All quality control measures were performed using PLINK. All SNPs with a genotyping success rate < 90%, those with MAF < 2% and subjects with genotyping success rate < 95% were excluded from the dataset. SNPs that were outside a Hardy-Weinberg Equilibrium threshold of p<0.0001 in control subjects were also removed. To correct for potential cryptic relatedness, the dataset was thinned to SNPs in linkage equilibrium (SNPs with r^2<0.25) and identity-by-descent of overall genome was calculated with a cut-off threshold of 12% shared with another individual. Population structure was determined by principal components analysis and outliers were removed.

Details of imputation of HLA alleles in EPIGEN Consortium samples:

We imputed HLA types across 5 loci (HLA-A, B, C, DR, DQ) to the level of 4 digits maximum. To achieve this, our full GWAS dataset was reduced to SNPs within the HLA locus (NCBI Build 36, base positions 25760204 to 33529555 on chromosome 6). All MPE cases and CBZ-tolerant controls were imputed together as one population. . Background European HLA allele frequencies were obtained from www.allelefrequencies.net [21] and through the Irish Blood Transfusion Service Bone Marrow Registry [22]. The quality of the imputation by MACH is described by an r^2 value for each HLA type imputed. The quality threshold was set to $r^2>0.9$. Summary information and quality scores per HLA type imputed are available upon request.

Background European HLA allele frequencies were obtained from www.allelefrequencies.net[21] and through the Irish Blood Transfusion Service Bone Marrow Registry[22]

HLA-A *3101 SSP genotyping:

In order to validate the association with the imputed HLA-A*3101 (in EPIGEN samples), we performed an established wet lab technique to confirm the presence of HLA alleles by amplification of genomic DNA with sequence specific primers (SSP) in a polymerase chain reaction. Sequence-specific primers for HLA-A*3101 were obtained with kind permission from Mary N. Carrington of the National Cancer Institute- Frederick (Maryland, USA) for detecting HLA-A*3101 across the entire EPIGEN cohort. The forward primer sequence is 5'-GATAGAGCAGGAGAGGCCT-3' and the reverse primer sequence is 5'-AGCGCAGGTCCTCGTTCAA-3'. Visualisation of confirmatory bands was possible by placing an electric current across a 2% agarose gel containing the samples (see Figure 4 below).

All samples were correctly imputed as each HLA-A*3101 allele carrier was positively identified by the SSP typing method.

Details of population stratification

Principle component analysis was performed to assess genetic markers for ethnicity. All participants were self reported Caucasans. Only individuals with genetically matching Caucasian ethnicity were included into the association analysis. We were working with Phase 1 1958 controls (n=1500), we removed 204 outliers from PCA plot leaving us with 1296 samples for case/control analysis. PCA plots for Liverpool HSS (n=22) in conjunction with healthy controls (n=2291) and EPIGEN MPE and EPIGEN controls are shown in Supp Fig 1 and Supp Fig 2 below respectively.

Results

Table 1. High resolution sequence-based HLA-A typing in patients with carbamazepine (CBZ)-induced hypersensitivity syndrome and CBZ-tolerant patients.

HLA allele[‡]	CBZ-tolerant controls	Allelic Frequency	Hypersensitive patients	Allelic Frequency	P-value	Odds Ratio (95% CI)
A*0101g[§]	17	0.19	12	0.27	0.3729	1.57 (0.6703-3.6595)
A*0201g	21	0.24	8	0.18	0.511	0.71 (0.2855-1.7605)
A*0301g	11	0.13	0	0	0.0601	0.08 (0.0044-1.3157)
A*1101g	11	0.13	3	0.07	0.3839	0.51 (0.1352-1.9399)
A*2301g	2	0.02	0	0	1	0.39 (0.0183-8.2745)
A*2402g	3	0.03	5	0.11	0.1162	3.63 (0.8263-15.969)
A*2403g	0	0	1	0.02	0.2576	6.10 (0.2436-152.9367)
A*2501g	5	0.06	1	0.02	0.6631	0.39 (0.0437-3.4093)
A*2601g	6	0.07	2	0.05	0.7182	0.65 (0.1259-3.365)
A*2608	1	0.01	0	0	1	0.66 (0.0262-16.419)
A*3001g	3	0.03	0	0	0.6622	0.27 (0.0139-5.4331)
A*3002	1	0.01	0	0	1	0.66 (0.0262-16.419)
A*3101g	**1**	**0.01**	**8**	**0.18**	**0.00064**	**19.33 (2.3325-160.2439)**
A*3201	3	0.03	1	0.02	1	0.66 (0.0665-6.5245)
A*3303g	0	0	2	0.05	0.1078	10.41 (0.489-221.697)
A*6801	0	0	1	0.02	0.2576	6.10 (0.2436-152.9367)
A*6801g	3	0.03	0	0	0.6645	0.27 (0.0139-5.4331)
Total alleles	88		44			

[‡]*Antigen recognition site allele*

[§]*Alleles bearing suffix 'g' in A locus have identical sequences in exon 2 and exon 3 antigen recognition sites.*

An association between HSS and HLA-A*3101 presence was identified (OR 24.6; 95% CI 2.8, 214.0); P=0.00004). No other HLA-A alleles showed statistically different allelic frequencies.

Figure 1. Population stratification detection using GWAS in WTCCC and patients with hypersensitivity syndrome (HSS)
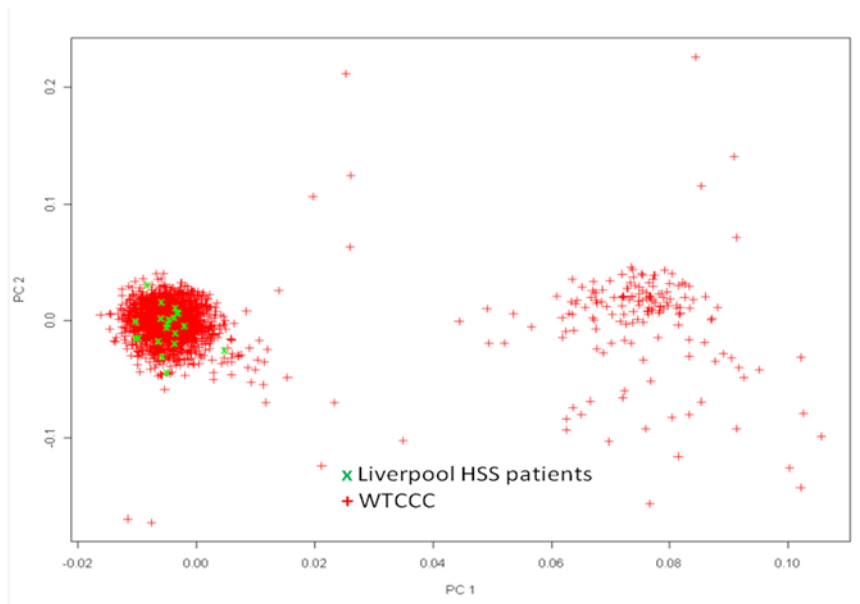

Figure 2. Population stratification detection using GWAS within the cohort of maculopapular exanthema (MPE) cases from the EPIGEN consortium and healthy controls from the 1958 cohort. All participants were self reported Europeans. Ethnicity was reported orally to clinician during investigations and recorded in clinical notes.

Principal Components Analysis was performed to reduce subjects to a homogenous cluster of apparent northern European ethnicity. The final cohort is shown below.
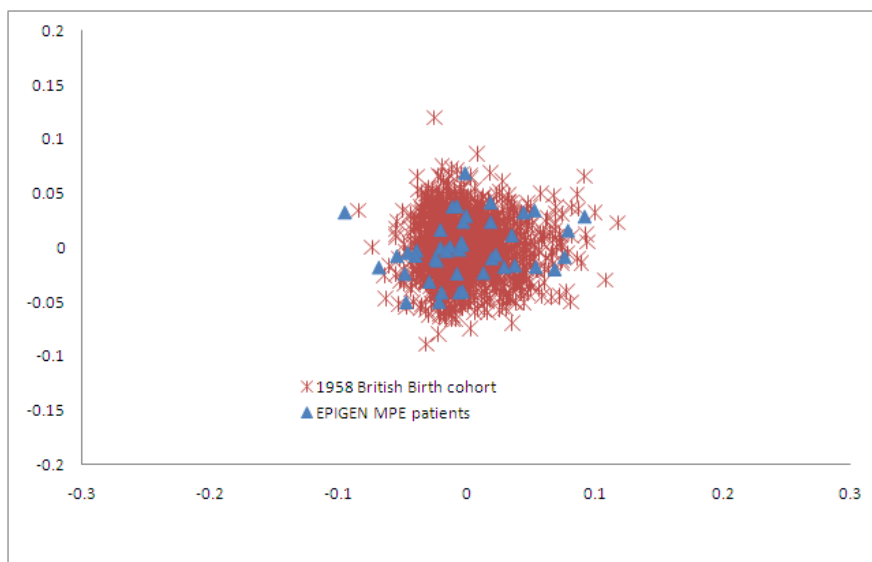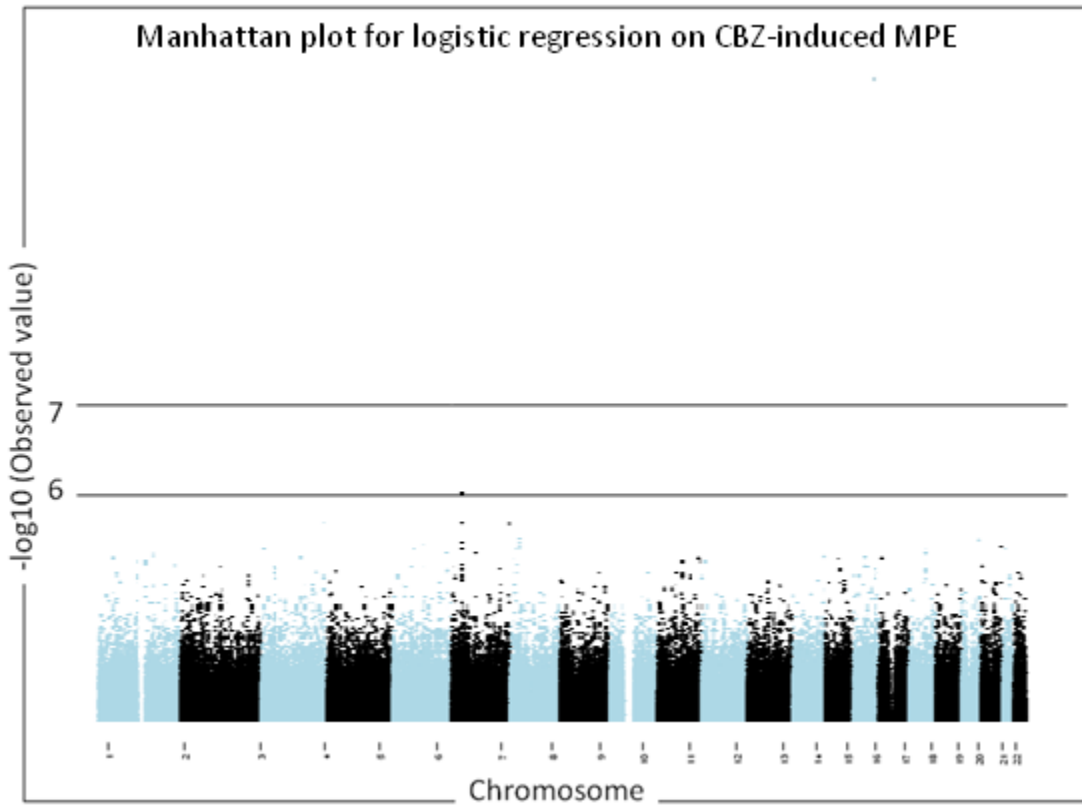
Figure 3. Results of MPE GWAS analysis

GWAS analysis involving MPE cases (n=43) and healthy controls from the 1958 British Birth Cohort (n=1296). Significant EIGENSTRAT covariates are included as covariates in logistic regression model. The top hit on chromosome six is HLA*3101 (P = 1.11 x 10^{-6}).

Figure 4: Selection of imputed HLA-A*3101 samples following PCR amplification with sequence specific primers.

Imputed positive sample lanes 1-4 contained a strong gel band as confirmation whereas the imputed negative sample shown here in lane 5 contained no band.