HLA-DPB1 and HLA Class I Confer Risk of and Protection from Narcolepsy

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Type 1 narcolepsy, a disorder caused by a lack of hypocretin (orexin), is so strongly associated with human leukocyte antigen (HLA) class II *HLA-DQA1*01:02-DQB1*06:02* (DQ0602) that very few non-DQ0602 cases have been reported. A known triggering factor for narcolepsy is pandemic 2009 influenza H1N1, suggesting autoimmunity triggered by upper-airway infections. Additional effects of other *HLA-DQ* alleles have been reported consistently across multiple ethnic groups. Using over 3,000 case and 10,000 control individuals of European and Chinese background, we examined the effects of other HLA loci. After careful matching of *HLA-DR* and *HLA-DQ* in case and control individuals, we found strong protective effects of HLA-DPA1*01:03-DPB1*04:02 (DP0402; odds ratio [OR] = 0.51 [0.38–0.67], $p = 1.01 \times 10^{-6}$) and HLA-DPA1*01:03-DPB1*04:01 (DP0401; OR = 0.61 [0.47–0.80], $p = 2.07 \times 10^{-4}$) and predisposing effects of *HLA-DPB1*05:01* in Asians (OR = 1.76 [1.34–2.31], $p = 4.71 \times 10^{-05}$). Similar effects were found by conditional analysis controlling for *HLA-DR* and *HLA-DQ* with DP0402 (OR = 0.45 [0.38–0.55] $p = 8.99 \times 10^{-17}$) and DP0501 (OR = 1.38 [1.18–1.61], $p = 7.11 \times 10^{-5}$). HLA-class-II-independent associations with *HLA-A*11:01* (OR = 1.32 [1.13–1.54], $p = 4.92 \times 10^{-4}$), *HLA-B*35:03* (OR = 1.96 [1.41–2.70], $p = 5.14 \times 10^{-5}$), and *HLA-B*51:01* (OR = 1.49 [1.25–1.78], $p = 1.09 \times 10^{-5}$) were also seen across ethnic groups in the HLA class I region. These effects might reflect modulation of autoimmunity or indirect effects of HLA class I and *HLA-DP* alleles on response to viral infections such as that of influenza.

Introduction

Type 1 narcolepsy (MIM 161400) is a life-long disorder characterized by sleepiness, cataplexy, and rapid-eye-movement sleep abnormalities. Onset usually occurs in children, adolescents, or young adults. The disease is caused by the loss of hypocretin-producing cells in the lateral hypothalamus.[1] Narcolepsy is strongly associated with a specific human leukocyte antigen (HLA) class II molecule, the $DQ\alpha_{0102}$–$DQ\beta_{0602}$ heterodimer (abbreviated DQ0602), which is shared by 98% of narcoleptics across ethnic groups and encoded by the *HLA-DQA1*01:02~DQB1*06:02* haplotype.[2,3] DQ0602 is present in 12%–38% of control individuals across ethnic groups. Genome-wide association studies (GWASs) in narcolepsy have also found associations with loci related to autoimmunity, such as T cell receptor (TCR) loci (*TRA* [MIM 186880], *TRB* [MIM 186930]), *IL10RB* [MIM 123889], *IFNAR1* [MIM 107450], *CTSH* [MIM 116820], *P2RY11* [MIM 602697], and *ZNF365* [MIM 607818].[4–6] These results suggest autoimmune-mediated hypocretin cell destruction that might involve antigen presentation by DQ0602 to $CD4^+$ T cells.

In addition, narcolepsy has a strong environmental component, and most monozygotic twins are discordant.[7] In children, where onset is often abrupt and more easily documented, narcolepsy is highly seasonal in that it peaks in the spring or summer.[8] Onset follows upper-airway infections, notably of influenza (MIM 614680) or

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Streptococcus pyogenes (MIM 607395), suggesting triggering effects of infections.[8,9]

A 4- to 6-fold increase in childhood narcolepsy onset was observed in the spring and summer of 2010, following the 2009 H1N1 swine pandemic flu in China.[5,10] Further, vaccination with Pandemrix, an AS03-adjuvanted pandemic H1N1 vaccine approved for use in Europe, was associated with a 3- to 17-fold increased risk of developing childhood narcolepsy in multiple countries, leading to increased incidence in Scandinavia.[11–17] For unclear reasons, increased risk of narcolepsy after the use of other H1N1 vaccines has not been reported, and is unlikely to be as strong as that following Pandemrix.[18] These findings, together with genetic evidence, suggest that narcolepsy is an autoimmune disease affecting hypocretin neurons and triggered by upper-airway infections.

Because of close physical proximity and a high degree of linkage disequilibrium (LD) observed for the *HLA-DRB1* (MIM 142857) and *HLA-DQB1* (MIM 604305) loci, it is difficult to assess additional effects of *HLA-DR* on susceptibility independently of *HLA-DQ*. In most ethnic groups, DQ0602 is exclusively associated with *HLA-DRB1*15:01*, but studies in Chinese and African Americans, two populations where LD between these two alleles is lower, demonstrate that the association is primarily with DQ0602.[3,19] Other minor associations have been reported for the DR locus (e.g., for rare *HLA-DRB1*04* subtypes[20–23]) but have never been confirmed on a large scale and across multiple ethnic groups.

Confirming the importance of *HLA-DQ*, additional *HLA-DQ* haplotypes consistently affect narcolepsy susceptibility when observed in *trans* of the major susceptibility haplotype *HLA-DQA1*01:02~DQB1*06:02*. Similar *trans* heterodimer effects have been reported for other autoimmune diseases, such as celiac disease (MIM 212750) and type 1 diabetes (MIM 222100).[24–26] In almost all cases, *trans* haplotypes that affect narcolepsy risk contain *HLA-DQ* alleles that are similar to *HLA-DQA1*01:02* or *HLA-DQB1*06:02* and, as a result, can cross-heterodimerize with DQ0602. Most notably, *HLA-DQA1*01:01~DQB1*05:01*, *HLA-DQA1*01:03~DQB1*06:03*, and *HLA-DQA1*01:03~DQB1*06:01* are protective against narcolepsy, whereas DQ0602 homozygosity increases risk in all ethnic groups.[2,3,22,26–29] We postulate that this is due to allele competition, a model where risk is proportional to the amount of DQ0602 available and its unique ability to present a putative autoantigen.[3,26] The model also predicts that any minor change in the DQ0602 antigen binding groove abolishes predisposition.

In addition to affecting allele competition, *HLA-DQB1*03:01* increases narcolepsy susceptibility when present in *trans* of DQ0602,[3,22,26,27,29] an effect unlikely to be explained by allele competition given that HLA-DQB1*03:01 does not heterodimerize with HLA-DQA1*01:02 and thus should not affect DQ0602 dosage.[30] Unlike DQ0602 dosage, *HLA-DQB1*03:01* also strongly reduces age of onset,[2,5] suggesting that it acts through a different mechanism, for example, development of the TCR repertoire.

The strong and consistent association between narcolepsy and *HLA-DQ* has obscured studies of other HLA loci, such as HLA class I loci and other class II loci, including *HLA-DP*. Additional HLA class I effects have been reported in many HLA-class-II-associated diseases, suggesting an involvement of $CD8^+$ T cells. For example, celiac disease and type 1 diabetes have weak HLA class I associations after HLA class II subtypes are controlled for. Type 1 diabetes also shows specific effects of *HLA-DRB1*04* subtypes in the presence of the same *HLA-DQ* heterodimer in Japan.[31] More recently, *HLA-DPA1* (MIM 142880) and *HLA-DPB1* (MIM 142858) have been associated with several autoimmune diseases primarily associated with *HLA-DR* or *HLA-DQ*, such as type 1 diabetes, multiple sclerosis (MIM 126200),[32,33] anti-glomerular basement membrane disease (MIM 233450),[34] and myasthenia gravis (MIM 254200).[35] Of notable interest are associations between *HLA-DP* and both influenza vaccine responses[36] and chronic viral infections, notably of hepatitis B virus.[37,38] To address the predisposition of HLA loci other than *HLA-DQ* in narcolepsy, we performed high-resolution class I and class II typing in *HLA-DQ*-matched narcoleptics versus control individuals and used imputation to replicate and extend our findings.

Material and Methods

HLA Typing and Selection of Samples

All narcolepsy-affected individuals were *HLA-DQB1*06:02* positive and had clear-cut cataplexy or documented low hypocretin-1 in the cerebrospinal fluid.[5,39,40] A subset of samples of Asian and white ethnicity (590 case and 692 control individuals) and sourced from the Stanford Center for Narcolepsy database were typed with deep sequencing (*HLA-DRB1*, *HLA-DQA1*, and *HLA-DQB1*) and IMGT/HLA Database version 3140.[41] With this information, a matched set of case and control individuals who shared the same ethnicity, country of origin, and *HLA-DQA1* and *HLA-DQB1* genotypes were selected, resulting in 322 case and 322 control individuals. For analysis of other loci, we further matched for *HLA-DRB1*, resulting in 304 case and 304 control individuals. These individuals were then typed for *HLA-A* (MIM 142800), *HLA-B* (MIM 142830), *HLA-C* (MIM 142840), *HLA-DPA1*, and *HLA-DPB1* with the Luminex xMAP Technology at Stanford Medical School Blood Center. This sample constituted the HLA-typed matched set.

Two other cohorts, one white and one Chinese, were also included in the analysis, but in these cases HLA genotypes were imputed from HLA region SNP data. These cohorts did not overlap the 644 HLA-typed samples and constituted the HLA-imputed matched set. The white matched sample was selected among 1,540 case and 10,421 control individuals.[39] Samples included previously published subjects sourced from the Stanford Center for Narcolepsy database and worldwide collaborators.[39] DNA samples were genotyped on the Illumina ImmunoChip array at the University of Virginia and Stanford University. UCSC Genome Browser hg18 mapping was used as a reference. Illumina manifest file Immuno_BeadChip_1149691_B.bpm was used in the majority

of cases. In cases where file Immuno_BeadChip_11419691_A was used, map positions were converted to be consistent with 1149691_B or were omitted from the analysis. Genotypes were called with Illumina GeneExpress (Illumina GenomeStudio GenTrain2.0 algorithm) with extensive additional curation.[39] Individuals with a call rate under 0.98 (147 case and 123 control individuals) and samples that were related ($\widehat{\pi} > 0.2$) were excluded from further analysis. Data from all sources were merged in forward-strand format. Using the PLINK suite of software,[42] we identified 142,054 high-quality SNPs with a call rate above 0.99 (in both case and control individuals separately) and passing Hardy-Weinberg equilibrium (HWE) filtering in control individuals ($p > 1 \times 10^{-5}$). Principal-component analysis for population stratification for this data set is shown in Figure S1.

The Chinese sample included a total of 1,189 narcolepsy subjects, 1,136[5] of whom were seen at the sleep laboratory of Peking University People's Hospital; this unit in the Department of Pulmonary Medicine evaluates patients with sleep disorders and receives referrals from all over China. In addition, 51 Asian samples came from Taiwan (Dr. Huang, National Taiwan University), and two came from Stanford. The individuals had hypocretin deficiency or clear-cut cataplexy and *HLA-DQB1*06:02*. Affected subjects were mostly of Han descent (0.87) and from North China (0.85). The majority of the subjects were male (0.67) and children (0.70). Control genotypes from China came from university employees and students (0.41 male). In addition, we had shared control individuals from GWASs underway for colon cancer (MIM 114500) and Sjögren syndrome (MIM 270150). The Chinese data set was genotyped on the Affymetrix Axiom CHB (Han Chinese in Beijing, China) array. Genotypes were called with the Affymetrix Genotyping Console. Individuals who had a call rate < 0.95, were outliers after principal-component analysis (n = 47), or were related (n = 53) were removed, leaving 1,189 case and 1,997 control individuals. For the main association study, we selected SNP variants with a minor allele frequency (MAF) ≥ 0.01, a call rate ≥ 0.90, and a HWE p value ≥ 0.001 in control individuals. Principal-component analysis for population stratification for this data set is shown in Figure S1.

Ethics Statement

Informed consent in accordance with governing institutions was obtained from all subjects. The research protocols were approved by institutional-review-board panels on medical human subjects at Stanford University and the Beijing University People's Hospital.

HLA Imputation in Samples with GWAS Data

HLA imputation for *HLA-A*, *HLA-B*, *HLA-C*, *HLA-DRB1*, *HLA-DQA1*, *HLA-DQB1*, *HLA-DPA1*, and *HLA-DPB1* was performed with the HIBAG package in R version 3.1.1 (July 10, 2014).[43] HIBAG is an HLA-imputation tool that uses attribute bootstrap aggregation of several classifiers (SNPs) to select groups of SNPs that predict HLA type.[44] For the ImmunoChip cohort, the imputation was performed with the European- and ImmunoChip-specific models from HIBAG. Imputation accuracy was verified by high-resolution typing in 177 individuals, resulting in imputation accuracy of 0.98 in *HLA-A*, 0.97 in *HLA-B*, 0.98 in *HLA-C*, 0.96 in *HLA-DRB1*, 1.00 in *HLA-DQA1*, 1.00 in *HLA-DQB1*, 1.00 in *HLA-DPA1*, and 0.92 in *HLA-DPB1*. The lower imputation quality of *HLA-DPB1* was due to incorrectly imputed *HLA-DPB1*20:01*, *HLA-DPB1*23:01*, and *HLA-DPB1*06:01* genotypes, which were rare. Because HIBAG did not have built-in haplotypes for *HLA-DPA1*, we first built a model for *HLA-DPA1* by using the type 1 diabetes consortium sample that had SNP and HLA information for 5,191 individuals from the SNP2HLA package.[45,46]

For the Chinese cohort, the Affymetrix CHB-specific chip reference panel was used for all loci but *HLA-DPA1*, for which a reference panel was built with HIBAG and publicly available Singapore Genome Variation Project (SGVP) data. One hundred Han Chinese individuals in SGVP have full 4-digit-level HLA typing and GWAS data available for *HLA-DPA1*. Imputation was verified for 254 individuals in the HLA class II genes, and the quality was high: 0.95 for *HLA-DRB1*, 0.94 for *HLA-DQA1*, and 0.98 for *HLA-DQB1*. Allele frequencies were within normal ranges according to dbMHC allele frequencies and earlier studies.[47]

Statistical Analysis

For stratified analysis, all samples were fully matched for country of origin and *HLA-DRB1*, *HLA-DQB1*, and *HLA-DQA1* genotypes (for analysis of HLA class I and *HLA-DP* loci) or for country of origin and *HLA-DQB1* and *HLA-DQA1* genotypes (for analysis of the *HLA-DR* locus).

The analysis was carried out with carrier frequencies and the chi-square test with package meta.MH in R version 3.1.1 (July 10, 2014).[43] Regional association plots were drawn with locus zoom.[48] Sub-analyses of HLA loci were carried out with the Mantel-Haenszel test and, in the case of the *HLA-DP* heterodimer analysis, with a "one-by-one" sequential analysis that removed the effect of the most significant variant. This latter technique is similar to relative predisposition-effect statistics.[49] Conditional analyses were performed on the full data sets with PLINK versions 1.7 and 1.9.[42] In the conditional analysis, individuals homozygous for *HLA-DQB1*06:02* were removed. Meta-analyses for conditional analysis were performed with GWAMA.[50] Nominal p values are reported for associations with $p < 0.0005$ after a Bonferroni correction for 100 tests. Other significant $p < 0.05$ associations are shown in Tables S1–S3, S4, S5, S6, S7 and S8, S9, S10–S16, and S17.

Results

HLA Class II Effects in *HLA-DQ*-Matched Narcolepsy Case and Control Individuals Reveal Strong Effects of *HLA-DP*

Genotype matching is the most conservative analytical method. The analysis of *HLA-DRB1* was done in an *HLA-DQ*- and country-matched sample composed of 1,221 case and 1,221 control individuals. No residual *HLA-DR* association with narcolepsy was seen, except for a nominal association with *HLA-DRB1*04:03* (Table S1). Because *HLA-DR* and *HLA-DQ* display extremely high LD, all subsequent analyses were performed in a *HLA-DRB1*-, *HLA-DQA1*-, and *HLA-DQB1*-matched sample for a total number of 1,063 case and 1,063 control individuals.

The strongest findings were seen in the HLA class II *HLA-DPB1* locus (Table 1), where *HLA-DPB1*04:02* conferred a strong protective effect against narcolepsy. In addition, *HLA-DPB1*05:01* increased the risk in Asians but not in whites (Table 1). Nominally protective effects were seen with *HLA-DPB1*04:01* and *HLA-DPB1*10:01* but not with other DPB1 alleles (Table S2). A nominally significant association was seen with *HLA-DPA1*01:03* (Table S3).

Table 1. Association between *HLA-DPB1* Alleles and Narcolepsy

	Asian				White				Mantel-Haenszel Test		
***HLA-DPB1* Allele**	**No. of Control Subjects (Freq)**	**No. of Case Subjects (Freq)**	**OR (CI)**	**p**	**No. of Control Subjects (Freq)**	**No. of Case Subjects (Freq)**	**OR (CI)**	**p**	**OR (CI)**	**p**	**p Heterogeneity Test**
04:02	49 (0.112)	23 (0.052)	0.44 (0.26–0.74)	0.0014	114 (0.29)	66 (0.17)	0.50 (0.35–0.70)	4.98×10^{-5}	0.50 (0.38–0.66)	6.105×10^{-07}	0.914
05:01	236 (0.538)	295 (0.67)	1.76 (1.34–2.32)	4.71×10^{-5}	34 (0.09)	30 (0.08)	1.29 (0.86–1.92)	0.221	1.48 (1.17–1.88)	0.001	0.106

Case and control individuals were matched for *HLA-DRB1*, *HLA-DQA1*, and *HLA-DQB1* alleles and for country and ethnicity. The p values were calculated with the Mantel-Haenszel test. Abbreviations are as follows: CI, confidence interval; Freq, carrier frequency; and OR, odds ratio.

In order to form functional HLA-DP molecules, the HLA-DPα and HLA-DPβ proteins (encoded by *HLA-DPA1* and *HLA-DPB1*, respectively) need to heterodimerize. Heterodimerization of HLA-DPα and HLA-DPβ can occur in *cis* (on the same haplotype) or in *trans* (encoded by different chromosomes), provided that HLA-DPα and HLA-DPβ are biochemically compatible. *HLA-DPA1* and *HLA-DPB1* encode distinct amino acid motifs in the peptide-binding region, and polymorphisms at these positions determine which peptides can be bound by specific HLA-DP α and β subtypes and how they are presented to T cells (the so-called peptide-binding repertoire). To examine for potential effects in both *cis* and *trans*, we next performed stepwise analysis of *HLA-DPA1-DPB1* heterodimers.

*HLA-DPB1*04:02* is in high LD with *HLA-DPA1*01:03*, whereas *HLA-DPB1*05:01* is in LD with *HLA-DPA1*02:02*. However, *HLA-DPB1*05:01* is also seen in *cis* with *HLA-DPA1*02:01*.[47,51] We thus tested a stepwise association of all possible heterodimers at the *HLA-DP* locus with narcolepsy across all samples. In the first pass analysis, HLA-DPA1*01:03-DPB1*04:02 (DP0402), followed by protective association with HLA-DPA1*01:03-DPB1*04:01 (DP0401), was most significantly associated with narcolepsy (Table 2). In addition, nominally significant associations were seen with HLA-DPA1*02:02-DPB1*19:01 and HLA-DPA1*02:02-DPB1*05:01 (DP0501) (Table 2).

In narcolepsy, the largest risk is seen in individuals homozygous for *HLA-DQB1*06:02* or heterozygous for *HLA-DQB1*03:01* and *HLA-DQB1*06:02*. The next largest risk is seen in individuals who are heterozygous but have neutral alleles on the other chromosome, whereas those who carry *HLA-DQA1*01* that is not *HLA-DQA1*01:02* in *trans* of *HLA-DQB1*06:02* are relatively protected.[26] In a final analysis, we tested whether the effect size of *HLA-DP* was affected by the *HLA-DQ* risk groups by dividing the sample into groups according to these previously known *HLA-DQ* risk subgroups.[22,26] The effects of *HLA-DP* did not differ across risk groups (Table S4).

Weak HLA Class I Associations in HLA-Class-II-Matched Narcolepsy Case and Control Individuals

We finally analyzed the effect of *HLA-A*, *HLA-B*, and *HLA-C* loci in *HLA-DR*- and *HLA-DQ*-matched subjects (Tables S5, S6, and S7). Nominally significant associations were seen with *HLA-A*02:07* (odds ratio [OR] = 1.66 [1.01–2.74], p = 0.046), *HLA-A*03:01* (OR = 0.79 [0.64–0.97], p = 0.024), *HLA-A*11:01* (OR = 1.43 [1.15–1.78], p = 0.001), *HLA-A*29:02* (OR = 0.50 [0.30–0.85], p = 0.008), *HLA-B*35:03* (OR = 2.30 [1.27–4.18], p = 0.005), *HLA-B*40:02* (OR = 0.54 [0.34–0.87]), *HLA-B*41:02* (OR = 0.14 [0.02–1.15], p = 0.33), *HLA-B*44:03* (OR = 0.55 [0.38–0.81], p = 0.002), *HLA-B*44:05* (OR = 0, p = 0.025), *HLA-C*05:01* (OR = 0.73 [0.54–0.99], p = 0.044), *HLA-C*14:03* (OR = 0.38 [0.15–1.01], p = 0.044), and *HLA-C*16:01* (OR = 0.42 [0.24–0.74]). Similar effects were also found after *HLA-DP* was matched between case and control individuals for the potential effect of extended haplotypes (Tables S8, S9, and S10).

Conditional Analysis Confirms Independent *HLA-DP* and Class I Effects

In order to study which *HLA-DR* and *HLA-DQ* alleles predispose to narcolepsy in the ImmunoChip and Asian data sets, we first performed stepwise analysis of *HLA-DRB1*, *HLA-DQB1*, and *HLA-DQA1* loci in whites and Asians. As expected, we saw a strong predisposing effect of the known narcolepsy risk locus *HLA-DQB1*06:02* in whites and Asians (Tables S11 and S12). Similarly strong associations were seen with *HLA-DRB1*15:01*, which is in strong LD with *HLA-DQB1*06:02*, and with *HLA-DQA1*01:02*, which is always present in *HLA-DQB1*06:02* haplotypes but is also found in other haplotypes. Figure 1A also shows GWAS data in the HLA region of whites (from ImmunoChip, see Faraco et al.[39]) and Asians (from Affymetrix CHB data, see Han et al.[5]) and a large association with the *HLA-DR-DQ* region, which obscured all other signals.

We next performed stepwise conditioning with *HLA-DQB1*06:02* to examine the effects of other *HLA-DRB1*, *HLA-DQA1*, and *HLA-DQB1* alleles. We did this after excluding subjects homozygous for *HLA-DQB1*06:02*. The *HLA-DRB1*, *HLA-DQA1*, and *HLA-DQB1* loci were analyzed independently. As previously reported in multiple studies,[2,3,22,27–29] we detected risk groups of *HLA-DRB1*, *HLA-DQA1*, and *HLA-DQB1* associations known to act in *trans* of *HLA-DRB1*1501~DQA1*01:02~DQB1**

Table 2. Association between DPA1-DPB1 Heterodimers and Narcolepsy in Stepwise Analysis

HLA-DPA1-DPB1 Heterodimer	No. of Control Subjects (Freq)	No. of Case Subjects (Freq)	Mantel-Haenszel Test		
			OR (CI)	p	p Heterogeneity Test
01:03-04:02	160 (0.15)	88 (0.083)	0.51 (0.38–0.67)	1.01×10^{-06}	0.852
01:03-04:01	516 (0.58)	459 (0.52)	0.61 (0.47–0.80)	2.07×10^{-04}	0.342
02:02-19:01	7 (0.020)	0 (0.00)	0 (0.00–NA)	0.008	NA
02:02-05:01	193 (0.57)	218 (0.64)	1.41 (1.02–1.95)	0.039	0.387

The p values were calculated with the chi-square test and Maentel-Haenszel test. The p heterogeneity test is Breslow-Day's p value. Abbreviations are as follows: CI, confidence interval; Freq, carrier frequency; NA, not available (the exact OR or p value could not be calculated); and OR, odds ratio.

06:02: (1) a set of protective alleles (*HLA-DRB1*13:01*, *HLA-DRB1*01:01*, *HLA-DRB1*08:03*, *HLA-DQB1*05:01*, *HLA-DQB1*06:03*, *HLA-DQA1*01:01*, and *HLA-DQA1*01:03*) in high LD with similar ORs in both ethnic groups (Tables S11 and S12); (2) additional predisposing effects of *HLA-DQA1*01:02*-bearing haplotypes (*HLA-DQA1*01:02* and *HLA-DQB1*05:02* in whites and *HLA-DQA1*01:02* in Asians); and (3) additional predisposing effects of *HLA-DQB1*03:01*-bearing haplotypes (Tables S11 and S12). These effects are well established and are consistent with the effect of *trans*-heterodimerization of DQ1 alleles on DQ0602 and an additional effect of *HLA-DQB1*03:01*. In addition, nominally significant effects were seen with *HLA-DQB1*02:02*, *HLA-DQB1*04:02*, and *HLA-DQB1*03:02* (Tables S11 and S12).

Associations were next conditioned on all significantly associated *HLA-DR* and *HLA-DQ* alleles and SNPs. As seen from residual HLA association effects, *HLA-DPB1*04:02* was again highly protective in both whites and Asians (Table 3). Similarly, *HLA-DPB1*05:01* also showed significant predisposing effects in narcolepsy (Table 3). Furthermore, *HLA-DPB1*02:01* was found as an additional association (Table 3; Tables S13 and S14). Figure 1B also shows GWAS data in the HLA region of whites (from ImmunoChip, see Franco et al.[39]) and Chinese (from Affymetrix CHB data, see Han et al.[5]) after conditioning for *HLA-DR* and *HLA-DQ*; it shows large residual association in the *HLA-DP* region and a main effect of *HLA-DPB1*04:02*.

In a final analysis, we examined HLA class I associations after conditioning on all identified HLA class II (*HLA-DR*, *HLA-DQ*, and *HLA-DP*) effects. Statistically significant predisposing associations were seen with *HLA-B*51:01*, *HLA-B*35:03*, *HLA-B*18:01*, *HLA-C*04:01*, and *HLA-A*11:01*, whereas *HLA-B*07:02* was protective (Table 4; Tables S15 and S16). Of special interest were associations with *HLA-A*11:01*, *HLA-B*51:01*, and *HLA-B*35:03* because these were in the same direction across ethnic groups, a finding more suggestive of a direct effect.

Figure 1C shows GWAS data for whites after conditioning for all class II (*HLA-DR*, *HLA-DQ*, and *HLA-DP*) effects; it shows complex residual association effects in the class I region. A common association is noted in both ethnic groups in the *HLA-B* region. In addition, a large association, peaking at rs2523882A (OR = 1.41 [1.26–1.57], p = 7.42×10^{-10}), is noted in whites in the *PSORS1* region. Surprisingly, several CHB panel SNPs with high LD with rs2523882 in Chinese were either weakly (rs2517474G, OR = 0.78 [0.64–0.96], p = 0.016) or not associated (rs3132564, rs62399065, and rs9263475). Because SNP coverage in this region is vastly superior in the ImmunoChip than in the CHB chip, additional fine typing will be needed to extend this observation.

Variation at the Amino Acid Level

In order to study whether amino acid polymorphisms across different HLA subtypes could affect the predisposition to narcolepsy, we imputed all amino acid polymorphisms in HLA alleles encoded by the different *HLA-A*, *HLA-B*, *HLA-C*, *HLA-DPA1*, and *HLA-DPB1* loci and performed association testing in the typed and imputed data sets that had been matched for HLA class II and country of origin.

At HLA-DPB1, no independent amino acid was associated with narcolepsy. At HLA-DPA1, Ala11 and Gln50 were weakly protective (OR = 0.65 [0.47–0.86], p = 0.0029, and OR = 0.68 [0.52–0.88], p = 0.0035, respectively), and these effects recapitulated effects of the protective HLA-DPA1*01:03 allele. These two HLA-DPA1 amino acids are present together in HLA-DPA1*01:03, the most frequent HLA-DPA1 allele, which is protective in the context of HLA-DPA1*01:03-DPB1*04:02 and HLA-DPB1*04:01. The lack of strong association with individual HLA-DPB1 amino acids suggests that larger binding motifs underlie the association with narcolepsy.

In the class I region, we found that HLA-A Tyr9 showed the strongest association with narcolepsy (OR = 1.35, [1.13–1.62], p = 0.0012), whereas only weak associations were seen with other amino acids. Interestingly, the predisposing HLA-A*11:01 allele has this polymorphism, and it is also found in HLA-A*25:01, which was detected with the conditional analysis.

Finally, we performed stepwise analysis with all class I alleles and HLA-A Tyr9 in the matched data set in order to see which alleles were driving the associations. The associations were nominally significant, and the strongest association was seen with *HLA-C*16:01*, followed by *HLA-A*11:01*, which explained in the stepwise analysis most of the HLA Tyr 9 association that was not significant after

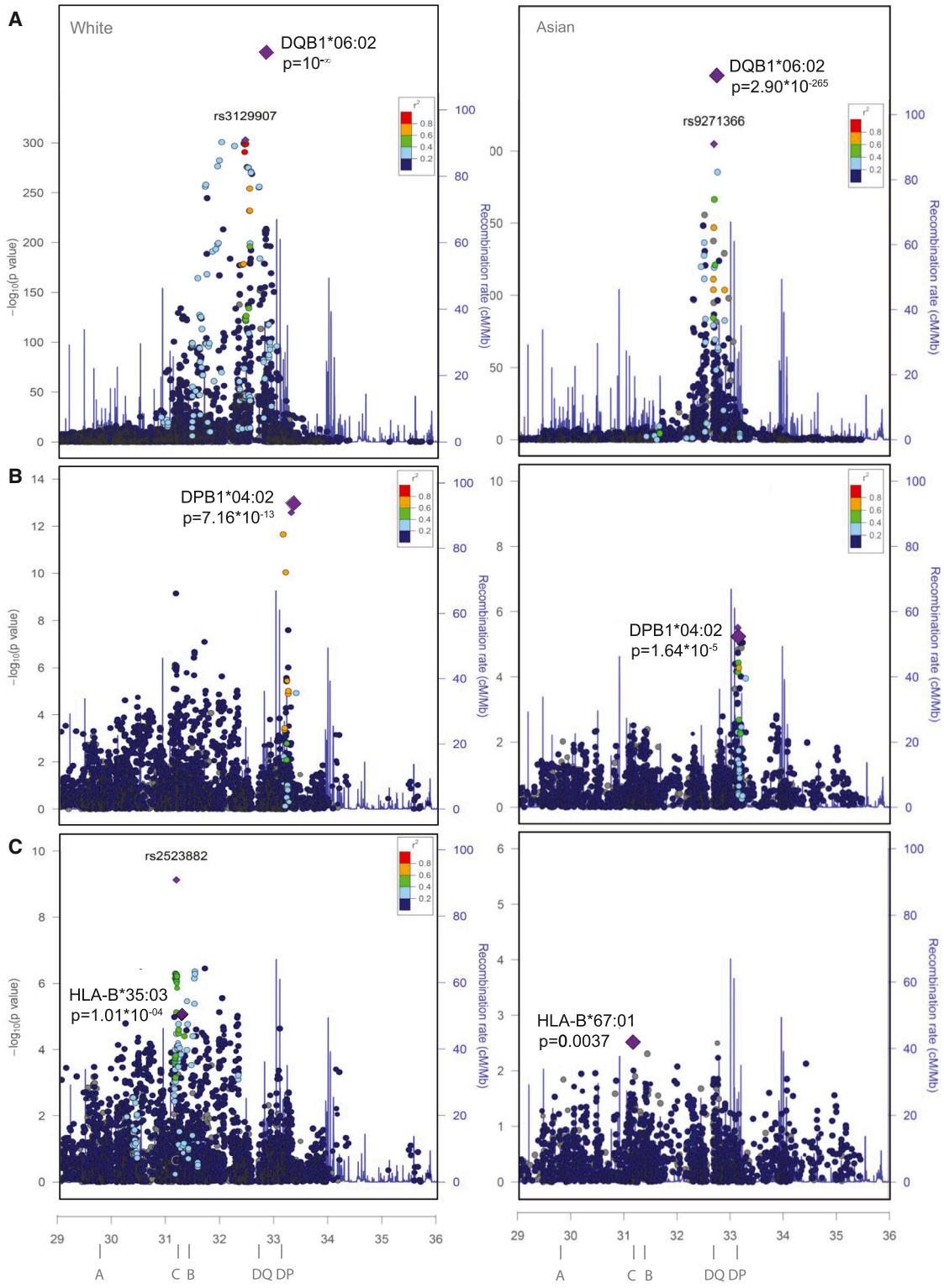

Figure 1. Association between HLA Loci and Narcolepsy

(A) Association of SNPs in the HLA region (Chr6: 29–36 Mb) reveals an overwhelming signal peaking at the level of *HLA-DQB1* in white (from Immunochip, see Faraco et al.[39]) and chinese (from Affymetrix CHB data, see Han et al.[5]) individuals. Extended LD and association signal within the *HLA-DR-DQ* region obscure all other signals.

(B) After conditioning for *HLA-DRB1*, *HLA-DQA1*, and *HLA-DQB1* (significant alleles from the stepwise analysis), a residual association is seen in the *HLA-DP* region.

(C) After conditioning for all significant HLA class II alleles, a remaining association is seen in the HLA class I region and is most visible proximal to *HLA-B*. A possible additional peak is seen in white individuals only in the vicinity of *PSORS1*.

Table 3. Association of *HLA-DPB1* Alleles after Conditioning for *HLA-DRB1*, *HLA-DQA1*, and *HLA-DQB1* Effects

ImmunoChip					Chinese GWAS				Meta-analysis		
***HLA-DPB1* Allele**	**No. of Control Subjects (Freq)**	**No. of Case Subjects (Freq)**	**OR (CI)**	**p**	**No. of Control Subjects (Freq)**	**No. of Case Subjects (Freq)**	**OR (CI)**	**p**	**OR**	**p Meta-analysis**	**I^2**
04:02	2,298 (0.22)	138 (0.090)	0.47 (0.38–0.58)	7.15×10^{-13}	258 (0.13)	57 (0.048)	0.38 (0.24–0.59)	1.64×10^{-05}	0.45 (0.38–0.55)	8.99×10^{-17}	0
02:01	2,527 (0.24)	347 (0.23)	1.39 (1.20–1.60)	1.14×10^{-05}	776 (0.39)	640 (0.54)	1.14 (0.93–1.39)	0.2037	1.30 (1.15–1.46)	1.74×10^{-05}	0.584
05:01	400 (0.038)	113 (0.073)	1.43 (1.08–1.89)	0.0123	1,187 (0.59)	776 (0.65)	1.35 (1.12–1.64)	0.00186	1.38 (1.18–1.61)	7.11×10^{-05}	0

Abbreviations are as follows: CI, confidence interval; I^2, heterogeneity in the meta-analysis as described in Higgins et al.[60] (0 means no heterogeneity); and OR, odds ratio.

removal of the *HLA-A*11:01* carriers. Similar to the conditioned analysis, nominally significant associations were also seen with *HLA-B*35:03*, *HLA-B*41:02*, and *HLA-B*51:01* (Table S17).

Discussion

In this study, we discovered HLA risk loci and protective variants for narcolepsy. These effects were independent of the well-established *HLA-DQ* effects in narcolepsy. The strongest protection was seen with *HLA-DPB1*04:02* across all ethnic groups and data sets. Further, *HLA-DPB1*05:01* predisposed to narcolepsy independently of *HLA-DPB1*04:02* in Chinese individuals, where it is a common allele, confirming a recently published study in Japanese subjects.[52] In addition, predisposing HLA class I associations were seen with *HLA-A*11:01*, *HLA-B*35:03*, and *HLA-B*51:01* across ethnic groups, although these effects were much weaker than *HLA-DP* effects. Finally, a possible remaining signal not explained by classic HLA gene polymorphisms was found near *PSORS1* in the class I region of white subjects.

Our strongest findings indicate an independent role for *HLA-DP* molecules in narcolepsy susceptibility. In narcolepsy, the effect of heterodimerization of HLA-DQA1 and HLA-DQB1 is well established.[26] In *HLA-DP*, there are only three common *HLA-DPA1* genes that have very conserved haplotypes with *HLA-DPB1*. Analysis of possible *cis* (in the same haplotype) and *trans* (on the other chromosome) heterodimers revealed that the most protective heterodimer was HLA-DPA1*01:03-DPB1*04:02, whereas HLA-DPA1*02:02-DPB1*05:01 conferred the largest risk. These haplotypes were observed in *cis*, and the analysis of *trans* associations did not improve statistical significance.

The *HLA-DP* loci are important in the development of autoimmune diseases such as multiple sclerosis (MS),[32,53] sarcoidosis (MIM 181000),[54] and type 1 diabetes.[33] Similar to in our findings, *HLA-DPB1*04:02* has been shown to be protective against type 1 diabetes and sarcoidosis,[33,54] whereas *HLA-DPB1*05:01* has been associated with increased risk of MS.[53,55] In addition, *HLA-DPB1*05:01* has been associated with non-clearance of viral infections such as that of chronic hepatitis B, whereas similar to in our study, *HLA-DPB1*04:02* is protective against this condition.[38]

The specific disease mechanisms underlying this new *HLA-DP* association in narcolepsy remain elusive. Narcolepsy was recently associated with pandemic H1N1 2009 vaccination[11–17] and infections.[8,10] In addition, streptococcal antibodies were found more frequently in narcoleptics than in matched healthy control individuals.[9] These findings suggest that environmental triggers, such as upper-airway winter infections, are strong effectors in the development of narcolepsy. It is thus interesting to speculate that the presence of *HLA-DP* risk alleles, such as *HLA-DPB1*05:01*, results in lower viral clearance or immune response, whereas the opposite might occur with protective alleles, such as *HLA-DPB1*04:02*. In this model, a lower clearance of the viral trigger could be critical to the development of autoimmunity. *HLA-DPB1*05:01* has also been shown to be more common in individuals who do not develop seroprotection after hepatitis B vaccination.[56]

We also observed consistent associations of HLA class I alleles *HLA-A*11:01*, *HLA-B*35:03*, and *HLA-B*51:01* (predisposing) after correction of all HLA class II effects, suggesting an independent role for these HLA alleles. These findings are similar to those found in other autoimmune diseases, such as MS[32,57,58] or type 1 diabetes,[59] where the main risk alleles are located in the HLA class II region but residual association is seen in HLA class I. Of notable interest is the fact that in type 1 diabetes, a disease where *HLA-DQB1*06:02* is strongly protective, opposite effects to type 1 diabetes of *HLA-A*11:01* are also seen in narcolepsy. HLA class I effects in these disease might suggest the involvement of $CD8^+$ T or natural killer cells, given that these three alleles are also known killer cell immunoglobulin-like receptor ligands.

To conclude, our findings suggest that the HLA associations in narcolepsy are more complex than previously thought and show that important high-risk variants reside

Table 4. Association of HLA Class I Alleles after Conditioning for *HLA-DRB1*, *HLA-DQA1*, *HLA-DQB1*, *HLA-DPA1*, and *HLA-DPB1* Alleles

HLA Allele	ImmunoChip				Chinese GWAS				Meta-analysis		
	No. of Control Subjects (Freq)	No. of Case Subjects (Freq)	OR (CI)	p	No. of Control Subjects (Freq)	No. of Case Subjects (Freq)	OR (CI)	p	OR (CI)	p Meta-analysis	I^2
*HLA-B*51:01*	1,114 (0.11)	189 (0.12)	1.51 (1.24–1.85)	5.45×10^{-5}	248 (0.12)	171 (0.14)	1.41 (0.97–2.07)	0.075	1.49 (1.25–1.78)	1.09×10^{-5}	0
*HLA-B*35:03*	158 (0.015)	65 (0.042)	1.96 (1.40–2.75)	1.01×10^{-4}	32 (0.016)	20 (0.017)	1.89 (0.62–5.64)	0.257	1.95 (1.41–2.70)	5.14×10^{-5}	0
*HLA-B*07:02*	2,368 (0.23)	886 (0.57)	0.78 (0.68–0.88)	8.70×10^{-5}	119 (0.060)	133 (0.11)	1.03 (0.69–1.54)	0.872	0.80 (0.71–0.90)	2.22×10^{-4}	0.441
*HLA-B*18:01*	1,047 (0.10)	251 (0.16)	1.46 (1.21–1.76)	8.43×10^{-5}	26 (0.013)	15 (0.013)	0.65 (0.61–1.65)	0.361	1.41 (1.17–1.69)	2.38×10^{-4}	0.369
*HLA-C*04:01*	2,203 (0.21)	259 (0.17)	1.42 (1.20–1.69)	6.89×10^{-5}	225 (0.11)	127 (0.11)	0.92 (0.21–1.38)	0.688	1.33 (1.13–1.56)	4.45×10^{-4}	0.732
*HLA-A*11:01*	1,206 (0.12)	199 (0.13)	1.28 (1.05–1.57)	0.0146	654 (0.33)	460 (0.39)	1.38 (0.13–1.07)	0.012	1.32 (1.13–1.54)	4.92×10^{-4}	0

Abbreviations are as follows: CI, confidence interval; Freq, carrier frequency; I^2, heterogeneity as described in Higgins et al.[60] (0 means no heterogeneity); and OR, odds ratio.

outside the known *HLA-DR-DQ* risk region, notably in the *HLA-DP* region, where *HLA-DPB1*04:02* and *HLA-DPB1* 05:01* have strong effects. We found additional HLA class I effects, some of which were most compatible with the direct effect of specific HLA alleles, and others will need further confirmation. Our study benefited from the evaluation of two ethnic groups, formal HLA typing, and HLA subtype imputation based on GWAS data. Combining these methods is likely to reveal a more precise picture of the role of the HLA region in autoimmune diseases such as narcolepsy.

Supplemental Data

Supplemental Data include one figure and 17 tables and can be found with this article online at http://dx.doi.org/10.1016/j.ajhg.2014.12.010.

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Web Resources

The URLs for data presented herein are as follows:

Online Mendelian Inheritance in Man (OMIM), http://www.omim.org
Singapore Genome Variation Project (SGVP), http://www.statgen.nus.edu.sg/~SGVP/
R project, http://www.r-project.org/
UCSC Human Genome Browser, http://genome.ucsc.edu/cgi-bin/hgGateway

References

1. Peyron, C., Faraco, J., Rogers, W., Ripley, B., Overeem, S., Charnay, Y., Nevsimalova, S., Aldrich, M., Reynolds, D., Albin, R., et al. (2000). A mutation in a case of early onset narcolepsy and a generalized absence of hypocretin peptides in human narcoleptic brains. Nat. Med. *6*, 991–997.
2. Pelin, Z., Guilleminault, C., Risch, N., Grumet, F.C., and Mignot, E.; US Modafinil in Narcolepsy Multicenter Study Group (1998). HLA-DQB1*0602 homozygosity increases relative risk for narcolepsy but not disease severity in two ethnic groups. Tissue Antigens *51*, 96–100.
3. Han, F., Lin, L., Li, J., Dong, S.X., An, P., Zhao, L., Liu, N.Y., Li, Q.Y., Yan, H., Gao, Z.C., et al. (2012). HLA-DQ association and allele competition in Chinese narcolepsy. Tissue Antigens *80*, 328–335.
4. Kornum, B.R., Kawashima, M., Faraco, J., Lin, L., Rico, T.J., Hesselson, S., Axtell, R.C., Kuipers, H., Weiner, K., Hamacher, A., et al. (2011). Common variants in P2RY11 are associated with narcolepsy. Nat. Genet. *43*, 66–71.
5. Han, F., Faraco, J., Dong, X.S., Ollila, H.M., Lin, L., Li, J., An, P., Wang, S., Jiang, K.W., Gao, Z.C., et al. (2013). Genome wide analysis of narcolepsy in China implicates novel immune loci and reveals changes in association prior to versus after the 2009 H1N1 influenza pandemic. PLoS Genet. *9*, e1003880.
6. Hallmayer, J., Faraco, J., Lin, L., Hesselson, S., Winkelmann, J., Kawashima, M., Mayer, G., Plazzi, G., Nevsimalova, S., Bourgin, P., et al. (2009). Narcolepsy is strongly associated with the T-cell receptor alpha locus. Nat. Genet. *41*, 708–711.
7. Dauvilliers, Y., Maret, S., Bassetti, C., Carlander, B., Billiard, M., Touchon, J., and Tafti, M. (2004). A monozygotic twin pair discordant for narcolepsy and CSF hypocretin-1. Neurology *62*, 2137–2138.
8. Han, F., Lin, L., Warby, S.C., Faraco, J., Li, J., Dong, S.X., An, P., Zhao, L., Wang, L.H., Li, Q.Y., et al. (2011). Narcolepsy onset is seasonal and increased following the 2009 H1N1 pandemic in China. Ann. Neurol. *70*, 410–417.
9. Aran, A., Lin, L., Nevsimalova, S., Plazzi, G., Hong, S.C., Weiner, K., Zeitzer, J., and Mignot, E. (2009). Elevated antistreptococcal antibodies in patients with recent narcolepsy onset. Sleep *32*, 979–983.
10. Wu, H., Zhuang, J., Stone, W.S., Zhang, L., Zhao, Z., Wang, Z., Yang, Y., Li, X., Zhao, X., and Zhao, Z. (2014). Symptoms and occurrences of narcolepsy: a retrospective study of 162 patients during a 10-year period in eastern China. Sleep Med. *15*, 607–613.
11. Partinen, M., Saarenpää-Heikkilä, O., Ilveskoski, I., Hublin, C., Linna, M., Olsén, P., Nokelainen, P., Alén, R., Wallden, T., Espo, M., et al. (2012). Increased incidence and clinical picture of childhood narcolepsy following the 2009 H1N1 pandemic vaccination campaign in Finland. PLoS ONE *7*, e33723.
12. Nohynek, H., Jokinen, J., Partinen, M., Vaarala, O., Kirjavainen, T., Sundman, J., Himanen, S.L., Hublin, C., Julkunen, I., Olsén, P., et al. (2012). AS03 adjuvanted AH1N1 vaccine associated with an abrupt increase in the incidence of childhood narcolepsy in Finland. PLoS ONE *7*, e33536.
13. O'Flanagan, D., Barret, A.S., Foley, M., Cotter, S., Bonner, C., Crowe, C., Lynch, B., Sweeney, B., Johnson, H., McCoy, B., and Purcell, E. (2014). Investigation of an association between onset of narcolepsy and vaccination with pandemic influenza vaccine, Ireland April 2009-December 2010. Euro Surveill. *19*, 15–25.
14. Persson, I., Granath, F., Askling, J., Ludvigsson, J.F., Olsson, T., and Feltelius, N. (2014). Risks of neurological and immune-related diseases, including narcolepsy, after vaccination with Pandemrix: a population- and registry-based cohort study with over 2 years of follow-up. J. Intern. Med. *275*, 172–190.
15. Miller, E., Andrews, N., Stellitano, L., Stowe, J., Winstone, A.M., Shneerson, J., and Verity, C. (2013). Risk of narcolepsy in children and young people receiving AS03 adjuvanted pandemic A/H1N1 2009 influenza vaccine: retrospective analysis. BMJ *346*, f794.
16. Dauvilliers, Y., Arnulf, I., Lecendreux, M., Monaca Charley, C., Franco, P., Drouot, X., d'Ortho, M.P., Launois, S., Lignot, S., Bourgin, P., et al.; Narcoflu-VF study group (2013). Increased risk of narcolepsy in children and adults after pandemic H1N1 vaccination in France. Brain *136*, 2486–2496.
17. Heier, M.S., Gautvik, K.M., Wannag, E., Bronder, K.H., Midtlyng, E., Kamaleri, Y., and Storsaeter, J. (2013). Incidence of narcolepsy in Norwegian children and adolescents after vaccination against H1N1 influenza A. Sleep Med. *14*, 867–871.
18. Montplaisir, J., Petit, D., Quinn, M.-J., Ouakki, M., Deceuninck, G., Desautels, A., Mignot, E., and De Wals, P. (2014). Risk of narcolepsy associated with inactivated adjuvanted (AS03) A/H1N1 (2009) pandemic influenza vaccine in Quebec. PLoS ONE *9*, e108489.
19. Matsuki, K., Grumet, F.C., Lin, X., Gelb, M., Guilleminault, C., Dement, W.C., and Mignot, E. (1992). DQ (rather than DR) gene marks susceptibility to narcolepsy. Lancet *339*, 1052.
20. Roh, E.Y., Park, M.H., Park, H., Park, D.H., Choi, J.B., Kim, S.J., and Jeong, D.U. (2006). Association of HLA-DR and -DQ genes with narcolepsy in Koreans: comparison with two control groups, randomly selected subjects and DRB1*1501-DQB1*0602—positive subjects. Hum. Immunol. *67*, 749–755.
21. Hong, S.C., Leen-Kim, Park, S.A., Han, J.H., Lee, S.P., Lin, L., Okun, M., Nishino, S., and Mignot, E. (2002). HLA and hypocretin studies in Korean patients with narcolepsy. Sleep *25*, 440–444.
22. Mignot, E., Lin, L., Rogers, W., Honda, Y., Qiu, X., Lin, X., Okun, M., Hohjoh, H., Miki, T., Hsu, S., et al. (2001). Complex HLA-DR and -DQ interactions confer risk of narcolepsy-cataplexy in three ethnic groups. Am. J. Hum. Genet. *68*, 686–699.
23. Mignot, E., Lin, L., Li, H., et al. (2006). HLA allele and microsatellite studies in narcolepsy. In HLA 2004, Immunobiology of the Human MHC, Proceedings of the 13th International Histocompatibility Workshop and Congress, J. Hansen and B. Dupont, eds. (Seattle: IHWG Press), pp. 817–823.
24. Megiorni, F., and Pizzuti, A. (2012). HLA-DQA1 and HLA-DQB1 in Celiac disease predisposition: practical implications of the HLA molecular typing. J. Biomed. Sci. *19*, 88.
25. Eerligh, P., van Lummel, M., Zaldumbide, A., Moustakas, A.K., Duinkerken, G., Bondinas, G., Koeleman, B.P., Papadopoulos, G.K., and Roep, B.O. (2011). Functional consequences of HLA-DQ8 homozygosity versus heterozygosity for islet autoimmunity in type 1 diabetes. Genes Immun. *12*, 415–427.
26. Ollila, H.M., Fernandez-Vina, M., and Mignot, E. (2014). HLA-DQ allele competition in narcolepsy: A comment on Tafti et al. DQB1 locus alone explains most of the risk and protection in narcolepsy with cataplexy in Europe. Sleep. Published online October 17, 2014.
27. Hong, S.C., Lin, L., Lo, B., Jeong, J.H., Shin, Y.K., Kim, S.Y., Kweon, Y., Zhang, J., Einen, M., Smith, A., et al. (2007). DQB1*0301 and DQB1*0601 modulate narcolepsy susceptibility in Koreans. Hum. Immunol. *68*, 59–68.

28. Hohjoh, H., Terada, N., Nakayama, T., Kawashima, M., Miyagawa, T., Honda, Y., and Tokunaga, K. (2001). Case-control study with narcoleptic patients and healthy controls who, like the patients, possess both HLA-DRB1*1501 and -DQB1*0602. Tissue Antigens *57*, 230–235.
29. Tafti, M., Hor, H., Dauvilliers, Y., Lammers, G.J., Overeem, S., Mayer, G., Javidi, S., Iranzo, A., Santamaria, J., Peraita-Adrados, R., et al. (2014). DQB1 locus alone explains most of the risk and protection in narcolepsy with cataplexy in Europe. Sleep *37*, 19–25.
30. Kwok, W.W., Kovats, S., Thurtle, P., and Nepom, G.T. (1993). HLA-DQ allelic polymorphisms constrain patterns of class II heterodimer formation. J. Immunol. *150*, 2263–2272.
31. Sugihara, S., Ogata, T., Kawamura, T., Urakami, T., Takemoto, K., Kikuchi, N., Takubo, N., Tsubouchi, K., Horikawa, R., Kobayashi, K., et al.; Japanese Study Group of Insulin Therapy for Childhood and Adolescent Diabetes (JSGIT) (2012). HLA-class II and class I genotypes among Japanese children with Type 1A diabetes and their families. Pediatr. Diabetes *13*, 33–44.
32. Patsopoulos, N.A., Barcellos, L.F., Hintzen, R.Q., Schaefer, C., van Duijn, C.M., Noble, J.A., Raj, T., Gourraud, P.A., Stranger, B.E., Oksenberg, J., et al.; IMSGC; ANZgene (2013). Fine-mapping the genetic association of the major histocompatibility complex in multiple sclerosis: HLA and non-HLA effects. PLoS Genet. *9*, e1003926.
33. Varney, M.D., Valdes, A.M., Carlson, J.A., Noble, J.A., Tait, B.D., Bonella, P., Lavant, E., Fear, A.L., Louey, A., Moonsamy, P., et al.; Type 1 Diabetes Genetics Consortium (2010). HLA DPA1, DPB1 alleles and haplotypes contribute to the risk associated with type 1 diabetes: analysis of the type 1 diabetes genetics consortium families. Diabetes *59*, 2055–2062.
34. Luo, H., Chen, M., Cui, Z., Yang, R., Xu, P.C., Zhou, X.J., and Zhao, M.H. (2011). The association of HLA-DQB1, -DQA1 and -DPB1 alleles with anti- glomerular basement membrane (GBM) disease in Chinese patients. BMC Nephrol. *12*, 21.
35. Horiki, T., Inoko, H., Moriuchi, J., Ichikawa, Y., and Arimori, S. (1994). Combinations of HLA-DPB1 and HLA-DQB1 alleles determine susceptibility to early-onset myasthenia gravis in Japan. Autoimmunity *19*, 49–54.
36. Moss, A.J., Gaughran, F.P., Karasu, A., Gilbert, A.S., Mann, A.J., Gelder, C.M., Oxford, J.S., Stephens, H.A., and Lambkin-Williams, R. (2013). Correlation between human leukocyte antigen class II alleles and HAI titers detected post-influenza vaccination. PLoS ONE *8*, e71376.
37. Nishida, N., Sawai, H., Kashiwase, K., Minami, M., Sugiyama, M., Seto, W.K., Yuen, M.F., Posuwan, N., Poovorawan, Y., Ahn, S.H., et al. (2014). New susceptibility and resistance HLA-DP alleles to HBV-related diseases identified by a trans-ethnic association study in Asia. PLoS ONE *9*, e86449.
38. Kamatani, Y., Wattanapokayakit, S., Ochi, H., Kawaguchi, T., Takahashi, A., Hosono, N., Kubo, M., Tsunoda, T., Kamatani, N., Kumada, H., et al. (2009). A genome-wide association study identifies variants in the HLA-DP locus associated with chronic hepatitis B in Asians. Nat. Genet. *41*, 591–595.
39. Faraco, J., Lin, L., Kornum, B.R., Kenny, E.E., Trynka, G., Einen, M., Rico, T.J., Lichtner, P., Dauvilliers, Y., Arnulf, I., et al. (2013). ImmunoChip study implicates antigen presentation to T cells in narcolepsy. PLoS Genet. *9*, e1003270.
40. American Academy of Sleep Medicine (2014). The International Classification of Sleep Disorders, Third Edition (Chicago: American Academy of Sleep Medicine).
41. Wang, C., Krishnakumar, S., Wilhelmy, J., Babrzadeh, F., Stepanyan, L., Su, L.F., Levinson, D., Fernandez-Viña, M.A., Davis, R.W., Davis, M.M., and Mindrinos, M. (2012). High-throughput, high-fidelity HLA genotyping with deep sequencing. Proc. Natl. Acad. Sci. USA *109*, 8676–8681.
42. Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M.A., Bender, D., Maller, J., Sklar, P., de Bakker, P.I., Daly, M.J., and Sham, P.C. (2007). PLINK: a tool set for whole-genome association and population-based linkage analyses. Am. J. Hum. Genet. *81*, 559–575.
43. R Development Core Team (2010). R: A language and environment for statistical computing (Vienna: R Foundation for Statistical Computing).
44. Zheng, X., Shen, J., Cox, C., Wakefield, J.C., Ehm, M.G., Nelson, M.R., and Weir, B.S. (2014). HIBAG—HLA genotype imputation with attribute bagging. Pharmacogenomics J. *14*, 192–200.
45. Jia, X., Han, B., Onengut-Gumuscu, S., Chen, W.M., Concannon, P.J., Rich, S.S., Raychaudhuri, S., and de Bakker, P.I. (2013). Imputing amino acid polymorphisms in human leukocyte antigens. PLoS ONE *8*, e64683.
46. Rich, S.S., Concannon, P., Erlich, H., Julier, C., Morahan, G., Nerup, J., Pociot, F., and Todd, J.A. (2006). The Type 1 Diabetes Genetics Consortium. Ann. N Y Acad. Sci. *1079*, 1–8.
47. Hollenbach, J.A., Madbouly, A., Gragert, L., Vierra-Green, C., Flesch, S., Spellman, S., Begovich, A., Noreen, H., Trachtenberg, E., Williams, T., et al. (2012). A combined DPA1~DPB1 amino acid epitope is the primary unit of selection on the HLA-DP heterodimer. Immunogenetics *64*, 559–569.
48. Pruim, R.J., Welch, R.P., Sanna, S., Teslovich, T.M., Chines, P.S., Gliedt, T.P., Boehnke, M., Abecasis, G.R., and Willer, C.J. (2010). LocusZoom: regional visualization of genome-wide association scan results. Bioinformatics *26*, 2336–2337.
49. Hollenbach, J.A., Mack, S.J., Thomson, G., and Gourraud, P.A. (2012). Analytical methods for disease association studies with immunogenetic data. Methods Mol. Biol. *882*, 245–266.
50. Mägi, R., and Morris, A.P. (2010). GWAMA: software for genome-wide association meta-analysis. BMC Bioinformatics *11*, 288.
51. Begovich, A.B., Moonsamy, P.V., Mack, S.J., Barcellos, L.F., Steiner, L.L., Grams, S., Suraj-Baker, V., Hollenbach, J., Trachtenberg, E., Louie, L., et al. (2001). Genetic variability and linkage disequilibrium within the HLA-DP region: analysis of 15 different populations. Tissue Antigens *57*, 424–439.
52. Miyagawa, T., Toyoda, H., Hirataka, A., Kanbayashi, T., Imanishi, A., Sagawa, Y., Kotorii, N., Kotorii, T., Hashizume, Y., Ogi, K., et al. (2014). New susceptibility variants to narcolepsy identified in HLA class II region. Hum. Mol. Genet.
53. Kira, J. (2003). Multiple sclerosis in the Japanese population. Lancet Neurol. *2*, 117–127.
54. Wennerström, A., Vlachopoulou, E., Lahtela, L.E., Paakkanen, R., Eronen, K.T., Seppänen, M., and Lokki, M.L. (2013). Diversity of extended HLA-DRB1 haplotypes in the Finnish population. PLoS ONE *8*, e79690.
55. Wu, X.M., Wang, C., Zhang, K.N., Lin, A.Y., Kira, J., Hu, G.Z., Qu, X.H., Xiong, Y.Q., Cao, W.F., and Gong, L.Y. (2009). Association of susceptibility to multiple sclerosis in Southern Han Chinese with HLA-DRB1, -DPB1 alleles and DRB1-DPB1 haplotypes: distinct from other populations. Mult. Scler. *15*, 1422–1430.

56. Wu, T.W., Chu, C.C., Liao, H.W., Lin, S.K., Ho, T.Y., Lin, M., Lin, H.H., and Wang, L.Y. (2014). HLA-DPB1 and anti-HBs titer kinetics in hepatitis B booster recipients who completed primary hepatitis B vaccination during infancy. Genes Immun. *15*, 47–53.
57. Sawcer, S., Hellenthal, G., Pirinen, M., Spencer, C.C., Patsopoulos, N.A., Moutsianas, L., Dilthey, A., Su, Z., Freeman, C., Hunt, S.E., et al.; International Multiple Sclerosis Genetics Consortium; Wellcome Trust Case Control Consortium 2 (2011). Genetic risk and a primary role for cell-mediated immune mechanisms in multiple sclerosis. Nature *476*, 214–219.
58. Link, J., Lorentzen, A.R., Kockum, I., Duvefelt, K., Lie, B.A., Celius, E.G., Harbo, H.F., Hillert, J., and Brynedal, B. (2010). Two HLA class I genes independently associated with multiple sclerosis. J. Neuroimmunol. *226*, 172–176.
59. Nejentsev, S., Howson, J.M., Walker, N.M., Szeszko, J., Field, S.F., Stevens, H.E., Reynolds, P., Hardy, M., King, E., Masters, J., et al.; Wellcome Trust Case Control Consortium (2007). Localization of type 1 diabetes susceptibility to the MHC class I genes HLA-B and HLA-A. Nature *450*, 887–892.
60. Higgins, J.P., and Thompson, S.G. (2002). Quantifying heterogeneity in a meta-analysis. Stat. Med. *21*, 1539–1558.