TISSUE ANTIGENS
IMMUNE RESPONSE GENETICS

Tissue Antigens ISSN 0001-2815

# HLA-DQ association and allele competition in Chinese narcolepsy

F. Han[1], L. Lin[2], J. Li[1], S. X. Dong[1], P. An[1], L. Zhao[1], N. Y. Liu[1], Q. Y. Li[1], H. Yan[1], Z. C. Gao[1], J. Faraco[2], K. P. Strohl[3], X. Liu[4], H. Miyadera[5] & E. Mignot[2]

1 Department of Pulmonary Medicine, Beijing University People's Hospital, Beijing, China
2 Stanford Center for Sleep Sciences and Medicine, Palo Alto, CA, USA
3 Division of Pulmonary, Critical Care and Sleep Medicine, Department of Medicine, Case Western Reserve University, and Cleveland Louis Stokes VA Medical Center, Cleveland, OH, USA
4 BFR Diagnostics LTD, Beijing, China
5 Department of Human Genetics, University of Tokyo, Tokyo, Japan

**Key words**
*DQB1*06:02*; *DRB1*15:01*; human leucocyte antigen; narcolepsy

**Correspondence**
Fang Han, MD
Department of Pulmonary Medicine
Beijing University People's Hospital
Beijing
China
Tel: +86 10 8832 4206
Fax: +86 10 8832 4207
e-mail: hanfang1@hotmail.com
and
Emmanuel Mignot, MD
Stanford Center for Sleep Sciences and Medicine
Palo Alto
CA
USA
Tel: +650 725 6517
Fax: +650 725 4913
e-mail: mignot@stanford.edu

Received 4 April 2012; revised 9 July 2012; accepted 16 July 2012

doi: 10.1111/j.1399-0039.2012.01948.x

## Abstract

In Japanese, Koreans and Caucasians, narcolepsy/hypocretin deficiency is tightly associated with the *DRB1*15:01-DQA1*01:02-DQB1*06:02* haplotype. Studies in African-Americans suggest a primary effect of *DQB1*06:02*, but this observation has been difficult to confirm in other populations because of high linkage disequilibrium between *DRB1*15:01/3* and *DQB1*06:02* in most populations. In this study, we studied human leucocyte antigen (HLA) class II in 202 Chinese narcolepsy patients (11% from South China) and found all patients to be *DQB1*06:02* positive. Comparing cases with 103 unselected controls, and 110 and 79 controls selected for the presence of *DQB1*06:02* and *DRB1*15:01*, we found that the presence of *DQB1*06:02* and not *DRB1*15:01* was associated with narcolepsy. In particular, Southern Chinese haplotypes such as the *DRB1*15:01-DQA1*01:02-DQB1*06:01* and *DRB1*15:01-DQA1*01:02-DQB1*05* were not associated with narcolepsy. As reported in Japanese, Koreans, African-Americans and Caucasians, additional protective effects of *DQA1*01* (non-*DQA1*01:02*) and susceptibility effects of *DQB1*03:01* were observed. These results illustrate the extraordinary conservation of HLA class II effects in narcolepsy across populations and show that *DRB1*15:01* has no effect on narcolepsy susceptibility in the absence of *DQB1*06:02*. The results are also in line with a previously proposed 'HLA-DQ allelic competition model' that involves competition between non-*DQA1*01:02*, non-*DQB1*06:02* 'competent' (able to dimerize together) DQ1 alleles and the major *DQα*01:02/ DQβ*06:02* narcolepsy heterodimer to reduce susceptibility.

## Introduction

Narcolepsy/hypocretin deficiency affects 1 in every 3000 individuals in most populations (1–6). It is caused by the loss of ~70,000 hypocretin (hcrt, also known as orexin)-producing neurons in the hypothalamus (7, 8). The loss of hypocretin neurons can be documented through the measure of hypocretin-1 in the cerebrospinal fluid (CSF) (9, 10). In almost all populations, narcolepsy is strongly associated with *DRB1*15:01-DQA1*01:02-DQB1*06:02* (11–17), suggesting an autoimmune mediation of the hypocretin cell loss. The human leucocyte antigen (HLA) association is even stronger when CSF hypocretin-1 has been evaluated (18). The disease also is notably rare in Israel (19) and Sardinia (20), where *DRB1*15:01-DQA1*01:02-DQB1*06:02* frequency is very low, suggesting the importance of this haplotype.

Genetic associations with other immune-modulating genes such as the T cell receptor alpha (17, 21) and P2RY11/DNMT1 polymorphisms (22) have been shown across multiple populations, including Chinese samples (23). Other associations have also been reported in Japan (14, 24). Renewed interest in this pathology has come from the recent observation that the autoimmune process is likely precipitated by winter upper airway infections such as H1N1 influenza (25, 26) and *Streptococcus pyogenes* (5, 27). Post pandemic 2009 H1N1 vaccination cases have also been reported in Northern Europe (25, 28), raising alarm.

Although almost all cases of narcolepsy carry *DRB1*15:01-DQA1*01:02-DQB1*06:02*, rare Caucasian cases carry unusual haplotypes with *DQB1*06:02* but not *DRB1*15:01*, while the reverse haplotypes are not found, suggesting the primacy of *DQB1*06:02* in this association (29). Furthermore, in African-Americans where linkage disequilibrium is not as high between *DQB1*06:02* and *DRB1*15:01*, association is stronger with *DQB1*06:02* (13, 30, 31), a phenomenon which was first noted using serological typing (32). In this population, *DRB1*15:01-DQA1*01:02-DQB1*06:02*, *DRB1*11:01-DQA1*01:02-DQB1*06:02* and *DRB1*15:03-DQA1*01:02-DQB1*06:02*, all common haplotypes in African-Americans, are all significantly increased in frequency in patients *versus* controls. Using *DQB1*06:02* as the disease marker, we estimate that less than 2% of cases with demonstrated hypocretin deficiency (low CSF hypocretin-1) do not have this allele (7, 10, 18, 33, 34), making narcolepsy one of the most tightly HLA-associated diseases known. These results show that *DQB1*06:02* alone can predispose to narcolepsy/hypocretin deficiency, but do not exclude the possibility that *DRB1*15:01* alone can also carry predisposition, as haplotypes with *DRB1*15:01* but without *DQB1*06:02* are not found in populations studied to date.

As in other HLA-associated diseases, the genetic association is not simply a dominant effect of *DQA1*01:02-DQB1*06:02*. First and foremost, association is highest with subjects homozygous for *DQB1*06:02* (about twofold to threefold higher risk than in heterozygotes) (35) a result likely explained by increased availability of the *DQβ*06:02* protein in immune cells of homozygous versus heterozygous patients (36). Furthermore, strong protective effects are noted with *DQA1*01:03-DQB1*06:01*, *DQA1*01:01-DQB1*05:01* and *DQA1*01:03-DQB1*06:03* (12, 13, 17). This result suggests that heterodimerization of *DQA1*01:02* and *DQB1*06:02* with other DQA1 and DQB1 alleles of the DQ1 groups may reduce the abundance of *DQα*01:02/DQβ*06:02*, possibly explaining decreased susceptibility (12). The only secondary association that cannot be explained by indirect effects on the availability of the *DQα*01:02/DQβ*06:02* heterodimer is a trans *DQB1*03:01* association that increases risk independent of DQA1 (12, 13, 37). Remarkably, all these complex effects are found consistently across multiple ethnic groups including African-Americans, Koreans, Japanese and Caucasians (12, 13).

Interestingly, recent studies have shown increased diversity of *DRB1*15:01-DQ6* haplotypes in the Chinese population, notably in South China and South East Asia where *DRB1*15:01* is associated not only with *DQB1*06:02* but also with *DQB1*06:01* (38–41), a protective allele for narcolepsy in Japan and Korea (12, 13, 42). Other haplotypes carrying *DRB1*15:01* with *DQB1*05:01*, *DQB1*05:02* and *DQB1*05:03* have also been reported but are less well documented (43). To test whether *DRB1*15:01* could predispose to narcolepsy independent of *DQB1*06:02*, we conducted a class II association study in Chinese narcolepsy *versus* control, with follow-up comparisons of DR2 haplotypes across cases and controls. Results confirm the primacy of the *DQA1*01:02-DQB1*06:02* association in narcolepsy and confirm additional HLA class II effects found in other populations.

## Subjects and methods

### Patients

Two hundred and two narcolepsy-cataplexy cases (Age, $13.55 \pm 9.33$; Female, 37.1%) were drawn from a sample of 906 cases with probable narcolepsy/hypocretin deficiency, with enrichment of the sample with subjects coming from South China. Probable hypocretin deficiency (called narcolepsy/hypocretin deficiency through the text) was defined as in prior studies by the presence of low CSF hypocretin-1 or by the presence of *DQB1*06:02* and definitive cataplexy (34). Using these criteria, we estimate that over 98% of these cases would have low CSF hyporetin-1 if all tested. All subjects were *DQB1*06:02* identified by codon 9–27 sequence-specific primer (SSP) polymerase chain reaction (PCR), as described in Hallmayer et al. (21), with follow up exon 2 sequencing. Most patients were Han (121/128, 95%) and came from North China (north of the Yangtze River) (179/202, 89%). The study was reviewed and approved by Stanford and University of Beijing Institutional Review Boards (ethics committees) and was conducted in accordance with the principles expressed in the Declaration of Helsinki.

### Controls

Controls were selected from several Chinese control panels and matched for North–south China sub-ethnicity. To establish a panel of randomly selected Chinese controls, we used 103 HLA class II oligotyped samples (DRB1, DQA1, DQB1) from two separately recruited North ($n = 91$) and South ($n = 102$) Chinese control populations, courtesy of Dr Jiabin An (UCLA VA hospital), matched for subethnicity (11% South Chinese). In additional comparisons, we selected *DQB1*06:02* positive or *DRB1*15:01* positive controls from Dr An's panels, adding a randomly selected sample of 41 *DQB1*06:02* positive control North Chinese subjects from Beijing University People's Hospital (screened using the codon 9–27 SSP PCR) and 50 *DRB1*15:01* positive North Chinese subjects randomly selected from the Chinese bone marrow registry, courtesy of Dr Xiangjun Liu. Using these panels, we constructed a sample of 110 samples of *DQB1*06:02* positive controls and 79 *DRB1*15:01* positive controls, both containing 89% subjects from Northern China and 11% subjects from South China. Of note, analyses were also performed separating South and North Chinese

samples, and although power was only sufficient to look at North Chinese samples only, results were virtually the same, with similar conclusions (Tables S2 and S3, *Supporting Information*).

### HLA typing

All patients and *DQB1*06:02* positive controls were DRB1, DQA1 and DQB1 exon 2 sequenced by Dr Xiangjun Liu in China. Some of the narcolepsy samples were also sequenced for exon 3, but as not all samples were sequenced, subtypes are only reported based on exon-2 sequencing. The random Chinese oligotyped controls from Dr Jiabin An (11% from South China) were exon-2 retyped by sequencing at Stanford. Unusual haplotypes were verified through repeat genotyping or sequencing.

### HLA class II haplotypes assignments

Two (DQA1-DQB1) and three (DRB1-DQA1-DQB1) locus haplotypes were assigned to all subjects on the basis of known HLA haplotype Chinese association. To do so: (i) It was assumed that the DRB1, DQA1 and DQB1 loci have no blanks. On the basis of this assumption, when a single HLA allele was found, the individual was considered homozygous for that allele; (ii) In the assignment of haplotypes, priority was given to combinations known to exist in homozygous B-cell lines or families, and to alleles having 100 per cent associations in the analysis of unrelated individuals; (iii) Rare associations were accepted when the other complementary haplotypes were well defined (i.e. fitted criteria).

### Statistical analysis

Chi-square or Fisher's exact tests were used for statistical comparison. Odds Ratios (OR) were reported to estimate the magnitude of the effects whenever the comparisons were significant ($P \leq 0.05$). Simple allele per allele comparisons of case *versus* control carrier (phenotype) frequencies were first performed between 202 patients and 103 geographically matched controls. Next, we compared DRB1-DQA1-DQB1 haplotype carrier frequencies for haplotypes carrying either *DQB1*06:02*, *DRB*15:01* (or both) in 202 cases *versus* (i) 103 unselected controls; (ii) 110 controls selected to be *DQB1*06:02* positive and (iii) 79 controls selected to be *DRB1*15:01* positive. Finally, we compared all *DQA1*01:02-DQB1*06:02/X* combinations in narcolepsy *versus* *DQA1*01:02-DQB1*06:02/X* controls (all possible genotype combinations). Next, based on the confirmation of prior findings in many other ethnic groups we compared the following genotypic combinations: *DQA1*01:02-DQB1*06:02* homozygotes, *DQA1*01:02-DQB1*06:02/DQA1*X-DQB1*03:01* (any DQA1), *DQA1*01:02-DQB1*06:02*/non*01:02 DQA1*01* and *DQA1*01:02-DQB1*06:02*/non-01:02, non-06:02 *DQB1*05/06* to all other combinations, identifying the most significant effect, then redoing the comparisons using the allele counts remaining after removing the most significant effects, a technique very similar to the relative predispositional effect used by Thomson et al. (44) in other HLA analyses.

This serial statistical approach is used to control for the fact all HLA allele effects are interdependent (13, 44). Indeed, for example, because *DQB1*06:02* is so strongly associated with narcolepsy, allele counts for all other alleles are artificially decreased, making them appear all artificially protective (Table 1). One way around this issue is to use a single parent transmission disequilibrium test approach as performed in our trio study during the 13th International Histocompatibility workshop (45). In this case, transmission of parental alleles is only analyzed in parents without *DQB1*06:02*. In case-control study, logistic regression or relative predispositional effect techniques are most appropriate (13, 44). In our analysis, the serial approach was used together with common sense allele pooling approach reflecting our allele competition model, i.e. pulling non-*DQA1*01:02* DQA1 and non-*DQB1*06:02 DQB1*05/06* alleles together.

## Results

### HLA class II allele phenotype frequencies in cases *versus* controls

Phenotype frequencies are shown in Table 1 for DRB1, DQA1 and DQB1 alleles. As can be seen, all 202 cases carried *DQA1*01:02* and *DQB1*06:02*, whereas 2 cases were *DRB1*15:01* negative. The *DQB1*03:01* allele was also significantly increased. Alleles that were significantly decreased included *DQA1*01:01/4/5* and *DQA1*01:03*. Of notable importance, *DQB1*06:01*, an allele strongly protective in Japanese and Korean populations was not strongly protective in Chinese. Further calculations with sequential removal of *DRB1*15:01*, non-01:02 *DQA1*01* and *DQB1*06:02* suggest that the predisposing effects of *DQB1*03:01* are independent of non-01:02 *DQA1*01* protective effects.

### Novel alleles detected by exon 3 sequencing

Three new alleles were found in narcoleptic subjects and named *DQA1*03:03:02* (*DQA1*03:03:01* like, except in exon 3: 108 CTC > CTT, no amino acid change) and *DQA1*01:08* (*DQA1*01:02* like, exon 3: 134 GGT > GCT p.G > A) and *DQA*01:09* (exon 3: 103 GGT > GAT p.G > D). Of note, *DQA1*01:08* was observed in a narcoleptic subject with *DRB1*15:01/DRB1*07:01*, *DQA1*01:08/DQA1*02:01*, *DQB1*02:02/DQB1*06:02*. In contrast, *DQA1*01:09* was observed in a narcolepsy subject also carrying the classical *DQA1*01:02*, *DQB1*06:02* predisposing haplotype *DRB1*15:01*/-, *DQA1*01:02/DQA1*01:09*,

**Table 1** Phenotype frequency in Chinese patients versus random controls

| DRB1 | Patients (202) | Controls (103) | OR (95% CI) | OR (95% CI) minus 15:01 |
|---|---|---|---|---|
| 01:01 | 0 | 5 | 0 (0–0.57) | |
| 01:02 | 0 | 1 | | |
| 03:01 | 3 | 6 | | |
| 04:01 | 4 | 4 | | |
| 04:02 | 0 | 1 | | |
| 04:03 | 3 | 2 | | |
| 04:04 | 1 | 1 | | |
| 04:05 | 8 | 10 | | |
| 04:06 | 3 | 9 | 0.16 (0.03–0.67) | |
| 04:07 | 1 | 0 | | |
| 04:10 | 0 | 1 | | |
| 07:01 | 25 | 24 | | |
| 08:02 | 1 | 2 | | |
| 08:03 | 3 | 7 | 0.21 (0.04–0.91) | |
| 09:01 | 23 | 26 | 0.31 (0.19–0.74) | |
| 10:01 | 1 | 3 | | |
| 11:01 | 28 | 12 | | 2.89 (1.35–6.28) |
| 11:04 | 7 | 1 | | |
| 12:01 | 13 | 5 | | |
| 12:02 | 17 | 12 | | |
| 13:01 | 2 | 3 | | |
| 13:02 | 7 | 1 | | |
| 14:01/14:54 | 1 | 8 | 0.06 (0.03–0.47) | |
| 14:02 | 0 | 1 | | |
| 14:03 | 1 | 2 | | |
| 14:05 | 2 | 1 | | |
| 15:01 | 200 | 23 | 348 (76–2195) | Not applicable |
| 15:02 | 4 | 17 | 0.10 (0.03–0.34) | |
| 16:02 | 1 | 9 | 0.05 (0.02–0.41) | |

| DQA1 | Patients (202) | Controls (103) | OR (95% CI) | OR (95% CI) minus 01:02 |
|---|---|---|---|---|
| 01:01/4/5 | 5 | 21 | 0.11 (0.04–0.33) | |
| 01:02/8/9 | 202[a] | 34 | inf (81–inf) | Not applicable |
| 01:03 | 4 | 19 | 0.10 (0.02–0.32) | |
| 02:01 | 23 | 24 | 0.48 (0.25–0.90) | |
| 03 | 46 | 50 | 0.41 (0.26–0.66) | |
| 04:01 | 0 | 2 | | |
| 05/non 05:02 | 52 | 24 | | 2.92 (1.63–5.27) |
| 06:01 | 17 | 12 | | |
| 01(non-01:02) | 9 | 40 | 0.10 (0.04–0.21) | 0.18 (0.08–0.41) |

| DQB1 | Patients (202) | Controls (103) | OR (95% CI) | OR (95% CI) minus 06:02 |
|---|---|---|---|---|
| 02 | 26 | 26 | 0.47 (0.26–0.87) | |
| 03:01 | 71 | 33 | | 2.84 (1.70–4.76) |
| 03:02 | 10 | 15 | 0.32 (0.13–0.78) | |
| 03:03 | 22 | 28 | 0.36 (0.19–0.67) | |
| 04:01 | 9 | 11 | | |
| 04:02 | 0 | 2 | | |
| 05:01 | 3 | 11 | 0.13 (0.03–0.5) | |
| 05:02 | 11 | 12 | | |
| 05:03 | 2 | 9 | 0.11 (0.02–0.54) | |
| 06:01 | 13 | 19 | 0.32 (0.15–0.71) | |
| 06:02 | 202 | 22 | inf (144-inf) | Not applicable |
| 06:03 | 1 | 2 | | |
| 06:04 | 4 | 0 | | |
| 06:09 | 2 | 1 | | |
| 05/06 (non 06:02) | 36 | 54 | 0.27 (0.16–0.44) | 0.56 (0.33–0.93)[b] |

OR, odds ratios; CI, confidence interval.
[a]One patient with *DQA1*01:08*, one patient with *DQA1*01:02/DQA1*01:09*.
[b]NS after correction of 06:02 and 03:01.

*DQB1*06:02*/-. Because not all subjects were sequenced for exon 3, *DQA1*01:08* and *DQA1*01:09* were considered *DQA1*01:02* for the purpose of further analysis. Similarly, *DQA1*03:03:02*, an allele observed in a subject with *DRB1*15:01/DRB1*14:54*, *DQA1*01:02/DQA1*03:03*, *DQB1*04:01/DQB1*06:02*, was considered *DQA1*03* for the analysis.

### Analysis of *DRB1* 15:01* and *DQB1* 06:02* carrying haplotypes

Carrier frequencies of various haplotypes containing either *DRB1*15:01* or *DQB1*06:02* are reported in various subgroups of controls matched for North South subethnicity in Table 2. As can be seen, all 202 narcolepsy patients carried at least one copy of *DQB1*01:02-DQB1*06:02*, almost always in the context of *DRB1*15:01-DQA1*01:02-DQB1*06:02* (two patients carried *DRB1*11:01-DQA1*01:02-DQB1*06:02*, and not *DRB1*15:01*, an haplotype also frequent in patients of African Ancestry). In controls, all 110 subjects selected as positive for *DQB1*06:02* were also *DRB1*15:01* positive (100%), while only 65 of 79 subjects selected as positive for *DRB1*15:01* were *DQB1*06:02* positive (82%). The results conclusively shows that in this population *DRB1*15:01* independent of *DQB1*06:02* does not predispose to narcolepsy. This was most striking when considering *DRB1*15:01-DQA1*01:02-DQB1*06:01*, a haplotype common in the South Chinese population (38) that was never found in isolation of *DQB1*06:02* in narcoleptic patients, but was frequent in controls.

### Effects of DQ alleles in trans of *DQB1* 06:02*

Further analysis focused on *DQB1*06:02* positive subjects only (narcolepsy versus controls, Table 3). These were done per individual haplotypes (Table S1, *Supporting Information*) and after grouping of the genotypes identified above and in prior publications as modifying narcolepsy risk (Table 3). As can be seen in Table 3, homozygosity for *DQA1*01:02-DQB1*06:02* and *DQA1*01:02-DQB1*06:02/DQA1*X-DQB1*03:01* had the highest predisposing ratios, doubling predisposition. We also

**Table 2** HLA DR-DQ carrier haplotype frequency in Chinese narcolepsy patients and controls

| Alleles/ haplotypes | Patients $n=202$ | | Unselected controls $n=103$ | | OR (95% CI) | Selected controls *DQB1*06:02+* $n=110$ | | Selected controls *DRB1*15:01+* $n=79$ | | OR (95% CI) versus 15:01 or 06:02 controls |
|---|---|---|---|---|---|---|---|---|---|---|
| | *n* | % | *n* | % | | *n* | % | *n* | % | |
| *DQA1*01:02* | 202[a] | 100 | 34 | 33 | inf (81-inf) | 110 | 100 | 78 | 99 | |
| *DQB1*06:02* | 202 | 100 | 22 | 21 | inf (144-inf) | 110 | — | 65 | 82 | |
| *DRB1*15:01* | 200 | 99 | 23 | 22 | 348 (76-2195) | 110 | 100 | 79 | — | inf (8.25-inf) |
| 15:01-01:02-06:02 | 200 | 99 | 21 | 19 | 390 (85.0-2479) | 110 | 100 | 65 | 82 | |
| 11:01-01:02-06:02 | 2 | 1.0 | | | | | | | | 21.5 (4.49–141.2) |
| 15:02-01:02-06:02 | | | 1 | 1.0 | | | | | | |
| 15:01-01:02-06:01 | 9 | 4.5 | 2 | 1.9 | | 3 | 2.6 | 9 | 11 | |
| 15:01-01:02-05:03 | | | | | | | | 4 | 5.1 | |
| 15:01-01:02-05:02 | | | | | | | | 2 | 2.5 | |
| 15:01-05-03:01 | 1 | 0.5 | | | | | | 1 | 1.3 | |

HLA, human leucocyte antigen; OR, odds ratios; CI, confidence interval.
[a]One with *DQA1*01:08*, one with *DQA1*01:02*, *DQA1*01:09*.

**Table 3** Predisposing and protecting allelic combinations in Chinese narcolepsy

| 01:02-06:02 | Other DQB1 | Other DQA1 | *DQB1*06:02* patients (202) | *DQB1*06:02* controls (110) | OR (95% CI) | OR (95% CI) |
|---|---|---|---|---|---|---|
| 01:02-06:02 | 06:02 | 01:02[a] | 27 | 7 | 2.27 (0.90–5.95)* | — |
| 01:02-06:02 | 06:02 | 01 but non 01:02 | — | — | — | — |
| 01:02-06:02 | 06:02 | Other | — | — | — | — |
| 01:02-06:02 | 05/06 (non 06:02) | 01:02 | 27 | 13 | 1.15 (0.54–2.48)[b,c] | — |
| 01:02-06:02 | 05/06 (non 06:02) | 01 but non 01:02 | 9 | 18 | 0.24 (0.10–0.59)*[,b] | — |
| 01:02-06:02 | 05/06 (non 06:02) | Other | 0 | 2 | inf (0.45-inf)[b] | — |
| 01:02-06:02 | Other | 01:02 | — | — | — | — |
| 01:02-06:02 | Other | 01 but non 01:02 | — | — | — | — |
| 01:02-06:02 | 03:01 | Other | 71 | 18 | 2.77 (1.50–5.18)*[,b] | 3.02 (1.54–5.96)*[,b] |
| 01:02[d]-06:02 | Other | Other | 68 | 52 | 0.57 (0.34–0.93)*[,b] | Ref |

OR, odds ratios; CI, confidence interval.
*$P < 0.05$.
[a]01:09 in one case.
[b]After removal of 06:02 homozygosity effects.
[c]After removal of both 06:02 homozygosity and DQ1 protective effects.
[d]01:08 in one case.

found that the *DQB1*03:01* effect was independent of DQA1 and DRB1 (Table 1 and Table S1, *Supporting Information*), as previously noted in other ethnic groups. In addition, we found 4× protective effects of *DQA1*01* alleles that are not *DQA1*01:02* when associated with *DQB1*05/06* alleles that are not *DQB1*06:02*. Interestingly, in cases where *DQA1*01:02* was present but associated with *DQB1*05/06* alleles that are not *DQB1*06:02*, predisposition was neutral (OR = 1.15, NS).

## Discussion

Our study is the first to examine HLA class II effects in Chinese narcolepsy. As in other ethnic groups, the findings show a remarkable conservation of effects previously reported in Japan, Korea, Caucasians and African-Americans, namely: (i) the requirement of at least one copy of *DQA1*01:02-DQB1*06:02* (estimated at more than 98% of cases); (ii) a predisposing effect of *DQA1*01:02-DQB1*06:02* homozygosity or the presence of *DQB1*03:01* in trans of *DQA1*01:02-DQB1*06:02*. (iii) Protective effects of *DQA1*01* (non-01:02) and *DQB1*05/06* (non-06:02) that could heterodimerize with *DQA1*01:02* or *DQB1*06:02*, reducing occurrence of the *DQα1*01:02/DQβ1*06:02* heterodimer.

The present study also showed several additional findings. First, in one case with typical narcolepsy and cataplexy, a rare new *DQA1*01:02* like allele, *DQA1*01:08* was found within a classic *DRB1*15:01-DQB1*06:02* susceptibility haplotype. The new polymorphism is in exon 3, in

a region not known to be functionally important, although it is bracketed by conserved amino acids associated with CD4 binding and DQ heterodimerization. Another rare exon 3 polymorphism on a *DQA1*01:02* (*DQA1*01:09*), was also found in one patient with narcolepsy. In this case, however, the patient also carried *DQA1*01:02*, thus it is uncertain if it could replace *DQA1*01:02* functionally in the context of the *DQα*01:02/DQβ*06:02* disease susceptibility dimer. These findings, made only in two cases, do not allow firm conclusions but suggest that additional sequencing of exon 3 in more Chinese cases could show additional diversity, helping to define key amino acids involved in susceptibility. As for *DQB1*02:01* and *DQB1*02:02* exon 3 differences and celiac disease, however, these polymorphisms are likely not functionally important, suggesting a primary role of the peptide-binding region of exons 1 and 2 for disease susceptibility. In addition to the two non-synonymous substitutions, we also report *DQA1*03:03:02* (*DQA1*03:03:01* like, except in exon 3: 108 CTC > CTT, no amino acid change) on a *DRB1*14:54-DQA1*03:03:01-DQB1*04:01* Chinese haplotype.

Second, our study excludes any significant effect of *DRB1*15:01*, something that had been difficult to do previously, as the allele is very tightly associated with *DQB1*06:02* in almost all ethnic groups, but less so in Chinese and South East Asians where is notably associated with *DQB1*06:01* (38–41, 43). Third, although the ranking of predisposition ORs for the various combinations (described in Table 3) was generally compatible with the allele competition model, variation was 10-fold between protective and predisposing combinations. This was more than expected if OR changes had been simply proportional to (i) variation in *DQα1*01:02/DQβ1*06:02* absolute amount (in theory 4× from homozygotes to protective heterozygotes) or (ii) % ratio of *DQα1*01:02/DQβ1*06:02* in relation to all possible dimers in a cell (also in theory varying up to 4× from homozygotes to protective heterozygotes). Additional analyses across ethnic groups are underway to test this model using bigger sample sizes. Interestingly, because there is a higher number of *DQA1*01:02* bearing haplotypes that do not carry *DQB1*06:02* in Chinese, it was possible to assess whether *DQA1*01:02* alone has some effect on predisposition in trans of *DQA1*01:02-DQB1*06:02* as predicted by the DQA1-DQB1 allelic competition model. Interestingly, predisposition for these combinations falls between *DQA1*01:02-DQB1*06:02*/other and that of *DQA1*01:02-DQB1*06:02* homozygotes also suggesting that both amount and ratio of *DQα1*01:02/DQβ1*06:02* may be important. On the basis of these findings, we propose a model involving competition between DQ1 compatible alleles with *DQα1*01:02/DQβ1*06:02* predisposing to narcolepsy, explaining the protective effects of the other DQ1 subtypes. This result is also compatible with a recent finding showing that the expression of the *DQB1*06:02* mRNA and *DQβ1*06:02* protein is 1.54-fold higher in *DQA1*01:02-DQB1*06:02* homozygotes versus heterozygotes (36), a finding that could explain why homozygotes are at increased risk for narcolepsy.

In contrast with this finding, the observation of a strong *DQB1*03:01* predisposing effect in the presence of *DQB1*06:02* remains difficult to explain. After removal of DQ1 protective alleles, *DQB1*03:01* predisposing effects were maintained and even slightly increased, indicating effects independent of our allele competition model. The *DQB1*03:01* predisposing effect was particularly strong in the Chinese population, potentially reflecting the fact most of our Chinese cases had onset as young children (70% of our sample) (26); whether *DQB1*03:01* positive patients have lower age of onset then *DQB1*03:01* negative subjects remains to be tested. Alternatively, the *DQB1*03:01* effect could also be due to linkage disequilibrium with another nearby polymorphism, but we believe this hypothesis to be unlikely considering the consistency of the association across very diverse ethnic groups. Furthermore, *DQβ1*03:01* is not known to pair with *DQα1*01:02 in vitro*, and should thus not interact with other DQβs, i.e. *DQβ1*06:02* (46, 47).

One possibility could be that *DQβ1*03:01*-TCR interactions stimulate T cells via preferential binding of some superantigen to *DQβ1*03:01*; an association with *Streptococcus* has been suggested to play a role as a cofactor in triggering onset and *Streptococcus* is known to be capable of generate superantigens (48). Another explanation could involve preferential competition of *DQβ1*03:01* and *DQβ1*06:02* with HLA-DM, DO (49, 50) or even peptides with similar binding motifs (47). These effects could somehow increase the loading of pathogenic peptides into the *DQα1*01:02/DQβ1*06:02* heterodimers for presentation. Finally, *DQβ1*03:01* could shape the T cell repertoire in ways that predispose to narcolepsy (51), or it could bind secondary antigens (other than those binding to *DQα1*01:02 DQβ1*06:02*) increasing disease predisposition through interaction with other infectious or immune factors. The relative simplicity and surprising conservation of HLA association effects in narcolepsy offers the opportunity to understand more complex HLA effects in other diseases, as illustrated by our proposed allelic competition model and resulting statistical approaches.

## Acknowledgments

This work was supported by research grants from the National Science Foundation of China (81070069), Sino-German Center for Research Promotion (GZ538), and Beijing Municipal Science and Technology Commission (D1011000050010029) to FH and by US National Institute of Health grants U01-NS073581 and P50-NS23724 to EM.

## Conflict of Interests

The authors have declared no conflicting interests.

## References


1. Silber MH, Krahn LE, Olson EJ, Pankratz VS. The epidemiology of narcolepsy in Olmsted County, Minnesota: a population-based study. *Sleep* 2002: **25**: 197–202.
2. Kaprio J, Hublin C, Partinen M, Heikkila K, Koskenvuo M. Narcolepsy-like symptoms among adult twins. *J Sleep Res* 1996: **5**: 55–60.
3. Heier MS, Evsiukova T, Wilson J, Abdelnoor M, Hublin C, Ervik S. Prevalence of narcolepsy with cataplexy in Norway. *Acta Neurol Scand* 2009: **120**: 276–80.
4. Shin YK, Yoon IY, Han EK et al. Prevalence of narcolepsy-cataplexy in Korean adolescents. *Acta Neurol Scand* 2008: **117**: 273–8.
5. Longstreth WT Jr, Ton TG, Koepsell T, Gersuk VH, Hendrickson A, Velde S. Prevalence of narcolepsy in King County, Washington, USA. *Sleep Med* 2009: **10**: 422–6.
6. Wing YK, Li RH, Lam CW, Ho CK, Fong SY, Leung T. The prevalence of narcolepsy among Chinese in Hong Kong. *Ann Neurol* 2002: **51**: 578–84.
7. Peyron C, Faraco J, Rogers W et al. A mutation in a case of early onset narcolepsy and a generalized absence of hypocretin peptides in human narcoleptic brains. *Nat Med* 2000: **6**: 991–7.
8. Thannickal TC, Moore RY, Nienhuis R et al. Reduced number of hypocretin neurons in human narcolepsy. *Neuron* 2000: **27**: 469–74.
9. Nishino S, Ripley B, Overeem S, Lammers GJ, Mignot E. Hypocretin (orexin) deficiency in human narcolepsy. *Lancet* 2000: **355**: 39–40.
10. Mignot E, Lammers GJ, Ripley B et al. The role of cerebrospinal fluid hypocretin measurement in the diagnosis of narcolepsy and other hypersomnias. *Arch Neurol* 2002: **59**: 1553–62.
11. Matsuki K, Juji T, Tokunaga K, Naohara T, Satake M, Honda Y. Human histocompatibility leukocyte antigen (HLA) haplotype frequencies estimated from the data on HLA class I, II, and III antigens in 111 Japanese narcoleptics. *J Clin Invest* 1985: **76**: 2078–83.
12. Hong SC, Lin L, Lo B et al. DQB1*0301 and DQB1*0601 modulate narcolepsy susceptibility in Koreans. *Hum Immunol* 2007: **68**: 59–68.
13. Mignot E, Lin L, Rogers W et al. Complex HLA-DR and -DQ interactions confer risk of narcolepsy-cataplexy in three ethnic groups. *Am J Hum Genet* 2001: **68**: 686–99.
14. Miyagawa T, Kawashima M, Nishida N et al. Variant between CPT1B and CHKB associated with susceptibility to narcolepsy. *Nat Genet* 2008: **40**: 1324–8.
15. Woo HI, Joo EY, Hong SB, Lee KW, Kang ES. Use of PCR with sequence-specific primers for high-resolution human leukocyte antigen typing of patients with narcolepsy. *Ann Lab Med.* 2012: **32**: 57–65.
16. Alaez C, Lin L, Flores AH et al. Association of narcolepsy-cataplexy with HLA-DRB1 and DQB1 in Mexican patients: a relationship between HLA and gender is suggested. *BMC Med Genet* 2008: **9**: 79.
17. Hor H, Kutalik Z, Dauvilliers Y et al. Genome-wide association study identifies new HLA class II haplotypes strongly protective against narcolepsy. *Nat Genet* 2010: **42**: 786–9.
18. Bourgin P, Zeitzer JM, Mignot E. CSF hypocretin-1 assessment in sleep and neurological disorders. *Lancet Neurol* 2008: **7**: 649–62.
19. Kwon OJ, Peled N, Miller K et al. HLA class II analysis in Jewish Israeli narcoleptic patients. *Hum Immunol* 1995: **44**: 199–202.
20. Molari A, Carcassi C. HLA and narcolepsy-cataplexy in the Sardinian population. *J Sleep Res* 2010: **19**: 624–5.
21. Hallmayer J, Faraco J, Lin L et al. Narcolepsy is strongly associated with the T-cell receptor alpha locus. *Nat Genet* 2009: **41**: 708–11.
22. Kornum BR, Kawashima M, Faraco J et al. Common variants in P2RY11 are associated with narcolepsy. *Nat Genet* 2011: **43**: 66–71.
23. Han F, Lin L, Li J et al. TCRA, P2RY11, and CPT1B/CHKB associations in Chinese narcolepsy. *Sleep Med* 2012: **13**: 269–72.
24. Shimada M, Miyagawa T, Kawashima M et al. An approach based on a genome-wide association study reveals candidate loci for narcolepsy. *Hum Genet* 2010: **128**: 433–41.
25. Dauvilliers Y, Montplaisir J, Cochen V et al. Post-H1N1 narcolepsy-cataplexy. *Sleep* 2010: **33**: 1428–30.
26. Han F, Lin L, Warby SC et al. Narcolepsy onset is seasonal and increased following the 2009 H1N1 pandemic in China. *Ann Neurol* 2011: **70**: 410–7.
27. Aran A, Lin L, Nevsimalova S et al. Elevated anti-streptococcal antibodies in patients with recent narcolepsy onset. *Sleep* 2009: **32**: 979–83.
28. Partinen M, Saarenpaa-Heikkila O, Ilveskoski I et al. Increased incidence and clinical picture of childhood narcolepsy following the 2009 H1N1 pandemic vaccination campaign in Finland. *PLoS One* 2012: **7**: e33723.
29. Mignot E, Kimura A, Lattermann A et al. Extensive HLA class II studies in 58 non-DRB1*15 (DR2) narcoleptic patients with cataplexy. *Tissue Antigens* 1997: **49**: 329–41.
30. Rogers AE, Meehan J, Guilleminault C, Grumet FC, Mignot E. HLA DR15 (DR2) and DQB1*0602 typing studies in 188 narcoleptic patients with cataplexy. *Neurology* 1997: **48**: 1550–6.
31. Matsuki K, Grumet FC, Lin X et al. DQ (rather than DR) gene marks susceptibility to narcolepsy. *Lancet* 1992: **339**: 1052.
32. Neely S, Rosenberg R, Spire JP, Antel J, Arnason BG. HLA antigens in narcolepsy. *Neurology* 1987: **37**: 1858–60.
33. Dalal MA, Schuld A, Pollmacher T. Undetectable CSF level of orexin A (hypocretin-1) in a HLA-DR2 negative patient with narcolepsy-cataplexy. *J Sleep Res* 2002: **11**: 273.
34. Han F, Lin L, Li J et al. Presentations of primary hypersomnia in chinese children. *Sleep* 2011: **34**: 627–32.
35. Pelin Z, Guilleminault C, Risch N, Grumet FC, Mignot E. HLA-DQB1*0602 homozygosity increases relative risk for narcolepsy but not disease severity in two ethnic groups. US Modafinil in Narcolepsy Multicenter Study Group. *Tissue Antigens* 1998: **51**: 96–100.
36. Weiner Lachmi K, Lin L, Kornum BR et al. DQB1*06:02 allele-specific expression varies by allelic dosage, not narcolepsy status. *Hum Immunol* 2012: **73**: 405–10.
37. Roh EY, Park MH, Park H et al. 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* 2006: **67**: 749–55.
38. Trachtenberg E, Vinson M, Hayes E et al. HLA class I (A, B, C) and class II (DRB1, DQA1, DQB1, DPB1) alleles and haplotypes in the Han from southern China. *Tissue Antigens* 2007: **70**: 455–63.
39. Wu Y, Liu B, Lin W et al. Human leukocyte antigen class II alleles and risk of cervical cancer in China. *Hum Immunol* 2007: **68**: 192–200.
40. Romphruk AV, Romphruk A, Kongmaroeng C, Klumkrathok K, Paupairoj C, Leelayuwat C. HLA class I and II alleles and haplotypes in ethnic Northeast Thais. *Tissue Antigens* 2010: **75**: 701–11.
41. Hei AL, Li W, Deng ZH et al. Analysis of high-resolution HLA-A, -B, -Cw, -DRB1, and -DQB1 alleles and haplotypes in 718 Chinese marrow donors based on donor-recipient confirmatory typings. *Int J Immunogenet* 2009: **36**: 275–82.
42. Hohjoh H, Terada N, Honda Y, Juji T, Tokunaga K. Negative association of the HLA-DRB1*1502-DQB1*0601 haplotype with human narcolepsy. *Immunogenetics* 2001: **52**: 299–301.
43. Trejaut J, Bhatia K, Greville WD et al. HLA-DR2 haplotypic diversity in populations of South-East Asia, northern China, Melanesia and Australian aborigines using PCR-RFLP for DRB1, DRB5, DQA1 and DQB1. A novel DRB1 allele: DRB1*16022. *EurJ Immunogenet* 1996: **23**: 437–49.
44. Thomson G, Barcellos LF, Valdes AM. Searching for additional disease loci in a genomic region. *Adv Genet* 2008: **60**: 253–92.
45. Mignot E, Lin L, Li H et al. HLA allele and microsatellite studies in narcolepsy. In: Hansen J, Dupont B, eds. *HLA 2004, Immunobiology of the Human MHC, Proceedings of the 13th International Histocompatibility Workshop and Congress*. *Seattle*: *IHWG Press*, *2006*, *817–23*.
46. Kwok WW, Kovats S, Thurtle P, Nepom GT. HLA-DQ allelic polymorphisms constrain patterns of class II heterodimer formation. *J Immunol* 1993: **150**: 2263–72.
47. Sidney J, Steen A, Moore C et al. Divergent motifs but overlapping binding repertoires of six HLA-DQ molecules frequently expressed in the worldwide human population. *J Immunol* 2010: **185**: 4189–98.
48. Llewelyn M, Sriskandan S, Peakman M et al. HLA class II polymorphisms determine responses to bacterial superantigens. *J Immunol* 2004: **172**: 1719–26.
49. van Lith M, McEwen-Smith RM, Benham AM. HLA-DP, HLA-DQ, and HLA-DR have different requirements for invariant chain and HLA-DM. *J Biol Chem* 2010: **285**: 40800–8.
50. Rinderknecht CH, Roh S, Pashine A et al. DM influences the abundance of major histocompatibility complex class II alleles with low affinity for class II-associated invariant chain peptides via multiple mechanisms. *Immunology* 2010: **131**: 18–32.
51. Ridgway WM, Fathman CG. MHC structure and autoimmune T cell repertoire development. *Curr Opin Immunol* 1999: **11**: 638–42.

## Supporting Information

The following supporting information is available for this article:

Table S1. DQA1-DQB1 genotype combinations on narcolepsy versus controls

Table S2. HLA DR-DQ haplotype carrier frequency in North Chinese Narcolepsy patients and controls

Table S3. Predisposing and protecting allelic combinations in Northern Chinese narcolepsy

Please note: Wiley-Blackwell is not responsible for the content or functionality of any supporting materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.