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DQB1*06:02 allele specific expression varies by allelic dosage, not narcolepsy status

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Abstract

The association of narcolepsy-cataplexy, a sleep disorder caused by the loss of hypocretin/orexin neurons in the hypothalamus, with DQA1*01:02-DQB1*06:02 is one of the tightest known single allele HLA associations. In this study, we explored genome wide expression in peripheral white blood cells of 50 narcolepsy versus 47 controls (half of whom were DQB1*06:02 positive) and found the largest differences between the groups to be in the signal from HLA probes. Further studies of HLA-DQ expression (mRNA and protein in a subset) in 125 controls and 147 narcolepsy cases did not reveal any difference, a result we explain by the lack of proper control of allelic diversity in Affymetrix HLA probes. Rather, a clear effect of DQB1*06:02 allelic dosage on DQB1*06:02 mRNA levels (1.65 fold) and protein (1.59 fold) could be demonstrated independent of the disease status. These results indicate that allelic dosage is transmitted into changes in heterodimer availability, a phenomenon that may explain increased risk for narcolepsy in DQB1*06:02 homozygotes versus heterozygotes.

Keywords

Narcolepsy; Human Leukocyte Antigen; DQB1*06:02; gene expression

Introduction

The human leukocyte antigen (HLA) plays a key role in the etiology of autoimmune diseases and orchestrates immune responses to infections, most notably through its ability to present antigens to the T-cell receptor [1-3]. The presence of HLA-DQB1*06:02 allele is associated with various autoimmune diseases, including narcolepsy [1, 4]; multiple sclerosis [5-7]; systemic lupus erythematosus [8-10]; sarcoidosis [11]; and primary sclerosing cholangitis [12]. Furthermore, HLA-DQB1*06:02 is associated with dominant protection against type I diabetes [13]. DQB1*06:02 is also associated [14] with protection against streptococcus septic shock (see discussion) and modulates disease presentation in leprosy and tuberculosis [15-16]. In most cases, however, because DQB1*06:02 is almost always present in the context of DRB1*15:01-DQA1*01:02-DQA1*06:02, it has been difficult to identify whether DR or DQ or both are involved. The presence of these specific alleles is a

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useful marker in risk assessment for some of these diseases, although in many cases the associations is not very strong.

One of the exceptions is narcolepsy-cataplexy where the DQA1*01:02-DQB1*06:02 association is extremely, strong, primary and independent of DRB1*15:01, as demonstrated through the study of African Americans [17-18]. Narcolepsy-cataplexy affects 1 in every 3,000 individuals and is primarily caused by the loss of around 70,000 hypocretin (hcrt, also known as orexin)-producing neurons in the hypothalamus [19-20]. Triggering factors likely involve winter infections such as H1N1 influenza [21-22] and *Streptococcus Pyogenes* infections [23-24]. The disease is not only associated with HLA-DQB1*06:02 [18] but also with the TCR α (encoding the T-cell receptor alpha chain) locus [25], suggesting an involvement of the MCH-peptide-TCR complex in development of the disease. Only a few cases of narcolepsy with demonstrated hypocretin deficiency worldwide have been shown to be DQB1*06:02 negative (and not sharing any specific allele), suggesting the single allele association may be as high as 99.5% when the disease is narrowly defined. Interestingly, all these cases, DQB1*06:02 positive, are also DQA1*01:02 positive, an effect secondary to the extremely tight linkage disequilibrium between these alleles across all ethnic groups. As a result, the association of narcolepsy is truly with the DQA1*01:02-DQB1*06:02 heterodimer, but best marked by DQB1*06:02.

As in most other HLA associated diseases, the genetic association is not solely mediated by a single dominant association with DQB1*06:02 [18]. Nonetheless, most of the other effects are also largely compatible with a primary effect of the DQA1*01:02-DQB1*06:02 heterodimer. First and foremost, association is highest with subjects homozygote for DQB1*06:02 (about 2-3 fold higher risk than in heterozygotes) [26]. Further, strong protective effects are noted with DQA1*01:03-DQB1*06:01, DQA1*01:03-DQB1*05:01 and DQA1*01:03-DQB1*06:03, suggesting that heterodimerization of DQA1*01:02 and DQB1*06:02 with other DQA1 and DQB1 alleles of the DQ1 group may reduce the abundance of DQA1*01:02-DQB1*06:02, possibly explaining decreased susceptibility [27]. The only secondary association that cannot be explained by indirect effects on the heterodimer availability is a DQB1*03:01 association that increases risk independent of DQA1 [18, 28]. Remarkably, all these complex effects are found consistently across multiple ethnic groups including African Americans, Koreans, Japanese and Caucasians.

In this study, we explored genome wide expression differences in peripheral blood mononuclear cells (PBMC) from narcolepsy patients versus healthy controls using the Affymetrix platform and found significant differences in probes interrogating HLA genes, a finding that was artifactual due to the nature of the HLA probes on the array and was secondary to the genomic association. Consequently, next we tested if HLA expression is truly different across these groups and also tested whether increased dosage of DQB1*06:02 at the genomic level is associated with increased availability of the protein at the surface of antigen presenting cells.

Subjects and Methods

Subjects

Patients and controls: A total of 272 samples composed of 147 narcolepsy- cataplexy cases (27.9 $\pm$ 2.8, 55.5% Female, 89.9% Caucasian, 100% DQB1*06:02 positive) and 125 controls (29.3 $\pm$ 3.2, 50.4% female, 93.6% Caucasian, 26.4% DQB1*06:02 positive) were used overall. Cases were all patients with definite narcolepsy/hypocretin deficiency, as described in Hallmayer, J. et al. [25]. All patients were DQB1*06:02 positive, while 26% of controls were DQB1*06:02 (enriched in the array experiments only, 53.33% DQB1*06:02). All patients and controls have been HLA DQB1 typed using HLA high resolution typing

method; HLA-DQB1 exon2 was amplified by DQB1 specific primers (SeCore Express DQB1 Locus, Life Technology, USA), then the exon 2 fragment was sequenced by using 3730 sequencer (Life Technology, USA). The sequencing data analysis was done by using the HLA SBT uType software (Life Technology, USA).

Genome wide expression in PBMC of narcolepsy patients versus healthy controls

50 patients (31.4 ± 3.6 years, 47% male, 100% Caucasian, 100% DQB1*06:02 positive) were selected and carefully matched with 45 controls (30.3 ± 2.3 years, 49% male, 100% Caucasian, 53.33% DQB1*06:02 positive). Total RNA (duplicate) was extracted from approximately 4×10^6 PBMCs using TRIzol (Invitrogen). RNA was amplified using the Ambion Amino Allyl MessageAmp II aRNA kit. We used Affymetrix GENECHIP® as described at www.Affymetrix.com. Data Analysis was performed using Affymetrix software and Expander software: <http://acgt.cs.tau.ac.il/expander/>. To assess genome wide significance, we use a false discovery rate (FDR method) that give a threshold p value based on permutations using the same data set. The corrected p-values is then compared to a threshold of significance at p-value at $p=0.05$.

Measurements of DQB1 expression using Real-Time PCR—147 patients (27 ± 2.8 , 55.5% Female, 89.9% Caucasian, 100% DQB1*06:02 positive) and 125 controls (29.3 ± 3.2), 50.4% female, 93.6% Caucasian, 26.4% DQB1*06:02 positive) were used. Total RNA was extracted from 4×10^6 PBMCs from each subject using Qiagen RNease mini kit (Qiagen #74104). cDNA was synthesized from 200 ng total RNA using High Capacity cDNA Reverse Transcription Kit (#4374966, Applied Biosystems). Gene expression was determined by RT-PCR (ABI 7000, Applied Biosystems) and TaqMan™ gene expression assays (Applied Biosystems). Probe and primers were custom designed to measure total DQB1 and allele specific DQB1*06:02 expression: Primers/probes amplifying all alleles included DQB1-F: CGCTTCGACAGCGACGTGG, DQB1-R: CAGCAGGTTGTGGTGGTTG, DQB1-probe: GAGTGGAGCCCACAGTGACC; DQB1*06:02 allele specific primers/probes: DQB1*06:02 specific F: CTGGCGATGCTGAGCTCCCT (-2,+5), DQB1*06:02 specific R: CCCCTGCGGCGTCACCGCG (+43,+54), DQB1*06:02 specific –probe: CCCGAGGATTTCGTGTTCCAG (+4,+10). The actin gene ACTB (#4333762F) was used as endogenous control gene. Relative quantities of target mRNAs were calculated using the comparative threshold method (Ct-method), with the ACTB expression as the endogenous controls. Standard deviations (SD) on fold changes were calculated as $SD = 2^{\Delta\Delta Ct} \cdot \ln 2 \cdot SD(\Delta Ct)$ with $SD(\Delta Ct)$ being the SD of ΔCt of all samples in the group. Efficiency for the DQB1*06:02 and DQB1 assays were 99.2% ($r^2=0.99$, $n=7$, CTs between ~22-35) and 85.5% ($r^2=1.00$, $n=7$, CTs between ~22 and 37) respectively.

FACS analysis of DQB1*06:02 allele-specific expression

40 patients (All DQB1*06:02 positive with 15% homozygous) and 40 controls (All DQB1*06:02 positive with 25% homozygous) were used. DQB1*06:02 allele-specific expression in homozygous vs. heterozygous was quantified in duplicate using Fluorescence Activated Cell Sorting (FACS, LSRII, BD Biosciences) and allele specific antibody DQ6 from abcom (ab42130) which we labeled using the antibody labeling kit -Lightning-Link™ Fluorescein from Innova Biosciences (#707-0030). To verify that DQ6 expression in these samples was measured in B-cells and not T-cells, we used the antibody panel: αCD3-Pacific Blue (#558117), and αCD19-APC (#555415) both from BD Biosciences, and Aqua Amine Live/Dead Cell Stain (L34957, Invitrogen). To avoid cross reactivity with other DQB1*06 subtypes, cells carrying DQ6 subtypes other than DQB1*06:02 were excluded. For data analysis we used the Fluorescence intensity (MFI) median of the B-cell (αCD19-APC). Data Analysis was performed using the software FlowJo 7.6.

Statistical analysis

For the statistical analysis of expression data, we used general linear regression models in Systat 12 Version 12.00.08, with control of relevant covariates (disease status, HLA-DQB1*06:02 copy number, age, sex and BMI), if significant.

Results

Microarray Affymetrix results

As shown in supplementary Table 1, no genes reached significance between controls (n=45) and patients (n=50) after multiple corrections using the false discovery method (FDR), thus only the top value are listed. Interestingly, however three of the top seven probes with differential expression were HLA genes DQ beta 1'' (HLA-DQB1) and "major histocompatibility complex, class II, DR beta 5" (HLA-DRB5) (marked as * in the Supplementary Table 1, first column). Subsequent analysis comparing HLA-DQB1*06:02 positive patients with HLA-DQB1*06:02 positive and negative controls revealed no differences in HLA probes when subjects were matched for DQB1*06:02 (second column). FDR corrected significant differences in HLA DR and DQ probes were also observed for HLA probes when HLA positive narcolepsy cases versus HLA negative controls were compared (marked as * in the Supplementary Table 1, third column). These results suggested strong allelic difference in HLA expression per DQB1*06:02 status. RT-PCR validation for the non-HLA genes did not confirm the microarray results.

DQB1 and DQB1*06:02 allele specific expression in narcolepsy vs. controls

Real-Time-PCR quantification of total HLA-DQB1 mRNA concentration in peripheral blood mononuclear cells (PBMCs) of 147 narcoleptic patients versus 125 controls showed no significant difference ($P=0.31$) in total expression of DQB1 between patients and controls (Fig. 1 A, B), or by DQB1*06:02 status (data not shown). To assess whether our DQB1*06:02 allele specific measures were reliable, we tested samples with various DQB1 allelic status, including other DQB1*06 subtypes, notably DQB1*06:01, DQB1*06:03, DQB1*06:04 and DQB1*06:09. Amplification was found to be specific for DQB1*06:02 except for DQB1*06:03. Individuals positive for DQB1*06:03 controls (n=19) and narcolepsy (n=2) were deleted from further analysis. As shown in Figure 1 C and D, expression was detectable in all patients with DQB1*06:02 while in the controls, a bimodal distribution was observed, with most individuals (all DQB1*06:02 negative) showing extremely low amplification. DQB1*06:02 was significantly lower in controls versus narcolepsy ($P<10^{-34}$).

DQB1*06:02 allele specific expression varies by allelic dosage, not disease status

Further analysis focused on DQB1*06:02 positive subjects only. These included 126 heterozygous (101 patient and 25 controls), and 26 homozygous (23 patient and 3 controls) for the DQB1*06:02 allele. Multivariate analysis including disease status and DQB1*06:02 dose (homozygous versus heterozygous) revealed a 1.65-fold higher expression in DQB1*06:02 homozygotes versus heterozygotes (adjusted means; $F=7.029$, $P<0.01$, Fig. 2B) but no effect of disease status (adjusted means; $F=1.447$ $P=0.231$) (Fig. 2C). We also examined whether disease duration correlated with higher DQB1*06:02 allele specific expression, but found no such effect after correcting for allelic dosage ($p=0.42$).

DQB1*06:02 protein expression in B cells varies by allelic dosage, not disease status

To confirm our finding at the protein level, a DQB1*06 allele-specific antibody (DQ6 from abcom, ab42130) was used to quantify surface protein expression using Fluorescence Activated Cell Sorting (FACS). The specificity of the DQ6 antibody was verified using cells

from subjects homozygous for DQB1*05, *03, and *02 genotypes. Sixteen DQB1*06:02 homozygous (6 narcoleptic and 10 controls) and 64 DQB1*06:02 heterozygous (34 narcoleptic and 30 controls not carrying another DQB1*06 subtype in trans) were used for this experiment (Fig. 3 A). The results showed significant difference ($F=147.183$, $p<0.0001$) between protein expression of DQB1*06:02 in homozygotes vs. heterozygous with a 1.59-fold higher expression in homozygotes (Fig. 3B). Protein expression did not differ ($F=0.004$, $P=0.949$) between narcoleptics and controls confirming the mRNA expression data (Fig. 3 C). Finally, we also explored whether DQB1*06:02 expression varies in DQB1*03:01/DQB1*06:02 ($n=9$) versus DQB1*02/DQB1*06:02 ($n=11$) and found no difference ($P=0.86$) (Fig. 3 D).

Discussion

Very few studies have examined allele specific expression of HLA-DQB1 in control and disease. Our interest stemmed from the very clear and definite primary association of narcolepsy with DQB1*06:02. Our initial finding, that HLA expression was higher in DQB1*06:02 using the Affymetrix platform was intriguing, but found likely to be the result of an artifact. Indeed, after further inquiry, it was discovered that most of the HLA sequences used in the design of Affymetrix probes came from a DRB1*15:01, DRB5*01:01, DQA1*01:02, DQB1*06:02 haplotype thus all subjects positive for these alleles show higher “apparent expression” most likely as the result of better probe matches. This may be relevant to many studies that rely on Affymetrix. For example, a recent study found a significant association of late onset Parkinson's disease with rs3129882G, a SNP strongly associated with DRB1*15:01, DRB5*01:01, DQA1*01:02, DQB1*06:02 though Genome Wide Association [29] and described a concomitant increase in HLA-DRA, HLA-DQA2, and HLA-DRB5 expression with this SNP ($P=10^{-7}$ to 10^{-4}) using a eQTL study that could merely represent preferential hybridization, as we demonstrated in this study.

In response to this study, we designed allele specific DQB1*06:02 primers and probe for RT-PCR evaluation. The assay distinguished DQB1*06:02 from all other common DQB1 subtypes except DQB1*06:03. Using this assay, we could not find any significant difference in DQB1 total and DQB1*06:02 expression between narcolepsy and controls once DQB1*06:02 status was controlled for. No difference was also found in total DQB1 expression. One issue could be that samples have been collected mostly in subjects with a long-standing disease, although we did not find significant effects of disease duration on DQB1 expression in narcolepsy. As narcolepsy is associated with a loss of hypocretin neurons that do not regenerate, it is possible the active disease process is already finished by the time patients are diagnosed and samples collected. In addition, the study of DQB1*06:02 in PBMCs or B-cells only may not reflect expression in cells that are more relevant to the disease process, for example brain microglia or antigen presenting dendritic cells.

A clear 1.65 fold higher mRNA expression was detected in DQB1*06:02 homozygous versus heterozygous, a difference that was also evident at the level of proteins on the surface of B cells (1.59 fold), one of the main HLA class II positive mononuclear cells present in PBMCs. One may however wonder why expression in homozygous was not twice that of heterozygous since promoter sequences at both DQA1 and DQB1 loci are the same in all chromatids. As efficiency for the DQB1*06:02 assay was 99.2%, and the effect observed both at the level of mRNA and proteins, we believe the finding has a biological basis. One possibility may be that in some cells there is partial promoter methylation and silencing of one of the DQB1*06:02 allele explaining that expression is not exactly twice overall. Methylation for HLA-DQ has been reported and it is difficult to exclude the possibility of a low degree of hemi methylation of DQ promoters in some PBMCs and B cells. Certainly for other loci, partial methylation and preferential expression of one allele versus the other

commonly occurs in some tissue even in the presence of identical promoter sequence. In the DQ locus, Nepon et al. [30] also found differential expression of DQB1*02 and DQB1*04 in an heterozygous cell line that was not obviously explained by differential promoter methylation or direct DNA-protein interactions in the X box region, suggesting the involvement of other regulatory proteins that could have low abundance and regulate differentially both promoters. In other cases where DQ allele specific expression has been looked at, focus has been directed at differential allelic expression in correlation with promoter differences not differential expression of the same allele, but it is clear from these studies as well that interindividual variation is not likely solely explain by DQ promoter variation [31, 32].

Whether or not the 1.5-1.6 fold increase for homozygous versus heterozygous expression is underestimated for technical or biological reasons, this finding is in accordance with the hypothesis that the 2-3 fold increased risk for narcolepsy in DQB1*06:02 homozygous versus other heterozygous is secondary to the number of antigen presenting molecules. The difference between 1.6 (PBMCs and B cells) and 2-3 folds may be secondary to the high prevalence of DQA1*01 (non DQA1*01:02) protective alleles not taken into consideration in this calculation. Whether or not this is also reflected in other antigen presenting cells such as dendritic cells is unknown, but likely.

Only a few studies have tried to compare expression of different DQB1 alleles in various cell populations. The DQB1 promoter is polymorphic and has been postulated to regulate allelic specific expression of various alleles differentially in cellular subtypes, although no definitive allelic hierarchy has been published [30-31]. Interestingly, Ferstl, et al. were studying cytokine driven maturation of monocytes into dendritic cells, a process known to involve increased HLA class II expression, and they found increased DQB1*03:01 expression in comparison to other alleles such as DQB1*06:02 and DQB1*05:01 in DQB1*03:01/DQB1*06:02 and DQB1*03:01/DQB1*05:01 heterozygotes [32], extending on a finding in primary human skin fibroblasts also showing increased expression of DQB1*03:01 versus other alleles [31],

Considering that DQB1*03:01 is one of the only other clear heterozygote effects in trans with DQB1*06:02 that increases narcolepsy risk, how could this allele be relevant to narcolepsy pathophysiology? One possibility could have been that DQB1*03:01 increases availability of DQB1*06:02, as homozygosity does. To explore this hypothesis, we compared protein expression of DQB1*06:02 in DQB1*02/DQB1*06:02 (a neutral risk combination for narcolepsy) versus DQB1*03:01/06:02 but found identical levels of DQB1*06:02 protein. Another possibility could be competition of peptide binding between DQA1*01:02-DQB1*06:02 and dimers with DQB1*03:01 as shown in Type I diabetes with DRB1*04:01 and DQ8 [33]. In this case, however, the increased expression of DQB1*03:01 would reduce availability of peptide to bind DQA1*01:02-DQB1*06:02 and thus be protective. Third, antigens presented by DQB1*03:01 but not DQB1*06:02 could modulate risk surprisingly however, DQB1*03:01/DQB1*06:02 heterozygosity increases risk independent of DQA1 [27]. A similar finding was made with Penphigoid Bullous association with DQB1*0301 independently of DQA1, a disease associated with anti laminin-332 antibodies. Effects independent of peptide presentation by DQB1*03:01 could be involved in some diseases, for example binding to superantigens, molecules known to directly bridge HLA to TCR molecules. With regard to narcolepsy, recent studies have linked narcolepsy occurrence with Streptococcus-A infections. Interestingly, DQB1*06:02 protects against Streptococcus A superantigen mediated septic shock while DQB1*03:01 may increase risk, [34]. Selected Streptococcus A superantigens can bind either DQA1 or DQB1 [35], and have shown allele specific interactions at the DQA1 level [36], suggesting a

similar interaction could occur preferentially with DQB1*03:01. DQB1*03:01 differs from all other common DQB1 allele at position 46 (G>E) [14].

In conclusion, we suggest that susceptibility to narcolepsy is primarily mediated by the availability of the DQA1*01:02-DQB1*06:02 to bind a specific unknown antigen. This would explain increased risk in DQA1*01:02-DQB1*06:02 homozygotes, protective effect of other DQ1 antigens (i.e. DQA1*01 other than DQA1*01:02 or DQB1*05:06 other than DQB1*06:02) limiting availability of DQA1*01:02-DQB1*06:02 through cross dimerization. Increased DQB1*06:02 expression in homozygote is consistent with this hypothesis. One remaining effect, that of DQB1*03:01, remains unexplained but could involve other mechanisms.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Doherty DG, Penzotti JE, Koelle DM, Kwok WW, Lybrand TP, Masewicz S, et al. Structural basis of specificity and degeneracy of T cell recognition: pluriallelic restriction of T cell responses to a peptide antigen involves both specific and promiscuous interactions between the T cell receptor, peptide, and HLA-DR. *J Immunol.* 1998; 161:3527–35. [PubMed: 9759873]
2. Gebe JA, Novak EJ, Kwok WW, Farr AG, Nepom GT, Buckner JH. T cell selection and differential activation on structurally related HLA-DR4 ligands. *J Immunol.* 2001; 167:3250–6. [PubMed: 11544312]
3. Nepom GT, Lippolis JD, White FM, Masewicz S, Marto JA, Herman A, et al. Identification and modulation of a naturally processed T cell epitope from the diabetes-associated autoantigen human glutamic acid decarboxylase 65 (hGAD65). *Proc Natl Acad Sci U S A.* 2001; 98:1763–8. [PubMed: 11172025]
4. Chabas D, Taheri S, Renier C, Mignot E. The genetics of narcolepsy. *Annu Rev Genomics Hum Genet.* 2003; 4:459–83. [PubMed: 14527309]
5. Hillert J, Olerup O. HLA and MS. *Neurology.* 1993; 43:2426–7. [PubMed: 8232981]
6. Hong SC, Hayduk R, Lim J, Mignot E. Clinical and polysomnographic features in DQB1*0602 positive and negative narcolepsy patients: results from the modafinil clinical trial. *Sleep Med.* 2000; 1:33–39. [PubMed: 10733618]
7. Oksenberg JR, Barcellos LF, Cree BA, Baranzini SE, Bugawan TL, Khan O, et al. Mapping multiple sclerosis susceptibility to the HLA-DR locus in African Americans. *Am J Hum Genet.* 2004; 74:160–7. [PubMed: 14669136]
8. Arnett FC, Reveille JD. Genetics of systemic lupus erythematosus. *Rheum Dis Clin North Am.* 1992; 18:865–92. [PubMed: 1455048]
9. Arnett FC, Reveille JD, Moutsopoulos HM, Georgescu L, Elkon KB. Ribosomal P autoantibodies in systemic lupus erythematosus Frequencies in different ethnic groups and clinical and immunogenetic associations. *Arthritis Rheum.* 1996; 39:1833–9. [PubMed: 8912505]
10. Cortes LM, Baltazar LM, Lopez-Cardona MG, Olivares N, Ramos C, Salazar M, et al. HLA class II haplotypes in Mexican systemic lupus erythematosus patients. *Hum Immunol.* 2004; 65:1469–76. [PubMed: 15603875]
11. Voorter CE, Drent M, van den Berg-Loonen EM. Severe pulmonary sarcoidosis is strongly associated with the haplotype HLA-DQB1*0602-DRB1*150101. *Hum Immunol.* 2005; 66:826–35. [PubMed: 16112030]

12. Spurkland A, Saarinen S, Boberg KM, Mitchell S, Broome U, Caballeria L, et al. HLA class II haplotypes in primary sclerosing cholangitis patients from five European populations. *Tissue Antigens*. 1999; 53:459–69. [PubMed: 10372541]
13. Pugliese A, Gianani R, Moromisato R, Awdeh ZL, Alper CA, Erlich HA, et al. HLA-DQB1*0602 is associated with dominant protection from diabetes even among islet cell antibody-positive first-degree relatives of patients with IDDM. *Diabetes*. 1995; 44:608–13. [PubMed: 7789622]
14. Bondinas GP, Moustakas AK, Papadopoulos GK. The spectrum of HLA-DQ and HLA-DR alleles, 2006: a listing correlating sequence and structure with function. *Immunogenetics*. 2007; 59:539–53. [PubMed: 17497145]
15. Singh M, Kothur K. Pulmonary sarcoidosis masquerading as tuberculosis. *Indian Pediatr*. 2007; 44:615–7. [PubMed: 17827640]
16. Singh UB, Arora J, Suresh N, Pant H, Rana T, Sola C, et al. Genetic biodiversity of *Mycobacterium tuberculosis* isolates from patients with pulmonary tuberculosis in India. *Infect Genet Evol*. 2007; 7:441–8. [PubMed: 17317334]
17. Matsuki K, Grumet FC, Lin X, Gelb M, Guilleminault C, Dement WC, et al. DQ (rather than DR) gene marks susceptibility to narcolepsy. *Lancet*. 1992; 339:1052. [PubMed: 1349071]
18. Mignot E, Lin L, Rogers W, Honda Y, Qiu X, Lin X, 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. [PubMed: 11179016]
19. Peyron C, Faraco J, Rogers W, Ripley B, Overeem S, Charnay Y, 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. [PubMed: 10973318]
20. Thannickal TC, Moore RY, Nienhuis R, Ramanathan L, Gulyani S, Aldrich M, et al. Reduced number of hypocretin neurons in human narcolepsy. *Neuron*. 2000; 27:469–74. [PubMed: 11055430]
21. Dauvilliers Y, Montplaisir J, Cochen V, Desautels A, Einen M, Lin L, et al. Post- H1N1 narcolepsy-cataplexy. *Sleep*. 33:1428–30. [PubMed: 21102981]
22. Mignot E, Dauvilliers Y, Montplaisir J. Comment on the Letter to the Editor By Dr. Marcus on the Association between Narcolepsy and H1N1 Exposure. *Sleep*. 34:689–90.
23. Koepsell TD, Longstreth WT, Ton TG. Medical exposures in youth and the frequency of narcolepsy with cataplexy: a population-based case-control study in genetically predisposed people. *J Sleep Res*. 19:80–6. [PubMed: 19732319]
24. Aran A, Lin L, Nevsimanova S, Plazzi G, Hong SC, Weiner K, et al. Elevated anti-streptococcal antibodies in patients with recent narcolepsy onset. *Sleep*. 2009; 32:979–83. [PubMed: 19725248]
25. Hallmayer J, Faraco J, Lin L, Hesselson S, Winkelmann J, Kawashima M, et al. Narcolepsy is strongly associated with the T-cell receptor alpha locus. *Nat Genet*. 2009; 41:708–11. [PubMed: 19412176]
26. 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. [PubMed: 9459509]
27. Hong SC, Lin L, Lo B, Jeong JH, Shin YK, Kim S, et al. DQB1*0301 and DQB1*0601 modulate narcolepsy susceptibility in Koreans. *Hum Immunol*. 2007; 68:59–68. [PubMed: 17207713]
28. Hor H, Kutalik Z, Dauvilliers Y, Valsesia A, Lammers GJ, Donjacour C, et al. Genome-wide association study identifies new HLA class II haplotypes strongly protective against narcolepsy. *Nat Genet*. 42:786–9. [PubMed: 20711174]
29. Hamza TH, Zabetian CP, Tenesa A, Laederach A, Montimurro J, Yearout D, et al. Common genetic variation in the HLA region is associated with late-onset sporadic Parkinson's disease. *Nat Genet*. 42:781–5. [PubMed: 20711177]
30. Nepom GT, Chung J, West KA. Differential expression of HLA-DQB1 alleles in a heterozygous cell line. *Immunogenetics*. 1995; 42:143–8. [PubMed: 7607705]
31. Beaty JS, West KA, Nepom GT. Functional effects of a natural polymorphism in the transcriptional regulatory sequence of HLA-DQB1. *Mol Cell Biol*. 1995; 15:4771–82. [PubMed: 7651394]

32. Ferstl B, Zacher T, Lauer B, Blagitko-Dorfs N, Carl A, Wassmuth R. Allele- specific quantification of HLA-DQB1 gene expression by real-time reverse transcriptase-polymerase chain reaction. *Genes Immun.* 2004; 5:405–16. [PubMed: 15175648]
33. Ge X, Piganelli JD, Tse HM, Bertera S, Mathews CE, Trucco M, et al. Modulatory role of DR4- to DQ8-restricted CD4 T-cell responses and type 1 diabetes susceptibility. *Diabetes.* 2006; 55:3455–62. [PubMed: 17130492]
34. Smeesters PR, Dreze PA, Perez-Morga D, Biarent D, Van Melder L, Vergison A. Group A *Streptococcus* virulence and host factors in two toddlers with rheumatic fever following toxic shock syndrome. *Int J Infect Dis.* 14:e403–9. [PubMed: 19828348]
35. Llewelyn M. Human leukocyte antigen class II haplotypes that protect against or predispose to streptococcal toxic shock. *Clin Infect Dis.* 2005; 41(Suppl 7):S445–8. [PubMed: 16237645]
36. Fraser JD, Proft T. The bacterial superantigen and superantigen-like proteins. *Immunol Rev.* 2008; 225:226–43. [PubMed: 18837785]

Figure 1.

Total DQB1 (A, B) and DQB1*06:02 allele-specific (C, D) expression in peripheral blood mononuclear cells (PBMCs) of narcolepsy versus controls. (A) Total DQB1 expression (delta Ct, with median of each group) in 147 cases versus 125 controls (PBMCs). (B) Total DQB1 expression was 1.18 fold higher in patients versus controls (mean $\pm$ s.e.m., $P=0.31$, panel B). (C) DQB1*06:02 allele specific expression (delta Ct in A, with median of each group) in 147 cases versus 125 controls. Note that in controls, there is bimodal distribution of amplifiers (subjects with DQB1*06:02 positive and thus amplifying with the primers) and non- amplifiers ((DQB1*06:02 negative). (D) DQB1*06:02 expression is 295 fold higher in patients versus controls (mean $\pm$ s.e.m, $P<10^{-34}$).

Ct= Fractional cycle number at which fluorescence passes the threshold. Delta Ct = Ct sample transcript - Ct of control transcript.

Figure 2.

Allele specific expression of DQB1*06:02 in controls and narcoleptics carrying one (DQB1*06:02 heterozygotes) or two alleles of DQB1*06:02 (DQB1*06:02 homozygotes). Studies were performed in peripheral blood mononuclear cells (PBMCs). Controls negative for DQB1*06:02 are not included and showed no expression (see Figure 1). (A) DQB1*06:02 expression in DQB1*06:02 heterozygous and homozygote subjects in narcolepsy (left) versus controls (right) (delta Ct with median of each group). (126 heterozygous; 101 patient and 25 controls, and 26 homozygous for the DQB1*06:02 allele; 23 patient and 3 controls). Note higher expression in homozygotes independent of disease status (B) Mean DQB1*06:02 expression (adjusted for disease status) is 1.65 higher in subjects with one versus two DQB1*06:02 allele ($F=7.029$, $P=0.009$). (C) Mean DQB1*06:02 expression in narcolepsy versus controls after control of allelic dosage (0.9 fold, $F=1.447$ $P=0.231$). To adjust for disease status or allelic dosage, a general linear regression analysis was performed using these covariates and residuals used to derive adjusted means (see methods). Ct= Fractional cycle # at which the fluorescence passes the threshold. Delta Ct = Ct sample transcript - Ct of control transcript.

Figure 3.

Allele specific protein amounts of DQB1*06:02 in controls and narcoleptics carrying one (DQB1*06:02 heterozygotes) or two alleles of DQB1*06:02 (DQB1*06:02 homozygotes). Studies were performed in peripheral blood mononuclear cells (PBMCs). Controls negative for DQB1*06:02 are not included and showed no expression (see text). (A) DQB1*06:02 protein amounts in DQB1*06:02 heterozygous and homozygote subjects in narcolepsy (left) versus controls (right) (F.C of Fluorescence intensity, MFI, Median from B cells). Note higher expression in homozygotes independent of disease status. (B) DQB1*06:02 protein amounts is 1.59 higher in subjects with two versus one DQB1*06:02 allele (means adjusted for disease status, $F=147.183$, $P<0.0001$). (C) DQB1*06:02 protein amounts in narcolepsy versus controls after control of allelic dosage (1 fold, adjusted means, $F= 0.004$, $P=0.949$). (D) DQB1*06:02 protein amounts in DQB1*03:01/DQB1*06:02 ($n=9$) versus DQB1*02/DQB1*06:02 ($n=11$) subjects ($P=0.86$).