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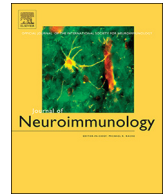
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HLA associations in narcolepsy type 1 persist after the 2009 H1N1 pandemic

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ABSTRACT

We aimed to compare HLA-DQB1-associations in narcolepsy type 1 (NT1) patients with disease onset before and after the 2009 H1N1 pandemic in a large Dutch cohort. 525 NT1 patients and 1272 HLA-DQB1*06:02-positive healthy controls were included. Because of the discussion that has arisen on the existence of sporadic and post-H1N1 NT1, HLA-DQB1-associations in pre- and post-H1N1 NT1 patients were compared. The associations between HLA-DQB1 alleles and NT1 were not significantly different between pre- and post-H1N1 NT1 patients. Both HLA-DQB1-associations with pre- and -post H1N1 NT1 reported in recent smaller studies were replicated. Our findings combine the results of studies in pre- and post-H1N1 NT1 and argue against considering post-H1N1 NT1 as a different entity.

1. Introduction

Narcolepsy type 1 (NT1) is a rare disorder of the regulation of sleep and wakefulness with an incidence of 1 per 100,000 person years and prevalence ranging between 20 and 50 per 100,000 individuals (Wijnans et al., 2013). NT1 is characterised by five core symptoms: excessive daytime sleepiness, cataplexy, hypnagogic hallucinations, sleep paralysis and disturbed nocturnal sleep. These symptoms arise as a result of the presumed destruction of over 90% of hypocretin (Hcrt)-producing neurons in the lateral hypothalamus hypothesized as being caused by an auto-immune response (Bassetti et al., 2019; Kornum et al., 2017).

95% of NT1 patients carry the *HLA-DQA1*01:02 / DQB1*06:02* haplotype encoding HLA-DQB1*06:02, an HLA-class II molecule expressed on antigen presenting cells (Tafti et al., 2014). HLA-DQB1*06:02 is also present in about 20% of the general European population. As a result, HLA-DQB1*06:02 has been considered as a

genetic factor necessary but not sufficient for development of NT1. Apart from the well-known association with HLA-DQB1*06:02, there are positive and negative associations between other HLA-DQB1 alleles and NT1: the frequency of HLA-DQB1*03:01 (HLA-DQ7) was found to be increased, whereas HLA-DQB1*02:01 (HLA-DQ2), HLA-DQB1*05:01, HLA-DQB1*06:01, HLA-DQB1*06:03 and HLA-DQB1*06:09 were decreased in NT1 patients compared with healthy controls (Hong et al., 2007; Ollila et al., 2015; Tafti et al., 2014).

An increase in the incidence of NT1 has been observed in several European countries since the 2009 influenza A(H1N1) pdm09 pandemic, and the subsequent vaccination campaign (Dauvilliers et al., 2013; Feltelius et al., 2015; Lind et al., 2014; Partinen et al., 2012). Recent studies have identified autoreactive T cells against hypocretin peptides (Latorre et al., 2018; Pedersen et al., 2019), but their reactivity was not HLA-DQB1*06:02 restricted. Reports on T cells recognizing both H1N1 and hypocretin peptides show conflicting results (Luo et al., 2018; Schinkelshoek et al., 2019), with detected T cell cross-reactivity

Abbreviations: H1N1, 2009 pandemic (H1N1)pdm09 influenza A strain; Hcrt, hypocretin; HLA-DQ2, Human leucocyte antigen molecule encoded by the HLA-DQB1*02:01 allele; HLA-DQ4, Human leucocyte antigen molecule encoded by the HLA-DQB1*04:01 allele; HLA-DQ7, Human leucocyte antigen molecule encoded by the HLA-DQB1*03:01 or 03:04 allele; HLA-DQ8, Human leucocyte antigen molecule encoded by the HLA-DQB1*03:02 or 03:05 allele; HLA-DQ9, Human leucocyte antigen molecule encoded by the HLA-DQB1*03:03 allele; ICSD-2/3, International Classification of Sleep Disorders, 2nd or 3rd edition; NT1, narcolepsy type 1

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in the first study not being replicated in the second.

Following these epidemiological and laboratory reports, a discussion on the existence of a post-H1N1 NT1 variant has arisen, with specifically Scandinavian countries making the distinction between sporadic and post-H1N1 NT1. Several recent Scandinavian studies report HLA-DQ-haplotypes associated specifically with post-H1N1 NT1 (HLA-DQ2, HLA-DQ7) (Juvodden et al., 2019; Lind et al., 2019). These reports started a discussion about whether different immunological mechanisms may be involved in post-H1N1 NT1.

Since these studies are small and only address HLA-DQB1-associations in a cohort of post-H1N1 NT1 patients and a healthy control population, our aim is to compare HLA-DQB1-associations in NT1 patients with disease onset before and after the 2009 H1N1 pandemic in a large Dutch cohort to assess whether differences can be seen which would test the hypothesis that sporadic and post-H1N1 NT1 should be regarded as separate entities based on different immunological mechanisms leading to the disease.

2. Materials and methods

2.1. Participants

We included all NT1 patients of European ancestry who, between 2005 and 2019, were HLA-genotyped for clinical care in the laboratory of the Leiden University Medical Center. Patients came from the sleep clinic of the Department of Neurology, Leiden University Medical Center, the Sleep-Wake Centre of Stichting Epilepsie Instellingen Nederland (SEIN), Heemstede and Zwolle, and the Sleep Centre of Kempenhaeghe, Heeze. All patients were diagnosed with either narcolepsy with cataplexy or NT1 according to the International Classification of Sleep Disorders (ICSD-2 or ICSD-3; (ICSD, 2005, 2014)). Age at symptom onset was assessed in all patients to separate those with symptom onset in childhood (< 16 years at symptom onset) or adulthood, and we also noted whether the symptoms of NT1 started before or after the 2009 H1N1 pandemic to be able to compare HLA-DQB1-associations before and after the H1N1 pandemic. We also included a cohort of HLA-DQB1*06:02 positive non-related healthy individuals from a panel of randomly selected Dutch individuals (van Rooijen et al., 2012) to be able to assess HLA associations in NT1.

2.2. HLA typing

HLA genotyping for HLA-DR and HLA-DQ was performed with a reversed approach of the PCR-sequence-specific oligonucleotide probe technique previously described (Verduyn et al., 1993). If a rare DRB1-DQB1 was found, typing results were confirmed by PCR-SBT, using the SBT Excellerator HLA-DRB and -DQB kit (Genome Diagnostics, Utrecht, The Netherlands).

2.3. Statistical analysis

Pearson's chi-square tests were used for comparisons. Odds ratios and corresponding 95% confidence intervals (CI) were calculated using Cochran-Mantel-Haenszel tests (Cochran, 1954; Mantel, 1963; Mantel and Haenszel, 1959). We compared the frequency of HLA-DQB1 alleles between NT1 patients and healthy controls who were HLA-DQB1*06:02 positive. We also compared the frequency of HLA-DQB1 alleles between NT1 patients with disease onset before and after the 2009 H1N1 pandemic. As the increase in NT1 incidence was reported predominantly in children with NT1, we assessed whether onset during childhood was associated with a change in HLA-DQB1-associations after the H1N1 pandemic.

Since one of our aims was specifically to replicate the results of other studies describing HLA-DQB1-associations in post-H1N1 NT1 patients, for these specific analyses corrections for multiple comparisons were not performed. For all other analyses, *p*-values were

Table 1
Narcolepsy type 1 patient characteristics.

	Pre-H1N1	Post-H1N1
N	397	128
Males	46%	49%
Age at disease onset (years)	19 (3–67)	14 (2–81)
Disease onset during childhood (< 16 years)	31%	52%
Hcrt-1 < 110 pg/mL	110/114 (97%)	40/43 (93%)
Hcrt-1 < 200 pg/mL	114/114 (100%)	43/43 (100%)
HLA-DQB1*06:02 positive (n)	396/397 (100%)	129/130 (99%)
H1N1 vaccination (n)	NA	13/29 (45%)
Proven H1N1 infection (n)	NA	1

Data on age at disease onset are shown as median and range. Data are shown as a percentage of patients in which data was available.

H1N1 = H1N1 influenza pandemic in 2009; Hcrt-1 = hypocretin-1; HLA-DQB1*06:02 = human leucocyte antigen molecule encoded by the HLA-DQB1*06:02 allele; NA = not applicable.

corrected for multiple comparisons according to the Šidák-Holm method (Šidák, 1967). Differences with *p*-values below 0.05 were deemed significant. All analyses were conducted using the IBM SPSS Statistics 25 software package. It must be noted that due to a high degree of linkage disequilibrium in the HLA complex, the tested alleles and loci are not independent.

3. Results

3.1. Participant characteristics

We included 525 NT1 patients (128 post-H1N1) and 1272 HLA-DQB1*06:02-positive controls. Participant characteristics are shown in Table 1. Notably, all but two NT1 patients were DQB1*06:02 positive. 158 patients had undergone a lumbar puncture for Hcrt-1 measurement; over 95% had Hcrt-1 values in the cerebrospinal fluid that were below the cut-off value of 110 pg/mL; all had Hcrt-1 values below 200 pg/mL.

3.2. The frequency of HLA-DQB1*06:02 homozygosity is unchanged between pre- and post-H1N1 NT1 patients

The important role of HLA-DQB1*06:02 in NT1 is demonstrated by its presence in almost all pre- and post-H1N1 NT1 patients (396/397 patients vs. 127/128 patients). Homozygosity for HLA-DQB1*06:02 is associated with NT1 with an odds ratio of 2.5 (7.2% in the healthy control population vs 16.2% in NT1 patients; Table 2). Furthermore, the percentage of NT1 patients homozygous for HLA-DQB1*06:02 is unchanged between pre- and post-H1N1 NT1 patients with 16.4% in pre-H1N1 NT1 patients and 15.6% in post-H1N1 NT1 patients (*p* = .79; Table 3).

3.3. Multiple HLA-DQ alleles other than HLA-DQB1*06:02 are associated with NT1

In addition to the association between HLA-DQB1*06:02 and NT1, Table 2 gives an overview of other HLA-DQ alleles and their association with NT1. HLA-DQ2, HLA-DQB1*05:01 and HLA-DQB1*06:03 are negatively associated with NT1, while HLA-DQ7 and HLA-DQB1*05:02 are more frequently found in NT1 patients than in healthy controls (Fig. 1).

3.4. No differences in HLA-DQB1 alleles in trans with HLA-DQB1*06:02 between pre- and post-H1N1 NT1 patients

HLA-DQ alleles in trans with HLA-DQB1*06:02 are shown for pre-

Table 2

Differences between HLA-DQB1 alleles in trans with HLA-DQB1*06:02 between NT1 patients compared with HLA-DQB1*06:02-positive healthy controls. P-values are derived from Pearson Chi-Square tests. Pc-values are calculated using the Šidák-Holm correction for multiple comparisons based on $k = 13$.

	Healthy controls	NT1	OR	95% CI	p-value	pc-value
N	1272	525	NA	NA	NA	NA
DQ2	315 (24.8%)	82 (15.6%)	0.56	0.43–0.73	< 0.001	< 0.001
DQ4	37 (2.9%)	24 (4.6%)	1.6	0.94–2.7	0.079	0.658
DQ7	216 (17.0%)	130 (24.8%)	1.6	1.3–2.1	< 0.001	0.002
DQ8	137 (10.7%)	56 (10.6%)	0.99	0.72–1.4	0.97	1
DQ9	67 (5.3%)	21 (4.0%)	0.75	0.45–1.2	0.251	0.977
DQB1*05:01	187 (14.7%)	35 (6.6%)	0.42	0.28–0.60	< 0.001	< 0.001
DQB1*05:02	20 (1.6%)	22 (4.2%)	2.7	1.5–5.0	0.001	0.011
DQB1*05:03	36 (2.8%)	18 (3.4%)	1.2	0.69–2.2	0.499	1
DQB1*06:01	5 (0.4%)	2 (0.4%)	0.97	0.19–5.0	0.966	1
DQB1*06:02	91 (7.2%)	85 (16.1%)	2.5	1.8–3.4	< 0.001	< 0.001
DQB1*06:03	86 (6.8%)	6 (1.1%)	0.16	0.07–0.37	< 0.001	< 0.001
DQB1*06:04	64 (5.0%)	41 (7.8%)	1.6	1.0–2.4	0.024	0.267
DQB1*06:09	11 (0.9%)	3 (0.6%)	0.66	0.18–2.4	0.516	1

HLA-DQ2/4/7/8/9 = human leucocyte antigen molecule encoded by the HLA-DQB1*02:01/-DQB1*04:01/-DQB1*03:01 or -03:04/-DQB1*03:02 or -03:05/-DQB1*03:03 allele; NT1 = narcolepsy type 1; NA = not applicable; OR = Mantel-Haenszel common odds ratio; pc-value = corrected p-value.

Table 3

Differences in distribution of HLA-DQB1 alleles in trans with HLA-DQB1*06:02 between pre- and post-H1N1 NT1 patients. P-values are derived from Pearson Chi-Square tests. Pc-values are calculated using the Šidák-Holm correction for multiple comparisons based on $k = 13$.

	Pre-H1N1	Post-H1N1	OR	95% CI	p-value	pc-value
N	397	128	NA	NA	NA	NA
DQ2	62 (15.6%)	20 (15.4%)	0.98	0.57–1.7	0.949	1
DQ4	18 (4.5%)	6 (4.6%)	1.0	0.40–2.6	0.969	1
DQ7	95 (23.9%)	35 (27.3%)	1.2	0.76–1.9	0.436	0.999
DQ8	41 (10.3%)	15 (11.5%)	1.1	0.60–2.1	0.697	1
DQ9	19 (4.8%)	2 (1.5%)	0.31	0.07–1.4	0.100	0.747
DQB1*05:01	29 (7.3%)	6 (4.6%)	0.61	0.25–1.5	0.285	0.987
DQB1*05:02	18 (4.5%)	4 (3.1%)	0.67	0.22–2.0	0.520	1
DQB1*05:03	12 (3.0%)	6 (4.7%)	1.6	0.58–4.3	0.368	0.997
DQB1*06:01	2 (0.5%)	0 (0%)	NA	NA	0.417	0.999
DQB1*06:02	65 (16.4%)	20 (15.4%)	0.93	0.54–1.6	0.790	1
DQB1*06:03	5 (1.3%)	1 (0.8%)	0.61	0.07–5.3	0.647	1
DQB1*06:04	28 (7.1%)	13 (10.0%)	1.5	0.74–2.9	0.276	0.985
DQB1*06:09	3 (0.8%)	0 (0.0%)	NA	NA	0.320	0.993

H1N1 = 2009 pandemic (H1N1)pdm09 influenza A strain; HLA-DQ2/4/7/8/9 = human leucocyte antigen molecule encoded by the HLA-DQB1*02:01/-DQB1*04:01/-DQB1*03:01 or -03:04/-DQB1*03:02 or -03:05/-DQB1*03:03 allele; NA = not applicable; NT1 = narcolepsy type 1; OR = Mantel-Haenszel common odds ratio; pc-value = corrected p-value.

and post-H1N1 NT1 patients separately in Table 3. For all alleles in trans with HLA-DQB1*06:02 no difference was shown between patients with symptom onset before the H1N1 pandemic and those with symptom onset after 2009 (Fig. 1).

3.5. HLA-DQB1-associations in post-H1N1 NT1 patients with disease onset during childhood are not different than those in other NT1 patients

No significant changes were demonstrated comparing those with disease onset after H1N1 and onset in childhood with all others with NT1 (Supplementary Table 1).

4. Discussion

The associations between HLA-DQB1 alleles and NT1 in this large Dutch cohort have not significantly changed since the H1N1 pandemic in 2009 and the vaccination program following the pandemic. The percentage of NT1 patients that is homozygous for HLA-DQB1*06:02 has remained unchanged and this strongly argues against considering sporadic and H1N1 NT1 to be separate entities with a separate (immune) aetiology.

One study in a Chinese cohort comparing HLA-DQB1-associations in pre- and post-H1N1 NT1 patients found a decrease in the percentage of NT1 patients that was homozygous for HLA-DQB1*06:02 since the 2009 H1N1 pandemic (Han et al., 2013). Differences in HLA-associations in populations from different ethnic background have been reported (Mignot et al., 2001), which may explain these contradictory findings. The positive association between NT1 and HLA-DQ7 and HLA-DQB1*05:02 persist after the H1N1 pandemic. The same goes for negative associations between NT1 and HLA-DQ2, HLA-DQB1*05:01 and HLA-DQB1*06:03.

These findings are in line with those of two recent reports describing these associations in small numbers of post-H1N1 NT1 patients that report positive associations of post-H1N1 with HLA-DQ7 and negative associations with HLA-DQ2 (Lind et al., 2019; Juvodden et al., 2019). Both these and other studies in NT1 patients before and after the H1N1 pandemic also describe the same associations that we found (Mignot et al., 2001; van der Heide et al., 2015; Hong et al., 2007; Tafti et al., 2014; Bomfim et al., 2017; Tafti et al., 2016; Han et al., 2013).

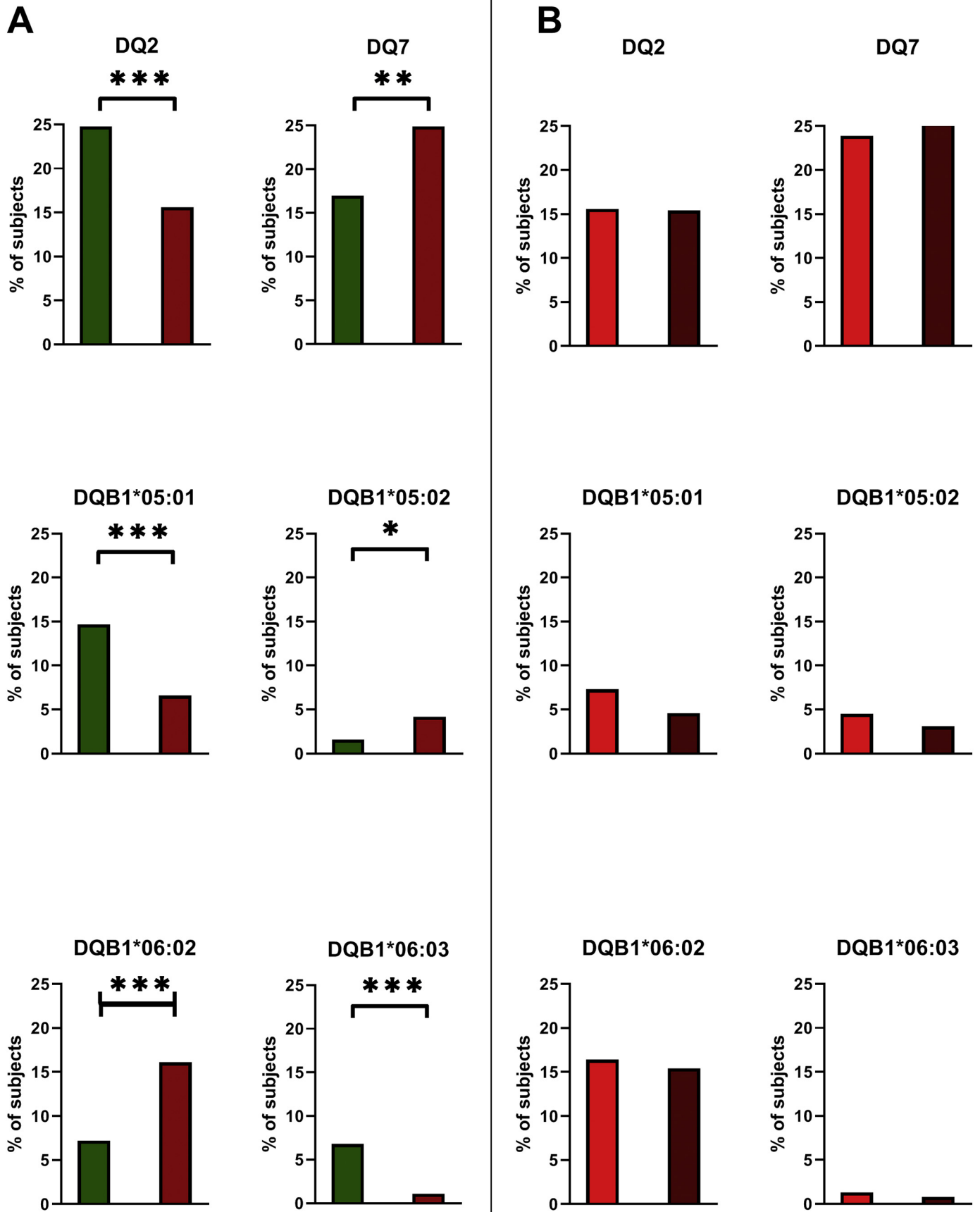
The negative association between NT1 and DQB1*06:01 previously reported (Mignot et al., 2001; Hong et al., 2007), were not replicated in the current study. This might be explained by the abundance of this HLA-DQ allele in the Asian population, compared to the almost absence of this allele in people of European ancestry. Other associations of NT1 (either positive or negative), with HLA-DQ8 (Hong et al., 2007; Bomfim et al., 2017), HLA-DQB1*06:04 (Hong et al., 2007) and HLA-DQB1*06:09 (Tafti et al., 2014; Bomfim et al., 2017) were also not replicated in the current study.

One of the limitations in our study is that we have not been able to distinguish between post-H1N1 NT1 patients and healthy controls who encountered the H1N1 influenza virus and those who did not. We have data on H1N1 exposure and vaccination for only a small subset of all participants included, which can be found in Table 1. Given the fact that the H1N1 vaccination coverage in the Netherlands was 30% for the general population and 74% for children (Mereckiene et al., 2012), it is likely that at least a significant percentage of patients with symptom onset after 2009 had encountered the H1N1 virus or were vaccinated against H1N1 during the vaccination campaign. Another factor to consider is the hypothesized post-vaccination risk window, that is defined as a period of two years after the H1N1 vaccination campaign in which the risk of developing narcolepsy type 1 was increased (Sarkanen et al., 2018). Only a small group of participants in the current study were diagnosed in 2010 and 2011, which makes a separate analysis on this group unfeasible. Data on this group is reported in Supplementary Table 2.

Our findings bridge the findings of studies in sporadic and post-H1N1. In addition to replicating HLA-associations reported in studies focusing on either sporadic NT1 or post-H1N1 NT1 patients, we show that these HLA-DQB1-associations can be found in both groups. Focusing on performing research on the autoimmune hypothesis of NT1 in NT1 patients shortly after symptom onset seems more promising than directing further research to the difference between sporadic and post-H1N1 NT1.

5. Conclusions

No differences in HLA-associations were found between NT1 patients with symptom onset before and those with symptom onset after



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Fig. 1. A. Visualisation of HLA-DQB1 allele frequencies that are significantly different between NT1 patients (red) and HLA-DQB1*06:02-positive healthy controls (green). B. Visualisation of HLA-DQB1 allele frequencies that are significantly different between pre- (bright red) and post-H1N1 (dark red) NT1 patients. HLA-DQ2/4/7/8/9 = human leucocyte antigen molecule encoded by the HLA-DQB1*02:01/-DQB1*04:01/-DQB1*03:01 or -03:04/-DQB1*03:02 or -03:05/-DQB1*03:03 allele; NT1 = narcolepsy type 1; * = $p < .05$, ** = $p < .01$, *** = $p < .001$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

the 2009 H1N1 pandemic. The positive association of HLA-DQ7 and negative associations of HLA-DQ2, HLA-DQB1*05:01 and DQB1*06:03 with both pre- and post-H1N1 described in other smaller studies were confirmed in this large Dutch cohort. These results therefore argue against considering sporadic and post-H1N1 NT1 to be separate entities.

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jneuroim.2020.577210>.

Declaration of Competing Interest

The authors confirm no conflict of interest to disclose.

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