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ORIGINAL ARTICLE

Distribution of HLA-DQB1 in Czech Patients with Central Hypersomnias

Milena Vrana[1] · Vera Siffnerova[1] · Pavla Pecherkova[1] · Eva Ratajova[1] · Karel Sonka[1,2]

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Abstract The aim of our study was to analyze the distribution of HLA-DQB1 in Czech patients with central hypersomnias and differences between diagnostic groups of narcolepsy type 1 (NT1), type 2 (NT2), idiopathic hypersomnia (IH) and no central hypersomnia subjects (no-CH). Statistical analysis of DQB1 genotyping was performed on the cohort of 716 patients (375 men, 341 women) with reported excessive daytime sleepiness. DQB1*06:02 allele was present in 94% of the NT1 patients. The decrease of DQB1*06:03 allele was also confirmed. No other DQB1*06 allele nor any other DQB1 allele group was differently distributed in the NT1. In the cohort of NT2 patients DQB1*06:02 allele was present in 43%. Allele group DQB*05 was detected with a significantly higher frequency in this diagnostic unit. Any differences in presence of DQB1*05 alleles in NT2 patients were not reported so far. The cohort of patients with IH did not show any difference in allele distribution of DQB1 alleles/allele groups comparing to healthy controls. DQB1*06:02 allele was significantly increased in the no hypersomnia group. No other DQB1 allele/allele group had any difference in distribution in patients comparing to healthy controls. The different distribution of DQB1*06:02 and other DQB1 alleles/allele groups was detected in analyzed diagnostic groups. These results indicate that DQB1 contributes to the genetic predisposition to NT1, NT2, IH and no-CH in different manners.

Keywords Narcolepsy · Idiopathic hypersomnia · Central hypersomnias · HLA-DQB1

Introduction

Central hypersomnias (CH) are neurological sleep disorders, characterized by periods of excessive daytime sleepiness (EDS). CH encompasses three main diagnoses: narcolepsy type 1, narcolepsy type 2, and idiopathic hypersomnia (IH). Narcolepsy with cataplexy (NC; new term Narcolepsy type 1: NT1) and narcolepsy without cataplexy (NwoC; new term Narcolepsy type 2: NT2) are chronic neurological diseases characterized by EDS which are divided into two independent entities based on different etiologies. Narcolepsy itself is a chronic neurological disease characterized by EDS, and is divided into narcolepsy with cataplexy (NC; new term Narcolepsy type 1: NT1) and narcolepsy without cataplexy (NwoC; new term Narcolepsy type 2: NT2) based on different etiologies [international classification of sleep disorders 3rd edition—ICSD3 (American Academy of Sleep Medicine 2014)]. NT1 occurs as a result of the loss of hypocretin (orexin) neurons in the lateral hypothalamus (Peyron et al. 2000). Although the exact mechanism of hypocretin deficiency is unknown, evidence from the past 20 years strongly favors either immune-mediated or autoimmune attack, specifically targeting hypocretin neurons in genetically predisposed individuals (Liblau et al. 2015). NT1 is very likely a T cell-mediated autoimmune disease, similar to other human leukocyte antigens (HLA)-associated diseases such as type 1 diabetes or celiac disease, with its onset triggered by

✉ Milena Vrana
milena.vrana@uhkt.cz

[1] HLA Department, Institute of Hematology and Blood Transfusion, U Nemocnice 1, 128 20 Prague 2, Czech Republic

[2] Department of Neurology and Center for Clinical Neuroscience, First Faculty of Medicine, Charles University and General University Hospital, Prague, Czech Republic

external stimulus, namely streptococcal infection or vaccination against H1N1 influenza (Aran et al. 2009; Maski and Owens 2016; Partinen et al. 2014). The leading symptoms of NT1 are EDS and cataplexy, and roughly half of patients experience hypnagogic hallucinations (HH) and sleep paralysis (SP). NT1 is characterized by a decrease of hypocretin in the cerebrospinal fluid. The cause of NT2 is not well understood; however, it is not characterized by cerebrospinal fluid hypocretin deficiency. Patients suffering from NT2 do not have cataplexy, and less frequently do they experience HH and SP. The number of sleep onset REM periods within a multiple sleep latency test (MSLT) and night polysomnography is two or more in the cases of both NT1 and NT2.

Another neurological disease including EDS as a primary symptom is IH. Subjects with IH have no cataplexy, have prolonged sleep (>660 min) and/or exhibit sleep latency in MSLT $\geq$ 8 min, and have less than two sleep onset REM periods (occurrence of REM sleep within 15 min after falling asleep) within MSLT and night polysomnography. The occurrence of IH is less frequent, when compared to NT1 and NT2.

The association of narcolepsy and HLA, namely HLA-DQB1*06:02, has been understood since the 1980s. The DQB1*06:02 allele is present in patients diagnosed with NT1 in nearly 100% of all cases (Mignot et al. 1994, 1998; Tafti et al. 2014). Homozygotes of the DQB1*06:02 allele are referred to have an additional 2–4-fold risk of narcolepsy when compared to heterozygotes, and may also exhibit more severe symptoms (Pelin et al. 1998). There are numerous alleles, including DQB1*03:01, DQA1*06, DRB1*08, DRB1*11, DRB1*12 and DRB*15 (Hong et al. 2007; Mignot et al. 2001; Nishino et al. 2000), which are known to increase predisposition. The most prominent of these susceptibility alleles is DQB1*03:01, which seems to yield consistently high relative risk in all ethnic groups (Hong et al. 2007).

Another series of HLA alleles has been identified that provides significant protective effects in trans position to DQB1*06:02. DQB1*06:01 was referred to have a major protective effect in the Chinese, Japanese and Korean populations; however, this allele is extremely rare in individuals of European ancestry (Han et al. 2012; Hohjoh et al. 2001; Hong et al. 2007). DQB1*06:03 is said to not occur in NT1 patients (Hor et al. 2010). The other three alleles (DQB1*06:09, DQB1*05:01 and DQA1*01/non-DQA1*01:02) were referred to have a protective effect (Tafti et al. 2014).

It was found that patients with mild, atypical, or no cataplexy exhibited an increased frequency of DQB1*06:02 (increase of 40–60%) in comparison to ethnically matched controls (24%) (Andlauer et al. 2012; Lin et al. 2001; Mignot et al. 1997). However, due to the high positivity of DQB1*06:02 in the general population, its testing is not sufficiently specific for NT2, and therefore is not generally recommended as a diagnostic tool for this diagnosis (Mignot et al. 2001).

No significant association between IH and DQB1*06:02 has been confirmed (Anderson et al. 2007; Miyagawa et al. 2015). On the contrary, a study of different populations has shown increased DQB1*06:02 in patients with IH (Martins-da-Silva et al. 2014).

The study was designed to investigate benefits of DQB1*06:02 screening for differential diagnostic of central hypersomnias. The aim of our study was to analyze the distribution of HLA-DQB1 alleles in Czech patients with central hypersomnias, and to confirm the usefulness of DQB1 examination as a part of the diagnostic approach investigating EDS.

Materials and Methods

Patients

The analyzed cohort consisted of 716 patients (375 men, 341 women). The biosamples from subjects were recruited at admission in the hospital before the assessment the final diagnosis. Complete subject examination included careful review of patient history, neurological clinical examination, night polysomnography (8 h) and a 5-nap MSLT (Littner et al. 2005). Hypocretin-1 in the cerebrospinal fluid was measured in a small number of the subjects.

All patients diagnosed with NT1, NT2, or IH fulfilled the diagnostic criteria of the ICSD3 (American Academy of Sleep Medicine 2014). The records of subjects diagnosed before 2014, the year of ICSD3 publication, were carefully reviewed, and conformity with the ICSD3 methodology was verified. The cohort was divided into four subject categories: NT1, NT2, IH, and no central hypersomnias (no-CH). The latter group was diagnosed with other sleep disorders, which included insufficient sleep syndrome, sleep related breathing disorders, periodic limb movement disorders or restless legs syndrome, circadian disturbances, EDS combined with Parkinson's disease, and REM sleep behavior disorders; there were also subjects in whom no EDS was ultimately diagnosed. The distinction of IH and no-CH membership of sleepy subjects is based on the presence of other diseases which can provoke sleepiness, because one of the diagnosis criteria of IH is the absence of any disease which can induce sleepiness.

The division of the cohort to the respective diagnostic groups was as followed: NT1 group included 137 subjects (71 males and 66 females); median of age at the diagnosis was 39 years, range 7–86. NT2 consisted of 61 subjects (32

males, 29 females); median of age at the diagnosis was 37 years, range 11–75. IH group included 66 subjects (31 males, 35 females); median of age at the diagnosis was 36 years, range 12–71. No-CH group comprised 452 subjects (244 males, 208 females), median of age at the diagnosis 47 years, range 6–83. The group consisted of 207 subjects with sleep relating breathing disorders, 89 subjects with parasomnias including idiopathic REM sleep behavior disorder, 59 subjects with restless legs syndrome and/or periodic limb movements in sleep, 23 pharmacologically induced hypersomnia, 17 subjects with insomnia, 14 subjects with hypersomnia related to other neurological disease (e.g. Parkinson disease), 10 subjects with disorders of circadian rhythm, and 33 subjects in whom no hypersomnia nor other sleep disorder was confirmed.

Controls

As a control group, data from the Czech National Marrow Donors Registry (NMDR) published at http://www.allelefrequencies.net were used (identification of the population is Czech Republic NMDR). That cohort consisted of 4049 Caucasians, with geographic location in the Czech Republic tested on the DQB1 locus. Typing was performed by Bone Marrow Registry in 2014 using polymerase chain reaction with sequence specific primers (PCR-SSP) and polymerase chain reaction with sequence specific oligonucleotide probes (PCR-SSOP) methods (Gonzalez-Galarza et al. 2015).

HLA Genotyping

HLA typing was performed using a commercially available PCR-SSP kit (Olerup SSP HLA Typing Kits) according to the manufacturer's recommendations. Low resolution typing of the HLA-DQB1 locus was performed for all samples. Subsequently, high resolution typing of all DQB1*06 positive samples was completed. The data were analyzed by Helmberg-SCORE software using the IMGT/HLA database (Robinson et al. 2015).

Statistical Analysis

Detailed analyses of DQB1 allele distribution in the particular diagnostic groups were calculated in two ways: (1) Calculating the numbers of individuals in whom the alleles/allele groups are present. This calculation is not dependent on the dose effect of distinctive alleles/allele groups; (2) Calculating the total numbers of copies of the alleles/allele groups. This calculation takes into an account the potential dose effect of distinctive alleles/allele groups.

Odd ratios (OR) and their asymptotic 95% confidence intervals (95% CI) were calculated by Gaussian approximation (DerSimonian and Laird 1986; Fleiss 1981). Cochran's Q analysis test of heterogeneity was performed (Deeks et al. 2005; Tate and Brown 1970). The complete data set was marginally insignificant because the P value 0.076 is more than the significance level 0.05, $I^2 = 44\%$ and 95% CI for $I^2 = \langle 0; 74 \rangle$.

Bonferroni multiple testing corrections were used to adjust statistical confidence measured (Noble 2009). P values were corrected using formula pcorr $= 1 -$ [$(1 - P)$^N], where $N = 4$ in Table 1, $N = 5$ for allele groups and $N = 9$ for single alleles in respective diagnostic groups (data mentioned in text).

Results

Based on the preliminary data (Dobrovolna et al. 2007) genotyping of HLA-DQB1 was introduced as a routine diagnostic tool for sleep disorders to support the differentiation of NT1 and other diseases in the Czech Republic. In this setting, the cohort of patients examined in the period of 04/2004–12/2015 was analyzed. The genotype of the HLA-DQB1 locus was tested on a high/medium resolution level, and the distribution of DQB1 alleles/allele groups was compared to final patient diagnoses.

The presence of DQB1*06:02 was confirmed in 297 cases (41% of the tested cohort), and in 41 individuals (5.7%) with a homozygous status. On the contrary, the protective allele DQB1*06:03 was detected in 74 individuals (10.3%), nine of which with a homozygous status. The combined genotype of DQB1*06:02 and DQB1*06:03 was detected in 11 individuals (1.5%).

An increase of DQB1*06:02 was statistically significant in NT1 and no-CH cohorts (NT1: OR $= 51.184$, $P < 0.0001$, no-CH: OR $= 1.381$, $P = 0.0036$). Subsequent analysis using the Bonferroni multiple testing corrections confirmed difference in DQB1*06:02 positivity in these diagnostic groups (NT1: $P < 0.0001$; no-CH: $P = 0.0142$).

The numbers of DQB1*06:02 positive patients in the particular diagnostic groups and a comparison with the healthy controls is displayed in Table 1.

Narcolepsy Type 1

The distribution of DQB1 alleles/allele groups in NT1 group and the comparison to healthy controls is reported in Table 2. DQB1*06:02 allele was present in 94% of the patients. The dominant difference in distribution of DQB1*06:02 allele was confirmed by the difference in the allele frequency in this group (OR $= 7.972$, $P < 0.0001$). Data are reported in Table 3. This difference was confirmed using Bonferroni multiple testing ($P < 0.0001$).

Another four alleles/allele groups were detected in significantly lower incidence in this diagnostic group

Table 1 DQB1*06:02 positive patients—comparison of diagnostic groups

Diagnosis	All	DQB1*06:02+		OR[a]	95% CI[b]		*P* value	Corrected *P* value[c]
	n	*n*	%					
NT1	**137**	**129**	**94.2**	**51.184**	**24.970**	**104.920**	**<0.0001**	**<0.0001**
NT2	61	21	34.4	1.666	0.978	2.840	0.0699	0.2516
IH	66	10	15.2	0.567	0.288	1.115	0.1095	0.3713
No-CH	**452**	**137**	**30.3**	**1.381**	**1.115**	**1.709**	**0.0036**	**0.0142**
controls	4049	970	24.0					

The cohort consisted of 716 patients (lines NT1, NT2, IH, no-CH) and 4049 healthy controls

Statistically significant results indicated in bold

[a] *OR* odds ratio

[b] *95% CI* asymptotic 95% confidence interval

[c] *P* value using Bonferroni multiple testing corrections

Table 2 NT1—allele distribution

	Patients		Controls		OR	95% CI		*P* value
	n	%	*n*	%				
***02**	**32**	**23.4**	**1606**	**39.7**	**0.464**	**0.311**	**0.692**	**0.0001**
***03**	**57**	**41.6**	**2222**	**54.9**	**0.586**	**0.415**	**0.827**	**0.0029**
*04	8	5.8	255	6.3	0.923	0.447	1.906	1.0000
***05**	**16**	**11.7**	**1264**	**31.2**	**0.291**	**0.172**	**0.493**	**<0.0001**
*06:01	1	0.7	72	1.8	0.406	0.056	2.944	0.7331
***06:02**	**129**	**94.2**	**970**	**24.0**	**51.184**	**24.970**	**104.920**	**<0.0001**
***06:03**	**3**	**2.2**	**624**	**15.4**	**0.123**	**0.039**	**0.387**	**<0.0001**
*06:04	6	4.4	202	5.0	0.872	0.380	2.001	1.0000
*06:09	2	1.5	33	0.8	1.803	0.428	7.591	0.3186

The cohort consisted of 137 NT1 patients and 4049 healthy controls

Statistically significant results indicated in bold

(DQB1*02: OR = 0.464, $P = 0.0001$; DQB1*03: OR = 0.586; $P = 0.0029$; DQB1*05: OR = 0.291, $P < 0.0001$ and DQB1*06:03: OR = 0.123, $P < 0.0001$).

The test of heterogeneity of NT1 was significant with P value 0.015 and $I^2 = 58\%$ CI = <12; 80>. This phenomenon is due to the big proportion of DQB1*06:02. The cohort was therefore analyzed excluding DQB1*06:02. Data are reported in Table 4.

The significant difference between NT1 patients and control group remained in the distribution of DQB1*02 (OR = 0.557, $P = 0.0053$), DQB1*05 (OR = 0.341, $P < 0.0001$) and DQB1*06:03 allele (OR = 0.142, $P < 0.0001$).

The allele frequencies excluding DQB1*06:02 were also analyzed (Table 5). The only significant difference between the NT1 patients and the control group remained in the distribution of the DQB1*06:03 allele (OR = 0.251, $P = 0.0063$). However, this result was not confirmed using Bonferroni multiple testing corrections (corrected $P = 0.0553$). The strong evidence of the protective effect of the DQB1*06:03 allele was confirmed; nevertheless, this protective allele was detected in our cohort in three NT1 patients. No other DQB1*06 allele nor any other DQB1 allele group was differently distributed in the NT1 patients and controls after the exclusion of the DQB1*06:02 effect in the analysis of allele frequencies.

Narcolepsy Type 2

In the cohort of NT2 patients, the DQB1*06:02 allele was present in 34% of subjects (see Table 6). Nevertheless, the difference comparing to healthy cohort was not statistically significant (OR = 1.666, $P = 0.0699$). An increased proportion of DQB1*06:02 was confirmed by its difference in allele frequency (OR = 1.749, $P = 0.0198$, see Table 7).

Allele group DQB*05 was detected with a significantly higher number in this diagnostic unit (OR = 1.869, $P = 0.0178$, see Table 6). Higher proportion of DQB1*05

Table 3 NT1—allele frequencies

	Patients		Controls		OR	95% CI		*P* value
	n	Allele freq[a]	*n*	Allele freq				
***02**	**32**	**0.117**	**1808**	**0.223**	**0.458**	**0.316**	**0.665**	**<0.0001**
***03**	**58**	**0.212**	**2658**	**0.329**	**0.547**	**0.408**	**0.734**	**<0.0001**
*04	8	0.029	259	0.032	0.908	0.444	1.854	1.0000
***05**	**16**	**0.058**	**1382**	**0.171**	**0.300**	**0.181**	**0.499**	**<0.0001**
*06:01	1	0.004	72	0.009	0.407	0.056	2.941	0.7343
***06:02**	**148**	**0.540**	**1037**	**0.128**	**7.972**	**6.231**	**10.199**	**<0.0001**
***06:03**	**3**	**0.011**	**624**	**0.077**	**0.132**	**0.042**	**0.414**	**<0.0001**
*06:04	6	0.022	202	0.025	0.873	0.384	1.983	1.0000
*06:09	2	0.007	33	0.004	1.792	0.428	7.506	0.3197

The cohort consisted of 137 NT1 patients and 4049 healthy controls

Statistically significant results indicated in bold

[a] Allele frequency: total number of copies of the allele in the population sample (Allele/2n) in decimal format

Table 4 NT1—allele distribution excluding DQB1*06:02

	Patients		Controls		OR	95% CI		*P* value
	n	%	*n*	%				
***02**	**32**	**27.4**	**1606**	**40.3**	**0.557**	**0.369**	**0.841**	**0.0053**
*03	57	48.7	2222	55.8	0.753	0.521	1.088	0.1320
*04	8	6.8	255	6.4	1.073	0.518	2.224	0.8472
***05**	**16**	**13.7**	**1264**	**31.7**	**0.341**	**0.200**	**0.580**	**<0.0001**
*06:01	1	0.9	72	1.8	0.468	0.065	3.399	0.7237
***06:03**	**3**	**2.6**	**624**	**15.7**	**0.142**	**0.045**	**0.447**	**<0.0001**
*06:04	6	5.1	202	5.1	1.012	0.440	2.329	1.0000
*06:09	2	1.7	33	0.8	2.082	0.494	8.780	0.2637

The cohort consisted of 117 NT1 patients and 3983 healthy controls

Statistically significant results indicated in bold

was also detected in analysis of allele frequencies (OR = 1.722, $P = 0.0110$, see Table 7). This difference was not confirmed using Bonferroni multiple testing (corrected $P = 0.0538$).

Idiopathic Hypersomnia

The cohort of patients with IH did not show any difference in the distribution of DQB1 alleles/allele groups, when compared to healthy controls. Data are reported in Table 8.

No Central Hypersomnias

The DQB1*06:02 allele was present in 30% of the subjects with no central hypersomnias. The presence of DQB1*06:02 differed significantly in this cohort compared to the healthy controls (OR = 1.381, $P = 0.0039$). No other DQB1 allele/allele group had any difference in distribution in these patients, when compared to the healthy controls. Data are reported in Table 9.

The increased number of DQB1*06:02 was confirmed by the analysis of allele frequencies (Table 10). The allele frequency of DQB1*06:02 was significantly higher, OR = 1.350, $P = 0.0022$. This difference was confirmed using Bonferroni multiple testing (corrected $P = 0.0196$).

Discussion

Narcolepsy Type 1

In concordance with other studies, the dominant DQB1 allele presenting in NT1 patients is DQB1*06:02. A similar percentage was documented in a study of DR2 occurrence in narcolepsy patients, performed nearly 30 years ago on a mainly Czech population (Roth et al. 1985).

Table 5 NT1—allele frequencies excluding DQB1*06:02

	Patients		Controls		OR	95% CI		*P* value
	n	Allele freq.	*n*	Allele freq.				
*02	32	0.254	1808	0.256	0.985	0.657	1.476	1.0000
*03	58	0.460	2658	0.378	1.406	0.987	2.002	0.0638
*04	8	0.063	259	0.037	1.775	0.858	3.670	0.1454
*05	16	0.127	1382	0.197	0.595	0.351	1.009	0.0536
*06:01	1	0.008	72	0.010	0.774	0.107	5.614	1.0000
***06:03**	**3**	**0.024**	**624**	**0.089**	**0.251**	**0.080**	**0.790**	**0.0063**
*06:04	6	0.048	202	0.029	1.692	0.737	3.887	0.1831
*06:09	2	0.016	33	0.008	3.424	0.813	14.426	0.1256

The cohort consisted of 117 NT1 patients and 3983 healthy controls

Statistically significant results indicated in bold

Table 6 NT2—allele distribution

	Patients		Controls		OR	95% CI		*P* value
	n	%	*n*	%				
*02	18	29.5	1606	39.7	0.637	0.366	1.108	0.1147
*03	30	49.2	2222	54.9	0.796	0.480	1.319	0.4371
*04	1	1.6	255	6.3	0.248	0.034	1.797	0.1809
***05**	**28**	**45.9**	**1264**	**31.2**	**1.869**	**1.125**	**3.107**	**0.0178**
*06:01	0	0.0	72	1.8	0.446	0.027	7.280	0.5710
***06:02**	**21**	**34.4**	**970**	**24.0**	**1.666**	**0.978**	**2.840**	**0.0699**
*06:03	5	8.2	624	15.4	0.490	0.196	1.228	0.1505
*06:04	1	1.6	202	5.0	0.317	0.044	2.302	0.3688
*06:09	1	1.6	33	0.8	2.028	0.273	15.073	0.3998

The cohort consisted of 61 NT2 patients and 4049 healthy controls

Statistically significant results indicated in bold

The 94% occurrence of the DQB1*06:02 allele in NT1 patients detected by our work was at the lower limit of those of the most recently published studies (Lin et al. 2001; Mignot et al. 2002; Tafti et al. 2014). The clinical difference between NT1 and NT2 is in the presence or absence of cataplexy; however, as this symptom is self-reported by patients, we can suspect that classification into NT1 or NT2 is not entirely accurate in all cases and in every patient of our and other studies, and this variation can mildly influence the percentage of HLA positive subjects diagnosed without a level of hypocretin in cerebrospinal fluid. The measurement of hypocretin levels was performed in very small number of subjects in this cohort.

Our finding of decreased DQB1*06:03 numbers in NT1 group is in correlation with published data about the protective effect of this allele (Hor et al. 2010; Mignot et al. 2001).

The strong evidence of the protective effect of the DQB1*06:03 allele was confirmed; nevertheless, this protective allele was detected in our cohort in higher percentage. These patients are candidates for the examination of hypocretin level in cerebrospinal fluid to confirm the diagnosis.

Typing at high resolution was not performed for other DQB1 allele groups. An impact of specific alleles (DQB1*03:01, DQB1*05:01) noticed by other groups might be hidden in a particular allelic group.

Narcolepsy Type 2

An increased number of DQB1*06:02 in the cohort of NT2 patients confirmed findings of other investigators (Andlauer et al. 2012; Lin et al. 2001; Mignot et al. 1997). The predisposing effect of this allele was not as dominant as it

Table 7 NT2—allele frequencies

	Patients		Controls		OR	95% CI		*P* value
	n	Allele freq.	*n*	Allele freq.				
*02	22	0.180	1808	0.223	0.763	0.479	1.213	0.2748
*03	35	0.287	2658	0.329	0.820	0.552	1.217	0.3821
*04	1	0.008	259	0.032	0.249	0.035	1.792	0.1888
***05**	**32**	**0.262**	**1382**	**0.171**	**1.722**	**1.146**	**2.588**	**0.0110**
*06:01	0	0.000	72	0.009	0.454	0.028	7.315	0.6289
***06:02**	**25**	**0.205**	**1037**	**0.128**	**1.749**	**1.122**	**2.728**	**0.0198**
*06:03	5	0.041	624	0.077	0.510	0.208	1.254	0.1686
*06:04	1	0.008	202	0.025	0.322	0.045	2.317	0.3750
*06:09	1	0.008	33	0.004	2.014	0.273	14.845	0.4000

The cohort consisted of 61 NT2 patients and 4049 healthy controls

Statistically significant results indicated in bold

Table 8 IH—allele distribution

	Patients		Controls		OR	95% CI		*P* value
	n	%	*n*	%				
*02	32	48.5	1606	39.7	1.432	0.880	2.330	0.1634
*03	35	53.0	2222	54.9	0.928	0.570	1.511	0.8037
*04	3	4.6	255	6.3	0.708	0.221	2.272	0.7974
*05	18	27.3	1264	31.2	0.826	0.479	1.426	0.5922
*06:01	1	1.5	72	1.8	0.850	0.116	6.208	1.0000
*06:02	10	15.2	970	24.0	0.567	0.288	1.115	0.1090
*06:03	5	7.6	624	15.4	0.450	0.180	1.124	0.0850
*06:04	3	4.6	202	5.0	0.907	0.282	2.913	1.0000
*06:09	0	0.0	33	0.8	0.970	0.059	16.090	0.9857

The cohort consisted of 66 patients and 4049 healthy controls

was in the NT1 group (NT1: OR = 51.184, NT2: OR = 1.666, see Table 1).

Our finding of increased frequency of DQB1*05 allele group in NT2 patients have not been reported so far. On the contrary, the protective effect of the DQB1*05:01 allele in NT1 has been established by others (Tafti et al. 2014). An association of DQB1*05:01 has been shown only with sleepwalkers (Lecendreux et al. 2003) and generally with NREM parasomnia (Heidbreder et al. 2016).

However, the pathological pathways of NT2 have not been discovered so far, the explanation of DQB*05 increase remained unclear and it should be further investigated.

The size of the cohort in this diagnostic group (66 subjects only) could have an impact on the results. Therefore, reanalysis of increased cohort of well-diagnosed patients is essential. Further detailed analysis of individual DQB1*05 alleles and other HLA loci (namely DQA1) is also necessary.

Idiopathic Hypersomnia

Our results are in concordance with published IH data (Anderson et al. 2007; Miyagawa et al. 2015). Neither DQB1*06:02, nor any other DQB1 allele, plays a role in predisposition to this diagnosis; however, this thesis is in contrast with the finding that the presence of DQB1*06:02 up-modulates the sleep tendency in humans (see next paragraph). Thus, one may expect that pathophysiology including EDS of IH is completely independent on HLA system.

Table 9 No-CH—allele distribution

	No-CH		Controls		OR	95% CI		*P* value
	n	%	*n*	%				
*02	164	36.3	1606	39.7	0.866	0.708	1.060	0.1706
*03	239	52.9	2222	54.9	0.923	0.759	1.121	0.4257
*04	21	4.7	255	6.3	0.725	0.459	1.144	0.1796
*05	147	32.5	1264	31.2	1.062	0.863	1.307	0.5930
*06:01	5	1.1	72	1.8	0.618	0.248	1.538	0.4412
***06:02**	**137**	**30.3**	**970**	**24.0**	**1.381**	**1.115**	**1.709**	**0.0039**
*06:03	63	13.9	624	15.4	0.889	0.672	1.176	0.4482
*06:04	22	4.9	202	5.0	0.974	0.621	1.530	1.0000
*06:09	4	0.9	33	0.8	1.087	0.383	3.081	0.7840

The cohort consisted of 452 no-CH subjects and 4049 healthy controls

Statistically significant results indicated in bold

Table 10 No-CH—allele frequencies

	Patients		Controls		OR	95% CI		*P* value
	n	Allele freq.	*n*	Allele freq.				
*02	189	0.209	1808	0.223	0.916	0.774	1.085	0.3321
*03	287	0.317	2658	0.329	0.948	0.818	1.099	0.5014
*04	22	0.024	259	0.032	0.753	0.484	1.170	0.2272
*05	159	0.176	1382	0.171	1.034	0.863	1.238	0.7100
*06:01	5	0.006	72	0.009	0.618	0.249	1.534	0.4435
***06:02**	**150**	**0.166**	**1037**	**0.128**	**1.350**	**1.120**	**1.628**	**0.0022**
*06:03	63	0.070	624	0.077	0.896	0.684	1.170	0.4678
*06:04	25	0.028	202	0.025	1.109	0.727	1.689	0.6543
*06:09	4	0.004	33	0.004	1.083	0.383	3.064	0.7848

The cohort consisted of 452 no-CH subjects and 4049 healthy controls

Statistically significant results indicated in bold

No Central Hypersomnias

In the no-CH subjects examined due to the daytime sleepiness, even if our group was heterogenous and it did not represent any defined cohort, the increased frequency of the DQB1*06:02 allele corresponds to the finding that the presence of DQB1*06:02 modulates human sleep tendencies (Mignot et al. 1999). Recently Goel et al. (2010) have determined this allele as a genetic biomarker for the prediction of inter-individual differences in sleep homeostasis, physiologic sleep, sleepiness, and fatigue in healthy adults.

Examinations of DQB1*06:02 and Hypocretin Levels in Cerebrospinal Fluid

Cataplexy which is a self-reported symptom is the single clinical difference between NT1 and NT2. Negativity of DQB1*06:02 in NT1 is a strong indication for the examination of hypocretin levels to clarify the differentiation between NT1 and NT2.

According to the work of Andlauer et al. (2012) 24% of patients diagnosed with narcolepsy without cataplexy (the former naming for NT2) are in fact hypocretin deficient. These patients should be more accurately classified as NT1.

Current diagnostic criteria rely heavily on the polysomnogram and MSLT (Berkowski and Shelgikar 2016). The distinction between NT2 and IH is in the different findings of MSLT and night polysomnograms; however, some studies have shown that repeated MSLT did not always confirm the initial diagnosis (Sonka et al. 2014; Trotti et al. 2013).

Based on these two findings, DQB1*06:02 positive subjects with clinical presentations of NT2 and IH exhibit an increased risk of misdiagnosis without further testing of hypocretin levels and concurrently subjects with negativity of DQB1*06:02 diagnosed with NT1 should undergo lumbar puncture to establish the diagnosis with full

evidence. Thus, DQB1*06:02 examination becomes a useful, and less invasive tool to select subjects for further cerebrospinal fluid hypocretin examination.

In conclusion, our study has shown in detail the different distributions of DQB1*06:02 and other DQB1 alleles/allele groups in NT1, NT2, IH and no-CH cohorts. While the frequency of DQB1*06:02 was increased in the NT1, NT2 and no-CH groups, no difference was found in the IH group. Additionally, the protective effect of DQB1*06:03 was detected only in the NT1 cohort. The presence of DQB1*05 was increased in our cohort of NT2 patients, where no differences in NT2 patient DQB1*05 allele frequency had previously been reported and further investigation is necessary. These results indicate that DQB1 contributes to the genetic predisposition to NT1, NT2, and no-CH in different manners, and that IH seems independent of DQB1. The examination of DQB1*06:02 can aid and inform clinicians in the better selection and classification of EDS patients for further invasive examination of hypocretin in their cerebrospinal fluid to determine the proper diagnosis.

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