ORIGINAL ARTICLE

Prevalence of the HLA-DQB1*0602 allele in narcolepsy and idiopathic hypersomnia patients seen at a sleep disorders outpatient unit in São Paulo

Prevalência do alelo HLA-DQB1*0602 em pacientes com narcolepsia e hipersonolência idiopática atendidos em ambulatório de sonolência em São Paulo

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Abstract

Objective: *Narcolepsy (with and without cataplexy) and idiopathic hypersomnia, are disorders with common features but with different HLA-DQB1*0602 allele prevalence. The present study describes the prevalence of HLA-DQB1*0602 allele in narcoleptics with and without cataplexy and in patients with idiopathic hypersomnia.* ***Method:*** *Subjects comprised 68 patients who were diagnosed for narcolepsy or idiopathic hypersomnia and 23 healthy controls according to the International Classification of Sleep Disorders-2. Subjects comprised 43 patients with narcolepsy and cataplexy, 11 patients with narcolepsy but without cataplexy, 14 patients with idiopathic hypersomnia and 23 healthy controls. Genotyping of HLA-DQB1*0602 allele was performed for all subjects.* ***Results:*** *The prevalence of the HLA-DQB1*0602 allele was increased in idiopathic hypersomnia and in narcoleptic patients with and without cataplexy when compared to healthy subjects ($p = 0.04$; $p = 0.03$ and $p < 0.0001$, respectively).* ***Conclusions:*** *This finding is in accordance with those of previous studies. The gold standard exam of narcolepsy with cataplexy is Hypocretin-1 dosage, but in patients without cataplexy and idiopathic hypersomnia, there are no specific diagnostic lab findings. The presence of the HLA-DQB1* 0602 allele may be important for the differential diagnosis of situations that resemble those sleep disorders such as secondary changes in sleep structure due to drugs' consumption.*

Descriptors: *HLA-DQB1*0602 allele; Narcolepsy; Hypersomnolence, idiopathic; Outpatients; Diagnosis/methods*

Resumo

Objetivo: *Narcolepsia (com e sem cataplexia) e hipersonolência idiopática são transtornos com características clínicas comuns, mas com prevalências do alelo HLA-DQB1*0602 diferentes. Este estudo descreve a prevalência do alelo HLA-DQB1*0602 em pacientes narcolépticos com e sem cataplexia e em pacientes com hipersonolência idiopática.* ***Método:*** *A amostra consistiu de 68 pacientes com diagnóstico de narcolepsia ou hipersonolência idiopática e 23 controles saudáveis segundo o* International Classification of Sleep Disorders-2. *A amostra foi composta de 43 pacientes com narcolepsia e cataplexia, 11 pacientes com narcolepsia e sem cataplexia, 14 pacientes com hipersonolência idiopática e 23 controles saudáveis. A análise da presença do alelo HLA-DQ*0602 foi realizada em todos os sujeitos.* ***Resultados:*** *A prevalência do alelo HLA-DQB1*0602 foi maior nos grupos de pacientes com hipersonolência idiopática e em pacientes narcolépticos com e sem cataplexia quando comparada com a dos sujeitos saudáveis ($p = 0,04$; $p = 0,03$ e $p < 0,0001$, respectivamente).* ***Conclusões:*** *Os resultados são compatíveis com o de estudos anteriores. O exame padrão-ouro para a confirmação da narcolepsia em pacientes com cataplexia é a dosagem de hipocretina, mas em pacientes sem cataplexia e hipersonolência idiopática não há testes laboratoriais específicos para o diagnóstico. A presença do alelo HLA-DQB1*0602 pode ser importante no diagnóstico diferencial de situações semelhantes a esses distúrbios do sono, como alterações secundárias na estrutura do sono causadas por consumo de drogas.*

Descritores: *Alelo HLA-DQB1*0602; Narcolepsia; Hipersonolência idiopática; Pacientes ambulatoriais; Diagnóstico/métodos*

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Introduction

Narcolepsy is characterized by excessive daytime sleep (EDS) and cataplexy. Sleep paralysis and hypnagogic hallucinations can be added to this clinical picture[1,2]. Two or more sleep onset rapid eye movement periods (SOREM) using a Multiple Sleep Latency Test performed after nocturnal polysomnography can be helpful in diagnosing narcolepsy in the absence of a convincing history of partial or complete attacks of cataplexy[2,3]. Narcolepsy studies show a frequent association of the HLA-DQB1*0602 allele with the presence of cataplexy[4].

The HLA-DQB1*0602 allele prevalence has been demonstrated in asymptomatic population: 25% of Caucasians, 12% in Japanese and 38% in African-Americans[1]. However, the prevalence of the HLA-DQB1*0602 allele has been described in 95% of Caucasian patients with cataplexy and in 40-50% of patients without cataplexy. This would strengthen the hypothesis of a genetic contribution for the disease. Other pathophysiological hypotheses derive from the environment, including infections and the immunological system response.

Idiopathic hypersomnia (IH) is a sleep disorder of the central nervous system characterized by prolonged nocturnal sleep and periods of daytime drowsiness. Affected individuals experience sleep drunkenness, automatic behavior, and memory disturbances. This condition differs from narcolepsy in that daytime sleep periods are longer, there is no association with cataplexy, and multiple sleep latency onset tests do not record SOREM[2]. The International Classification of Sleep Disorders-2 (ICSD-2)[2] divided IH in patients with and without long sleep duration. Some authors failed to describe the increase of the HLA-DQB1*0602 allele in idiopathic hypersomnia[5,6].

Patients with cataplexy have a greater prevalence of the HLA-DQB1*0602 allele along with a marked diminution of Hypocretin-1 in the cerebral spinal fluid when compared with patients without cataplexy or IH[7]. There is a dearth of immunological studies in the literature which focus on patients with narcolepsy in which clinical and laboratory differences were assessed[8]. These differences, which also include the frequency of cataplexy, prevalence of the HLA-DQB1*0602 allele and lower levels of Hypocretin-1 in the cerebral spinal fluid, encouraged some investigators to posit a pathophysiological discrepancy in sleep disorders[8].

The investigation of the HLA-DQB1*0602 allele is not a routine procedure. The presence of the HLA DQB1*0602 allele could be important for the differential diagnosis of situations that resemble those sleep disorders such as drugs changing sleep structure and others.

Our study was to investigate the prevalence of the HLA-DQB1*0602 allele in patients with narcolepsy with cataplexy, patients with narcolepsy without cataplexy, and patients with IH with a long sleep duration.

Method

The study was prospective and was undertaken between November 2003 and February 2008 at the Sleep Institute, Department of Psychobiology, Universidade Federal de São Paulo (Unifesp-EPM), São Paulo.

The study received the seal of approval from the Ethics and Research Committee of Unifesp, identified by the registered code number 1139/03. All patients gave their signed consent after agreed to participate in the study.

1. Subjects

Twenty-three healthy subjects and 68 patients were submitted to an interview for initial assessment. All patients were diagnosed for narcolepsy and IH using ICSD-2[2]. The patients were divided into 3 groups: 43 with narcolepsy and cataplexy, 11 patients with narcolepsy without cataplexy and 14 patients with IH with long sleep duration. Others patients with others diagnoses were excluded: narcolepsy due to a medical condition and IH without long sleep duration for example. The identification of the presence of the HLA-DQB1*0602 allele was performed in all healthy subjects and patients.

2. HLADQB1*0602 allele analysis

HLADQB1*0602 allele analysis was carried out in the Genetic Laboratory which collaborates with our Sleep Institute. The presence of DQB1*0602 was determined using the following procedure: The DNA was extracted from white blood cells. The region where the DQB1*0602 allele is located was amplified by a polymerase chain reaction (PCR) using primers of DQBF (5'- CCCGCAGAGGATTTCGTGTT - 3') and DQBR (5'- AACTCCGCCCGGGTCCC - 3'). These primers amplified DQB 1 *0602 and also the rare DQB1*0610, DQB1*0613, and DQB1*0614 alleles as a product of 218 base pairs.

As an internal control for the amplifications were used in the same reaction of PCR, the following primers amplified the exon 3 of the gene DRBI: EX3f (5'-TGCCAAGTGGAGCACCCAA - 3') and EX3r (5'- GCATCTTGCTCTGTGCAGAT - 3'). PCR analyses were performed using 35 cycles of 95ºC for 30 seconds, 35 cycles of 63ºC for 30 seconds and 35 cycles of 72ºC for 60 seconds.

3. Statistical analysis

Variable distribution was verified using the Kolmogorov-Smirnov test with values presented as averages and standard deviation.

The Chi-squared test was used to test the differences between groups of patients. The t-Student's test for independent samples was used for comparison of age between the groups. Statistical significance was attributed when $p \leq 0.05$.

4. Results

Demographic characteristics (age and gender) were similar among all groups studied – Table 1.

The prevalence of the HLA-DQB1*0602 allele increased in IH and in narcolepsy patients with and without cataplexy when compared with healthy subjects ($p = 0.04$; $p = 0.03$ and $p < 0.0001$, respectively). Our data showed no difference regarding the HLA-DQB1*0602 allele prevalence between IH and narcolepsy without cataplexy ($p = 0.9$) – Table 2.

5. Discussion

Our understanding of narcolepsy has improved in recent years. The greater prevalence of the HLA-DQB1*0602 allele in narcoleptic patients with cataplexy is well known[8,9]. The discovery of Hypocretin-1 and its reduction through cell loss in the lateral hypothalamus in patients with frequent cataplexy is one possible explanation for the main clinical symptoms in narcolepsy[9].

Our study found that the prevalence of the HLA-DQB1*0602 allele was increased in the narcolepsy population, being unanimous in patients affected by frequent cataplexy. This finding is in accordance with those of a previous study[3]. The inherent limitation of our study could be due to the small number of patients and healthy subjects used. Further, it was impossible to study patients according to their ethnic origins.

In our population the HLA-DQB1*0602 allele prevalence in patients with IH was less prevalent than that in narcolepsy patients with cataplexy and was similar to that in narcolepsy patients

Table 1 - Narcoleptic patients

Patient	Cataplexy	HLA DQ 0602	Age	Sex	Hypnagogic hallucinations	Sleep paralysis	Sleep latency	SOREM
1	Yes	Negative	15	F	Yes	No	2.2	5
2	Yes	Negative	17	F	Yes	No	0.3	2
3	Yes	Negative	60	M	Yes	Yes	5	2
4	Yes	Negative	33	M	Yes	Yes	14	2
5	Yes	Negative	67	M	No	No	9	2
6	Yes	Positive	28	M	Yes	Yes	1	4
7	Yes	Positive	30	M	Yes	Yes	0.1	4
8	Yes	Positive	40	F	Yes	Yes	3	2
9	Yes	Positive	33	M	Yes	No	0.2	4
10	Yes	Positive	36	F	Yes	No	2	3
11	Yes	Positive	5	M	Yes	Yes	2	4
12	Yes	Positive	29	F	Yes	Yes	2	2
13	Yes	Positive	29	M	Yes	Yes	5	2
14	Yes	Positive	77	M	Yes	Yes	7	2
15	Yes	Positive	23	F	Yes	Yes	4	2
16	Yes	Positive	36	M	Yes	Yes	3	2
17	Yes	Positive	56	M	No	No	1	5
18	Yes	Positive	42	F	Yes	Yes	10	3
19	Yes	Positive	52	F	Yes	Yes	1	5
20	Yes	Positive	19	F	Yes	Yes	2	3
21	Yes	Positive	65	F	Yes	Yes	1	5
22	Yes	Positive	41	M	Yes	Yes	2	5
23	Yes	Positive	29	F	Yes	No	10	2
24	Yes	Positive	42	M	No	No	7	2
25	Yes	Positive	19	M	Yes	No	1	5
26	Yes	Positive	32	M	Yes	No	0,5	2
27	Yes	Positive	25	F	Yes	Yes	2	4
28	Yes	Negative	51	M	No	No	5	3
29	Yes	Negative	42	F	No	Yes	4	3
30	Yes	Negative	60	F	Yes	Yes	9	2
31	Yes	Negative	23	F	No	Yes	7	2
32	Yes	Negative	17	F	Yes	Yes	8	2
33	Yes	Negative	35	F	Yes	Yes	7.5	2
34	Yes	Negative	36	F	No	Yes	6	4
35	Yes	Negative	23	F	Yes	Yes	3	3
36	Yes	Negative	29	N	No	No	6	2
37	Yes	Positive	25	F	Yes	Yes	1	2
38	Yes	Positive	19	M	Yes	No	9	2
39	Yes	Positive	24	F	No	No	4	2
40	Yes	Positive	39	F	Yes	Yes	6.5	3
41	Yes	Positive	29	F	No	No	2	2
42	Yes	Positive	17	M	No	No	3.1	2
43	Yes	Positive	39	M	No	No	5	2
44	No	Negative	56	F	No	No	5	4
45	No	Negative	25	M	No	No	1	3
46	No	Negative	21	F	No	Yes	5	2
47	No	Negative	25	F	No	Yes	5	3
48	No	Negative	27	F	No	Yes	4	4
49	No	Negative	28	F	No	No	5	2
50	No	Positive	27	F	No	No	1	3
51	No	Positive	44	F	No	Yes	5	4
52	No	Positive	29	M	No	No	5	2
53	No	Positive	37	F	No	Yes	7	2
54	No	Positive	23	M	Yes	No	1	5

Table 2 - Presence of the HLA-DQB1*0602

	Healthy subjects (H)	Narcolepsy with cataplexy (T)	p
Presence of the HLA-DQB1*0602	4	29	< 0.0001
Absence of the HLA-DQB1*0602	20	14	
	Healthy subjects (H)	**Narcolepsy without cataplexy (A)**	
Presence of the HLA-DQB1*0602	4	6	0.03
Absence of the HLA-DQB1*0602	20	5	
	Healthy subjects (H)	**Idiopathic hypersomnia (I)**	
Presence of the HLA-DQB1*0602	4	6	0.04
Absence of the HLA-DQB1*0602	20	8	
	Narcolepsy with cataplexy (T)	**Narcolepsy without cataplexy (A)**	
Presence of the HLA-DQB1*0602	29	6	0.63
Absence of the HLA-DQB1*0602	14	5	
	Narcolepsy with cataplexy (T)	**Idiopathic hypersomnia (I)**	
Presence of the HLA-DQB1*0602	29	6	0.26
Absence of the HLA-DQB1*0602	14	8	
	Narcolepsy without cataplexy (A)	**Idiopathic hypersomnia (I)**	
Presence of the HLA-DQB1*0602	6	6	0.9
Absence of the HLA-DQB1*0602	5	8	

without narcolepsy. The two disorders share several common features[5,6]. There is no difficulty of differential diagnosis between narcolepsy with cataplexy and narcolepsy without cataplexy. ICSD-2 characterizes narcolepsy patients without cataplexy when the patient presents sleepiness along with two or more narcolepsy symptoms. IH is an exclusion diagnosis. Some psychiatric diseases, such as depression, can be associated with excessive sleepiness. An increased prevalence of some HLA allele types has been described in other psychiatric disorders. Depression and other psychiatric disorders are common in narcoleptic and IH patients[10].

Some autoimmune diseases such as Systemic Lupus Erythematosus and Rheumatoid Arthritis present a higher prevalence of the HLA-DQ*0602 and HLA DRB1 alleles, respectively[11]. The correlation of narcolepsy and rheumatoid arthritis with some HLA alleles could suggest a similar pathophysiology for both diseases. Clinical and laboratory differences suggest a possible pathphysiology discrepancy between the subgroups of patients with narcolepsy[11,12].

In clinical practice, an important step to make differential diagnoses is to genotype the HLA-DQB1*0602 allele in patients with diseases that are similar to narcolepsy such as epilepsy, psychiatric disorders, sleep apnea and/or in patients who take drugs which affect REM sleep. Some patients taking antidepressives drugs, dopamine agonists, and other drugs can simulate characteristics of narcolepsy. Clinical and electrophysiological differentiation could be difficult in these situations[13-15].

The gold standard exam for narcolepsy with cataplexy is Hypocretin-1 dosage, but in patients without cataplexy and in IH, diagnosis is not yet automatic. HLA-DQB1*0602 allele plays a role in increasing information where differential diagnosis is concerned.

Disclosures

Writting group member	Employment	Research grant[1]	Other research grant or medical continuous education[2]	Speaker's honoraria	Ownership interest	Consultant/ Advisory board	Other[3]
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** Modest*
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Note: UNIFESP = Universidade Federal de São Paulo; AFIP = Associação Fundo de Incentivo à Psicofarmacologia; FAPESP = Fundação de Amparo à Pesquisa do Estado de São Paulo.
For more information, see Instructions for authors.

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