Original article

The contribution of HLA molecules to Dupuytren's contracture in a Southeast Brazilian population

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ABSTRACT

Purpose: The aim of the present study was to investigate the HLA phenotype in Dupuytren's contracture (DC) patients in order to verify the correlation of these alleles with risk factors for development of DC in the Brazilian population.

Methods: This was a case-controlled study of 25 DC patients and 443 healthy individuals with no history of HLA-associated diseases. HLA class I and class II typing was performed using the polymerase chain reaction sequence-specific primer method. Results: The HLA-B*18 phenotype was observed in 32% of the patients and 10.5% of controls. However, P values did not remain significant after correction.

Discussion: Although we observed an increased tendency of DC patients to possess the HLA-B*18 allele, the results were not statistically significant after correction. This allele was higher in patients of Italian and/or Spanish ethnicity, localities with frequencies higher than 18.0% and 14.0% respectively. Further investigation with a larger cohort of DC patients is required to confirm the potential role of HLA in this disease.

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Contribuição das moléculas de antígeno de histocompatibilidade leucocitária (HLA) para a contratura de Dupuytren em uma população do Sudeste do Brasil

RESUMO

Palavras-chave:
Contratura de Dupuytren
HLA
Complexo principal de
  histocompatibilidade
Associação da doença
Imunogenética

Objetivo: O objetivo deste estudo foi investigar o fenótipo do HLA em pacientes com contratura de Dupuytren (CD) para verificar a correlação desses alelos com os fatores de risco para o desenvolvimento da CD na população brasileira.

Métodos: Este foi um estudo de caso-controle de 25 pacientes com CD e 443 indivíduos saudáveis sem histórico de doenças associadas ao HLA. As tipagens classe I e classe II do HLA foram feitas utilizando o método iniciador de sequências específicas da reação em cadeia da polimerase. Resultados: O fenótipo HLA-B*18 foi observado em 32% dos pacientes e 10,5% do grupo controle. Contudo, os valores de p não permaneceram significativos após correção.

Discussão: Apesar de termos observado um aumento na tendência de os pacientes com CD terem o alelo HLA-B*18, os resultados não foram estatisticamente significativos após correção. Esse alelo foi maior em pacientes de etnia italiana e/ou espanhola, locais com frequências superiores a 18% e 14%, respectivamente. São necessárias investigações adicionais com uma coorte maior de pacientes com CD para confirmar o possível papel do HLA nessa doença.

Introduction

Dupuytren's contracture (DC) is a connective tissue disease that presents abnormal fibroblastic proliferation of the palmar fascia. It is characterized by the formation of nodules and fibrous cords causing longitudinal digital contracture. This leads to deformities that determine the characteristic anatomical features of the disease and, in an advanced stage, can cause loss of motor function of the hand.[1,2] Although there is a high rate of recurrence of the lesions, the most effective treatment is surgical excision.[3] Many risk factors have been associated with DC, such as smoking, alcohol, local trauma, epilepsy, and diabetes mellitus.[4-7] The incidence of DC is higher in Caucasians of European origin, predominantly affecting men over 50-years old.[5] An autoimmune component for DC was proposed in 1972, and studies have confirmed the presence of antibody anti-collagen, but to date the exact etiology of DC has remained unknown.[8-10]

Genetic susceptibility to DC is supported by epidemiological observations, as its prevalence is quite high in people of Northern European origin and is only rarely seen in African and Asian populations. Observations from twin and family studies, examining Dupuytrens diathesis, in particular, have also supported a genetic basis.[11,12] Several studies were performed to identify candidate genes for susceptibility to DC, including the cytokine transforming growth factor-β (TGF-β)[13-15]; the transcription factors Zf9[16], Mafb[17] and Zic1[18]; matrix metalloproteinases (MMPs)[19]; and nucleotide oligomerization domain/caspase recruitment domain (NOD/CARD) genes.[20] Although there is evidence of genetic predisposition to DC development, no susceptibility gene has been recognized as a risk factor for the disease. It is unclear whether DC is a simple monogenic Mendelian disorder or a complex oligogenic condition[10]. Due to the late onset of symptoms, it is difficult to observe the positive family history in many patients, suggesting that the role of genetics in DC can be greater than the observed.[16,21]

After the discovery and description of the functions of the Major Histocompatibility Complex (MHC), Histocompatibility Leukocyte Antigens (HLA) in humans, several associations with different diseases have been described, which are linked to particular characteristics of this complex. The MHC is the most polymorphic genetic system in the human genome that participates in antigen presentation to T lymphocytes. The T cells are activated by specific peptides presented by HLA molecules, resulting in a specific immune response. Variations in responses to antigens have implicated the MHC as a factor in disease susceptibility.[10,22] MHC diversity does not change over time in an individual, but alleles may differ significantly between individuals, making the MHC complex a promising biomarker target.[20] Regarding this, studies have associated autoantibodies with variation in genes involved in antigen presentation. Pereira et al. reported that auto-antibodies to denatured collagen type II were more prevalent in HLA-DR4-positive DC patients than in the control population.[23] In contrast, Neumüller et al. found the association of HLA-DR3 and auto-antibodies in the connective tissue of DC patients.[24] However, Spencer et al. observed a higher (not statistically significant) incidence of HLA-DR4 in individuals with DC than in those without it. They also found that HLA-DR3-positive DC patients almost always carried HLA-A1 and HLA-B8 alleles, indicating that the HLA A1-B8-DR3 haplotype, which has also been associated with other autoimmune disorders, could be important in DC.[25] These results provided evidence of an immunogenic component, although no specific HLA allele has been linked to the disease.

The recent study by Brown et al. revealed significant association of the frequency of HLA-DRB1*15 with the pathogenesis of DC in Caucasian patients of European origin.[10]

Although the above results are controversial, the literature suggests that this disease is multifactorial in origin, with familial traits. There are no genetic studies of DC in the Brazilian population, which is characterized by major miscegenation according to Parra et al.[26] Thus, the purpose of this study was to identify the frequency of HLA class I (loci A* and B*) and class II (loci DRB1* and DQB1*) in DC patients in the Brazilian population in a case-controlled model and to investigate HLA allele as a risk factor for disease development.

Methods

Patients and controls

Twenty-five unrelated patients diagnosed with DC (17 men and 8 women), with a mean age of 54.24 years (range: 40–70 years old) from Bauru and Jaú cities of São Paulo State-SP, Southeast of Brazil, were enrolled in this study. The classification of ancestry was based on the phenotype through interviews in the approach to patients. The sample size calculation was not applied, due the rarity of this disease in the population studied, thus all of the patients with DC and who agreed to participate were included in the study. All of them were diagnosed by physicians specialized in hand surgery at the Lauro de Souza Lima Research Institute (ILSL), Bauru-SP. The control group was composed of 443 healthy individuals with no history of HLA-associated diseases (200 men and 243 women), with a mean age of 37.65 years (range: 20-68 years old). They were matched for ethnic background and other demographic patterns. All samples were typed at the Immunogenetics Laboratory of the ILSL, after the participants signed informed consent forms, and the study was approved by the ILSL's Ethics Committee on research involving human beings.

Typing of the HLA alleles

Genomic DNA was extracted using the salting-out method.[27] The HLA alleles, class I locus A* (A*01-74) and B* (B*07-81), class II DRB1* locus (DRB1*01-18), and DQB1* (DQB1*02-09) were typed using the polymerase chain reaction sequence-specific primer (One-Lambda, Canoga Park, CA, USA).

Statistical analysis

HLA class I and class II alleles were counted to determine allelic frequencies. The distribution of these frequencies was evaluated for Hardy–Weinberg disequilibrium using Arlequin software, version 3.1.[28]

The analysis of associations of HLA with DC was achieved using the chi-square test with Yates' correction or Fisher's exact test. P-values ≤ 0.05 were considered statistically significant. Odds ratio (OR) was calculated with a 95% confidence interval (95% CI). P values were corrected (Pc) by multiplying the P value by the number of antigens tested (Bonferroni test), with 5% significance level, using the statistical program SISA (http://home.clara.net/sisa/). The power of the study was calculated using the software OpenEpi version 3 with bilateral confidence interval of 95% (http://www.openepi.com/v37/Power/PowerCC.htm).

Results

This study compared HLA allele frequencies of DC patients with healthy controls in a southeast region of Brazil.

Table 1 shows that 68% (17) of the patients were men and these 76.5% (13) were aged 50 years or older. Manifestation of DC in men was at a mean age of 52 years (range: 40–63 years old) while disease onset occurred latter in women, with the mean age of 57.9 years (range: 46–74 years old). With respect to manual activities, 68% (17) of patients reported high levels of moderate-to-heavy activities. From the total of patients studied, 44% (11) had both their hands affected by the disease, and from these 63.6% (7) were men. Of the 25 patients, 24 were of European ancestry, with 70.8% being of Italian descent (17).

Direct counting provided the frequency of class I and class II alleles, and their distribution was confirmed by the Hardy–Weinberg equilibrium using Arlequin 3.1 software. The HLA class I and class II allele frequencies in both DC and the control group are showed in Table 2-5. For the majority of HLA-A,-B, DR and DQ allele frequencies were found to be similar in both groups. However the frequency of HLA-B*18 allele was found in 32.0 % (8) of the 25 of DC patients compared to 10.5% (46) of the non-affected controls (P = 0.003, OR: 4.02 and CI 95%: 1.64–9.83), although P values were not significant after correction by Bonferoni test multiplying the P value by the number of antigens, as shown in Table 3.

Discussion

Although DC has been described for centuries, its etiology and pathology remain poorly understood.[29] To date, several environmental risk factors have been implicated in DC, including smoking, alcohol intake, diabetes, epilepsy, and trauma. Many patients with DC believe that their condition was caused by heavy labor or trauma. Dupuytren, himself, initially believed this to be the cause of the disease because many of the patients he studied were laborers.[12] In the present study, moderate-to-heavy manual activity was predominant.

Epidemiological studies indicate a high prevalence of DC among Northern European Caucasian men aged 50 years or older, and this is cited as the most common inherited connective tissue disorder in this population.[2,9,30] Previous studies have shown that men are more commonly affected than women, with the disease presenting at an earlier age. Our study also found that most patients were men aged above 50 years, and the mean age of disease manifestation in women was higher than in men, furthermore bilateral disease were more common in men, in agreement with the literature.[31]

Although there is evidence of genetic involvement on the onset of DC, there is no consensus regarding the specific role of genetics in susceptibility to DC. To date, no specific gene has been described as a risk factor for this disease, although some studies have been conducted to elucidate the role of genetics in the immunopathogenesis of the disease.[10,32,33]

Currently, it is known that T lymphocytes recognize antigens when presented with HLA molecules on the surface of antigen presenting cells, where a specific immune response is initiated, such as the production of interleukins, proliferation,

Table 1 – Characterization of patients with Dupuytren´s contracture in the study.

Patient	Gender	Age	Ocupation	Ancestry
1	M	40	cutlery	Spanish
2	M	50	farmer	Portuguese
3	F[a]	49	housewife	German/ Portuguese
4	M	46	farmer	Italian
5	M	50	professor	Portuguese
6	M[a]	43	driver	German/Italian
7	M	50	bricklayer	Spanish
8	M	55	economist	Italian/Spanish
9	M	55	machine operator	Italian/Brazilian
10	F[a]	63	housewife	Brazilian
11	F	46	sales representative	Italian/Portuguese
12	M	63	farmer	Italian/Portuguese
13	F	74	farmer	Italian/Brazilian
14	M[a]	55	businessman	Italian/Brazilian
15	F[a]	57	housewife	Italian
16	F	64	housewife	Italian
17	M	58	farmer	Italian/Spanish
18	F	47	shoemaker	Italian/Brazilian
19	M[a]	46	guard	Italian
20	M	63	businessman	Spanish
21	F	63	housewife	Italian
22	M[a]	57	driver	Italian
23	M[a]	50	vigilant	German
24	M[a]	51	banking	Italian/Chinese
25	M[a]	52	designer	Italian/Brazilian

M, male; F, female.
[a] Bilateral disease.

Table 2 – HLA-A* allele frequency in patients with Dupuytren´s contracture patients and control group.

Alleles	Dupuytren´s contracture (N = 25)		Control group (N = 443)		p
	n	Fa (%)	n	Fa (%)	
A*01	08	32.0	79	18.0	0.107
A*02	07	28.0	203	46.0	0.098
A*03	02	8.0	71	16.0	0.142
A*11	04	16.0	47	10.7	0.163
A*23	00	0.0	44	10.0	0.077
A*24	07	28.0	66	15.0	0.089
A*25	02	8.0	15	3.4	0.174
A*26	00	0.0	40	9.1	0.098
A*29	03	12.0	41	9.3	0.225
A*30	03	12.0	51	11.5	0.245
A*31	00	0.0	33	7.5	0.150
A*32	04	16.0	25	5.7	0.060
A*33	00	0.0	24	5.5	0.255
A*34	00	0.0	07	1.6	0.676
A*36	01	4.0	04	0.9	0.217
A*66	01	4.0	07	1.6	0.296
A*68	02	8.0	46	10.4	0.265
A*69	00	0.0	00	0.0	1.000
A*74	00	0.0	01	0.2	0.946
Blank	06	24.0	71	16.0	0.118

N, number of individuals; n, number of alleles; Fa, frequency of alleles; p value, Fisher test p ≤ 0.05.

and control of effector molecules, and regulatory functions of lymphocytes.[34]

Nowadays, many diseases have established genetic links with the HLA complex, such as narcolepsy and HLA-DR2, rheumatoid arthritis and HLA-DR4 [35,36], hemochromatosis and HLA-A3, psoriasis and HLA-Cw6[37], multiple sclerosis and HLA-DR2, and celiac disease and HLA-DR3.[38] The mechanisms of these associations with the particular alleles are still not fully understood. It is believed that for some of these diseases there is a failure in the expression of the HLA molecule, resulting in disabled molecules. In other cases, defects in other genes may influence the expression of HLA molecules on the cell surface.[39,40]

According to Ottenhoff et al.,[41] the same antigens are recognized in individuals who fall ill and healthy individuals, but there are differences in the ability of the individuals to re-

Table 3 – HLA-B* allele frequency in patients with Dupuytren´s contracture and control group.

Alleles	Dupuytren´s contracture (N = 25)		Control group (N = 443)		p
	n	Fa (%)	n	Fa (%)	
B*07	01	4.0	57	13.0	0.122
B*08	05	20.0	50	11.3	0.099
B*13	01	4.0	10	2.3	0.346
B*18	08	32.0	46	10.5	0.003[a]
B*27	01	4.0	18	4.1	0.385
B*35	04	16.0	104	23.5	0.142
B*37	00	0.0	10	2.3	0.571
B*38	01	4.0	23	5.2	0.368
B*39	04	16.0	20	4.5	0.082
B*41	00	0.0	14	3.2	0.455
B*42	00	0.0	10	2.3	0.571
B*44	04	16.0	85	19.2	0.199
B*45	00	0.0	14	3.2	0.455
B*46	01	4.0	00	0.0	0.060
B*47	00	0.0	01	0.2	0.946
B*48	00	0.0	01	0.2	0.946
B*49	02	8.0	25	5.7	0.263
B*50	00	0.0	21	4.8	0.304
B*51	04	16.0	79	17.8	0.211
B*52	02	8.0	28	6.4	0.278
B*53	00	0.0	12	2.7	0.510
B*54	00	0.0	00	0.0	1.000
B*55	00	0.0	15	3.4	0.430
B*56	00	0.0	02	0.4	0.895
B*57	01	4.0	13	3.0	0.374
B*58	00	0.0	06	1.4	0.715
B*60	01	4.0	18	4.1	0.385
B*61	01	4.0	15	3.4	0.383
B*62	02	8.0	33	7.5	0.289
B*63	01	4.0	15	3.4	0.383
B*64	00	0.0	09	2.0	0.604
B*65	02	8.0	38	8.6	0.287
B*70	01	4.0	23	5.2	0.368
B*75	00	0.0	01	0.2	0.946
B*76	00	0.0	00	0.0	1.000
B*81	00	0.0	07	1.6	0.676
Blank	04	16.0	55	12.5	0.195

N, number of individuals; n, number of alleles; Fa, frequency of alleles; p value - Fisher test p ≤ 0.05.
[a] Odds Ratio (OR): 4.02, Confidence Interval (IC 95%): 1.64-9.83, Pcorrected (Pc) = 0.11.

Table 4 – HLA-DRB1* allele frequency in patients with Dupuytren´s contracture and control group.

Alleles	Dupuytren´s contracture (N = 25)		Control group (N = 443)		p
	n	Fa (%)	n	Fa (%)	
DRB1*01	03	12.0	83	18.7	0.164
DRB1*04	05	20.0	85	19.2	0.200
DRB1*07	07	28.0	107	24.2	0.164
DRB1*08	03	12.0	55	12.4	0.244
DRB1*09	00	0.0	14	3.2	0.458
DRB1*10	00	0.0	22	5.0	0.290
DRB1*11	10	40.0	123	27.8	0.073
DRB1*12	00	0.0	14	3.2	0.458
DRB1*13	05	20.0	112	25.3	0.166
DRB1*14	02	8.0	34	7.7	0.289
DRB1*15	07	28.0	76	17.2	0.078
DRB1*16	02	8.0	32	7.2	0.287
DRB1*17	05	20.0	69	15.6	0.172
DRB1*18	01	4.0	10	2.3	0.344

N, number of individuals; n, number of alleles; Fa, frequency of alleles; p value - Fisher test p ≤ 0,05.

Table 5 – HLA-DQB1* allele frequency in patients with Dupuytren´s contracture and control group.

Alleles	Dupuytren´s contracture (n = 25)		Control group (n = 443)		p
	n	Fa (%)	n	Fa (%)	
DQB1*02	12	48.0	180	40.7	0.125
DQB1*04	03	12.0	56	12.7	0.243
DQB1*05	09	36.0	154	34.8	0.167
DQB1*06	10	40.0	213	48.2	0.121
DQB1*07	11	44.0	147	33.2	0.090
DQB1*08	03	12.0	41	9.3	0.224
DQB1*09	02	8.0	12	2.7	0.135

N, number of individuals; n, number of alleles; Fa, frequency of alleles; p value-Fisher test p ≤ 0,05.

spond to these proteins. It is known that HLA varies according to patients' ethnicity and geographic location. Brazil shows an extremely mixed ethnicity spread across a large geographic region. Because of these peculiarities, it is extremely important to study the correlation of HLA alleles and DC in this population.[42]

Discordant or non-replicated results for the association of HLA with DC appear in the literature. Shih et al.[29] suggested a possible role of the ancestral haplotype HLA A1-B8-DR3 in DC. Studies by Neumüller et al.[24] and Brown et al.[10] describe that HLA-DR3 and HLA-DRB1*15 respectively are risk factors for DC; however, this genetic association has not yet been pathophysiologically explained.

The results of the present study do not confirm the findings described above, once we found increased of the HLA-B *18 in patients with DC when compared to the low incidence in the control group in a Brazilian population. Although these data lose significance after statistical correction, the increased frequency of this allele in individuals with DC should not be neglected, given the small number of patients studied. However, these results cannot be interpreted as lack of effect since the study had a power of 100%.

The disagreement between the results obtained in this study and the literature data may originate from the genetic background of patients, as well as the different ethnic groups and geographic regions studied, as also the technique used to determine the HLA specificities.[42]

The HLA-B* 18 allele is found in Italian and Spanish population with a frequency higher than 18.0% and 14.0%, respectively, however in the Brazilian population this allele is observed only in 8.0%.[43] It is interesting to note that of the 25 patients studied, 20 were of Italian and/or Spanish descent, which could suggest an association of ethnicity with a higher incidence of this disease, although our results do not show a strong association of this allele with DC.

Future studies with a larger cohort of DC patients could confirm the potential role of HLA in this disease and help to understand the immunopathogenesis as well as to contribute for the development of models for diagnosis, prevention, and appropriate treatment regimens for patients.

Conflicts of interest

The authors declare no conflicts of interest.

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