

Association of HLA-DRB1*01 with Dupuytren's disease

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Objectives: To explore the human leucocyte antigen (HLA)-DRB1 allele frequency in Dupuytren's disease (DD).

Method: HLA-DRB1 genotypes were analysed by sequence-specific primers (SSPs) in samples collected from 172 men participating in a nested case–control study on the clinical manifestations and progression of DD. Of those, 121 had signs of DD while 51 did not. Of the 121 men with DD, 49 had contracted fingers or had been operated on, while 72 had nodules or fibrous cords in the palms. Odds ratios (ORs) and 95% confidence interval (CIs) were used to evaluate the results.

Results: The HLA-DRB1*01 allele was observed in 26 of the 121 affected men (23.7%) but in only four of the controls (7.8%) (OR 3.22, 95% CI 1.06–9.75). The HLA-DRB1*01 allele frequency in those affected was 11%, while in the control group it was 4% (OR 3.07, 95% CI 1.05–9.03).

Conclusions: This observation indicates a possible association of HLA-DRB1*01 with DD, but further studies are needed for confirmation.

Dupuytren's disease (DD) is one of the most common connective tissue disorders in humans, affecting about one in five males of northern European ancestry (1). Immunological deviations have been described in DD patients, involving both the humoral and the cellular immune system (2, 3). It is also known that in some cases the disease may have a strong familial predisposition (4, 5). The prevalence of human leucocyte antigen (HLA)-DR3 (HLA-DRB1*03) has been shown in relatively small studies to be increased in DD patients with raised levels of autoantibodies (6). An association with HLA-DR4 has also been reported (7), especially with the coexistence of antibodies to collagen type II (8), and more recently, an association between the HLA-DRB1*15 genotype and DD in 67 Caucasians patients has been revealed (9). The aim of the present study was to investigate the association between HLA-DRB1 genotypes and DD in a well-defined randomly selected population cohort.

Method

A large-scale population-based health survey (The Reykjavík Study) was started in 1967 by the Icelandic Heart Association. Participants in the study were males and females born in the years 1907 to 1935 with residence in the Reykjavík area of Iceland. Along with the main

survey, several smaller studies have been carried out focusing on other disorders than cardiovascular diseases. These include diabetes mellitus, malignancy and DD. In 1981–82 a total of 1297 males were examined for the presence of DD. Of these, 249 (19.2%) were found to have clinical signs of DD (1).

A nested case–control study was carried out on those participants of the 1981–82 study who were still alive and willing to participate in a follow-up study of DD (10). An equally large group of symptom-free men, matched for age and smoking habits, from the 1981–82 study were invited to participate as controls. The participants were invited by letter, and those not responding were contacted by telephone. The participants answered a structured questionnaire about past and present health status, with special reference to DD, family history and known risk factors of this condition. The same physician examined all participants. The hands and feet were evaluated for clinical signs of DD. The hands were graded as follows: (a) those with fibrotic nodules or fibrous cords in the palms were classified as having stage 1 DD and (b) those with contracted fingers or operated on for DD were classified as stage 2 of the disease. The present study included 172 men, 121 with clinical signs of DD and 51 without any clinical signs of DD.

Blood samples, including ethylenediaminetetraacetic acid (EDTA) blood, were collected from the study participants. DNA was extracted from the samples for HLA typing. The method chosen for the HLA typing was polymerase chain reaction (PCR) with sequence-specific primers (SSPs; Invitrogen, Carlsbad, CA, USA).

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Accepted 17 July 2012

Table 1. HLA-DRB1 allele frequency in patients with Dupuytren's disease (DD) and matched controls.

HLA-DRB1*	Controls (n = 51)		DD patients (n = 121)		p-value
	n (%)	Allele frequency	n (%)	Allele frequency	
01	4 (7.8)	0.04	26 (23.7)	0.11	0.02
03	7 (13.7)	0.07	21 (15.3)	0.09	0.37
04	16 (31.4)	0.17	44 (36.4)	0.21	0.22
07	13 (25.5)	0.14	20 (17.8)	0.09	0.11
08	10 (19.6)	0.10	12 (10.2)	0.05	
09	4 (7.8)	0.04	8 (6.8)	0.03	
10	0	0.00	2 (1.7)	0.01	
11	1 (2.0)	0.01	6 (5.1)	0.02	
12	3 (5.9)	0.03	7 (5.9)	0.03	
13	18 (35.3)	0.20	36 (29.7)	0.17	0.30
14	2 (3.9)	0.02	0	0.00	
15	19 (37.3)	0.20	43 (35.6)	0.19	0.47
16	0	0.00	1 (0.8)	0.00	

n refers to the number of subjects with the given HLA-DRB1* allele. The percentage (%) within parentheses shows the proportion of subjects with that given allele in either a homozygous or a heterozygous state. For example, 13 of the 51 control subjects were found to have the HLA-DRB1*07 allele or 25.5%. Most individuals were compound heterozygous. Allele frequency was calculated from the total number of alleles observed. Twelve were heterozygous and one was homozygous for HLA-DRB1*07. Hence the allele frequency $14/102 = 0.14$.

Statistical evaluation was performed, applying Fisher's exact test, and is presented as odds ratios (ORs) and 95% confidence intervals (CIs). This was only estimated for HLA-DRB1 groups with at least 10% ($n > 12$) of the DD patients. The investigation was retrospective and designed with a hypothesis-generating approach and p-values (one-sided) were not corrected for multiple testing (11, 12). The study was approved by the State Medical Ethical Committee and the State Data Protection Committee.

Results

Of the 172 participants of this study, 121 had signs of DD while 51 had no signs of the disease. Of the 121 participants with signs of DD, 72 had nodules or fibrous cords in the palms of the hands (stage 1 disease) while 49 had contracted fingers or had been operated on due to DD (stage 2 disease).

The HLA-DR1 allele frequencies in both DD patients and the control group are summarized in Table 1. For the majority of HLA-DRB1*01 groups allele frequencies were found to be similar in both groups. The HLA-DRB1*01 allele was found in 26 (23.7%) of the 121 of DD patients, of whom one was homozygous, compared to four (7.8%) of the non-affected controls who all were heterozygous (OR 3.22, 95% CI 1.06–9.75). The HLA-DRB1*01 allele frequency in those affected was 11%, while in the control group it was 4% (OR 3.07, 95% CI 1.05–9.03). The previously reported association of HLA-DRB1*15 with DD was not replicated. HLA-DRB1*15 allele frequency in the DD group was 35.6%, and 37.3% in the control group.

Discussion

We have previously described an increase in activated HLA-DR-positive T lymphocytes in the peripheral blood of DD patients (2) and various inflammatory cells are

Table 2. Studies analysing the associations between HLA and Dupuytren's disease (DD).

Study (year)	Reference	No. of patients	HLA class analysed	Association observed
Hunter et al (1981)	14	31	HLA-A, B, C	No association observed
		20	HLA-DR	No association observed
Tait and Mackay (1982)	15	100	HLA-A, B	Positive association with HLA-B12
Williams et al (1983)	16	40	HLA-A, B, DR	No association observed
Spencer and Walsh (1984)	7	37	HLA-A, B, C, DR	Positive association with HLA-A1 and DR4
				Also positive with haplotype HLA-DR3, A1, B8
Pereira et al (1986)	8	16	HLA-A, B, DR	Increase of HLA-DR4 if anti-collagen type II antibodies were elevated
Neumuller et al (1994)	6	33	HLA-A, B, C, DR	Positive association with HLA-DR3, especially if elastin or collagen antibodies were also elevated
Brown et al (2008)	9	67	HLA-DRB1*01-16	Positive association with HLA-DRB1*15 (HLA-DR2)
Present study (2011)		118	HLA-DRB1*01-16	Positive association with HLA-DRB1*01 (HLA-DR1)

found in affected DD tissue, including large numbers of HLA-DR-positive T lymphocytes (3). The results of the current study show that the HLA-DRB1*01 allele is found more frequently in men with DD than in those with no signs of the disease. In Table 2 seven previously published association studies of HLA in Dupuytren's disease since 1981 are summarized. In four studies different associations between HLA-DR and DD have been observed (6–9). Spencer and Walsh reported an association between DD and the haplotype HLA-DR3, A1, B8 (7). An association with HLA-DR3 was also noted for patients with raised levels of antibodies against elastin and/or collagen (6). In another study a positive association with HLA-DR4 was found for DD patients with elevated anti-collagen type II antibodies (8). In the present study we did not test for elastin or collagen auto-antibodies, but it should be noted that the overall prevalence of HLA-DR4 (HLA-DRB1*04) and HLA-DR3 (HLA-DRB1*03) was very similar in the DD patients and healthy controls (Table 1). Furthermore, in a previous study we found the prevalence of positive rheumatoid factor to be lower in DD patients than in healthy controls (13). Thus, both our previous and present findings do not support a strong positive association between the HLA-DR3 or DR4 antigens, autoantibodies and DD. In several of these studies relatively few patients were studied. The present study was also only moderately powered to confirm an association of HLA with DD, but as with all previous investigations in this field as cited in Table 2, this was first and foremost designed as an exploratory exercise in a well-defined and homogeneous population. Several approaches for correcting for multiple testing in HLA studies have been proposed including hierarchical testing, but in the phase of early investigation too strict significance levels might mask any potential association (12). Of note, Brown et al (9) reported a positive association of HLA-DRB1*15 with DD in Caucasians. The strength of our present study is that it is based on a population cohort and is the largest to date. Our main finding of a putative association between DD and HLA DRB1*01 rather than HLA DRB1*15 suggests that the HLA-DRB1 genes do not directly determine disease susceptibility. It is more likely that if a true association exists, it describes a linkage disequilibrium with the DRB1 locus, and a large population-based approach may be needed to confirm this finding.

Acknowledgements

We thank N Sigfússon and the Icelandic Heart Association for support in carrying out this study.

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