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Common variants of the *ALDH2* and *DHDH* genes and the risk of Dupuytren's disease

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Abstract
The object of this study was the investigation of four common variants of single nucleotide polymorphisms of the aldehyde dehydrogenase H2 gene and dihydrodiol dehydrogenase gene and their association with the occurrence of Dupuytren's disease. DNA samples were obtained from the peripheral blood of 300 consecutive patients. The control group comprised 300 healthy adults who were age matched with the Dupuytren's patients. All four common variants were analysed using TaqMan® genotyping assays and sequencing. The differences in the frequencies of variants of single nucleotide polymorphisms in patients and the control group were statistically tested. No significant differences were found in the frequencies of the variants of the aldehyde dehydrogenase H2 and dihydrodiol dehydrogenase genes between the groups. Likewise, no significant differences were found in the frequencies of the variants of the genes between men and women. We noted a statistically significantly higher prevalence of the variants of the dihydrodiol dehydrogenase gene (*rs2270941* and *rs11666105*) in the group with a positive familial history of Dupuytren's disease, in comparison with the group with a negative familial history.

Keywords
Dupuytren's disease, aetiology, genetics, snp genotyping

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Introduction

The prevalence of Dupuytren's disease (DD) is related to geography and race. It is more common in Northern Europe and affects whites more than any other ethnicities (Gudmundsson et al., 2000). Family clusters affected by the disease have been identified as well as chromosome abnormalities, suggesting a possible genetic component (Bonnici et al., 1992). A positive familial history has been noted in 41% of DD patients by the use of the sibling recurrence risk method, suggesting a familial aggregation of the disease (Hindocha et al., 2006). Other genetic investigations have indicated an autosomal dominant pattern of inheritance, with variable penetrance of the predisposing genes (Bonnici et al., 1992; Burge 1999; Satish et al., 2008). However, no single gene has yet been identified and it is thought that DD may have a complex aetiology, arising from a combination of environmental and multiple genetic factors; in this type of genetic predisposition, the occurrence of the disease depends on the simultaneous presence of multiple alleles (Burge, 1999; Capstick et al, 2012; Kloen, 1999).

Dysregulation of specific genes may have an effect on the characteristics of fibroblasts in the palmar aponeurosis, leading to their progressive differentiation into myofibroblats and over-production of type III

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collagen. Pan et al. (2003) found 23 genes to be significantly dysregulated in fibroblasts from DD palmar aponeurosis compared with fibroblasts from the normal palmar fascia. Recent studies have revealed as many as 55 different genes with a dysregulated expression profile (Michou et al., 2012). This included two genes involved in alcohol metabolism: the aldehyde dehydrogenase H2 gene (*ALDH2*) and the dihydrodiol dehydrogenase gene (*DHDH*). It is believed that lack of expression of these two genes may be associated with the possible increased incidence of DD in alcohol abusers (Pan et al. 2003). The *ALDH2* gene has been identified as a focus gene and listed as a target in various signalling cascades through the Ingenuity Knowledge Based Analysis (Satish et al., 2008).

There is continuing interest in identifying low penetrance genes that are associated with an increased susceptibility to common diseases. There are several approaches to this problem, including the use of single nucleotide polymorphism (SNP) arrays to interrogate a large number of genes simultaneously and preselecting candidate genes of interest. Single nucleotide polymorphism is a DNA alteration (point mutation) in which a single nucleotide is deleted or replaced by another nucleotide within the coding sequences of genes, non-coding regions of genes or in the intergenic regions. Some SNPs may change the structure of the protein produced by the gene resulting in its dysfunction and a subsequent disease. Other SNPs may influence gene expression (i.e. the activity of the gene measured by the amount of its RNA or protein product). An abnormal amount of normal protein can lead to a disease (e.g. overexpression of the oncogene or down-regulation of tumour suppressor gene can lead to the development of neoplasm). This methodology is widely used in studies on hereditary neoplasms and familial aggregations of malignancies.

The object of this study was to investigate four common variants of single nucleotide polymorphisms of the *ALDH2* and *DHDH* genes and their association with the occurrence of Dupuytren's disease in a series of consecutive patients and controls from the authors' native population.

Patients and methods

Patients and controls

Over a period of 4 years (2008–2011), 300 patients with DD, 253 men (84%) and 47 women (16%) with a mean age of 56 years (range 38–76) were recruited. One hundred and twelve patients had undergone surgery for DD and were invited to participate by mail, whereas 188 were recruited during their stay in hospital for operation. The approval of the Bioethical Council of the local Medical University was obtained and informed consent was taken from all participants before enrolment. During an interview the goals of the study were explained, genetic counselling was given and a blood sample was taken for DNA analysis. A detailed family history and the duration of DD were recorded. A neoplasm risk factor questionnaire was also completed.

The control group comprised 300 healthy adults, 200 men and 100 women with a mean age of 53 years (range 36–74) who were age matched (+/−2 years) with the DD patients. The healthy adults were assessed as having a negative family history for cancer after answering a questionnaire about their family medical history, which was part of a population-based study of the 1.5 million residents of West Pomerania province to identify familial aggregations of malignancies. However, these participants were not reviewed for the presence of DD and their (possible) familial history of DD was not recorded. This may be considered a weakness in the control group, but, considering the incidence of DD in our region of Europe (approximately 1% in the general population), the likelihood of its occurrence among controls is low, amounting to three cases. A blood sample was taken for DNA analysis from all controls.

Methods

Peripheral blood samples were used as the source of DNA (not tissue samples as was the case in most other studies), because we were looking for germline (inherited) mutations in which DNA changes are present in all the patient's cells, not only in the affected tissue. These hereditary mutations can be identified in patients' family members even when they are free of the disease. All four common variants of single nucleotide polymorphisms were analysed by real-time polymerase chain reaction (PCR), using the LightCycler480 (Roche, Rotkreutz, Switzerland). The analyses were carried out using TaqMan® genotyping assay (Life Technologies Corp, Foster City, CA, USA), consisting of sequence specific primers and oligonucleotide fluorescent labelled probes, which enabled amplification of the examined fragments and further allele discrimination. Two non-synonymous exonic common changes in the *DHDH* gene (*rs2270941–S66N, rs11666105–V247A*) and two common intronic variants of the *ALDH2* gene *(rs7296651 and rs11066028)* were genotyped. Due to technical difficulties and the poor quality of DNA, the genotypic results were not obtained in some cases. In Table 1 the actual number of successful genotypic analysis is given (i.e. *rs2270941* n=291).

Table 1. Frequency of the examined SNPs in Dupuytren's disease patients and controls

DD patients n=300	Controls n=300	Odds ratios and p-coefficients
rs2270941 n=291	**rs2270941 n=291**	
GG 182 (62%)	GG 164 (56%)	Baseline
AG 94 (32%)	AG 108 (37%)	OR=0.8 *p*=0.1
AA 15 (5%)	AA 19 (7%)	OR=0.8 *p*=0.3
rs7296651 n=294	**rs7296651 n=288**	
CC 193 (66%)	CC 193 (67%)	Baseline
CG 93 (32%)	CG 82 (28%)	OR=1.2 *p*=0.2
GG 8 (3%)	GG 13 (5%)	OR=0.6 *p*=0.1
rs11066028 n=292	**rs11066028 n=284**	
AA 156 (53%)	AA 158 (56%)	Baseline
AC 117 (40%)	AC 101 (36%)	OR=1.2 *p*=0.1
CC 19 (7%)	CC 25 (9%)	OR=0.7 *p*=0.2
rs11666105 n=281	**rs11666105 n=282**	
TT 190 (68%)	TT 188 (67%)	Baseline
CT 78 (28%)	CT 79 (28%)	OR=0.9 *p*=0.5
CC 13 (5%)	CC 15 (5%)	OR=0.8 *p*=0.4

A – Adenine, C – Cytosine, G – Guanine, T – Thymine

Statistical methods

The basic statistical analysis included comparison of the four allele frequencies in DD patients (n=300) and in controls (n=300). Over and above this we analysed the frequency of the SNP variants in selected subgroups of patients: men (n=253) vs women (n=47) and with a positive (n=100) vs negative (n=200) familial history of DD, compared to the similar matched controls. A report of at least one first- or second-degree relative of the proband affected with DD was considered to be a positive family history.

For the analysis of the differences in genotype frequencies between the controls and the DD patients, a Chi-squared test was used. Each SNP is considered separately for the generation of individual odds ratios. The odds ratios were expressed in relation to the most common variant, and – if any statistically significant differences were identified – were used together with the 95% CI as an estimation of the relative risk.

Results

The assessed allele distributions for all analysed SNPs were in Hardy-Weinberg equilibrium for both the DD and control groups. The Hardy-Weinberg equilibrium model assumes that both allele/genotype frequencies in a population remain constant from generation to generation, unless specific disturbing factors are introduced. A study population is in Hardy-Weinberg equilibrium if the occurrence of some combinations of alleles or genetic markers is the same frequency as would be expected from a random formation of haplotypes from alleles based on their frequencies.

As shown in Table 1, the frequencies of the *ALDH2* and *DHDH* variants in the unselected DD group when compared with the respective control population failed to reveal any association between the individual SNPs and disease. Next we evaluated the frequencies of the examined variants among men and women but we failed to identify any statistically significant differences (Table 2). Finally, we compared the frequency of SNPs in DD patients having a positive (n=100) vs negative family history (n=200).

We found an increased prevalence of both variants of the *DHDH* gene (*rs2270941* and *rs11666105*) in patients with a positive familial history. The *rs227094_GG* genotype was present in 9/100 familial cases (9%), in 6/187 sporadic cases (3.2%) and in 19/291 of the controls (6.5%). The difference between familial and sporadic DD patients was statistically significant (OR=2.9, *p*=0.04, 95%CI 1.03 to 8.6), whereas between familial patients and controls it was insignificant (OR=1.4, *p*=0.4).

The *rs11666105_CC* genotype was present in 9/100 familial cases (9%), in 4/179 sporadic cases (2.2%) and in 15/282 of controls (5.3%). The difference between familial and sporadic DD patients was statistically significant (OR=4.4, *p*=0.009, 95%CI 1.3 to 14.4), whereas between familial cases and controls it was insignificant (OR=1.8, *p*=0.17). We did not find any differences in the allele distribution of both variants of the *ALDH2* gene among familial, sporadic and control cases.

Table 2. Incidence of the SNPs in men and women in the patients and control groups

Women DD patients n=47	Women controls n=100	Odds ratios p-coefficents	Men DD patients n=253	Men controls n=200	Odds ratios p-coefficents
rs2270941=42	**rs2270941 =41**		**rs2270941=249**	**rs2270941=250**	
GG 21 (50%)	GG 25 (61%)	Baseline	GG 161(65%)	GG 139 (55%)	Baseline
AG 18 (43%)	AG 13 (32%)	OR=1.6 *p*=0.2	AG 76 (30%)	AG 95 (38%)	OR=0.7 *p*=0.09
AA 3 (7%)	AA 3 (7%)	OR= 1.0 *p*=0.5	AA 12 (5%)	AA 16 (6%)	OR=0.7 *p*=0.2
rs7296651=42	**rs7296651 =39**		**rs7296651 =252**	**rs7296651=249**	
CC 23 (55%)	CC 26 (67%)	Baseline	CC 170 (67%)	CC 167 (67%)	Baseline
CG 15 (36%)	CG 11 (28%)	OR=1.4 *p*=0.2	CG 78 (31%)	CG 71 (28%)	OR=1.1 *p*=0.3
GG 4 (9%)	GG 2 (5%)	OR=1.9 *p*=0.2	GG 4 (2%)	GG 11 (4%)	OR=0.3 *p*=0.09
Rs11066028 =42	**rs11066028 =38**		**rs11066028=250**	**rs11066028=246**	
AA 17 (40%)	AA 25 (66%)	Baseline	AA 139 (56%)	AA 133 (54%)	Baseline
AC 18 (43%)	AC 9 (24%)	OR= 2.4 *p*=0.1	AC 99 (40%)	AC 92 (37%)	OR=1.1 *p*=0.3
CC 7 (17%)	CC 4 (10%)	OR= 1.7 *p*=0.2	CC 12 (5%)	CC 21 (8%)	OR=0.5 *p*=0.09
Rs11666105 =38	**rs11666105 =37**		**rs11666105=243**	**rs11666105=245**	
TT 23 (60%)	TT 25 (68%)	Baseline	TT 167 (69%)	TT 163 (66%)	Baseline
CT 14 (37%)	CT 11 (30%)	OR=1.4 *p*=0.3	CT 64 (26%)	CT 68 (28%)	OR=0.9 *p*=0.4
CC 1 (3%)	CC 1 (3%)	OR=1.0 *p*=0.5	CC 12 (5%)	CC 14 (6%)	OR=0.9 *p*=0.4

A – Adenine, C – Cytosine, G – Guanine, T – Thymine

Discussion

The results of our study show that common variants of the *ALDH2* gene are not associated with higher susceptibility to Dupuytren`s disease. However, due to the small number of cases and controls we were unable to determine the prevalence of the SNPs and clinical characteristics of the participants, such as duration of the disease, alcohol consumption, smoking, simultaneous occurrence of co-morbidities etc. Although some of these data was collected (e.g. duration of the disease was a mean of 5 years (range 1–27), 28 DD patients (9%) were diabetics and none had symptomatic liver cirrhosis), we did not investigate the relationship of common variants of the *ALDH2* and *DHDH* genes and these factors. This may be considered a weakness of the present study, but we are able to examine this problem in a future study.

In contrast, our data suggest possible association of both variants of the *DHDH* gene with familial Dupuytren's disease, as over-representation of the *rs2270941* and *rs11666105* alterations was identified among 100 patients with a familial history of the disease. Additionally we observed a tendency of over-representation of the *rs11066028* change in affected women, but with only 47 women examined we cannot exclude a type II statistical error. Thus, it is justifiable to carry out additional studies with a larger number of participants to verify our findings and explore any possible predisposition to DD in a given clinical subgroup.

The onset of DD is associated with abnormal proliferation of fibroblasts, their differentiation into myofibroblasts and excessive production of type III collagen. This process may be induced and maintained by many factors, both environmental and genetic, including alterations of the expression profile of some genes (Howard et al., 2004; Satish et al., 2008). Among others, two metabolic genes, *ALDH2* and *AKR1C1* have been identified by two microarray platforms to be down regulated in the DD palmar fascia, relative to healthy tissue (Satish et al., 2008). It has been suggested that altered expression of these genes might play a role in the initiation and progression of DD. Our findings based on a single nucleotide polymorphism array analysis failed to confirm the relationship between common variants of the *ALDH2* gene and an increased risk of DD. Our method of genetic analysis differed from those used in the studies of Pan et al. (2003) and Satish et al. (2008). These authors analysed gene expression profiles in fibroblasts isolated from the affected tissue, whereas our investigations concerned germline (inherited) mutations, which are present in each body cell. This methodology is widely used in studies on hereditary neoplasms and familial aggregations of malignancies. To our minds, it is more appropriate and reliable and better reflects the actual genetic susceptibility to the disease than analysis of gene expression profiles in the affected tissue.

The next gene investigated in our study was *DHDH*. The product of this gene catalyses the nicotinamide adenine dinucleotide phosphate (**+**) (NADP +) linked oxidation of trans-dihydrodiols of aromatic hydrocarbons to

the corresponding catechols. It plays a role in the detoxification of various oxidative stress molecules, including superoxide and hydroxyl radicals. Toxic free radical production increases with age as a result of localised hypoxia, gradual restriction of capillaries, smoking and other environmental factors. It is suggested that down-regulation of the *DHDH* gene may reduce the ability of fibroblasts to detoxify free radicals, which may induce their transformation into myofibroblasts (Satish et al., 2008). Our findings did not confirm this supposition, but provide a different basis of evidence that two *DHDH* gene variants may be involved in the development of DD, as they were found to be over-represented in patients with a positive familial history for the disease.

Most oncological studies show down-regulation of *ALDH2* and *DHDH* genes to be associated with a higher risk of gastrointestinal tract cancer in alcohol consumers (Chang et al., 2009; Wu et al., 2012). As alcohol abuse has been believed to increase the risk of occurrence of DD, this relationship might confirm the involvement of the *ALDH2* and *DHDH* genes in the development of DD. However, an association between ethanol consumption and Dupuytren's disease has not been definitively proven and remains a matter of speculation. However there is continuing interest in molecular and genetic studies of possible links between diseases in which a hereditary component is proven or highly suspected. Using our institutional register of familial aggregations of malignancies and the same methodology, we have started to investigate the possible association of DD with common malignancies, such as digestive tract cancer, breast cancer and malignant melanoma.

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Conflict of interests

None declared.

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