Genetic Origin of Dupuytren's Disease and Associated Fibromatosis

Guido H.C.G. Dolmans

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Publication of this thesis was financially supported by: Department of plastic surgery University Medical Center Groningen, University of Groningen, Pfizer, Nederlandse Vereniging voor Plastische Chirurgie (NVPC), Groningen University Institute for Drug Exploration (GUIDE), Allergan, Kortjakje, Junior Vereniging Plastische Chirurgie (JVPC), Carl Zeiss, ABN AMRO

ISBN: 978-90-367-7154-2 (printed version)
ISBN: 978-90-367-7153-5 (electronic version)

Cover: photos by Bert Tebbes, design by Guido Dolmans and graphic editing by Tim Hoffman, Guido Dolmans and Ridderprint BV, Ridderkerk, the Netherlands
Lay out and Printed by: Ridderprint BV, Ridderkerk, the Netherlands

rijksuniversiteit
groningen

Genetic Origin of Dupuytren's Disease and Associated Fibromatosis

Proefschrift

ter verkrijging van de graad van doctor aan de

Rijksuniversiteit Groningen
op gezag van de
rector magnificus prof. dr. E. Sterken
en volgens besluit van het College voor Promoties.

De openbare verdediging zal plaatsvinden op

maandag 24 november 2014 om 16.15 uur

door

Guido Hubertus Carolina Gerardus Dolmans

geboren op 1 mei 1979
te Maastricht

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ter nagedachtenis aan mijn vader

Contents

Chapter 1


Unraveling the molecular genetic aspects of intestinal inflammatory disorders
Therapy-resisting Dupuytren's disease new perspectives in adjuvant treatment
AL KINEMATICS
Thybout M.
s of asthma in childhood: an approach by longitudinal wheezing phenotypes
Inocarpal Region of the Wrist: Morphological, Biomechanical, Radiological and Surgical Considerations
gement of Peripheral Nerve Problems
s of fractures of the hand
F.J.T. van
Horseshoe kidneys
O.B. Stroosma
M.D.E. KOOPMANN
FACIALIS VERLAMMING
ect of peri-operative innovations in free flap reconstruction
l risk factors of breast reconstruction and their clinical implications - Leonie A.E
ENGINEERING OF SKELETAL MUSCLE
MEREL KO
FREE FLAP PRESERVATION: AN EXPERIMENTAL STUDY ON COLD
ISCHAEMIC TOLERANCE IN RATS
P.M.N. WERKER 1992
OLE OF LIPOPOLYSACCHARIDE BINDING PROTEIN IN INNATE IMMUNITY IN INFECTED PARTIAL THICKNESS BURNS
Outcomes of Major Trauma
C.K. van der Sluis
BIOLOGY OF ADAMs IN RENAL DISEASE
WYNAND B.W.H. M
Reorganization and Motor Imagery after Flexor Tendon Repair
OUS NEEDLE FASCIOTOMY FOR DUPUYTREN'S DISEASE:
A RANDOMIZED CLINICAL TRIAL AND RELATED RESEARCH
Festen - Shared Genetic Background of Inflammatory Diseases
cs of celiac disease and its diagnostic value
Unlocking the genetics of coeliac disease

Introduction &
Outline of thesis

Dupuytren's disease (DD) is a benign, progressive fibromatosis of the palmar fascias of the hand and fingers, which leads to the formation of nodules and cords, and may lead to disabling extension deficits of the finger joints (Figure 1). Fibromatosis is a pathological condition characterized by local proliferation of fibroblasts and manifested clinically by soft tissue thickening.[1]

The most common form is DD, with a prevalence that is reported between 0.6% and 31.6%.[2] A study in Flanders (Belgium) revealed a prevalence of 32% in individuals over 50 years old, however, DD with finger contraction was only present in 8% of this age group.[3] In a study in the northern part of the Netherlands, a random sample of 1,360 individuals was investigated: in 17.9% of individuals there were nodules and cords present and 4.2% of individuals had flexion contractures of the fingers.[4] The prevalence of DD rises with increasing age[5] and is most frequently found in Caucasian males.[6]

Treatment consists of division (fasciotomy) or surgical excision (fasciectomy) of the pathological cords, but other treatments are emerging.[7,8] Recently Collagenase (collagenase clostridium histolyticum) injection has been approved for the treatment of

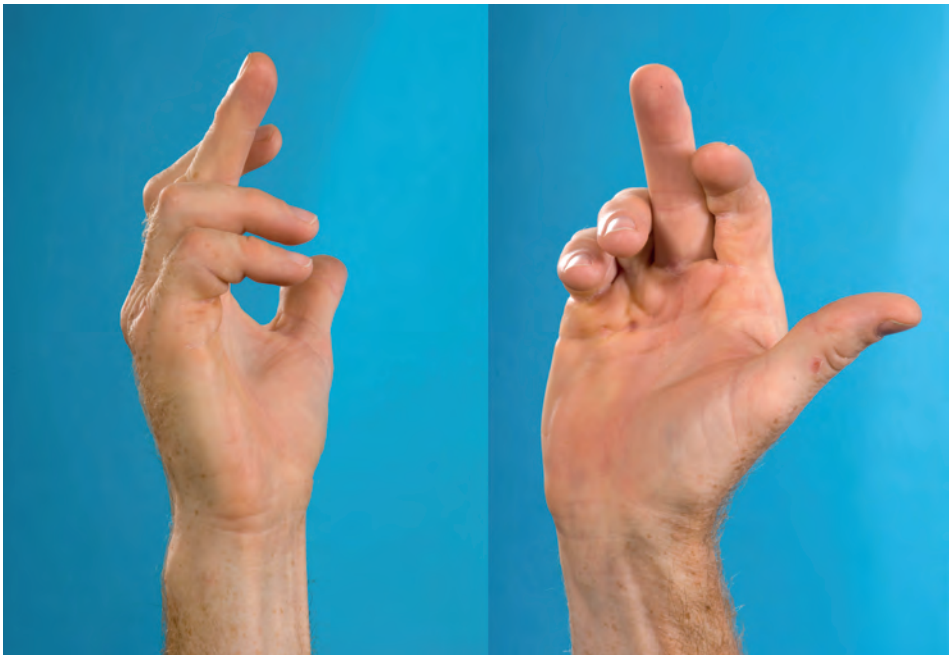

Figure 1. Extensive flexion contractures of the metacarpalphalangeal and proximal interphalangeal joints of the index-, ring-, and little fingers are evident in the right hand of a patient with recurrent Dupuytren's disease. All joints are at maximal possible extension.

DD[7] and lipofilling is being used in combination with percutaneous needle fasciotomy.[9] Radiotherapy can also be used in the early stages of the disease.[10] However, there is no cure for the disease and reported recurrence rates vary from 8% to 85%, depending on the treatment modality.[11-13]

Even though DD has been recognized for several centuries and was first described as a medical condition by Plater of Basle in 1614,[14] its pathogenesis remains unclear. The proliferating fibroblast and myofibroblast are the cells responsible for the flexion contraction in DD.[15,16] These cells are characterized by the presence of α-smooth muscle actin, an actin isoform typical of vascular smooth muscle cells.[17] Transforming growth factor (TGF)-ß is an important factor involved in the development of myofibroblasts and also promotes collagen formation, with a decrease of the ratio of type III to type I collagen.[18]

DD is associated with other fibromatoses, such as Peyronie's disease (PD) and Ledderhose's disease (LD). PD is a localized fibromatosis of the penile tunica albuginea, leading to pain and/or a variety of deformities, which are most evident during erection.[19] LD is a rare fibromatosis of the plantar aponeurosis, leading to nodules and pain in the foot.[20] DD is also associated with factors such as smoking, use of alcohol, anti-epilepsy drugs, and medical conditions including liver disease and diabetes.[21,22]

The way DD develops in time varies. Some clinical characteristics of patients with DD are related to a more aggressive course of the disease or diathesis. In 1963, Hueston formed a hypothesis with the concept of a DD diathesis and pinpointed four factors to be involved: early onset of disease, bilateral involvement, positive family history, and the presence of ectopic lesions (knuckle pads, Ledderhose's disease and Peyronie's disease).[23] Refinements of the DD diathesis have been made by Hindocha et al. and Abe et al.[5,24]

The clustering of DD in families, its prevalence in individuals of northern European descent, and its association with similar diseases (PD, LD) suggest a genetic influence on the development of the disease. Family studies mostly showed an autosomal dominant mode of inheritance for DD.[25-28] Hu et al. established genetic linkage at a 6 cM region on chromosome 16 in a single Swedish family.[29] DD has also been suggested to be a complex genetic condition, in which several genes and risk factors are involved, each conferring a certain (limited) risk to developing the disease. To date, a small number of candidate gene association studies have been performed in DD,[30-32] nonetheless, no causal variants have been found so far.

In recent years technical developments have made it possible to study genetic variation in genes throughout the genome on a single chip, and the costs of these genotyp-

ing platforms are becoming more affordable for researchers. It is widely recognized that a better understanding of the genetic background of a disease may yield more insight into its pathogenesis. This may then lead to the development of new treatment modalities in the future.

Aim of this thesis

The general aim of this thesis was to investigate the genetic background of Dupuytren's disease and its associated fibromatosis. This knowledge will ultimately lead to a better understanding of the disease and possibly to alternative treatments. In this thesis the following questions are addressed:

1. What is known about the genetic basis of Dupuytren's disease and which tools are available for genetic studies?
2. What is the mode of inheritance of familial Dupuytren's disease?
3. Which common genetic variants are associated with Dupuytren's disease?
4. Which common genetic variants are associated with Peyronie's disease?
5. Is there a shared genetic background for Dupuytren's disease and its associated fibromatosis?
6. Are clinical characteristics (including the DD diathesis) in patients with Dupuytren's disease associated with a higher genetic risk for the disease?

Outline of this thesis

Chapter 2 provides an introduction to basic genetics and explains the available genetic techniques. It gives an overview of the genetic studies that have been performed in DD to date and gives an insight into the plans for our future research.

In **chapter 3**, we describe eleven families (475 family members and 66 subjects diagnosed with DD) in order to investigate the mode of inheritance of familial DD.

To identify susceptibility genes for DD we performed a genome-wide association study, described in **chapter 4**. Initially, 960 Dutch patients with DD and 3,117 control persons were tested for association with the use of more than 300k single nucleotide polymorphisms (SNPs). Subsequently, we tested the 35 SNPs most strongly associated with DD in three more independent cohorts, comprising 1,365 patients with DD and 8,445 control persons in total.

Chapter 5 describes how we examined if the DD-associated variants identified in the genome-wide association study (chapter 4) also play a role in the genetic susceptibility of PD. We tested 111 men with PD and 490 controls for association for the nine DD SNPs identified in the genome-wide association study.

In **chapter 6** the association between clinical characteristics (including the DD diathesis features) of patients with DD and their genetic risk for developing the disease (using the SNPs identified in the genome-wide association study) are investigated.

The main findings of this thesis are discussed in **chapter 7** and future perspectives for studies in DD are given.

Finally, a summary and a Dutch translation of the summary can be found in **chapter 8**

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Chapter 2

Introduction in genetics and the genetic basis of Dupuytren's disease

Guido H.C.G. Dolmans
Hans C. Hennies

Dupuytren's Disease and Related Hyperproliferative Disorders. Springer Berlin Heidelberg, 2012:87-91. Text adapted for this thesis.

Introduction

The application of Mendelian principles to heredity in man[1] has opened the particular field of human genetics and the experimental study of human traits. It was not before 2001, when the draft sequence of the human genome was published and thus the sequence of the nucleotides, the chemical base pairs that make up DNA (and genes), was mostly determined,[2,3] that we could study the human genome in its entirety rather than one gene at a time.

Genetic variation, mostly represented by single nucleotide polymorphisms (SNPs) and copy number variants (CNVs), is frequent; any two humans are different in about 0.5% of their DNA sequence. Mutations, i.e. permanent changes in the genome including point mutations affecting single nucleotides and structural changes of chromosomes, can predispose to developing disease, modify the course of disease, or cause the disease itself. Virtually any disease is the result of the combined action of genes and environment but the relative role of the genetic component may be large or small. In a simplistic way we can distinguish monogenic and multifactorial diseases.

Monogenic disease is the result of a single mutated gene. These diseases, such as cystic fibrosis and Huntington's disease, usually exhibit obvious inheritance patterns in families according to the laws of Mendel. These patterns can be autosomal dominant, recessive or X-linked. Most of such single-gene diseases are rare, with a frequency as high as 1 in 500 or 1,000 individuals but usually much less. Genetic disorders may also be complex, meaning that they are associated with the effects of multiple genes (polygenic) in combination with environmental factors (multifactorial). Multifactorial diseases include, among many others, forms of diabetes, cancer, cardiovascular disease, and psoriasis.

Dupuytren's disease can be considered as a multifactorial disease as well. Although complex genetic diseases often cluster in families, they do not have a clear (Mendelian) pattern of inheritance. Multifactorial diseases have a major impact on the entire population. The identification of genes involved in Dupuytren's disease will further our understanding of the pathogenesis and also provide insight into new treatment modalities.

Two fundamental approaches are available for identifying genes involved in diseases or, more generally, in traits: linkage analysis and association analysis.[4,5]

Linkage analysis

The first approach, linkage analysis, is family based. Linkage analysis is a method that allows us to determine regions of chromosomes that are likely to contain a risk gene, and rule out areas where there is a low chance of finding a risk gene. This technique works by investigating the segregation of genetic markers to reveal recombination events between any two chromosomal loci. Currently, mostly SNPs are used as genetic markers. The location of approximately 3 million SNPs is presently known in the human genome. This means that there is one genetic marker (SNP) available in every 1,000 base pairs of our DNA.

Linkage analysis involving specific genetic disease models is especially powerful for the study of rare single-gene diseases. In linkage studies, researchers are searching for a marker locus that is consistently inherited with a disease. To this end marker alleles are experimentally determined and their segregation followed in a pedigree. When a marker is found that co-segregates with the disease, the marker locus and the disease locus are said to be linked and assumed to be located near each other on a chromosome. The statistical estimate of whether two loci are linked to each other and therefore likely inherited together is called a LOD score. A LOD score above 3 is considered significant.

Association analysis

The second approach, association analysis, is population based. Association analysis is performed to determine whether a genetic variant (SNP) is associated with a disease. This technique is especially powerful for more common, genetically complex diseases.[6,7] Most often a case/control study design is used. The allele frequencies of hundreds of thousands of markers (SNPs) spread over the genome are compared between cases (individuals with the disease) and controls from the same population. A significant difference in the frequency of an allele of a SNP between cases and controls indicates that the SNP is associated with the disease and may increase the risk of developing this disease. However, an associated SNP identified in an association study is usually not a disease causing variant but located on a stretch of DNA adjacent to this variant. This concept is called linkage disequilibrium (LD): the non-random association of alleles at two ore more loci in a population. An associated SNP therefore pinpoints a very small region of DNA with strong LD, which usually harbors only a few genes.

Because hundreds of thousands of markers have to be tested in a genome-wide association study, the possibility to detect false positive results is high. The easiest way

to reduce false positive findings is a stringent significance threshold. Generally a value $p < 5.0 \times 10^{-8}$ is accepted in studies of up to 1×10^6 markers. Another drawback in association analysis is population stratification. Population stratification is the presence of a systematic difference in allele frequencies between subpopulations, usually due to a different ancestry. Hence cases and controls must be from the same population. In order to provide evidence that associations are not false positive, it is furthermore important to replicate results in other study cohorts preferably from different populations.

Genetic basis of Dupuytren's disease

Dupuytren's disease is a multifactorial disease. It has a strong genetic component, as demonstrated by concordance rates in twin studies and familial clustering.[8] The recurrence risk for persons with an affected sibling, for instance, was determined as $\lambda_s = 2.9$ in a population from England.[9]

There are few reports of extended pedigrees with a transmission of Dupuytren's disease, which demonstrate an autosomal dominant inheritance with reduced penetrance. In one pedigree from Sweden, a whole-genome linkage analysis has been conducted.[10] It has localized a candidate region on chromosome 16, an underlying mutation, however, has not yet been identified. Other studies have used a case/control design for the analysis of an association with particular functional candidate genes such as those involved in the TGF-b pathways. Markers in the genes for TGF-b1, TGF-b2, or TGF-b receptors have not proven an association so far. Only a SNP in the gene for transcription factor Zf9, which may activate TGF-b1, showed an association with an odds ratio of 1.9.[11]

In order to systematically elucidate the genetic basis for Dupuytren's disease, we have decided to use our study cohorts for genome-wide association studies in a case/control study design. As outlined above, large sample numbers are necessary for whole-genome association studies, in order to gain sufficient power to obtain significant results. The 2010 International Symposium on Dupuytren's Disease has initiated the collaboration of research groups from the Netherlands, Germany and UK, involving the institutions of the authors, departments from Oxford (UK), and several others. Until now we have incorporated more than 1,000 Dutch cases, more than 600 German cases, and more than 700 UK cases. This joint endeavour made it possible to perform a large scale genome-wide association study including the chip-based analysis of very large numbers of SNPs, i. e., up to 1×10^6 markers distributed over the human genome.

Experimental procedures and data analysis are done in an automated manner. The data analysis also involves several statistical tools to remove false positive findings and test against systematic genotyping errors, genetic selection, and population stratification. These and other problems are for instance addressed by strictly taking account of the Hardy-Weinberg equilibrium (HWE), which regards the genotype distribution at a single locus in the population. Finally, our collaborations also allow for powerful replication studies, which are considered as an important part of a strong genome-wide association study. The most significant results of this cooperative study identifying several loci associated with Dupuytren's disease will soon be published elsewhere. These approaches are only first steps to characterize the genetic basis for Dupuytren's disease. However, they are an important undertaking, since only the subsequent characterization of the molecular etiology will lead to the development of more effective and possibly causal treatment opportunities for Dupuytren's disease.

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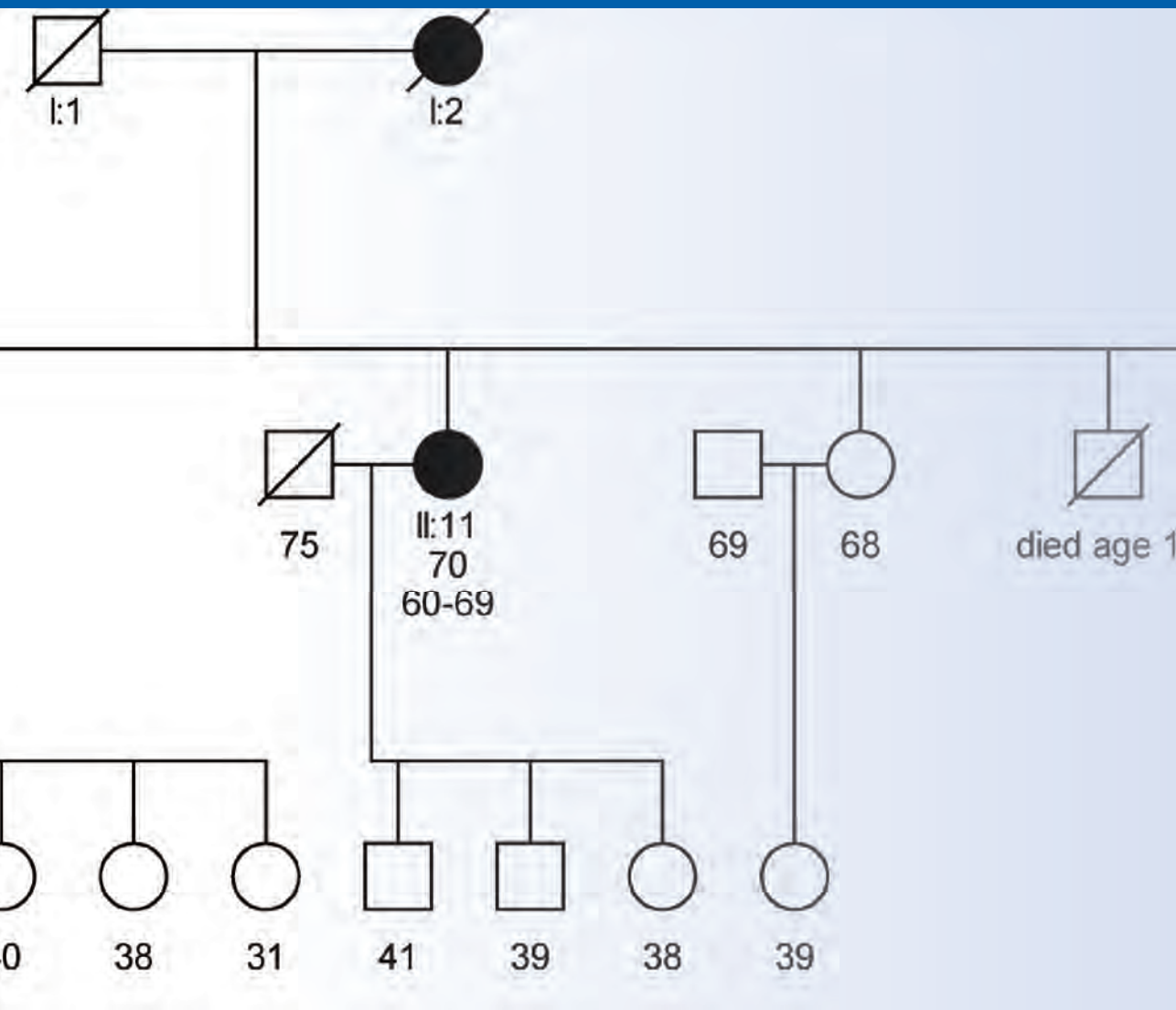
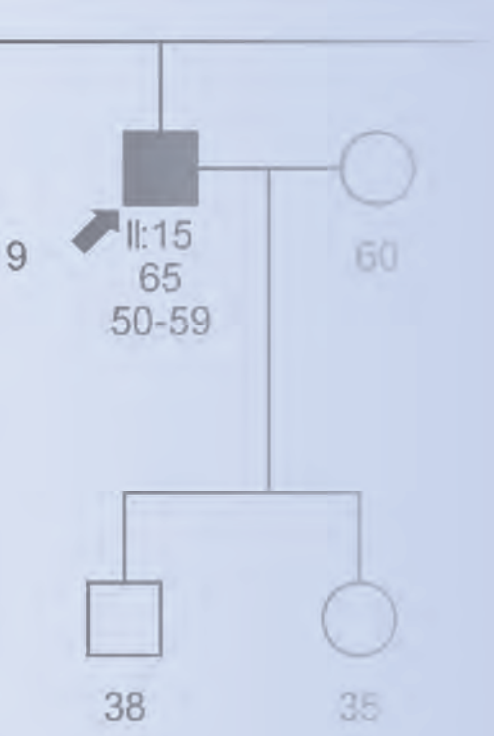
Chapter 3



A clinical genetic study of familial Dupuytren's disease in the Netherlands



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Dupuytren's Disease and Related Hyperproliferative Disorders. Springer Berlin Heidelberg, 2012:115-122

Abstract

Although several studies on the genetic origin of Dupuytren's disease have been performed, the mode of inheritance is still not clear. We describe 11 pedigrees with familial Dupuytren's disease from the Netherlands.

Patients with Dupuytren's disease with two or more affected first-degree relatives were asked to participate in the study together with their family. In five families, all members were clinically examined. In the six other families, the diagnosis of Dupuytren's disease was based on information provided by the proband. All participants completed an extensive questionnaire.

Eleven pedigrees, consisting of 475 family members and including 66 subjects diagnosed with Dupuytren disease, were studied. Of the affected family members, 67% were male and 33% female. Paternal transmission was observed in 45% of cases and maternal transmission in 55% of cases. Fifty-two percent of the offspring of the affected individuals also have Dupuytren's disease.

Our data suggest an autosomal dominant mode of inheritance for familial Dupuytren's disease, but with a reduced penetrance. This confirms previous studies.

Introduction

Dupuytren's disease is a benign, progressive fibrosing disorder of the palmar fascias of the hand and fingers, which leads to the formation of nodules and cords, and may lead to disabling extension deficits of the finger joints. Treatment consists of division or surgical excision of pathologic cords, but there is a high recurrence rate after surgery in patients with the Dupuytren's disease diathesis.[1]

Dupuytren's disease has been recognized for several centuries and was first described as a medical condition by Plater of Basle in 1614.[2] Its pathogenesis remains largely unknown. The reported prevalence of Dupuytren's disease varies from 3-42%,[3] with the highest incidence in Caucasians, while it is rarely seen in Africans.[4] Men are seven times more often affected than women, but in later life the incidence in women increases to the same as in men.[3] Some suggest that the disease symptoms are milder in women and therefore may remain unnoticed for a longer period.[5]

The clustering of Dupuytren's disease in families suggests a genetic influence on the onset of the disease. Several studies on the genetic origin of Dupuytren's disease have been performed,[5-15] but its mode of inheritance is still not clear. Multiple reports describe Dupuytren's disease as an autosomal dominant disease with varying penetrance.[16-18] An allele with a reduced penetrance means that some disease gene carriers do not develop the disease phenotype. Table 1 presents an overview of the family studies performed on Dupuytren's disease.

Table 1. Review of the English-language literature

Author	Year	Journal	No. of families	Affected/total no. family members	Mode of inheritance
Ling et al.	1963	J Bone Joint Surg Br	34	151/832	Autosomal dominant
Maza et al.	1968	J Hered	1	4/16	Autosomal dominant
Matthews et al.	1979	Br J Plast Surg	1	13/42	Autosomal dominant*
Hu et al.	2005	Clin Genet	1	18/50	Autosomal dominant

** Matthews et al. reported a family with predominantly affected females (Males:Females = 4:9).*

The most recent one was published by Hu et al. who presented a single Swedish family with a clear autosomal dominant mode of inheritance.[13] But there are many sporadic (non-familial) cases of the disease that are not compatible with a Mendelian inheritance pattern.[5] In a review in 1999, Burge et al. suggested that recessive inheritance still remains a viable hypothesis for Dupuytren's disease.[5] In two candidate-gene association studies by Bayat et al., the genes for TGFßR and Zf9 were only significantly associated with Dupuytren's disease when using a recessive model.[9,10] Another pos-

sible hypothesis is that Dupuytren's disease is a complex trait in which several genes and risk factors are involved, each conferring a certain (limited) risk to developing the disease. It is known that factors such as smoking, use of alcohol, anti-epilepsy drugs, and medical conditions including liver disease and diabetes, are associated with a higher prevalence of the disease,[3,19] complicating the picture even further. Since Dupuytren's disease is considered to be one of the most common hereditary connective tissue disorder in Caucasians,[12] finding genes for this disease is of utmost importance to understanding the disease pathogenesis and for developing diagnostic and prognostic protocols.

We investigated the clinical characteristic of families with Dupuytren's disease and examined the mode of inheritance. Here we describe 11 pedigrees with familial Dupuytren's disease from the Netherlands. The results of this work will significantly aid in designing proper genetic studies to identify the underlying gene(s).

Material and Methods

We identified Dupuytren patients visiting the outpatient clinics, who had two or more first-degree relatives with the disease. They were asked to participate in this study and family members were contacted via the proband. All participants signed an informed consent and this study was approved by the Medical Ethics Committees.

All the subjects (including probands, affected and unaffected family members) of five families were personally examined by trained clinical researchers or plastic surgeons with substantial clinical experience in treating Dupuytren's disease. The diagnosis was based on the presence of characteristic Dupuytren nodules and/or cords in the palm of the hand and/or digits, with or without contractures of the digits. All probands and affected family members were asked to complete a questionnaire on the age of onset, presence of suggested risk factors (diabetes, alcohol consumption, liver disease, anti-epileptic medication), occupation, hobbies, and the presence of recurrent disease. In most cases the hands of the affected individuals were also photographed. Information about deceased family members was gathered via the proband and other family members.

In the six remaining families, the diagnosis of Dupuytren's disease was based on information provided only by the proband; the family members were not clinically examined.

Data were entered into a database (Excel 2003; Microsoft, US). To investigate the genetic transmission of Dupuytren's disease in these families, pedigree charts were reconstructed from the data obtained from all 11 families using pedigree-drawing software (Cyrillic; Cyrillic software, UK). The mean age of onset of Dupuytren's disease

in patients with a positive family history in a study by Hindocha et al. was 49 years.[12] Therefore, unaffected family members under the age of 49 were included in the pedigrees, but were not considered in the analysis of the inheritance pattern, because of the age-dependent penetrance of Dupuytren's disease. Healthy family members suspected of being a carrier of the disease gene were used in the analysis of the transmission pattern. These unaffected carriers have at least one affected first-degree family member and one affected child. All the families who participated in this study between January and December 2007 are described in this paper.

The pedigrees encompassed 475 family members, with 66 subjects diagnosed with Dupuytren's disease. Of the affected family members, 44 (67%) were male and 22 (33%) were female. Five of the pedigrees spanned three generations and six pedigrees spanned four generations.

Results

Demographics and mode of inheritance

We describe the mode of inheritance of 11 pedigrees with Dupuytren's disease (Table 2) and the detailed clinical characteristics of five of these families (Table 3). All family members were Caucasians and originated from the northern part of the Netherlands.

In the selected pedigrees, two individuals were thought to be unaffected carriers because they had transmitted the disease to the next generation. This suggested an incomplete penetrance in at least 3% (2/68) of the Dupuytren carriers, assuming a dominant model of inheritance. All types of transmission were observed (male to male, male to female, female to female, female to male). Paternal transmission was observed in 45% of cases and maternal transmission in 55%. Fifty-two percent of the offspring of the affected individuals have Dupuytren's disease. The inheritance pattern of Dupuytren's disease in these pedigrees was compatible with an autosomal dominant mode of inheritance with slightly reduced penetrance (95%). Penetrance is the proportion of individuals carrying a disease gene that also expresses this disease.

Description of selected pedigrees

Family 1

This family spanned four generations and consisted of 76 family members, with nine affected. Of the affected family members, five were male and four were female. The family members in the fourth generation were all younger than 40 years. Family

Table 2. Characteristics of 11 Dupuytren families

Fam	Generation	No. of affected members	Unaff. carriers	Affected, M:F	Ethnicity	Total no. of family members	Family members, M:F	Paternal transmission	Maternal transmission	% offspring of affected parents who also have DD*	Compatible with autosomal dominant transmission
1	4	9	0	5:4	Caucasian	76	38:38	2	6	40% (8/20)	+
2	3	3	0	2:1	Caucasian	33	16:17	3	0	60% (3/5)	+
3	3	8	0	6:2	Caucasian	54	28:26	1	6	54% (7/13)	+
4	3	7	1	5:1	Caucasian	48	21:27	5	0	38% (5/13)	+
5	4	5	0	3:2	Caucasian	19	8:11	2#	4#	50% (4/8)	+
6	3	5	1	3:2	Caucasian	56	27:29	2	3	44% (4/9)	+
7	3	5	0	3:2	Caucasian	25	14:11	3	3	60% (3/5)	+
8	4	6	0	5:1	Caucasian	44	27:17	?	?	75% (6/8)	+
9	4	5	0	3:2	Caucasian	52	28:24	4	0	29% (4/14)	+
10	4	9	0	4:5	Caucasian	45	25:20	0	8	80% (8/10)	+
11	4	4	0	4:0	Caucasian	23	9:14	3	0	43% (3/7)	+
Total	39	66	2	44:22		475	241:234	25 (45%)	30 (55%)	52%	+

 # transmission of Dupuytren disease from the second to third generation could be paternal, maternal, or both

** unaffected individuals under the age of 49 were not considered in the analyses, unaffected carriers were used.*

DD: Dupuytren's disease

Table 3. Clinical characteristics of affected individuals in 5 families with Dupuytren's disease

Family	Individual	Sex	Age of onset	Recurrent disease	Manual laborer	Other disease locations	Co-morbidity	Smoking
1	I:2	f	60-69	-	+	-		
1	II:1	m	60-69	-	-	-	-	-
1	II:4	f	70-79	-	-	-	-	-
1	II:5	m	70-79	-	-	-	-	-
1	II:10	f	30-39	-	+	-	-	-
1	II:11	m	30-39	+	+	-	-	-
1	III:1	m	50-59	-	+	-	-	-
1	III:30	f	30-39	-	+	-	-	+
1	III:32	m	30-39	-	+	-	-	-
2	II:4	f	50-59	+	+	-	-	+
2	II:5	m	50-59	+	-	-	-	-
2	II:7	m	40-49	-	-	-	-	+
3	II:3	m	60-69	+	+	kn	-	-
3	II:4	m	60-69	-	-	-	-	-
3	II:6	m	40-49	+	-	-	-	-
3	II:11	f	60-69	-	+	-	-	-
3	II:15	m	50-59	+	-	kn	-	-
3	II:17	m	50-59	-	+	-	epilepsy	stopped 1974
3	III:9	m	30-39	-	-	-	-	-
4	II:5*	m	?	?	?	?	?	?
4	III:1	m	40-49	+	+	-	diabetes	-
4	III:3	m	40-49	-	+	-	-	+
4	III:5	m	40-49	+	-	pd	-	stopped 1998
4	III:21	f	50-59	-	-	-	-	+
4	III:24	m	40-49	+	-	-	-	stopped 2000
5	II:1	m	50-59	+	+	-	-	-
5	II:2	f	60-69	-	+	-	-	-
5	III:1	m	40-49	+	-	kn/ld	-	-
5	III:3	m	30-39	+	+	kn/ld/pd	-	-

** The clinical characteristics of individual II:5 in family 4 are unknown.*

Kn: Knuckle pads, Ld: Ledderhose's disease, pd: Peyronie's disease

member II:11 and his children III:30 and III:32 displayed an early onset of the disease (before age 40).

Family 2

This family spanned three generations, with 33 family members, of whom three were affected. In the first generation the grandparents, who were unrelated, did not develop the disease, however the grandfather (I:1) died at the early age of 39 years. Except for individual III:1 (50 yrs,) the entire third generation was younger than 49 years old.

Family 3

In this three-generation family, 8 out of 54 family members were affected. In the second generation, 6 out of 12 individuals were affected. Individual III:9 (42 yrs), who developed the disease before the age of 40, was the only affected family member in the third generation. His father, II:6, was the only affected person in the second generation who demonstrated Dupuytren's disease before the age of 50.

Family 4

This three-generation family had 48 members, of whom six were affected. It is not known whether the grandparents were affected. Individual II:1 was presumed to be an unaffected carrier. The fourth generation is not shown, since they are all younger than 40 years and so far unaffected.

Family 5

In this small family, both parents in the second generation (II:1 and II:2) were affected by Dupuytren's disease, with the father having an age of onset between 50 and 59 years and the mother between 60 and 69 years. Individuals III:1 and III:3 showed the first signs of the disease before the age of 44. They have both been treated three times with a selective fasciectomy. III:1 and III:3 not only display the disease in both hands including knuckle pads, but also in their feet (Ledderhose's disease), while individual III:3 also has a benign progressive fibrosing disorder affecting the penis (Peyronie's disease).

All pedigrees were compatible with an autosomal dominant mode of inheritance.

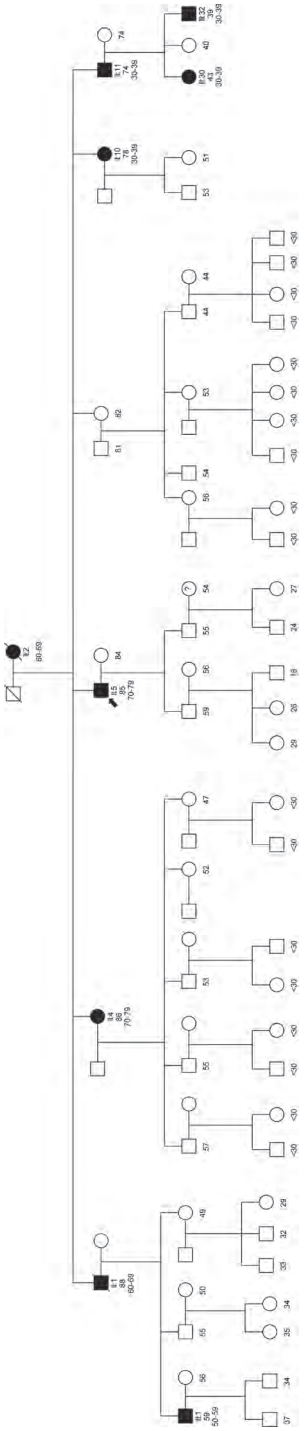

Figure 1. Family 1

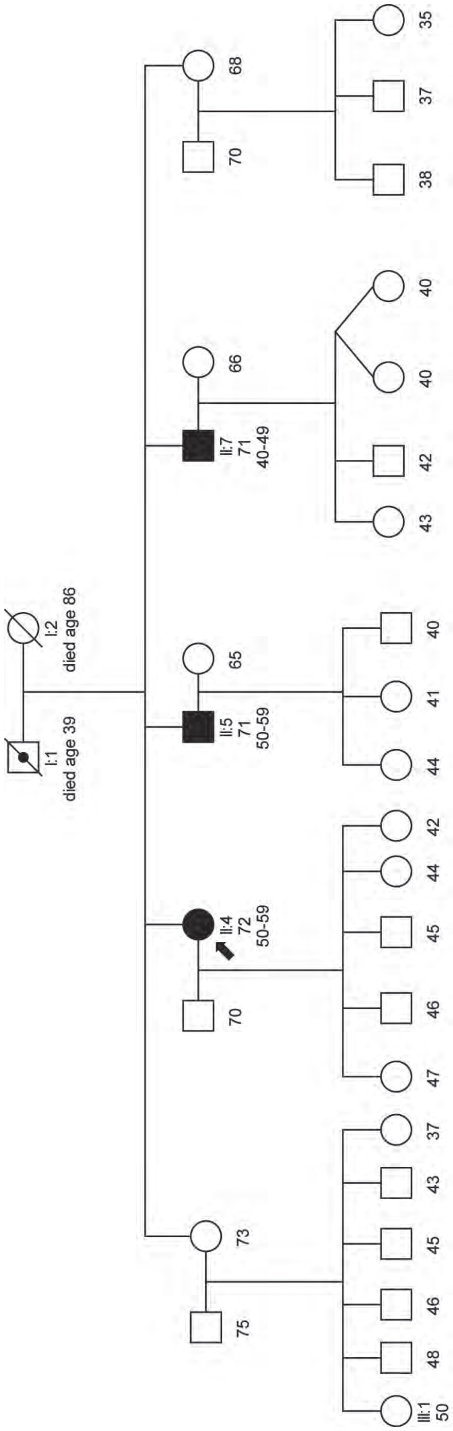

Figure 2. Family 2

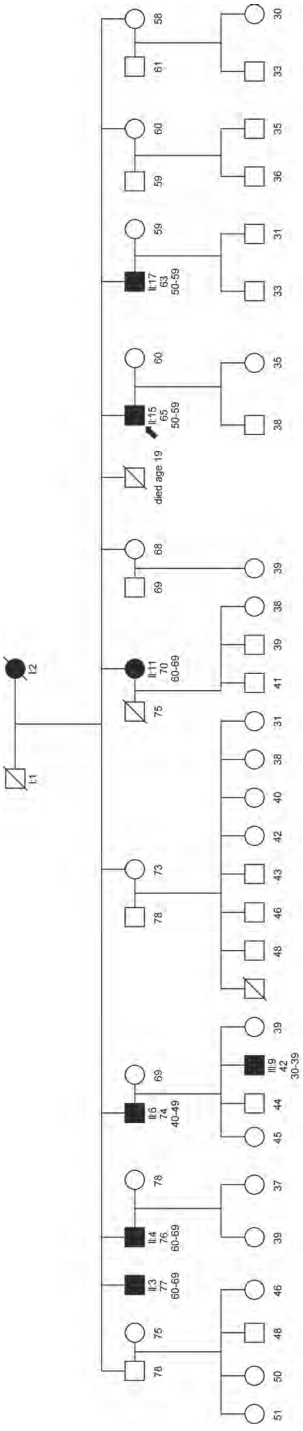

Figure 3. Family 3

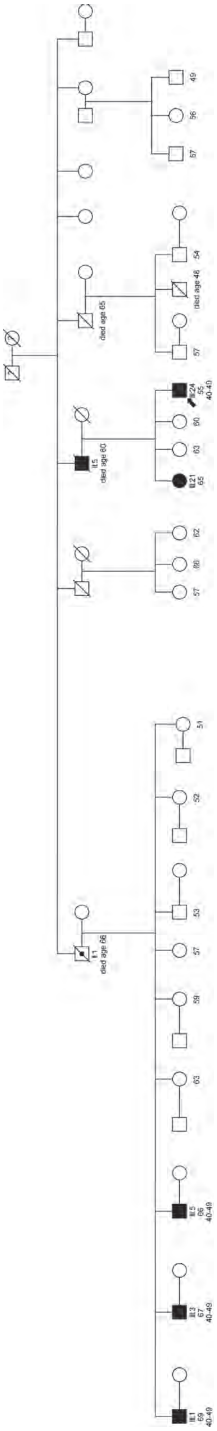

Figure 4. Family 4

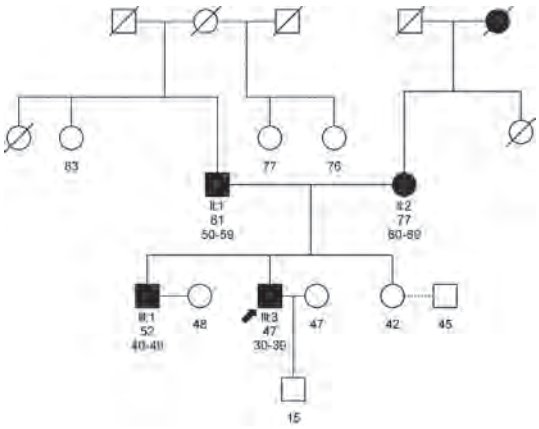

Figure 5. Family 5

Discussion

We describe the mode of inheritance and the clinical characteristics of families with Dupuytren's disease in order to further elucidate the genetics of this familial disorder. Probands and their relatives of five families were interviewed and examined. Information on six additional families was collected via six other probands.

The sex-distribution of affected individuals male:female was 2:1 (67%:33%). As noted by Ross, women develop Dupuytren's disease approximately a decade later and the sex-distribution will consequently be nearly equal by the ninth decade of life.[3] This explains the relatively small difference in the sex-distribution found in this study.

Paternal and maternal transmission was observed in 45% and 55% of cases, respectively. Fifty-two percent of the offspring of affected individuals was also affected at age 49 years or older. These data, in combination with the appearance of the pedigrees, are compatible with an autosomal dominant mode of inheritance for a late-onset disease in these families.

From the families described, it is apparent that the decade of onset of the disease seen in the parents also predicts the age of onset for their offspring. For instance, in family 1, individual II:11 developed Dupuytren's disease in the fourth decade (30-39 yrs) just as his offspring (III:30 and III:32). In family 3, III:9 was the only affected person in the third generation so far. His father (II:6) was the only individual affected under the age of 50 in the second generation of this family.

In family 2, the grandfather (I:1) might have been a carrier of the disease. He died aged 39 and had probably not developed Dupuytren's disease at that stage.

In family 5 individuals III:1 and III:3 not only displayed an earlier onset than their parents, but also a far more aggressive form and at different locations. The co-existence of Dupuytren's disease with Ledderhose's disease and Peyronie's disease has been noted frequently. Since both their parents are affected, individuals III:1 and III:3 could be homozygous for the mutation of this apparently dominant disease. Ling et al. also observed a more severe clinical manifestation of the disease in individuals with both parents affected.[16]

Table 3 shows the clinical characteristics of the affected individuals in the five families, but there are no apparent patterns. Two factors complicate the resolving of the genetic mechanism of Dupuytren's disease: the late age of onset and its fairly high incidence in the general population. Our data suggest that the most likely mode of inheritance in these families with Dupuytren's disease is autosomal dominant with a reduced penetrance, which confirms previously published data.

Since only Dupuytren patients with two or more affected first-degree relatives were asked to participate in this study, there will have been an ascertainment bias. Sporadic cases of the disease were excluded in this design, hence alternative modes of inheritance cannot be ruled out. To prevent this bias, all Dupuytren patients presenting at the outpatient clinics should have been included. Furthermore, to improve the power of this study more families need to be included and all family members should be examined by an experienced clinician.

The large pedigrees with multiple affected patients with Dupuytren's disease will be instrumental in the genetic mapping and identification of genetic factors involved in Dupuytren's disease by using classical linkage approaches. Genes identified in this way may also help to resolve the disease etiology of the sporadic form of Dupuytren's disease. This knowledge will ultimately lead to improved diagnosis and possibly to alternative treatments.

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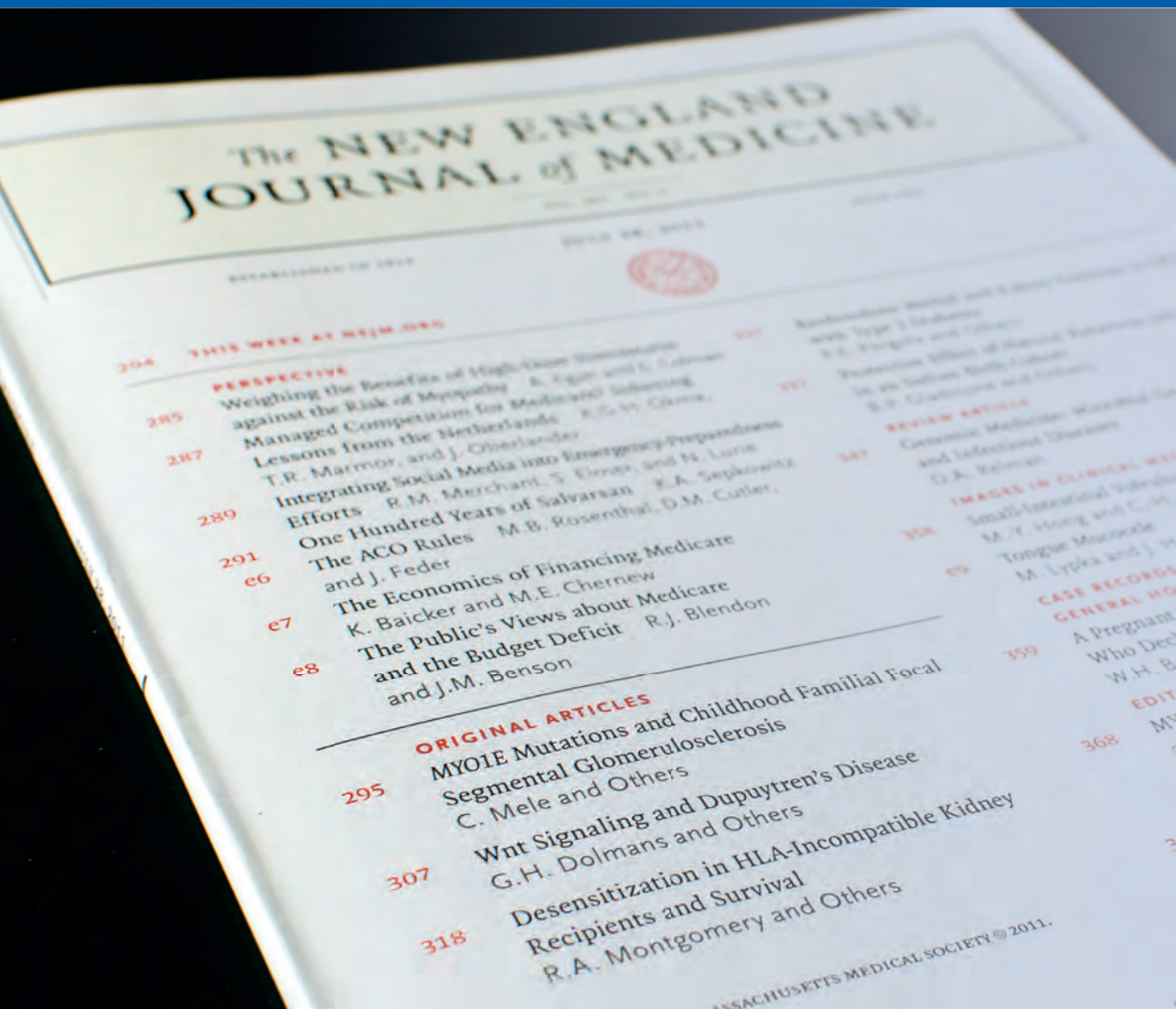
Chapter 4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REVIEW ARTICLE
347
IMAGES IN CLINICAL MEDICINE
358
CASE RECORDS
359
368
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Wnt-signaling and Dupuytren's Disease

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N Engl J Med 2011;365:307-17

Abstract

Background

Dupuytren's disease is a benign fibromatosis of the hands and fingers that leads to flexion contractures. We hypothesized that multiple genetic and environmental factors influence susceptibility to this disease and sought to identify susceptibility genes to better understanding its pathogenesis.

Methods

We conducted a genome-wide association study of 960 Dutch persons with Dupuytren's disease and 3,117 controls (the discovery set) to test for association between the disease and genetic markers. We tested the 35 single nucleotide polymorphisms (SNPs) most strongly associated with Dupuytren's disease ($P<1x10^{-4}$) in the discovery set in three additional, independent case series comprising a total of 1,365 affected persons and 8,445 controls from Germany, the United Kingdom and the Netherlands.

Results

Initially, we observed a significant genome-wide association between Dupuytren's disease and 8 SNPs at three loci. Tests of replication and joint analysis of all data from 2,325 patients with Dupuytren's disease and 11,562 controls yielded an association with 11 SNPs from nine different loci ($P<5.0x10^{-8}$). Six of these loci contain genes known to be involved in the Wnt-signaling pathway: *WNT4* (rs7524102, $P = 2.81x10^{-9}$, OR 1.28), *SFRP4* (rs16879765, $P = 5.63x10^{-39}$, OR 1.98), *WNT2* (rs4730775, $P =3.02x10^{-8}$, OR 0.83), *SULF1* (rs2912522, $P = 2.01x10^{-13}$, OR 0.72), *RSPO2* (rs611744, $P = 7.87x10^{-15}$, OR 0.75), and *WNT7B* (rs6519955, $P = 3.24x10^{-33}$, OR 1.54).

Conclusions

This study implicates nine different loci involved in genetic susceptibility to Dupuytren's disease. The fact that six of these nine loci harbor genes encoding proteins in the Wnt-signaling pathway suggests that aberrations in this pathway are key to the process of fibromatosis in Dupuytren's disease.

Introduction

Dupuytren's disease (DD) is a benign fibromatosis of the hands and fingers, giving rise to the formation of nodules and cords and often leading to flexion contractures. The prevalence of DD is reported to be between 0.2% and 56%.[1] Men are more often affected than women, but by the ninth decade of life, the incidence in women is the same as that in men.[2,3] Standard treatment consists of surgical excision or transection of pathologic nodules and cords, but other methods of treatment are emerging.[4,5] There is no cure for the disease, however, and reported recurrence rates range from 8% to 66%, depending on the treatment.[6,7] The pathogenesis of DD is not fully understood.

The clustering of cases of DD in families suggests a genetic influence on the onset of disease; however, it is probably a complex condition, in which several genetic and environmental risk factors are involved, each contributing in small part to susceptibility to the disease. To date, a limited number of small candidate-gene association studies have been performed,[8,9] but no causal genes have been identified. To identify common genetic variants associated with this disease, we carried out a genome-wide association study involving 960 persons with DD and 3,117 controls, all from the Netherlands and of European descent.

Methods

Subjects

Participants provided written informed consent, and we obtained approval from an institutional review board to carry out the study. Between 2007 and 2010 we recruited 960 patients with DD through the outpatient clinics of the plastic surgery departments of six hospitals in the Netherlands. All 3,117 controls for the discovery set were drawn from LifeLines, a large, population-based cohort study being conducted in the northern Netherlands.[10] We obtained blood samples for replication studies from 189 Dutch patients with DD, as well as from 561 Dutch controls who were newly enrolled in LifeLines and for whom genotyping data were already available; from 711 British patients with DD, as well as from 5,984 controls from the Wellcome Trust Case Control Consortium 2 (WTCCC, 1958 Birth Cohort and UK National Blood Service) for whom genotyping data were already available,[11] and from 465 German patients with DD, as well as from 1,900 controls, for 1,618 of whom genotyping data were already available (1,164 from the PopGen study at the University of Kiel and 454 from KORA [Cooperative Health Research in the Region of Augsburg] at the Helmholtz Center Munich in Neuherberg)(Table 1). Participants reported ancestry by means of a multiple-choice

Table 1. Sample collections and genotyping platforms for the genome-wide association study and replications in patients with Dupuytren's disease and control subjects.

		Patients with Dupuytren's disease (N=2,325)			Control Subjects (N=11,562)		
Collection No.	**Country**	No. of Samples before QC[a]	No. of Samples after QC	Platform	No. of Samples before QC	No. of Samples after QC	Platform
GWAS							
1	The Netherlands	960	856	Illumina HumanCytoSNP-12	3,117	2,836	Illumina HumanCytoSNP-12
Replication							
2	The Netherlands	189	184	KASP by design	561	500	Illumina HumanCytoSNP-12
3	United Kingdom	711	665	KASP by design	5,984	4,765	Illumina 1.2M, Affymetrix 6.0
					8,935[b]	8,274	Immunochip
4	Germany	465	449	Affymetrix 6.0	1,618	1,604	Affymetrix 6.0
					282[c]	267	GenomeLab SNPstream
Total		2,325	2,154		11,562	9,972	

a: QC denotes quality control b: In the original analysis, rs611744 was imputed in the UK control series. Subsequently, we directly genotyped this SNP in another set of 8,935 UK controls (WTCCC) with the use of the Immunochip array. c: In the German case series, several replication SNPs were genotyped in a separate control series that included 282 persons, and these were included in the main study (for the SNP numbers, see Table 2).

questionnaire, the choices being Dutch, European (specify country), and other (specify country). DNA samples were obtained from either blood samples (in the Dutch and German case series) or saliva (in the UK case series).

Tests for Association

We genotyped the Dutch samples (discovery set) and control samples (LifeLines) with Illumina HumanCytoSNP-12 arrays, comprising 301,232 SNPs, and called SNPs with the use of the Illumina algorithm (Genome Studio version 2.10.1). To test for replication of association, we selected SNPs that showed an association in the discovery set with a P value of less than 10^{-4}. We confirmed the integrity of these associations by manually inspecting genotype clusters and selected two SNPs to represent each independent locus. We genotyped the SNPs selected for tests of replication in the Dutch and British persons by means of KASP by design assays (KBioscience). We used the Human SNP Array 6.0 (Affymetrix) to genotype these SNPs in the German persons. To test for replication of SNPs for which no direct or tag SNPs were present on the Affymetrix 6.0 platform, we genotyped the selected SNPs in both the German persons affected with DD and a separate control series, using GenomeLab SNPstream (Beckman Coulter).

Statistical analysis

We excluded from the analysis specific SNPs and data from specific samples as described previously.[12,13] SNPs with call rates of less than 95%, a minor-allele frequency of less than 0.01, or deviation from Hardy-Weinberg equilibrium (P<0.0001) were excluded, as were samples with call rates below 99% or with a discrepancy between recorded sex and genotype-inferred sex. We also excluded relatives and ethnic outliers. Because genome-wide association studies are performed in patients and controls in the same ethnic group, we used multidimensional scaling in the study participants and persons in HapMap to identify nonwhites in our study population (i.e., ethnic outliers). We compared genotype prevalence in cases and controls with the use of a basic chi-square allelic test with 1 degree of freedom and calculated the over-dispersion factor of association test statistics (genomic control inflation factor, λ_{GC}) with the use of observed versus expected values for all SNPs by means of the PLINK software package (version 1.07). Principal component analysis was performed with the use of the EIGENSTRAT software package to control for population stratification. We conducted conditional analysis with the use of SNPTEST, version 2, when more than one SNP with a significant genome-wide association clustered at a certain region.

We excluded SNPs from tests of replication if they had a call rate below 98% or deviated from Hardy-Weinberg equilibrium (P<0.0001), and we excluded samples with call rates below 95%. We carried out a joint analysis of the discovery and replication phases

Table 2. The 35 SNPs selected for replication and the tag SNPs used in the UK and German series are shown (with r2 value).

Chr	SNP	UK series		German series	
		Tag SNP	r^2	Tag SNP	r^2
1	rs7524102	–	–	–	–
3	rs1123148	–	–	–	–
3	rs2323206	–	–	–	–
3	rs1356802	–	–	–	–
4	rs6824106	–	–	–	–
5	rs11743146	–	–	–	–
5	rs11745128	rs11743146[b]	0.98	rs11743146[a]	1.00
6	rs7747741[a]	–	–	–	–
6	rs2179367	–	–	–	–
6	rs237018	rs237012	1.00	–	–
7	rs16879765	–	–	–	–
7	rs1668357	–	–	–	–
7	rs4730775	rs6951125	1.00	rs6951125	1.00
7	rs4719773[a]	–	–	–	–
8	rs1365415	rs13269711	1.00	rs13269711	1.00
8	rs611744	rs423940	0.84	rs423940	0.84
8	rs2912522[a]	–	–	–	–
9	rs10809642	–	–	rs7863802	1.00
9	rs10809650	–	–	–	–
10	rs7072865	rs11188849[b]	0.87	rs11188849	0.88
12	rs638791	rs616559	1.00	–	–
12	rs2073950	–	–	–	–
12	rs12372139	–	–	–	–
15	rs4932194[a]	–	–	–	–
15	rs6496520	–	–	rs7168492	0.96
15	rs2171286	–	–	rs17302219	1.00
17	rs4789939[a]	–	–	–	–
18	rs504302	rs474605	0.87	rs474605	0.87
18	rs1944967	–	–	rs625896	1.00
19	rs11672517[a]	–	–	–	–
20	rs6029273	rs742745	1.00	rs742745	1.00
20	rs8124695	rs6093338	1.00	rs6093338	1.00
22	rs8140558	rs6519955[b]	0.87	rs6519955[a]	0.96
22	rs4820663[a]	–	–	–	–
22	rs6519955[a]	–	–	–	–

[a] SNPs genotyped with GenomeLab SNPstream in a separate German control series of 282 individuals. The other replication SNPs were present in the German control series genotyped on the Affymetrix 6.0 platform (1,604 individuals). [b] SNPs imputed from WTCCC control data. This imputed data was generated with BEAGLE Genetic Analysis Software Package based on HapMap 2 in CEU (individuals of European ancestry).

with the use of Cochran-Mantel-Haenszel stratification. In the UK control series, not all SNPs selected for replication were available on the Illumina 1.2M and Affymetrix 6.0 genotyping platforms, which were used by WTCCC; in some cases, we used tag SNPs, and for four replication SNPs, we used imputed WTCCC control data (Table 2) generated with BEAGLE Genetic Analysis Software Package 3.2 and based on the HapMap phase 2 reference of the Centre d'Etude du Polymorphisme Humain (CEPH) persons of European ancestry (CEU). In the German series, several SNPs selected for replication were not available on the Affymetrix 6.0 platform; in these cases, tag SNPs were used as well (Table 2). We used multiple genotyping platforms for the control cohorts in the replication phase. Since the replication signals were in the same direction and of the same magnitude as the discovery results, it is unlikely that the confirmatory results were due to biased genotyping. We checked for inter-platform reproducibility by comparing genotypes of the same samples between different platforms and showed concordance rates of more than 99.99%.

We were not able to correct for population stratification in the samples used to test for replication because we genotyped only a limited number of SNPs in this phase. Meta-analysis of the discovery and replication data was also performed with PLINK software. We performed an analysis with the use of the Gene Relationships across Implicated Loci (GRAIL) statistical strategy involving hg18 and PubMed data sets (December 2006), with the 11 SNPs that had a significant genome-wide association as query regions.[14]

Results

Genome-wide Associations

Data obtained through genome-wide genotyping of affected persons and controls are stored at the European Genome-Phenome Archive (accession number, EGAS00000000043). We excluded 66,293 SNP genotypes because they did not meet quality-control criteria, leaving 234,939 SNPs typed in 856 patients with DD and in 2,836 controls (Table 1). The call rate for the remaining SNPs was 99.9%. There was moderate evidence for inflation in the test statistic (λ_{GC} = 1.21). Adjustment for differential population stratification with the use of the first five components on the basis of a principal component analysis of uncorrelated SNPs reduced the inflation to λ_{GC} = 1.19. Figure 1 shows that the case and control groups were well matched for population stratification after correction for these components. We found that the inflation was caused by genetic heterogeneity between persons in the north and south of the Netherlands and noted differences in case patients between the clinics (Figure

2). After exclusion of case patients from the most southern and eastern hospitals in the Netherlands, the inflation decreased to 1.07 (Figure 3 and 4). There were no signs of differences in SNP call rates between case patients and controls. After correction for the inflation factor, the quantile-quantile plots of the logarithms of our genome-wide P-values shows 83 data points above the expected diagonal line (Figure 5a).

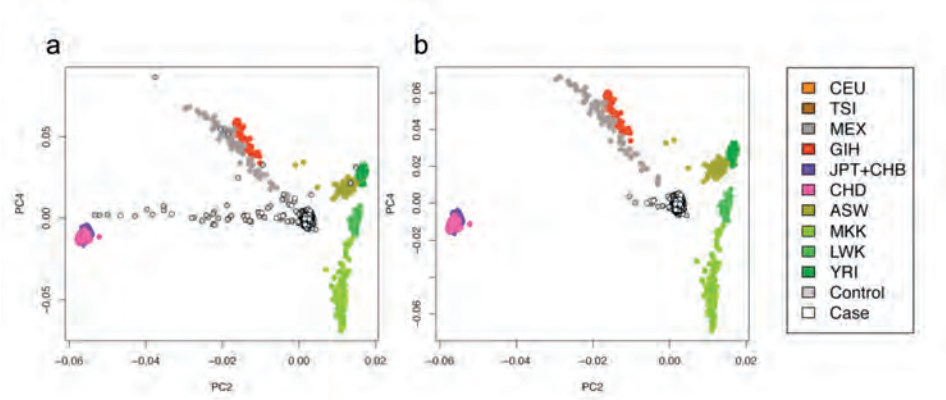


Figure 1. Plots of principal components 2 and 4 from the PCA, including all subjects of the GWAS and HapMap 2.

a: before removal of the first 5 components. b: after removal of the first 5 components.

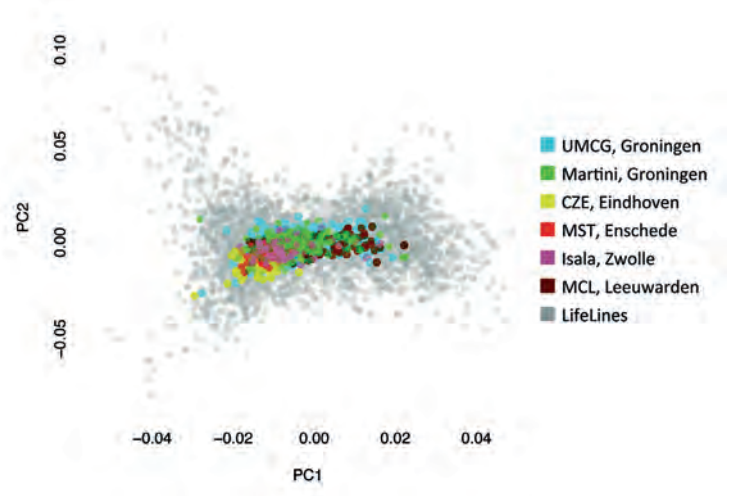


Figure 2. Principal component analysis of our DD cases, shown per clinic.

In the genome-wide association study, we identified eight SNPs at three loci that showed significant association ($P< 5x10^{-8}$)(Figure 5b). On chromosome 7, we identified a locus with four significantly associated SNPs. Association was strongest at rs16879765, which lies within the gene encoding ependymin-related protein 1 (*EPDR1)* ($P = 1.9x10^{-16}$; odds ratio (OR) = 1.94). The three other associated SNPs were in linkage disequilibrium with the top SNP: rs1668357 ($r^2 = 0.57$), rs1668347 ($r^2 = 0.59$), rs952368 ($r^2 = 0.44$). Similarly, three significantly associated SNPs were identified at a single locus on chromosome 22. The most significant SNP on 22q, rs6519955 ($P = 2.75x10^{-13}$;

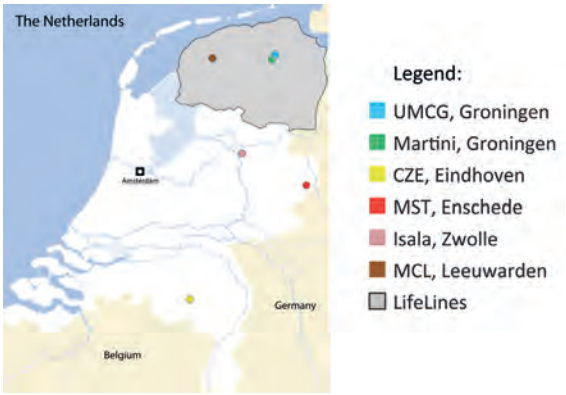


Figure 3. Location of the six participating clinics and the region from which the 'LifeLines' cohort is being built up (shaded grey) in the Netherlands.

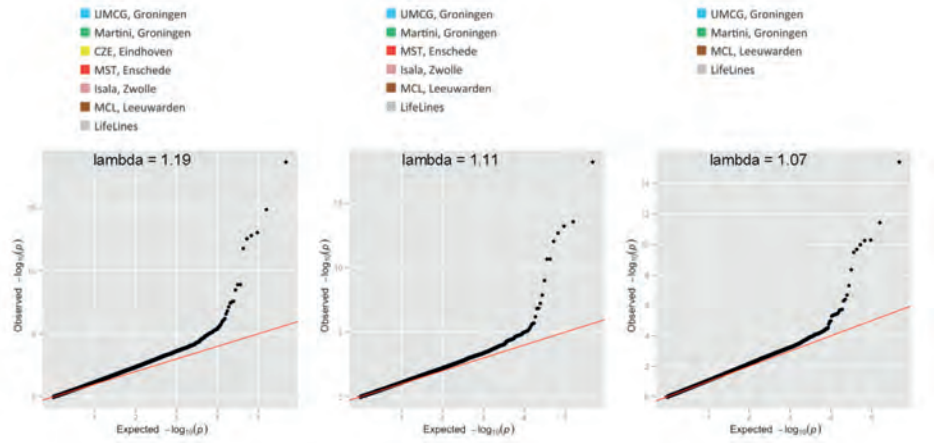


Figure 4. QQ-plots of the full discovery set, after excluding CZE, and after excluding CZE, Isala, and MST.

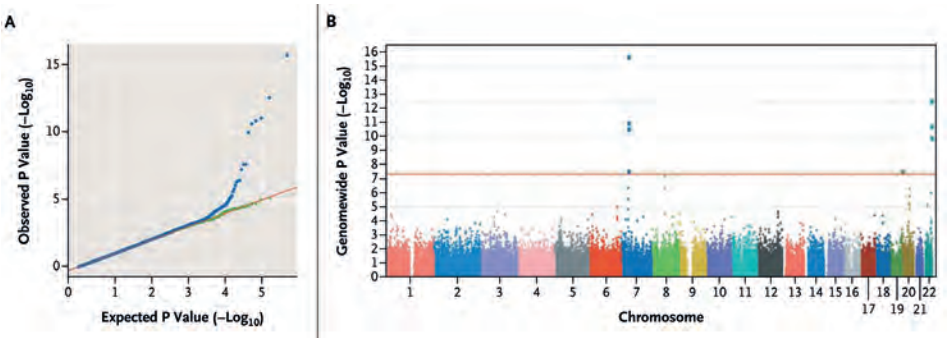


Figure 5. Results of the Genome-wide Association Study in Dupuytren's Disease.

The genome-wide P-values were obtained with the use of a basic chi-square allelic test with 1 degree of freedom, corrected for genomic inflation, for 234,939 SNPs in 856 DD patients with DD and 2,836 controls from the Netherlands. Panel A is a quantile-quantile plot of observed P values for association (blue) and after removal of SNPs within 1 megabase of the nine regions identified as significant after meta-analysis (green). The red line represents concurrence of the expected and the observed P values. Values above the red line indicate a signal in the data. Panel B is a Manhattan plot showing the genome-wide P values (-log$_{10}$) plotted against their respective positions on each chromosome. The horizontal red line shows the genome-wide significance threshold of 5.0 x 10^{-8}.

OR = 1.56), is located between Wingless-type MMTV integration site family member 7B (*WNT7B*) and *LOC100271722*, a hypothetical non-coding RNA gene. The two other associated SNPs on chromosome 22 were in linkage disequilibrium with the top SNP: rs8140558 (r^2 = 0.96), rs4072455 (r^2 = 0.75). A conditional analysis to adjust for the top SNPs for these two loci showed no independent signals, suggesting that there is one pivotal genetic variation that drives the association of the neighboring SNPs. One additional putative DD associated locus was identified on chromosome 19, with a single significant SNP (rs11672517)(P = 2.76x10^{-8}; OR = 1.46).

Tests of Replication

To test for replication of our initial findings, we selected 35 SNPs from 24 independent loci that met the significance threshold of P<1x10^{-4} in the discovery phase. We collected genotype data for the 35 SNPs in three different populations of case patients and controls, from the Netherlands, the United Kingdom and Germany (1,365 case patients with DD and 8,445 controls before quality control). One SNP (rs10809642) failed on genotyping in the Dutch and UK replication series and four SNPs (rs1123148, rs2179367, rs638791 and rs12372139) failed genotyping because of a laboratory error in the German series. One SNP (rs1668357) was out of Hardy-Weinberg equilibrium

(P<0.0001) in all three replication series and was therefore excluded from further analysis. Eleven SNPs from nine different regions showed clear evidence of replication after correcting for the 35 tested SNPs (P<0.0014 and association with the same allele and in the same direction as in the discovery phase) and reached genome-wide significance in a meta-analysis (P< 5.0×10^{-8})(Table 3). All loci that showed significant associations in the discovery set also showed significant associations in the replication set: rs16879765 on 7p14.1 (P=5.63×10^{-39}, OR 1.98), rs6519955 (P=3.24×10^{-33}, OR 1.54) and rs8140558 (P=1.20×10^{-22}, OR 1.39) on 22q13 and rs11672517 on 19q13.4 (P=6.81×10^{-14}, OR 1.34). Two SNPs of borderline significance in the discovery set showed significant association in the tests of replication: rs2912522 (P=2.01×10^{-13}, OR 0.72) on 8q13, and rs8124695 (P=7.60×10^{-10}, OR 1.48) on 20q11.2-q13.1. Four additional SNPs also reached genome-wide significance: rs611744 on 8q23.1 (P=7.87×10^{-15}, OR 0.75), rs10809650 (P=6.15×10^{-9}, OR 0.80) and rs10809642 (P=1.21×10^{-8}, OR 1.35) on 9p24.3, and rs7524102 (P=2.81×10^{-9}, OR 1.28) on 1p36.23-p35.1.

For 2 of the 11 SNPs with a genome-wide significant association, we used tag SNPs with less than complete linkage disequilibrium or imputed SNPs in the meta-analysis (Table 2). We genotyped one of these SNPs, rs611744, on the Immunochip platform in 8,274 UK controls (Table 1) and observed association (P=1.8×10^{-14}) on meta-analysis. The other SNP, rs8140558, is one of two SNPs at the *WNT7B* locus. In addition, a meta-analysis for this SNP that excluded the data from the UK and German case series (since these data were only indirectly genotyped [Table 2]) showed a significant genome-wide association (P=4.8×10^{-16}) (Table 4).

Regional plots of the nine DD risk loci are shown in Figure 6. To gain insight into the biological mechanisms and to find genes functionally related at these regions, we applied GRAIL analysis. The 11 SNPs at the nine regions that had a significant genome-wide association were used as query regions, resulting in the analysis of 22 unique genes. We found a total of seven associations with SNPs (P<0.05), including four SNPs implicating four WNT genes (P< 0.0001 for each) (Table5). When these results were corrected for multiple testing (22 tests), the associations with SNPs implicating the four WNT genes (rs7524102-*WNT4*, rs4730775-*WNT2*, rs6519955-*WNT7B* and rs611744-*RSPO2*) remained significant (P<0.003). We observed no association between the identified SNPs with a significant genome-wide association and gene expression in six quantitative-trait-locus data sets.

Table 3. The 35 Single Nucleotide Polymorphisms (SNPs) selected for replication.

Chromosome	SNP[a]	Base-Pair Position	Minor allele	Minor allele frequency[b]	P_{GWAS} 856 Cases, 2,836 Controls	$P_{replication}$ 1,365 Cases, 8,445 Controls	P_{meta} 2,325 Cases, 11,562 Controls	OR[c] (95% CI)	Genes of interest and GRAIL annotation[d]
1	rs7524102	22571034	G	0.21	2.9×10^{-5}	**1.0×10^{-4}**	**2.8×10^{-9}**	1.28 (1.17-1.41)	*RP11-415K20.1, WNT4*
3	rs1123148[f]	73973835	A	0.23	1.1×10^{-4}	0.39	4.8×10^{-4}	0.87 (0.80-0.95)	*RP11-20B7.1, PDZRN3*
3	rs2323206	74033842	A	0.36	5.4×10^{-5}	0.81	4.3×10^{-3}	0.91 (0.84-0.98)	*RP11-20B7.1, PDZRN3*
3	rs1356802	133320767	C	0.31	3.2×10^{-5}	0.36	1.6×10^{-4}	0.86 (0.80-0.93)	*CPNE4*
4	rs6824106	179038012	G	0.37	3.4×10^{-4}	0.12	1.4×10^{-4}	0.87 (0.81-0.94)	*RP11-162G9*
5	rs11743146	36844388	A	0.14	6.0×10^{-5}	0.78	1.2×10^{-3}	1.14 (1.02-1.27)	*CTD-2653M23.2, C5orf42*
5	rs11745128	36863776	G	0.13	9.9×10^{-5}	0.76	2.5×10^{-3}	1.12 (1.02-1.24)	*CTD-2653M23.2, C5orf42*
6	rs7747741	18409482	G	0.45	3.1×10^{-5}	0.46	0.02	0.92 (0.86-0.99)	*AL138825.1, AOF1*
6	rs2179367[f]	149804230	G	0.42	9.3×10^{-6}	0.01	2.5×10^{-7}	1.23 (1.14-1.32)	*ZC3H12D, TAB2*
6	rs237018	149785623	A	0.46	6.0×10^{-5}	0.08	1.7×10^{-5}	0.86 (0.79-0.92)	*TAB2*
7	rs16879765	37955620	A	0.19	**1.9×10^{-16}**	**2.0×10^{-22}**	**5.6×10^{-39}**	1.98 (1.78-2.18)	*EPDR1, SFRP4*
7	rs1668357[g]	37970931	C	0.27[h]	**9.5×10^{-12}**	–	–	–	*EPDR1, SFRP4*
7	rs4730775	116704354	A	0.41	4.7×10^{-5}	**3.7×10^{-4}**	**3.0×10^{-8}**	0.83 (0.77-0.88)	*WNT2*
7	rs4719773	24528683	G	0.17	7.1×10^{-5}	0.70	0.02	0.89 (0.82-0.97)	*AC005084.1, OSBPL3*
8	rs1365415	70142382	A	0.25	3.7×10^{-7}	0.09	2.8×10^{-7}	1.28 (1.17-1.39)	*C8orf34, SULF1*
8	rs611744	109297184	G	0.40	4.4×10^{-5}	**7.0×10^{-11}**	**7.9×10^{-15}**	0.75 (0.70-0.81)	*EIF3E, RSPO2*
8	rs2912522	70154934	G	0.20	6.3×10^{-8}	**3.0×10^{-6}**	**2.0×10^{-13}**	0.72 (0.66-0.78)	*C8orf34, SULF1*
9	rs10809642[e]	1189448	A	0.25	2.6×10^{-5}	**6.9×10^{-4}**	**1.2×10^{-8}**	1.35 (1.19-1.53)	*RP11-341G2.1, DMRT2*
9	rs10809650	1192371	G	0.24	1.4×10^{-4}	**4.5×10^{-5}**	**6.2×10^{-9}**	0.80 (0.74-0.88)	*RP11-341G2.1, DMRT2*
10	rs7072865	98354534	A	0.14	7.3×10^{-5}	0.53	5.9×10^{-4}	0.86 (0.77-0.95)	*PIK3AP1*

Chromosome	SNP[a]	Base-Pair Position	Minor allele	Minor allele frequency[b]	P_{GWAS} 856 Cases, 2,836 Controls	$P_{replication}$ 1,365 Cases, 8,445 Controls	P_{meta} 2,325 Cases, 11,562 Controls	OR[c] (95% CI)	Genes of interest and GRAIL annotation[d]
12	rs638791[f]	110473619	G	0.19	1.9×10^{-5}	0.27	5.5×10^{-5}	0.85 (0.78-0.94)	*SH2B3, ATXN2*
12	rs2073950	110378455	A	0.19	4.9×10^{-5}	0.42	2.5×10^{-4}	0.87 (0.80-0.96)	*SH2B3, ATXN2*
12	rs12372139[f]	130679608	A	0.44	3.1×10^{-4}	0.18	3.5×10^{-5}	0.81 (0.74-0.90)	*AC117500.2*
15	rs4932194	87046243	C	0.31	2.9×10^{-4}	1.9×10^{-3}	8.1×10^{-7}	0.82 (0.77-0.89)	*ISG20*
15	rs6496520	87039259	C	0.30	3.3×10^{-4}	2.9×10^{-3}	1.2×10^{-6}	0.82 (0.76-0.89)	*ISG20*
15	rs2171286	57265777	A	0.21	6.2×10^{-5}	0.19	7.5×10^{-5}	0.85 (0.78-0.93)	*MYO1E*
17	rs4789939	74393298	A	0.14	3.5×10^{-5}	7.0×10^{-3}	6.0×10^{-7}	0.82 (0.74-0.90)	*AC100788.1, TIMP2*
18	rs504302	37916515	G	0.50	3.9×10^{-5}	0.86	2.9×10^{-3}	1.11 (1.04-1.20)	*PIK3C3*
18	rs1944967	37785137	G	0.39	1.3×10^{-4}	0.94	4.1×10^{-3}	1.11 (1.03-1.20)	*PIK3C3*
19	rs11672517	62370006	A	0.28	**2.8×10^{-8}**	**2.7×10^{-6}**	**6.8×10^{-14}**	1.34 (1.25-1.45)	*DUXA, ZNF264*
20	rs6029273	38747104	G	0.12	1.6×10^{-6}	0.07	1.2×10^{-6}	0.76 (0.68-0.86)	*MAFB*
20	rs8124695	38461850	A	0.10	4.9×10^{-7}	**8.8×10^{-4}**	**7.6×10^{-10}**	1.48 (1.30-1.68)	*AL049691.1, MAFB*
22	rs8140558	44818937	G	0.47	**1.5×10^{-11}**	**3.8×10^{-11}**	**1.2×10^{-22}**	1.39 (1.30-1.48)	*RP11-398F12.1, WNT7B*
22	rs4820663	24684655	C	0.08	7.1×10^{-6}	0.56	3.5×10^{-3}	1.20 (1.06-1.35)	*MYO18B*
22	rs6519955	44800506	A	0.47	**2.8×10^{-13}**	**1.7×10^{-19}**	**3.2×10^{-33}**	1.54 (1.44-1.65)	*RP11-398F12.1, WNT7B*

a: The SNP positions are according to Build 36.3. SNPs meeting the significance threshold ($P<5.0x10^{-8}$ for P_{gwas} and P_{meta} or $P<0.0014$ for $P_{replication}$) are shown in bold type. b: Minor allele frequency are shown for all samples in the meta-analysis. c: Odds ratios are shown for the meta-analysis with 95% confidence intervals (CI). d: Selected named genes within or adjacent to the same linkage disequilibrium block as the associated SNPs are shown; causality is not proven. There may be other genes and other causal mechanisms. Gene names underlined were identified with the use of GRAIL analysis (P<0.01). e: The SNP rs10809642 failed genotyping in the Dutch and UK replication series. f: Four SNPs (rs1123148, rs2179367, rs638791 and rs12372139) failed genotyping in the German series. g: The SNP rs1668357 was out of Hardy-Weinberg equilibrium in the replication phase (P<0.0001) and was therefore excluded. h: The minor allele frequency for the discovery set is shown

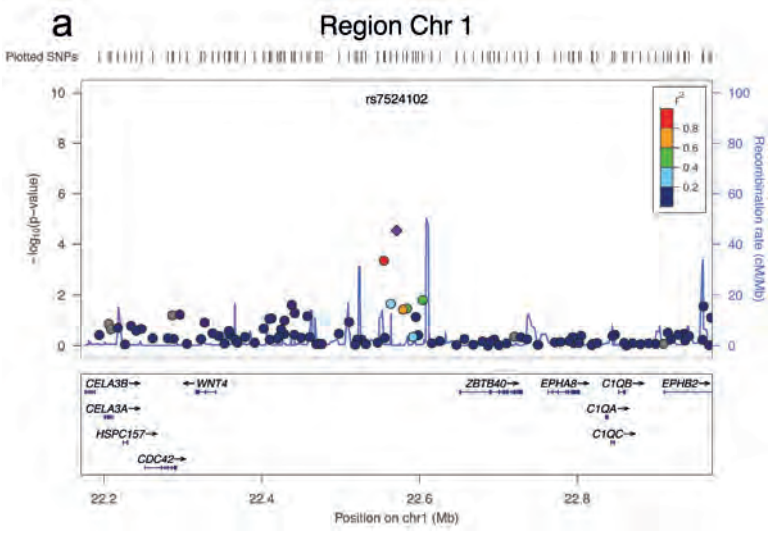
Table 4. Results for rs611744 using 8,274 UK controls on the Immunochip platform and the results for rs8140558 without using the UK and German case series.

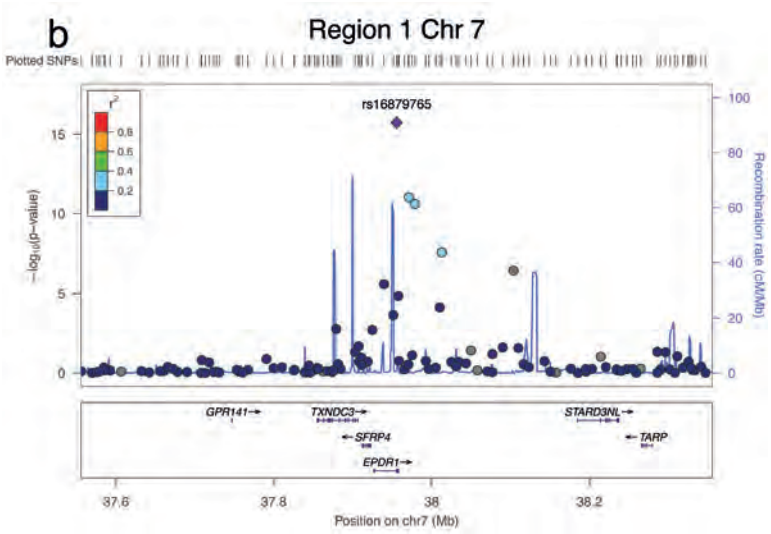
			P-values					
Chr	SNP	Locus	GWAS	Dutch	UK	German	Follow-up	Meta
8	rs611744	*RSPO2*	4.4×10^{-5}	6.5×10^{-3}	9.2×10^{-9}	NA	2.1×10^{-10}	1.8×10^{-14}
22	rs8140558	*WNT7B*	1.5×10^{-11}	5.7×10^{-4}	NA	NA	5.7×10^{-4}	4.8×10^{-16}

Table 5. The genome-wide significant SNPs with a GRAIL P-value < 0.05

SNP	Gene	GRAIL P-value*
rs7524102	*WNT4*	5.2×10^{-6}*
rs8140558	*WNT7B*	2.2×10^{-5}*
rs6519955	*WNT7B*	2.2×10^{-5}*
rs4730775	*WNT2*	2.3×10^{-5}*
rs611744	*RSPO2*	1.1×10^{-4}*
rs8124695	*MAFB*	0.02
rs10809642	*DMRT1*	0.04
rs10809650	*DMRT1*	0.04
rs16879765	*EPDR1*	0.04

**P-values are uncorrected. Correction for 22 tests requires a P-value < 0.0023. P-values indicated with an asterisk withstand the multiple testing correction.*

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Plotted SNPs
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r²
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0.4
0.2
−log10(p-value)
Recombination rate (cM/Mb)
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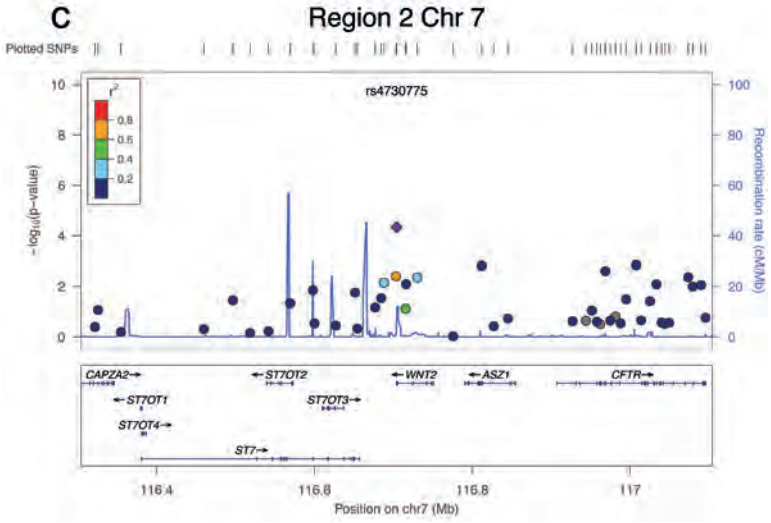
TXNDC3
SFRP4
EPDR1
STARD3NL
TARP
37.6
37.8
38
38.2
Position on chr7 (Mb)


C
Region 2 Chr 7
Plotted SNPs
rs4730775
r²
0.8
0.6
0.4
0.2
- log10(p-value)
Recombination rate (cM/Mb)
CAPZA2
ST7OT1
ST7OT4
ST7OT2
ST7OT3
ST7
WNT2
ASZ1
CFTR
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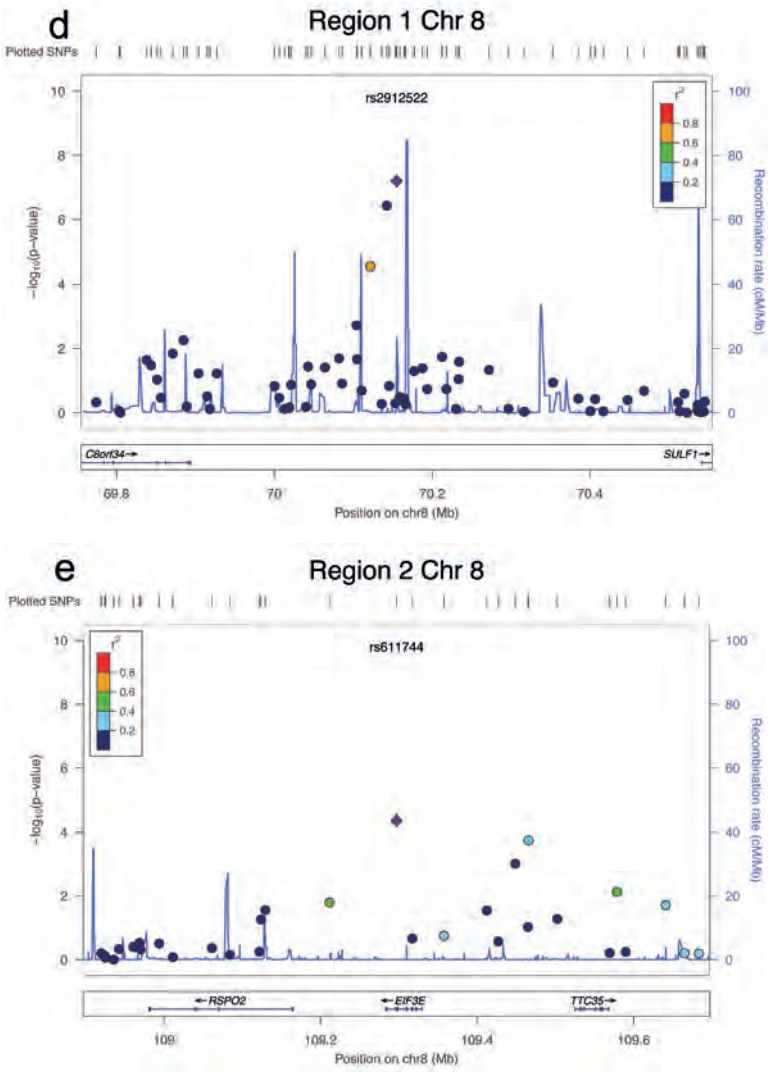
116.6
116.8
117
Position on chr7 (Mb)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0.6
0.4
0.2
−log10(p−value)
Recombination rate (cM/Mb)
C8orf34
SULF1
69.8
70
70.2
70.4
Position on chr8 (Mb)
e
Region 2 Chr 8
Plotted SNPs
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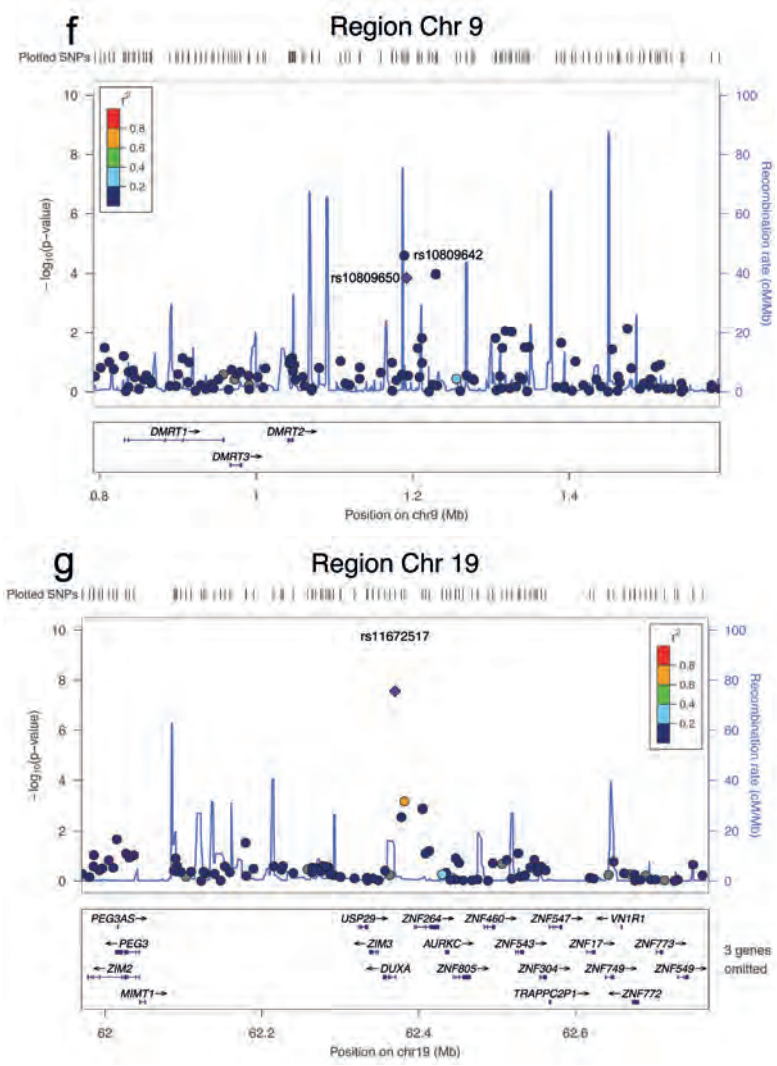
RSPO2
EIF3E
TTC35
109
109.2
109.4
109.6
Position on chr8 (Mb)


f
Region Chr 9
Plotted SNPs
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rs10809650
DMRT1
DMRT2
DMRT3
Position on chr9 (Mb)
Recombination rate (cM/Mb)
g
Region Chr 19
Plotted SNPs
rs11672517
PEG3AS
PEG3
ZIM2
MIMT1
USP29
ZNF264
ZNF460
ZNF547
VN1R1
ZIM3
AURKC
ZNF543
ZNF17
ZNF773
DUXA
ZNF805
ZNF304
ZNF749
ZNF549
TRAPPC2P1
ZNF772
3 genes omitted
Position on chr19 (Mb)
Recombination rate (cM/Mb)

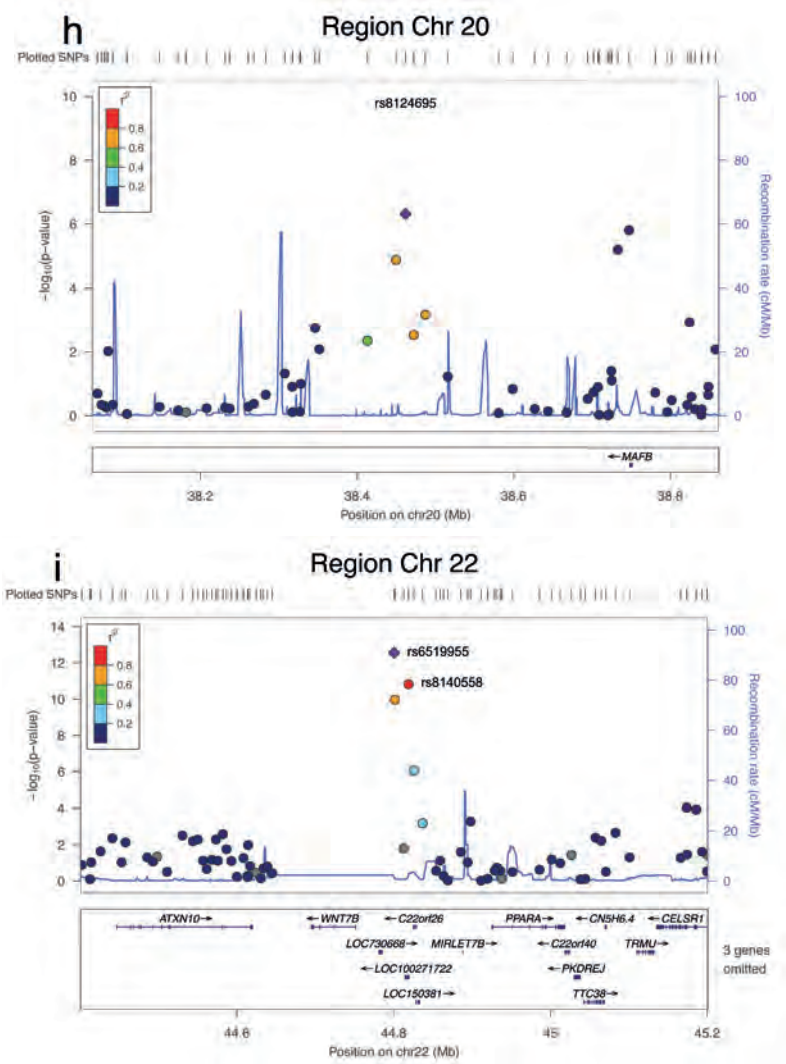

Figure 6. Regional plots of the nine Dupuytren's disease risk loci identified in this study.

The P-values obtained in the discovery phase using a 1-degree-of-freedom basic χ^2 allelic test corrected for genomic inflation (y-axis) were plotted against their chromosomal map positions (x-axis). Per region, the most significant SNP in the meta-analysis is plotted in purple and in a diamond shape. The color of each SNP spot reflects its r^2 linkage disequilibrium value. Estimated recombination rates were plotted in blue.[3] a: region on chromosome 1. b: region 1 on chromosome 7, EPDR1 and SFRP4 are located near rs16879765. c: region 2 on chromosome 7. d: region 1 on chromosome 8. e: region 2 on chromosome 8. f: region on chromosome 9. g: region on chromosome 19. h: region on chromosome 20. i: region on chromosome 22, SNPs rs6519955 and rs8140558 are in linkage disequilibrium with each other (r^2 = 0.96).

Discussion

We identified nine chromosomal loci associated with susceptibility to DD. Very little is known about the heritability of this disease, since there are only a few reports from family and twin studies.[15-17] Our findings suggest that common genetic variants have an important causative role in DD in Northern European populations.

A GRAIL analysis showed that four different DD risk loci contain genes that encode proteins in the Wnt-signaling pathway: 1p36.23-p35.1, containing *WNT4* (rs7524102); 7q31.2, containing *WNT2* (rs4730775); 22q13, containing *WNT7B* (rs6519955); and 8q23.1, containing *RSPO2* (rs611744). Three other associated loci also contain WNT genes, although they were not implicated on GRAIL analysis: 7p14.1, containing *SFRP4* (rs16879765); 8q13, containing *SULF1* (rs2912522); and 6q25.1, containing *TAB2* (rs2179367). However, the last of these three did not reach genome-wide significance ($P_{meta}=2.47x10^{-7}$).

The WNT gene family consists of structurally related genes that encode glycoproteins, extracellular signaling molecules. Abnormal Wnt-signaling is linked to a range of diseases, especially cancer. The best understood Wnt-signaling pathway is the canonical pathway, which activates the nuclear functions of ß-catenin, leading to changes in gene expression that influence cell proliferation and survival.[18] Abnormal proliferation of fibroblasts is a key feature of early development of DD. The disease can be divided into three histological stages: stage 1, proliferation of fibroblasts; stage 2, differentiation of fibroblasts into myofibroblasts; and stage 3, formation of mature type 1 collagen.[19,20] Wnt-signaling is known to regulate the proliferation and differentiation of fibroblasts in both cancer and fibromatosis.[21] Most of our knowledge of Wnt-signaling is derived from studies of cancer. In colon cancer, up-regulation of WNT-signaling causes intestinal crypt cells to proliferate for longer than usual before they migrate and differentiate.[22] This prolonged proliferation phase results in the formation of polyps and confers a predisposition to cancer.

The involvement of the Wnt-signaling pathway in the pathogenesis of DD is consistent with features of the disease and with established aspects of Wnt-signaling. An imbalance of Wnt-signaling in DD could cause fibroblasts in the fascia of the hand to proliferate and form nodules. Indeed, increased levels of ß-catenin have been observed in DD primary cell cultures in vitro,[23] suggesting that the Wnt-signaling pathway is overstimulated in DD.

The WNT proteins, WNT2, WNT4 and WNT7B, which were identified on GRAIL analysis, bind to Frizzled receptors, leading to a cascade of events that eventually result in a decrease in the rate of β-catenin degradation (Figure 7).[18] Secreted frizzled-related proteins, such as SFRP4, antagonize the Wnt-signaling pathway by binding to either

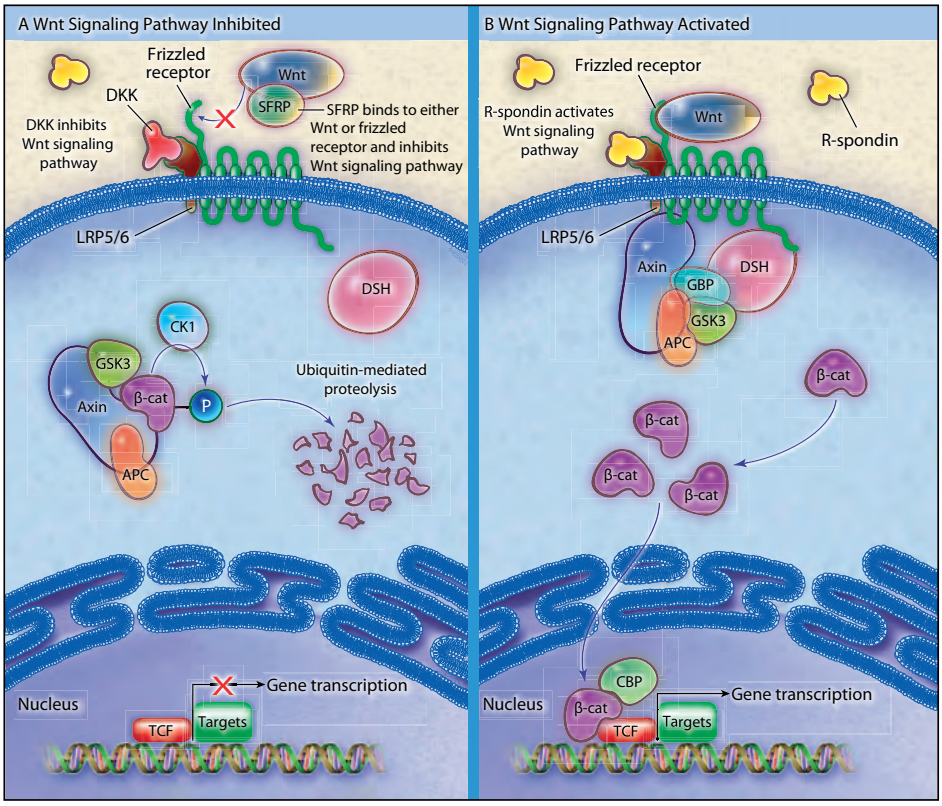

Figure 7. Signaling pathways of WNT and ß-catenin.

The nine susceptibility regions in DD include three WNT genes, one gene for secreted frizzled-related protein (SFRP), and one gene for R-spondin (RSPO2) genes. Panel A shows that in the absence of WNT protein, ß-catenin (ß-cat) is degraded, and forthcoming target genes are in a repressed state. Panel B shows that if WNT signaling is active, ß-catenin degradation is reduced. SFRPs antagonize the Wnt-signaling pathway by binding to either WNT or frizzled receptor, thereby affecting receptor occupancy. R-spondin positively regulates ß-catenin signaling by interacting with the frizzled receptor and the low-density lipoprotein-receptor-related protein (LRP5/6) and by competing with dickkopf protein (DKK).[18-31] APC denotes adenomatous polyposis coli, CBP cyclic AMP response-element– binding (CREB) protein–binding protein, CK1 casein kinase 1, DSH disheveled protein, GBP GSK3-binding protein, GSK3 glycogen synthase kinase 3, P phosphorylation, SFRP secreted frizzled-related protein, and TCF T-cell factor.

WNTs or Frizzled receptors, thereby affecting receptor occupancy. In the absence of active WNT, ß-catenin is degraded, and potential target genes will not be activated.

Another DD risk locus contains *RSPO2*, encoding an R-spondin; members of the R-spondin family interact with Frizzled receptors and LRP5/6 to induce ß-catenin

signaling. Furthermore, R-spondins induce canonical Wnt-signaling by competing with dickkopf (DKK) protein for binding to LRP5/6. The DKK protein is an inhibitor of Wnt-signaling; it hinders the formation of a complex between WNT, Frizzled receptor and LRP5/6 (Figure 7).[24] SULF1, a Heparan sulfate 6-O-endosulfatase, is known to influence canonical Wnt-signaling. How it does so is not clear, but 6-O-desulfation of heparan sulfate proteoglycans may alter the binding of WNT to its Frizzled receptor.[25,26] Increased activity of these WNT and R-spondin genes or a decreased activity of SFRP could stimulate Wnt-signaling and reduce intracellular ß-catenin degradation. This mechanism could trigger fibroblasts to proliferate, leading to the development DD.

Also supporting a role for Wnt-signaling in DD is the microRNA (miRNA) expression profiles of fibroblasts and palmar fascia in persons with this disease, as compared with those of healthy controls. These miRNAs regulate genes related to the ß-catenin pathway: *WNT5A*, *ZIC1*, and TGFB1.[27]

The three remaining significant loci lack an obvious connection to the WNT pathway. An interesting candidate gene from these regions is *MAFB*. The RNA of *MAFB* has been shown to be up-regulated in the excised cord tissue from persons with DD, as compared to fascia of the hand in healthy controls.[28] Maf proteins are known for their role in fibrosarcoma and are believed to influence tissue development and cellular differentiation.[29] MAFB can transform primary fibroblasts in vitro.[30] The gene might therefore be involved in stage 2 of DD (the differentiation of fibroblasts into myofibroblasts). The miRNA expression profile associated with DD implicated some miRNAs in influencing the expression of *MAFB* as well.[27]

The results of our study indicate that genetic factors have a major role in the development of DD. Associations with variations in genes that encode proteins in the Wnt-signaling pathway suggests that aberrations in this pathway confer susceptibility to the disease. Further genetic and basic research in this field is required to fully unravel the pathogenesis of Dupuytren's disease.

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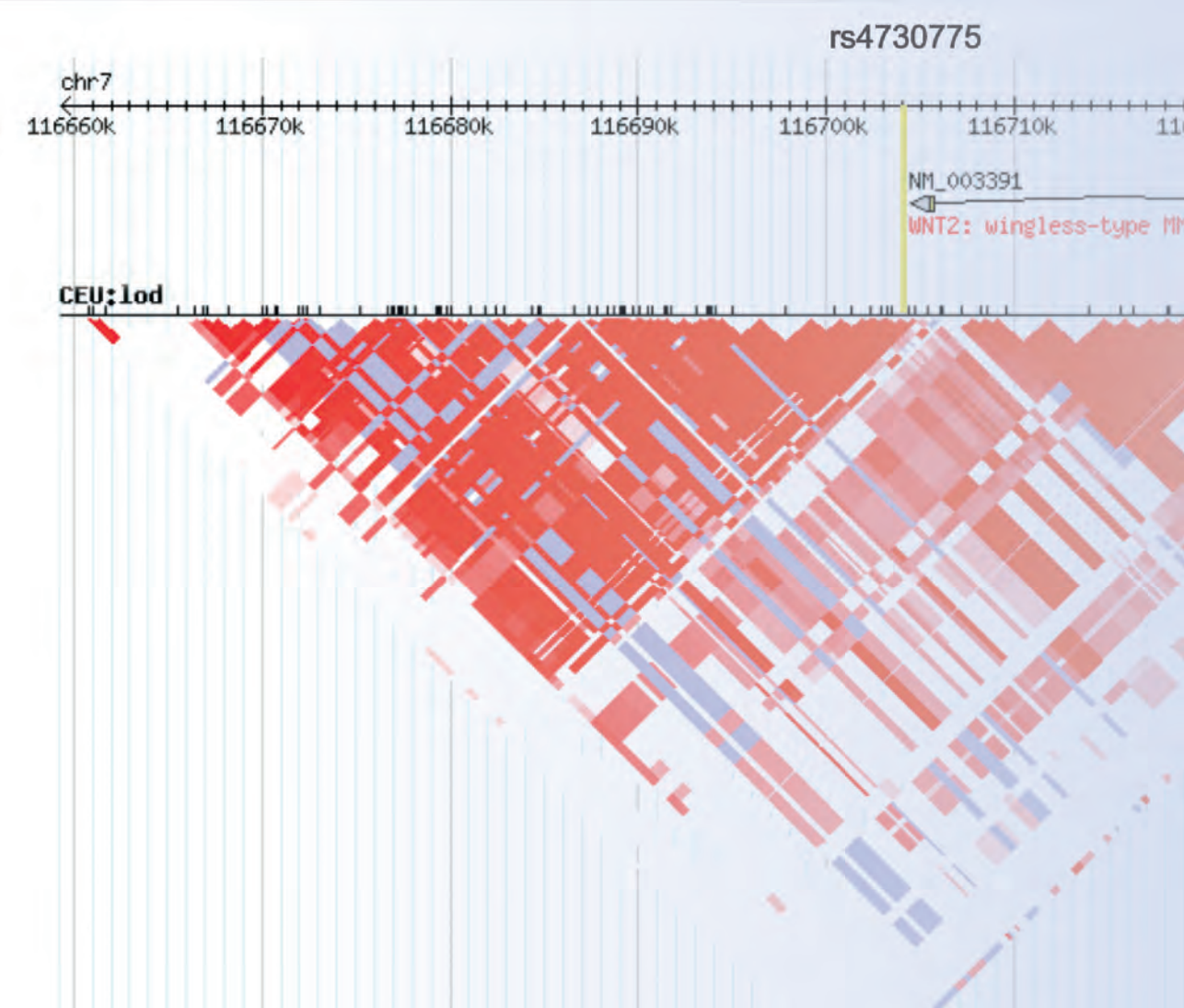
Chapter 5


rs4730775
chr7
116660k
116670k
116680k
116690k
116700k
116710k
NM_003391
WNT2: wingless-type
CEU:lod

WNT2 locus is involved in genetic susceptibility of Peyronie's disease

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J Sex Med 2012;9:1430-4

Abstract

Introduction

Peyronie's disease (PD) is a fibromatosis of the penis, with a pathology very similar to what is seen in the hand (palmar fascia) in Dupuytren's disease (DD). Recently, we performed a genome-wide association study and identified nine genetic loci containing common variants associated with DD. Seven of these loci mapped within or near genes of the canonical WNT pathway and each locus yielded relatively large odds ratios (ORs) for DD disease status.

Aim

Given the clinical overlap between PD and DD, we examined whether the nine DD susceptibility loci are also involved in PD.

Methods

An association study was performed using a case/control design. From 2007 to 2010, we prospectively included 111 men who had been clinically diagnosed with PD. Control subjects (n=490 males) were randomly drawn from a population-based cohort from the same region of the Netherlands. Allele frequencies in the 111 PD cases and 490 controls were compared using a 1-degree-of-freedom basic chi square test. A P-value < 0.05 after Bonferroni correction for the nine tested single nucleotide polymorphisms (SNPs) was considered statistically significant (i.e. $P < 0.0056$).

Main Outcome Measures

Association of genetic markers (SNPs) with PD

Results

We observed significant association with SNP rs4730775 at the wingless-type MMTV integration site family member 2 (*WNT2)* locus on chromosome 7 (P = 0.0015, OR 0.61), but found no evidence for the other eight loci being involved with PD despite the large effect size seen for some of these variants in DD. The *WNT2* association was even more significant after we removed 15 patients with co-morbid DD.

Conclusions

WNT2 is a susceptibility locus for PD and our finding provides evidence for a partly shared genetic susceptibility between PD and DD.

Introduction

Peyronie's disease (PD) is a localized fibromatosis of the penile tunica albuginea (TA), the fibrous layer of connective tissue covering the corpora cavernosa of the penis. PD may present with pain and/or a variety of deformities, which are most evident during erection as TA compliance is compromised at the plaque. The curvature and loss of rigidity may compromise sexual intercourse and thereby seriously affect the quality of life of patients and their partners.

The prevalence of PD in the general population is estimated to be as high as 3-9%.[1,2] PD is thought to be under-reported because of embarrassment, poor screening, or because it is considered an unavoidable consequence of aging.[3] The etiology of PD is still not fully understood. Repetitive minor trauma during sexual intercourse, followed by abnormal wound healing and scar formation, has been suggested as a mechanism of plaque formation.[4] A genetic predisposition to the development of PD has also been proposed. Nyberg et al. showed that PD was transmitted in an autosomal dominant pattern in three families.[5] However, no susceptibility locus has been identified so far.

A variety of co-morbidities have been associated with PD, including cigarette smoking, radical prostatectomy, diabetes mellitus, hyperlipidemia, hypertension and Dupuytren's disease (DD).[6-8] In a recent cross-sectional study, DD was shown to be the only co-morbidity factor which was significantly associated with PD, with a co-morbidity rate ranging widely, from 1.5–39%.[9]

DD is characterized by fibrosis of certain fascias of the hand and fingers with similar fibrotic alterations to those seen in PD. As the myofibroblast is an essential cellular component of DD nodules, a common pathophysiology of PD and DD has been suggested. This was substantiated by the observation of similar alterations in gene expression in PD and DD.[10]

We recently performed a genome-wide association study (GWAS) in 2,325 DD cases and 11,562 population controls; this revealed nine susceptibility loci for DD with relatively large effect sizes (odds ratio (OR) of 1.25-1.98).[11] Given these results, we examined whether the DD-associated variants identified in the GWAS also play a role in PD susceptibility.

Materials and Methods

Patient population

We had 121 PD patients available for this study; they were prospectively recruited at the outpatient clinic of the Urology Department, University Medical Center Groningen,

the Netherlands from 2007 to 2010. Written informed consent was given by all patients, and the Institutional Review Board approved the study. PD was diagnosed by an experienced urologist based on palpation of a plaque and available photographs of the erect penis. The diagnosis of DD was based on the presence of characteristic nodules and/or cords in the palm of the hand, with or without contracture of the digits. We collected clinical information, including age of onset, and treatment modality. We also collected 10 ml of venous blood from each subject for DNA extraction using standard methods.

Five hundred male control subjects, of whom genotype data were already available, were randomly drawn from the 'LifeLines' cohort, a large population-based study currently being conducted in the northern Netherlands.[12] No phenotypic information with regard to DD or PD was available for these subjects; there was no overlap between the control subjects for the genome-wide association study on DD[11] and those used for this study.

Genotyping

Nine SNPs that were previously associated with DD[11] were genotyped using KASP assays (KBioscience, Hoddesdon, Herts, UK) (Table 1). For all the control individuals we had Illumina CytoSNP-12 (Illumina, San Diego, CA, USA) data, comprising more than 300,000 SNPs and including the nine SNPs associated with DD. To corroborate that no genotyping bias is introduced due to difference by genotyping platform, we performed individual genotyping of these nine SNPs in 96 individuals using KASP and different Illumina SNP arrays and observed 100% concordance rate.

Statistics

We used PLINK 1.07 for quality control and statistical analysis.[13] SNPs were excluded when the call rate < 98%, or when deviation from Hardy Weinberg equilibrium (HWE) was observed ($P < 0.05$); samples were removed from further analysis when call rates < 95%. Quality control was performed separately in the case and control groups and repeated after merging the genotype data. We were not able to correct for population stratification, since only the nine SNPs were genotyped in the PD patient group. However, all patients were Caucasians from the Netherlands.

Allele frequencies in the PD cases and controls were compared using a 1-degree-of-freedom basic chi square test. A P-value < 0.05 after Bonferroni correction for the nine tested SNPs was considered statistically significant (i.e. $P < 0.0056$).

Statistical power calculations using the odds ratios and allele frequencies of the previous DD GWAS indicated that, given the sample size of this study, the power ranged between 26% and 98% for the different loci (Table 1).

Table 1. Nine SNPs associated with Dupuytren's disease (DD) in a GWAS by Dolmans et al.[11]

Chr	SNP	Position	Minor allele	Allele frequencies		P	OR (95% CI)[a]	Power[b]
				DD Cases	Controls			
1	rs7524102	22571034	G	0.21	0.17	2.8×10^{-9}	1.28 (1.17-1.41)	29%
7	rs16879765	37955620	A	0.19	0.11	5.6×10^{-39}	1.98 (1.78-2.18)	98%
7	rs4730775	116704354	A	0.40	0.46	7.0×10^{-9}	0.79 (0.74-0.86)	26%
8	rs2912522	70154934	G	0.20	0.25	2.0×10^{-13}	0.72 (0.66-0.78)	57%
8	rs611744	109297184	G	0.40	0.47	7.9×10^{-15}	0.75 (0.70-0.81)	38%
9	rs10809650	1192371	G	0.24	0.28	6.2×10^{-9}	0.80 (0.74-0.88)	29%
19	rs11672517	62370006	A	0.28	0.23	6.8×10^{-14}	1.34 (1.25-1.45)	36%
20	rs8124695	38461850	A	0.10	0.07	7.6×10^{-10}	1.48 (1.30-1.68)	46%
22	rs6519955	44800506	A	0.47	0.37	3.2×10^{-33}	1.54 (1.44-1.65)	43%

a: OR = odds ratio, CI = confidence interval. b: power calculation assuming similar minor allele frequencies in PD as in DD for these SNPs; 111 cases; 490 controls; PD prevalence of 9%; significance threshold of 0.05. Power calculations were performed using the Genetic Power Calculator (http://pngu.mgh.harvard.edu/~purcell/gpc/).[21]

Results

The mean age of patients at the time of diagnosis was 56.8 years (standard deviation (SD) ± 9.7) and the mean age of PD onset was 51.9 years (SD ± 10.2). Fifteen patients (13.5%) were also affected with DD.

We excluded 10 PD subjects and 10 controls because of low genotyping rates for the 9 SNPs, leaving 111 patients and 490 control subjects for further analysis. There were no signs of differences in SNP call rates between cases and controls in the data. The genotype rate in the remaining individuals was 100% with no genotype exclusion based on HWE criteria.

The results of the association analysis are listed in Table 2. We observed significant association with SNP rs4730775, which is located at wingless-type MMTV integration site family member 2 (*WNT2*) with an uncorrected P = 0.0015, and OR of 0.61 (95% confidence interval (CI) 0.45-0.83) (Figure 1, Table 2). Excluding the 15 PD cases with co-morbidity of DD revealed an even more significant association, with an uncorrected P = 0.00084 and OR 0.58 (95% CI 0.42-0.80).

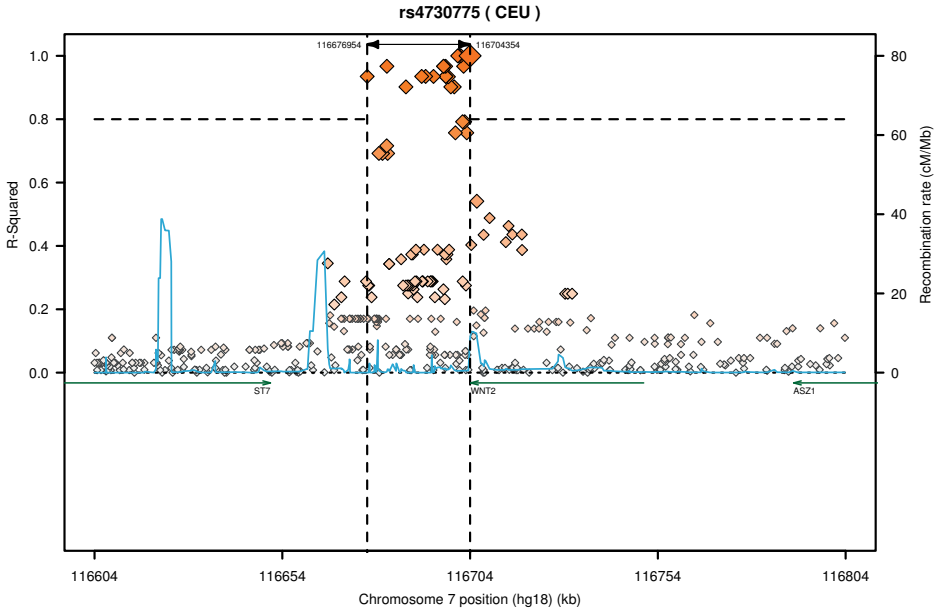

Figure 1. Regional linkage disequilibrium plot for SNP rs4730775 on chromosome 7 (based on 1000 Genomes data, pilot 1 in CEPH [CEU]). This figure was generated with SNP Annotation and Proxy Search.[20]

Table 2. The association results for Peyronie's disease.

Chr	SNP	Position	Minor allele	Allele frequencies		P	OR (95% CI)[a]	Gene[b]
				PD Cases	Controls			
1	rs7524102	22571034	G	0.17	0.15	0.48	1.15 (0.78-1.71)	*WNT4*
7	rs16879765	37955620	A	0.13	0.12	0.85	1.04 (0.67-1.62)	*SFRP4*
7	**rs4730775**	**116704354**	**A**	**0.35**	**0.46**	**0.0015**	**0.61 (0.45-0.83)**	***WNT2***
8	rs2912522	70154934	G	0.22	0.24	0.53	0.89 (0.63-1.27)	*SULF1*
8	rs611744	109297184	G	0.46	0.48	0.61	0.93 (0.69-1.24)	*RSPO2*
9	rs10809650	1192371	G	0.28	0.30	0.55	0.91 (0.66-1.25)	*DMRT2*
19	rs11672517	62370006	A	0.26	0.22	0.18	1.26 (0.90-1.76)	*ZNF264*
20	rs8124695	38461850	A	0.08	0.07	0.79	1.08 (0.62-1.87)	*MAFB*
22	rs6519955	44800506	A	0.38	0.36	0.51	1.11 (0.82-1.49)	*WNT7B*

a: OR = odds ratio, CI = confidence interval. b: selected named genes within or adjacent to the same linkage disequilibrium (LD) block as the associated SNPs; causality is not proven. The one SNP meeting the significance threshold is shown in bold.

Discussion

To examine whether the DD-associated variants identified in the GWAS also play a role in PD susceptibility, we performed this association study.

We observed significant association with SNP rs4730775 (*WNT2*). The association was even more significant after we removed 15 patients with co-morbid DD, which proves that the association is caused by PD and not by DD. None of the other loci yielded a significant result with PD, even though power calculations had indicated a very high likelihood of finding a significant association for rs16879765 (98%, Table 1) assuming similar ORs between PD and DD.

The fact that we find rs4730775 to be associated with PD in this relatively small cohort (with an a priori power of 26% based on DD findings) may suggest that this locus has more effect on the origin of PD than DD. For the remaining seven loci (not including rs16879765 with a power of 98%), the power was < 57% indicating that we were less likely to replicate these loci in this study anyway.

An OR < 1 for the A-allele at rs4730775 (0.61) means that this allele is protective for developing PD as it is also protective for developing DD. However we do not know yet if this improves or deteriorates the function of *WNT2* or other genes at this locus. *WNT2* is a strong candidate gene for PD pathogenesis. *WNT2* is a member of the *WNT* gene family, which consists of structurally related genes that encode glycoproteins. These act as extracellular signaling factors. WNT2 is especially associated with gastrointestinal cancer and is also used as a tumor marker of gastric and colorectal cancer.[14] The best understood Wnt-signaling pathway is the canonical pathway, which activates the nuclear functions of ß-catenin, leading to changes in gene expression that influence cell proliferation and survival.[15]

A recent study identified increased levels of ß-catenin, the end-product of Wnt-signaling, in TA-derived cells from PD patients compared to cells from normal TA tissue.[16] This suggests that the Wnt-signaling cascade is over-stimulated in PD. Wnt-signaling is known to regulate proliferation and differentiation of fibroblasts in both cancer and fibromatosis.[17] This mechanism may trigger fibroblasts to proliferate excessively as observed in the process of developing PD.

Flanking *WNT2* we found two other genes (Figure 1): *ST7* that encodes for a low-density lipoprotein receptor-related protein that interacts with proteins related to signal transduction pathways,[18] and *ASZ1* (or *GASZ*) that encodes for a germ cell protein, which is essential for male meiosis.[19] There is no functional data at this time that would support involvement of these genes in the susceptibility of PD or DD, however.

The strong aspects of this study are the prospective design and the fact that all PD patients were examined by a single experienced urologist. Limitations are the sample size and the absence of a replication cohort.

We have identified *WNT2* as susceptibility locus for PD and provide evidence for a partly shared genetic susceptibility between PD and DD. The fact that we did not find evidence for involvement of the *SFRP4* locus with PD despite a statistical power of 98% based on previous DD findings suggests that there may also be some distinct genetic susceptibility factors between the diseases. However, larger follow-up studies are required to establish this more firmly. The strong genetic findings for these disorders warrant further genome-wide efforts to determine the genetic bases of PD and DD.

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Chapter 6

Dupuytren Diathesis and Genetic Risk

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Hand Surg Am 2012;37:2106-11

Abstract

Purpose

Dupuytren's disease (DD) is a benign fibrosing disorder of the hand and fingers. Recently, we identified 9 single nucleotide polymorphisms (SNPs) associated with DD in a genome-wide association study. These SNPs can be used to calculate a genetic risk score for DD. The aim of this study was to test whether certain clinical characteristics (including the DD diathesis features) of patients with DD are associated with a high genetic risk score.

Methods

Between 2007 and 2010, we prospectively invited all DD patients (1,120 in total) to participate. Clinical characteristics were noted using patient- and doctor-completed questionnaires, and blood was obtained for DNA analysis. We analyzed a total of 933 subjects with genetic and clinical data. The 9 previously identified DD SNPs were used to calculate a weighted genetic risk score. Patients were categorized into high and low genetic risk score groups, according to their weighted genetic risk score. Logistic regression was performed to study the association of clinical characteristics with a high genetic risk score.

Results

In a univariate regression model, patients with an age of onset of DD younger than 50 years, a family history positive for DD, knuckle pads, and Ledderhose's disease were statistically significantly associated with a high genetic risk score. In an additional analysis using high and low genetic risk groups that deviate further from the median, Ledderhose's disease was no longer significantly associated with DD.

Conclusions

Patients with DD who present with these diathesis features, and predominantly patients with knuckle pads, are more likely to carry more risk alleles for the discovered DD SNPs than patients without these diathesis features.

Clinical Relevance

These markers may prove useful in predicting disease progression or recurrence.

Introduction

Dupuytren's disease (DD) is a benign fibrosing disorder of palmar fascias of the hand and fingers leading to the formation of nodules and cords. Often these cords contract, causing flexion contractures of the fingers. The prevalence of DD has been reported to vary between 0.2 and 56%.[1] The prevalence of DD rises with increasing age,[2] and DD is found most frequently in Caucasian males.[3] Standard treatment consists of collagenase injection, and percutaneous division or surgical excision of the nodules and cords.[4,5] At present, the disease is incurable and recurrence rates following treatment vary from 8% to 66%, depending on the treatment modality and definition of recurrence.[5-7] DD is associated with several environmental factors, such as alcohol consumption, smoking, and antiepileptic drug use, as well as with diseases such as diabetes mellitus and liver disease.[8]

The way DD develops varies over time. Some clinical characteristics of patients with DD are related to a more aggressive course of the disease or diathesis. In 1963, Hueston[9] postulated the idea of a DD diathesis and described 4 factors defining this subset of disease: early onset of disease, bilateral involvement, positive family history, and the presence of ectopic lesions (knuckle pads, Ledderhose's disease, and Peyronie's disease). In 2006, male sex as a diathesis factor was added, "early onset of disease" was refined to age of onset younger than 50 years, and the ectopic lesions were restricted to the presence of knuckle pads only.[2] Features of the DD diathesis were used also in a scoring system by Abe et al. in 2004 to evaluate the risk of recurrence and extension of DD for a Japanese population, implicating a more aggressive course of the disease.[10] These authors suggested the addition of radial side involvement and little finger involvement to the diathesis scoring list.

The clustering of DD in families has long been recognized and most genetic studies have reported an autosomal dominant inheritance pattern.[11,12] Recently, we suggested that DD is a complex genetic disorder, in which several genetic and environmental risk factors are involved, each contributing to disease susceptibility.[13] Nine SNPs associated with DD were identified in a 2-stage genome-wide association study in 2,325 DD patients and 11,562 population controls. These SNPs represent the presently known genetic DD profile and can be used to calculate a genetic risk score for DD in each patient; the more risk alleles a patient carries, the higher the genetic risk score will be for that patient.[14]

We hypothesized that there is an association between certain clinical characteristics and the genetic risk score of patients with DD. Therefore, the aim of this study was to test whether clinical characteristics (including the diathesis features) of DD patients are associated with a high genetic risk score.

Material and Methods

Between 2007 and 2010, we prospectively invited all patients evaluated for a diagnosis of DD at the outpatient clinics of the plastic surgery departments of 6 hospitals in the Netherlands to participate. In this period a total of 1,120 patients gave their consent (see flow chart, Figure 1). Patients were diagnosed by plastic surgeons with substantial clinical experience in treating DD. The diagnosis was based on the presence of characteristic nodules and/or cords in the palm of the hand and/or digits, with or without contracture of the digits. Written informed consent was acquired from all patients, with institutional review board approval. Patients were asked to complete a questionnaire inquiring about details concerning their clinical characteristics including age of onset, familial involvement, level of education, hand labor, medical history, and medications. A positive family history was defined as the presence of at least 1 other affected family member as noted by the patient. For 58 patients the patient's questionnaire was not available (Figure 1). The plastic surgeons completed a separate

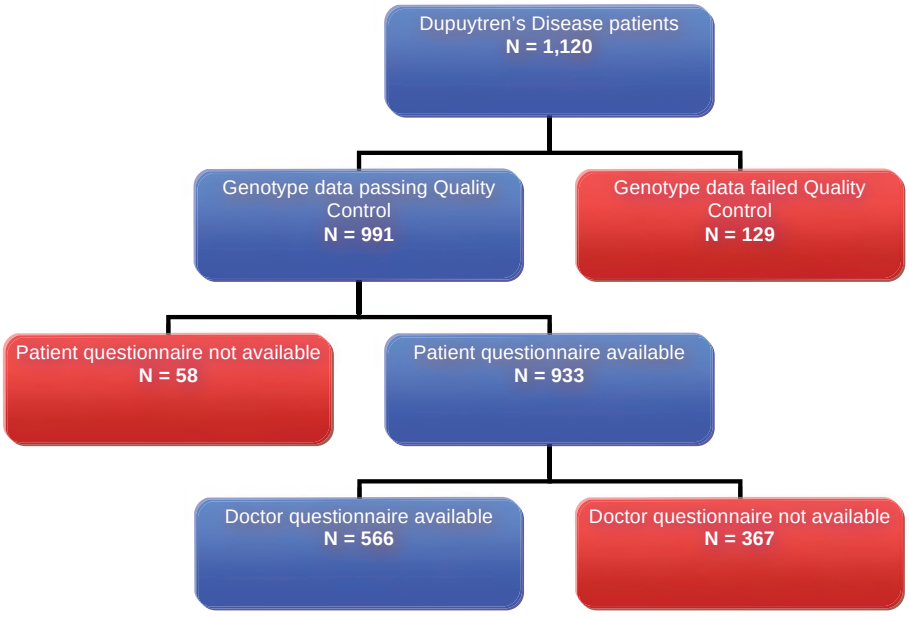

Figure 1. Inclusion flow chart.

questionnaire on the clinical characteristics of these patients, including passive extension deficits in metacarpophalangeal (MCP), proximal interphalangeal (PIP), and distal interphalangeal (DIP) joints, unilateral or bilateral disease, the number of rays involved, and the presence of ectopic deposits (knuckle pads, Ledderhose's disease, and Peyronie's disease). For 367 patients the doctor's questionnaire was not available (Figure 1). We did not collect clinical data concerning radial side involvement and little finger involvement as used in the scoring system by Abe et al.[10] Blood was obtained from all patients for DNA analysis.

Genetic and statistical analysis

Details about genotyping and quality control steps have been described previously.[13] One hundred twenty-nine DNA-samples did not pass standard quality control and were excluded from further analysis (Figure 1). The 9 SNPs that were found to be associated with DD were at an individual basis used to calculate a weighted genetic risk score (wGRS).

The wGRS of each patient was calculated by multiplying the number of risk alleles per SNP by the weight for that SNP, taking the sum across the SNPs, and dividing this number by the 9 SNPs according to the following formula:

$$wGRS = \frac{\sum_{i=1}^{n} w_i X_i}{n}$$

where i is the SNP, n is the number of SNPs, w_i is the weight for SNP i, and X_i is the number of risk alleles. The natural log of the odds ratio (OR) for each allele was used for the weight. For this wGRS calculation, PLINK software (version 1.07),[15] a tool set for genetic analysis, was used.

The more risk alleles that are carried by a patient, the higher the wGRS. The wGRS scores were primarily divided in 2 categories (low and high score), where all scores below the median were considered as low score and all the scores equal to or higher than the median were considered as high score. An additional analysis was performed in which the groups were subdivided into categories that deviated further from the median. The low genetic risk score group was defined as a wGRS lower than 1 standard deviation (SD) from the median and the high genetic risk score group as a wGRS higher than 1 SD from the median. These categories were designed arbitrarily.

All data were transcribed categorically by using binary variables. Because the doctor's questionnaire was not available for all patients, a chi-square test was used to

compare the patient groups with and without a doctor's questionnaire. We thereafter performed univariate logistic regression to study the association of clinical characteristics with the presence of a high genetic score and calculated ORs and 95% confidence intervals. $P < 0.05$ was considered as statistically significant.

Results

An overview of the clinical characteristics noted in the patient's and doctor's questionnaires is shown in Table 1 and Table 2, respectively. Of the 933 patients who completed the patient's questionnaire, 711 (76%) were male and 222 (24%) were female. There were no differences in the frequencies of clinical characteristics between the patients with or without a completed doctor's questionnaire (Table 1).

Table 1. Relevant clinical characteristics, patient's questionnaire (N = 933)

Clinical Characteristics	Full Group N (%)	Doctor Questionnaire Present N=566 (%)	Doctor Questionnaire Absent N=367 (%)	Chi -square
Sex				
Male	711 (76%)	421 (74%)	290 (79%)	*P*=0.104
Female	222 (24%)	145 (26%)	77 (21%)	
Age of onset				
< 50	355 (39%)	208 (37%)	147 (41%)	*P*=0.251
≥ 50	561 (61%)	350 (63%)	211 (59%)	
Family history for DD				
Positive	461 (50%) w	270 (48%)	191 (52%)	*P*=0.208
Negative	468 (50%)	293 (52%)	175 (48%)	

DD, Dupuytren's disease.

The median wGRS of the 933 patients was 0.009 (ranging from -0.044 to 0.064, see also Figure 2). Based on the wGRS, 461 patients were below the median and classified as the low genetic risk score group (including 282 patients with a completed doctor's questionnaire), and 472 patients were above the median and classified as the high genetic risk score group (including 284 patients with a completed doctor's questionnaire).

Table 2. Relevant clinical characteristics, doctor's questionnaire (N = 566)

Clinical Characteristics	N (%)
Number of Affected Rays	
<3	390 (74%)
≥ 3	139 (26%)
Total passive Extension Deficit	
<45°	298 (54%)
≥ 45°	253 (46%)
Knuckle pads present	
Yes	82 (15%)
No	465 (85%)
Ledderhose's disease present	
Yes	72 (13%)
No	475 (87%)
Peyronie's disease present	
Yes	22 (4%)
No	339 (60%)
Bilateral involvement	
Yes	330 (59%)
No	226 (41%)

The OR of having a high genetic risk score were significantly greater in patients with an age of onset of DD younger than 50 years of age, a family history positive for DD, knuckle pads, and Ledderhose's disease (Tables 3 and 4). An additional analysis was performed, in which the low genetic risk score group was defined as a wGRS lower than 1 SD from the median and the high genetic risk score group as a wGRS higher than 1 SD from the median. Age of onset of DD younger than 50 years of age, a family history positive for DD, and knuckle pads significantly predicted a high genetic score in this additional analysis (Table 5).

Discussion

The goal of this study was to test whether selected clinical characteristics of patients with DD were associated with a high genetic risk score. Patients were categorized into high and low genetic score groups according to their wGRS. In a univariate regression model, age of onset of DD younger than 50 years of age, a family history positive for

Table 3. Prediction of characteristics patient's questionnaire on high genetic risk score using a univariate logistic regression analysis.

Variable	OR	95% CI	*P*-value
Sex			
Male	1.28	0.94-1.73	0.11
Female	1		
Age of onset			
<50	1.35	1.04-1.77	**0.03**
≥ 50	1		
Family history for DD			
Positive	1.58	1.22-2.04	**0.001**
Negative	1		

CI, confidence interval; DD, Dupuytren's disease; OR, odds ratio.

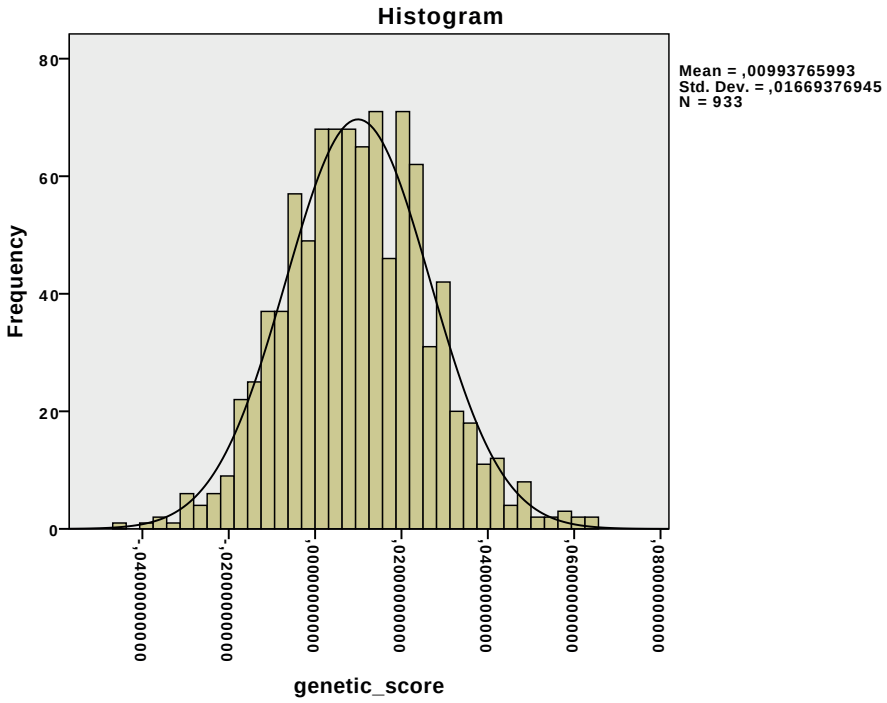

Figure 2. Histogram of genetic risk scores.

Table 4. Prediction of characteristics doctor's questionnaire on high genetic risk score using a univariate logistic regression analysis.

Variable	OR	95% CI	*P*-value
Number of Affected Rays			
≥ 3	0.87	0.59-1.28	0.47
<3	1		
Total passive Extension Deficit			
≥ 45°	1.22	0.87-1.71	0.24
<45°	1		
Knuckle pads present			
Yes	1.95	1.20-3.18	**0.01**
No	1		
Ledderhose's disease present			
Yes	1.66	1.00-2.76	**0.05**
No	1		
Peyronie's disease present			
Yes	1.26	0.53-3.03	0.60
No	1		
Bilateral involvement			
Yes	1.29	0.92-1.80	0.15
No	1		

CI, confidence interval; OR, odds ratio.

DD, knuckle pads, and Ledderhose's disease were significantly associated with a high genetic risk score.

The OR's of the significant diathesis features varied from 1.35-1.95, each having only a moderate effect on predicting a high genetic risk score. This is related to the fact that the study population was primarily divided into 2 large subgroups (genetic risk scores higher or lower than the median), in which most patients had a genetic score with a value almost equal to the median. When the groups were further subdivided into categories that deviated 1 SD from the median, age of onset of DD younger than 50 years of age, a family history positive for DD, and knuckle pads remained significant with, as expected, larger effect sizes (Table 5). The presence of Ledderhose's disease-ceased to be significant in the additional analysis and was only just significant in the primary analysis. Further research has to demonstrate if Ledderhose's disease is really associated with a high genetic risk score.

Table 5. Prediction of the significant diathesis features on high genetic risk score in an additional analysis using univariate logistic regression*

Variable	OR	95% CI	*P*-value
Age of onset			
<50	1.92	1.18-3.12	**0.009**
≥ 50	1		
Family history for DD			
Positive	1.92	1.20-3.10	**0.007**
Negative	1		
Knuckle pads present			
Yes	4.40	1.76-10.98	**0.001**
No	1		
Ledderhose's disease present			
Yes	1.44	0.59-3.51	0.42
No	1		

CI, confidence interval; OR, odds ratio.

** For this additional analysis, the low genetic risk score group was defined as a wGRS lower than 1 SD from the median and the high genetic risk score group as a wGRS higher than 1 SD from the median.*

The clinical characteristics that were statistically significant in this study are all features of the DD diathesis defined by Hueston[9] and later revisited by Hindocha et al.[2] In the revisited DD diathesis, the definition of ectopic lesions was restricted to the presence of knuckle pads.[2] In our study, the presence of knuckle pads revealed the highest OR of all the significant clinical characteristics. Therefore, the presence of knuckle pads can be seen as the diathesis feature predominantly associated with a high genetic risk score.

Male sex and bilateral disease, 2 other DD diathesis features, were not associated with a high genetic score. The former can be easily explained, because the 9 DD susceptibility SNPs are not located on the sex chromosomes. Therefore, an analysis using these SNPs will not show differences related to sex. The latter we consider as a less specific characteristic, because bilateral disease occurs in many patients over time and the survey time point influenced this factor. Peyronie's disease was also not associated with a high genetic score. In 36% of doctor's questionnaires Peyronie's disease was not scored (Table 2), perhaps because of hesitance of the doctor to ask about this issue. This might have had an effect on the results.

The strengths of this study were the prospective design and the large cohort of patients who have been characterized. Limitations of this study were the lack of follow-up, the use of nonvalidated questionnaires, and the fact that, in only 566 patients, the doctor's questionnaire was completed. Because patients presented to the outpatient clinics for evaluation of their disease, presumably because of concern of their condition, there might have been a selection bias.

DD patients who present with an age of onset younger than 50 years, a positive family history, or ectopic disease (particularly with knuckle pads) are more likely to carry more risk alleles for the discovered DD SNPs than patients without these diathesis features. It is reassuring to find that there was a relation between certain diathesis features and the recently identified DD risk genotypes.

We know that the diathesis features of DD can lead to a more aggressive disease. It is to be expected that the more risk alleles a patient carries (the higher the genetic risk score), the more aggressive the disease will be. We are currently planning the follow-up for patients in this study to answer this question. It would be interesting to investigate the relationship between genetics and the course of the disease and especially the occurrence of recurrent disease.

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Chapter 7

General Discussion

Introduction

In this thesis we investigated the genetic background of Dupuytren's disease (DD) and its associated fibromatosis. Although DD has been recognized for centuries, its aetiology and pathogenesis have not been fully elucidated. The clustering of DD seen in families, its prevalence in individuals of northern European descent, and its association with other fibromatosis, like Peyronie's disease (PD) and Ledderhose's disease, suggest a genetic influence on the development of this disease. Understanding the genetic background of DD could provide insight into the pathogenesis of the disease. Genetic knowledge about fibromatosis could also aid in disease prediction, facilitate diagnosis, lead to the development of alternative, non-surgical, treatments, and even to interventions that prevent fibromatosis from occurring or re-occurring.

To this end, we have studied the mode of inheritance of familial DD and performed a genome-wide association study (GWAS). This resulted in the discovery of nine genetic loci associated with DD that show strong evidence for the involvement of Wingless-type MMTV integration site family (Wnt) signaling in disease susceptibility. Subsequent analysis of these susceptibility loci provided evidence for a shared genetic risk factor between DD and PD. Finally, we discovered that patients with certain DD diathesis features are more likely to carry more risk alleles for the DD single nucleotide polymorphisms (SNPs) than patients without these clinical features. In the following discussion, we interpret what these results mean for disease prediction, treatment and pathogenesis.

Disease prediction and the role of genetics

The current situation

Several clinical characteristics of patients with DD have been found to be related to a more aggressive course of the disease or diathesis and can be used to predict the progression of the disease. In 1963, Hueston postulated the DD diathesis and suggested that four factors are involved: early onset of disease, bilateral involvement, a positive family history, and the presence of ectopic lesions.[1] In 2006, male gender was added as a diathesis factor, "early onset of disease" was refined to age of onset under 50 years, and the ectopic lesions were restricted to the presence of knuckle pads only.[2] Features of the DD diathesis were also used in a scoring system by Abe et al. in 2004 to evaluate the risk of recurrence and extension of DD in a Japanese population who showed a more aggressive course of the disease.[3] They suggested the addition of radial side involvement and previous surgery to the little finger to the diathesis scoring list. In

chapter 6 of this thesis, we show that patients with an age of onset of DD younger than 50 years, a family history positive for DD, knuckle pads and Ledderhose's disease were more likely to carry more risk alleles for the nine DD SNPs we discovered (chapter 4) than patients without these diathesis features. The question remains whether these genetic variants can also help us with disease prediction.

Disease prediction in complex genetic disease

Complex genetic diseases such as DD result from the joint effects of multiple genetic factors, mostly with small effect sizes, and environmental factors. This is in contrast to monogenetic diseases where mutations in a single gene induce a disease or lead to a distinctively higher risk for that disease. Risk of a complex genetic disease differs only slightly between a carrier and a non-carrier of a risk variant of a single susceptibility gene. Hence, prediction of disease based on a single variant is not informative.[4] Prediction of complex diseases instead requires the simultaneous testing of multiple genetic variants known as genetic profiling. Furthermore, the value of this genetic profiling depends on the heritability: the proportion of phenotypic variation in a disease that is due to genetic variation. Assuming that the heritability of a disease is 50% (a hypothetical assumption) and that all genetic variants affecting this trait are known and their effects can be estimated without error, genetic profiling would still predict only half of the phenotypic variation. To measure the efficacy of a disease predictor, whether genetic or clinical, the area under the receiver operator curve (AUC) is typically reported; a high AUC value means that there is high predictive power.

In a systematic review of risk prediction based on genetic markers in type 2 diabetes, the AUCs among the 23 studies included did not improve substantially with the addition of genetic markers compared to the conventional risk prediction based on clinical risk factors (median AUC 0.79 versus median AUC 0.78, respectively).[5] In another study, the investigators used genetic risk reclassification for type 2 diabetes by age of onset (below or above 50 years) using 40 SNPs. In people younger than 50 years, the genetic risk score improved upon that from prediction based on clinical risk factors alone by 10%, compared to 0.4% in people 50 years or older.[6] This example shows that a more accurate risk classification can improve the predictive power of genetic markers when using them in an appropriate subgroup of individuals.

Since, for most complex diseases, the susceptibility loci discovered so far only explain a small proportion of the heritability, they consequently have low predictive power. It has been suggested that a substantial proportion of heritability comes from a large number of common SNPs, each with very small effect sizes, which, as a consequence, cannot be detected at the stringent genome-wide significance levels with current sample sizes. Prediction performance could be improved by using a polygenic

approach that includes many SNPs that do not reach genome-wide significance.[7] Stahl and colleagues estimate that by using polygenetic risk score analysis in type 2 diabetes, an additional 49% of disease risk can be explained.[8] A similar effect is seen in studies on the quantitative trait of height: in a large GWAS involving more than 100,000 subjects, 200 loci were identified that explain only 10% of the variance.[9] However, when another study simultaneously considered 294,831 common SNPs in predicting adult height, 45% of the variance could be explained.[10] Therefore, a polygenetic risk score approach could be more useful for disease prediction in DD as well.

Heritability of DD

Little was known about the heritability of DD until recently, with just a few reports from family and twin studies.[11-13] More recently, Capstick et al. calculated that nine loci account for 12.1% of the total heritability of DD, based on a sibling recurrence risk (λ_s) in the UK of 4.5.[14,15] In their study, 47% of siblings of DD patients had DD compared with 10% of randomly selected siblings. In another study, λ_s for DD was 2.9.[13] However, in this study, λ_s was calculated by dividing the sibling recurrence risk in DD patients (10.3) by the population prevalence of DD in north-western England (3.5). Since the reported prevalence of DD varies considerably, between 0.6% and 31.6%,[16] one must be careful with the interpretation of these data.

Another method of calculating the genetic variance explained by the nine DD loci is to estimate the population attributable risk (the proportion of cases that would not

Table 1. The calculated population attributable risk (PAR) for Dupuytren's disease assuming a prevalence of the disease of 5%, 8% and 32%.

Chromosome	SNP	Position	Allele	PAR% Prevalence 0.05	PAR% Prevalence 0.08	PAR% Prevalence 0.32
1	rs7524102	22571034	G/A	1.2	1.9	5.3
7	rs16879765	37955620	A/G	4.0	6.1	15.6
7	rs4730775	116704354	A/G	0.9	1.4	4.3
8	rs611744	109297184	G/A	1.4	2.2	6.5
8	rs2912522	70154934	G/A	1.4	2.3	6.9
9	rs10809650	1192371	G/A	0.9	1.5	4.4
19	rs11672517	62370006	A/G	1.5	2.3	6.7
20	rs8124695	38461850	A/C	1.8	2.8	7.7
22	rs6519955	44800506	A/C	2.1	3.3	9.5
	PAR% combined			14.4	21.5	50.4

occur if the factor where eliminated, PAR). However, PAR is also estimated based on prevalence. In Table 1, we calculated PAR for DD assuming a prevalence of the disease of 5%, 8% and 32%, resulting in a PAR of 14.4%, 21.5% and 50.4%, respectively. These prevalence numbers are based on a study in Flanders, where 32% of individuals with an age above 50 years had DD (at least one nodule) and 8% of individuals had a flexion contracture of the fingers.[17] In a more recent prevalence study in the northern part of the Netherlands, a random sample of 1,360 individuals was investigated: in 17.9% of individuals of over 50 years of age, nodules and cords were present and 4.2% of individuals had flexion contractures of the fingers.[18] What our calculations show is that there are still uncertainties as to how much of the genetic variance is explained by the known DD susceptibility loci.

Is disease prediction relevant for DD?

DD is not a life-threatening disease and there are no preventative measures as yet. Therefore, it could be argued that it is not healthcare's highest priority to predict who will develop DD in the future. Nevertheless, for a clinician it remains difficult to accurately predict the course of DD. In some patients the disease remains stable and does not progress into disabling flexion contractures.[19] An intervention in these cases is not necessary. In other patients the disease can be very aggressive and a more extensive treatment may be appropriate,[20] since the type of treatment influences the chance of recurrence of the disease. For example, Van Rijssen and colleagues reported a recurrence rate of 84.9% after five years for needle fasciotomy and of 20.9% for limited fasciectomy.[21] Since secondary surgery has more complications and a worse outcome,[22] it would be helpful for the physician, and beneficial to patients, to accurately predict the course of the disease prior to deciding on the type of treatment.

How to further study and improve prediction in DD

Designing a prospective cohort study

In order to test if the currently-known genetic variation can be used for disease prediction, a follow-up study of the probands included in the GWAS or a new prospective cohort study is desirable. LifeLines, a large, population-based, cohort study in the northern Netherlands,[23] would be a suitable cohort for studying this topic. LifeLines aims to collect prospectively a wide spectrum of data from 165,000 individuals over a period of 30 years, to allow the longitudinal study of multiple diseases. DNA samples from more than 3,000 individuals from LifeLines were used as control individuals for the GWAS in chapter 4. Unfortunately for our study, DD had not been included as a phenotype for study in the LifeLines cohort. Merely adding a standardized photograph

of the hands and adding several questions to the current questionnaires, in combination with the already available genetic data, would make this an ideal cohort to study disease progression and the relation with genetics. In the light of the current digital and mobile era, we could even ask LifeLines participants to photograph their hands and upload these to their private LifeLines folder. Additionally, the LifeLines cohort could also be used for studying DD prevalence, and investigating the relationship of certain comorbidities and medication with the disease.

Finding more genetic variation involved in DD

Increasing sample size

As explained above, the ability to predict the course of DD will increase as we identify more of the genetic variation involved in the disease. To date, only one medium-sized GWAS on DD has been performed that discovered nine loci associated with DD (chapter 4). For PD, we are just at the beginning of genetic studies, with only our small association study revealing a shared DD locus at *WNT2* (chapter 5). By increasing sample size, and thereby statistical power, future GWAS should identify more genetic associations. Since large numbers of SNPs are being tested in GWAS, an association must reach a stringent threshold ($P < 5 \times 10^{-8}$) to achieve statistical significance.[24] Most GWAS are underpowered to identify significant SNPs with smaller effect sizes. From recent studies we know that increasing the sample size has led to the discovery of many more common variants with modest effects in complex diseases.[9,25,26] A study by Parkes and colleagues showed a strong relationship between sample size and the expected number of detectable associated loci ($r^2 = 0.94$).[27].

To give an example of the sample sizes used in GWAS: the first study in 2005, in more than 1,000 individuals with inflammatory bowel disease (IBD), found only one associated locus.[28] A large GWAS meta-analysis in 2011, in almost 45,000 individuals, revealed 99 IBD loci,[29] and the most recent, even larger, meta-analysis in 75,000 individuals identified 163 IBD loci.[30]

The Oxford team who collaborated with us in the replication phase of our DD GWAS (chapter 4) is currently building an extensive new cohort including more than 5,000 cases for a larger second generation GWAS in DD. Since we too are continuing to collect blood samples of DD patients, we will be able to contribute to the further expansion of future GWAS studies. Combining data from individual association studies in a meta-analysis increases the power to find SNP associations for common alleles of modest effect size. However, different studies may have been executed on different array platforms and using different numbers of SNPs. A useful tool to harmonize GWAS datasets for meta-analysis is imputation. This technique is used to predict the

genotypes at SNPs that are not directly genotyped in the study sample, but based on existing knowledge of haplotype structure, such as from HapMap or the Genome of the Netherlands.[31,32] Imputation can improve the power for GWAS in a single study,[33] and can be instrumental in the meta-analysis of GWAS as it facilitates combining data from different studies and/or different genotyping platforms. On the other hand, imputing genotypes can also introduce errors in the dataset depending on the quality of the reference genome and the linkage disequilibrium between genotyped and predicted SNPs.

Another approach to gaining power for GWAS would be to perform a cross disease meta-analysis, using diseases with a presumed similar background. In the case of DD this could, for instance, be another type of fibromatosis or other diseases in which a potential role for Wnt-signaling has been identified (for instance in colon cancer[34]). Such an approach has been successful for other complex human traits: in a meta-analysis of celiac disease and rheumatoid arthritis GWAS studies identified 14 new shared disease loci.[35]

Selecting SNPs in a pathway

Instead of hypothesis-free testing of genetic markers for association, an alternative option is to use a candidate-pathway approach that consists of genes encoding proteins that are known to form a functional network.[36] Only testing genes in a pre-defined pathway seriously decreases the number of markers to be used, and consequently the number of tests to be carried out, thereby reducing the need for statistical correction. The disadvantage of this kind of approach is its reliance on existing knowledge about the known or presumed biology of the phenotype under investigation. For DD it would be interesting to use this pathway approach for genes involved in Wnt-signaling. A Wnt-signaling pathway-association-analysis was recently performed in HCV-infected patients.[37] They identified 58 candidate genes involved in the Wnt-signaling pathway and found 3,016 SNPs with a minor allele frequency (MAF) > 0.05 on the Illumina HumanOmni chip to be used for this analysis. Polymorphisms in several genes involved in the Wnt-signaling pathway were associated with hepatic fibrosis or inflammation risk in HCV-infected patients.

Low frequency and rare variants

Part of the genetic susceptibility for DD could be explained by variants with low MAF, defined as 0.5% < MAF < 5%, or by rare variants (MAF < 0.5%).[38] These rare variants are not present on many of the commercially available GWAS chips (e.g. the chip we used for the DD GWAS), which mainly consist of variants with MAF greater than 5%. Due to the low allele frequency of rare variants, case-control cohorts need to be very large

to gain enough power to find significant association, e.g. more than 10,000 cases are needed to generate the 80% statistical power necessary to detect a variant with a MAF of 0.1% and odds ratio of 2 (disease prevalence 0.05).[39] On the other hand, the effect sizes of these rare variants are often not sufficient to be detected via classical linkage analysis in family studies (Figure 1, lower left). Figure 1 shows the relation between MAF and effect size; the lower the frequency of a genetic variant, the higher the effect size. We performed linkage analysis (unpublished data) in six families (including the families described in chapter 3). In all, we investigated 32 affected individuals and found no significant linkage. Nor could we replicate the locus on 16q identified using linkage analysis in one large Swedish family by Hu et al.[11] The fact that we did not find significant linkage could be the result of the lack of multigenerational data. Since DD is a late onset disease, with a mean age of onset of 49 years in patients with a positive history of DD,[13] it is difficult to find families where more than two generations are affected. Other explanations could be lack of power or extensive genetic heterogeneity.

The most comprehensive technique for detecting rare sequence variants is by sequencing (the process of determining the full nucleotide order of a given DNA segment). Recently, the possibility of high-resolution high-throughput sequencing of a

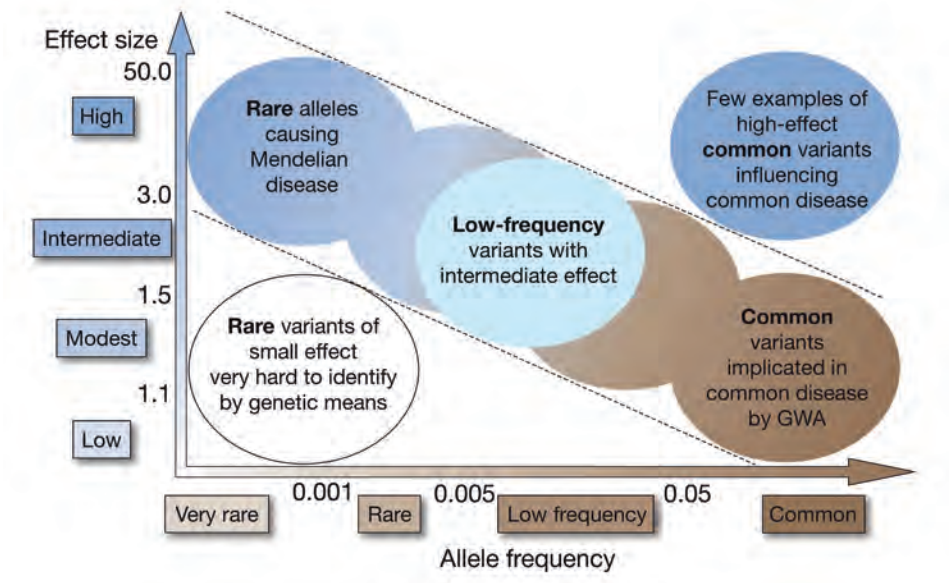

Figure 1. Feasibility of identifying genetic variants by risk allele frequency and strength of genetic effect (odds ratio). Reprinted by permission from Macmillan Publishers Ltd: [Nature] (Manolio et al.), copyright (2009).[38]

whole exome, or even a whole genome, has become financially feasible. Sequencing studies on, for instance, IBD have identified multiple rare variants.[40,41] However, Hunt and colleagues concluded from their large sequencing study of 25 GWAS risk genes of auto-immune diseases in 24,892 cases and 17,019 controls that rare variants have a minor role in disease susceptibility.[42] It has been postulated that the remaining heritability results from many common variant loci of weak effect in type 2 diabetes and autoimmune diseases.[8,42-44]

Structural variation and other interactions

Structural variation, such as copy number variants (CNVs, insertions and deletions) and copy neutral variation (inversions and translocations) could also account for a part of the unexplained heritability. CNVs can be detected using commercially available SNP genotyping arrays, however they are often under-represented.[45] Shih et al. made the first attempts to find CNVs in DD and found significantly higher copy numbers of CNVs at chromosome 7p14.1 and 14q11.2.[46,47] The strongest associated SNP in the DD GWAS (rs16879765; P = 5.6×10−39; odds ratio, 1.98) near *SFRP4* is located at chromosome 7p14.1.

The missing heritability could also be explained by *de novo* mutations, epigenetic effects and gene-environmental interactions.

In contrast to DD patients with a positive family history for DD, we also see many sporadic cases, which could be caused by *de novo* mutation events rather than inherited variants. *De novo* mutations in DD could be identified by sequencing exomes of sporadic DD patients and their unaffected parents and by investigating whether *de novo* mutations affecting protein sequences occur at higher than expected rates. It would be interesting to see if these mutations implicate genes in specific biological processes, as done by a study in schizophrenia, which found *de novo* mutations in genes encoding postsynaptic proteins.[48]

Epigenetic effects are reversible, heritable changes to the way cells respond to environmental cues and regulate gene expression. Epigenetic effects occur without changing the DNA sequence itself. The partially reversible process of switching part of the genome on or off is controlled by DNA methylation, histone modification, RNA interference and chromatin structure. The Encyclopaedia of DNA Elements (ENCODE) project has systematically mapped these regions of the genome.[49] It is not yet known if these epigenetic effects are tissue-specific and therefore it is not clear how these effects should be investigated in DD.

For the investigation of gene-environmental interactions very large sample sizes are typically needed to generate adequate power to perform the analysis while taking into account multiple confounders. The LifeLines cohort study would be an ideal cohort

to investigate these interactions. DD is known to be associated with factors such as smoking, use of alcohol, anti-epilepsy drugs, and medical conditions including liver disease and diabetes.[18,50,51]

The role of genetics in the treatment of DD

The current situation

The standard treatment of DD consists of surgical excision of the nodules and cords, collagenase injection, or percutaneous division.[21,52] At present, the disease is incurable and recurrence rates following treatment vary from 8% to 85%, depending on the treatment modality and definition of recurrence.[20,21,53,54] At the moment, there is no medication available to treat for DD.

Treatments based on genetic knowledge

Genetic knowledge could be helpful in developing new treatment strategies for disease. Some GWAS findings have identified previously unknown pathogenic pathways, such as complement-mediated inflammation in macular degeneration[55] and autophagy in Crohn's disease.[56] Clinical applications to interact with these pathways in these diseases still have to be developed. In the DD GWAS (chapter 4), we identified nine genome-wide significant loci for DD. At six of these loci, genes involved in Wnt-signaling are present. It is important to note that thus far we have only identified genome-wide significant associations with SNPs near *WNT* genes. We did not formally prove that Wnt-signaling is involved in DD.

Wnt-signaling is also a critical regulatory component in the control of bone formation and bone resorption. WNT induces osteoblastogenesis and thereby enhances bone formation and suppresses osteoclastogenesis. This evidence forms the basis for new anabolic approaches to the treatment of osteoporosis.[57] Antibodies to sclerostin, which prevent the binding of sclerostin to the Lrp-5-Lrp6-co-receptor, enhance Wnt-signaling and increase bone mass in rodent and in non-human primates.[58] Clinical trials with romosozumab (AMG 785), a monoclonal humanized antibody to sclerostin are currently underway.[59] Anti-Dkk-1 antibodies that stimulate Wnt-signaling have also been developed for human use. Information on their tolerability and effectiveness is not available yet.[60] These drugs that influence Wnt-signaling could also have an effect on DD, and could potentially be used in its treatment.

Drug targets for DD

At the moment, more information is needed about the presumed role of Wnt-signaling in DD. Investigation of the downstream effects of the associated DD loci are needed. Also, the identification of new genetic variants and the pathways involved may provide new drug targets for DD. In a recent paper on rheumatoid arthritis (RA), the investigators found 98 RA risk genes, 27 of these genes are the pharmacological targets of known and approved RA drugs.[61]

It would be interesting to know if there are also patients with DD in the romosozumab drug trial. The potential effect on DD could then simultaneously be investigated. The incidence of DD in individuals treated with WNT drugs could potentially be lower and individuals with DD could have a less aggressive phenotype.

The role of genetics in the pathogenesis of DD

The current situation

The pathogenesis of DD remains uncertain. The proliferating myofibroblasts are the cells thought to be responsible for the flexion contraction in DD.[62,63] These cells are characterized by the presence of α-smooth muscle actin, an actin isoform typical of vascular smooth muscle cells.[64] Transforming growth factor (TGF)-ß is an important factor involved in the development of myofibroblasts and also promotes collagen formation, with a decrease of the ratio of type III to type I collagen.[65]

Insight in pathogenesis based on genetic findings

As already mentioned, genetic associations can yield insight into previously unknown pathogenic pathways, such as complement-mediated inflammation in macular degeneration[55] and autophagy in Crohn's disease.[56] The GWAS findings in DD (chapter 4) highlighted Wnt-signaling as a possible pathway involved in DD pathogenesis. It must be noted that in the light of all the GWAS performed since 2005, it is a unique finding to discover such a clear pathway in a single moderately powered GWAS.

The *WNT* gene family is an essential mediator in the cell-cell communication during embryogenesis.[66] It consists of structurally related, highly conserved genes that encode glycoproteins, which have a function as extracellular signaling molecules. The best-known Wnt-signaling pathway is the canonical pathway, which activates the nuclear functions of ß-catenin, leading to changes in gene expression that influence cell proliferation and cell survival.[67]

Abnormal Wnt-signaling has also been linked to a range of other diseases such as colorectal cancer,[34] schizophrenia,[68] kidney disease,[69-71] leukaemia,[72] osteoarthritis,[73,74]

osteoporosis,[75] and fibromatosis.[67,76] Except for the associations of DD with other fibromatosis, liver disease and diabetes,[51] no other comorbidities are yet known. It would be interesting to investigate if DD is more common in patients with one of the above diseases also linked to abnormal Wnt-signaling.

Most of our knowledge is derived from studies of cancer. In colon cancer, up-regulation of Wnt-signaling causes intestinal crypt cells to proliferate for longer than usual before they migrate and differentiate.[34] This prolonged proliferation phase results in the formation of polyps and can predispose to cancer. Abnormal proliferation of myofibroblasts/fibroblasts is a key feature in the early development of DD[77] and therefore fits well with our current ideas of the origin of the disease. An imbalance of Wnt-signaling in DD could cause fibroblasts in the hand to proliferate and form nodules.

Translation into function

Attempts should be made to proceed from disease-associated SNPs to function. By identifying downstream effects, more insight will be gained into DD's pathogenesis.

In GWAS, associations are found with common variants, which are generally not the disease-causing variant. A follow-up approach would be to search for disease-causing variants, which could be done by DNA sequencing. Sequencing of affected individuals in families with DD or patients with extreme phenotypes would maximize the chances of positive results.

Limiting the sequencing effort to a limited target region optimizes efficiency and cost-effectiveness. We could start with the loci that were pinpointed by the GWAS in DD or prioritize on candidate genes in these loci. Genes can be prioritized based on known gene characteristics, however, we tend to prefer genes and pathways with particular functions that connect to the disease in question. There are also tools available to prioritize genes in an associated LD block, such as GRAIL (Gene Relationships Across Implicated Loci), which is based on text-mining.[78] There are multiple other ways to prioritize genes, for example, in a recent paper on rheumatoid arthritis, genes were prioritized based on eight criteria.[61]

Based on our findings in chapter 4, I would suggest focusing on the *WNT* gene members at the loci discovered. An interesting candidate gene at the non-WNT containing loci is *MAFB* on 20q. In a study by Lee et al. RNA of *MAFB* was shown to be up-regulated in excised cord tissue from persons with DD, compared with fascia of the hands in healthy controls.[79] When they used immunochemistry staining, MAFB protein was only identified in DD tissue and not in the fascia of healthy controls, implicating a possible role in DD development.

Another option to gain more insight into downstream affects would be to investigate expression quantitative trait loci (eQTL) for DD. These are loci at which genetic al-

lelic variation is associated with variation in certain gene expression levels. Studies on eQTLs have shown that common variants can affect gene expression levels of nearby genes (*cis* eQTLs)[80] and also of genes further away (*trans* eQTLs).[81,82]

Differential gene expression studies could also help in dissecting disease pathogenesis. Obviously, for DD these studies should be performed in the DD tissue itself. The most cell-rich tissue is found in the nodule in comparison to cord tissue.[65] The cell type known to be involved in DD is the myofibroblast/fibroblast.[83,84] As control tissue, the transverse palmar ligament[85,86] (Skoog's fibres) or the A1 pulley have been used, since these are not involved in DD. Samples of DD tissue are easily available, since the treatment of DD consists of excision of the affected tissue. However, direct isolation of RNA from DD tissue has proven to be very difficult in our experience. An alternative is to culture cells from DD tissue and subsequently isolate RNA out of these cells. The next step is to study the protein, the end product of a gene. It is currently possible to selectively knock out a gene in a cell by using the bacterial clustered regularly interspaced short palindromic repeats (CRISPR)-Cas9 system.[87] Investigating the properties of myofibroblast/fibroblast with selective knock out of one of the *WNT* genes could yield more insight into the Wnt-signaling pathway and the function of Wnt proteins in DD.

In summary

Our results have shown that DD is a complex disorder with genetic factors playing an important role in disease susceptibility. This new insight provides avenues for additional research into the molecular basis of DD and the associated fibromatosis. Below we have outlined several ways to continue this mission:

- Designing a prospective cohort study to investigate how disease progression is related to the DD susceptibility SNPs and investigate gene-environmental interactions
- Identifying more common variants using a larger second-generation GWAS, meta-analysis with future GWAS studies or a candidate-pathway approach; expanding the genetic studies to include structural variations (CNV) analysis
- Identifying rare variants using (exome) sequencing techniques or linkage analysis in multigenerational families
- Performing functional studies in cell lines of fibroblasts/myofibroblasts that focus on products of the *WNT* gene family

With the research presented in this thesis we have made a large leap forward in the genetic studies of DD. However, there is still a lot of work to do to completely dissect the origin of DD. In our department, and in collaboration with the Departments of Medical Genetics (Groningen and Los Angeles) and Molecular Biology (Groningen), we are working on the perspectives discussed above. These future studies have the potential to make personalised medicine possible and to improve the clinical care of patients with DD. They may also, ultimately, lead to a cure for DD.

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Chapter 8

Summary

Nederlandse Samenvatting

Summary

Dupuytren's disease (DD) has been recognized for centuries, but its aetiology and pathogenesis has not been fully elucidated. The clustering of DD in families, its prevalence in individuals of northern European descent, and its association with other fibromatoses (Peyronie's disease (PD) and Ledderhose's disease) suggest a genetic influence on the development of this disease. The general aim of this thesis was to investigate the genetic background of DD and its associated fibromatosis. This knowledge could lead to a better understanding of the disease and might ultimately lead to alternative treatments.

First, an introduction is given into basic genetic principles and an overview of the pre-existing genetic knowledge of Dupuytren's disease is provided.

Then we studied the mode of inheritance of familial DD, for which we analysed the pedigrees of 11 families, consisting of 475 family members and 66 patients. We confirmed the work of previous studies and showed that the mode of inheritance was most compatible with an autosomal dominant mode of inheritance for a late onset disease with variable penetrance. However, the inheritance did not completely follow the pattern of a Mendelian disease and we therefore hypothesized that DD is a complex genetic disease influenced by a combination of common genetic variants and environmental factors.

In order to identify common genetic variants associated with this disease, we carried out a genome-wide association study (GWAS), in which we initially analysed 234,939 single nucleotide polymorphisms (SNPs) in 856 patients with DD and 2,836 controls. We identified eight SNPs at three loci that had genome-wide significance. Tests of replication and a further joint analysis of 2,325 patients with DD and 11,562 control individuals from three different populations yielded genome-wide significant associations with eleven SNPs at nine different loci. Six of these loci contain genes known to be involved in the Wnt-signaling pathway, suggesting that abnormalities in this pathway are key to the process of fibromatosis in DD.

Given the clinical overlap between DD and PD, we then examined if the nine DD susceptibility loci were also involved in PD, by genotyping 111 patients with PD and 490 controls. We found a significant association at one of the DD susceptibility loci (*WNT2* locus), thus providing evidence that PD and DD share some genetic susceptibility factors. We did not find any evidence for involvement of the *SFRP4* locus (the most strongly associated locus with DD) with PD, despite having statistical power of 98%

based on previous DD findings. Our result implies that the two diseases could also have some distinct genetic susceptibility factors.

Finally, we investigated if there was an association between the clinical characteristics of patients with DD and their genetic risk score (based on the nine SNPs identified). We analysed the genetic and clinical data of 933 patients. They were categorized into high and low risk groups, according to their weighted genetic risk score. We used logistic regression to study the association of clinical characteristics with a high genetic risk score and found that patients with DD who present with a young age of onset (<50 years) and a positive family history for DD, Ledderhose's disease, or knuckle pads were likely to carry more risk alleles for our nine SNPs than patients without these diathesis features.

The research work in this thesis represents a major leap forward in the study of the genetics of DD. However, there is still a lot of work needed to fully dissect the origin of DD.

Future studies include a larger second-generation GWAS to identify more common variants, and also studies aimed to find rare variants and structural variations. A prospective cohort study could be designed in order to investigate the relationship between disease progression and genetic variation and to study gene-environmental interactions. Furthermore, functional studies are needed to investigate how these genetic variants influence protein function.

These future studies may lead to improved clinical care, to a more personalised treatment and, ultimately, to a cure for Dupuytren's disease.

Nederlandse Samenvatting

De ziekte van Dupuytren is een aandoening, waarbij zich bindweefsel vormt (fibromatose) in de handpalm en vingers. Dit kan leiden tot een kromstand van de vingers. De ziekte van Dupuytren is eeuwen geleden voor het eerst beschreven. Echter, de ontstaanswijze is nog steeds niet goed bekend. De ziekte komt veel in families voor en wordt vooral gezien bij mensen van Noord-Europese afkomst. Tevens zien we de ziekte van Dupuytren vaker samen met vergelijkbare fibromatosen op andere lichaamsdelen (de ziekte van Peyronie in de penis en de ziekte van Ledderhose in de voet). Bovenstaande kenmerken suggereren een genetische achtergrond voor de ziekte van Dupuytren en vergelijkbare fibromatosen.

Het voornaamste doel van dit proefschrift was het onderzoeken van de genetische achtergrond van de ziekte van Dupuytren en vergelijkbare fibromatosen. Deze kennis zou kunnen leiden tot een beter begrip van de ziekte en dit zou uiteindelijk kunnen resulteren in nieuwe behandelingen.

In dit proefschrift wordt eerst een introductie gegeven over de basis principes van de genetica en er wordt een overzicht gegeven van de aanwezige genetische kennis van de ziekte van Dupuytren.

Vervolgens werd de manier van overerven onderzocht in families waar de ziekte van Dupuytren voorkomt. Elf families bestaande uit 475 familieleden en 66 patiënten werden onderzocht. De manier van overerven kwam overeen met hetgeen voorgaande onderzoekers hadden geconcludeerd: een autosomaal dominante overerving. Dit betekent dat één van de ouders de aandoening kan doorgeven aan gemiddeld de helft van de kinderen. Echter, de overerving paste niet geheel in dit patroon en derhalve werd verondersteld dat de ziekte van Dupuytren mogelijk veroorzaakt zou kunnen worden door het samenspel van vele genen die elk een klein risico met zich mee brengen in combinatie met omgevingsfactoren. Men noemt dit een complex genetische ziekte.

Om te onderzoeken of er meerdere genen betrokken zijn bij het ontstaan van de ziekte werd een zogeheten associatie studie uitgevoerd, waarbij 234.939 gen-varianten onderzocht werden in 856 patiënten en 2.836 controle personen. Er werden acht gen-varianten gevonden verspreid over drie locaties, die geassocieerd zijn met de ziekte van Dupuytren. Daarna werd een vervolg studie gedaan in 2.325 patiënten en 11.562 controle personen afkomstig uit drie verschillende populaties (Nederland, Engeland en Duitsland). Gezamenlijke analyse van al deze gegevens leverde elf gen-varianten op in negen verschillende genetische locaties. Zes van deze locaties bevatten genen die

betrokken zijn bij de zogenaamde Wnt-signalering. Dit suggereert dat afwijkingen in deze groep van genen een belangrijke rol spelen bij de ziekte van Dupuytren.

Gezien de klinische overlap tussen de ziekte van Dupuytren en de ziekte van Peyronie, onderzochten we of de negen geïdentificeerde gen-varianten ook een rol spelen bij de ziekte van Peyronie. Hiervoor werden 111 patiënten en 490 controle personen onderzocht. Eén van de gen-varianten (gelegen bij het *WNT2* gen) was eveneens geassocieerd met de ziekte van Peyronie. Deze bevinding levert bewijs voor een overlap in de genetische gevoeligheid voor deze ziektes.

Tenslotte hebben we onderzocht of er een relatie bestaat tussen klinische kenmerken van patiënten met de ziekte van Dupuytren en hun genetische score. Deze genetische score is gebaseerd op de negen geïdentificeerde gen-varianten en is als het ware een optelsom van de gen-varianten, die een individuele patiënt bij zich draagt. Dus hoe meer van deze varianten je hebt, hoe hoger je genetische score. We hebben de genetische en klinische gegevens van 933 patiënten geanalyseerd. Hierbij vonden we dat patiënten met de ziekte van Dupuytren met de volgende kenmerken: aanvang van de ziekte voor het vijftigste levensjaar, bloedverwanten met de ziekte, de ziekte van Ledderhose of ‘knuckle pads’ (knobbels op de strekzijde van de vingers) een hogere genetische score hadden.

Met de studies in dit proefschrift is een grote stap gemaakt in het onderzoek naar de genetica van de ziekte van Dupuytren. Echter, er is nog een hoop werk te doen om de exacte ontstaanswijze te achterhalen.

Toekomstig onderzoek kan bestaan uit een grotere tweede generatie associatie studie, waarbij ook naar andere soorten gen-varianten gekeken kan worden. Verder zou een studie uitgevoerd kunnen worden, waarbij patiënten in de tijd gevolgd worden en de relatie tussen de voortgang van de ziekte en de aanwezige gen-varianten en omgevingsfactoren wordt bekeken. Tevens is er onderzoek nodig naar de effecten van de geïdentificeerde gen-varianten op de functie van de uiteindelijke eiwitten en cellen.

Deze toekomstige onderzoeken zouden kunnen leiden tot een verbeterde, gepersonaliseerde behandeling en uiteindelijk een manier om de ziekte van Dupuytren te genezen.


Witteke-ke
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Dankwoord

About the author

Dankwoord

Het is alweer ruim 7 jaar geleden dat ik begon met mijn promotie onderzoek in het kader van een AGIKO constructie (gecombineerde opleiding tot medisch specialist en promotie onderzoek). Na 2 jaar als ANIOS plastische chirurgie in het Catharina ziekenhuis te Eindhoven gewerkt te hebben, ging ik solliciteren voor een opleidingsplek plastische chirurgie in het Universitair Medisch Centrum Groningen. Echter tijdens het gesprek bleek het om een zogenaamde AGIKO plek te gaan. Na een korte aarzeling besloot ik om deze mogelijkheid met beide handen aan te pakken.

Het team bestond uit Prof. dr. Paul Werker, Prof. dr. Roel Ophoff, Prof. dr. Cisca Wijmenga en ik. Roel en Paul hadden elkaar ontmoet op een internationaal congres en daar was het idee ontstaan om een genetische studie op te gaan zetten naar de ziekte van Dupuytren. Bij Roel zit de ziekte van Dupuytren in de familie en Paul heeft een grote expertise in de behandeling van de ziekte van Dupuytren. Cisca was net benoemd als hoofd van de afdeling genetica in het UMCG en was bereid om mee te werken aan het project.

Prof. dr. Werker, beste Paul, ik bewonder jouw energie en passie voor ons vak en natuurlijk de ziekte van Dupuytren. Ik wil je bedanken voor het vertrouwen dat je in mij had om dit grote project van de grond te krijgen. Tijdens onze vele gesprekken wist je mij altijd te stimuleren en was je altijd eerlijk en recht door zee. Genetica was voor ons beiden een onbekend gebied, dankzij de onmisbare expertise van onze topgenetici zijn we een heel eind gekomen. Onze uitstapjes naar het buitenland waren erg plezierig.

Prof. dr. Ophoff, beste Roel, ik wil je bedanken als initiator van het project. Ik heb je ervaren als een integer persoon en ik heb het idee dat we vaak op een lijn zaten. Onze long distance-samenwerking werd mogelijk gemaakt door Skype en onze Macbooks, hierdoor leek Utrecht en zelfs Los Angeles dichtbij.

Prof. dr. Wijmenga, beste Cisca, jij zette hoog in voor de genoomwijde associatie studie, minstens 1000 patiënten moesten verzameld worden. Het was een zeer ambitieus plan. Ik heb van jou geleerd dat je groot moet denken als je iets wilt bereiken. Dankzij jou en de knappe koppen op jouw afdeling heb ik mij een deel van de genetica eigen kunnen maken.

De leescommissie bestaande uit Prof. dr. de Bakker, Prof. dr. Hovius en Prof. dr. Snieder, hartelijk dank voor het beoordelen van dit proefschrift.

Beste Eric en Esther, ik werd altijd welkom ontvangen in het lab in Utrecht. We hebben samen van alles uitgeprobeerd om het stugge Dupuytren weefsel klein te krijgen, zodat we hieruit RNA en DNA konden isoleren. Het bleef tot het einde toe lastig en vaak frustrerend. Ik wil jullie bedanken voor jullie hulp en natuurlijk de gezelligheid.

Beste Kristel, we hebben vaak gebrainstormd over de Linkage studie, helaas hebben we deze studie nog niet kunnen publiceren. Ik wil je bedanken voor de analyses die je hebt uitgevoerd en wens je veel succes met jouw onderzoek.

Beste Monique en Ron, bedankt voor jullie goede hulp. Monique heeft me de kneepjes van het DNA isoleren bijgebracht en later kwam ik je weer tegen als operatieassistent. Ron heeft mij begeleid bij het isoleren van meer dan 1500 samples, wat een karwei!

Beste Marcel, Mathieu en Soesma, bedankt voor jullie hulp. Marcel en Mathieu waren betrokken bij het opzetten van de logistiek van de studie. En later kwam ik Marcel weer tegen bij LifeLines. Mathieu, als ik ook maar iets nodig had in het lab kon ik altijd bij jou terecht. Bedankt hiervoor. Soesma, bedankt voor het hybridiseren van de samples, deze klus had ik zelf niet kunnen klaren.

Beste Pieter, bedankt voor je goede hulp in het lab. Wat had ik zonder jou en je robot gemoeten.

Dear Gosia, Asia and Jihane, thank you for your help with the genetic analysis; using PLINK, R-package and performing genetic risk scoring. Good luck with your scientific carriers.

Beste Noortje, bedankt voor je hulp bij onder andere de ‘principal component’ analyse. Ik vond het plezierig om een medestander te hebben als clinicus op de genetica afdeling.

Beste Harm-Jan, bedankt voor de hulp bij de imputatie van de GWAS en ook de uitleg in de bio-informatica. Succes in Boston!

Beste Lude, ik wil je graag bedanken voor alle overleg momenten. Met name jouw steun en hulp tijdens de NEJM rebuttal fase is heel waardevol geweest.

Beste Cleo, bedankt voor je hulp met name in de laatste fase van mijn promotie.

Beste Jacky, ik heb het als een ontzettende luxe ervaren dat mijn teksten door jou ge-edit zijn. Hartelijk dank hiervoor.

I would like to thank all the other people from the celiac group and genetic department who have been involved in this project.

Beste Jitske, je was een van de eerste studenten betrokken bij het genetisch Dupuytren onderzoek. Jij bent onder andere druk bezig geweest om families met Dupuytren uit te pluizen. Bedankt voor je goede werk.

Hanna, Nirvana en Sterre, beste dames, zonder jullie geweldige ondersteuning was het nooit gelukt om de administratie van zo'n groot project te verwerken. Hartelijk dank voor jullie hulp.

Beste Freek, bedankt voor het digitaliseren van het merendeel van de vragenlijsten. Jammer dat het niet gelukt is om samen tot een publicatie te komen.

Graag wil ik alle collega's bedanken, die betrokken zijn geweest bij het includeren van Dupuytren patiënten. Zonder jullie was het allemaal niet mogelijk geweest!!!

Apart wil ik graag de contactpersonen noemen van de externe klinieken: Auke de Boer en Marius Kemler (Martini Ziekenhuis), Hein ter Linden (Isala klinieken), Anneke Knepper (Medisch Centrum Leeuwarden), Oliver Zöphel (Medisch Spectrum Twente), Maarten Hoogbergen en Luitzen de Boer (Catharina Ziekenhuis).

Dear Hans Hennies and Dominic Furniss, thank you for collaborating with us on the GWAS. By working together, we were able to make our study so successful! I hope we can continue our cooperation.

Dr. van Driel, beste Mels, ik wil je graag hartelijk bedanken voor de enthousiaste medewerking aan de Peyronie studie! De inclusie van al deze patiënten is volledig aan jou te danken.

Prof. dr. van der Sluis, beste Corry, graag wil ik je hartelijk bedanken voor het uitvoeren van de belangrijke taak als onafhankelijk arts binnen het GODDAF onderzoek. Deze functie is een stuk drukker gebleken dan ik aanvankelijk voor ogen had.

Graag wil ik het LifeLines team ruimhartig bedanken voor de samenwerking! Zonder het gebruik van de LifeLines data als controles hadden we de GWAS niet kunnen uit-

voeren. Ik hoop dat we in de toekomst verder kunnen samenwerken. In hoofdstuk 7 (general discussion) zijn een aantal voorbeelden voor toekomstig onderzoek gegeven.

Beste Evert-Jan, graag wil ik je bedanken voor je hulp bij mijn onderzoek en met name bij het maken van prachtige illustraties en flitsende keynote presentaties. Je geeft nu vervolg aan het genetisch Dupuytren onderzoek. Ik ben erg benieuwd naar de resultaten en wens je veel succes!

Beste Tim Hoffman, met veel enthousiasme begon je aan functionele studies als vervolg op mijn onderzoek. Helaas heb je dit door omstandigheden niet af kunnen maken. Ik wil je hartelijk danken voor de grote hulp bij het maken van de illustraties voor de kaft van dit proefschrift.

Beste Bert Tebbes en Judith Bender, hartelijk dank voor alle foto's die jullie gemaakt hebben van de Dupuytren patiënten. Zie ook de prachtige opname die gebruikt is voor de kaft.

Ik wil alle Dupuytren patiënten die wilden deelnemen aan het onderzoek bedanken voor de medewerking.

Beste Hildo, de vele overleg momenten en ook dart sessies werkten heel relativerend, hiervoor wil ik je bedanken. Ik wens jou veel succes met de afronding van je eigen proefschrift en hoop dat we in de toekomst goed contact houden.

Mede AIOS plastische chirurgie: Steven, Vic, Patrick, Marijn, Mireille, Johan, Ellen, Ilona, Rinze en Merel. Bedankt voor de fijne samenwerking.

Beste mede onderzoekers plastische chirurgie: Rinze, Johan, Evert-Jan, Ellen, Rosanne, Dieuwke en Anna. Bedankt voor de gezelligheid en de vele thee momenten. Succes met jullie onderzoek!

Collega's van de afdeling plastische chirurgie in het UMCG. Alle secretaresses, medewerkers, verpleegkundigen en artsen wil ik bedanken voor de jarenlange goede samenwerking.

Medewerkers van de dienst bloedafname. Hartelijk dank voor de jarenlange medewerking.

En niet onbelangrijk qua productiviteit wil ik Apple (Steve Jobs) bedanken voor het ontwikkelen van zo'n fijne spullen.

Mijn paranimfen. Wat ben ik blij dat jullie mij vandaag bij willen staan.

Lieve Merel, we hebben heel wat overleg momenten met elkaar doorgebracht. Zowel op de zaak als op de golfbaan, racefiets of BOOT-OX. Je hebt alle pieken en dalen van het promoveren meegemaakt. Bedankt voor alle steun, vriendschap en gezelligheid.

Beste Hugo, breur, ondanks dat je thuis al te veel medische verhalen vrijwillig verplicht moest aanhoren, vind ik het extra bijzonder dat je mij vandaag wilt bijstaan als paranimf. Ik hoop dat we in de toekomst nog veel meer activiteiten en reizen mogen beleven.

Beste Patrick, we hebben voor het eerst kennis gemaakt in het kader van het onderzoek naar families met de ziekte van Dupuytren. Je reisde samen met Olga het hele land door om deze families in kaart te brengen. We hebben sindsdien vele leuke activiteiten meegemaakt en we zijn goede vrienden geworden. Bedankt voor jullie hulp en interesse en natuurlijk de vele relativerende 'sport' sessies.

Lieve familie, bedankt voor jullie interesse.

Pap en mam, bedankt voor jullie onvoorwaardelijke steun en vertrouwen. Van jongs af aan hebben jullie ons (Valérie, Hugo en ik) een warm en veilig nest geboden en gestimuleerd om te bereiken wat we voor ogen hadden. Pap, ik draag dit boek aan jou op, je had er zo graag bij willen zijn.

Lieve Denise, zonder jou was dit boekje er nooit gekomen. Je bent de belangrijkste persoon in mijn leven, samen met onze prachtige kinderen Julie en Jens. Bedankt voor alle ruimte die je mij hiervoor gegeven hebt. Ik zie uit naar ons leven samen zonder promotietraject!

About the author

Guido Hubertus Carolina Gerardus Dolmans was born on 1st May 1979 in Maastricht, the Netherlands. After finishing high school (*Gymnasium, Stedelijke Scholengemeenschap Maastricht*), he studied medicine at Maastricht University from 1997 to 2003 and graduated *Cum Laude* for both his MSc as well as his MD degree. During this period he developed an interest in surgery and a passion for travel. He went to Groote Schuur Hospital in Cape Town, South Africa, for a research internship at the renal transplant unit and a clinical internship at the trauma department. He also did a dermatology internship abroad, in Kuala Lumpur, Malaysia. After his final internship in plastic surgery at the Atrium Medical Center, Heerlen, he decided to pursue a career in plastic surgery. In 2004, he started as a plastic surgery resident-not-in-training at the Catharina Hospital in Eindhoven and published his first research paper under the supervision of Dr. J.H.A. van Rappard and Dr. M.M. Hoogbergen. In 2007, he started as *AGIKO*, combining his specialist training in plastic, reconstructive and hand surgery with a PhD project, under the supervision of Prof. P.M.N. Werker, at the University Medical Center Groningen. From 2008 to 2010, he completed his two years of general surgery at the Martini Hospital in Groningen under the supervision of Dr. P.C. Baas. He received the Esser prize and the International Dupuytren Award from respectively the NVPC (*Nederlandse Vereniging voor Plastische Chirurgie*) and the International Dupuytren Society for his paper on 'Wnt signaling and Dupuytren's disease', which was published in the New England Journal of Medicine in 2011. In 2013, he went to Perth, Australia, on a 6-month fellowship at Sir Charles Gairdner Hospital under the supervision of Dr. Lip Teh, where he focused on microsurgery, reconstructive surgery and hand surgery. He will complete his specialist training at the University Medical Center Groningen in December 2014.