ORIGINAL ARTICLE

Wnt Signaling and Dupuytren's Disease

Guido H. Dolmans, M.D, Paul M. Werker, M.D., Ph.D., Hans C. Hennies, Ph.D.,
Dominic Furniss, M.D., Ph.D., Eleonora A. Festen, M.D., Ph.D.,
Lude Franke, Ph.D., Kerstin Becker, M.S., Pieter van der Vlies, B.S.,
Bruce H. Wolffenbuttel, M.D., Ph.D., Sigrid Tinschert, M.D.,
Mohammad R. Toliat, Ph.D., Michael Nothnagel, Ph.D., Andre Franke, Ph.D.,
Norman Klopp, Ph.D., H.-Erich Wichmann, M.D., Ph.D., Peter Nürnberg, Ph.D.,
Henk Giele, M.D., Ph.D., Roel A. Ophoff, Ph.D., and Cisca Wijmenga, Ph.D.,
for the Dutch Dupuytren Study Group, the German Dupuytren Study Group,
the LifeLines Cohort Study, and the BSSH–GODD Consortium*

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 understand its pathogenesis.

METHODS

We conducted a genomewide association study of 960 Dutch persons with Dupuytren's disease and 3117 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 ($P<1\times10^{-4}$) in the discovery set in three additional, independent case series comprising a total of 1365 affected persons and 8445 controls from Germany, the United Kingdom, and the Netherlands.

RESULTS

Initially, we observed a significant genomewide association between Dupuytren's disease and 8 SNPs at three loci. Tests of replication and joint analysis of all data from 2325 patients with Dupuytren's disease and 11,562 controls yielded an association with 11 SNPs from nine different loci ($P<5.0\times10^{-8}$). Six of these loci contain genes known to be involved in the Wnt-signaling pathway: *WNT4* (rs7524102) ($P=2.8\times10^{-9}$; odds ratio, 1.28), *SFRP4* (rs16879765) ($P=5.6\times10^{-39}$; odds ratio, 1.98), *WNT2* (rs4730775) ($P=3.0\times10^{-8}$; odds ratio, 0.83), *RSPO2* (rs611744) ($P=7.9\times10^{-15}$; odds ratio, 0.75), *SULF1* (rs2912522) ($P=2.0\times10^{-13}$; odds ratio, 0.72), and *WNT7B* (rs6519955) ($P=3.2\times10^{-33}$; odds ratio, 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.

DUPUYTREN'S DISEASE IS A BENIGN FIBRO-matosis of the hands and fingers, giving rise to the formation of nodules and cords and often leading to flexion contractures (Fig. 1 in the Supplementary Appendix, available with the full text of this article at NEJM.org). The prevalence of Dupuytren's disease is reported to be between 0.2% and 56.0%.[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 Dupuytren's disease is not fully understood.

The clustering of cases of Dupuytren's disease 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 genomewide association study involving 960 persons with Dupuytren's disease and 3117 controls, all from the Netherlands and of European descent.

METHODS

STUDY PARTICIPANTS

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 Dupuytren's disease through the outpatient clinics of the plastic surgery departments of six hospitals in the Netherlands. All 3117 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 Dupuytren's disease, 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 Dupuytren's disease, as well as from 5984 controls from the Wellcome Trust Case Control Consortium 2 (WTCCC,

1958 British Birth Cohort, and U.K. National Blood Service) for whom genotyping data were already available[11]; and from 465 German patients with Dupuytren's disease, as well as from 1900 controls, for 1618 of whom genotyping data were already available (1164 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). (A detailed description of case patients and controls is provided in the Supplementary Appendix.) Participants reported ancestry by means of a multiple-choice questionnaire, the choices being Dutch, European (specifying country), and other (specifying country). DNA samples were obtained from either blood samples (in the Dutch and German case series) or saliva (in the U.K. 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 (see the Supplementary Appendix). 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 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 Dupuytren's disease 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 (see the Supplementary Appendix). Because genomewide 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 overdispersion 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 EIGENSTRAT software to control for population stratification. We conducted conditional analysis with the use of SNPTEST, version 2, when more than one SNP with a significant genomewide 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 with the use of Cochran–Mantel–Haenszel stratification. In the U.K. 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 1 in the Supplementary Appendix) generated with BEAGLE Genetic Analysis Software Package 3.2 and based on the HapMap phase 2 reference of 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 1 in the Supplementary Appendix). 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 interplatform reproducibility by comparing genotypes of the same samples between different platforms and found concordance rates of more than 99.99% (see the Supplementary Appendix).

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 genomewide association as query regions.[14]

RESULTS

GENOMEWIDE ASSOCIATIONS

Data obtained through genomewide 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 Dupuytren's disease and in 2836 controls (Table 1). The call rate for the remaining SNPs was 99.9%. There was moderate evidence for inflation in the test statistic ($\lambda_{gc}=1.21$). 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 $\lambda_{gc}=1.19$. Figure 2 in the Supplementary Appendix 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 in the Netherlands and noted differences in case patients between the clinics (Fig. 3 in the Supplementary Appendix). After exclusion of case patients from the most southern and eastern hospitals in the Netherlands, the inflation decreased to 1.07 (Fig. 4 and 5 in the Supplementary Appendix). 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 genomewide P values showed 83 data points that were above the expected diagonal line (Fig. 1A).

In the genomewide association study, we identified eight SNPs at three loci that showed significant association (P<5×10⁻⁸) (Fig. 1B). On chromosome 7, we identified a locus with four significantly associated SNPs. Association was strongest at rs16879765, which lies within the gene

Table 1. Sample Collections and Genotyping Platforms for the Genomewide Association Study (GWAS) and Replications in Patients with Dupuytren's Disease and Control Subjects.*

Collection No.	Country	Patients with Dupuytren's Disease (N = 2325)			Control Subjects (N = 11,562)		
		No. of Samples before QC	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	3117	2836	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	5984; 8935†	4765; 8274	Illumina 1.2 M, Affymetrix 6.0; Immunochip
4	Germany	465	449	Affymetrix 6.0	1618; 282‡	1604; 267	Affymetrix 6.0; GenomeLab SNPstream
Total		2325	2154		11,562	9972	

* QC denotes quality control.
† In the original analysis, rs611744 was imputed in the U.K. control series. Subsequently, we directly genotyped this SNP in another set of 8935 U.K. controls (WTCCC) with the use of the Immunochip array.
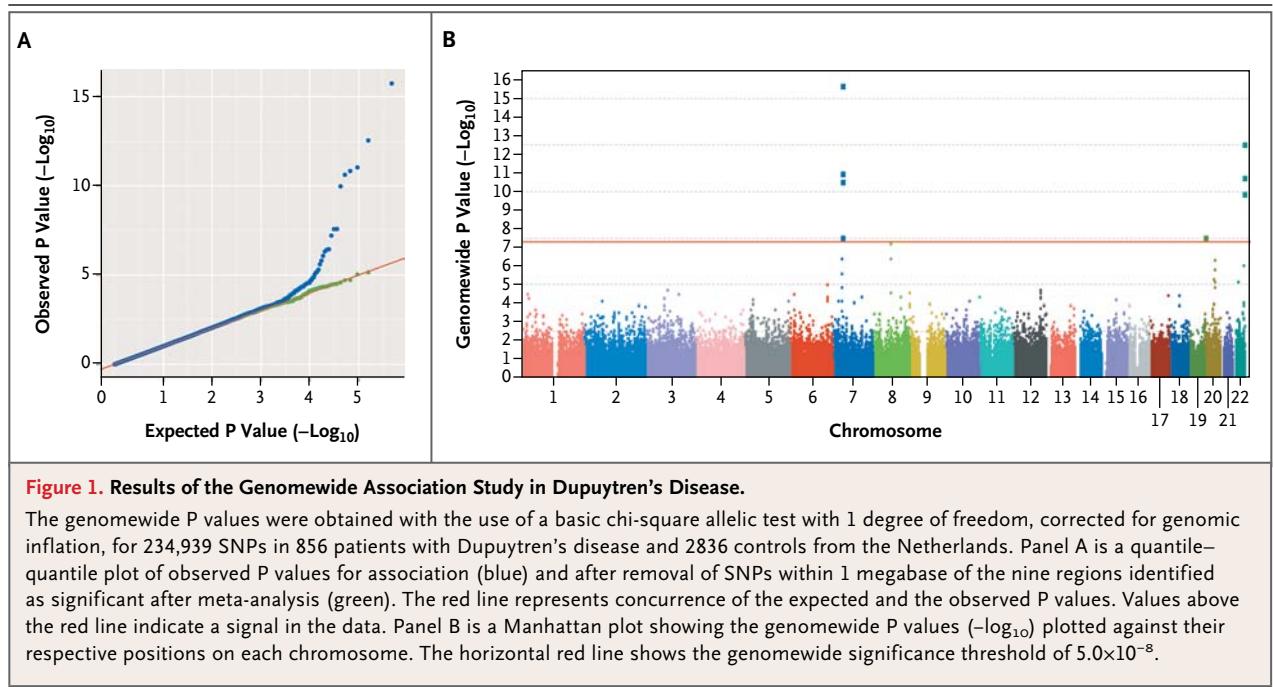‡ In the German case series, several replication SNPs were genotyped in a separate control series that included 282 persons, and these were included in the main study. (For the SNP numbers, see Table 1 in the Supplementary Appendix.)

encoding ependymin-related protein 1 (*EPDR1*) (P=1.9×10⁻¹⁶; odds ratio, 1.94). The three other associated SNPs were in linkage disequilibrium with the top SNP: rs1668357 (r²=0.57), rs1668347 (r²=0.59), and rs952368 (r²=0.44). Similarly, three significantly associated SNPs were identified at a single locus on chromosome 22. The most significant SNP on 22q, rs6519955 (P=2.8×10⁻¹³; odds ratio, 1.56), is located between wingless-type mammary-tumor virus integration site family member 7B (*WNT7B*) and *LOC100271722*, a hypothetical noncoding RNA gene. The two other associated SNPs on chromosome 22 were in linkage disequilibrium with the top SNP: rs8140558 (r²=0.96) and rs4072455 (r²=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 Dupuytren's disease–associated locus was identified on chromosome 19, with a single significant SNP (rs11672517) (P=2.8×10⁻⁸; odds ratio, 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<1×10⁻⁴ 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 (1365 case patients with Dupuytren's disease and 8445 controls before quality control). One SNP (rs10809642) failed on genotyping in the Dutch and U.K. 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 correction 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 genomewide significance in a meta-analysis (P<5.0×10⁻⁸) (Table 2, and the Supplementary Appendix). All loci that showed significant associations in the discovery set also showed significant associations in the replication set: rs16879765 (P=5.6×10⁻³⁹; odds ratio, 1.98) on 7p14.1, rs6519955 (P=3.2×10⁻³³; odds ratio, 1.54) and rs8140558 (P=1.2×10⁻²²; odds ratio, 1.39) on 22q13, and rs11672517 (P=6.8×10⁻¹⁴;

Figure 1. Results of the Genomewide Association Study in Dupuytren's Disease.

The genomewide 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 patients with Dupuytren's disease and 2836 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 genomewide P values ($-\log_{10}$) plotted against their respective positions on each chromosome. The horizontal red line shows the genomewide significance threshold of 5.0×10^{-8}.

odds ratio, 1.34) on 19q13.4. Two SNPs of borderline significance in the discovery set showed significant associations in the tests of replication: rs2912522 ($P = 2.0 \times 10^{-13}$; odds ratio, 0.72) on 8q13 and rs8124695 (($P = 7.6 \times 10^{-10}$; odds ratio, 1.48) on 20q11.2–q13.1. Four additional SNPs also reached genomewide significance: rs611744 ($P = 7.9 \times 10^{-15}$; odds ratio, 0.75) on 8q23.1, rs10809650 ($P = 6.2 \times 10^{-9}$; odds ratio, 0.80) and rs10809642 ($P = 1.2 \times 10^{-8}$; odds ratio, 1.35) on 9p24.3, and rs7524102 ($P = 2.8 \times 10^{-9}$; odds ratio, 1.28) on 1p36.23–p35.1.

For 2 of the 11 SNPs with a significant genomewide association, we used tag SNPs with less than complete linkage disequilibrium or imputed SNPs in the meta-analysis (Table 1 in the Supplementary Appendix). We genotyped one of these SNPs, rs611744, on the Immunochip platform in 8274 U.K. controls (Table 1) and observed an association ($P = 1.8 \times 10^{-14}$) 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 U.K. and German series (since these data were only indirectly genotyped [Table 1 in the Supplementary Appendix]) showed a significant genomewide association ($P = 4.8 \times 10^{-16}$) (Table 3 in the Supplementary Appendix).

Regional plots of the nine Dupuytren's disease risk loci are shown in Figure 6 in the Supplementary Appendix. To gain insight into the biologic mechanisms and to find genes functionally relat-

ed at these regions, we applied GRAIL analysis. The 11 SNPs at the nine regions that had a significant genomewide 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) (Table 4 in the Supplementary Appendix). 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 genomewide association and gene expression in six quantitative-trait-locus data sets (see the Supplementary Appendix).

DISCUSSION

We identified nine chromosomal loci associated with susceptibility to Dupuytren's disease. 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 Dupuytren's disease in Northern European populations.

A GRAIL analysis showed that four different Dupuytren's disease risk loci contain genes that

Table 2. The 35 Single-Nucleotide Polymorphisms (SNPs) Selected for Replication.

Chromosome	SNP*	Base-Pair Position	Minor Allele	Minor-Allele Frequency†	P Value			Odds Ratio (95% CI)‡	Genes of Interest and GRAIL Annotation§
					GWAS (856 Case Patients and 2836 Controls)	Replication (1365 Case Patients and 8445 Controls)	Meta-Analysis (2325 Case Patients and 11,562 Controls)		
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	rs11231148¶	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¶	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‖	37970931	C	0.27**	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

Chr	SNP	Allele	Freq	P_{gwas}	$P_{replication}$	P_{meta}	Odds Ratio (CI)	Gene(s)
9	rs10809642††	A	0.25	2.6×10^{-5}	$\mathbf{6.9\times10^{-4}}$	$\mathbf{1.2\times10^{-8}}$	1.35 (1.19–1.53)	*RP11–341G2.1, DMRT2*
9	rs10809650	G	0.24	1.4×10^{-4}	$\mathbf{4.5\times10^{-5}}$	$\mathbf{6.2\times10^{-9}}$	0.80 (0.74–0.88)	*RP11–341G2.1, DMRT2*
10	rs7072865	A	0.14	7.3×10^{-5}	0.53	5.9×10^{-4}	0.86 (0.77–0.95)	*PIK3AP1*
12	rs638791¶	G	0.19	1.9×10^{-5}	0.27	5.5×10^{-5}	0.85 (0.78–0.94)	*SH2B3, ATXN2*
12	rs2073950	A	0.19	4.9×10^{-5}	0.42	2.5×10^{-4}	0.87 (0.80–0.96)	*SH2B3, ATXN2*
12	rs12372139¶	A	0.44	3.1×10^{-4}	0.18	3.5×10^{-5}	0.81 (0.74–0.90)	*AC117500.2*
15	rs4932194	C	0.31	2.9×10^{-4}	1.9×10^{-3}	8.1×10^{-7}	0.82 (0.77–0.89)	*ISG20*
15	rs6496520	C	0.30	3.3×10^{-4}	2.9×10^{-3}	1.2×10^{-6}	0.82 (0.76–0.89)	*ISG20*
15	rs2171286	A	0.21	6.2×10^{-5}	0.19	7.5×10^{-5}	0.85 (0.78–0.93)	*MYO1E*
17	rs4789939	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	G	0.50	3.9×10^{-5}	0.86	2.9×10^{-3}	1.11 (1.04–1.20)	*PIK3C3*
18	rs1944967	G	0.39	1.3×10^{-4}	0.94	4.1×10^{-3}	1.11 (1.03–1.20)	*PIK3C3*
19	rs11672517	A	0.28	$\mathbf{2.8\times10^{-8}}$	$\mathbf{2.7\times10^{-6}}$	$\mathbf{6.8\times10^{-14}}$	1.34 (1.25–1.45)	*DUXA, ZNF264*
20	rs6029273	G	0.12	1.6×10^{-6}	0.07	1.2×10^{-6}	0.76 (0.68–0.86)	*MAFB*
20	rs8124695	A	0.10	4.9×10^{-7}	$\mathbf{8.8\times10^{-4}}$	$\mathbf{7.6\times10^{-10}}$	1.48 (1.30–1.68)	*AL049691.1, MAFB*
22	rs8140558	G	0.47	$\mathbf{1.5\times10^{-11}}$	$\mathbf{3.8\times10^{-11}}$	$\mathbf{1.2\times10^{-22}}$	1.39 (1.30–1.48)	*RP11–398F12.1, WNT7B*
22	rs4820663	C	0.08	7.1×10^{-6}	0.56	3.5×10^{-3}	1.20 (1.06–1.35)	*MYO18B*
22	rs6519955	A	0.47	$\mathbf{2.8\times10^{-13}}$	$\mathbf{1.7\times10^{-19}}$	$\mathbf{3.2\times10^{-33}}$	1.54 (1.44–1.65)	*RP11–398F12.1, WNT7B*

* The SNP positions are according to Human Build 36.3. SNPs meeting the significance threshold ($P<5.0\times10^{-8}$ for P_{gwas} and P_{meta} or $P<0.0014$ for $P_{replication}$) are shown in bold type.

† Minor-allele frequencies are shown for all samples in the meta-analysis. (Minor-allele frequencies for the separate case series are provided in the Supplementary Appendix.)

‡ Odds ratios are shown for the meta-analysis, with 95% confidence intervals (CI).

§ 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 that are underlined were identified with the use of GRAIL analysis ($P<0.01$).

¶ Four SNPs (rs1123148, rs2179367, rs638791, and rs12372139) failed genotyping in the German series.

‖ The SNP rs1668357 was out of Hardy–Weinberg equilibrium in the replication phase ($P<0.0001$) and was therefore excluded.

** The minor-allele frequency for the discovery set is shown.

†† The SNP rs10809642 failed genotyping in the Dutch and U.K. replication series.

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 genomewide significance ($P_{meta} = 2.5 \times 10^{-7}$).

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 β-catenin, leading to changes in gene expression that influence cell proliferation and survival.[18] Abnormal proliferation of fibroblasts is a key feature in the early development of Dupuytren's disease. The disease can be divided into three histologic 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.
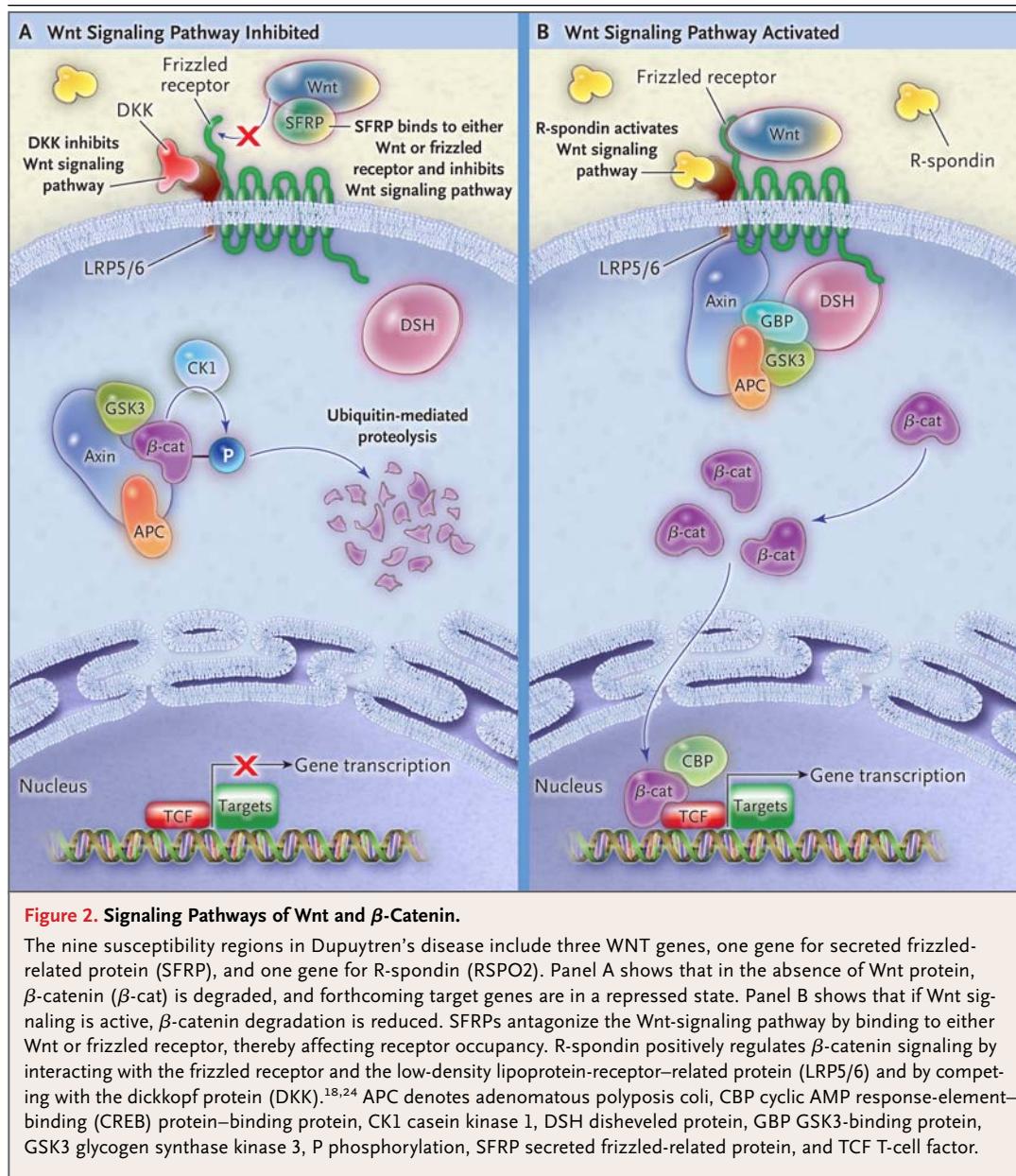
The involvement of the Wnt-signaling pathway in the pathogenesis of Dupuytren's disease is consistent with features of the disease and with established aspects of Wnt signaling. An imbalance of Wnt signaling in Dupuytren's disease could cause fibroblasts in the fascia of the hand to proliferate and form nodules. Indeed, increased levels of β-catenin have been observed in primary cell cultures in vitro,[23] suggesting that the Wnt-signaling pathway is overstimulated in Dupuytren's disease.

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[18] (Fig. 2). Secreted frizzled-related proteins, such as SFRP4, antagonize the Wnt-signaling pathway by binding to either Wnts or frizzled receptors, thereby affecting receptor occupancy. In the absence of active Wnt, β-catenin is degraded, and potential target genes will not be activated.

Another Dupuytren's disease risk locus contains *RSPO2*, encoding an R-spondin; members of the R-spondin family interact with frizzled receptors and LRP5/6 to induce β-catenin signaling. Furthermore, R-spondins induce canonical Wnt signaling by competing with the dickkopf (DKK) protein for binding to LRP5/6. The DKK protein is an inhibitor of Wnt signaling; it hinders the formation of a complex among Wnt, frizzled receptor, and LRP5/6 (Fig. 2).[25] 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.[26,27] Increased activity of these WNT and R-spondin genes or decreased activity of SFRP could stimulate Wnt signaling and reduce intracellular β-catenin degradation. This mechanism could trigger fibroblasts to proliferate, leading to the development of Dupuytren's disease.

Also supporting a role for Wnt signaling in Dupuytren's disease is the microRNA (miRNA) expression profiles of fibroblasts and palmar fascia in persons with this disease, as compared with those in healthy controls. These miRNAs regulate genes related to the β-catenin pathway: *WNT5A*, *ZIC1*, and *TGFB1*.[28] 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 Dupuytren's disease, as compared with fascia of the hand in healthy controls.[29] Maf proteins are known for their role in fibrosarcoma and are believed to influence tissue development and cellular differentiation.[30] MAFB can transform primary fibroblasts in vitro.[31] The gene might therefore be involved in stage 2 of Dupuytren's disease (the differentiation of fibroblasts into myofibroblasts). The miRNA expression profile associated with Dupuytren's disease implicated some miRNAs in influencing the expression of *MAFB* as well.[28]

Figure 2. Signaling Pathways of Wnt and β-Catenin.

The nine susceptibility regions in Dupuytren's disease include three WNT genes, one gene for secreted frizzled-related protein (SFRP), and one gene for R-spondin (RSPO2). Panel A shows that in the absence of Wnt protein, β-catenin (β-cat) is degraded, and forthcoming target genes are in a repressed state. Panel B shows that if Wnt signaling is active, β-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 β-catenin signaling by interacting with the frizzled receptor and the low-density lipoprotein-receptor–related protein (LRP5/6) and by competing with the dickkopf protein (DKK).[18,24] 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.

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

APPENDIX

The authors' affiliations are as follows: Departments of Plastic Surgery (G.H.D., P.M.W.), Genetics (G.H.D., E.A.F., L.F., P.V., C.W.), Gastroenterology (E.A.F.), and Endocrinology (B.H.W.), University Medical Center Groningen and University of Groningen, Groningen; and the Department of Medical Genetics and Rudolf Magnus Institute of Neuroscience, University Medical Center Utrecht, Utrecht (R.A.O.) — all in the Netherlands; the Cologne Center for Genomics, Division of Dermatogenetics (H.C.H., K.B., M.R.T., P.N.), the Cluster of Excellence on Cellular Stress Responses in Aging-associated Diseases (H.C.H., K.B., P.N.), and the Center for Molecular Medicine (H.C.H., P.N.), University of Cologne, Cologne; the Institute of Clinical Genetics, Faculty of Medicine Carl Gustav Carus, Dresden University of Technology, Dresden (S.T.); the Institute of Medical Informatics and Statistics (M.N.) and the Institute of Clinical Molecular Biology (A.F.), Christian-Albrechts-Universität, Kiel; the Institute of Epidemiology, Helmholtz Zentrum München, Neuherberg (N.K., H.-E.W.); and the Institute of Medical Informatics, Biometry and Epidemiology, Ludwig-Maximilians-Universität and Klinikum Grosshadern, Munich (H.-E.W.) — all in Germany; the Nuffield Department of Surgical Sciences, University of Oxford, and the Department of Plastic and Reconstructive Surgery, West Wing, John Radcliffe Hospital, Oxford, United Kingdom (D.F., H.G.); and the Center for Neurobehavioral Genetics, University of California, Los Angeles (R.A.O.)

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