Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

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Supplementary Appendix

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Contractures of the hand in recurrent Dupuytren's disease

Supplementary Figure 1. Extensive flexion contractures of the metacarpal–phalangeal 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.

Detailed Description of case and control subjects

Between 2007 and 2010 we recruited 960 DD patients through the outpatient clinics of the plastic surgery departments of six hospitals in the Netherlands. Written informed consent was given by all patients, with Institutional Review Board approval. DD patients were diagnosed by plastic surgeons with substantial clinical experience in treating DD. The clinical diagnosis of DD was based on the presence of characteristic DD nodules and/or cords in the palm of the hand and/or digits, with or without contractures of the digits. Patients were asked to complete a questionnaire on the age of onset, presence of possible risk factors (diabetes, alcohol consumption, liver disease, anti-epileptic medication), occupation, leisure activities, the presence of recurrent disease and related fibromatosis (Ledderhose's disease and Peyronie's disease). All 3,117 controls for the discovery set were drawn from 'LifeLines', a large, population-based cohort study being conducted in the northern Netherlands.[1]

Samples for replication studies were drawn from: (i) 189 Dutch DD cases and 561 new Dutch control samples from LifeLines; genotyping data was already available for all the controls, (ii) 711 UK DD cases and 5,984 controls from the Wellcome Trust Case Control Consortium 2 (WTCCC, 1958 Birth Cohort and UK National Blood Service controls); genotyping data was available for all the controls,[2] (iii) 465 German DD cases and 1,900 control individuals. Genotype data from 1,618 German controls were already available (Table 1): 1,164 of these were part of the Popgen study (University of Kiel, Germany) and 454 were from KORA (Helmholtz Center Munich, Neuherberg, Germany).

Dutch DD cases in the replication phase were collected in the same fashion as in the discovery phase. Subjects from the UK were collected by the 'British Society for Surgery of the Hand Genetics of Dupuytren's Disease Consortium' UK (BSSH GODD Consortium). The UK cases had all undergone surgery for DD and were identified by surgeons at their respective institutions between January 2003 and December 2009. All patients gave written informed consent. The UK DD study was given nationwide approval by the Oxford Research Ethics Committee B (09/H0605/65). Subjects from the German DD case series were recruited and classified by hand surgeons in 'The German Dupuytren Study Group' between 2007 and 2010 and clinical data were obtained using a standardized questionnaire. Written informed consent was obtained from all patients, with Institutional Review Board approval.

Inspection of genotype clusters

The integrity of the SNP genotypes selected for replication was confirmed by visual inspection of the raw genotype data. Three clear genotype clusters per SNP should be visible, permitting us to check whether the genotype calling algorithm had correctly assigned the genotypes to each of the samples.

Exclusion of relatives and ethnic outliers

Close relatives and duplicates were identified by computing identity-by-state (IBS) probabilities for all pairs, using a cut-off pi-hat value of 0.4. We excluded the control from the case-control pairs. Ethnic outliers were identified by computing IBS scores between participants and individuals in HapMap and by using multidimensional scaling. These ethnic outliers were then excluded from further analysis.

Use of tag and imputed SNPs

The 35 SNPs selected for replication and the use of tag or imputed SNPs in the UK and German series are shown (with their r^2 value) in Supplementary Table 1.

Supplementary Table 1. The 35 SNPs selected for replication and the tag SNPs used in the UK and German series are shown (with r^2 value).

		UK series		German series	
Chr	SNP	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).

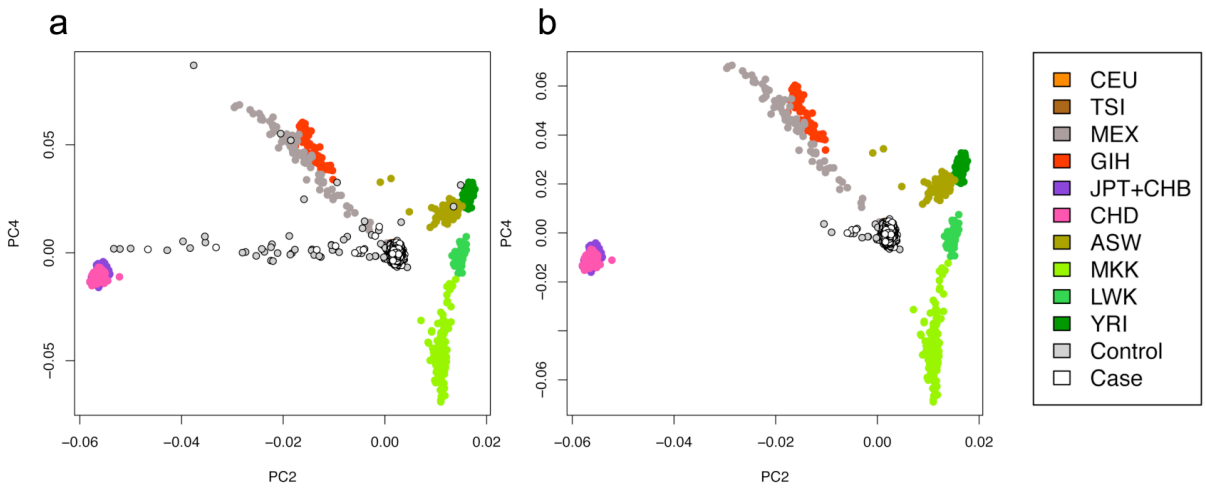
Multiple platform concordance

We checked for inter-platform reproducibility by comparing genotypes of 110 'LifeLines' samples, which were genotyped on both the Illumina CytoSNP-12 platform and the Illumina Immunochip platform (Illumina, San Diego, CA). 6,245 SNPs were present in both datasets after quality control steps (with the

same thresholds as in the GWAS dataset). The inter-platform concordance rate was > 99.99%.

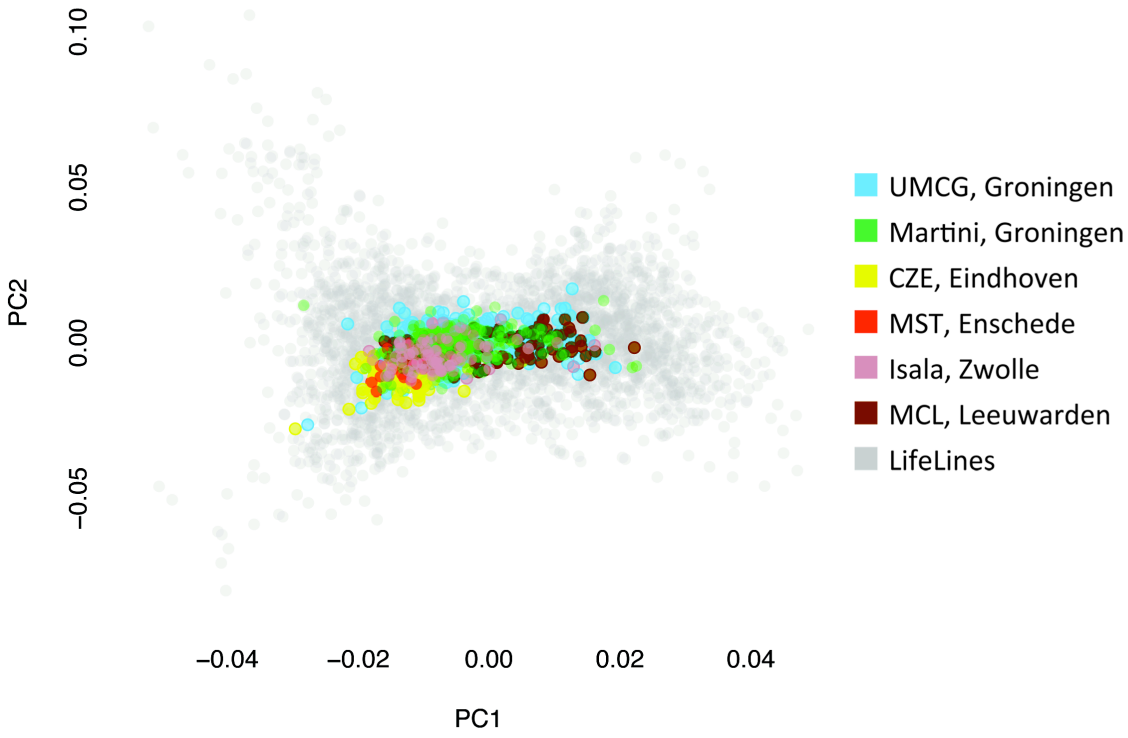
Population stratification and genomic inflation

There was moderate evidence for inflation in the test statistic (λ_{GC} = 1.21). Adjustment for differential population stratification using the first five components based on a principal components analysis (PCA) of uncorrelated SNPs reduced the inflation to λ_{GC} = 1.19. Figure 2 in the Supplementary Appendix shows that the case and control groups were well matched for population stratification after correcting for these components.



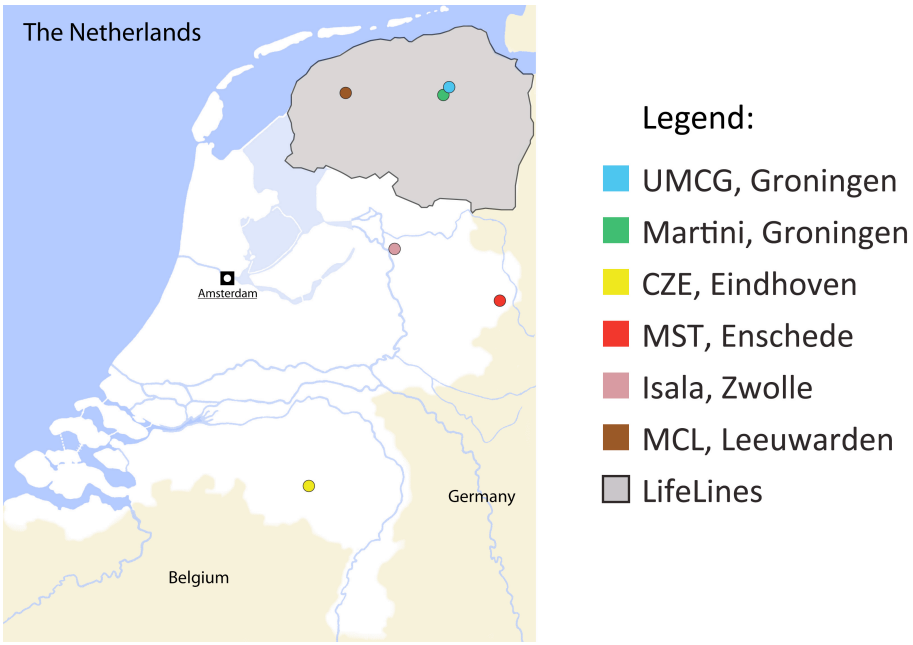
Supplementary Figure 2. Plots of principal components 2 and 4 from the PCA, including all subjects of the GWAS and HapMap 2. a: before removal of the first 5 components. b: after removal of the first 5 components.

We investigated the remaining cause of the inflation by analysing each of the sub-populations that reflect individuals from different regions in the Netherlands. We re-ran the PCA, confining this solely to the Dutch samples. Even though the Netherlands is a small country and considered to be genetically quite homogenous, we found differences between the cases per clinic (Supplementary Figure 3).



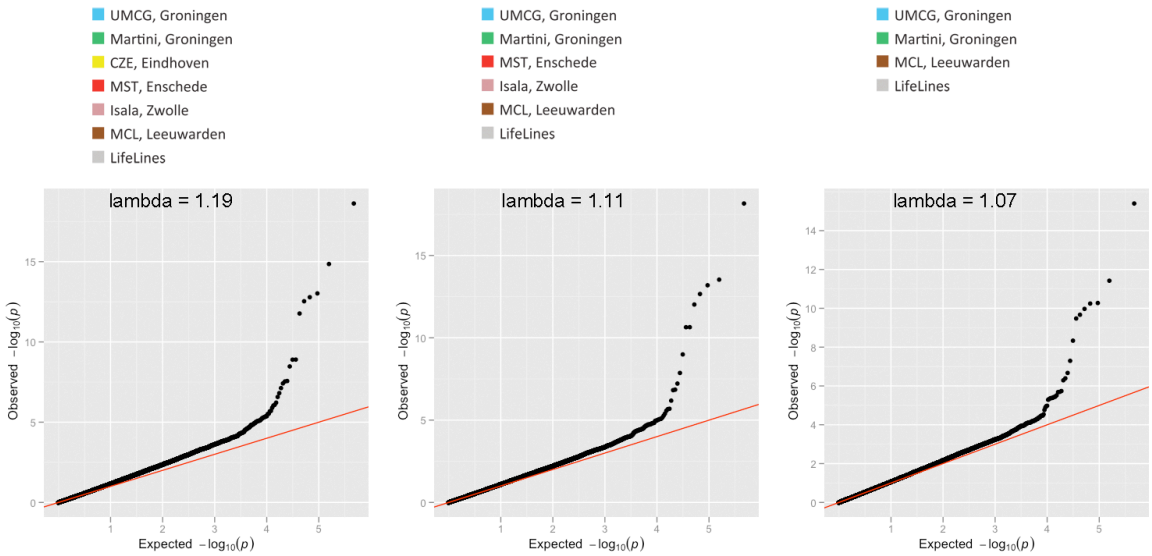
Supplementary Figure 3. Principal component analysis of our DD cases, shown per clinic.

Cases for the discovery phase were included from six major hospitals in the Netherlands, most of which are located in the north of the country. The control individuals ('LifeLines') mainly originated from the northern provinces (Supplementary Figure 4).



Supplementary Figure 4. Location of the six participating clinics and the region from which the 'LifeLines' cohort is being built up (shaded grey) in the Netherlands.

After excluding 121 cases from the most southern hospital (CZE, Eindhoven), the inflation decreased to 1.11. When we also excluded 133 individuals from the eastern hospitals (MST and Isala), the inflation dropped to 1.07 (Supplementary Figure 5)



Supplementary Figure 5. QQ-plots of the full discovery set, after excluding CZE, and after excluding CZE, Isala, and MST.

Supplementary table 2 shows the P-values and odds ratios (OR) in the discovery phase of the eleven SNPs that were significant after meta-analysis. After excluding cases from the southern hospitals, the ORs remained the same, which indicates that the samples from the non-northern hospitals do not cause spurious associations.

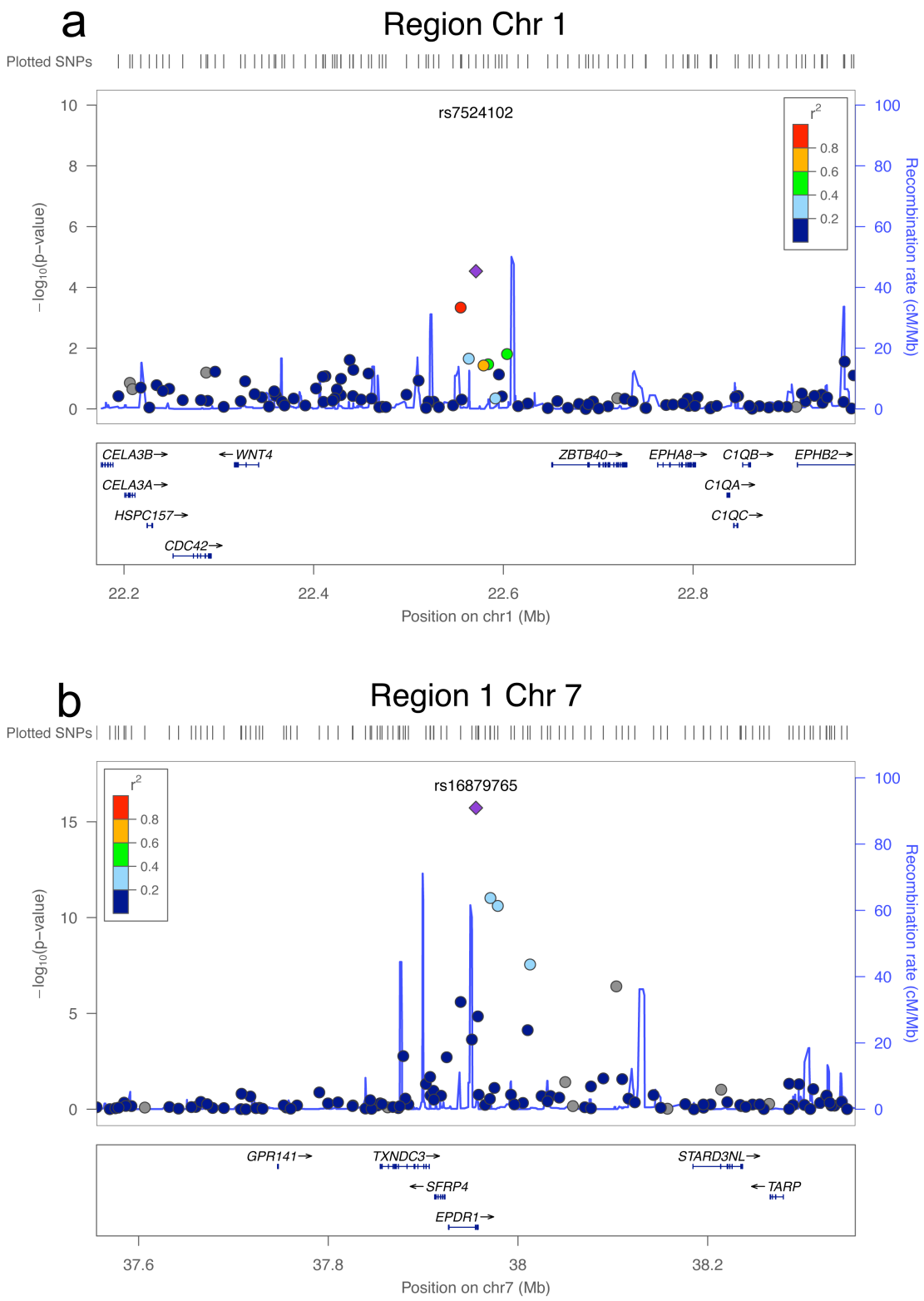
			Full set		Excluded: CZE		Excluded: Isala, MST	CZE,
Chr	SNP	Position[a]	Uncorrected P-value	OR	Uncorrected P-value	OR	Uncorrected P-value	OR
1	rs7524102	22571034	4.9×10^{-6}	1.38	2.1×10^{-4}	1.32	2.3×10^{-3}	1.29
7	rs16879765	37955620	2.4×10^{-19}	1.94	7.0×10^{-19}	2.00	4.0×10^{-16}	1.99
7	rs4730775	116704354	8.5×10^{-6}	0.78	4.0×10^{-6}	0.76	5.2×10^{-6}	0.74
8	rs2912522	70154934	3.4×10^{-9}	0.66	1.4×10^{-7}	0.67	4.0×10^{-7}	0.66
8	rs611744	109297184	7.9×10^{-6}	0.78	1.8×10^{-3}	0.83	3.8×10^{-3}	0.83
9	rs10809642	1189448	4.3×10^{-6}	1.35	1.7×10^{-4}	1.30	6.4×10^{-3}	1.24
9	rs10809650	1192371	3.2×10^{-5}	0.77	1.9×10^{-3}	0.81	2.1×10^{-3}	0.80
19	rs11672517	62370006	1.3×10^{-9}	1.46	6.0×10^{-8}	1.44	2.1×10^{-6}	1.41
20	rs8124695	38461850	3.9×10^{-8}	1.69	4.3×10^{-5}	1.54	1.5×10^{-4}	1.54
22	rs6519955	44800506	1.4×10^{-15}	1.56	2.9×10^{-14}	1.58	3.8×10^{-12}	1.57
22	rs8140558	44818937	1.6×10^{-13}	1.51	9.6×10^{-13}	1.53	5.7×10^{-11}	1.53

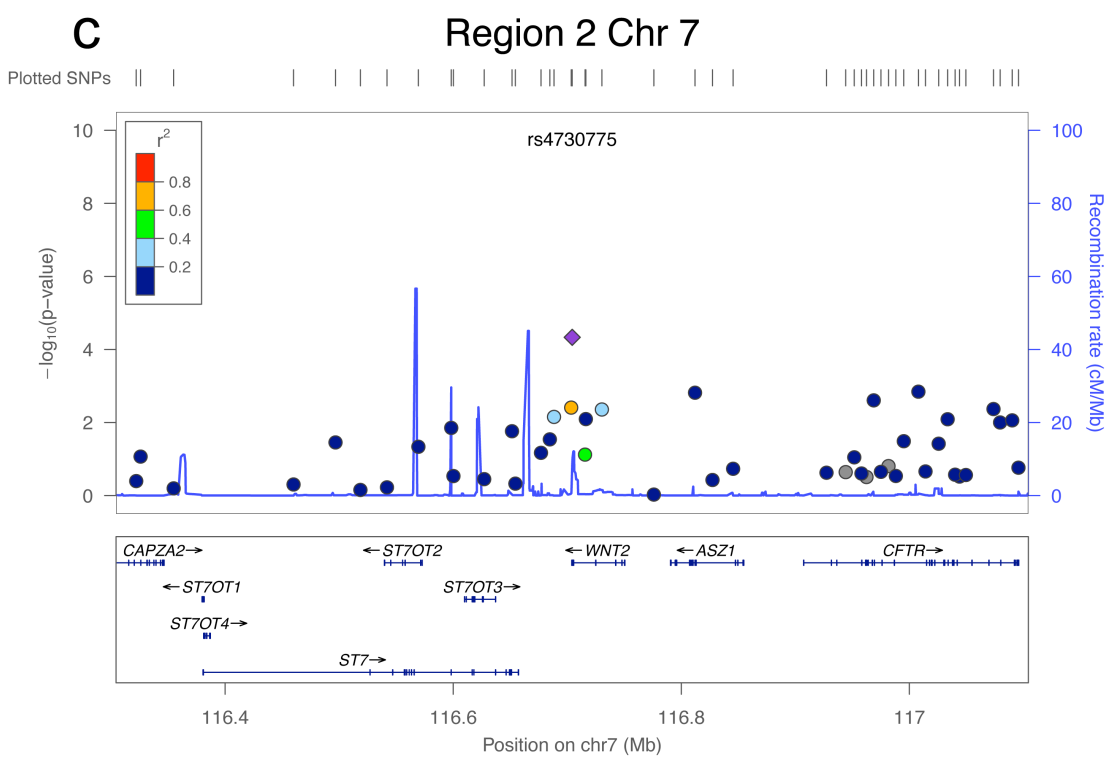
Supplementary Table 2. Results in the discovery phase of the 11 SNPs, which were significant after meta-analysis of the full set from the Netherlands, after excluding the southern hospital (CZE, Eindhoven), and after excluding the three southern and eastern hospitals (CZE Eindhoven; Isala Zwolle; MST, Twente). [a] Positions according to build Human Build 36.3.

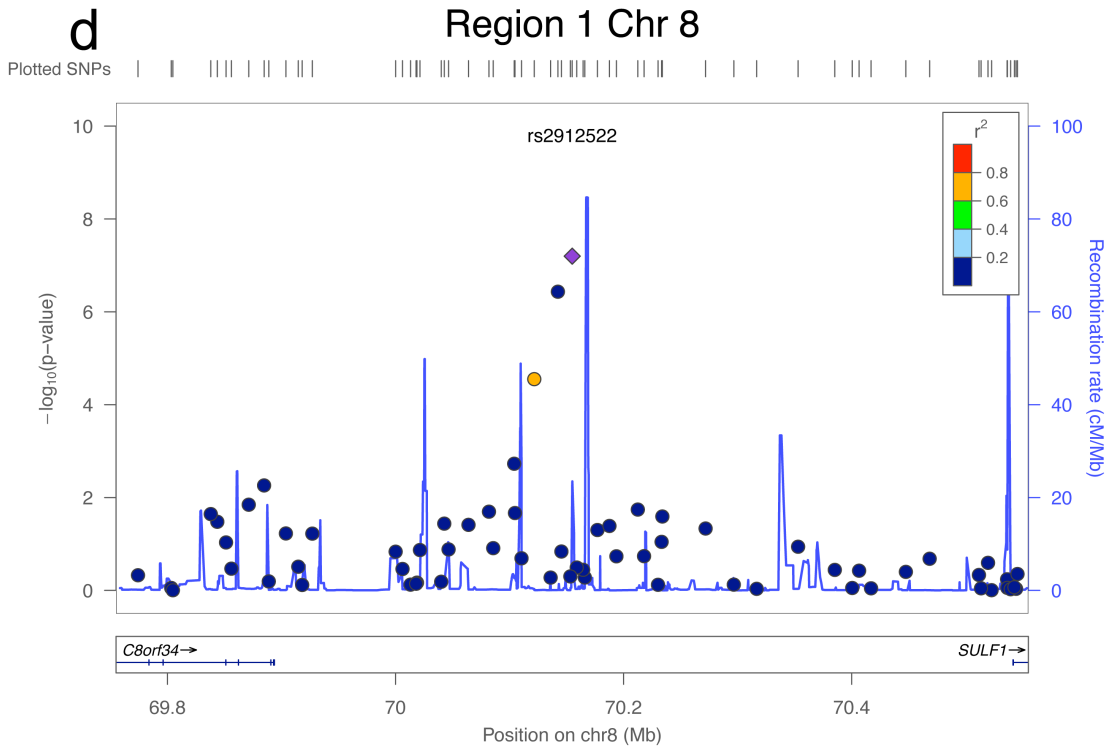
Regional plots

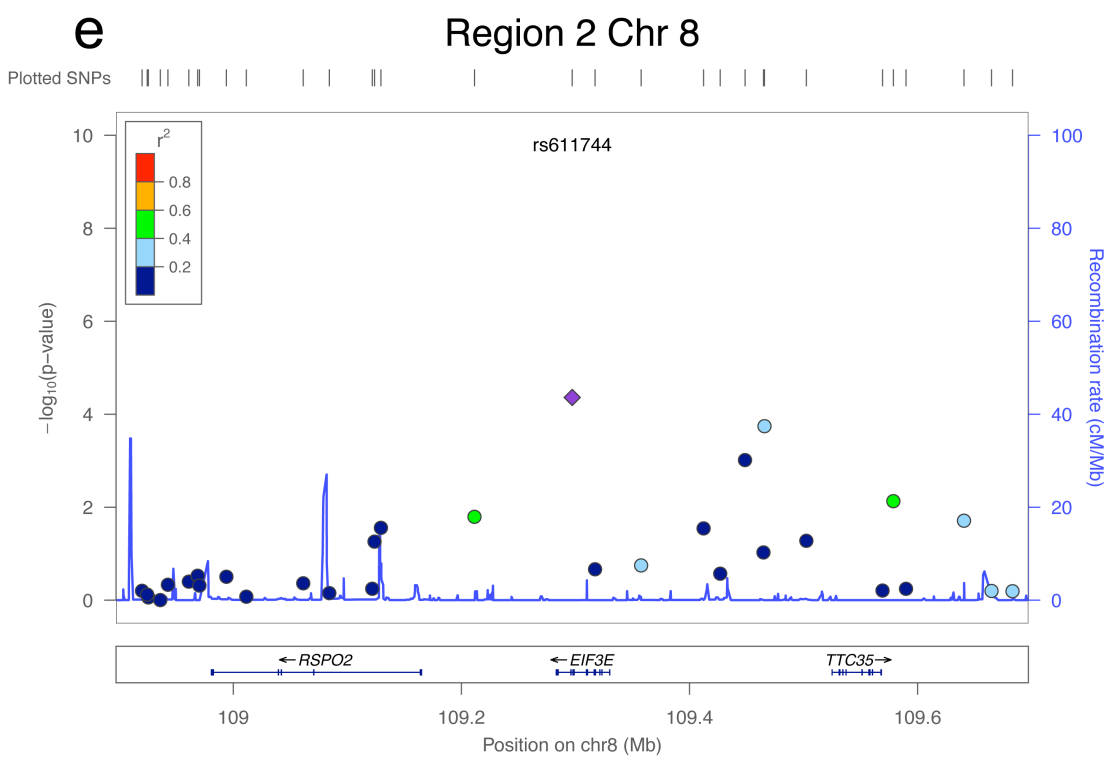
Regional plots of the nine DD risk loci are shown in Supplementary 6.

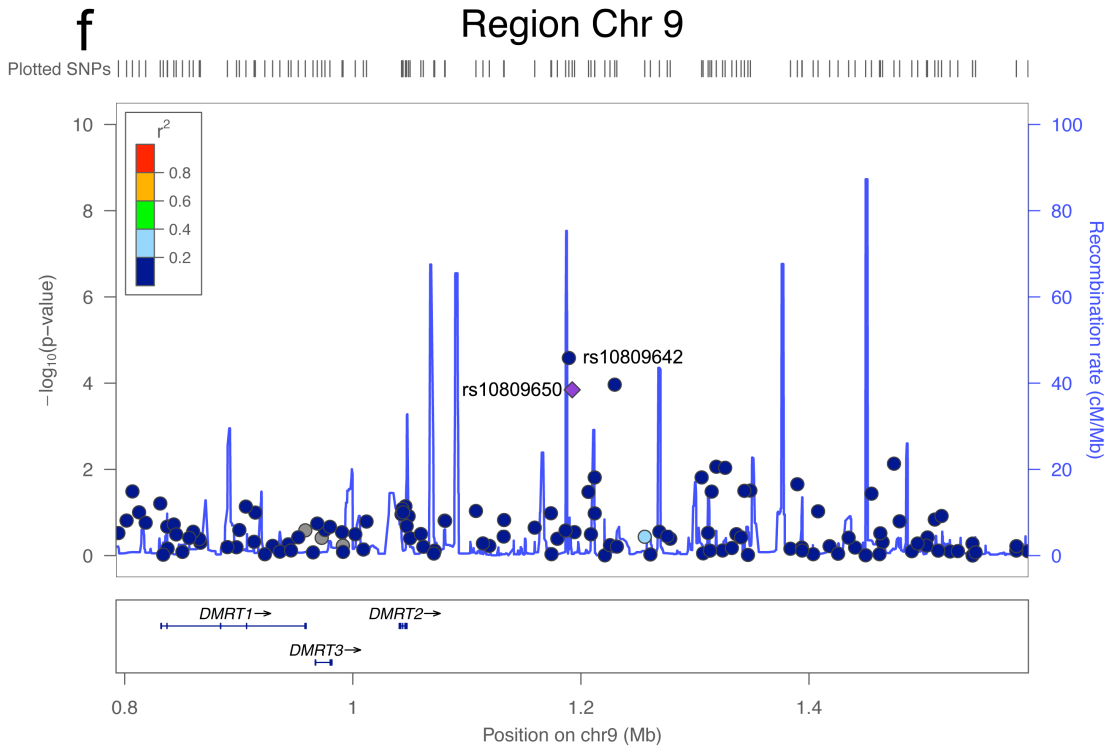
Supplementary Figure 6. Regional plots of the nine Dupuytren's disease risk loci identified in this study.




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→
←ST7OT2
←WNT2
←ASZ1
CFTR→
←ST7OT1
ST7OT3→
ST7OT4→
ST7→
116.4
116.6
116.8
117
Position on chr7 (Mb)


d
Region 1 Chr 8
Plotted SNPs
rs2912522
r²
0.8
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
rs611744
r²
0.8
0.6
0.4
0.2
−log10(p−value)
Recombination rate (cM/Mb)
RSPO2
EIF3E
TTC35
109
109.2
109.4
109.6
Position on chr8 (Mb)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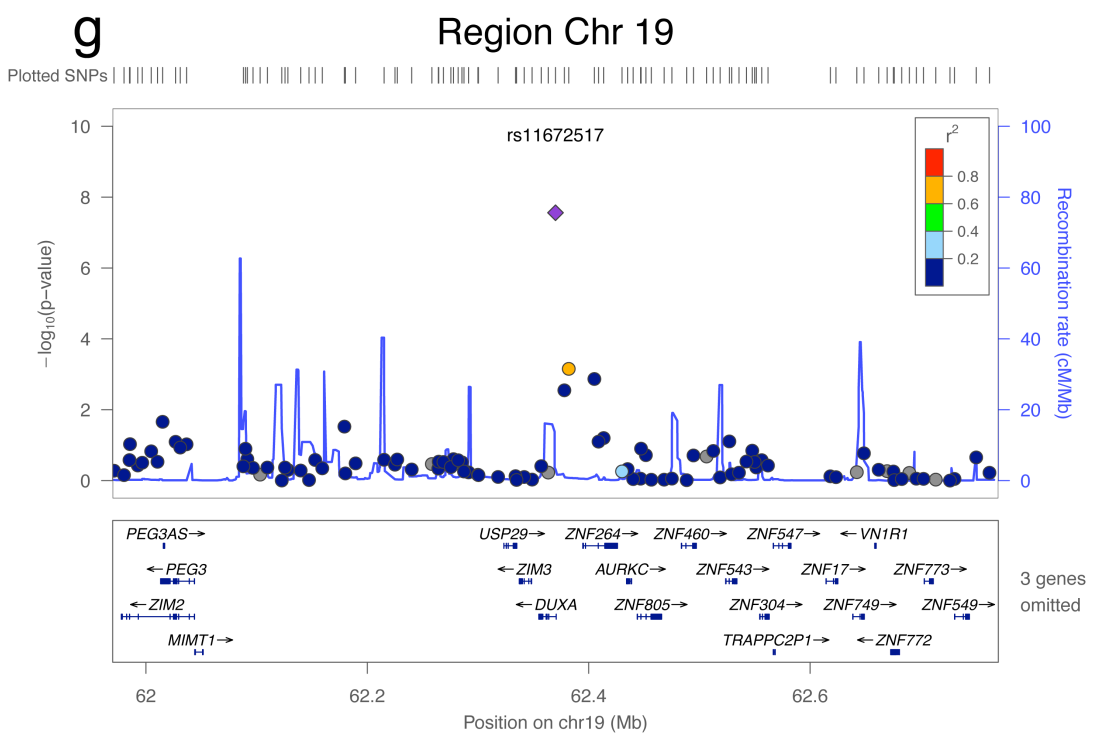
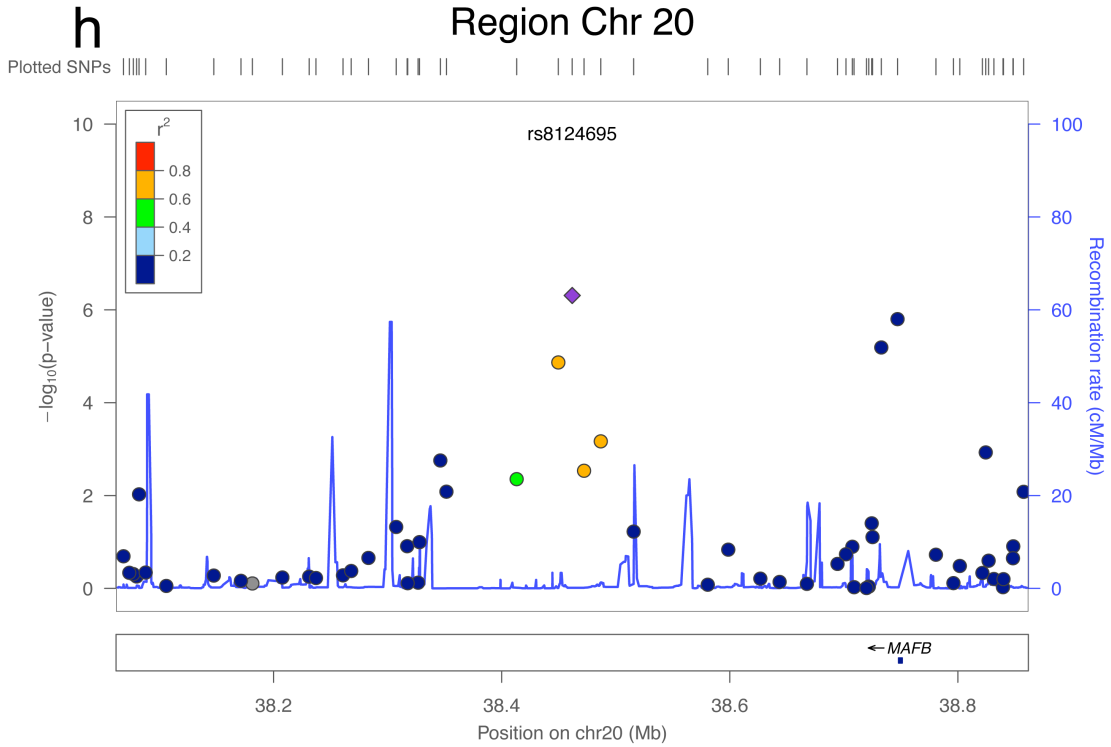
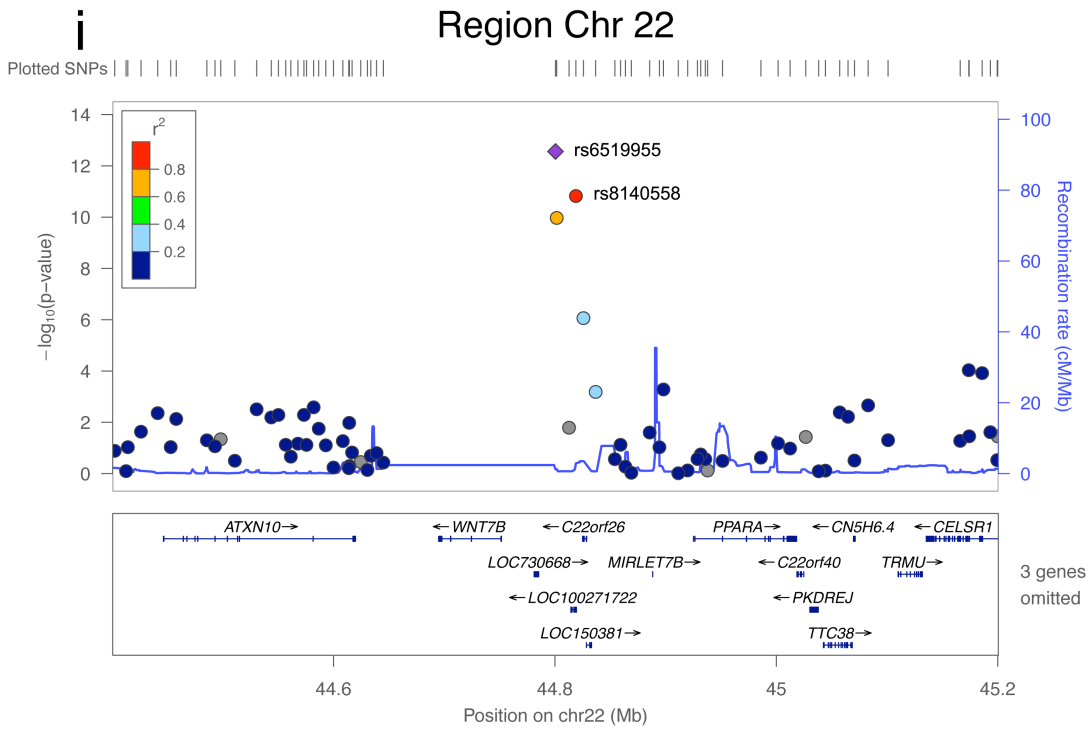
Region Chr 9
Plotted SNPs
rs10809642
rs10809650
r²
0.8
0.6
0.4
0.2
−log10(p−value)
Recombination rate (cM/Mb)
DMRT1
DMRT2
DMRT3
0.8
1
1.2
1.4
Position on chr9 (Mb)


g
Region Chr 19
Plotted SNPs
rs11672517
r²
0.8
0.6
0.4
0.2
−log10(p−value)
Recombination rate (cM/Mb)
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)


h
Region Chr 20
Plotted SNPs
rs8124695
r²
0.8
0.6
0.4
0.2
−log10(p−value)
Recombination rate (cM/Mb)
←MAFB
Position on chr20 (Mb)



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).

Additional analysis indirectly genotyped SNPs

For two of the eleven genomewide significant SNPs, tag SNPs with less than complete LD or imputed SNPs were used in the meta-analysis. In addition, we therefore genotyped rs611744 directly on the Immunochip platform in 8,274 UK controls, and rs8140558 was also genomewide significant when we excluded the UK and German case series. (Supplementary Table 3)

			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}

Supplementary Table 3. 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.

GRAIL analysis

GRAIL analysis was performed. The 11 genomewide significant SNPs in the nine regions were used as query regions, resulting in the analysis of 22 unique genes. The genes with a P-value < 0.05 are shown in Supplementary Table 4. (n.b. These 22 genes are not listed, only those with P < 0.05)

Supplementary Table 4. The genomewide 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.

eQTL analysis

We assessed whether the genomewide significant SNPs did indeed affect gene expression. We investigated six eQTL datasets (references given below: 4-9) that studied lymphoblastoid B-cell line samples (Zhang et al, 176 samples; Choy et al, 246 samples; Stranger et al, 269 samples), peripheral blood samples (Dubois et al, 1490 samples; Heinzen et al, 80 samples) or brain samples (Heinzen et al, 93 samples; Webster et al, 356 samples). In each of these datasets we ran a *cis*-eQTL analysis using a SNP-probe distance of at most 250 kb, using linear regression and controlled the false-discovery rate at 0.05, by running 100 permutations. Full details of the methodology are provided in Dubois et al. (2010). However, we did not observe *cis*-eQTL effects for any of the SNPS that were genomewide significant.

Details regarding funding and acknowledgements of control cohorts

The LifeLines Cohort Study, and generation and management of GWAS genotype data for it, is supported by the Netherlands Organization of Scientific Research (*NWO*, grant 175.010.2007.006), the Dutch government's Economic Structure Enhancing Fund (*FES*), the Ministry of Economic Affairs, the Ministry of Education, Culture and Science, the Ministry for Health, Welfare and Sports, the Northern Netherlands Collaboration of Provinces (*SNN*), the Province of Groningen, the University Medical Center Groningen, the University of Groningen, the Dutch Kidney Foundation and Dutch Diabetes Research Foundation. We thank Behrooz Alizadeh, Annemieke Boesjes, Marcel Bruinenberg, Ilja Nolte, and Mitra Valimohammadi for their help in creating the GWAS database, and Rob Bieringa, Joost Keers, René Oostergo, Rosalie Visser, and Judith Vonk for their work related to data collection and validation. We are grateful to the study participants, the staff from the LifeLines Cohort Study and Medical Biobank Northern Netherlands, and all the participating general practitioners and pharmacists.

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