University of Groningen

WNT2 Locus Is Involved in Genetic Susceptibility of Peyronie's Disease

Dolmans, Guido H.; Werker, Paul M.; de Jong, Igle J.; Nijman, Rien J.; Wijmenga, Cisca; Ophoff, Roel A.; Lifelines Cohort Study

Published in:
Journal of Sexual Medicine

DOI:
[10.1111/j.1743-6109.2012.02704.x](https://doi.org/10.1111/j.1743-6109.2012.02704.x)

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2012

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):

Dolmans, G. H., Werker, P. M., de Jong, I. J., Nijman, R. J., Wijmenga, C., Ophoff, R. A., & Lifelines Cohort Study (2012). WNT2 Locus Is Involved in Genetic Susceptibility of Peyronie's Disease. *Journal of Sexual Medicine*, 9(5), 1430-1434. <https://doi.org/10.1111/j.1743-6109.2012.02704.x>

Copyright

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license. More information can be found on the University of Groningen website: <https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment>.

Take-down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

ORIGINAL RESEARCH—PEYRONIE'S DISEASE

WNT2 Locus Is Involved in Genetic Susceptibility of Peyronie's Disease

Guido H. Dolmans, MD,^{*,†} Paul M. Werker, MD, PhD,^{*} Igle J. de Jong, MD, PhD,[‡] Rien J. Nijman, MD, PhD,[‡] LifeLines Cohort Study,^{**} Cisca Wijmenga, PhD,[†] and Roel A. Ophoff, PhD^{§¶}

^{*}Department of Plastic Surgery, University Medical Center Groningen and University of Groningen, Groningen, the Netherlands; [†]Department of Genetics, University Medical Center Groningen and University of Groningen, Groningen, the Netherlands; [‡]Department of Urology, University Medical Center Groningen and University of Groningen, Groningen, the Netherlands; [§]Center for Neurobehavioral Genetics, University of California, Los Angeles, CA, USA; [¶]Department of Psychiatry, Rudolf Magnus Institute of Neuroscience, University Medical Center Utrecht, Utrecht, the Netherlands; ^{**}The LifeLines Cohort Study

DOI: 10.1111/j.1743-6109.2012.02704.x

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 Measure. 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 comorbid DD.

Conclusions. *WNT2* is a susceptibility locus for PD and our finding provides evidence for a partly shared genetic susceptibility between PD and DD. **Dolmans GH, Werker PM, de Jong IJ, Nijman RJ, LifeLines Cohort Study, Wijmenga C, and Ophoff RA. *WNT2* locus is involved in genetic susceptibility of Peyronie's disease. J Sex Med 2012;9:1430–1434.**

Key Words. Peyronie's Disease; Dupuytren's Disease; WNT Signaling; Genetic Susceptibility; Association Study; Fibromatosis of the Penis

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 underreported 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 comorbidities 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 comorbidity factor which was significantly associated with PD, with a comorbidity rate ranging widely from 1.5% to 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 GWAS 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

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

Combined set GWAS Dupuytren's disease 2,352 cases; 11,562 controls								
Chr	SNP	Position	Minor allele	Allele frequencies		P	OR (95% CI) [†]	Power* (%)
				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

*Power calculation assuming similar minor allele frequencies in Peyronie's disease (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/>) [13]. OR = odds ratio; CI = confidence interval; GWAS = genome-wide association study

Table 2 The association results for Peyronie's disease (PD)

Association results for PD 111 cases; 490 controls

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

*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. OR = odds ratio; CI = confidence interval

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 (<http://pngu.mgh.harvard.edu/purcell/plink/>; Purcell S, et al. 2007. A toolset for whole-genome association and population-based linkage analysis. *American Journal of Human Genetics* 81.) for quality control and statistical analysis [14]. 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).

Results

The mean age of patients at the time of diagnosis was 56.8 years (standard deviation [SD] $\pm$ 9.7) and the mean age of PD onset was 51.9 years

(SD $\pm$ 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 comorbidity of DD revealed an even more significant association, with an uncorrected $P = 0.00084$ and OR 0.58 (95% CI 0.42–0.80).

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 comorbid 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

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 [15].

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 [16]. 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 [17].

A recent study identified increased levels of β -catenin, the end product of WNT signaling, in

TA-derived cells from PD patients compared with cells from normal TA tissue [18]. This suggests that the WNT-signaling cascade is overstimulated in PD. WNT signaling is known to regulate proliferation and differentiation of fibroblasts in both cancer and fibromatosis [19]. 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 [20], and *ASZ1* (or *GASZ*) that encodes for a germ cell protein, which is essential for male meiosis [21]. 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.

Corresponding Author: Roel A. Ophoff, PhD, Center for Neurobehavioral Genetics, University of California, Los Angeles, CA 90095-1761, USA. Tel: (310) 794-9602; Fax: (310) 794-9613; E-mail: ophoff@ucla.edu

Conflict of Interest: None.

Statement of Authorship

Category 1

(a) Conception and Design

Guido H. Dolmans; Paul M. Werker; Roel A. Ophoff; Cisca Wijmenga

(b) Acquisition of Data

Guido H. Dolmans

(c) Analysis and Interpretation of Data

Guido H. Dolmans; Roel A. Ophoff; Cisca Wijmenga

Category 2

(a) Drafting the Article

Guido H. Dolmans

(b) Revising It for Intellectual Content

Paul M. Werker; Roel A. Ophoff; Cisca Wijmenga; Igle J. de Jong; Rien J. Nijman

Category 3

(a) Final Approval of the Completed Article

Guido H. Dolmans; Paul M. Werker; Roel A. Ophoff; Cisca Wijmenga; Igle J. de Jong; Rien J. Nijman

References

- Mulhall JP, Creech SD, Boorjian SA, Ghaly S, Kim ED, Moty A, Davis R, Hellstrom W. Subjective and objective analysis of the prevalence of Peyronie's disease in a population of men presenting for prostate cancer screening. *J Urol* 2004;171:2350-3.
- Schwarzer U, Sommer F, Klotz T, Braun M, Reifenrath B, Engelmann U. The prevalence of Peyronie's disease: Results of a large survey. *BJU Int* 2001;88:727-30.
- Akkus E, Carrier S, Baba K, Hsu GL, Padma-Nathan H, Nunes L, Lue TF. Structural alterations in the tunica albuginea of the penis: Impact of Peyronie's disease, ageing and impotence. *Br J Urol* 1997;79:47-53.
- Mulhall JP. Expanding the paradigm for plaque development in Peyronie's disease. *Int J Impot Res* 2003;15(suppl 5):S93-102.
- Nyberg LM Jr, Bias WB, Hochberg MC, Walsh PC. Identification of an inherited form of Peyronie's disease with autosomal dominant inheritance and association with Dupuytren's contracture and histocompatibility B7 cross-reacting antigens. *J Urol* 1982;128:48-51.
- El-Sakka AI. Prevalence of Peyronie's disease among patients with erectile dysfunction. *Eur Urol* 2006;49:564-9.
- Tal R, Heck M, Teloken P, Siegrist T, Nelson CJ, Mulhall JP. Peyronie's disease following radical prostatectomy: Incidence and predictors. *J Sex Med* 2010;7:1254-61.
- Bjekic MD, Vlajinac HD, Sipetic SB, Marinkovic JM. Risk factors for Peyronie's disease: A case-control study. *BJU Int* 2006;97:570-4.
- Rhoden EL, Riedner CE, Fuchs SC, Ribeiro EP, Halmen-schlager G. A cross-sectional study for the analysis of clinical, sexual and laboratory conditions associated to Peyronie's disease. *J Sex Med* 2010;7:1529-37.
- Qian A, Meals RA, Rajfer J, Gonzalez-Cadavid NF. Comparison of gene expression profiles between Peyronie's disease and Dupuytren's contracture. *Urology* 2004;64:399-404.
- Dolmans GH, Werker PM, Hennies HC, Furniss D, Festen EA, Franke L, Becker K, van der Vlies P, Wolffenbuttel BH, Tinschert S, Toliat MR, Nothnagel M, Franke A, Klopp N, Wichmann HE, Nurnberg P, Giele H, Ophoff RA, Wijmenga C, Dutch Dupuytren Study Group, German Dupuytren Study Group, LifeLines Cohort Study, BSSH-GODD Consortium. Wnt signaling and Dupuytren's disease. *N Engl J Med* 2011;365:307-17.
- Stolk RP, Rosmalen JG, Postma DS, de Boer RA, Navis G, Slaets JP, Ormel J, Wolffenbuttel BH. Universal risk factors for multifactorial diseases: LifeLines: A three-generation population-based study. *Eur J Epidemiol* 2008;23:67-74.
- Purcell S, Cherny SS, Sham PC. Genetic power calculator: Design of linkage and association genetic mapping studies of complex traits. *Bioinformatics* 2003;19:149-50.
- Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, Maller J, Sklar P, de Bakker PI, Daly MJ, Sham PC. PLINK: A tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet* 2007;81:559-75.
- Johnson AD, Handsaker RE, Pulit SL, Nizzari MM, O'Donnell CJ, de Bakker PI. SNAP: A web-based tool for identification and annotation of proxy SNPs using HapMap. *Bioinformatics* 2008;24:2938-9.
- Kato M. WNT2 and human gastrointestinal cancer (review). *Int J Mol Med* 2003;12:811-6.
- Moon RT, Kohn AD, Ferrari GVD, Kaykas A. WNT and beta-catenin signalling: Diseases and therapies. *Nat Rev Genet* 2004;5:691-701.
- De Young LX, Bella AJ, O'Gorman DB, Gan BS, Lim KB, Brock GB. Protein biomarker analysis of primary Peyronie's disease cells. *J Sex Med* 2010;7:99-106.
- Degreef I, De Smet L, Sciort R, Cassiman JJ, Tejpar S. Immunohistochemical evidence for Zic1 coexpression with beta-catenin in the myofibroblast of Dupuytren disease. *Scand J Plast Reconstr Surg Hand Surg* 2009;43:36-40.
- Battle MA, Maher VM, McCormick JJ. ST7 is a novel low-density lipoprotein receptor-related protein (LRP) with a cytoplasmic tail that interacts with proteins related to signal transduction pathways. *Biochemistry* 2003;42:7270-82.
- Ma L, Buchold GM, Greenbaum MP, Roy A, Burns KH, Zhu H, Han DY, Harris RA, Coarfa C, Gunaratne PH, Yan W, Matzuk MM. GASZ is essential for male meiosis and suppression of retrotransposon expression in the male germline. *PLoS Genet* 2009;5:e1000635.