**ORIGINAL RESEARCH—PEYRONIE’S DISEASE**

**WNT2 Locus Is Involved in Genetic Susceptibility of Peyronie’s Disease**

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**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.


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

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**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...
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] ± 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 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
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.

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