SNPs Previously Associated with Dupuytren’s Disease Replicated in a North American Cohort

Eric R. Anderson, DO; Zhan Ye, PhD; Michael D. Caldwell, MD, PhD; and James K. Burmester, PhD

Objective: Dupuytren’s disease is a progressive fibrosis of the hand that often results in debilitating flexion contractures. Its etiology is not completely understood but likely involves both genetic and environmental factors. A recent study performed in Europe identified DNA variants that associate with Dupuytren’s disease. Given the likelihood for genetic variation among populations, we planned to validate the genetic variants identified by this study in a North American population.

Methods: In the Marshfield Clinic’s Personalized Medicine Research Project, 296 cases with Dupuytren’s disease were identified and matched 3-to-1 to controls without Dupuytren’s disease. Clinical data were abstracted from the electronic medical record. The top 12 single nucleotide polymorphisms (SNPs) from the European study were selected and tested in a multiplex assay using the MassArray Analyzer 4 (Sequenom, Inc., San Diego, CA). Differences in allele frequency were determined, and variants with a $P$ value of $<0.004$ were considered significant.

Results: We replicated 5 of the 12 SNPs previously reported to be associated with Dupuytren’s disease.

Conclusion: Our findings support a role for the Wnt signaling pathway in the development of Dupuytren’s disease, and suggest that further study of this pathway may result in early diagnosis and non-surgical treatments for Dupuytren’s disease.

Keywords: Dupuytren’s disease; Wnt signaling pathway; Single nucleotide polymorphism; Fibrotic hand disease; Genetic replication

Dupuytren’s disease (DD) is a progressive fibrosis of the hand that may lead to severe flexure deformities and limit the ability to perform normal activities. It is a common hereditary connective tissue disorder affecting individuals of Northern European descent.1,2 Inheritance of DD occurs with variable penetrance.2,3 The prevalence of DD ranges from 0.5% to 11% in the United States, with the majority of cases over age 60. Men are affected up to five times more often than women.1,3

At the present time, there is no cure for DD. Treatment options include excision of the diseased fascia, percutaneous fasciotomy, and steroid or collagenase injection.4-6 Recurrence of DD ranges from 8% to 66%, dependent on the severity and treatment of disease.2 The high recurrence rate results from failure to treat the underlying cause of DD, which is not completely understood.7

Both genetic and environmental factors are likely to contribute to DD.8-10 Environmental factors known to affect
the development of DD include repeated hand injuries, diabetes, epilepsy, alcohol consumption, and tobacco use.\textsuperscript{11-13} These factors play a role in inflammation, fibrosis, cellular trauma, and ischemia, affecting the interaction between the myofibroblast and collagen matrix. Altered regulation of growth factor pathways, such as transforming growth factor-β and epidermal growth factor, stimulate extracellular matrix formation and myofibroblast activity, increasing the severity of DD.\textsuperscript{14,15}

Several studies have investigated the possible genetic causes of DD. One study has shown an association with regions of chromosomes 6, 11, and 16, with alterations in copy number and structure of chromosomes and possible linkage with DUPC1 at 16q.\textsuperscript{8} Other studies identified the candidate genes MafB, MMPs, TGF-β, ADAM 12, POSTN, TNC, ADAMTS3, BMP5, collagenases, α-smooth muscle actin, fibronectin, β1 integrin, laminin, tenascin C, Hsp47, collagen 1, ALDH1A1, IRX6, PRG4, ZF9, PDGF-β, and c-myc.\textsuperscript{15-21} Most recently, a whole genome-wide association study performed in Europe, identified eleven single nucleotide polymorphisms (SNPs) from nine different loci, with six of the loci known to be involved in the Wnt signaling pathway.\textsuperscript{22} Because many genetic studies fail replication, and the findings may differ among populations of people, we set out to confirm these findings in a North American population.

**Methods**

The study was approved by Marshfield Clinic’s Institutional Review Board for human subjects protection. We identified 519 cases of DD in the Marshfield Clinic data warehouse by using International Classification of Diseases Ninth Revision (ICD-9) code 728.6 for palmar fascia contracture. Of those, 296 were found to have genetic data and DNA available in the Marshfield Clinic Personalized Medicine Research Project (PMRP).\textsuperscript{23} There were 919 age- and gender-matched controls without the diagnosis of DD also identified in PMRP. The PMRP bio-bank contains DNA, plasma, and serum from more than 20,000 subjects residing in central Wisconsin and is linked to the electronic medical record of each subject.\textsuperscript{23} As genetic data are collected, the data are deposited into the genetic repository and made available for approved studies. Clinical data were abstracted for each case and control including race, gender, age at DD diagnosis, hand affected, dominant hand, alcohol use, tobacco use, presence of epilepsy, presence of diabetes, and family history of DD. Eight non-Caucasian subjects were removed at the time of data analysis to prevent spurious associations that could occur from admixture.

**Genetic Testing**

Genetic testing was performed using the MassArray Analyzer 4 (Sequenom, Inc., San Diego, CA) and the iPLEX assay that uses primer extension reaction chemistry designed to detect sequence differences at the single nucleotide level. DNA samples were amplified in a multiplexed reaction and extended with a primer specific for the target SNP sequence. The top 12 SNPs from the Dolmans et al\textsuperscript{22} study were selected for this replication study. Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry was used to detect the difference in mass that differentiates SNP alleles. Twenty-three samples from the HapMap-CEU CEPH DNA collection (Coriell Institute, Camden, NJ) were tested as positive controls. These HapMap samples were chosen to represent examples of heterozygous and each homozygous genotype. All genotyping results were compared to the Genotype and Allele Frequency Report from NCBI dbSNP when available for the 12 SNPs included in the

| Gene               | Chromosome | SNP #          | $P$ value | Anderson | Dolmans |
|--------------------|------------|----------------|-----------|----------|---------|
| Wnt7Ba*            | 22         | rs8140558      | 4.104×10^{-5} | 1.2E-22  |
|                    |            | rs6519955      | 1.3×10^{-4}  | 3.2E-33  |
| C8orf34,SULF1a*    | 8          | rs1365415      | 2.096×10^{-4} | 2.8E-07  |
|                    |            | rs2912522      | 5.361×10^{-2} | 2.0E-13  |
| EPDR1,SFRP4a*      | 7          | rs16879765     | 1.336×10^{-3} | 5.6E-39  |
| DUXA, ZNF264       | 19         | rs11672517     | 3.058×10^{-3} | 6.8E-14  |
| EIF3E,RSPO2        | 8          | rs611744       | 2.348×10^{-1} | 7.9E-15  |
| MAFB               | 20         | rs8124695      | 9.156×10^{-1} | 7.6E-10  |
| DMRT2              | 9          | rs10809650     | 4.930×10^{-1} | 6.2E-09  |
| WNT2               | 7          | rs4730775      | 5.834×10^{-1} | 3.0E-08  |
| DMRT2              | 9          | rs10809642     | 9.866×10^{-1} | 1.2E-08  |
| TIMP2              | 17         | rs4789939      | 9.247×10^{-1} | 6.0E-07  |

*SNPs with a $P$ value less than 0.004 and are considered replicated.
Sequenom assay. In addition to the study samples, each 384-well plate also included six of the HapMap samples with two no-template controls that were compared to the expected genotypes for quality control.

Statistical Analysis
Quality assurance was performed on the genetic data prior to statistical analysis. This included ensuring data were available for each SNP on at least 90% of the subjects and that genotypes were within Hardy-Weinberg equilibrium set at $10^{-3}$. Differences in allele frequency were determined, and the $P$ values from logistic model and Fisher exact tests were reported. The SNPs with a $P$ value <0.004 were considered replicated to account for multiple testing (Bonferroni correction). We incorporated the genetic models (additive, dominant and recessive), since we do not know the mode of inheritance of Dupuytren disease. All the procedures and calculations were done using the PLINK software.\textsuperscript{24} In addition, we calculated the odds ratios estimates from each of the markers with the confidence intervals.

Results
The PMRP population with DD was made up of 62% men and 38% women, of which 98% were white non-Hispanic, 34% were diabetic, 18% reported regular use of alcohol, 29% reported tobacco use, and 4% had epilepsy. The distribution of white non-Hispanic subjects in PMRP is Germany (74.4%), Poland/Czech Republic (7%), Sweden/Norway (5.8%) and British and Irish Isles (12.8%).\textsuperscript{25} Approximately 6% of our cases had a family history of DD which is much less than the 18% previously reported in one study\textsuperscript{1} and approximately 40% in another study.\textsuperscript{8} It is possible that our rate is an underestimate do to inability to capture a complete family history through the electronic medical record where some family members may not be diagnosed or treated and a complete family history may not have been collected by the medical staff.

Of the 12 SNPs tested, we were able to replicate 5. Table 1 shows the $P$ values for the 12 SNPs in both our cohort and that of Dolmans et al (the meta-analysis $P$ values).\textsuperscript{22} It highlights the 5 SNPs with $P$ values <0.004 that were considered replicated.

Figure 1 shows the forest plot generated to show the final results for the 12 selected SNPs. The odds ratio reports the ratio between two proportions, which, in the context of our study, are the proportion of individuals in the DD group having a specific allele and the proportions of individuals in the matched control group having the same allele. An odds ratio higher than 1 indicates that the allele frequency in the DD group is much higher than in the control group, that also shows the tested SNPs are associated with the condition. The confidence interval estimation illustrates the precision of the
estimation of the odds ratio. In our study, we showed that all five identified significant SNPs have higher odds ratios (>1), and also have confident intervals above 1, showing the estimated odds ratios were precisely calculated.

**Discussion**

Dupuytren’s disease can be difficult to diagnose, and patients may present with advanced disease, making non-operative therapy difficult and ineffective. Additionally, with operative therapy associated with risk of neurovascular injury and recurrence rates between 8% to 66%, early detection is critical. The inability of current treatments to target the unknown, underlying pathology of the disease underscores the importance of identifying an etiology that may allow for earlier detection and better treatments.

The discovery and replication of a genetic cause for DD should allow for long-term development of disease prediction models before the onset of any symptoms, allowing individuals at higher risk of DD to have the option of modifying their activities and limiting exposure to environmental factors associated with DD. Also, understanding the molecular pathways that predispose individuals to DD should lead to a better understanding of the disease pathophysiology, allowing for development of novel targeted interventions.

Although it is well-established that fibrosis is important in DD, little is known about the underlying mechanisms that lead to the fibrotic changes seen in DD. It has been hypothesized that a combination of genetic and environmental interactions results in the disease. Therefore, a single genome-wide search for underlying genetic variants that may predispose individuals to DD was performed by Dolmans et al. in a European population. It identified eleven SNPs from nine different loci, with six of the loci known to be involved in the Wnt signaling pathway. However, many genetic association studies cannot be replicated due to artifacts in the design of the original study, and replication in multiple cohorts often fails to show a true association with disease. We were able to replicate the findings of Dolmans et al in a North American population, with the identification of five SNPs with significant P values. However, our cohort was relatively small and some of the tested SNPs may not have reached statistical significance due to our small sample size. Additional studies in larger cohorts will be necessary to investigate interactions between the SNPs and to understand how the individual SNPs contribute to disease.

Of the nine loci identified in the Dolmans et al study, six are involved in the Wnt signaling pathway, suggesting that misregulation of this pathway contributes to fibrosis seen in DD. Within this pathway, β-catenin is a key signaling molecule regulated by Wnt whose expression is dysregulated in DD. Currently, it is unknown how the SNPs affect gene expression within the Wnt pathway. One study demonstrated down-regulation of Wnt11 in surgically removed tissue, and another study demonstrated an increase in Wnt5a, with a third study demonstrating that the microRNA profile in DD is altered, including that for Wnt5a. The Wnt family of proteins regulates cell growth, proliferation and differentiation and has been linked to abnormal proliferation of fibroblasts, and appears necessary for TGF-β-mediated fibrosis seen in fibrotic diseases. Modulation of Wnt signaling ultimately leads to changes in gene expression that impact cell proliferation and survival.

Several additional studies implicated the Wnt pathway in DD, although they did not identify specific SNPs that predispose to genetic disease. Our study supports the findings of the Dolmans et al study. Larger consortium studies can utilize these results to develop risk stratification and, potentially, novel detection methods and treatments for DD.

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