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Evaluation of a Mitochondrial DNA Mutation in Maternally Inherited and Sporadic Cases of Dupuytren Disease

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Objective: The purpose was to test the hypothesis that Dupuytren disease (DD) is associated with a previously reported mutation in mitochondrial DNA at position 2839.

Methods: Two hundred sixty-nine cases of DD and an equal number of matched controls were identified in Marshfield Clinic's Personalized Medicine Research Project (PMRP). Clinical data used to describe the cohort were abstracted from the electronic medical records of the population. Genetic analysis of all the cases and controls was done using a custom synthesis TaqMan assay, while genetic analysis of sixteen of the above cases with a familial history of DD was performed by mitochondrial DNA sequencing at position C2839A.

Results: Cases and controls were evenly distributed with 167 (62%) men and 102 (38%) women. The majority, 264 (98%) of the cases and controls were white non-Hispanic. Of the 269 cases, 16 were found to have a familial history of DD. Two cases had a maternal history, eight a paternal history, five an affected sibling, and one a paternal grandfather. All cases and controls were found to have only the C allele at the site of the reported mitochondrial C2839A polymorphism.

Conclusions: The previously reported mitochondrial mutation was not present in our small, maternally inherited cohort or in the total population of 538 cases and controls. This finding does not support the reported incidence of this polymorphism in 90% of the affected population with a maternal inheritance, and calls into question the role of the C2839A mitochondrial DNA polymorphism in familial or sporadic cases of DD.

Keywords: Mitochondrial DNA; Dupuytren disease; Mutation

Dupuytren disease (DD) is a progressive and irreversible fibrosis of the palmar fascia of the hand. The fibrotic process begins with palmar nodules followed by rope-like cord formation that over time produces progressive contraction of the digits at the metacarpalphalangeal and proximal interphalangeal joints. The ring finger is most frequently affected, followed by the small, thumb, long, and index fingers. The symptoms are usually unilateral but may progress to bilateral disease.[1,2] In some patients, the subsequent flexion deformities of the hand severely limit the ability to perform normal activities and negatively impact quality of life.

In Caucasians, DD is considered one of the most common hereditary connective tissue disorders, predominantly affecting individuals of Northern European descent. Approximately 40% of DD patients have an affected relative based on several familial aggregation studies.[2] Prevalence of DD in the United States ranges from 0.5% to 11%, with the annual number of new cases of physician-diagnosed DD in the year 2007 estimated at 3 per 10,000.[1] Most patients diagnosed with DD are over age 60, and men are affected 3 to 5 times more often than women.[1-3]

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Currently, there is no cure for DD, and treatment options include open excision of the diseased fascia, percutaneous needle fasciotomy, and injections with steroids or collagenase.[1,3] However, these treatment options are associated with risk of neurovascular injury and have recurrence rates between 8%–66%, depending on the treatment and severity of disease.[3] The potential for recurrence underscores the inability of current treatments to target the underlying pathology as the etiology of disease is not completely understood.

We do know that environmental factors such as alcohol consumption, tobacco use, repeated hand injuries, diabetes, and epilepsy have been associated with DD.[1-3] These factors are thought to play a role in the cellular trauma and ischemia that result in tissue inflammation and fibrosis seen in DD. The process by which cellular transformation occurs, and to what degree, remains unknown. The primary cell type involved in DD is the myofibroblast, and its direct interaction with the extracellular matrix of collagen is linked to the development of DD.[2,4] Studies have shown that growth factors such as transforming growth factor beta (TGF-β) and epidermal growth factor (EGF) can stimulate excess myofibroblast activity and fascial contraction in DD.[4,5] Cell adhesion glycoproteins like fibronectins and catenins (ie, β-catenin) are modified and/or over-expressed.[4] Additionally, extracellular proteins of the metalloproteinase family exhibit many dysfunctions responsible for collagenic proliferation.[4]

The genetic mode of DD inheritance appears to be heterogeneous, and mostly autosomal dominant with variable penetrance.[2,3,6] Numerous genetic studies have been performed in order to more clearly understand the genetic basis of disease. These studies have shown an association of DD with regions of chromosomes 6, 11, and 16, alterations in copy number and structure of chromosomes, and a possible linkage with the *DUPC1* locus at 16q.[2] Other studies have identified several candidate genes associated with DD, including *MafB, MMPs, TGF-β, ADAM 12, POSTN, TNC, ADAMTS3, BMP5, ALDH1A1, IRX6, PRG4, PDGF-B,* and *C-MYC*.[4,7-13]

It has also been suggested that given the role of mitochondria in cellular metabolism and apoptosis, a mutation in mitochondrial DNA may have a role in the development of DD.[6] Normally, mitochondria produce the majority of cellular ATP and generate physiologically appropriate levels of reactive oxygen species (ROS), which are further moderated by antioxidant enzymes. As tissues age and/or become diseased, mitochondrial function becomes impaired and increased levels of ROS are generated while antioxidant enzyme activities and ATP production decrease.[6,14]

Mitochondrial DNA is more susceptible to damage than nuclear DNA due to a lack of protective histones, limited DNA repair capacity, lack of introns, and its exposure to ROS.[14] Additionally, mutations in mitochondrial DNA have been shown to accumulate with age. These mutations can induce nuclear stress signaling pathways leading to decreased energy production, increased ROS, deleterious protein modifications, abnormal phenotypic changes, apoptosis, and even more mutations.[6,15,16] A combination of mitochondrial DNA mutations and increased levels of ROS could lead to the over expression of TGF-β leading to excess myofibroblast activity, fascial contraction, and DD.[6]

Mitochondrial DNA and ROS also play roles in the regulation of apoptosis. Increased levels of ROS trigger mitochondrial-mediated apoptosis, and mutated mitochondrial DNA can alter the regulation of apoptosis.[6,17] Compared to normal tissue, DD tissue contains larger numbers of myofibroblasts, which in turn contain abnormally high numbers of mitochondria as confirmed by electron microscopy.[2,6] This situation allows more opportunity for increased levels of ROS and potential for mitochondrial DNA mutations.

Pursuing the role(s) that mitochondrial DNA may play in the development of DD, a group of researchers was able to identify a previously unknown heteroplasmic mutation within the 16s rRNA region of the mitochondrial genome. Using a multiplex denaturing high-performance liquid chromatography technique, the mutation at position C2839A was present in 90% of their subjects with maternally inherited DD and 0% of controls.[6] In the present study, we tested the hypothesis that DD is associated with the reported mitochondrial mutation by analyzing the DNA of those with and without a familial pattern of DD. Validating the results of the prior study and finding the mutation in sporadic cases of DD could further our understanding of the disease.

Methods

After obtaining approval from the Marshfield Clinic's Institutional Review Board, we electronically identified 269 cases of DD in the Marshfield Clinic's Personalized Medicine Research Project (PMRP) by utilizing the ICD-9 code 728.6 for contracture of palmar fascia. The PMRP is a large population-based biobank containing DNA, plasma, and serum, along with a growing database of genetic information from more than 20,000 patients residing in central Wisconsin.[18] Enrollment into PMRP began in 2002 and was mostly completed by 2004; with enrollment continuing to this date.[19] In 2005, the migration rate into PMRP was 4.7% of the population, excluding births, while the migration rate out of PMRP was 3.6%, excluding deaths.[19]

An equal number of age- and gender-matched controls without the diagnosis of DD were also selected from PMRP. Clinical data for the cases and controls were abstracted from the dynamic electronic medical records of the PMRP population. These data included race, gender, age at DD diagnosis, hand dominance, hand affected, tobacco use, alcohol use, presence of diabetes, presence of epilepsy, and a family history of DD.

Table 1. Study population distribution of age, gender, and race.

	Men	Women	White Non-Hispanic	Diabetic	Regular alcohol use	Tobacco Use	Epilepsy
Cases (n=269)	62%	38%	98%	34%	18%	29%	4%
Controls (n=269)	62%	38%	98%	24%	8%	23%	2%

Genetic analysis of all samples (cases and controls) was performed using a custom synthesis TaqMan assay from Applied Biosystems (Applied Biosystems; Carlsbad, CA). TaqMan assays are custom designed by Applied Biosystems for genotyping of specific SNPs and have the advantage of being able to test a large number of samples for less cost than DNA sequencing. Unlabeled PCR primers and TaqMan probes labeled with Fam and VIC dye were used to amplify genomic DNA in PCR using TaqMan Universal PCR Master Mix with No AmpErase UNG. Each assay allows scoring of both the FAM and VIC alleles in a single well. Water controls are used on each plate to ensure absence of DNA contamination.

Genetic analysis of 16 of the 269 cases found to have a familial history of DD was performed by mitochondrial DNA sequencing at position C2839A of NC_012920. Sequence analysis is generally considered the gold standard since it provides direct sequence of the putative polymorphism as well as sequence around the putative polymorphic site. For this analysis, target DNA was amplified by polymerase chain reaction, and the products were electrophoresed through a 1% agarose gel. They were then extracted, purified, and sequenced using a BigDye Terminator v3.1 cycle sequencing kit (Applied Biosystems) and an Applied Biosystems 3100 Genetic Analyzer.

The two methodological approaches were used to validate each other and ensure the accuracy of the genetic testing. The results were consistent between the TaqMan and DNA sequencing; neither method identified the putative polymorphism.

Results

Cases and controls were evenly distributed based on age, gender, and race (table 1). Two hundred sixty-four (98%) of the cases and controls were white non-Hispanic, and the majority of both cases and controls were of German descent. Of the 269 cases, 62% were men, 38% were women, 34% were diabetic, 18% reported regular consumption of alcohol, 29% reported regular use of tobacco products, and 4% had epilepsy. Sixteen were found to have a familial history of DD; two cases had a maternal history, eight a paternal history, five an affected sibling, and one a paternal grandfather (table 2). All 269 cases and 269 controls were found to have only the C allele, not the mutation, at the site of the mitochondrial C2839A polymorphism found by Bayat and colleagues.[6]

Discussion

Despite multiple studies, the genetic basis of DD remains unknown. Several candidate genes have been identified by studies utilizing small sample sizes;[4,7-13] however, no specific gene has yet been identified. A promising study into the role that mitochondrial mutations play in DD identified a mutation at position C2839A in the mitochondrial genome 16s rRNA region present in 90% of their subjects and 0% of the controls.[6] If validated, this finding could have a significant impact on our understanding of DD and in the development of new diagnostic tests, medications, and treatments for DD.

By utilizing the PMRP database, we have been able to analyze the largest population of DD patients to date in the United States. We found that 6% of our cases had a familial history of DD, much less than the 18% reported in a study by DiBenedetti et al[1] and also less than the approximately 40% found in several familial aggregation studies[2], thus reflecting the genetic heterogeneity of DD. The 6% rate for family history of DD is based on the data we have available from the PMRP database. It is possible this rate is an underestimate of the familial cases of DD in our study population. There likely are cases of DD in which we were unable to capture a complete family history through the electronic medical record secondary to family members not being diagnosed, treated or a complete family history was not obtained by the diagnosing physician. However, we do not expect the rate to be substantially higher than the 6% we report. In addition, men in our study population were affected twice as often as women compared to the estimated 3 to 5 times found in other studies.[1-3]

Our results do not support the reported incidence of the previously reported polymorphism in 90% of the affected

Table 2. Family history of disease in study population.

	Family History			
Cases	**Maternal**	**Paternal**	**Affected Sibling**	**Paternal Grandfather**
269	2	8	5	1

population with a maternal inheritance and call into question the role of the C2839A mitochondrial DNA polymorphism in familial or sporadic cases of DD. This may be due to several differences that exist between our study and that of Bayat et al.[6] First, in reflecting the heterogeneity of DD, genetic variances between the study populations could explain the results, since the majority of our population is of German descent as compared to the Northern England population studied by Bayat.[6] Other genetic studies have shown genetic differences between similar populations. Using genotype-based tests of population differentiation, Medici et al[20] found major differences in the frequencies of several Crohn disease-associated polymorphisms between German and Norwegian populations. Muller et al[21] examined RHD mutations of the blood group D antigen and found mutation patterns varied between the populations of the Tyrol region of Austria, Northern Germany, and Southwestern Germany. It is thus possible that our population of study did not have a similar disease pattern as those studied by Bayat and colleagues.[6] Second, our cases were confirmed through an electronic search of medical records and were not personally examined or interviewed. This is a potential limitation of the study, as patients may present with mild disease without contractures and therefore are not diagnosed with DD. Thus, the number of DD cases in our study population may be under-represented in the electronic medical records and PMRP biobank. Lastly, 16 of 269 subjects were confirmed to have a familial history of DD; of those, only 2 had a maternal history of disease. This is compared to the 20 maternally inherited cases identified and studied by Bayat et al.[6]

Despite these differences, we believe our inability to find the mitochondrial mutation reported by Bayat et al[6] in any of our subjects or controls does not support the reported incidence of this mitochondrial mutation in 90% of the affected population with a maternal inheritance and calls into question the role of the C2839A mitochondrial DNA mutation in the pathogenesis of familial or sporadic DD. As the pathogenesis of DD is most certainly based within the genetic makeup of those affected, and often in concert with other known risk factors, our understanding of this disease and the future of treatment depends on further genetic study.

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