Short Report

Mapping of an autosomal dominant gene for Dupuytren’s contracture to chromosome 16q in a Swedish family


Dupuytren’s contracture (DC) (OMIM 126900) is the most common connective tissue disease of mankind and has both heritable and sporadic forms. The inherited form is most frequently observed among the xanthochroi peoples of Northern Europe where its most common manifestations are thickening of the palmar fascia and contracture of the fingers. We ascertained a five-generation Swedish family in which DC is inherited in an autosomal dominant manner with high, but incomplete, penetrance by the end of the fifth decade. Blood was collected from all affected and informative unaffected family members for the performance of a genome-wide scan at a resolution of approximately 8 cM for all autosomes. Linkage was established to a single 6 cM region between markers D16S419 and D16S3032 on chromosome 16. A maximal two-point logarithm of odds (LOD) score of 3.18 was achieved at microsatellite marker D16S415 with four other markers in the region producing LODs of >1.5.

Dupuytren’s contracture (DC) (OMIM 126900) is the most common heritable disorder of connective tissue (OMIM); however, sporadic cases are also common. It is a disease of the soft tissues of the palm and fingers characterized by a progressive thickening and shortening of the fascial structures that normally provide support to the glabrous skin of the palm. In its forme fruste, DC presents as a thickening in the palm which can progress to a hard, tender subcutaneous nodule that limits the ability to fully extend one or several digits. As the condition becomes more advanced, it interferes with the ability to grasp and manipulate objects; however, the hand, as an organ of human contact, can be affected even in the early stages of the disease.

Clinical signs of DC are commonly first noted in the fifth or sixth decade of life, and disease progression is variable. The generally late onset of symptoms, incomplete penetrance, and variations in severity all contribute to the high percentage of patients without a positive family history and have contributed to difficulties in disease gene identification (1–3). The onset at a younger age is associated with an aggressive disease course and an increased risk for recurrence after treatment.

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The hereditary form is most prevalent in Scandinavia and among Scandinavian immigrant populations, but DC is found among all ethnic groups at lower prevalence rates (6) including Black Africans (7) and Japanese (8).

DC is, in part, a fibroproliferative disease in which fibroblasts display an abnormal morphology, and the lesions have increased collagen III deposition with elevated levels of hydroxylation and glycosylation (9, 10). These phenomena are characteristic of granulomatous disease, and several lines of evidence suggest that DC results from a defect in the wound repair mechanism: DC has a male-to-female ratio of greater than 3:1 (4, 11, 12); the hypertrophic cellular responses leading to increased collagen deposition; and the fact that DC is more highly penetrant among persons with chronic hand trauma (13, 14). The association between wound repair and DC in its sporadic form has been observed in numerous epidemiological studies in which chronic substance abusers such as alcoholics and tobacco users, who have decreased wound healing responses secondary to their lifestyle, have been demonstrated to have increased rates of the disease (15–19). Similarly, links between diabetes and decreased liver function in the absence of alcoholism have also been made with DC (15, 20). Multiple fibroproliferative conditions including Peyronie disease, knuckle pads, congenital generalized fibromatosi, juvenile fibromatosis, plantar fibromatosis, and frozen shoulder (OMIM; 21) have been associated with DC at rates higher than can be explained by chance alone, suggesting a common underlying wound repair defect.

DC patients seek medical advice at various stages of disease progression, but there is presently no alternative to surgical treatment, and surgical complications are common. Numerous surgical techniques have been described for the treatment of DC, all of which have a high incidence of complications, including recurrence of the disease, and none have proven to be consistently more effective than others (22). The post-operative course often requires physical therapy over extended periods of time for optimal results, and manual workers rarely are able to return to work within 2 months (23). Identification of the gene(s) associated with DC would provide insight into the fundamental pathogenesis of the disease, and suggest targets for prevention or medical intervention.

**Materials and methods**

**Patients and specimen collection**

IRB approval was obtained from the University of Umea, Umea, Sweden to conduct a family-based linkage-mapping project. All initial family contacts were made by the proband’s hand surgeons (A Nystrom and I Mossberg), and subsequent family contact was made by these individuals and by a medical professional identified in the family. Pedigrees were ascertained at the time of initial family contact, and all participants were asked to verify their relationship within the family during phenotyping and blood draws. Blood was collected from all affected and informative unaffected family members (n = 26) via peripheral venipuncture into vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ). DNA was extracted using Puregene DNA kits (Gentra Systems, Minneapolis, MN) according to the manufacturer’s recommendations. DNAs were quantitated by ultraviolet spectrophotometry and adjusted to 50 ng/μl.

**Genotyping**

A microsatellite-based genome-wide scan, excluding the sex chromosomes, was performed at a resolution of approximately 8 cM as described (24). Briefly, a multiplex amplification and gel analysis format was used in which all of the markers from a given ABI panel were amplified in three reactions, grouped by fluorescent dye, and analyzed on a single gel. Amplification conditions were as follows: 50 mM KCl; 10 mM Tris-HCl (pH 8.3); 2.5 mM MgCl₂; 400 μM of each dNTP (dATP, dCTP, dGTP, and dUTP) (Pharmacia, Piscataway, NJ); 50 nM of each forward and reverse primer; 0.12 U of Ampli-Taq Gold (Perkin-Elmer, Wellesley, MA, USA); and 50 ng of DNA. Polymerase chain reaction (PCR) conditions were: 1 cycle at 95 °C for 12 min; 10 cycles at 94 °C for 15 s, 55 °C for 15 s, 72 °C for 30 s; 20 cycles at 89 °C for 15 s, 55 °C for 15 s, 72 °C for 30 s; 1 cycle at 72 °C for 10 min; and 1 cycle at 4 °C on hold. After amplification, the three dye-based multiplex reactions were combined as follows: 3 μl of 5-Carboxyfluorescein (FAM); 3 μl of 6-Hexachlorofluorescein (HEX); and 6 μl of NED; denatured at 99 °C for 2 min with formamide (1:2) in the presence of 0.2 μl of ROX-labeled-size standards from 70 to 400 basepairs, (BioVentures, Murfreesboro, TN) and 0.3 μl tracking dye solution; quenched on ice; and loaded on a 0.2 mm thick 5% LongRanger gel (FMC Bioproducts, Rockland, ME). All gels were run on ABI 377 PRISMs using the GENESCAN software package.

Fine structure mapping of the candidate region was accomplished by identifying additional markers, within 10 cM of the marker giving the highest
logarithm of odds (LOD) score. All primer pairs for confirmational analyses were synthesized with FAM, HEX or TET on the forward primer by Research Genetics (Huntsville, AL), and PCR conditions were established by adjusting MgCl\textsubscript{2} concentration and then annealing temperature, if necessary.

Allele calling, haplotyping, and linkage analyses

We utilized a suite of VISUAL BASIC computer programs written for Macintosh (24) to assist in: 1) allele calling; 2) binning of alleles; 3) inheritance checking; 4) the performance of data transformations necessary to compile the genotypic data in a form suitable for LINKAGE; and 5) LOD score data extraction from the LINKAGE output files. These programs are available for downloading from the CGS website (www.centerforgenomicsciences.org). All haplotyping and phasing were performed by visual inspection.

Two-point linkage analyses were carried out on all autosomal markers using MLINK (25, 26). DC was modeled as an autosomal dominant disorder with two liability classes for penetrance. Liability class 1 was assigned to persons up to the age of 45 and was set at 0.0 penetrance; liability class 2 was assigned to persons above 45 and was set at 0.9 penetrance. The phenocopy rate was set at 0.01 due to the high prevalence rate in the target population (approximately, 6% among Scandinavians >55 years of age) (27), and the fact that approximately 30% of cases are diagnosed as sporadic, although the actual rate of sporadic cases is thought to considerably less based on inadequate familial ascertainment. Because the true allele frequencies were unknown, equal frequencies for all alleles were used in the analysis.

Results

A five-generation Swedish family was ascertained in which there were multiple persons of both sexes affected with DC over four generations. A diagnosis of DC was made for all members of the family evidencing either palmar thickening or contracture of the distal digits. A family tree was constructed (Fig. 1) which indicated that DC was inherited in an autosomal dominant manner with high, but incomplete, penetrance by 50 years of age. Both male-to-male and male-to-female transmissions were observed ruling out both X-linked and mitochondrial inheritances in this family. There was one instance (pedigree numbers IV-1 and IV-2) in which two affected persons, both with a family history of DC, married and produced affected offspring (V-1–V-2); their younger children (V-3–V-5) are below the age of penetrance.

A genome-wide microsatellite-based approach was used to identify a single region of approximately 30 cM on chromosome 16, bounded by markers D16S3131 and D16S514, that produced LOD score of >1.5, no other region produced a LOD score of >1. We then ascertained another branch of the family made up of four siblings; the proband’s second cousins (pedigree numbers IV-17–IV-20) of whom two were affected. Genotyping of these persons together with the use of additional microsatellite markers supported linkage to the region and produced a maximal LOD score of 3.18 for D16S415 with four other markers producing LODs of >1.5 (Table 1).

The telomeric limit for the DC gene is most likely defined by a recombination in pedigree number V-1 that occurred between markers D16S3053 and D16S3032. The haplotyping and phasing for this branch of the family can be constructed with a high degree of confidence as genotyping data are available from both parents and five children. As both of V-1’s parents are affected, it is possible that he could be inheriting the disease gene from the paternal (marry in) chromosome. However, this scenario is highly unlikely as his affected brother, V-2, did not receive the risk haplotype from the mother; therefore, it can be assumed that he inherited his father’s disease haplotype. Because V-1 and V-2 received different paternal chromosomes, we can exclude the hypothesis that V-1 received a risk chromosome from the father, and thus, the recombination observed between his grandmaternal chromosomes most probably defines the telomeric limit of the DC locus. Another clue to the telomeric limit is the observation (assuming maximum parsimony in constructing haplotypes when intervening relatives are not available) that there was an apparent recombination in one of the ancestors of the proband’s second cousins (pedigree numbers IV-17–IV-20), three of whom appear to share a 4-marker haplotype with the main branch of the family. If this is a true [identical-by-descent (IBD)] shared haplotype, then this would fix the telomeric limit at D16S3032. Defining the centromeric limit is more problematic; however, it is most plausible that it is located between D16S419 and D16S415. Affected pedigree member IV-16 appears to have a recombination at this point, but the lack of phasing data makes it possible that the
Fig. 1. Pedigree of a Swedish family with Dupuytren’s contracture inherited as an autosomal dominant disorder with incomplete penetrance. Squares = male; circles = female; filled icons = affected status; open icons = unaffected status; a diagonal line through an icon = deceased; a line between male and female indicates marriage; arrowhead = proband. The black bars beside the allele numbers denote the risk haplotype (or the inferred risk haplotype for those family members for whom we do not have phasing data). In the branch of the family that contains IV-17, IV-18, IV-19, and IV-20, missing ancestral genotypes make it impossible to establish identity-by-descent (IBD) for any haplotype in relation to the other branches of the pedigree. In this branch, black bars indicate an inferred risk haplotype that is at least identical-by-state (IBS) to the risk haplotype in the proband’s branch. Other haplotypic hatchings in the different branches of the pedigree were chosen for graphical convenience and are not meant to suggest either IBD or IBS between different branches. In IV-1, V-2, and V-5, the inferred paternal risk haplotype is denoted by bars with horizontal lines (see text). Unaffected individual V-5 is a member of the non-penetrant liability class 1 (see text).
constructed risk haplotype is not necessarily IBD in relation to the proband’s branch of the pedigree. However, the fact that it is possible to construct for her affected sister a 10-marker haplotype that is identical to that of the proband makes it quite likely that this is the centromeric limit.

Discussion

A five-generation family with DC was ascertained in which the disease gene was passed in an autosomal dominant mode, and all possible transmissions were observed with the exception of female to female. This finding is in contrast to the report of Bayat et al. (3) who claim to have identified a heteroplasmic mitochondrial mutation associated with DC. These investigators also claim to have identified associations between polymorphisms in the human TGFbetaR and Zf9 genes with DC. These findings raise the possibility that DC may be produced by multiple genetic lesions, however, it is clear that none of these other proposed DC mutations are present in the members of the family described herein.

The pathophysiology of DC may result from a defect in the wound repair process (15) or from an exuberant response to wounding (28). These hypotheses are based upon biochemical characterizations of affected tissues that show increased deposition of collagen III relative to collagen I and increased levels of collagen hydroxylation and glycosylation (9). A search for genes associated with wound healing, tissue remodeling, and inflammation near the DC locus reveals a number of candidates including several matrix metalloproteinases and NOD/CARD genes. The former are associated with tissue remodeling throughout life and are involved in the normal response to trauma, while the latter are associated with inflammatory responses, particularly those associated with infection. Ongoing studies in this laboratory are directed toward the identification of mutations in several genes in the region of the DC locus that are inherited with the phenotype.

Acknowledgements

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References


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cM, centiMorgan; LOD, logarithm of odds; map distance, the distance in cM from the p-arm telomere according to the Marshfield map; θ, the recombination fraction. LOD scores of 1.5 or greater are shown in boldface.

Table 1. Two-point LOD scores for the Dupuytren’s contracture locus and microsatellite markers on chromosome 16 for a Swedish family.