OULU 2016
D 1353

ACTA UNIVERSITATIS OULUENSIS

Minna Forsman

HISTOLOGICAL CHARACTERISTICS AND GENE EXPRESSION PROFILING OF DUPUYTREN'S DISEASE

UNIVERSITY OF OULU GRADUATE SCHOOL;
UNIVERSITY OF OULU, FACULTY OF MEDICINE;
BIOCENTER OULU;
OULU UNIVERSITY HOSPITAL;
UNIVERSITY OF HELSINKI, HAARTMAN INSTITUTE

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HISTOLOGICAL CHARACTERISTICS AND GENE EXPRESSION PROFILING OF DUPUYTREN'S DISEASE

Academic dissertation to be presented with the assent of the Doctoral Training Committee of Health and Biosciences of the University of Oulu for public defence in Auditorium F101 of the Faculty of Biochemistry and Molecular Medicine (Aapistie 7), on 13 May 2016, at 12 noon

UNIVERSITY OF OULU, OULU 2016

Acta Univ. Oul. D 1353, 2016

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ISBN 978-952-62-1166-4 (Paperback)
ISBN 978-952-62-1167-1 (PDF)

ISSN 0355-3221 (Printed)
ISSN 1796-2234 (Online)

Cover Design
Raimo Ahonen

JUVENES PRINT
TAMPERE 2016

Forsman, Minna, Histological characteristics and gene expression profiling of Dupuytren's disease.

University of Oulu Graduate School; University of Oulu, Faculty of Medicine; Biocenter Oulu; Oulu University Hospital; University of Helsinki, Haartman Institute

Acta Univ. Oul. D 1353, 2016
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Abstract

Dupuytren's disease is a Caucasian male-dominant disease that affects the palmar fascia. Incidence grows with age, but persons with strong diathesis seem to develop the disease at an earlier age than the majority of the diseased. Myofibroblasts are histopathologically the main cell type in DD tissue. Despite scientific research, the aetiology of the disease is still unrevealed. Only genetic susceptibility is generally accepted as predisposing to DD. Available treatment has thus far been unsatisfactory, because only symptoms can be cured to date.The disease recurs. With genetic susceptibility the recurrence rates are high (even up to 70%) and the time to recurrence is inevitably shorter. This behaviour is considered the aggressive type of DD.

To be able to predict the behaviour of DD, whether it is an aggressive or conventional type, twenty-one Dupuytren samples were gathered and compared with five controls by means of immunohistochemical stainings. It was found that cellularity was better expresented in aggressive and recurred samples. Alfa-SMA and Ki-67 showed more activity in the aggressive tissue type of DD. Tenascin was vaguely expressed in aggressive-type samples.

To compare the gene and protein expressions and to obtain a more profound understanding of the disease, a microarray technique was used. With a microarray it is possible to compare nucleotide pair hybridisations to resolve the genome of the tissues. In this study RT-PCR was used to examine mRNA levels to determine gene expression changes. Twelve DD palmar fascia samples were compared with three healthy control samples. Both myoglobin and ROR2, which we considered as the most valuable results, were found in the DD samples. ROR2 acts as a receptor or co-receptor for the Wnt system. The Wnt signalling pathway transfers signals from outside of the cell through cell surface receptors, and plays a significant role in proliferation processes such as in fibrotic conditions.

To evaluate a possible chromosomal imbalance behind the aetiology of the disease, eighteen DD palmar fascia samples were compared with two reference samples. However, we were not able to detect any chromosomal imbalance in the DD samples. The method used was Oligonucleotide aCGH Agilent's 60-mer oligonucleotide-based microarray according to the manufacturer's instructions, which can reveal gains and losses of approximately 35 kilobases in the whole genome. The result does not exclude copy number changes entirely; a small presence of aberrant cells will not be detected if the change is less than 50%.

In conclusion, we revealed elements in DD tissue that would enable us to predict the nature of the disease; whether the disease is aggressive with a stronger tendency to recur. Histological differences could be detected, and this can be used to benefit patients. As a new element, ROR2 was discovered in DD tissue.The genome-wide analysis with the 44K oligonucleotide-based array method revealed no changes of DNA number sequences.

Keywords: Dupuytren's disease, gene copy number variations in DD, gene expression of DD, histopathology of DD

Forsman, Minna, Kämmenkalvon kutistumataudin solu-j a geenitason löydökset.
Oulun yliopiston tutkijakoulu; Oulun yliopisto, Lääketieteellinen tiedekunta; Biocenter Oulu; Oulun yliopistollinen sairaala; Helsingin yliopisto, Haartman-instituutti
Acta Univ. Oul. D 1353, 2016
Oulun yliopisto, PL 8000, 90014 Oulun yliopisto

Tiivistelmä

Kämmenkalvon kuroumatauti eli Dupuytrenin kontraktuura on valkoihoisen miehen kämmenkalvon sairaus. Sairastumisen todennäköisyys lisääntyy ikääntymiseen liittyen, mutta vahva sukurasitus poikkeuksellisesti altistaa sairaudelle jo tavanomaista nuoremmalla iällä. Myofibroblastit ovat tärkein ja edustetuin solutyyppi Dupuytren kudoksessa. Huolimatta runsaasta tutkimustyöstä ei etiologiaa ole saatu vielä selvitettyä. Sukurasitus näyttää selkeästi altistavan taudille. Toistaiseksi kyetään hoitamaan ainoastaan sairauden aiheuttamat seuraukset, mutta ei perussyytä. Lisäksi tauti uusiutuu. Dupuytrenin sukurasitus lisää uusiutumista suurella todennäköisyydellä. Myös uusiutumisaika on tuolloin tavanomaista nopeampi, ja kyseessä katsotaan olevan ns.aggressiivisempi taudin muoto.

Väitöskirjatyössäni pyrittiin löytämään mahdollisia tekijöitä, joiden perusteella voitaisiin ennustaan onko kyseessä aggressiivisempi vai tavanomainen taudin muoto. Tätä varten tutkittiin kaksikymmentä yksi Dupuytren kudosnäytettä ja viisi tervettä kämmenkalvon näytettä immunohistologisilla värjäyksillä, ja voitiin todeta, että soluisuus oli selkeästi koholla aggressiivisten ja taudin uusineiden potilaiden näytteissä. Tulos oli samanlainen myös alfa-SMA ja Ki-67 suhteen. Tenaskiiniä voitiin löytää edellisiä niukemmin aggressiivisista näytteistä.

Dupuytrenin taudin luonteen lisäselvittelemiseksi geeni- ja proteiinitasolla tehtiin mikroarray, jossa emäsparien pariutumisen avulla selvitetään taudin genomia ja myös sitten tästä aiheutuvien proteiinien ilmentymistä. Kahtatoista Dupuytren potilaan kämmenkalvon kudosnäyttettä verrattiin kolmeen terveeseen verrokki kudosnäytteeseen ja voitiin todeta myoglobiinin ja ROR2:n selkeät pitoisuuden muutokset terveisiin näytteisiin verrattaessa. ROR2 toimii solujen välisten viestien välityksen reseptorina, eli siirtää signaalin solun ulkopuolelta sen sisäpuolelle solun pinnalla olevan kiinnittymiskohdan avulla. Sillä on selkeä merkitys ja tehtävä proliferatiivisissä tapahtumissa, kuten sidekudoksen lisääntymisessä.

Mahdollisia kromosomin määrän muutoksia Dupuytren kudoksessa selviteltiin kahdeksantoista kudosnäytteen tutkimisella ja löydösten tulosta verrattiin sitten kahteen normaaliin verrokki kudosnäytteen tulokseen. Tutkimuksessa ei saatu selville kromosomien määrän muutosta, kun muutosten kokonaismäärä on vähäinen tai ainakin alle 50 % kokonaismäärää alhaisempi.

Yhteenvetona voidaan todeta, että löytyi histologisia kudoselementtejä, joiden perusteella voidaan ennustaa, onko Dupuyrenin tauti aggressiivisempi ja todennäköisemmin uusiutuva luonteeltaan. ROR2 ei ole aikaisemmin yhdistetty Dupuytrenin kontraktuuraan. Dupuytren kudoksesta ei voitu 44K oligonukleotide mikroarray tekniikalla paljastaa geenimäärien muutoksia.

Asiasanat: Dupuytrenin taudin histopatologia, Dupuytrenin tauti, Dupytrenin taudin geeni ekspressio, geenimäärän muutokset Dupuytrenin taudissa

Acknowledgements

This study was carried out at the Department of Surgery, Oulu University Hospital, Department of Pathology Oulu University Hospital, Department of Dentistry, Oulu University Hospital and Department of Pathology Haartman Institute and HUSLAB University of Helsinki with contribution from the Microarray Core Facility Biocenter Oulu Finland.

I am grateful to my supervisor Docent Jorma Ryhänen who has been giving me the assistance, guidance and support to accomplish my thesis during these years.

I also expess my warm thanks to Ph.D. Matti Kallioinen, Prof. Sakari Knuutila, Prof. Tuula Salo and Ph.D. Jussi Vuoristo for the invaluable and expertise co-operation this study was enabled.

I owe my thanks also to other article co-authors and for their contributions: Ph.D. Virve Pääkkönen, M.D. Leena Kallioinen, post-doctoral researcher Sippy Kaur and Ph.D. Marcello L. Larramendy.

I thank the personnel of the laboratories in the Department of Dentistry and Pathology of Oulu University Hospital and in the Department of Pathology of Haartman Instutute for their technical assistance.

I also wish to thank Docent Jyrki Parkkinen and Ph.D. Hanna-Stiina Taskinen for reviewing the present manuscript and for their useful and expert criticism and comments for the completion of the work.

Finally, I express my warm gratitude to my parents for the everlasting support, love and patience during these years.

I also warmly thank my friends for their friendship and optimism.

Abbreviations

ACP5	acid phosphatase 5
ADAMTS	a disintegrin and metalloproteinase with thrombospondin motifs
Alfa –SMA	alfa-smooth muscle actin
AKR1C1	aldo-keto reductase family1 member 1
ALDH2	aldehydedehydrogenase 2 family
APP	amyloid precursor protein
ARCN1	archain 1
ATP7B	Cu++ transporting beta polypeptide
b-FGF	basic fibroblast growth factor
CCL5	chemokine (c-c motif) ligand 5
CD14	cluster of differentiation 14, a human gene
CGH	comparative genomic hybridisation
CLEC3B	C-type lectin domain family 3 member B
COL5A2	type 5 collagen alpha-2
CSF1	colony stimulating factor 1
CTN1	cardiac troponin 1
CTNNA1	cattiness cadherin-associated protein alpha 1
CYB5A	cytochrome B5 type A
DAD1	dolichyl-diphosphooligosaccharide-protein glycosyltransferase subunit DAD1
DCM	DNA cytosine-C5 methyltransferase
DD	Dupuytren disease
DNA	deoksiribonucleic acid
c-RNA	complimentary ribonucleic acid
ECHS1	enoyl CoA hydratase shory chain 1
EGF	epidermal growth factor
EPB49	erythrocyte membrane protein band 4.9
FZD	frizzled family receptor
GDF11	growth differentiation factor 11
GDP1	glyceraldehyde-3-phosphate dehydrogenase
GM-CSF	granulocyte macrophage-colony stimulating factor
GNAS	guanine nucleoatide binding protein alpha stimulating
HIV	human immunodeficiency virus
HSPB1	heat shock protein beta 1
IgA	immunoglobulin A

IGF	insulin-like growth factor
IgG	immunoglobulin G
IgM	immunoglobulin M
IL	Interleukin
LAMA3	Laminin alpha3 protein
LEF	lymphoid enhancing factor
LIPE	hormone-sensitive lipase gene
LPA	lysophosphatic acid
LRRC17	Leucine rich repeat containing 17 gene
LSP1	Lymphocyte-specific Ca2+ binding protein
Ki-67	nuclear protein and cellular proliferation marker
MAFB	musculoaponeurotic fibrosarcoma oncogene homolog B
MCP joint	metacarpophalangeal
microRNA	a small non-coding RNA molecule (containing about 22 nucleotides)
MMP	matrix metalloproteinase
NRH1	neurotrophin receptor homolog protein
P4HA2	prolyl 4-hydroxylase alpha polypeptide II
PDGF	platelet-derived growth factor
PIP joint	proximal interdigital phalangeal PNFpercutaneous needle fasciectomy
PRKX	protein kinase X-linked
PTK7	protein-tyrosine kinase 4
PTN	Pleiotrophin protein, a coding gene
SHANK2	SH3 and multiple Ankyrin repeat domains protein 2 is encod by SHANK2 gene
RHOA	Ras homolog gene family memeber A
ROR2	tyrosine kinase-like orphan receptor 2
RT-PCR	reverse transcriptase polymerase chain reaction
RUNX2	runt-related transcription factor 2
Ryk	related to receptor tyrosine kinase, a gene that encodes the protein Ryk
SYMPK	a gene that codes symplekin protein
TCF	T-cell factor
TGF	transforming growth factor
TFP12	methylated tissue factor Pathway inhibitor 2
TMSB10	thymosin beta10

TNF	tumor necrosis factor
TNFSF12	tumor necrosis Factor ligand super family member 12
TIMP	MMP inhibitors
TMSB4X	thymosin Beta-4 X- chromosome
TUBA1A	tubulin alpha 1A
WNT5A	Wingless type MMTV interation site family member 5A
ZF9	zinc-finger 9 protein

List of original articles

This thesis is based on the following articles, which are referred to in the text by their Roman numerals (I–III).

I Forsman M, Kallioinen L, Kallioinen M & Ryhänen J (2005). Dupuytren's contracture; increased cellularity – proliferation, is there equality? Scand J Surg 94(1): 71–75.

II Forsman M, Pääkkönen V, Tjäderhane L, Vuoristo J, Kallioinen L, Salo T, Kallioinen M & Ryhänen J (2008). The expression of myoglobin and ROR2 protein in Dupuytren's disease. J Surg Res 15: 146(2): 271–275.

III Kaur S, Forsman M, Ryhänen J, Knuutila S & Larramendy ML (2008). No gene copy number changes in Dupuytren's contracture by array comparative genomic hybridisation. Cancer Genet Cytogenet 183(1): 6–8.

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1 Introduction

Dupuytren's contracture is a benign fibroproliferative disease that affects the palmar fascia and deforms the fingers while maturing. The disease was presented by and named after surgeon Baron Guillaume Dupuytren in 1831; he proposed that it was of fibrotic origin (Murrell 1992). Dupuytren's disease affects mostly elderly white Caucasian men, typically at the age of 60 years or more. Men are seven times more likely to have DD than women. The ratio equalises with age. Nonetheless, it also affects other human races, and even newborns (Burge 1999, Foucher 2001, Chinyama *et al*. 2000, Wilbrand *et al*. 1999, Anwar 2007, Pan *et al*. 2003). DD can appear bilaterally in approximately 55% of cases, and ectopic Dupuytren-like lesions are found in the plantar fascia (Mb Ledderhose), in the male genitals (Mb Peyron) and on the dorsum of the digits (Garrod's nodes) (Connelly 1999, Donato & Morrison 1996, Kulkarni & Elliot 2005, Brenner *et al.* 2001, Birks *et al.* 2013.) The disease has been well studied, but despite the vast amount of thorough scientific work, it still remains an enigma (Shih *et al.* 2010, Johnston *et al.* 2007, Ojwang *et al.* 2010). It seems that DD is strongly hereditary, yet environmental factors, some diseases and traumas seem to have an impact, probably by triggering the disease and enhancing it to an apparent disease (Chansky *et al.* 1999, Hindocha *et al.* 2006, Rayan 2005, Mc Farlane 1991). It has been postulated that DD is inherited by means of a dominant autosomal pattern (Gudmundsson *et al.* 2000). Smoking and alcohol consumption are considered to be among the main risk factors that increase the morbidity of DD besides the more significance factors like male gender, age and diathesis (Burke *et al.* 1997, Gudmundsson *et al.* 2001). Diabetes, especially medicinally treated, is strongly associated with the disease. Epilepsy or antiepileptic medication, on the contrary, are no longer undisputably considered to be related to DD (Geoghegan 2004). In some manual labour occupations that expose the hands to vibration and mini traumas, the incidence of a somewhat milder type of DD also seems to be abundant. DD is also clearly represented in HIV infection despite the patients' notably younger age (McFarlane 1991, Descatha *et al.* 2011, Bower *et al.* 1990).

Although DD is a benign fibroproliferative disease, it also has some aggressive features. It infiltrates the skin and therefore does not respect tissue margins. It also tends to recur after resection in 2% up to 42% of cases, depending on the operating technique and straining risk factors (Picardo & Khan 2012, Anwar *et al*. 2007). Interestingly, it is related to sarcoma and other cancers. Epitheloid sarcoma in the palm region resembles Dupuytren's disease both clinically and histopathologically.

In epidemiological studies it also seems that cancer mortality increases simultaneusly with the prevalence of DD (Wilbrand *et al.* 2000, 2002, Gudmundson *et al.* 2002, Erdmann *et al.* 1995). Ki-67 is typically high in all cell proliferating conditions, such as in carcinomas, where mitose activity and proliferation are emphasised in the same way as in fibrotic conditions like DD (Vujic *et al.* 2014). The relation to the wound healing process is beyond controversy. Because of the many similar characteristics, it has been postulated that it is an exaggerated response to wound healing, yet without a real existing wound (Howard *et al.* 2004). The key elements in DD are considered to be myofibroblasts—cells that share characteristics with both smooth muscle cells and fibroblasts. They have the ability to contract, which causes the fingers to develop the typical fibrotic nodules and cords on the palmodigital fascia, leading to fixed flexion contractures of the joints (Picardo *&* Khan 2012). Histopathologically, three stages can be recognised in the development of the disease: proliferation, involution and the final residual stage. Certain features and events can be pointed out in every stage. It is known that for some reason growth factors begin to stimulate fibroblasts to proliferate and transform into myofibroblasts (Badalamente *et al.* 1996). It has been suggested that local hypoxia or free radicals or both are the triggers that start the complicated chain of reactions leading to DD (Hart *&* Hooper 2005, Hueston *&* Murrell 1990). Because of the immunal response, DD is related to infectious conditions, too. Especially IgA and IgM immunoglobulin antibodies are found in DD patients' tissue and blood in high amounts, compared with normal reference tissue (Józsa *et al.* 1988, Houghton *et al.* 1983).

Clinically, the disease matures through different stages that can be distinguished histologically, although overlapping occurs. At the beginning, a separate growing nodule arises from the palmar fascia in the ray of a finger on the palm. Typically, at the end the nodule develops a cord that will gradually restrict extension of the finger or fingers. This progression is common but not inevitable, and is estimated to take less than 10 years from a nodule to maturation into a restricting cord (Reilly *et al.* 2005).

Some young males with diathesis develop DD earlier than the majority, and the disease recurs aggressively despite accurate excision of the affected palmar fascia. Diathesis is defined as a family history of the disease or a genetic susceptibility to DD. It seems that if both parents are affected, the probability of early appearance of DD is even stronger (Becker *et al*. 2014). In these cases the patients often have ectopic lesions, DD is bilateral and the disease may typically also affect the radial

fingers instead of typically only the ulnar fingers (Wilbrand *et al.* 2002, Gudmundsson *et al.* 2000).

The main procedure for treating contractures of the fingers is an operation to excise or cut, or to dissolve, the diseased fascia. Conservative treatment options are far too often inconvenient to implement *in vivo*, they are complicated, have side effects and, most importantly, are insufficient. They include splinting, different injection treatments (e.g., cortisone, steroids), radiation, physiotherapy, external devices, anti-inflammatory drugs and interferon. More effective operative options are limited fasciectomy, dermatofasciectomy with skin grafting and percutaneus needle fasciotomy. Collagenase injection is an alternative method to surgery (Stanbury & Hammert 2011, Misra *et al.* 2007, Dias & Braybrooke 2006, Badalamente & Hurst 2007). No actual cure for the disease has yet been discovered; to date only its symptoms can be cured.

The histology of DD has largely been examined by means of basic cellular stainings, and nowadays also the genome of DD has been a target of the microarray technique. Both of these instruments have made it possible to understand the disease and its different elemental connections more deeply. Nucleotide hybridisation (DNA and mRNA) studies have elucidated that there is a large amount of changed gene and protein expressions in DD tissue compared with normal control tissues (Shih *et al.* 2012, Lee *et al.* 2006, Quian *et al.* 2004). Myofibroblasts undoubtedly are the core structure of the disease with a connection to chromosomal alterations detected. More accurate investigations have also been done with microRNA studies (post-transcriptional regulation) at the mitochondrial level, and these results connect Wnt/B-catenin pathways to DD pathology (Michou *et al*. 2012).

The purpose of this thesis project was to evaluate the histopathological differences between the tissues of more aggressive cases with high recurrence risk and less aggressive conventional DD cases. We wanted to more accurately study the histology of the disease using the microarray technique in addition to sample stainings to discover and detect the differences between DD and healthy references.

2 Review of the literature

2.1 Pathology of Dupuytren's contracture

A myofibroblast is considered to be the main element in Dupuytren's disease. In DD, fibroblasts are triggered to proliferate and transform into myofibroblasts, which continue to proliferate still more. A fibroblast, which is found in connective tissue, constitutes an elongated cell and a slender, fusiform and smoothly contoured nucleus. It has a scattered mitochondria and well-developed secretory organelles, a large Golgi apparatus and a rough endoplasmic retinaculum.The structure has no actual basal lamina, fibronectin fibrils or fibronexus. A myofibroblast resembles both a fibroblast and a myoblast. A myoblast is a smooth muscle cell, and therefore it has the ability to contract. In stainings myofibroblasts contain alfa-smooth muscle actin (α-SMA), which particularly owns this contracting quality (Hinz 2015**,** Rayan *&* Tomasek 1994, Tomasek *et al.* 1999).

Other opinions have been published; J.J. Tomasek detected that nodular DD cells stained for nonmuscle myosin and fibronectin, but not for smooth muscle myosin or laminin, indicating that myofibroblasts in DD consist of non-muscle types of cells. Nevertheless, these cytoskeletal proteins interact with actin and thereby are able to form contraction too. The DD type of myosin also has the ability to contract, even though it is not considered a real muscle type of myosin (Tomasek *et al.* 1986). The fibronexus is a specialised attachment site situated in a myofibroblast, and is formed by extracellular fibrils and fibronectin at the plasmalemma. It has been proposed that it enables contraction by transmitting the force of actin to the surrounding extracellular matrix and tissues **(**Tomasek *et al.* 1999). Fibrogenic cytokines and transforming growth factors (TGF-β1 and -β3) are stimulating factors that enhance the formation of myofibroblasts (Bisson *et al.* 2003, Wilutzky *et al.* 1998).

A variety of cells are found in and connected to DD tissue. They can be considered elements of the disease, yet simultaneously they contribute to the development of DD by being stimulated, inhibited or both. Many cytokines and proteins are involved in DD (TGF-alfa, PDGF, EGF, ZF9, GM-CSF, free radicals, metalloproteinases, collagen and fibronectine). It is a complex system with delicate but well controlled interactions between these elements. The immune system is also associated with palmar fibromatosis (Cordova *et al.* 2005). Large amounts of IgG, IgA and IgM can be indicated from DD tissue (Menzel *et al.* 1979). The type and

amount of collagen in DD changes in the same manner as it changes in the healing process of a wound. Collagen type III is abundantly represented, while it is virtually absent from the normal palmar fascia. With the presence of a raised collagen III level, hexosamine content also rises in DD tissue (Brickley-Parsons *et al.* 1981). Metalloproteinases (MMP) and their inhibitors (TIMP) are involved in different fibrotic conditions and therefore also in the wound healing process and similar processes. In DD, the balance between these two elements is disturbed. The upgrading of TIMPs and some MMP groups seems to provoke fibrosis (Dietmar *et al.* 2009, Bains 2003).

2.2 Pathogenesis of Dupuytren's disease

Genetic diathesis indisputably plays a key role in DD. It has been postulated that it is maternally inherited. Hereditary predisposition causes a person to develop the disease assumably under certain circumstances. The cellular environment, both extra- and intracellular, and its chemical controlling factors, are disturbed and programmed to behave out of normal cell control. The same reactions as in the normal wound healing process will happen, with the exception that normal scars are not formed, but rather tumour-like scar accumulation with a more invasive quality (Hueston *&* Murrell 1990). The same particular and uncontrolled features are to be seen in the development of cancers, especially in children and epitheloid sarcoma, which resembles DD in the proliferative stage (Erdmann *et al.* 1995, Rhomberg *et al.* 2002, Fitzgerald *et al.* 1999).

Ischaemia in tissue caused by microvascular narrowing of vessels for different reasons (smoking, ageing, diabetes) is accepted as one theory for DD. (AL-Quattan 2006, Hart *&* Hooper 2005*).* In hypoxic tissue, free radicals emerge through a self-perpetuating cycle when adenosine triphosphate breaks down and increases conversion of hypoxanthine into xanthine and xanthine into uric acid. Xanthine dehydrogenase then converts into xanthine oxidase and furthermore into free radicals like superoxide and hydrogen peroxide. The free radicals are toxic and continue to damage the tissue further. In proportional concentrations it stimulates fibroblast proliferation. This eventually leads to abnormally proliferated fibrotic tissue with changes in collagen composition (Yi *et al.* 1999). Xanthine dehydrogenase is normally located in the cytoplasm of endothelial cells of small capillaries and is catalysed to convert into xanthine oxidase. Eventually free radicals and their breakdown products are caused by the hypoxia. High alcohol consumption is assumed to enable free radical production in the same way by

changing xanthine dehydrogenase into free radicals, producing xanthine oxidase. (Hueston & Murrell 1990). Free radicals induce cytokines like IL1, which stimulates the Langerhans cells and the immune system and lymphosytes (CD-45 positive T cells and other inflammatory cells). This arouses fibroblast proliferation and growth factors to promote differentiation of myofibroblasts, which further induce the amount of collagen type III, and fibronectin and metalloproteinase inhibitors (TIMP) (AL-Quattan 2006, Benson *et al.* 1998).

All these processes linked to each other finally produce DD and contraction of the actin-myocin complex situated in myofibroblasts—the phenomenon that ultimately causes contracture and a lack of extension in the finger (Yi *et al.* 1999, Nunn & Schreuder 2014).

Lipid metabolism is also linked to the development of DD. Alcohol and phenobarbitones increase cholesterol metabolism and produce high levels of triglycerine and LPA. The LPA binds to myofibroblast receptors which cause the cell to respond to TGF-β, furthermore decreasing the amount of cyclic adenosine monophosphate (cAMP) and increasing intracellular calcium levels. Calcium binds with calmodulin and together they activate the myosin light chain kinase, enabling contraction of the actin-myocin complex (Al-Qattan 2006). In conclusion, the genes involved in cytoskeleton development and lipid metabolism seem to be affected abnormally (Rehman *et al.* 2008).

Trauma and injuries of the hand also trigger the repair processes by causing microruptures of the tissues. In the same manner heavy labour is assumed to activate IL1 with a series of events and thereby predispose to DD (Picardo & Khan 2012, Lanzetta & Morrison 1996*)*.

The ischaemia theory has steady support, though there is a fair possibility that the immune system also has a significant role in the development of DD. IgG and IgM antibodies to human collagen types can be detected from DD tissue. This is interesting and puts DD in the same group of anti-immune disorders as rheumatoid arthritis, where a similar phenomenon of collagen antibody formation can be detected. This likelihood places DD in the range of connective tissue disorders in which collagen auto-immunity is found (Pereira *et al.* 1986).

The aetiology triggering these pathologic events that indicate DD are still under study. It has been assumed that DD is caused by hypoxia or a change in the immune system, or because of continuous physical stress or both, assuming that there are some genetic predisposing factors present beneath. However, it has been postulated that heredity is not always evident or even necessary for DD. Continuous physical exposure in the manner of hard manual work or any other stressful situation to the

hand (fracture, operation, vibration) may potentially contribute to DD. Purely environmental and external aetiology is under debate (Descatha *et al.* 2011, Becker *et al.* 2014). Whether these are the causes or mainly the triggering elements of DD is uncertain.

Cultured fibroblasts from DD tissue, previously quiescent, can be reactivated to transform into myofibroblasts by TGF-β stimulation, and this may explain the reason for the high recurrence rates after surgery or injury (Bisson *et al.* 2003). The debate over the expression of the disease—whether it is genetic or a result of an occupational practice—has evoked the suggestion that it should be called either a disease or a condition of palmar fibromatosis, a situation brought about by mechanical microtrauma. A total revelation of the aetiology would eventually explain DD and the accuracy of these contradictory allegations.

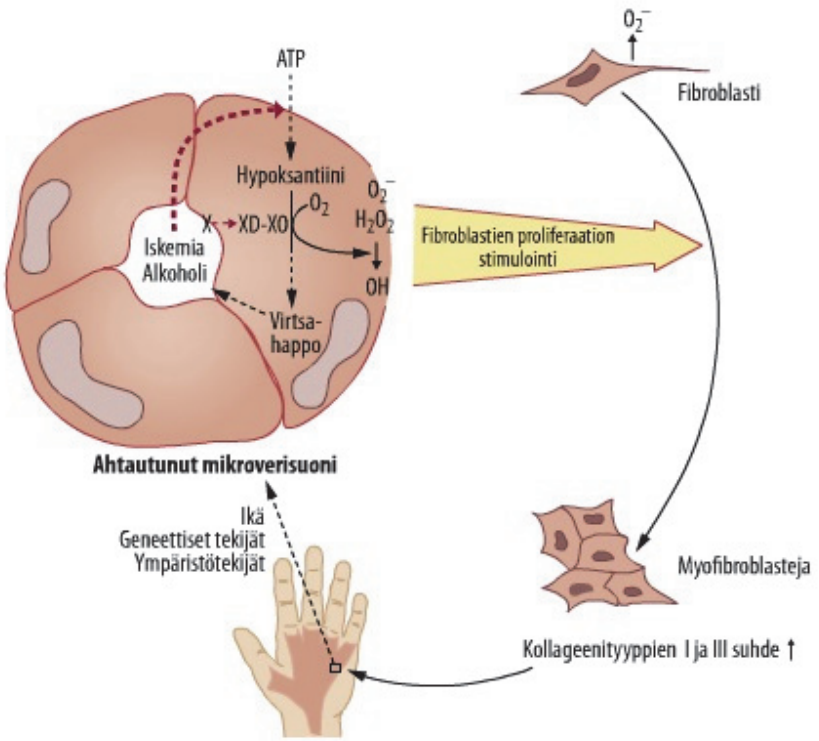

Fig. 1. A theory of the aetiology of DD caused by ischaemia. Different environmental and genetic elements narrow the micro blood circulation and local ischaemia breaks adenosinetriphosphatase (ATP) into hypoxanthine and further into xanthine (X) and uric acid. The process releases oxygen radicals (O and OH) which induce proliferation of fibroblasts and change them into myofibroblasts. The amount of collagen type I diminishes compared with collagen III, which further narrows the blood vessels with this feedback mechanism. (reprinted from Duodecim 2012;128:4 by permission)

Three different partially overlapping phenomena form three different and separate stages of DD and can be pointed out histopathologically and roughly also clinically. In the proliferative phase the amount of myofibroblasts increases. In the next phase, which is involutional, these cells align along the longitudinal lines of tension. In the residual phase there are less cells and bundles of collagen III, which gives the tissue a scar-like appearance (Hart & Hooper 2005).

2.2.1 Proliferative phase

Dupuytren's disease can be sectioned into different stages that describe the maturity of the disease. The first stage is the proliferating stage, or the phase where histopathologically the tissue changes from a normal palmar fascia into altered and pathological fibrotic tissue. The triggering aetiology is considered to be multifactorial, requiring certain factors such as genetic susceptibility, and a widely accepted theory of free radicals produced by local ischaemia caused by different reasons. The free radicals emerge in hypoxid conditions as an end product of the xanthine oxidase pathway. Because of these elements the fibroblasts begin to proliferate and turn into myofibroblasts to further accelerate the process of fibrosis. The source of the myofibroblasts remains unknown. One presumable suggestion is that they originate from mesenchymal fibroblasts. Therefore, excision of palmar fat and skin in a fasciectomy indisputably reduces the recurrence rate of the disease. For this reason when a skin graft is applied and used after excision of the DD aponeurosis, recurrence is significantly diminished (Hueston 1990, Murrell 1987, Hueston 1985*)*. Growth factors like TGF-B and cytokines play the main role in this proliferating procedure and in the contraction of the DD tissue (Tse *et al.* 2004). Periostin probably also has a role in the formation of DD (Picardo *&* Khan 2012, Vi L *et al.* 2009).

Every phase has a major cell type. In the first, proliferative phase, fibroblasts and myofibroblasts as well as increased extracellular matrix (ECM) depositions are well represented. Clinically this phase may be identified by tumour-like nodules forming tense clusters. However, they do not necessarily correlate completely with the histological nodules, which can be more scattered. Eventually the histological nodules expand to the surface of the skin and replace subcutaneous adipose and attach to the deep layers of the skin. This phase is rich in cells, depositing into the vascularized nodules with intense metabolic activity (Benson *et al.* 1998, Rehman *et al.* 2011). The mechanism that enables progression from a nodule to a fibrotic cord is not yet fully understood. Nevertheless, two theories have been proposed. In one theory proliferation and outward migration of the diseased cells from the nodules to the adjacent fascia mediate population of the disease. The end result is a contractible cord. The other theory favours the role of the secreted factors from the nodules, which alter and trigger the quiescent phenotypically normal cells in the fascia to change into the contractile phenotype (Picardo *&* Khan 2012*)* The latter theory, with the idea of extracellular matrix interactions, is somewhat more favoured than the first one (Vi *et al.*2009).

Table 1. The phases of DD with their main elements; richly represented in the first phase, gradually diminishing in the development of the process to the end phase.

Proliferative phase	Involutional phase	Residual Phase
fibroblasts > myofibroblasts	myofibroblasts > apoptosis	acellularity fibrosis few fibroblasts
macrofages >	variable amounts of cytokines;	no TGF-β2
TGF-β1,TGF-β2,TGF-α	TGF-β1, TGF-β2	TGF-β1
IL-1B,IL-6,TNF-α	EGF	EGF
PDGF, EGF		
Factor XIIIa	variable amounts of	
Langerhans cells	inflammatory cells	
MMP1, MMP-2, MMP9, MMP13, MMP14	variable amounts of	-
ADAMTS14, TIMP1, TIMP2	metalloproteinases and	
	their inhibitors	
Collagen I > Collagen III	Collagen III	Collagen III degreases small amounts of extracellular Collagen I, III, IV

Myofibroblasts

According to one theory, fibroblasts that proliferate and differentiate into myofibroblasts are assumed to be of microvascular origin. It is thought that they are derivered from damaged local microvascular intima more probably than from blood circulation (Hueston *&* Murrell 1990, Iqbal *et al.* 2014). This has been indicated in lung fibrosis. Interestingly, carcinoma-associated fibroblasts are derived from mesenchymal stem cells in bone marrow. In DD it has been observed that myofibroblasts potentially originate from mesenchymal stem cells (= MSC) situated in perinodular palmar fat and skin. DD tissue contains significantly higher amounts of MSCs in the skin overlying a Dupuytren nodule and in perinodular fat rather than in control tissue. This was indicated by MSC markers in DD tissue. MSCs are pluripotential and progenitor-type cells, subsequently capable of differentiating into myofibroblasts (Iqbal *et al.* 2012). Dupuytren contracture can be considered a disturbed and exaggerated process of wound healing—a continuous fibrous accumulation similar to a tumour, without normal tissue control and with an absence of a healing wound (Moulin *et al.* 1998). The ability to contract is due to muscle actin cells; it has a significant role in the wound healing process in addition to collagen synthesis (Spector 2001).

Like healing wound tissue, DD apparently contains an inflammatory part including immune cells and macrofages. The same histological and biochemical phenomena can be detected in other fibrotic conditions, too. Macrofages seem to be able to produce cytokines (TGF-β1) and proinflammatory cytokines (IL-1B, IL-6 and TNF-α), which further promote myofibroblast development (Golbar *et al.* 2013, Verjee *et al.* 2013). TGF-β1, in particular, stimulates mesenchymal cells and is found to abundantly localise intracellularly in myofibroblasts and fibroblasts, especially in the proliferative stage and also in the involutionary stage of DD. TGF-β1 and TGF-β2 increase proliferation of myofibroblasts, induce extracellular matrix synthesis and modulate myofibroblast activity (Magro *et al.* 1997, Picardo *et* Khan 2012, Cordova *et al.* 2005). By using immunohistochemical techniques, myofibroblasts can be detected in DD tissue by their expression of intracellular smooth muscle actin (α-SMA) microfilaments. α-SMA is most richly presented in the nodules in DD tissue (Bisson *et al.* 2003, Verjee *et al.* 2009).

Growth factors

Transforming growth factors are a large group of proteins that stimulate and control differentiation and growth of fibroblasts and promote synthesis of collagen and deposition of the extracellular matrix. They bind on the surface of proteins of specific target receptors and regulate several reactions between cells. But, contrary to classic peptide hormones, they act more like local mediators. Growth factors are a family of signalling polypeptides present and influential in many cancers and other diseases (Alioto *et al.* 1994, Zhang *et al.* 2008).

TGF-β especially is a protein with a significant role in DD. Seven isoforms of TGF-β are known, and each has a certain function in either stimulating and/or inhibiting fibroblasts and myofibroblasts (Cordova *et al.* 2005, Satish *et al.* 2011, Alioto *et al.* 1994). TGF-β1 has been shown to have firm localisation in myofibroblasts in all stages of DD and to increase the force of contraction. They are also present in the normal palmar fascia, but in much lower concentration (Picardo *&* Khan 2012). TGF-β2 has been detected in fibroblasts in all other stages except the residual stage and in the normal palmar fascia. Furthermore, it also has the most significant proliferating effect. TGF-β stimulates collagen synthesis and fibronectin in addition to producing fibrosis (Badalamente et *al.* 1996). Granulocyte macrophage-colony stimulating factor (GM-CSF) is another cytokine and the only one besides TGF-β that induces alfa-actin synthesis in myofibroblasts

and induces fibrosis formation. It has been postulated that it is important in DD, too (Cordova *et al.* 2005).

Epidermal growth factor (EGF) is present in clinically mature and advanced DD tissue; it also has an important role in the development of DD. TGF-α acts through EGF receptors and is present in DD tissue in the proliferative stage, stimulating proliferation. Platelet-derived growth factor (PDGF) is also involved in fibromatosis, being a major mitogen for cells of connective tissue origin. It binds to cell membrane receptors on myofibroblasts and potentially also induces cell proliferation. PDGF is suspected to be controlled by TGF-β (Cordova *et al.* 2005). PDGF co-exists also in wound healing, in chronic inflammations and in sarcomatous conditions (Alioto *et al.* 1994). Myofibroblasts have high-affinity binding sites for basic fibroblast growth factors (bFGF) and they also proliferate in response to exogenous bFGF. This is considered to enable the mitogenic effect. Other growth factors, e.g., insulin-like growth factor (IGF), also take part in DD, but are less significant than the latter ones mentioned above. Peculiarly, myofibroblasts accelerate their own fate by secreting both growth factors and cytokines in an autocrine and paracrine fashion (Yi & Moneim 1999).

Zinc-Finger 9 protein

ZF9 is a protein synthesised inside the cell nucleus. It binds the genes of TGF-β1 and TGF-β2 to their receptors, and thereby promotes transcription of the RNA messengers of these molecules (Cordova *et al.* 2005). The zinc-finger protein family is evolved in many other genetic processes and therefore in the development of different diseases. These proteins are reported to destabilize mRNA, and by decaying mRNA they create changes in immune responses (Yang C *et al.* 2015). Studies indicate that gene polymorphism of ZF9 protein and genotype allele frequencies seem to add to the risk for DD (Bayat *et al.* 2003).

Inflammatory cells

It is implied that DD is like a disturbed wound healing process, besides being a tumour-like process, and therefore it is connected to inflammation and inflammatory cells with different phases (Howard *et al.* 2004, Bianchi *et al.* 2015). It has been suggested that DD may be an immune-mediated or even an atypical auto-immune disease. Langerhans cells (an epidermal cell of dendritic lineage) are found to place beside diseased Dupuytren tissue and form a network of antigen-

trapping attendant cells that ingest and process antigens. The Langerhans cells migrate from the dermis into the DD tissue hypothetically because of dysregulation of the immune system, further because of a change in the levels of inflammatory cytokines. DD tissue also contains CD45-positive cells as an indication and a clear mark of an inflammatory state. CD45 detects all lymphocytes present in the tissue. Langerhans cells cluster around derma-epidermal junctions and capillaries and in nodules (Qureshi *et al.* 2001).

Factor XIIIa-positive dermal dendrocytes can be indicated in DD tissue among other inflammatory cells and their products. Cytokines are assumed to increase the expression of Factor XIIIa and this further probably releases TGF-B1, which regulates dendritic cell maturation in this vicious cycle. Factor XIIIa is also a vital element in the wound healing process, because it induces fibroblast proliferation and regulates collagen synthesis (Quatresooz *et al.* 2008*)*.

A patient case, in which DD seems to have been triggered by some kind of infection itself, has been presented (Mandal *&* Fahmy 2006*)*.

Myofibroblasts are involved in both DD and inflammatory conditions (Meek *et al.* 2002, Meek *et al.* 1999, Zawahir *et al.* 2015). Macrophages, the main inflammatory cells, are largely found in DD nodules but also in the dermis and subdermis above the nodules. On the other hand, lymphocytes (B and T) are less present around the nodules, but found in the tissue at lower levels (Andrew *et al.* 1991). Macrophages release growth factors, which induce the myofibroblasts to further produce fibrosis. Furthermore, growth factors also promote the survival of inflammatory cells. The pro-inflammatory cytokines IL -1 and tumour necrosis factor alpha (TNFα) inhibit apoptosis of monocytes and increase the proliferating processes (Meek *et al.* 2002). Exogenous addition of TNF also induces the differentiation of palmar dermal fibroblasts into myofibroblasts in DD patients (Verjee *et al.* 2013).

It has been verified that steroids decrease the amount of integrin of inflammatory cells in DD tissue, and thereby also TGF-β levels. It has also been clinically verified that steroid injections injected into DD tissue softened and flattened the nodules and modified progression of the disease. Steroids have been used to resolve scar tissue and keloid, and it is common knowledge that they down-regulate inflammation. This proves the significance of these inflammatory elements in the fibrotic processes (Meek *et al.* 1999, Ketchum *et al.* 2000).

Collagen

Collagen type III is richly presented in DD tissue (Menzel *et al.* 1979, Brinckley-Parsons *et al.* 1981, Pereira *et al.* 1985, Melling *et al.* 2000). This change from the normal palmar fascia structure (mostly collagen I) to DD tissue is caused by a cascade of many different events. The density of fibroblasts, stimulation by fibroblasts, decreased apoptosis and imbalance of matrix matalloproteinases (MMPs) all together as part of the fibrosis process produce and change the quality and amount of collagen in the palmar fascia, enhancing collagen type III. The collagen in DD is newly synthesised and immature and is abundant in the tissue, resembling collagen in a healing wound (Brickley-Parsons *et al.* 1981, Notbohm *et al.* 1995*)*. Collagen I is implicated in the regulation of B-catenin accumulation and the modification of TGFB-1-induced proliferation and contraction of myofibroblasts in DD (Vi *et al.* 2009).

In other fibrotic musculoskeletal syndromes resembling DD—as well as in DD—there is a broad spectrum of TIMPs present in the tissue. TIMPs block MMP-mediated collagenolysis. An increase in ADAMTS 14, which is found abundantly in DD tissue, and TIMPs both affect together to increase synthesis and deposition of collagen, leading to fibrosis (Johnston *et al.* 2007, Picardo *&* Khan 2012). The same phenomenon of an increased amount of collagen type III is present in the wound healing process. The contractile force is transmitted intracellularly from activated myosin /actin components via fibronectin receptors at the fibronexus site *(= adhesion complex that connects intracellular actin microfilaments and extracellular fibronectin fibrils together. Fibronectin is an extracellular glycoprotein.)* to extracellular collagen bundles. This finally leads to the typical contracture of the finger. Longitudinal bands, formed mostly by collagen fibres, are represented in more advanced and matured DD tissue (Al-Quattan 2006).

There are two theories proposing an explanation for the contracted fingers in DD caused by collagen. In the intrinsic theory inner changes in the collagen structure itself result in a shortening of the collagen and thereby cause the contraction. The same phenomenon is to be seen when the triple helical configuration of the molecules is shattered during denaturation. The extrinsic theory explains the phenomenon of contractured fingers by a shortening of the distal extremities of the palmar fascia tissue, in the same way as in the wound healing process, where the edges heal by migrating towards the centre of the defect and simultaneously synthesising new collagen. The plausible explanation for

enhancement of the contraction is myofibroblasts, having the proven ability to contract.

Still, no contraction is possible without the strong adhesions and cellular structures produced by collagen together with fibronectine, allowing the shortening forces to be transmitted (Brickley-Parsons *et al.* 1981).

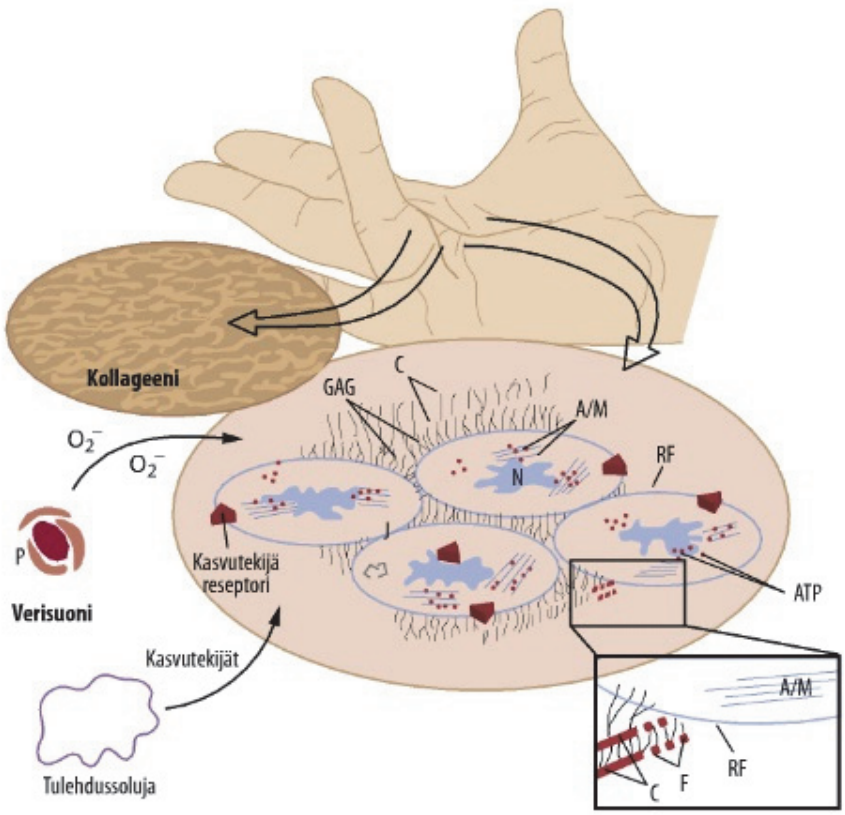

Fig. 2. Cell, molecule and collagen components in DD.The actin-myocin filaments (AM), which have the ability to contract inside the myofibroblasts, adhere via the fibronexus (RF) to the surface of the membrane of the cell, to the extramatrix collagen fibres (C), glycosaminoglycan (GAG) and fibronectin (F). ATP is the fuel in the contraction of actin-myocin filaments. (reprinted from Duodecim 2012;128:4 by permission).

Immune system

The HLA system (human leucocyte antigens complex), or in other words MHC, is the most polymorphic genetic system in the genomy of all mammals; it presents a range of antigens to which T lymphocytes will respond. Therefore, the system is essential and convenient when revealing diseases and genetic linkages. Associations between certain HLA alleles and diseases have been pointed out, and there is a positive association between Class II HLA DR loci as a coherent biomarker and DD, too (McCarty *et al.* 2010). Furthermore, the HLA system has been shown to have a strong association with fibrotic disorders such as sarcoidosis or systemic sclerosis and is therefore convenient for use (Brown *et al.* 2008).

Human individuals with HLA-DR3 seem to have a 2, 94 times higher risk to develop DD compared to references. It has been postulated that people with HLA-DR3 have a tendency to form autoantibodies against components of the extracellular matrix. It might be that these antibodies then induce the release of profibrotic cytokines like TGF-B and GM-CSF (= granulate-macrophage stimulating factor) to furthermore produce fibrosis (Cordova *et al.* 2005).

There also seems to be a connection between the allele of the HLA-DR group in the form of HLA-DRB1 and DD. Caucasoids of European origin have this allele and seem to have an increased risk of developing DD. Scandinavian studies confirm this observation; the HLA-DRB1*01 allele is well presented among DD patients (Jónsson *et al.* 2013). However, no specific HLA gene can be pointed out as a direct cause of DD because of the extensive linkage disequilibrium between the genes and their interactions (Brown *et al.* 2008).

People affected with have shown to have autoantibodies against collagen type I–IV. After surgical excision of the diseased fascia, the level of autoantibodies is diminished to undetectable, most probably because of the removal of the antigens. Immune cell infiltrates can be detected in DD nodules in certain stages of the disease, suggesting that the immune system is involved in its progress (Picardo *&* Khan 2012).

Metalloproteinases and metalloproteinase inhibitors

Matrix metalloproteinases are a group of zinc-dependent endopeptidases that tear down and dissolve the extracellular matrix as a part of controlled matrix remodelling.These peptidases, a family of 23 enzymes in humans, are produced by myofibropblasts, macrophages, endothelial cells and keratinocytes. Each MMP has

a certain role in facilitating tissue remodelling and changes. MMPs and TIMPs, a family of four specific inhibitors, take part in many fibrotic processes and can be detected, e.g., in conditions like frozen shoulder, wound healing and cancers and carcinomas. TIMPs regulate MMP function. MMPs are also found in normal tissue cells, but at low levels.The activity of these proteinases is controlled by TIMPs and a variety of cytokines and growth factors and endogenous inhibitors. The ratio balance is disturbed in DD, as it can be detected to be in many other conditions like rheumatoid arthritis, tumours, chronic obstructive pulmonary disease and so on. A reduction in the MMP:TIMP ratio is postulated to be the reason for emerged collagen amounts and connective tissue. MMP activity has been shown *in vitro* to have a critical role in the contraction of fibroblast-populated collagen lattices (Cordova *et al.* 2005, Townley *et al.* 2008, Hutchinson *et al.* 1998, Tarlton *et al.* 1998, Johnston *et al.* 2007).

The levels of interstitial collagenase MMP1 and gelatinases MMP2 and MMP9 as well as MMP13 and MMP14 are found to increase in DD. The level of ADAMTS 14, which is closely linked to the MMP and TIMP system, also increases in DD.

The ADAMTS family consists of 19 different types of members and they are implicated in extracellular matrix metabolism. TIMP1 and TIMP2 levels are also up-regulated in DD. TIMPs promote fibrosis by inhibiting the normal cleaving process by MMPs. Marimastat, a synthetic inhibitor, was given as a treatment to gastric adenocarcinoma patients, and they developed palmar fibrosis similar to DD (Johnston *et al.* 2007, Cordova *et al.* 2005, Dietmar *et al.* 2009, Tarlton *et al.* 1998, Townley *et al.* 2008).

Wnt pathway and B-catenin dysregulation

The Wnt gene family encodes glucoproteins and extracellular signalling molecules. They play a significant role in cell proliferation, differentiation, survival, apoptosis and migration (Wodarz & Nusse 1998**).** The Wnt pathway is, so to say, a group of signal transduction pathways consisting of proteins that pass signals from the outside to the inside the cell. Wnts are upstream regulators of cellular beta-catenin accumulation. The Wnt -1 gene works the same way as retroviruses, for example, which carry oncogenes from cell to cell. The canonical Wnt that is the Beta-catenin pathway is the type of pathway that causes accumulation of Beta-catenin in the extracellular cytoplasm and eventually in the nucleus. It works as a transcriptional co-activator of transcription factors that belong to the TCF/LEF (T-cell factor/lymphoid enhancing factor) family. TCF/LEF transcription factors bind to

DNA and enable beta-catenin to act on genes they target (Brantjes *et al.* 2005). Beta-catenin protein has a bifunctional purpose. The first part is to impact between-cell adhesions and the second part is to co-activate transcription of Wnt target genes via Wnt pathway signalling. It co-ordinates events and also is involved in cell adhesions. It reorganises the cytoskeleton and transmission of mechanical forces, which is important for cell migration and contraction. The Wnt pathway regulates gene transcription (Varallo *et al.* 2003). The elevated levels of B-catenin in DD cell lattices are shown to be more sensitive to isometric tension and therefore also provoke contraction of actin filaments and in the end, DD cells (Howard *et al.* 2003). The Wnt pathway is also involved in tumourgenesis, and its deregulation contributes to a number of cancers, too (Bruise Alberts *et al.* 1994, Picardo *&* Khan 2012, Varallo *et al.* 2003, Howard *et al.* 2003).

It is still somewhat unknown how the Wnt /B catenin cascade actually works in the pathogenesis of DD. Wnt stimulation leads to increased intracellular accumulation of B catenin, which further works as a trigger for transactivation of several target genes like fibronectin (O'Gorman *et al.* 2006, Al-Quattan 2006). In DD, Wnt genes 5a, 9a, 10b and 11 are the major Wnt family members expressed (Degreef *et al.* 2009). B catenin is richly accumulated in the cytoplasm in fibroproliferative diseases, as in Dupuytren's disease (Bowley *et al. 2007)*. However, some studies do not confirm this theory or suggestion of the Wnt genes' connection to the aetiology of DD without doubt. (Picardo *&* Khan 2012*)*. The majority of studies about Wnt pathways and the connected proteins are *in vitro* studies and animal studies, mostly by mouse experiments. Recent studies of the Wnt pathway and keloid pathogenesis have elucidated the beeta-canonial pathway and it's involvement in keloid pathogenesis. TGF-beeta induces up-regulation of canonial pathway signalling in hypertrophic scar and keloid fibroblasts (Shih *et al.* 2012). Wnt pathways have been shown to be critical for the fate of mesenchymal stem cells *in vitro*. Furthermore, it has been detected that stem cells originally derived from palmar skin and subcutaneous fat are a potential source of myofibroblasts, the key element of DD (Hidocha *et al.* 2011, Igbal *et al.* 2012). The Wnt pathway is divided into three categories according to the manner of the working process used.

The Wnt pathway gives an interesting aspect for revealing the possible aetiology of DD. The Wnt signalling pathway also has a significant role in the wound healing process (Zhang *et al.* 2009).

The non-canonical planar cell polarity pathway (PCP) does not involve Beta - catenin. This pathway regulates the cytoskeleton and is responsible for the shape

of the cell. It is thought to use NRH1, Ryk, PTK7 or ROR2 as a mediator and effector in the cell. The Wnt5A-ROR2 signalling pathway has been suggested as being necessary for the expression of MMP13, which is also overexpressed in DD (Shih *et al.* 2012). The Wnt5a-mediated non-canonical signalling network potentially participates in inflammation-induced angiogenesis (Zhang *et al.* 2009). Both β-catenin and ROR2 seem to influence the mesenchymal stem cells through regulating pathways (Cai *et al.* 2014).

The third pathway is the non-canonical Wnt/calcium pathway, which doesn't stimulate the accumulation of β-catenin. It helps regulate calcium release from the endoplasmic retinaculum (ER) in order to control intracellular calcium levels, by which it then regulates cell adhesion, migration and tissue separation (Shi-Xia Cai *et al.* 2014*)*.

All three of these pathways are activated by the binding of a Wnt-protein ligand to a Frizzled family receptor (FZD) on the cell membrane, thereby passing the biological signal to the protein inside the cell. These intracellular events can produce mutations that lead to a variety of diseases clinically (Guo *et al.* 2014).

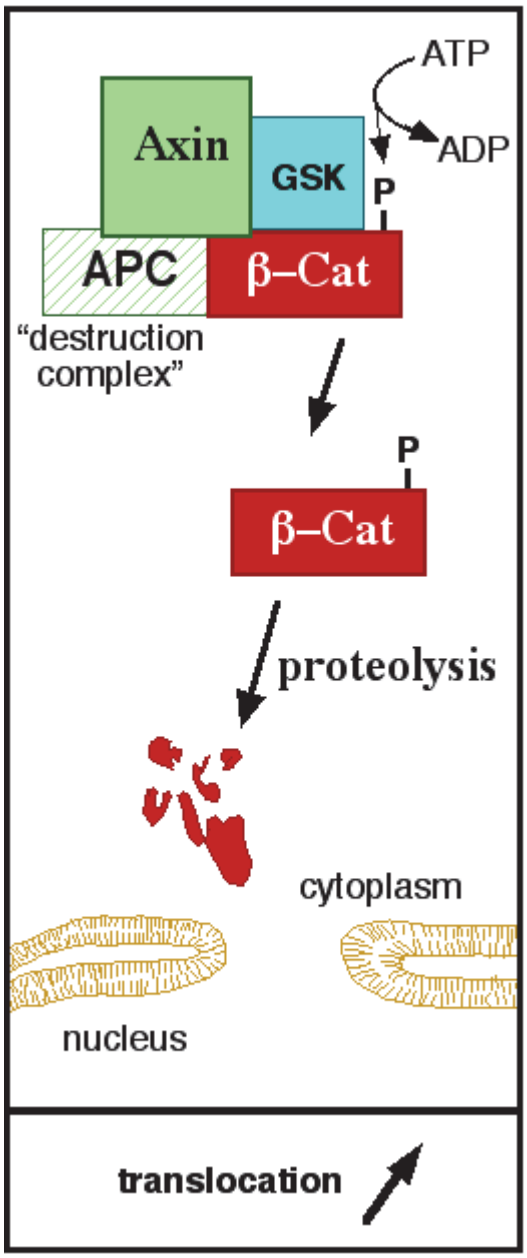

Fig. 3. No Wnt binding into the receptor and therefore β- Cat is destroyed. (picture from Wikipedia).

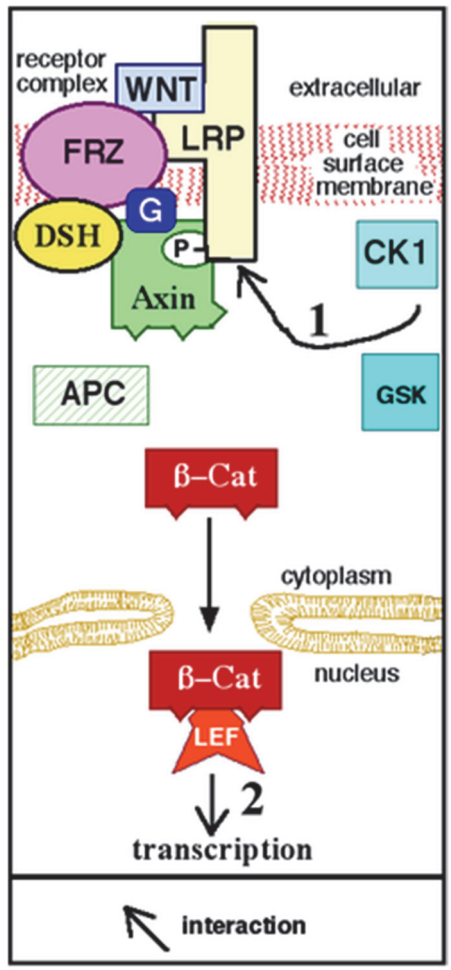

Fig. 4. Wnt binds into receptors and β-Cat moves into the nucleus and activates protein transcription. (picture from Wikipedia).

ROR2

Tyrosine-protein kinase transmembrane receptor ROR2 is a protein belonging to the ROR subfamily and is located in chromosome 9. It is evolved in bone and cartilage development and mutations of the gene will cause skeletal disorders such as brachydactyl type B and Robinow's syndrome, which both cause hand deformities (Schwabe *et al.* 2000). Furthermore, it is linked to cell remodelling by apoptosis and signal transduction between cells (Malemud 2004). It is a receptor or

co-receptor of the Wnt5a non-colonial pathway, inhibiting the Beeta-catenin-TFC pathway, and has a role in activating the Wnt/JNK pathway, which results in polarised cell migration. In summary, ROR2 is a regulator of cell proliferation, differentiation, migration and death (Oishi *et al.* 2003).

It is acknowledged that mesenchymal stem cells (MSCs) are multipotential and have the ability to develop into different directions and tissue types. Most MSC studies have been *in vitro* studies because of the unstable condition of the *in vivo* element and therefore the difficulty to control alterations. The palmar skin and fat-derived MSCs are suspected to be possible resources of DD (Hidocha *et al.* 2011). A connection between ROR2 and MSCs has been detected. It seems that ROR2 plays a critical role via Wnt5a in the proliferation and migration of MSC (Cai *et al.* 2014, Many & Brown 2014).

2.2.2 Involutional phase

The second histopathological stage in DD is called the involutional phase. Myofibroblasts are still the dominating cellular phenotype, yet their level decreases because of organised cell apoptosis (cell death), and hypocellular areas can be detected. The nodules became smaller and less defined.The phenomenon is an important initiation and may be considered the final step of myofibroblast phenotype evolution (Wilutzky *et al.* 1998). Furthermore, the myofibroblasts and actin microfilaments begin to arrange in longitudinal linear lines of tension surrounding collagen bundles. The latter then form a fibronexus, which is the attachment site on the surface of the cell membrane and transmits the contractile force from intracellular to extracellular tissue. An early formation of contraction is formed (Tomasek & Haaksma 1991, Picardo & Khan 2012). The TGF-β2 isoform from the TGF family has intense intracellular localisation within myofibroblasts in both the proliferative and involutional phases. TGF-β induces significant effects that are the same as in the wound healing process via fibrosis formation, collagen type III formation, angiogenesis and glycosaminoglycan synthesis. These all are at the same time elements of DD (Badalamente *et al.* 1996).

The formation of cords continues to mature, and in the early stage it can be difficult to determine the margins between the nodule and the cord (Margo *et al.* 1997). As the maturing process advances, the amount of cells diminishes even more in the cords and the amount of collagen increases and the cord begins to contract the finger.

2.2.3 Residual Phase

The final stage in the formation of DD is called the residual phase. The tissue is less cellular—almost acellular and fibrotic—and therefore resembles mature scar tissue. There are less myofibroblasts and locally they are even absent from parts of the tissue. The few remaining cells are mainly fibrocytes. The nodules disappear and tendon-like fibrotic cords become more and more pronounced. The amount of type III collagen decreases, although it is still well represented in the tissue; actually, extracellular deposits of collagen type I, III and IV can be detected. The most powerful proliferating effect of TGF-β2 vanishes and fibroblasts do not produce TGF-β2 anymore. Clinically, the flexion contracture forms rigid extension lags of the finger joints (Wilutzky *et al.* 1998, Fitzgerald *et al.* 1999, Badalamente *et al.* 1996, Michou *et al.* 2012).

2.3 Aetiology

Despite the vast amount of scientific research, the main reason for DD is still unknown. The disease seems to have both a heritable and a sporadic form. Estimates of the prevalence of DD have varied immensely in selected populations, ranging from 2% up to 42% (Godtfredsen *et al.* 2004). Many different factors have been named that predispose a person to the disease. The final triggering element or elements, however, have not yet been discovered. Thus, heredity seems to have the most essential role in the development of the disease. The disease does not directly pass down in the family, but more likely the predisposition to it. Caucasian and northwestern European origin gives a strong tendency to inherit the disease (Gudmundsson *et al.* 2000). A sporadic form of DD is also acknowledged. It is connected to palmar microtraumas caused by, e.g., manual labor. It is currently accepted that DD develops because of an interplay between some endogenous and/or exogenous factors that contribute to intrinsic susceptibility (Wilbrand *et al.* 2000). Several environmental or life habit factors seem to promote advancement of the disease (Geoghegan *et al.* 2004*)*.

The period of time from the beginning of appearance of the disease to restricted extension of the finger/-s has been estimated to take a little less than 10 years (Reilly *et al.* 2005). Clinically it has been noticed that some patients tend to develop the disease in a much shorter time, and certain risk factors for more rapid enhancement of DD have been pointed out. These high-risk patients seem to develop the disease within only a few years, definitely in a shorter period of time

than is conventionally assumed. The onset of DD at a younger age is associated with the aggressive course of the disease (Hu *et al.* 2005).

2.3.1 Epidemiology

DD is a fibrotic disease that affects the palmar fascia. Similar to Mb Ledderhose, there is fibrosis in the plantar fascia, and in Mb Peyron, in the penile fascia or corpora cavernosa (Hart & Hooper 2005, Cutts *et al.* 2005). All these forms can be found among high-risk patients (Gudmundsson *et al.* 2013, Kan & Hovius 2012). DD is exceptionally well represented in the Caucasian race, but can also be detected in other human races, although in much fewer numbers (Sladicka *et al.* 1996). The prevalence of the disease increases with ageing and race (Mikkelsen *et al.* 1999). Gudmundsson and collaborators have elucidated that its prevalence among young male Caucasians aged 45–49 years is as high as 7.2%, but with ageing it rises as high as 39.5% (age 70–74 years) (Gudmundsson *et al.* 2000). Its global prevalence has been estimated to be about 3–5%, but the prevalence estimates vary from 0.2% to 56% depending on race, age and method of data collection. Geographic variability inevitably plays an important role in this (Dibenedetti *et al.* 2011, Lanting *et al.* 2013). This indicates that DD is a racial issue, and therefore it is recognized as the "Viking disease" (Nunn & Schreuder 2014). Caucasians seem to have a higher frequency of a positive presence of the HLA-DR1 phenotype, which has been reported to probably predispose to DD (Brown *et al.* 2008, Michou *et al.* 2012*)*.

Genetic susceptibility is well supported by epidemiological observations and studies, and because of the obvious prevalence among the northern European stock. Within the UK population the risk of the disease is found to be about 2.9-fold higher in individuals with an affected sibling than in the general population (Picardo *et al.* 2012). Also, the sibling DD recurrence-risk ratio equaled 2.9 based on 95% confidence intervals for the population prevalence (Hidocha *et al* 2006). According to some studies the recurrence risk for DD with diseased siblings is even higher, being 4,5-fold (Capstick *et al.* 2013*)*. This also supports the high genetic basis for the causation of DD.

A lower age of onset and the greater severity of DD is associated significantly with a positive family history of the disease (Hindocha *et al.* 2006, Dolmans *et al.* 2012). Initially it was proposed that the disease is inherited as an autosomal recessive trait, but after more research has been done, it has been found to be inherited as an autosomal dominant trait with variable and incomplete penetrance,

rarely autosomally recessive. Mitochondrial maternal heredity has been suggested. It is possible that DD is a complex oligogenic or simple monogenic Mendelian disorder (Picardo *et al.* 2012, Anderson *et al.* 2012, Yi *et al.* 1999, Michou *et al.* 2012). An autosomal dominant gene for DD susceptibly has been mapped to chromosome 16q in a Swedish family with a five-generation inheritance, although no specific causative gene could be identified (Hu FZ *et al.* 2005). Other chromosomes like 6 and 11 may also contain genes for DD. Nonetheless, a probable genetic association has been indicated in these chromosomes. Ultimately, the disease is considered to be an oligogenic disorder of combined actions of alleles of more than one gene (Ojwang *et al.* 2010, Michou *et al.* 2012).

Although the disease affects both genders, it is more richly represented in the male gender. The male-to-female ratio varies between 4:1 and 10:1 and depends on age. Ageing eventually equalises the ratio (Cutts *et al.* 2005, Stahl *&* Calif 2008*)*. It has been shown that androgen receptors co-localise with alfa-actin in myofibroblasts in DD in both cell cultures and tissue samples. Myofibroblasts are therefore target cells for androgens. This is proposed to explain the male predominancy in the disease. It is accepted that testosterone can increase alfa-actin synthesis and stimulate smooth muscle cell proliferation (Pagnotta *et al.* 2000).

The prevalence of DD increases with ageing. Males start to develop the disease in the fifties, females a decade later

(Wilbrand *et al.* 2002, Ross 1999, Mikkelsen 1972). DD is considered to be a disease of aged people in general, but it can be diagnosed even congenitally or in children or adolescents (Foucher *et al.* 2001). Patients with a family history, bilateral disease, ectopic lesions and radially expressing disease in the hand tend to atypically have the aggressive form of DD at an early age. They also have a high recurrence level (Ojwang *et al.* 2010). Without exception, these patients are young men, with onset of DD before 50 years of age (Michou *et al.* 2012).

About 5% of DD patients also have diabetes. Its prevalence varies from 14% to 56% depending on age and ethnic origin. Diabetes is admitted to be a significant risk factor for DD. This especially concerns people with DM type I. It is accepted that diabetes acts as a triggering factor for DD. It is assumed that ischaemia caused by microangiopathy and increased collagen production promotes the disease. Nonetheless, DD among diabetic patients is described as milder than among non-diabetic patients with DD (Picardo *et al.* 2012, Ross 1999**,** Wilbrand *et al.* 2002, Chammas *et al.* 1995). The adjusted relative risk for DD in the presence of diabetes was considered to be 1.8 (1.2–2.6). With insulin-treated DM patients the risk was as high as 4.4 (2.1–9.1) (Burke *et al.* 2007).

Smoking is considered to raise the risk for DD, although the evidence is somewhat contradictory. Still, smokers seem to have a three times higher prevalence of DD than non-smokers. This has been demonstrated with rigorous statistical methods in several studies (Burge *et al.* 1997, Ross 1999, Godtfredsen *et al.* 2004). The mechanism that leads to DD is microvascular changes that create hypoxic conditions in tissue by narrowing the vessels (Picardo *et al.* 2012*)*. The adjusted relative risk is assumed to be 1.3 (1.2–1.5) when more than 20 cigarettes are smoked per day (Burke *et al.* 2007*)*.

Alcohol consumption 40 g up to 80 g of alcohol per day is undisputedly considered to predispose to DD. Therefore, alcoholics tend to have DD twice as often as controls. According to some studies this is not related statistically to liver diseases. However, it is accepted that estimating the influence of alcohol is difficult because of the vast amount of confusing factors. It is difficult to separate smoking from alcohol intake because they often go together. There are no good data to show the dose-response relationship or the effect of cumulative consumption in the development of DD. According to Burke *et al.* the relative risk for DD is 1.6 (1.5–1.7) if more than 22 drinks are consumed per week (Burke *et al.* 2007). Free radicals are considered to be the predisposing factor that links alcohol consumption and DD. Alcohol is assumed to mediate the conversion of xanthine dehydrogenase into xanthine oxidase, which can oxidise hypoxanthine and thereby produce free radicals. This is accepted as the hypothesis explaining the prevalence of DD among people with high alcohol consumption (Picardo *et al.* 2012, Burke *et al.* 1997, Ross 1999, Gudmundsson *et al.* 2001).

Epilepsy and/or anticonvulsive drugs like phenobarbitone has been considered to predispose to DD. This presumption is controversial according various studies and has been proved to be inconsistent nowadays (Geoghegan *et al.* 2004).

HIV (human immunodeficiency virus) has also been associated with DD. It was noticed that AIDS patients of relatively young age got DD among other rare infections and diseases because of their weakened immunity. According to studies, apparently its prevalence is about 6% and somewhat the same as in the normal population. Indisputably there is a marked discrepancy between the relatively young age of AIDS patients and DD (Ross 1999). In fact, one study found that 36% of HIV patients were affected by DD. This patient group with advanced infection possibly has a disturbed free radical metabolism (Picardo *et al.* 2012). Weakened immunity may hold unrevealed effects in the development of DD.

A vast amount of data has been obtained concerning risk factors and DD. A large study of 2919 operated DD patients, from 1956 to 2006, did not confirm any

significant statistical correlation between DD and diabetes mellitus, alcohol consumption, heavy smoking or epilepsy and the stage of the disease. An only slightly higher prevalence risk for DD was detected compared with the normal population. However, the amount of smoking and alcohol consumption are often difficult to accurately determine, which has to be acknowledged (Loos *et al.* 2007, Becker *et al.* 2014). The conclusion of the study confuses the theory of DD, making it more complicated to explain.

2.3.2 Occupation and trauma

A manual occupation is proposed as a cause a DD besides other risk factors. Repetitive trauma of the hands, like vibration exposure, is assumed to produce lesions and microruptures and haemorrhaging in tissue and therefore produce scar and collagen fibres that eventually lead to DD. However, no specific work pattern has been related to DD, nor has it been accurately determined what dose of trauma or what time period or cumulativity is needed for correlation and to increase the prevalence of DD. Many confusing matters disturb the conclusion and a consensus has not been reached (Liss & Stock 1996). Manual work as a concept is not sufficiently precise to be related to DD. According to this, it should be possibly to get DD through other manual activities, like hobbies. The debate about trauma and the aetiology of DD was started already by Baron Guillaume Dupuytren himself and is still somewhat continuing (Descatha *et al.* 2011, Khan *et al.* 2004, Burke *et al.* 2007). Some support for the hypothesis of an association between high manual work exposure, vibration and DD thus does exist (Palmer *et al.* 2014, Gudmundsson *et al.* 2000, Descatha *et al.* 2012). However, it is difficult to distinguish when trauma caused by manual work should actually be considered an injury behind DD (Rayn & Moore 2005). DD is not included as an occupational disease to date.

A single injury to the hand causing DD has been reported. Factors like wrist or hand fractures, previous surgery of the hand (trigger, carpal tunnel) or sometimes hand infections seem to have a role in promoting DD (Rayan 2005, Elliot & Ragoowansi 2005). Well-documented patient cases of 309 patients from Mc Farlane's series seem to confirm the possibility that occasionally a single injury can precipitate the onset of DD (Mc Farlane 1991). Other confirming studies exist. In a series of 235 patients with distal radius fractures, about 9% developed DD, and all except one patient were females with only three having diathesis (Kelly *et al.* 1992, Wichelhaus *et al.* 2015). A causal relationship can be established only in

young people, because the tendency to get DD increases with age. In other words, it includes men diseased before age 40 and women at 50 without diathesis. The following criteria somewhat define the concept of trauma possibly causing DD: the disease is in the injured hand and unilateral, there is objective evidence of the injury in the hand, the DD is in the area of the injury in the hand and the DD appears within two years of the injury. There must also be histological evidence of DD besides the previous factors (Mc Farlane 1991*)*. Nonetheless, this theory has a weak point; what is the role of a possible sporadic form of DD and its correlation with trauma? This nongenetic form of the disease, more fibromatosis-like, is considered to be non-progressive, sometimes even regressive and spontaneously healing, unilateral without ectopic lesions, and in line with a single digit but generally without finger contracture (Rayan 2005). This kind of palmar fibromatosis is to be separated from actual DD, which is a progressive disease with diathesis affecting the palmar fascia and which apparently requires operative actions to liberate the flexion of fixed fingers. The non-genetic disease is believed to be less severe and is considered not to cause finger contractions.DD patients with diathesis tend to have ectopic lesions and more aggressive excursion. Understandably, it is sometimes a vague and confusing line to separate these two groups *in vivo*. These aetiologies can be overlapping.

Table 2. Epidemiology.

Epidemilogy /Risk factors
Caucasian race
genetic susceptibility
age
gender
diabetes mellitus,
epilepsy (phenobarbitones)?
smoking, alcohol consumption?
occupation/trauma
HIV?

2.4 Clinical presentation

In its early stages the disease can be difficult to diagnose. The disease is perceived to be asymptomatic, and in the beginning there can be a small, palpable, but non-annoying nodule on the palm of the hand or a thickening of the skin. Sometimes there are skin pits and dimpling of the skin can be seen, while the dermis and

fibrosis retract the skin above and it becomes tethered. The skin is pulled inward. As the disease advances, the nodules grow bigger and proliferate, and then unite with each other, forming a cord. The nodules are rich in cells and vascular compared with cords. The main cell type is a myofibroblast, and apparently they vanish in the maturing process, making way for collagen. The cord will go on expanding from the palmar region towards a finger or fingers, parallel with the tendons above them (Watson &Palm 1991). Before maturing to a constricting scar-like cord, there is a mixed situation with both nodules and cords together in a tight combination. At this point it is difficult to say where the nodules end and the cord begins. With progression of the disease the nodules gradually regress, giving space for the forming cord. However, this apparently is not always clearly noticeable *in vivo*. Subcutaneous fat is replaced by fibrosis and eventually the cord longitudinally reaches the finger and forms flexion deformity (Rayan 1999).

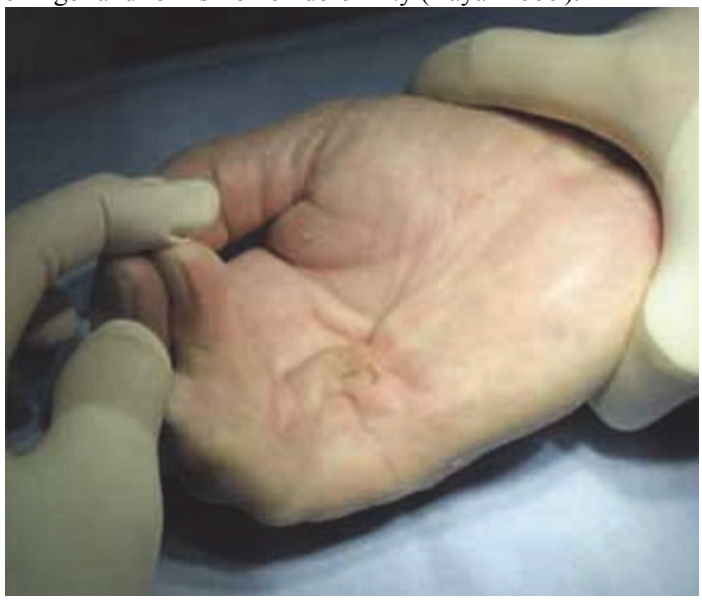

Fig. 5. Clinical presentation of typical DD contracture appearing in the ulnar digits. (reprinted from Duodecim 2012;128:4 by permission)

The disease may sometimes begin and remain only in the digit area. (Rayan 1999) The most affected finger is the ring finger (with 85.1%), but the little finger is also quite often presented (with 45.4%) (Reilly *et al.* 2005, Misra *et al.* 2007). In some studies the little finger holds first place (Dias & Braybrooke 2006). The middle finger is affected less often (with 28.3%), then the thumb (with 3.0%) and least

often the index finger (with 1.2%) (Mikkelsen 1976). Patients with a strong diathesis tend to have ectopic regional lesions on the dorsum of the digits, called Garrod's nodules or knuckle pads. These fibrotic lesions rarely cause joint contracture. They are situated on the dorsum of the PIP joints. Plantar fibrosis is called Mb Ledderhose and penile fibrosis is called Mb Peyronie and they are typically ectopic lesions involved in DD (Nugteren *et al.* 2011). The prevalence of Mb Ledderhose is about 6% and Mb Peyronie, about 2% in DD patients. Something like 50% of DD patients have a bilateral disease, and it typically involves the two ulnar digits. In very rare cases the disease has been described to extend to the wrist and forearm region in the upper limp (Rayan 1999). There is no relation to handedness.

Luckily, this flexion contracture of the fingers tends to take years to form (Picardo *et al.* 2012).

Table 3. Presentation of DD in the hand.

Finger	Percentage
4.finger	85.1%
5.finger	45.5%
3.finger	28.3%
1.finger	3.0%
2.finger	1.2%

Frozen shoulder syndrome, a fibrotic adhesive capsulitis of the shoulder, has same histopathological features as DD (Raykha et al. 2014). *In vivo* these two diseases may both be found together in the same patient. In a study by Smith et al, DD was 8.27 times more common in patients with frozen shoulder than in the general population (Smith *et al.* 2001).

2.5 Recurrency

Despite the meticulous research, DD remains unsolved. As long as the aetiology remained unrevealed, the treatment options will continue to be unsatisfactory. The main problem is that, to date, only the causes of the disease but not the ultimate reason for it can be cured. There are different methods for eliminating the rigid cord and freeing the digit for extension. The problem connected to this matter is the high level of recurrence regardless of the excision method chosen. Recurrence is to be distinguished from a lack of extension after operation; recurrence is a new disease

emerging in a previously operated area, with distinguishable nodule or cord formation. A lack of extension can be caused by many reasons, e.g., scar tissue, but not necessarily by the disease. In both situations the deformity more or less disables everyday functions (Gudmundsson *et al.* 2000, Dias & Braybrooke 2006). Still, there is the problem of the variety of definitions of recurrence. (Werker et al. 2012)

A consensus on this has been established by 24 hand surgeons from different countries by using the Delphi method. In brief, the agreement is as follows: a cord or a nodulus with finger contracture of a joint > 20 degrees is passive extension deficit after treatment. This should ease comparisons between different treatment methods in the future (Felici *et al.* 2014).

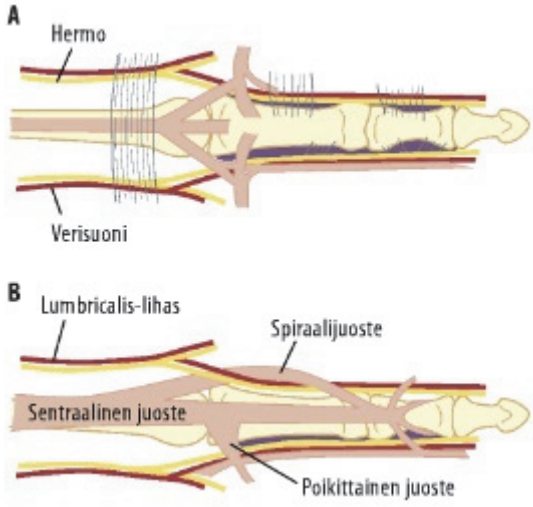

Fig. 6. Normal structure of a digit B.DD contracture in a digit yellow line = a digital nerve red line = a digital blood vessel (reprinted from Duodecim 2012 by permission).

The result of surgery often shows a poor functional outcome if the deformity is severe at the time of the procedure (Ebskov *et al.* 2000, Rodrigo *et al.* 1976). In such cases expectations should be cautious, and even more so if already a few operations have been done previously in the same area. Also, the proximal interphalangeal joint seems to be more demanding than the metacarpal joint. Residual flexion contracture at the time of surgery is a predictor of a worse outcome, especially in a recurrent PIP contracture (Misra *et al.* 2007, Honner *et al.* 1971). The result after the operation is also a somewhat subjective opinion and therefore complicates estimation and comparisons of functional outcomes and

situations between patients. By measuring extension lag, it it possible to get an objective result of the operation.Thus, even a mild contracture may cause a disability if many digits are affected, or even more so if the disease is bilateral. Not to mention that poor surgical techniques or incomplete excision will lower the good level of the outcome of the excision (Dias & Braybrooke 2006). Skin tension is known to stimulate proliferation of Dupuytren's tissue, and this assumably also has an impact on recurrence *in vivo* if the skin closure is tight (Citron *et al.* 2003). Recurrence rates after dermofasciectomy have been noticed to diminish obviously (Abe *et al.* 2007). The disease is disabling when well matured and with possibly several fingers affected and severely contracted.

The risk and high ratio of recurrence it is connected to patients with DD diathesis. The risk increases according to some studies by about 71% compared with the baseline risk of 23% in patients without diathesis (Hindocha *et al.* 2006). Also the aggressive behaviour of the disease comes along with diathesis. DD is estimated to progress from a nodular form to a constricting cord in an average of about ten years after diagnosis made with a single nodule. With the aggressive type of DD, progression is more rapid and a constricting cord may develop in only a few years (Hueston 1985). Some specific factors have been evaluated that distinctly raise the risk of recurrence (Reilly *et al.* 2005). Bilateral disease, a family history of DD, ectopic lesions (DD found outside the palmar fascia) and ethnicity are considered to raise the risk of a greater tendency for recurrence and make the disease behave aggressively (Hueston 1985). Bilateral expression of DD increases the odds of recurrence by 48% compared with a unilateral disease. Also, young age at the onset of DD, male gender and somewhat radial expression of the disease in the hand suggest a raised risk for more aggressive behaviour (Abe *et al.* 2004). There is acceptance that the recurrence rate after surgery varies from 22% with no known risk factors to 71% with many risk factors. According to studies, the higher the risk, the more diathesis factors are present that predispose to DD (Hindocha *et al.* 2006).

2.6 Treatment options

The deforming cord is tense and consists of very strong material that does not rupture easily. The very first therapeutic approach was implemented in 1831 by Guillaume Dupuytren, who did the first fasciotomy for a DD patient. Nonoperative treatment is available, but with progressing disease these options are often all too insufficient, time-consuming or difficult or complicated to carry out. The present

opinion about treatment methods favours surgical treatments and collagenase injection treatment. Clinically, all other methods are only supportive and connectable to operative choices. The nonsurgical methods include, e.g., digit extension with devices, splints, radiation, dimethyl sulfoxide, physical therapy, ultrasonic therapy, steroids, 5-fluorouracil, Tamoxifen and anti-gout medications (Bulstrode *et al.* 2004, Ketchum *et al.* 2000, Kuhn *et al.* 2002, Betz *et al.* 2010). Gamma-interferon has been cautiously promising, but no long-term studies have been done and it involves the problem of possible long-time complications. *In vitro* model studies with Interferon have shown inhibited contraction of Dupuytren's fibroblasts (Sanders *et al.* 1999*)*. Radiation in the early proliferative stage of DD has shown variable success according to studies and is more commonly used in Germany (Bentz *et al.* 2010). Yet, disagreements on the results in long-term studies still exist. Because of the side effects, it is not considered a normal treatment tradition in other countries, and also because radiation is considered to be a treatment for malignant diseases, which DD is not (Hurst *&* Badalamente 1999).

It is possible to achieve the best correction of the contracture surgically or with collagenase (Badalamente *et al.* 1999, Badalamente *et al.* 2002, Ball 2013). Yet, to date, only the consequences but not the disease can be cured.

The guidelines and criteria for operation have been established. The tabletop test is positive when the patient can no longer place the palm of the hand and the finger/fingers flat on a table surface. This test is considered a simple test to evaluate the option of an operation. The test correlates quite well with the situation of the MCP joint being contracted at least 30–40 degrees, which is agreed on as the reasonable limit for operative treatment. With the PIP joint, even less contraction is allowed as the threshold because of the difficulty to achieve and maintain full extension after the operation. This is especially so if the situation is already severe at the time of the operation. The rate of complication increases with the severity of the disease, particularly when the PIP joint exceeds the angle of 60 degrees before surgery (Benson *et al.* 1998, Bulstrode *et al.* 2005). Also the amount of previous operations on the same finger obviously raise the rate of complication because of scar tissue.

The available surgical techniques to be chosen from depend on the severity of the disease, the age and demands of the patient, and no less, somewhat also the surgeon's preference for a certain type of technique.

Percutaneous needle fasciotomy (PNF) is simple, cheap and easy to perform even as an outpatient procedure in the office. It involves surprisingly few complications if done by a person acquainted with the method and finger anatomy.

It is done with a 25-gauge needle; the cord is cut with the needle percutaneously or through a mini wound, preferably as a percutaneous fasciotomy with a blade. There are a few ways to do the cutting, but eventually the cord is sliced into two pieces so that the tethering and tightening effect is undone. PNF works best with low-demanding patients with high operation risks, because it is less invasive and it can be done as an outpatient procedure. It works best only in mild cases affecting the MCP joint. The cost of the treatment is understandably low (Rijssen *et al.* 2006). The problem is the high rate of recurrence. According to different studies, the risk of recurrence can be up to 65% within a 33-month follow-up. According to van Rijssen et al., the risk of recurrence is up to 84.9% at a five-year follow-up. Even higher recurrence risk has been proposed (Rijssen *et al.* 2006, Stanbury *et al.* 2011*,* Ball *et al.* 2013).

Open palm surgery where the wounds are left open to heal over time is no longer favoured because of the long healing time and discomfort. Also, the radical technique where the whole palmar fascia including the macroscopically healthy part of it is excised has been abandoned. Invasiveness increases complications. However, it is accepted that DD invades the whole aponeurosis of the affected hand in addition to the visible cords and nodules (Quaglino *et al.* 1997).

Limited fasciectomy is less invasive than open palm techniques or radical fasciectomy, as a limited skin wound is required. Only the cord and nodules causing contractura are excised through a zigzag incision, not the entire fascia. This technique is considered to be a standard, although the method is always patient- and disease-dependent. Complications are reported to be about 17% overall. A complication can be a wound-healing problem, like infection or skin necrosis, or more permanent such as a digital nerve irritation or damage or arterial damage. Even CRPS (Complex regional pain) syndrome is possible. Tendon ruptures are rare. Recurrence rates vary widely in different series because of the threshold of acceptance of the concept of recurrence. The nature of the disease probably also affects to recurrence. The rate also rises over time, suggesting that it is the disease progressing rather than the operation that determines recurrence. With limited fasciectomy recurrence is about 33% and up to 50% at the five-year follow-up. But there is variation in the numbers of recurrence; even rates as minimal as 20.9% have been proposed (Ball *et al.* 2013*).* The large variation in the numbers exists because of the lack of good randomised studies.

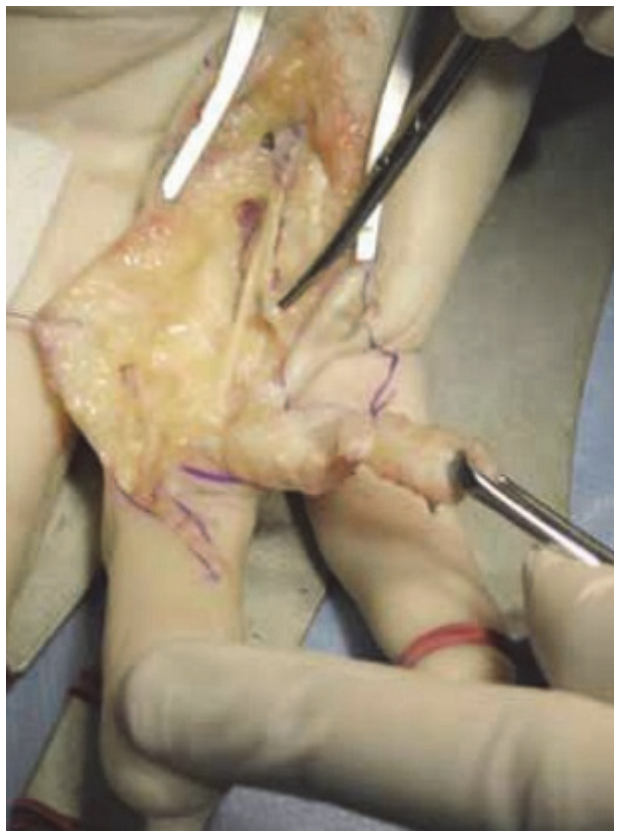

Fig. 7. Fasciectomy of a digit. (reprinted from Duodecim 12/4 by permission).

Dermatofasciectomy includes excision of the involved fascia and the skin above it. A skin graft or a regional hand tissue flap is used to cover and close the wound. This is needed when the local skin is too tethered or diminished to cover the wound, especially in recurred cases or very severe cases (Ekerot 1995). It is also advisable to use skin grafts for closure in young patients with a high risk of recurrence due diathesis, even at the first operation. The rate of recurrence is much lower, being about 30%, and according to some studies no recurrence underneath a skin graft could be indicated (Brotherston *et al.* 1994). This method highly supports the theory that skin tension and mechanical stress may enhance the production of fibrosis, and furthermore also the stem cells which have the ability to change into fibroblasts and myofibroblasts to promote fibrosis. Stem cells are colonised in subcutaneous tissue and the dermis (Hidocha *et al.* 2011, Iqbal *et al.* 2012). By

eliminating the possible factors of recurrence, patients will assumebly have a longer disease-free time period. DD may recur at the edges of the skin graft, but rarely if never underneath it (McGrouther 1999).

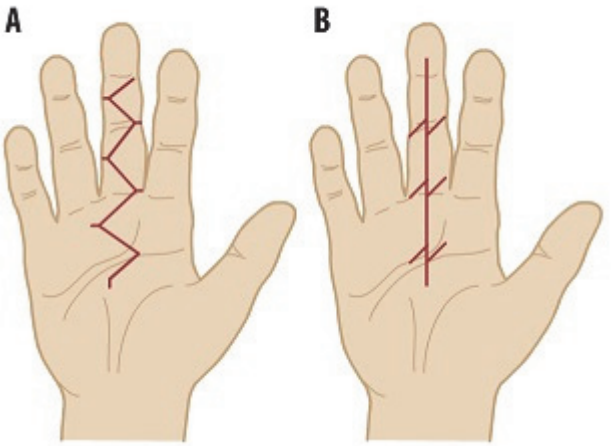

Fig. 8. Two different alternatives for making the skin insicions to avoid skin tension. (reprinted from Duodecim 2012/4 by permission).

Collagenase injection is an alternative choice besides the operative and non-operative options. The criteria for using it are the same as with surgery. Purified collagenase is an enzyme, derived from the bacterium Clostridium histolyticum, and it has collagenolytic properties (Starkweather *et al.* 1996). Collagenase in an adequate dose injected into the cord will rupture the cord formed of collagen type III after a period of incubation. After a certain time (minimum 24 h), the finger is manipulated to full extension and by this manner the cord is ruptured. Only elements consisting of the same type of collagen as DD, like tendons or ligaments, are at risk of a rupture too if accidentally injected. Thus, with experience with the injection technique, this is a possible but rare complication. More often skin tears, haematoma, pain, blisters, swelling, regional lymphadenopathy, pruritus, tenderness or oedema will emerge. Collagenase is considered an enzymatic fasciotomy (Gilpin et al. 2010).

The complications are mostly short-term, and therefore as an outpatient and noninvasive method, it is a reasonable treatment option for certain patients. The therapeutic results are also comparable with fasciectomy. Recurrence is still

somewhat under surveillance as are postoperative complications, which at the moment are considered mostly short-term. As a relatively fresh method, injection therapy has been studied meticulously and recurrence has been determined more accurately compared with conventional operative methods. Recurrence after collagenase in a treated MCP joint revealed no high recurrence with 0% (50 degrees or more contracture in the joint), and low recurrence (5 - 50 degrees of contracture) was found in about 33.3% in a follow-up of 12 months. With the PIP joints the results were not this good (Sood *et al.* 2014, Hurst *&* Badalamente 1999, Badalamente *&* Hurst 2000). The study by CORDLESS (= Collagenase option for reduction of Dupuytren long-term evaluation of safety study) has evaluated the five-year results of collagenase injection treatment to date. More than 20% and up to 39% of the treated MCP joints and 66% of the PIP joints recurred.

The overall recurrence rate was 47% and it is considered to be comparable with the rates after surgical treatments (Peimer *et al.* 2015).

3 Aims of the study

1. To evaluate whether there are differences in the palmar fascia between conventionally progressing DD and aggressively progressing DD and to find out if there is a factor or factors which could predict the behaviour of the disease.
2. To evaluate and compare the gene expression profile of the palmar fascia in Dupuytren's disease and a health one to reveal the pathogenesis of DD and to identify gene expression changes associated with the disease.
3. To evaluate if there is specific chromosomal imbalance indicated by changes in DNA copy numbers when comparing DD samples and healthy references.

4 Materials and methods

4.1 Increased cellularity and proliferation in Dupuytren's disease and correlation with the aggressive progression of the disease

The material was derived from the palmar fascia of twenty-one Dupuytren's disease patients and five control patients. The control patients had carpal tunnel disease, and a suitable piece of their palmar fascia was taken in connection with a normal carpal tunnel neurolysis. The operations were carried out by a hand surgeon or a resident according normal practice for limited fasciectomy and the criteria for the surgical protocol. Contracture of the MCPJ of more than 20 degrees or an annoying nodulus in the mid palm was the threshold for the surgery. The operations were done in Oulu University Hospital between October 1999 and October 2001 according the guidelines and approval of the Faculty of Medicine at the University of Oulu. The DD patients were divided into two groups, one with patients without previous history of DD surgery and the other group with a previous fasciectomy and a recurrence of the disease within about two years after the operation. This determination was a somewhat cautious estimation based on the medical history of the patient (the previous fasciectomy was done two years before the next one done in the study) or after two years of a follow-up period, after which the situation was checked by a surgeon by phone. This way all the operated patients in the study and possible recurrence were controlled. In the beginning there were seven patients with previous operations and 14 patients with no history of DD operation. During the follow-up the disease recurred with four patients originally in the non-aggressive group, and therefore in the end there were altogether 11 patients in the aggressive group and 10 patients in the conventional or non-aggressive group. The operations were done under a tourniquet and brachial plexus block or general anaesthesia.

Eighteen of the patients were men and three were females; no females were included in the non-aggressive group. There were three males and two females in the control group.

The mean age in the non-aggressive group was 63 years (range from 43 years to 81 years). In the aggressive group the mean age was 55 years (range from 37 years to 75 years).

4.1.1 Immunohistochemistry

The excised samples consisted of the entire diseased tissue, which was preserved immediately in the operating theatre in a formalin solution and delivered to the pathology department for further processing. The samples were cut, fixed and handled according to the standard routine.

All the specimens were first stained with HE staining to determine cellularity and the amount of collagen in the samples.The results were categorised into three groups depending on the cellularity and amount of collagen. (+ = minor, ++ = moderate, +++ = major)

Immunohistochemical stainings were performed on formalin-fixed and paraffin-embedded tissue sections. Then they were prepared for antibody application by deparaffination and rehydration. α-SMA antibody was retrieved by heating the tissue section in a sodium citrate buffer. For Ki-67 application the specimens were kept in a Tris/EDTA buffer, and for Factor

XIIIa, in trypsin at room temperature. For MSA and tenascin antibody they were incubated in pepsin at 37 °C. An ultra vision kit was used for anti-α-SMA, anti-MSA, anti factor XIIIa and tenascin. A non-biotinylated Envision kit was used for Ki-67. Haematoxylin was used for counterstaining and the stainings were divided into three categories according to the intensity of the positive reaction. The classification was - for no positive reaction, + for weak, ++ for moderate and +++ for a strong positive reaction.

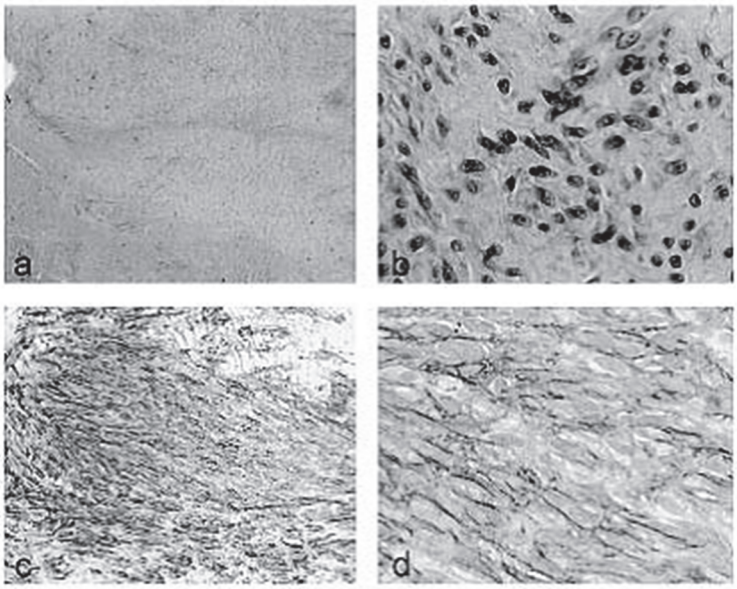

Fig. 9. a) Haematoxylin- eosinofil staining shows the nodular structure of Dupuytren's tissue (HE, original magnification × 20). b) HE staining shows the myofibroblasts in the nodules (HE, original magnification × 400). c) and alfa SMA (original magnification × 100). d) Spindle-shaped myofibroblasts express anti-α-SMA proteins in Dupuytren's contracture alfa SMA, original magnification × 400).

4.2 The discovery of ROR2 protein and myoglobin in Dupuytren's disease

The samples were recruited from the palmar fascia of 12 Dupuytren patients in normal limited fasciectomy. The operations were carried out by two hand surgeons between April 2002 and August 2003 in Oulu University Hospital. All the patients were men, and three healthy hand trauma patients were accepted as control patients. Their ages were 18, 19 and 31 years. The mean age of the Dupuytren patients was 59 years (range 31 yr to 78 yr). Nine of the patients had a recurred disease and eight of them also had a positive diathesis. The protocol for the surgery was according to the normal custom; 30 degrees of extension limitation in the MCPJ or an annoying mid palmar cord. The fasciectomy was performed under general anaesthesia and a tourniquet. The samples were then divided into two halves to be used for immunohistochemical staining and RNA and protein isolation. For the RNA and protein analysis, the samples were further divided into two more randomized pools

(D1 and D2) to improve the reliability of the study. Both of these pools contained six Dupuytren patients. There were three patients in the healthy control pool. The study protocol was approved by and according to the Ethical Committee of the Faculty of Medicine.

4.2.1 Isolation of total RNA and microarray analysis

A DNA microarray (DNA chip or biochip) is a collection of microscopic DNA spots attached to a solid surface. When the surface is glass or silicon, the chip is known as an Affy chip or an Affymetrix. A microarray measures the expression levels of a large number of genes simultaneously or the genotypes of multiple regions of a genome. A specific DNA sequence is included in the spot, which can be a short section of a gene or other DNA element to hybridize a cDNA or cRNA sample. A DNA microarray can be used to detect RNA (cDNA after reverse transcription) that may or may not be translated into proteins.

All the recruited samples were cut into 15-micron cryosections and pulverized. Total RNA was isolated from the samples by using a Trizol protocol (GIBCO-BRL, Gaithersburg, MD) and then purified with a Qiagen RNeasy Mini Kit (Qiagen, Hilden,

Germany) according to the manufacturer's instructions. For further analysis with a microarray, the RNA was pooled into a healthy pool and into the two diseased sample pools, D1 and D2. Affymetrix GeneChip expression Analysis Technical Manual (Affymetrix, Santa Clara, CA) was used in the experiment. Affymetrix HGU133A contains more than 22 000 probe sets for over 14 500 genes, which includes almost the whole human genome. The samples were hybridised once.

4.2.2 Analysis of protein expression with Western Blot and Dot Blot

The proteins were isolated from the samples according to the Trizol reagent protocol. The pooled samples (H, D1 and D2) were run on a 10% sodium dodecyl sulfate-polyacrylamide gel and then filtered with an Immobilon P filter (Millipore Corp, Bedford, Ma). To prevent non-specific binding, the samples were kept incubated with phosphate-buffered saline supplemented with 5% nonfat dry milk (Valio, Helsinki, Finland) for 60 min. The filter was first incubated overnight at room temperature with primary antibodies against myoglobin (Novocastra Laboratories Ltd., Newcastle upon Tyne, United Kingdom), then treated with

peroxidase-conjugated secondary antibody (DAKO A/S, Glostrup, Denmark), followed by incubating with ABComplex solution (DAKO) including avidin and biotinylated horseradish peroxidase. For final protein detection, ECL Western blotting reagents (Amersham Pharmacia Biotech, Buckinghamshire, United Kingdom) were used according to the product protocol.

Individual samples were analysed for myoglobin protein expression by dot blotting. One healthy and two DD samples had to be excluded because of low protein content. So the final dot blot was performed to two healthy and ten DD samples. The proteins (18 microg) were transferred onto Protran filters (Schleicher and Schuell GmbH, Dassel, Germany) and handled similarly to the Western filters above. The intensities of the dots were then measured using Scion Image software (Scion Corporation, Frederick, MA).

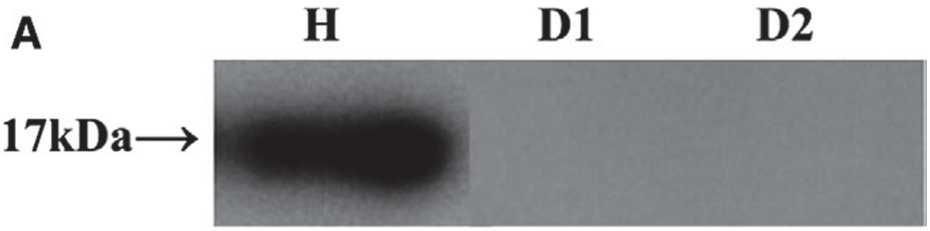

Fig. 10. (A) Myoglobin Western blot showing the expected 17 kDa band in the healthy sample (H) but not in either of the pooled diseased samples (D1 and D2).

4.2.3 Immunohistochemical analysis

Immunohistochemical staining was performed on 3-µm-thick formalin-fixed paraffin sections. The samples were then prepared for the primary antibodies of ROR2; first by deparaffination and rehydration, after which they were microwaved for 15 min (300 W) in 10 mM Tris/EDTA (ph9) for epitope retrieval. Endogenous peroxidase activity was inhibited by incubation with peroxidase blocking solution (ChemMate; DakoCytomation, Glostrup, Denmark) for 5 min. The primary antibodies of ROR2 (1:100) were incubated for 30 min at RT. A ChemMate Dako enVision Detection Kit (ChemMate) and Labvision Autostainer (Labvision Corp, Fremont, CA) were used for detection.

The sections were counterstained with Mayer's haematoxylin. A section incubated with a non-immune primary antibody was used as a negative control.

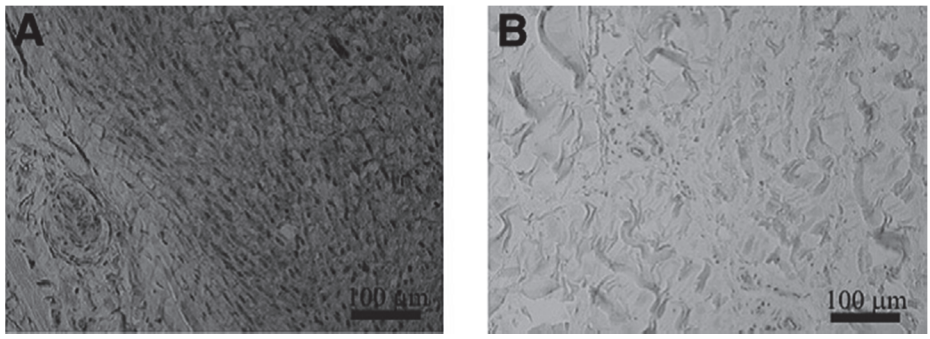

Fig. 11. Immunohistochemical analysis of ROR2 protein expression showed expression in diseased tissue (A) but not in healthy control tissue (B). 300 x magnification was used.

4.3 Gene copy number changes can be detected in Dupuytren's disease by array analysis

Palmar fascia samples were obtained from 18 patients with Dupuytren's disease between 2003 and 2006 in the University Hospital of Oulu by two hand surgeons without any specific patient preselection. All the patients had DD and were entitled to fasciectomy. The criteria and protocol to proceed with the fasciectomies were as in th previous studies, and with the approval of the Ethical Committee of the Faculty of Medicine. Only one of the diseased samples was from a female, the rest were male samples, and there were two samples taken from the same male patient (total 19 samples). Reference male and female DNAs were extracted from pooled peripheral blood cells of healthy donors. The mean age was 66.3 years (between 51 yr and 81 yr). Four males had diathesis. The samples were maintained in liquid N2 until DNA extraction was performed. Oligonucleotide aCGH and data analyses were conducted. CGH means comparative genomic hybridisation, which is a molecular cytogenetic method for analysing copy number variations in the DNA of a test sample compared with a reference sample, without the need for culturing cells. This is a quick and efficient techniquefor detecting either gains or losses in terms of either whole chromosomes or subchromosomal regions (a portion of a whole chromosome). A DNA microarray combined with CGH techniques gives a more specific form of array; the aCGH, which can allow a locus-by-locus measure of copy number variations with increased resolution even as low as 100 kilobases.

aCHG was performed using Agilent's 60-mer oligonucleotide-based microarray according to the manufacturer's instructions (Human Genome CGH Microarray Kit 44B; Agilent Technologies, Palo Alto, CA, USA). DNA from the samples was extracted via the normal protocol. After hybridisation and post-hybridisation washes the arrays were scanned with an Agilent laser confocal scanner and analysed using CGH Analytics software (version 3.1: Agilent Technologies).

5 Results

5.1 Cellularity, collagen, Alfa-SMA and Ki-67

Cellularity was examined in the Dupuytren samples and was found to be scant in four aggressive cases, moderate in five and abundant in two cases. It was noteworthy that cellularity was presented less in the non-aggressive cases than in the aggressive samples. In one case the amount was the same as in the controls, and in eight cases it was scant and in one case moderate and in one abundant.

The amount of collagen found was small in four aggressive cases and moderate in seven cases; there was no large amount of collagen in any of these cases. In non-aggressive cases, the amount of collagen was small in two cases, moderate in six cases and large in two cases. There was a small tendency for larger presentation of collagen in the non-aggressive group. In our study no macrophages or lymphocytes existed in the DD samples. No cells or any other findings were discovered in the normal palmar fascia of the control specimens.

The Dupuytren's tissue samples indicated more positive results in the immunohistochemical stainings for α-SMA. These spindle- shaped cells contain alpha smooth muscle actin, which can be detected and is therefore a reliable marker for myofibroblasts. The HE staining showed a greater intensivity of α-SMA within the aggressive population; there were two weak reactions, nine moderate and no strong reactions. On the contrary, the non-aggressive samples showed no reaction in three cases, weak reaction in four, moderate reaction in three cases and no strong reaction.

MSA (a muscle specific antigen) did not show a distinct difference between the samples. A minor tendency for larger presentation was detected in the aggressive sample group. There was no scant reaction, eight mild, three moderate and no strong reaction. In the non-aggressive group, one scant, nine mild and no moderate or strong reactions were found.

Ki-67 is a marker that indicates all cell proliferation, e.g., in cancers but also in various active cell actions like wound healing. This protein was found in cellular areas and was presented in aggressive samples; weakly in seven cases and moderately in four. There were no strong cases. In non-aggressive sample staining the protein was scantly found in four cases, mildly in four and moderately in two. As with the aggressive cases, there were no strong findings. The result indicates activity in the aggressive type of Dupuytren's tissue by its larger presentation.

There are four different tenascin members of the tenascin gene family. Tenascins are highly expressed during embryonic development, tissue repair and pathological situations such as chronic inflammation and cancer. Some of them have the ability to bind to fibronectin and block interaction with specific syndicans. Tenascins are extracellular matrix proteins. Their expression is located in the cellular areas. *(Midwood and Orend 2009)* In this study expression was weak in five aggressive samples and moderate in six, and there was no strong reaction. There were nine weak, one moderate and no strong reactions in the non-aggressive samples. A mildly stronger expression may be considered to exist in the aggressive group.

Factor XIII is a plasma transglutaminase enzyme of the blood coagulation system that crosslinks fibrin. It is a fibrin-stabilising factor and it requires calcium as a cofactor to be active. *(Byrnes et al. 2015*) Stellate dendrocytic cells are expressed in the areas surrounding Dupuytren nodules and they are positive for factor XIIIa. Dendrocytes are associated with fibrotic conditions. In aggressive cases there were four mild and seven moderate cases but no strong or scant cases. In non-aggressive cases there were similarly no scant or strong cases but eight mild and two moderate cases.

5.2 ROR2 protein and myoglobin

In an mRNA microarray analysis, 127 genes had a clear, at least eight-fold change in the expression profile in one pooled diseased sample from pools D1 and D2 (At the time of the study, the D1 pool consisted of diseased DD patients and the D2 pool consisted of aggressive-type DD patients) compared with healthy control samples. At least a four-fold change was indicated when the healthy control sample was compared with a diseased sample (D1 or D2). The alteration was either an increase or a decrease in the gene's expression. The genes were divided into different main groups according to the role or function of the gene in cell behaviour. The categorised groups were named as follows: immune response, angiogenesis, apoptosis, carbohydrate metabolism, cell adhesion and cell-matrix adhesion, cell cycle and proliferation, cell differentiation, transcription, development, signalling and signal transduction, protein synthesis and folding, oxygen transport, muscle-specific genes and other genes. A gene from every category was brought up, according to its potential contribution to the development of DD, and to emphasize the possible worthiness of some genes in the pathogenesis of DD. This was done especially when the category was large. If the genes were summarised into the same

paragraph, it could have diminished the importance of a single gene. Of the total 127 genes, 27 genes were present in both the healthy samples and the DD samples. Four belonged to the immune response category (S100A8, IL6, PTX3, SERPINA 1), two each were from the cell adhesion (DAM12, DSG2), others (RNU17D, COX6A2) and development categories (LIM, ADAMTS9), three each were from the signalling (CXCL2, CCL2, CALCA) and oxygen transport categories (HBA1, HBB, HBA2), and eleven were from the muscle category (TTN, KBTBD10, MB, NEB, MYBPC1, TTID, ACTC, DES, CASQ1, MYOZ1, TPM1) . There were genes that were totally absent from the diseased samples and vice versa. Most genes absent from DD tissue belonged to the muscle category, like myoglobin, although it was exceptionally present in both healthy and diseased samples. The other genes absent from the diseased samples belonged to the immune response category and others category (e.g. STIP1, AMPD1). Genes involved in carbohydrate metabolism exhibited the greatest changes in expression levels (e.g. ENO3, PGAM2).

5.2.1 Confirmation of microarray results at the protein level

Despite the gene expression, the genetic code does not always process further into the protein level. Although genes contain the information required to make a cell, it is the proteins that determine the shape and structure of the cells. A Western blot and immunohistochemistry were done to verify protein expression. With the Western blot it was confirmed that myoglobin protein was detected from the pooled healthy sample, but in neither of the pooled diseased samples. To further analyse the individual samples, a dot blot was performed using two healthy samples, five of the D1 samples and five of the D2 samples. The diseased samples showed varying but lower expression than the healthy samples with the strongest expression level. The dot blot therefore confirmed the findings of the Western blot.

Since ROR2 is linked to other hand deformities, it was selected for further study at the protein level. This was done by immunohistochemical staining, which revealed ROR2 protein expression in diseased samples but not in healthy samples, thus confirming the microarray results.

5.3 No chromosomal imbalance in the analysed samples

The study detected no chromosomal imbalance in any of the 19 samples. Thus far 46 gene expression alterations have been found in other studies, yet no common pattern has been discovered. Most of these expressions are involved in tissue

development and cellular differentiation (e.g., collagen degradation, ossification and myofibroblast differentiation). Twenty-four of the altered genes were found to be overexpressed (i.e., CSF1, TMSB10, COL5A2, WNT5A, RHOA, P4HA2, CTNNA1, RUNX2, HSPB1, LRRC17, PTN, SHANK2, ARCN1, CTN1, TUBA1A, DAD1, MMP2, TCF4, MAFB, GNAS, APP, TMSB4X, PRKX and GD11) and the remaining twenty-two were found to be underexpressed (i.e., ECHS1, CLEC3B, CD14, TNF, TFP12, EPB49, AKR1C1, CD81, LSP1, WNT11, GDP1, DCN, ALDH2, ATP7B, TNFSF12, CCL5, LAMA3, LIPE, CYB5A, ACP5, SYMPK and 1CAM1). In contrast, none of these genes was affected in our study by either DNA copy number gains or losses within the samples. (http://www.cangem.org/)

The CGH method can reveal gains and losses of genes of approximately 35 kilobases in the whole genome, and the indicated changes should be present in at least 50% of the cells. Alterations outside this resolution threshold can exist, and it is also possible that the frequency of aberrant cells was lower than 50% within the samples. The presence of nonrecurrent numerical/structural clonal and sporadic (non-clonal) numerical/structural changes in up to 50% of the metaphases in previous conventional cytogenetic and interphase *in situ* hybridisation studies has been analysed. This has been reported especially among +7 and +8. It is quite possible that these alterations are therefore most probably the result of abnormal growth of trisomic fibroblasts with a selective growth advantage over cytogenetically normal fibroblasts in the *in vitro* condition. Taking these observations into account and including the absence of DNA copy number changes in our study, it can be suggested that the presence of trisomies in DD is most probably related to a Darwinian model *in vitro* with a higher proliferative capacity over cells with a normal karyotype.

Table 4. Clinical characteristics of the palmar fascia of 18 DD patients studied by aCGH; M = male, F = female.

Sample no.	Age/Sex	Smoking	Hereditary trait	Recurrence	Patient features
DC1	M/68	-	-	-	healthy
DC2	F/62	-	-	-	coronary heart disease, hypertension
DC3	M/68	-	-	-	hypertension
DC4	M/70	-	brother	-	hypertension, bronchial asthma, hypercholesterolinemia,

Sample no.	Age/Sex	Smoking	Hereditary trait	Recurrence	Patient features
					DMII
DC5	M/71	-	-	+	healthy
DC6	M/73	-	-	-	hypertension
DC7	M/57	-	-	-	healthy
DC8	M/65	-	-	+	healthy
DC9	M/72	-	-	-	bronchial asthma, hypercholesterolinemia
					DMII, prostata hyperplasia
DC10	M/78	-	-	-	healthy
DC11	M/80	-	-	-	healthy
DC12	M/62	-	-	-	Charcot-Marie-toothdisease, MB Menier
DC13	M/51	+	-	-	coronary heart disease, hypercholesterolinemia
DC14	M/52	+	brother,sister,father	-	healthy
DC15	M/67	-	mother	-	healthy
DC16	M/81	+	-	-	hypertension
DC17	M/60	+	brothers	-	arrhytmia
DC18A	M/59	-	-	-	healthy
DC18B	M/59	-	-	-	healthy

5.4 Microarray analysis

To more thoroughly understand the pathogenesis of DD, investigations have been done to reveal the genetics behind the disease. Microarray analysis and reverse transcription polymerase chain reaction (RT-PCR) microarray analysis have been used to examine the expression of a focused panel of genes and more profoundly solve gene expressions found in DD tissue (Sheils *et al.* 2003, *Rehman* et al. *2008).*

A DNA micoarray is a collection of microscopic DNA spots attached to a solid surface. A probe (a short section of a gene or other DNA element) containing a specific DNA sequence is used to hybridise a cDNA or cRNA sample under high-stringency conditions. This process can be detected and quantified by detecting fluorophore-, silver- or chemiluminescense-labeled targets to determine the relative abundance of nucleic acid sequences in the target. This method uses the normal coupling process of nucleotides as a tool to reveal the forming proteins. RT-PCR, for instance, clones expressed genes by reverse transcribing the desired RNA into its DNA complement by the use of reverse transcriptase. Then the cDNA is amplified using traditional PCR. Different types of arrays can be used; for example,

when the probe is on a silicon surface it is called an affymetrix. Glass or plastic is also used as the surface to which the probes can attach *(Burgess 2001)*. The microarray technique presents data that only give expression ratios, and therefore only trends can be deduced from it. A large number of expression levels is obtained, and this vast amount of data can be difficult to analyse unless a large number of genes are to be elucidated simultaneously for comparison or the total genome is to be harvested. The method involves several distinct stages and analysis of the data before the final information is ready. Finally, to verify the results they must be further processed to detect the absolute gene expression levels and proteins. This is usually done with Northern blots, Western blots or immunohistochemical staining. On the other hand, the power of microarray data indeed comes from analysis of not just one but several subjects and identification of common patterns of gene expression (Pan *et al*. 2003). Microarrays differ in fabrication, workings, accuracy, efficiency and cost. Some are qualitative and some quantitative in the manner of detecting gene expressions.

6 Discussion

In this study we wanted to find and examine elements in DD tissue that could help predict the nature of the disease in affected patients. Even recently it has been proposed that it can be demanding to point out histological differences between aggressive and conventional DD tissue (Degree *et al.* 2009, Wilbrand *et al. 2003)*. The problem stands especially with younger males who tend to develop an aggressively behaving disease with a strong tendency to recur in a short time despite meticulous surgical techniques. Recurrence can take place within a few years compared with the normal time period of several years and extension lag. All too often these patients seem to form contractures that are difficult to treat finally. With this kind of medical history, the probability of multiple operations and treatments, in the end, promises no good results.

We started by collecting DD samples and employing immunnohistochemical stainings to compare the results between diseased, healthy control and aggressively recurred samples. We did get results, as the study reveals. Immunohistochemical staining is simple to carry out. It is also, if appropriately implemented, quite reliable and a relatively cheap and very common procedure for examining samples. Histological alterations have been confirmed by other studies too, concerning differences between aggressively and conventionally behaving DD (Balaguer *et al.* 2009).

To continue, gene and protein expressions were examined for possible differences between healthy and DD samples. The method chosen was the mRNA microarray technique, because of the vast information it delivers. This on the other hand, hides the dilemma it includes. It is always a decision to justify which results you consider meaningful and important in the end. ROR2 is undoubtedly a significant discovery because of its close relation to the Wnt pathway. Via this pathway it has an impact on different cell processes. Even though no chromosomal imbalances were found in this study, it still revealed that these possible changes in genomes do not require large amounts of cells to happen. More accurate microarrays have detected these alterations and confirmed that they require only a minimal amount of cells to enable chromosomal imbalances.

The number of patients and samples derived can be considered not large according to some opinions. This has to be considered. The sample material was sought to be relatively optimal; the control samples were from patients critically chosen to be as healthy as possibly, so that no confusing elements could disturb the results. On the contrary, the diseased samples were chosen to be average

presentations of DD patients. Some logistical problems with sample transportation unexpectedly emerged. So, a few of the original samples were lost for this reason, diminishing the total amount of material. In the end, some of the patients declined to take part in the study for different reasons.

We were able to show differences between conventional and aggressive-type DD tissues. Even with the present studies, despite microarray techniques, it has been difficult to expose the elements behind aggressively recurring DD. On the contrary, plenty of epidemiological and clinical studies have been published about the aggressiveness of the disease and the possible issues predicting it (Hidocha *et al.* 2006, Tubiana 1999, Couto-Gonzalezet *et al.* 2010, Degreef *et al.* 2009, Wilbrand *et al.* 2003).

6.1 Increased cellularity and α-SMA of the DD samples

Stronger expression of alpha-SMA in aggressive DD tissue was indicated in our study along with greater cellularity. Although the recruited samples were limited in number, the same results have recently been discovered and confirmed by a study from Lukas A Holzer in 2013 (Holzer *et al.* 2013). In DD, histological studies are mainly small randomised samples, which does not necessarily diminish the value of the result when the studies have been meticulously and properly carried out, and therefore they are comparable with epidemiological studies with a larger amount of material. Nonetheless, the limited number always raises a question of valid generalisation of the results. Still, many similar studies with same the information support each other and the conclusion. The amount of alpha-SMA was shown to be clearly higher in active DD tissue than in nonactive residual DD tissue along with the expression of VEGF and HIF-1 alpha. These seem to behave in the same manner as alpha-SMA according a study by Latha Satish (Satish 2013). Alpha-SMA was investigated in DD samples in the involutional phase; nonetheless the main observation was that alpha-SMA reflects activity in contracting tissue. It can be assumed that the more contraction, the more advanced the disease is also clinically, and therefore the tissue can be considered a more aggressive form. Alpha-SMA is a marker for myofibroblasts and therefore it is logical that a large amount of it will raise the amount of cellularity in the tissue. Myofibroblasts are the core for contraction and in enhancing the disease (Türker *et el* 2013).

Concerning the other results in the study of the first stage, clear but vaguer results of increased expressions of tenascin and Factor XIIIa were seen. These expressions were moderate in our results and therefore not treated further in the

study. According to other studies, higher levels of tenascin have been observed in more aggressive forms of DD. It is connected to fibrotic diseases and cell migration (Shih *et al.* 2009, 2012). Although not as evident, the amount of tenascin still did appear to be expressed in the same manner in our study. Ki-67 showed more expression and a tendency to be present in aggressive tissue, also predicting recurrence of DD. This result did support other studies (Vujic *et al.* 2014).

6.2 Expression of ROR2 protein

Ror2 was considered to be the most important discovery in the study of the second stage; it revealed differences between normal and DD tissue by means of a microarray. We were able to reveal ROR2 protein in Dupuytren tissue and therefore reinforce the presence of this protein in pathologic fibrotic conditions such as DD for the first time. It has been suggested that ROR2 and the Wnt5a pathway have a certain role in controlling the Wnt canonial pathway by down- or up-regulating by working as an antagonist or agonist (Minami *et al.* 2010).

The canonial pathway has been revealed and connected firmly to fibrotic conditions in many studies (Bowley *et al.* 2007, Degreef *et al.* 2009, Degreef *et al.* 2009**).** ROR2 regulates the canonial Wnt pathway at the level of TCF/LEF-mediated transcription. Hypoxia initiates a shift of ROR1 to a more invasive ROR2 phenotype in melanoma, for example. ROR2 contributes to the invasiveness of cells, respectively (O'Connell *et al.* 2013). Hypoxia is considered to promote DD, so this aspect of a microenvironmental change and a lack of oxygen can guide the phenotypic plasticity of cells via Wnt pathways. A weakness in our study is that we did not investigate the role of ROR2 and its possible connection to the aggressiveness of DD. Furthermore, in malignant diseases like osteosarcoma, Wnt5a/Ror2 induces invasive properties by inducing matrix metalloproteinase 13 (MMP-13) (Enomoto *et al.* 2009). Increased amounts of metalloproteinases (MMPs) have been discovered in DD samples, among these MMP13 (Johnston *et al.* 2008). ROR2 acts as a receptor to the Wnt pathway. Wnt5a is well expressed in human inflammatory diseases, as in keloid conditions and abnormal wound healing. In the study by Shinichi Igota et al., treatment of normal and keloid tissue fibroblasts with recombinant Wnt5a protein increased the level of total Beta-catenin. This may indicate that Wnt5a is a potential trigger for the canonial pathway (Igota *et al.* 2013). Furthermore, this will open the possibility to approach the key of the pathology of DD in a new manner and through a new connection. In mouse studies, ROR2 induces renal fibrosis by epithelial-to-mesenchymal transition of

cells via the Wnt5a pathway. The induced fibrosis is linked to the induced amount of MMP-2, respectively. MMP-2 is associated with the transition phase of the epithelial-to-mesenchymal process and Wnt5a-ROR2 seems to work via it (Li *et al.* 2013). A connection to fibrosis has been detected.

Myoglobin, the protein found in muscle tissue, has the ability to bind iron and oxygen and is therefore similar and related to haemoglobin, which works through same function in red blood cells. Myoglobin is not generally accepted as being found in smooth muscle. In our study, myoglobin expression was reduced from 52.0- to 90.5-fold. Interestingly, the sample pool containing recurrent DD samples showed stronger expression of myoglobin, which confirms the result of the first study. The smooth muscle myosin in DD is proposed to be of different quality than conventional smooth muscle myosin or laminin; although it is categorised in a non-muscle group, it is able to contract (Tomasek *et al.* 1986). The result reflects this: DD tissue contains some levels of myoglobin; recurrent tissue more abundantly.

6.3 No chromosomal imbalance attributed to DNA copy number changes

In this study for the very first time we used the 44K oligonucleotide-based array comparative genomic hybridisation to detect any gene number changes. This method accurately reveals the number of changes of approximately 35 kilobases in the whole genome when at least 50% of the cells have the alteration. We were not able to find any gains or losses of genes to reinforce the claim of copy number changes responsible for DD, when the change is not present in the majority of the DNA sequences. The same result was proposed in a study by Michael Pampel *et al. (*2010) with a single nucleotide polymorphism array analysis that revealed the chromosomal locus of hereditary gingival fibromatosis (Pampel *et al.* 2010). Moreover, the chromosomal imbalances found in previous studies may be caused by trisomies related to a Darwinian in the culturing and a selection of trisomy-carrying cells with a higher proliferative capacity over normal controls. Trisomies occur most often in chromosomes 7 and 8 (Dal Cin *et al.* 1999, Bonnici *et al.* 1992). This so-called trisomy theory has been the explanation for the genetic imbalance in DD so far. Since this, other studies have been done with more higher-resolution genome-wide screening to provide more number variations and their associations with DD (Shih *et al.* 2010, Shih *et al.* 2012). Gene copy number variations have been detected in 10q22, 16p12.1 and 17p12. Nine other chromosomal regions including 6p21 (HLA locus) may contain gene copy polymorphisms in DD (Shih

et al. 2010, Michou *et al.* 2012). In the light of these new studies with information from larger arrays, we are aware now that copy number alterations exist.

By now it has been evidenced that DD includes genetic alterations compared with healthy controls. The connection between DD and changes in chromosomes number 6, 11 and 16 has been proved by genome-wide studies (Michou *et al.* 2012). It has also been admitted that it is an inheritable disorder. The problem in resolving the disease is partly because of the lack of firm information on the possible pattern of inheritance and where the possible genetic change or mutation takes place in the cell. Autosomal dominance with variable penetrance is considered the most likely mode of inheritance, but whether it is a complex oligogenic or a simple monogenic Mendelian disorder is still somewhat under debate (Michou *et al.* 2012, Wurster-Hill *et al.* 1988, Burge P 1999). Clinically, the difficulty of studying the disease arises from the fact that the incidence of Dupuytren's disease grows with age and it can emerge sporadically and exclude a generation to express itself again in the next generation in the future. What's more, the limited number of members affected in the family also complicates the research. The disease seems to behave somewhat unforeseeably (Bobinski *et al.* 2004). To recall family history requires good and precise documentation and gathering of knowledge. Other occurring hand deformities may confuse the truth. It has been demonstrated that mutations such as duplications or deletions, which both impact the number of the human chromosomes, or even qualitative changes as aberrations, will cause abnormal protein expressions and lead to anomalies, different diseases and syndromes (Choy *et al.* 2010).

DD is a difficult target to study despite the new gene array technology. Mostly because it still remains without a confirmed pattern of genetic inheritance. No single gene has been detected to cause the disease. Whether it can be actually divided into two different subgroups—the inherited type and the sporadic type caused by mutations or possibly triggered by different traumas and repetitive physical stress of the hand or a systemic disease like diabetes mellitus or some factor—is still not recognised. After resolving the aetiology we will know if these are even to be separated.

Microarrays have evidenced that there are hundreds of genes with significantly higher expression levels and even more than a thousand genes with lower gene expressions in DD compared with normal controls. Many of the identified genes are, in principal, potential candidates for triggering DD. The increased fibrosis and cell adhesions because of the lack or because of the induced affect of this factor or factors in control, is the key in the disease (Forrester *et al.* 2013).

Depending on the microarray technology or application, the resolution and sensitivity to elucidate genes varies. Furthermore, the data obtained by microarray depends, e.g., on the number of probes under examination. All abnormalities such as reciprocal translocations, inversions or ring chromosomes do not affect gene copy number, but they measure the relative amount of mRNA expressed in experimental conditions. Yet, difficulties exist in correlating changes between protein expression and mRNA expression even when the complete genome is known. It is possible that in some phases of the disease mRNA expressions are stronger than the expected protein expression it represents in the same phase.

Despite the knowledge of expression profiles of DD to date, more valid information may still be held in the introns of genes (microRNA) or in qualitative changes in addition to quantitative changes in chromosomes. Probably the aetiology of DD is a result of simultaneous occurrences of aberrations in several networks containing both internal and environmental factors.

7 Conclusions

On the basis of the present study, the following codutions can be made:

1. Alpha-SMA, a marker for myofibroblasts, was more frequently expressed in the aggressive type of tissue of DD than in conventional DD tissue. Myofibroblasts are the main cell type in DD and especially present in the proliferative stage. More vast representation of it in samples was connected to the aggressiveness of the disease. Ki-67, the actual marker for proliferation, was also more widely represented in aggressive tissue samples. Some difference, with vaguely stronger expression of tenascin, was found in the aggressive DD sample group in our study. High cellularity in the DD samples clearly predicted the aggressiveness and recurrence of DD.
2. It was indicated that more than 2000 genes were altered in the comparison between DD and healthy reference samples, and there was at least an eight-fold expression difference in 127 genes. This result was to be verified to define the actual protein level, and genes considered to be interesting, such as ROR2 and myoglobin, were chosen. ROR2 plays an important part in the Wnt5a pathway through which it effects and controls many cell functions and structures. ROR2 inevitably was richly presented in DD tissue also at the protein level. Muscle-related genes were the largest category with the greatest changes among differentially expressed genes. Myoglobin was detected in both healthy and DD samples, but showed a 90-fold down-regulation in DD samples.
3. No chromosomal gains or losses could be detected with 44K oligonucleotide-based array comparative genomic hybridisation. Despite this we can not rule out the possibility that such alterations can exist outside the solution threshold used in the study nor that the frequency of aberrant cells was lower than 50% within the samples.

References

Abe Y, Rokkaku T, Ofuchi S, Tokunaga S, Takahashi K & Moriya H (2004) An objective method to evaluate the risk of recurrence and extension of Dupuytren's disease. J of Hand Surg 29B(5): 427-430.

Abe Y, Rokkaku T, Kuniyoshi K, Matsudo T & Yamada T (2007) Clinical results of dermofasciectomy for Dupuytren's disease in Japanese patients. J Hand Surg 32(4): 407-10.

Al-Qattan MM (2006) Factors in the pathogenesis of Dupuytren's Contracture. J Hand Surg 31A : 1527-1534

Alioto RJ, Rosier RN, Burton RI & Puzas EJ (1994) Comparative effects of growth factors on Dupuytren's tissue and normal palmar fascia. J Hand Surg 19A: 442-452.

Anderson ER, Burmester JK & Caldwell MD (2012) Evaluation of a mitochondrial DNA mutation in maternally inherited and sporadic cases of Dupuytren disease. Clin Med Res 10(3):122-6.

Andrew JG, Andrew SM, Ash A & Turner B (1991) An investigation into the role of inflammatory cells in dupuytren's disease. J Hand Surg 16B: 267-271.

Anwar MU, Al Ghazal SK & Boome RS (2007) Results of surgical treatment of Dupuytren's disease in women: A review of 109 consecutive patients. J Hand Surg 32A: 1423-1428.

Badalamente MA, Sampson SP, Hurst LC, Dowd A, Misaka K & Brook S (1996) The role of transforming growth factor Beta in Dupuytren's disease. J Hand Surg 21A: 210-215.

Badalamente MA & Hurst LC (1999) Nonoperative Treatment of Dupuytren's disease. Hand Clinics Vol15 (1): 97-107.

Badalamente MA & Hurst LC (2002) Collagen as a clinical target: nonoperative treatment of Dupuytren' disease. J Hand Surg 27(5): 788-98

Badalamente MA & Hurst LC (2007) Efficacy and safety of injectable mixed collagenase subtypes in the treatment of Dupuytren's contracture. J Hand Surg 32(6): 767-74.

Badalamente MA & Hurst LC (2000) Enzyme injection as nonsurgical treatment of Dupuytren's disease. J Hand Surg 25A: 629-636.

Bains W (2003) Vasoprotective VEGF as a candidate for prevention of recurrence of fibrotic diseases such as Dupuytren's contracture. Med Hypotheses 60(6): 793- 996.

Balaguer T, David S, Ihrai T, Cardot N, Daideri G & Lebreton E (2009) Histological staging and Dupuytren's disease recurrence or extension after surgical treatment: a retrospective study of 124 patients. J Hand Surg Eur 34(4): 493-6.

Ball C, Pratt AL & Nanchahal J (2013) Optimal functional outcome measures for assessing treatment for Dupuytren's disease: a systematic review and recommendations for future practice. MMC Musculoskeletal Disorders 14:131.

Bayat A, Watson JS, Stanley JK, Ferguson MW, Ollier WE (2003) Genetic susceptibility to dupuytren disease: association of Zf9 transcription factor gene.Plast Reconstr Surg 111(7): 2133-9.

Becker K, Tinschert S, Lienert A, Bleuler PE, Staub F, Meinel A, Röβler J, Wach W, Hoffmann R, Kühnel F, Damert H-G, Nick H-E, Spicher R, Lenze W, Langer M, Nürnberg P & Hennies HC (2014) The importance of genetic susceptibility in Dupuytren's disease Clin Genet 87: 483-487.

Benson LS, Williams CS & Kahle M (1998) Dupuytren's Contracture. J Am Acad Orthop Surg 6: 24-35.

Betz N, Ott OJ, Adamietz B, Sauer R, Fietkau R & Keilholz L (2010) Radiotherapy in early-stage Dupuytren's contracture.Long-term results after 13 years. Strahlenther Onkol 186(2): 82-90.

Bianchi E, Taurone S, Bardella L, Signore A, Pompili E, Sessa V, Chiappetta C, Fumagalli L, DI Gioia C, Pastore FS, Scarpa S & Artico M (2015) Involvement of pro-inglammatory cytokines and growth factors in the pathogenesis of Dupuytren's contracture: a novel target for a possible future therapeutic strategy? Clin Sci (Lond) 1:129(8): 711-20.

Birks M, Bhalla A (2013) Dupuytren'sdisease. Orthopedics III: UpperLimb Surgery 31:4: 177-180.

Bisson MA, McGrouther DA, Mudera V, & Grobbelaar AO (2003) The different characteristics of Dupuytren's disease fibroblasts derived from either nodule or cord: expression of alpha-smooth muscle actin and the response to stimulation by TGF- beta1. J Hand Surg 28B(4): 351-356.

Bobinski R, Olczyk K, Wisowski G & Janusz W (2004) Genetic aspect of Dupuytren's disease. Wiad Lek 57:(1-2): 59-62.

Bonnici AV, Birjandi F, Spencer JD, Fox SP & Berry AC (1992) Chromosomal abnormalities in Dupuytren's contracture and carpal tunnel syndrome. JHand Surg 17(3): 349-355.

Bower M, Nelson M & Gazzard BG (1990) Dupuytren's Contractures in patients infected with HIV. Br Med J 300:164-5.

Bowley E, O'Gorman DB & Gan BS (2007) Beta-catenin signaling in fibroproliferative disease. J Surg Res 138(1): 141-50.

Brantjes H, Barker N, van Es J & Clevers H (2005) TCF: lady justice casting the final verdict on the outcome of Wnt signalling. Biol Chem 383 (2): 255-61.

Brenner P, Krause-Bergmann A & Van VH (2001) Dupuytren contracture in North Germany Epidemiological study of 500 cases. Unfallchirurg 104(4): 303-11.

Brickley-Parsons D, Glimcher MJ, Smith RJ, Albin R & Adams JP (1981) Biochemical changes in the Collagen of the palmar fascia in patients with Dupuytren's Disease. J Bone Joint Surg 63A:787-797.

Brotherston TM, Balakrishnan C, Milner RH & Brown HG (1994) Long term follow-up of dermatofasciectomy for Dupuytren's contracture. Br J Plast Surg 47(6): 440-3.

Brown JJ, Ollier W, Thomson W & Bayat A (2008) Positive association of HLA-DRB1*15 with Dupuytren's disease in Caucasians. Tissue Antigens 72(2): 166-70.

Bulstrode NW, Bisson M, Jemec B, Pratt AL, McGrouther DA & Grobbelaar AO (2004) A prospective randomized clinical trial of the intra-operative use of 5-Fluorouracil on the outcome of Dupuytren's disease. J Hand Surg 29B(1):18- 21.

Bulstrode NW, Jemec B & Smith PJ (2005) The complicationsof Dupuytren's contracture Surgery. J Hand Surg Am 30A: 1021-1025.

Burge P, Hoy G, Regan P & Milne R (1997) Smoking, alcohol and the risk of Dupuytren's contracture. J Bone Joint Surg 79B(2): 206-10.

Burge P (1999) Genetics of Dupuytren's disease. Hand Clin 1(1): 63-71.

Burge PD, Proud G, Lawson IJ, McGeoch KL & Miles JNV (2007) An assessment of the effects of exposure to vibration, smoking, alcohol and diabetes on the prevalence of Dupuytren's disease in 97 537miners. J Hand Surg 32B(4): 400-406.

Burgess JK 2001. Gene expression studies using microarrays. Clin Exp Pharmacol Physiol 28(4):321-8.

Byrnes JR, Duval C, Wang Y, Hansen CE, Ahn B, Mooberg MJ, Clark MA, Johnsen JM, Lond ST, Lam WA, Meijers JC, Ni H, Ariëns RA & Wolberg AS (2015) Factor XIIIa-dependent retention of red blood cells in clots is mediated by fibrin alfa-chain crosslinking.Blood 126(16):1940-8.

Cai SX, Liu AR, He HL, Chen QH, Yang Y, Guo FM, Huang YZ, Liu L & QB (2014) Stable genetic alterations of Beta-Catenin and ROR2 regulate the Wnt pathway, affect the fate of MSCs. J Cell Physiol 299: 791-800.

Capstick R, Bragg T, Giele H & Furniss D (2013) Sibling recurrence risk in Dupuytren's disease. J Hand Surg 38B(4): 424-9.

Chammas M, Bousquet P, Renard E, Poirier J-L, Jaffiol C & Allieu Y (1995) Dupuytren's Disease, Carpal tunnel syndrome, Trigger finger, and Diabetes mellitus. J Hand Surg 20A: 109-114.

Chansky HA, Trumble TE, Conrad EU 3rd, Wolff JF, Murray LW, Raskind WH (1999) Evidence for a polyclonal etiology of palmar fibromatosis. J Hand Surg Mar; 24(2): 339-44.

McCarty S, Syed F & Bayat A (2010) Role of the HLA system in the pathogenesis of Dupuytren' disease. Hand (N Y) 5(3): 241-50.

Chinyama CN, Roblin P, Watson SJ, Evans DM (2000) Fibromatoses and related tumors of the hand in children. A clinicopathlogic review. Hand Clin 16(4): 625-35, ix.

Choy KW, Setlur SR, Lee C & Lau TK (2010) The impact of human copy number variation on a new era of genetic testing. BJOG 117(4): 391-8.

Citron N & Hearnden A (2003) Skin tension in the etiology of Dupuytren's disease; A prospective trial. J Hand Surg 28B (6): 528-530.

O'Connell MP, Marchbank K, Webster MR, Valiga AA, Kaur A, Vultur A, Li L,Herlyn M, Villanueva J, Liu Q, Yin X, Widura S, Nelson J, Ruiz N, Camilli TC, Indig FE, Glaherty KT, Wargo JA, Frederick DT, Cooper ZA, Nair S, Amaravadi RK, Schuchter LM, Karakousis GC, Xu W, Xu Weeraratna AT (2013) Hypoxia induces phenotypic plasticity and therapy resistance in melanoma via the tyrosine kinase receptors ROR1 and ROR2. Cancer Discov 3(12): 1378-93.

Connelly TJ (1999) Development of Peyronie's and Dupuytren's diseases in an individual after single episodes of trauma: a case report and review of the literature. J Am Acad Dermatol. 41(1): 106-8.

Cordova A, Tripoli M, Corradino B, Napoli P & Moschella F (2005) Dupuytren's contracture: An update of bimolecular aspects and therapeutic perspectives. J Hand Surg 30B (6): 557-562.

Couto-Gonzalez I, Brea-Garcia B, Taboada-Suárez A & González-Álvarez E (2010) Aggressive Dupuytren's diathesis in a young woman. BMJ Case Rep 20:1-5.

Cutts S, Dias R & Rajaratnam V (2005) Dupuytren's disease – A review. J Orthopaedic Nursing 9: 230-234.

Dal Cin; DeSmet L, Sciot R, Van Damme B & Van den Berghe H (1999) Trisomy 7 and trisomy 8 in dividing and non-dividing tumor cells in Dupuytren's disease. Cancer Genet Cytogenet 15; 108(2): 137-40.

Degreef I, De Smet L, Sciot R, Cassiman J-J & Tejpar S (2009) Beta-Catenin over expression in Dupuytren's diseases unrelated to disease recurrence. Clin Orthop Relat Res 467: 838-845.

Degreef I, De Smet L, Sciot R, Cassiman JJ & Tejpar S (2009) Immunohistochemical evidence for Zic1 coexpression with beta-catenin in the myofibroblast of Dupuytren disease. Scand J Plast Reconstr Surg Hand Surg 43(1): 36-40.

Descatha A, Bodin J, Ha C, Goubault P, Lebreton M, Chastang JF, Imbernon E, Leclerc A, Goldberg M & Roquelaure Y (2012) Heavy manual work, exposure to vibration and Dupuytren's disease? Results of a surveillance program for musculoskeletal disorders. Occup Environ Med 69(4): 296- 9.

Descatha A, Jauffret P, Chastang J-F, Roquelaure Y & Leclerc A (2011) Should we consider Dupuytren's contracture as work-related? A review and meta-analysis of an old debate. BMC Musculoskelet Disord. 12(96): 1-10.

Dias JJ & Braybrooke J (2006) Dupuytren's contracture: An audit of the outcomes of surgery. J Hand Surg 31B(5): 514-521.

Dibenedetti DB, Nguyen D, Zografos L, Ziemiecki R & Zhou X (2011) Prevalence, incidence, and treatments of Dupuytren's disease in the United States: results from a population-based study. Hand (N Y) 6(2): 149-58.

Dietmar U, Franziska U, Andrzej P & Norbert P (2009) Expression of matrix metalloproteinases and their inhibitors in cords and nodules of patients with Dupuytren's disease. Arch of Orthop &Trauma Surg 129(11): 1453-1459.

Dolmans GH, de Bock GH & Werker PM (2012) Dupuytren diathesis and genetic risk. J Hand Surg 37A(10): 2106-11.

Donato RR & Morrison WA (1996) Dupuytren's disease in the feet causing flexion contractures in the toes. J Hand Surg 21B(3): 364-6.

Eaton C (2014) Evidence-based medicine: Dupuytren contracture. Plast Reconstr Surg 133(5): 1241-51.

Ebskov LB, Boeckstyns MEH, Sorensen AI & Soe-Nielsen N (2000) Results after surgery for severe Dupuytren's contracture: does a dynamic extension splint influence outcome? Scand J Plast Reconstr Hand Surg 34: 155- 160.

Elliot D & Ragoowansi R (2005) Dupuytren's disease secondary to acute injury, infection or operation distal to the elbow in the ipsilateral upper limb - a historical review. J Hand Surg 30B: 148-156.

Ekerot L (1995) The distally-based dorsal hand flap for resurfacing skin defects in Dupuytren's contracture. J Hand Surg 20B(1): 111-114.

Enomoto M, Hayakawa S, Itsukushima S, Ren DY, Matsuo M, Tamada K, Oneyama C, Okada M, Takumi T, Nishita M & Minami Y (2009) Autonomous regulation of osteosarcoma cell invasiveness by Wnt5a/Ror2 signaling. Oncogene 10;28(36): 3197-208.

Erdmann MW, Quaba AA & Sommerlad BC (1995) Epitheloid sarcoma masquerading as Dupuytren's disease. Br J Plast Surg 48(1): 39-42.

McFarlane RM (1991) Dupuytren's disease: Relation to work and injury. J Hand Surg 16A: 775-9.

Felici N, Marcoccio L, Giunta R, Haerle M, Leclercq C, Pajardi G, Willbrand A, Georgescu V & Pess G (2014) Dupuytren Contracture recurrence project: Reaching consensus on a Definition of recurrence. Handchir Mikrochir Plast Chir 46: 350- 355.

Fitzgerald AMP, Kirkpatrick JJR & Naylor IL (1999) Dupuytren's disease: the way forward? J Hand Surg 24B(4): 395-399.

Forrester HB, Temple-Smith P, Ham S, de Kretser D, Southwick G & Sprung CN (2013) Genome-wide analysis using exon arrays demonstrates an important role for expression of extra-cellular matrix, fibrotic control and tissue remodeling genes in Dupuytren's disease. PLoS One Vol 8(3): 1-16.

Foucher G, Lequeux C, Medina J, Garcia RN & Nagel D (2001) A congenital hand deformity: Dupuytren's disease. J Hand Surg 26A: 515-517.

Geoghegan JM, Forbes J, Clark DI, Smith C & Hubbard R (2004) Dupuytren's disease risk factors. J Hand Surg 29B(5): 423- 6.

Gilpin D, Coleman S, Hall S, Houston A, Karrasch J & Jones N (2010) Injectable collagenase Clostridium histolytic: a new nonsurgical treatment for Dupuytren's disease. J Hand Surg 35A(12): 2027-38.

Godtfredsen NS, Lucht H, Prescott E, SØrensen TIA & GrØnbaek M (2004) A prospective study linked both alcohol and tobacco to Dupuytren's disease. J Clin Epidemiol 57(8): 858-63.

Golbar HM, Izawa T, Juniantito V, Ichikawa C, Tanaka M, Kuwamura M & Yamate J (2013) Immunohistochemical characterization of macrophages and myofibroblasts in fibrotic liver lesions due to Fasciola infection in cattle. J Vet Med Sci 31;75(7): 857-65.

O'Gorman DB, Wu Y, Seney S, Zhu RD & Gan BS (2006) Wnt expression is not correlated with beta-catenin dysregulation in Dupuytren's disease. J Negat Results Biomed 30;5: 13.

McGrouther DA (1999) Green's operative Hand Surgery/17 fourth edition. 1: 563-591.

Gudmundsson KG, Jónsson T & Arngrimsson R (2013) Association of Morbus Ledderhose with Dupuytren's contracture. Foot Ankle Int 34(6):841-5.

Gudmundsson KG, Arngrimsson R, & Jónsson T (2001) Eighteen years follow-up study of the clinical manifestations and progression of Dupuytren's disease. Scand J Rheumatol 30: 31-4.

Gudmundsson KG, Arngrimsson R & Jónsson T (2001) Dupuytren's disease, alcohol consuption and alcoholism. Scand J Prim Health Care Sep; 19(3):186-90.

Gudmundsson KG, Arngrimsson R, Sigfússon N, Björnssón A & Jónsson T (2000) Epidemiology of Dupuytren's disease: clinical, serological and social assessment. The Reykjavik study. J Clin Epidemiol 1;53(3): 291-6.

Gudmundsson KG, Arngrimsson R, Sigfússon N & Jónsson T (2002) Increased total motrality and cancer mortality in Dupuytren's disease; a 15- year follow –up study. J Clin Epidemiol 55 (1): 5-10.

Guo H, Nagy T & Pierce M (2014) Post-translational glycoprotein modifications regulate colon cancer stem cells and colon adenoma progression in Apc(min/+) mice through altered Wnt receptor signaling. J BIol Chem 7;289(45): 31534- 49.

Hart M, & Hooper G (2005) Clinical associations of Dupuytren's disease. Postgrad Med J 81(957): 425- 428.

Hidocha S, Iqbal SA, Farhatullah S, Paus R & Bayat A (2011) Characterization of stem cells in Dupuytren's disease. Br J Surg 98(2): 308-15.

Hindocha S, Stanley JK, Watson S & Bayat A (2006) Dupuytren's diathesis revisited: Evaluation of prognostic indicators for risk of disease recurrence. J Hand Surg 31A: 1626-1634.

Hinz B, Myofibroblasts (2015) Exp Eye Res (15): 00228-9.

Holzer LA, Cör A, Pfandlsteiner G & Holzer G (2013)

Expression of VEGF,its receptors, and HIF-1alfa in Dupuytren's disease. Acta Orthop 84(4): 420-5.

Honner R, Lamb DW & James JI (1971) Dupuytren's contracture. Long term results after fasciectomy. J Bone Joint Surg 53(2)240-6.

Houghton S, Holdstok G, Cockerell R & Wright R (1983) Dupuytren's contracture, chronic liver disease and IgA immune complexes. Liver 3(4): 220-4.

Howard JC, Varallo VM, Ross DC, Faber KJ, Roth JH, Seney S & Gan BS (2004) Wound healing-associated proteins Hsp47 and fibronectin are elevated in Dupuytren's contracture. J Surg Res 117(2): 232-8.

Howard JC, Varallo VM, Ross DC, Roth JH, Faber KJ, Alman B & Gan BS (2003) Elevated levels of beta-catenin and fibronectin in three-dimensional collagen cultures of Dupuytren's disease cells are regulated by tension in vitro. BMC Musculoskelet Disord 16;4:16.

Hu FZ, Nystrom A, Ahmed A, Palmquist M, Dopico R, Mossberg I, Gladitz J, Rayner M, Post JC, Ehrlich GD &Preston RA (2005) Mapping of an autosomal dominant gene for Dupuytren's contracture to chromosome 16q in a Swedish family. Clin Genet 68(5): 424-9.

Hueston J (1985) The role of the skin in Dupuytren's disease. Annals of the Royal College of Surgeons of England 67: 372 -375

Hueston JT & Murrell GA (1990) Cell-controlling factors in Dupuytren's contracture. Ann Chir Memb Super 9(2): 135-7.

Hurst LC & Badalamente MA (1999) Nonoperative treatment of Dupuytren's disease. Hand Clin 15(1): 97-107.

Hutchinson JW, Tierney GM, Parsons SL & Davis TR (1998) Dupuytren's disease and frozen shoulder induced by treatment with a matrix metalloproteinase inhibitor. J Bone Joint Surg Br 80(5): 907-8.

Igota S, Tosa M, Murakami M, Egawa S, Shimizu H, Hyakusoku Hiko & Ghazizadeh M (2013) Identification and characterization of Wnt signaling pathway in keloid Pathogenesis. Int J Med Sci 10(4): 344-354.

Iqbal SA, Manning C, Syes F, Kolluru V, Hayton M,Watson S & Bayat A (2012) Identification of mesenchymal stem cells in perinodular fat and skin in Dupuytren's disease: A potential source of myofibroblasts with implications for pathogenesis and therapy. Stem Cells and Development 21(4): 609-622.

Iqbal SA, Hayton MJ, Watson JS, Szczypa P & Bayat A (2014) First identification of resident and circulating fibrocytes in Dupuytren's disease shown to be inhibited by serum amyloid P and Xiapex. PloS One 16:9(6): e99967.

Johnson P, Larson D, Clark IM & Chojnowski AJ (2008) Metalloproteinase Gene expression correlates with clinical outcome in Dupuytren's disease. J Hand Surg 33A (7): 1160-7.

Johnston P, Chojnowski AJ, Davidson RK, Riley GP, Donell ST & Clark IM (2007) A complete expression profile of matrix-degrading metalloproteinases in Dupuytren's disease. J Hand Surg 32A(3): 343- 51.

Jonsson T, Gudmundsson KG, Bjarnadòttir K, Hjàlmarsdotti IB, Gudmundsson S & Arngrimsson R (2013) Association of HLA-DRB1*01 with Dupuytren's disease. Scand J Rheumatol 42(1): 45-7.

Józsa L, Demel S, Pintér T, Renner A & Rétry A (1988) Immunopathological study on palmar aponeurosis in Dupuytren's disease. Acta Histochem 83 (2):153-8.

Kan HJ & Hovius SE (2012) Long-term follow-up of flps for extensive Dupuytren's and Ledderhose disease in one family. J Plast Reconstr Aesthet Surg 65(12): 1741-5.

Kelly S, Burke F & Elliot D (1992) Injury to the distal radius as a trigger to onset of Dupuytren's disease. J Hand Surg 17B: 225-229.

Ketchum LD, Donahue TK & Park O (2000) The injection of nodules of Dupuytren's disease with Triamcinolone Acetonide. J Hand Surg Am 25A: 1157-1162.

Khan AA, Rider OJ, Jayadev CU, Heras-Palou C, Giele H & Goldacre M (2004) The role of manual occupation in the etiology of dupuytren's disease in men in England and Wales. J Hand Surg Br 29B(1) :12-14.

Kuhn MA, Wang X, Payne WG, Ko F & Robson MC (2002) Tamoxifen decreases fibroblast function and down regulates TGF-beeta2 in Dupuytren's affected palmar fascia. J of Surgical research 103: 146-152.

Kulkarni M, Elliot D (2005) Dupuytren's disease of the big toe. J Hand Surg 30B(6): 567-9.

Lanting R, van den Heuvel ER, Westerink B & Werker PM (2013) Prevalence of Dupuytren disease in the Netherlands. Plast Reconstr surg 132(2):394-403.

Lanzettà M & Morrison WA (1996) Dupuytren's disease occurring after a surgical injury to the hand. J Hand Surg 21B(4): 481-3.

Lee LC, Zhang AY, Chong AK, Pham H & Longaker MT (2006) Expression of a novel gene, MafB, in Dupuytren's disease.J Hand Surg 31(2):211-8.

Liss GM & Stock SR (1996) Can Dupuytren's contracture be work-related?: review of the evidence. Am J Ind Med 29(5): 521-32.

Li X, Yamagata K, Nishita M, Endo M, Arfian N, Rikitake Y, Emoto N, Hirata K,Tanaka Y & Minami Y (2013) Activation of Wnt5a-Ror2 signaling associated with epithelial-to-mesenchymal transition of tubular epithelial cells during renal fibrosis. Genes to Cells 18: 608-619.

Loos B, Puschkin V & Horch RE (2007) 50 years experience with Dupuytren's contracture in the Erlangen University Hospital - A retrospective analysis of 2919 operated hands from 1956 to 2006. BMC Musculoskeletal Disorders Jul 8(60): 1-9.

Malemud CJ (2004) Protein kinases in chondrocyte signaling and osteoarthritis. Clin Orthopedics and related research 427S: S145-S151.

Mandal A & Fahmy F (2006) Infection as a trigger to the onset of Dupuytren's disease. J Hand Surg 31B(4): 459-60.

Many AM & Brown AM (2014) Both canonial and non-canonical Wnt signaling independently promote stem cell growth in mammospheres. PLoS One14; 9(7): 1-9.

Margo G, Lanteri E, Micali G, Paravizzini G, Travali S & Lanzafame S (1997) Myofibroblasts of palmar fibromatosis co-express transforming growth factor-alpha and epidermal growth factor receptor. J Pathol 181(2): 213-217.

Margo G, Fragetta F, Colombatti A & Lanzafame S (1997) Myofibroblasts and extracellular matrix glycoproteins in palmar fibromatosis. Gen Diagn Pathol 142(3-4): 185-90.

Meek RMD, McLellan S & Crossan JF (1999) Dupuytren's disease: A model for the mechanism of fibrosis and its modulation by steroids. J Bone Joint Surg Br 81B(49): 732-738.

Meek RDM, McLellan S, Reilly J & Crossan JF (2002) The effect of steroids on Dupuytren's disease: role of programmmed cell death. J Hand Surg 27B(3): 270-273.

Melling M, Karimian-Teherani D, Mostler S, Behnam M, Sobal G & Menzel EJ (2000) Changes of biochemical and biomechanics properties in Dupuytren disease. Arch Pathol Lab Med 124:1275-1281.

Menzel EJ, Piza H, Zielinski C, Endler AT, Steffen C & Millesi H (1979) Collagen types and anticollagen-antibodies in Dupuytren's disease. The Hand 11(3): 243-248.

Midwood KS & Orend G (2009) The role of tenascin-C in tissue injury and tumorigenesis. J Cell Commun Signal 3(3-4): 287-310.

Minami Y, Oishi I, Endo M & Nishita M (2010) Ror-family receptor Tyrosine Kinases in non canonical Wnt Signaling : Their implications in developmental morphogenesis and human diseases. Dev Dyn 239:1-15.

Michou L, Lermusiaux JL, Teyssedou JP, Bardin T, Beaudreuil J & Petit-Teixeira E(2012) Genetics of Dupuytren's disease. J Bone Spine 79(1): 7-12.

Mikkelsen OA, HØyeraal HM & Sandvik L (1999) Increased mortality in Dupuytren's disease. J Hand Surg 24B(5): 515-518.

Mikkelsen OA (1976) Dupuytren's disease - a study of the pattern of distribution and stage of contracture in the hand. The Hand Oct: 265- 271.

Mikkelsen OA (1972) The prevalence of Dupuytren's disease in Norway. A study in a representative population sample of the municipality of Haugesund. Acta Chir Scand 138(7):695-700.

Misra A, Jain A, Ghazanfar R, Johnston T & Nanchahal J (2007) Predicting the outcome of surgery for the proximal interphalangeal joint in Dupuytren's disease. J Hand Surg 32A: 240-245.

Moulin V, Castilloux G, Auger FA, Garrel D, O'Connor-McCourt MD & Germain L (1998) Modulated response to cytokines of human wound healing myofibroblasts compared to dermal fibroblasts. Exper Cell Res 238: 283-293.Murrell GA (1992) An insight into Dupuytren's contracture. Ann R Surg Engl. 74(3): 156-60.

Murrell GA , Francis MJ & Brompley L (1987) Free radicals and Dupuytren's contracture. Br Med J (Clin Res Ed) 28: 295(6610) 1373-5.

Notbohm H, Bigi A, ROveri N, Hoch J, Acil Y & Koch MH (1995) Ultrastructural and biochemical modifications of collagen from tissue of morbus Dupuytren patients. J Biochem 118(2): 405-10

Nugteren HM, Nijman JM, de Jong IJ & van Driel MF (2011) The association between Peyronie's and Dupuytren's disease. Int J Impot Res 23(4): 142-5.

Nunn AC & Schreuder FB (2014) Dupuytren's contracture: emerging insight into a Viking disease. Hand Surg 19(3): 481-90.

O'Connell MP, Marchbank K & Webster MR et al.(2013) Hypoxia induces Phenotypic plasticity and therapy resistance in melanoma via theTyrosine Kinase Receptors ROR1 and ROR2. Cancer Discov 3: 1378-1393.

Oishi I, Suzuki H,Onishi N, Takada R, Kani S, ohkawara B, Koshida I, Suzuki K,Yamada F, Schwabe GC, Mundlos S, Shibuya H, Takada S & MinamiY (2003) The receptor tyrosine kinase Ror2 is involved in non-canonical Wnt5a/JNK signalling pathway. Genes Cells 8: 645-654.

Ojwang JO, Adrianto I, Gray-McGuire C, Nath SK, Sun C, Kaufman KM, Harley JB & Rayan GM (2010) Genome- wide association scan of Dupuytren's disease. J Hand Surg Vol 35A: 2039-2045.

Pagnotta A, Specchia N & Greco F (2000) Androgen receptors in Dupuytren's contracture. J Orthop Res 20: 163-168. Pagnotta A, Specchia N, Soccetti A, Manzotti S & Greco F (2003) Responsiveness of Dupuytren's disease fibroblasts to 5alpha-dihydrotestosterone. J Hand Surg 28A: 1029-1034.

Palmer KT, D'Angelo S, Syddall H, Griffin MJ, Cooper C & Coggon D (2014) Dupuytren's contracture and occupational exposure to hand-transmitted vibration. Occup Environ Med 71(4): 241-5.

Pampel M, Maier S, Kreczy A, Weirich-Schwaiger H, Utermann G & Janecke A.R (2010) Refinement of the GINGF3 locus for hereditary gingival fibromatosis. Eur J Pediatr 169: 327- 332.

Pan D, Watson H K, Swigart C, Thomson J G, Honig S C & Narayan D (2003) Microarray gene analysis and expression profiles of Dupuytren's contracture. Ann Plast Surg 50(6): 618-622 .

Peimer CA, Blazar P, Coleman S, Kaplan TD,Smith T & Lindau T (2015) Dupuytren contracture recurrence following treatment with Collagenase Clostridium Histolyticum (CORDLESS [Collagenase option for reduction of Dupuytren long-term evaluation of safety study]): 5- Year Data.J Hand Surg Am 40(8): 1597-605.

Pereira RS, Black CM, Turner SM & Spencer JD (1986) Antibodies to Collagen Types I-VI in Dupuytren's Contracture. J Hand Surg Vol 11B: 58-60.

Picardo NE & Khan WS (2012) Advances in the understanding of the etiology of Dupuytren's disease. The Surgeon Jun: 10(3): 151-8.

Quaglino D, Bergamini G, Croce A, Boraldi F, Barbieri D, Caroli A, Marcuzzi A, Tiozzo R, Ronchetti IP (1997) Cell behavior and cell-matrix interactions of human palmar aponeurotic cells in vitro. J Cell Physiol 173: 415-422.

Quatresooz P, Paquet P, Hermanns-Lê T, Piérard G (2008) Molecular mapping of Factor XIIIa-enriched dendrocytes in the skin (Review) Int J Mol Med 22: 403-409.

Quian A, Meals RA, Rajfer J & Gonzalez-Cadavid NF (2004) Comparison of gene expression profiles between Peyronie's disease and Dupuytren's contracture. Urology 64(2): 399-404.

Qureshi FI, Hornigold R, Spencer JD, Hall SM (2001) Langerhans cells in Dupuytren's contracture. J Hand Surg 26B(4): 362-367.

Rayan GM & Moore J (2005) Non-Dupuytren's disease of the palmar fascia. J Hand Surg 30B(6): 551-556.

Rayan GM (2005) Commentary: Dupuytren's disease vs Non- Dupuytren's contracture J Hand Surg 30A(5): 1019- 1020.

Ryan GM (1999) Clinical presentation and types of Dupuytren's disease. Hand Clin 15(1): 87-96.

Rayan GM & Tomasek JJ (1994) Generation of contractile force by cultured Dupuytren's disease and normal palmar fibroblasts. Tissue and Cell 26(5): 747-756.

Raykha CN, Crawford JD, Burry AF, Drosdowech DS, Faber KJ, Gan BS & O'Gorman DB (2014) IGF2 expression and B-catenin levels are increased in Frozen Shoulder Syndrome. Clint Invest Med 1; 37(4); E262-7.

Rehman S, Goodacre R, Day PJ, Bayat A & Westerhoff HV (2011) Dupuytren's: a systems biology disease. Arthritis Res There 13(5): 238.

Rehman S, Salway F, Stanley JK, Ollier WER, Bayat A (2008) Molecular phenotypic descriptors of Dupuytren's disease. Defined using informatics analysis of the transcriptome. J Hand Surg 33A: 359-372.

Reilly RM, Stern PJ & Goldfarb CA (2005) A retrospective review of the management of Dupuytren's nodules. J Hand Surg 30A: 1014-1018.

Rhomberg M, Rainer C, Gardetto A, Piza-Katzer H (2002) Dupuytren's disease in children - differential diagnosis. J Pediatric Surg 37: 1-6.

van Rijssen AL, Gerbrandy FSJ, Linden HT, Klip H, Werker PMN (2006) A comparison of the direct outcomes of percutaneous needle fasciotomy and limited fasciectomy for Dupuytren's disease: A 6- week follow-up study. J Hand Surg 31A: 717-725.

Rodrigo JJ, Niebauer JJ, Brown RL & Doyle JR (1976) Treatment of Dupuytren's contracture. Long-term results after fasciotomy and fascial excision. J Bone Joint Surg Am 58(3): 380-7.

Ross DC (1999) Epidemiology of Dupuytren's disease. Hand Clin 15(1): 53-61.

Sanders JL, Dodd C, Ghahary A, Scott PG, &Tredget EE (1999) The effect of interferon-alfa2b on an In Vitro model of Dupuytren's Contracture. J Hand Surg 24A: 578-585.

Satish L, Gallo PH, Baratz ME, Johnson S & Kathju S (2011) Reversal of TGF-B1 stimulation of alfa-smooth muscle actin and extracellular matrix components by cyclic AMP in Dupuytren' s - deriver fibroblasts. BMC Musculoskelet Disord 25(12): 113.

Satish L, O'Gorman DB, Johnson S, Raykha C, Gan Bing S, Ang James HC & Kathju S (2013) Increased CCT-eta expression is a marker of latent and active disease and a modulator of fibroblast contractility in Dupuytren's contracture. Cell Stress Chaperones 18(4): 397-404.

Schwape GC, Tinschert S, Buschow C, Meinecke P, Wolff G, Gillessen-Kaesbach G, Oldridge M, Wilkie AO, Kömec R & Mundlos S (2000) Distinct Mutations in the receptor Tyrosine kinase gene ROR2 cause Brachydactyly Type B. Am J Hum Gen 67: 822-831.

Sheils O, Finn S, & O'Leary J (2003) Nucleic acid microarrays: an overview. Curr Diagn Pathol 9: 155-158.

Shih BB, Tassabehji M, Watson JS, McGrouther AD & Bayat A (2010) Genome-Wide High-Resolution Screening in Dupuytren's Disease reveals common regions of DNA copy number alterations. J Hand Surg 35A(7): 1172-1183.

Shih B, Tassabehji M, Watson JS & Bayat A (2012) DNA Copy number variations at chromosome 7p14.1 and chromosome 14q11.2 are associated with Dupuytren's disease: potential role for MMP and Wnt Signaling Pathway. Plast Reconstr Surg 129(4):921-32.

Shih B, Watson JS & Bayat Ardeshir (2012) Whole genome and global expression profiling of Dupuytren's disease: systematic review of current findings and future perspectives. Ann Rheum Dis 71:1440- 1447.

Shih B, Wijeratne D, Amstrong DJ, Lindau T, Day P& Bayat A (2009) Identification of biomarkers in Dupuytren's disease by comparative analysis of fibroblasts versus tissue biopsies in disease-specific phenotypes. J Hand Surg 34A(1):124-36.

Sladicka S J, Benfanti P, Raab M & Becton J (1996) Dupuytren's contracture in the black population : A case report and review of the literature. J Hand Surg 21A: 898-899.

Smith SP, Devaraj VS & Bunker TD (2001) The association between frozen shoulder and Dupuytren's disease. J Shoulder Elbow Surg 10(2): 149-51.

Sood A, Therattil PJ, Paik AM, Simpson MF & Lee ES (2014) Treatment of Dupuytren disease with injectable collagenase in a veteran population: a case series at the department of veterans affairs New Jersey Health Care System. Eplasty Mar14: 97-105.

Spector M (2001) Musculoskeletal connective tissue cells with muscle: Expression of muscle actin in and contraction of fibroblasts, chondrocytes, and osteoblasts. Wound Rep Reg 1(9): 11-18.

Stahl S & Calif E (2008) Dupuytren's palmar contracture in women. Isr Med Assoc J 10(6): 445-7.

Stanbury SJ & Hammert WC (2011) Dupuytren Contracture. J Hand Surg 36A: 2038-2040.

Starkweather KD, Lattuga S, Hurst LC, Badalamente MA, Guilak F, Sampson SP, Dowd A & Wisch D (1996) Collagenase in treatment of Dupuytren's disease : an in vitro study. J Hand Surg 21A(3): 490-5.

Tarlton JF, Meagher P, Brown RA, McGrouther DA, Bailey AJ & Afolke A (1998) Mechanical stress in vitro induces increased expression of MMPs 2 and 9 in Excised Dupuytren's disease tissue. J Hand Surg 23B(3): 297- 302.

Tomasek JJ, Schultz RJ, Episalla CW & Newman SA (1986) The Cytoskeleton and extracellular matrix of the Dupuytren's disease "myofibroblast": an immunofluorescence study of a non muscle cell type. J Hand Surg 11A(3): 365-71.

Tomasek JJ, Vaughan MB & Haaksma CJ (1999) Cellular structure and biology of Dupuytren's disease. Hand Clin Vol 15(1): 21-34.

Tomasek JJ & Haaksma CJ (1991) Fibronectin filaments and actin microfilaments are organized into a fibronexus in Dupuytren's diseased tissue. Anat Rec 230(2): 175-82.

Townley WA, Cambrey AD, Khaw PT & Grobbelaar AO (2009) The role of an MMP inhibitor in the regulation of mechanical tension by Dupuytren's disease fibroblasts. J Hand Surg 34B(6):783-7.

Townley WA, Cambrey AD, Khawn PT & Grobbelaar AO (2008) Matrix metalloproteinase inhibition reduces contraction by dupuytren fibroblasts. J Hand Surg 33A(9): 1608-16.

Tse R, Howard J, Wu Y & Gan BS (20049 Enhanced Dupuytren's disease fibroblast populated collagen lattice contraction is independent of endogenous active TGF- beta2. BMC Musculoskelet Disord 12;5(1): 41.

Tubiana R (1999) Dupuytren's disease of the radial side of the hand. Hand Clin 15(1): 149-59.

Türker T, Murphy E, Kaufman CL, Kutz JE, Meister EA & Hoying JB (2013) Response of Dupuytren fibroblasts to different oxygen environments. J Hand Surg 38A(12): 2365-9.

Varallo VM, Gan BS, Seney S, Ross DC, Roth JH, Richards RS, McFarlan RM, Alman B & Howard JC (2003) Beta-catenin expression in Dupuytren's disease: potential role for cell-matrix interactions in modulating beta-catenin levels in vivo and in vitro. Oncogene 12;22(24): 3680-4.

Verjee LS, Verhoekx JS, Chan JK, Krausgruber T, Nicolaidou V, Iz Davidson D, Feldmann M, Midwood KS & Nanchahal J (2013) Unraveling the signaling pathways promoting fibrosis in Dupuytren's disease reveals TNF as a therapeutic target. Proc Natl Acad Sci U S A 5;110(10) E928- 37.

Verjee LS, Midwood K, Davidson D, Essex D, Sandison A & Nanchahal J (2009) Myofibroblast distribution in Dupuytren's cords: correlation with digital contracture. J Hand Surg 34A(10): 1785-94.

Vi L, Gan BS & O'Gorman DB (2009) The potential roles of cell migration and extra-cellular matrix interactions In Dupuytren's disease progression and recurrence. Med Hypothesis 74(3): 5 : 10-2.

Vi L, Feng L, Zhu RD, Wu Y, Satish L, Gan BS & O'Gorman DB (2009) Periostin differentially induces proliferation, contraction and apoptosis of primary Dupuytren's disease and adjacent palmar fascia cells. Exp Cell Res 10:315(20):3574-86.

Vichelhaus A, Wendt M, Mielsch N, Gradl G & Mittlmeier T (2015) Manifestation of Dupuytren nodules following fracture of the distal radius. Handchir Mikrochir Plast Chir 47(1): 38-43.

Vujic I, Marker M, Posch C, Mühlehner D, Monshi B, B Ortiz- urda S & Rappersberger K (2015) Merkel Cell carcinoma: mitoses, expression of Ki-67 and bcl-2 correlate with disease progression. J Eur Acad Dermatol Venereol 29(3):542-8.

Watson HK & Paul H (1991) Pathologic anatomy. Hand Clin 7(4):661-8.

Wilbrand S, Ekbom A & Gerdin B (1999) The sex ratio and rate of reoperation for Dupuytren's contracture in men and women. J Hand Surg 24B(4): 456-9.

Wilbrand S, Ekbom A & Gerdin B (2000) Cancer incidence in patients treated surgically for Dupuytren's contracture. J Hand Surg 25B(3):283-7.

Wilbrand S, Ekbom A & Gerdin B (2002) Dupuytren's contracture and sarcoma. J Hand Surg 27B(1): 50-2.

Wilbrand S, Flodmark C, Ekbom A & Gerdin B (2003) Activation markers of connective tissue in Dupuytren's contracture: relation to postoperative outcome. Scand J Plast Reconstr Surg Hand Surg 37(5): 283-92.

Wilutzky B, Berndt A, Katenkamp D & Koshmehl H (1998) Programmed cell death in nodular palmar fibromatosis (Morbus Dupuytren) Histol Histopathol 13 (1): 67-72.

Wodarz A & Nusse R (1998) Mechanisms of Wnt signaling development. Annu Rev Cell Dev Biol 14:59-88.

Wurster-Hill DH, Brown F, Park JP & Gibson SH (1988) Cytogenetic studies in Dupuytren contracture. Am J Hum Genet 43(3): 285-92.

Yang C, Huang S, Wang X & Gu Y (2015) Emerging roles of CCCH-Type Zinc finger proteins in destasbilizing mRNA encoding inflammatory factors and regulating immune responses. Crit Rev Eukaryot Gene Expr 25(1): 77-89.

Yi IS, Johnson G & Moneim MS (1999) Etiology of Dupuytren's disease. Hand Clin 15 (1): 43-51.

Zawahir S, Li G, Banerjee A, Shiu J, Blanchard TG & Okogbule-Wonodi AC (2015) Inflammatory and Immune Activation in intestinal Myofibroblasts in developmentally regulated. J Interferon Cytokine Res 35(8): 634-40.

Zhang AY, Fong KD, Pham H, Nacamuli RP, Longaker MT & Chang J (2008) Gene expression analysis of Dupuytren's disease: the role of TGF-beta 2. J Hand Surg Eur Vol 33(6): 783-90.

Zhang DL, Gu LJ, Liu L, Wang CY, Sun BS, Li Z & Sung CK (2009) Effect of Wnt signaling pathway on wound healing. Biochem Biophys Res Commun 378: 149-151.

Original articles

This thesis is based on the following articles, which are referred to in the text by their Roman numerals (I–III).

I Forsman M, Kallioinen L, Kallioinen M & Ryhänen J (2005). Dupuytren's contracture; increased cellularity – proliferation, is there equality? Scand J Surg 94(1): 71–75.

II Forsman M, Pääkkönen V, Tjäderhane L, Vuoristo J, Kallioinen L, Salo T, Kallioinen M & Ryhänen J (2008). The expression of myoglobin and ROR2 protein in Dupuytren's disease. J Surg Res 15: 146(2): 271–275.

III Kaur S, Forsman M, Ryhänen J, Knuutila S & Larramendy ML (2008). No gene copy number changes in Dupuytren's contracture by array comparative genomic hybridisation. Cancer Genet Cytogenet 183(1): 6–8.

Original publications are not included in the electronic version of the dissertation

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ACTA UNIVERSITATIS OULUENSIS

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ISBN 978-952-62-1166-4 (Paperback)
ISBN 978-952-62-1167-1 (PDF)
ISSN 0355-3221 (Print)
ISSN 1796-2234 (Online)