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The Doctoral Programme in Population Health (DocPop)

THE SEARCH FOR GENETIC FACTORS IN HAND OSTEOARTHRITIS

Satu Hämäläinen

ACADEMIC DISSERTATION

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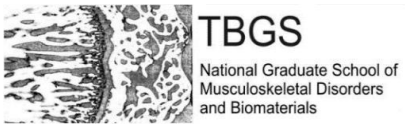

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LIST OF ORIGINAL PUBLICATIONS

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I **Hämäläinen S**, Solovieva S, Hirvonen A, Vehmas T, Takala EP, Riihimäki H, Leino-Arjas P. *COL2A1* gene polymorphisms and susceptibility to hand osteoarthritis in Finnish women. Ann Rheum Dis 2009 Oct;68(10):1633-7

II **Hämäläinen S**, Solovieva S, Vehmas T, Leino-Arjas P, Hirvonen A. Variations in the *TNFα* gene and their interactions with the *IL4R* and *IL10* genes in relation to hand osteoarthritis. BMC Musculoskelet Disord 2014 Sep 24;15:311.

III **Hämäläinen S**, Solovieva S, Vehmas T, Leino-Arjas P, Hirvonen A. Adipokine genes in hand osteoarthritis among Finnish women. (Submitted)

IV **Hämäläinen S**, Solovieva S, Vehmas T, Luoma K, Leino-Arjas P, Hirvonen A. Genetic influences on hand osteoarthritis in Finnish women - A replication study of candidate genes. PLoS ONE 2014 May 13;9(5):e97417.

ABBREVIATIONS

A1AT	Alpha-1-Antitrypsin / SERPINA1 Serpin Peptidase Inhibitor, Clade A (Alpha-1 Antiproteinase, Antitrypsin), Member 1
A1ACT	Alpha 1-antichymotrypsin
A2BP1	ataxin 2 binding protein 1
ACAN	aggrecan
ACE	Angiotensin I Converting Enzyme
ADAM	A Disintegrin And Metalloproteinase Domain
ADAMTS	A Disintegrin-Like And Metalloprotease (Reprolysin Type) With Thrombospondin Type 1 Motif
ADIPOQ	adiponectin
ALDH1A2	aldehyde dehydrogenase 1 family member A2
APLN	apelin
ASPN	asporin
BCAP29	B cell receptor-associated protein 29
BMI	body mass index
BMP5	bone morphogenetic protein 5
CA10	Carbonic Anhydrase X
CI	confidence interval
cM	centimorgan
CMC	carpometacarpal
CMC1	first carpometacarpal joint
CNV	copy number variation
COG5	component of oligomeric golgi complex 5
COL2A1	collagen, type II, alpha 1
COL9A1	Collagen, Type IX, Alpha 1
COMT	catechol-O-methyltransferase
COX2	Cyclooxygenase
CRP	C-reactive protein
CRTL1/HAPLN1	Hyaluronan And Proteoglycan Link Protein 1
CRTM/MATN1	Matrilin 1, Cartilage Matrix Protein
DIO2	iodothyronine-deiodinase enzyme type 2 (D2)
DIP	distal interphalangeal
DNA	deoxyribonucleic acid
DUS4L	dihydrouridine synthase 4-like

DVWA	dual von Willebrand factor A domains / COL6A4P1 (Collagen, Type VI, Alpha 4 Pseudogene 1)
ECM	extra cellular matrix
ENPP1	ectonucleotide pyrophosphatase/phosphodiesterase 1
EOA	erosive osteoarthritis
ER/ESR1	estrogen receptor 1
ESR2	Estrogen Receptor 2 (ER Beta)
FOS	Framingham Osteoarthritis Study
FRZB	Frizzled-Related Protein
GARP	Genetics, Arthrosis and Progression
GDF5	growth differentiation factor 5
GWAS	genome wide association study
HFE	hemochromatosis gene
HH	hereditary hemochromatosis
HLA/BTNL2	human leukocyte antigen / butyrophilin-like 2 (MHC class II associated)
HWE	Hardy-Weinberg equilibrium
IGF1	insulin-like growth factor 1
IL1	interleukin 1
IL1A	interleukin 1 alpha
IL10	interleukin 10
IL1B	interleukin 1 beta
IL13	interleukin 13
IL1RN	interleukin 1 receptor antagonist
IL4	interleukin 4
IL4R	interleukin 4 receptor
IL6	interleukin 6
IP	thumb interphalangeal
ITLN	intelectin/omentin
KCND3	Potassium Channel, Voltage Gated Shal Related Subfamily D, Member 3
K-L	Kellgren and Lawrence grading score
LD	linkage disequilibrium
LEP	leptin
LEPR	leptin receptor
LOD	logarithm (base 10) of odds
LRP5	Low Density Lipoprotein Receptor-Related Protein 5
LPR6	Low Density Lipoprotein Receptor-Related Protein 6

nsSNP	non-synonymous SNP
MAF	minor allele frequency
MAMDC2	MAM Domain Containing 2
MATN3	matrilin 3
MCP	metacarpophalangeal
ME3	Malic Enzyme 3, NADP(+)-Dependent, Mitochondrial
MetS	metabolic syndrome
MGP	matrix Gla protein
MHC	major histocompatibility complex
MHT	menopause hormone therapy
MICAL3	microtubule associated monooxygenase, calponin and LIM domain containing 3
MMP	matrix metalloproteinase
MRI	magnetic resonance image
MrOS	Osteoporotic Fractures in Men Study
NAMPT	nicotinamide phosphoribosyltransferase, visfatin
NOA	nodal osteoarthritis
OA	osteoarthritis
OR	odds ratio
PARD3B	partitioning defective 3 homolog B
PC	principle component
PCA	principle component analysis
PCR	polymerase chain reaction
PF	patellofemoral joint
PIP	proximal interphalangeal
POSTN	Periostin, Osteoblast Specific Factor
PTGS2	prostaglandin-endoperoxide synthase 2
RARRES2	retinoic acid receptor responder (tazarotene induced) 2, chemerin
RETN	resistin
RFLP	restriction fragment length polymorphism
RHOA	radiographic hand osteoarthritis
RNA	ribonucleic acid
ROA	radiographic osteoarthritis
RR	risk ratio
rs	reference single nucleotide polymorphism
SD	standard deviation

SERPINA12	Serpin Peptidase Inhibitor, Clade A (Alpha-1 Antiproteinase, Antitrypsin), Member 12, vaspin
S-HsCRP	Serum- High-sensitivity C-reactive Protein
SMAD	Sma (small body size) and Mad (mothers against decapentaplegic) related protein
SNP	single nucleotide polymorphism
SOA	symptomatic osteoarthritis
SOF	Study of Osteoporotic Fractures study
TF	tibiofemoral joint
TGFB1	transforming growth factor beta 1
TINAG	Tubulointerstitial Nephritis Antigen
TNF	tumor necrosis factor
TNFα	tumor necrosis factor alpha
TNKS	Tankyrase, TRF1-Interacting Ankyrin-Related ADP-Ribose Polymerase
TREAT-OA	Translational Research in Europe – Applied Technologies for Osteoarthritis
TRIB1	tribles homolog 1
TS	trapezio-scaphoid joint
VDR	Vitamin D (1,25- Dihydroxyvitamin D3) Receptor
VNTR	variable number tandem repeat

TIIVISTELMÄ

Nivelrikko on nivelten yleisin sairaus ja kaikkein useimmin sitä esiintyy sormien nivelissä. Se aiheuttaa kipua ja toiminnanrajoitteita ja voi heikentää työkykyä kättä paljon kuormittavissa tehtävissä. Nivelrikko on monitekijäinen sairaus, jonka syntyyn vaikuttaa yksilöllinen geneettinen herkkyys yhdessä ympäristötekijöiden kanssa. Nivelrikon riski kasvaa iän myötä ja yli 50-vuotiailla sitä esiintyy enemmän naisilla kuin miehillä.

Tässä tutkimuksessa tarkasteltiin sorminivelrikon mahdollisia geneettisiä riskitekijöitä aiemmin tunnettujen riskimekanismien pohjalta, joita ovat nivelruston epäedullinen kuormitus, tulehdus ja ylipainoon tai lihavuuteen liittyvät tekijät. Lisäksi tarkasteltiin aiemmin löydettyjen sorminivelrikon alttiusgeenien sekä muiden nivelten nivelrikon alttiusgeenien yhteyttä sorminivelrikkoon aineistossamme.

Tutkimusaineistona (n=542) oli 45-63 -vuotiaita naisia, jotka edustivat kahta ammattiryhmää, hammaslääkäreitä (n=294) ja opettajia (n=248) pääkaupunkiseudulta. Tutkimuskäynnillä heiltä otettiin molemmista käsistä röntgenkuvat sekä verinäyte geneettisiä analyysejä varten. Taustatiedot kerättiin kyselylomakkeella. Sorminivelrikon osoittimena käytimme pääasiassa vähintään lievän radiologisen nivelrikon esiintymistä vähintään kolmessa sorminivelessä.

Ensin tutkimme nivelruston yleisimmän kollageenin *COL2A1*-geenin monimuotoisuuskohtia. *COL2A1* rs2276455:n muuntunut alleeli, joka on perheittäin esiintyvän varhaisen nivelrikon alttiusgeeni, lisäsi sorminivelrikon riskiä myös meidän tutkimusaineistossamme. Hammaslääkäreillä, joilla oli tämä alleeli, sorminivelrikon riski oli kaksinkertainen verrattuna muuntumattomaan alleeliin. Hammaslääkärin työtehtävien vähäinen vaihtelu työhistorian aikana yhdistettynä muuntuneen alleelin kantajuuteen lisäsi sorminivelrikon riskiä entisestään. Myös eri geenimuotoja yhdistelevä haplotyyppianalyysi tuki *COL2A1*-geenin yhteyttä sorminivelrikon etiologiaan.

Tulehdukseen liittyvän *TNFα:*n rs1799964 ja rs1800630 -monimuotoisuuskohdat ja näiden haplotyyppi olivat yhteydessä lisääntyneeseen sorminivelrikon riskiin. Nämä yhteydet olivat riippumattomia *IL1β* ja *IL6* -geenien riskialleelien esiintymisestä, joiden yhteys sorminivelrikkoon löytyi aiemmin tutkimusaineistossamme. Lisäksi löytyi yhteisvaikutus tiettyjen *TNFα*-, *IL4R*- ja *IL10*-geenien monimuotoisuuskohtien välillä, mikä viittaa siihen, että *IL4R* ja *IL10* muokkaavat *TNFα:*n ja sorminivelrikon välistä yhteyttä.

Adipokiinigeeni *RETN:*n muuntuneet alleelit sekä haplotyyppi, jossa nämä alleelit olivat, vähensivät riskiä sorminivelrikkoon. Yhteys vähentyneeseen

riskiin oli selvempi ylipainoisilla/lihavilla kuin normaalipainoisilla naisilla. *RARRES2* varianttialleeli ja *LEPR* haplotyyppi puolestaan lisäsivät sorminivelrikon riskiä. *LEP* haplotyyppi oli yhteydessä vähentyneeseen sorminivelrikon riskiin vain ylipainoisilla naisilla, mikä viittaa siihen, että BMI muokkaa monimuotoisuuskohtien ja sorminivelrikon välistä yhteyttä.
Toistotutkimuksessa sorminivelrikon alttiusgeenin *A2BP1* rs716508:n muuntunut alleeli vähensi sorminivelrikon riskiä myös meidän aineistossamme ja näin varmisti alkuperäisen tuloksen. Selkärangan nivelrikon alttiusgeenin *TGFB1* rs1800470:n ja oireisen sorminivelrikon välillä havaittiin yhteys aineistossamme, jossa muuntunut alleeli lähes kaksinkertaisti sorminivelrikon riskin. Lisäksi opettajilla, joilla oli *ESR1* rs9340799:n muuntunut alleeli, oli miltei kolminkertainen oireisen sorminivelrikon riski. Havaitsimme geenien monimuotoisuuskohtien välisen yhdysvaikutuksen: *COG5* rs3757713:n muuntunut alleeli nosti sorminivelrikon riskiä vain naisilla joilla oli homotsygoottinen genotyyppi *BCAP29* rs10953541-varianttialleelin suhteen. Oireisen sorminivelrikon riski oli koholla henkilöillä, jotka olivat joko *HFE*-geenin rs179945:n tai *ESR1*-geenin rs9340799:n muuntuneen alleelin kantajia.
Yhteenvetona voidaan todeta, että tässä tutkimuksessa tarkasteltiin 27 kandidaattigeenin 43 monimuotoisuuskohtaa ja kahdeksasta geenistä (*COL2A1, TNFα, LEP, LEPR, RARRES2, RETN, A2BP1, ja TGFB1*) löydettiin yhteys sorminivelrikkoalttiuteen. Lisäksi yhteisvaikutuksia löydettiin geenimuotojen, työperäisen kuormituksen ja BMI:n väliltä.

ABSTRACT

Osteoarthritis (OA) is the most common joint disease and a frequent cause of pain and disability with the joints of the hand being the most frequently affected site. OA is a complex disorder that results from the interplay of genetic and environmental factors. It becomes more prevalent with age, and after the age of 50 a higher proportion of women are affected than men.

We examined some possible genetic risk factors of hand OA with consideration of previously proposed risk mechanisms, namely: loading of cartilage structure, inflammation, and overweight. We also attempted to verify some hand OA susceptibility genes and to replicate in our material the genetic associations found at other joint sites in other studies.

The participants in this study (n=542) were women aged 45 to 63, who represented two occupations, dentists (n=294) and teachers (n=248), and were from the Helsinki metropolitan region. Both hands of each participant were radiographed and blood samples were taken for genetic analyses in a clinical examination. Information on background variables were collected by questionnaire. The main outcome variable in the present study was at least mild radiographic OA (ROA) in at least three finger joints.

We first studied the variation in the *COL2A1* gene, which encodes for the main collagen present in normal cartilage. The *COL2A1* rs2276455 SNP minor allele, previously associated with generalised OA, was also associated with ROA in the hand; among dentists the risk was doubled in carriers of this allele compared with those with the major allele. A history of repetitive hand loading tasks combined with carrying the minor allele further increased the risk. Haplotype analyses supported the role of variation in the *COL2A1* gene in the aetiology of hand ROA.

Similarly, the minor alleles of the pro-inflammatory *TNFα* rs1799964 and rs1800630 SNPs, and their haplotype, were associated with an increased risk of ROA. These associations were independent of the variants in the *IL1β* and *IL6* genes previously shown to be associated with hand OA in our study material. Further, interactions between *TNFα*, *IL4R*, and *IL10* SNPs were found, which suggest that the effect of the *TNFα* polymorphisms on ROA was modified by the *IL4R* and *IL10* gene variants.

The minor alleles of the two adipokine *RETN* SNPs, and the haplotype containing these minor alleles, decreased the risk of ROA. The association of the haplotypes with the deceased risk of ROA was stronger in overweight

women. In contrast, the *RARRES2* SNP minor allele and *LEPR* haplotype increased the risk of ROA. The *LEP* haplotype, however, was associated with a lower risk of ROA only among overweight women.

The replication-study found that the *A2BP1* rs716508 SNP minor allele decreased the risk of ROA, which confirms the original finding. The minor allele of the spinal OA candidate gene *TGFB1* rs1800470 almost doubled the risk of symptomatic DIP OA. Teachers with the minor allele of *ESR1* rs9340799 SNP had an almost tripled risk of suffering symptomatic DIP OA. Furthermore, carrying the *COG5* rs3757713 minor allele raised the risk of ROA to 2.6-fold only among the women with the homozygous *BCAP29* rs10953541 minor allele genotype, and those carrying the minor allele of either *HFE* rs179945 or the *ESR1* rs9340799 SNP had a doubled risk of symptomatic DIP OA.

In conclusion, 43 SNPs from 27 candidate genes were analyzed in this study and SNPs in eight of them (*COL2A1, TNFα, LEP, LEPR, RARRES2, RETN, A2BP1,* and *TGFB1*) were found to be associated with susceptibility to hand OA. Interactions between the genotypes, occupational loading and BMI were also observed.

1 INTRODUCTION

Osteoarthritis (OA) is the most common joint disease with the hand being the site most frequently affected (1). OA causes work disability particularly among ageing manual workers and it is the most important cause of disability in daily activities among the elderly (2, 3). The occurrence of OA displays a steep socioeconomic gradient: lower socioeconomic groups have a higher prevalence of the disease (2, 3).

Although the multifactorial aetiology of OA has been extensively studied, it is still not fully understood. OA becomes more prevalent with age, and after the age of 50 years a higher proportion of women than men are affected (4). Other well-known risk factors include obesity, injury, and repetitive joint loading (5).

In all its heterogeneous forms, OA appears to be strongly genetically determined (6-9). A study of British elderly female twins estimated that the heritability of hand OA was 65%, when hand OA was assessed as a sum score of joint space narrowing and osteophytes (9). It has been suggested that genetic susceptibility may be more relevant in women than in men (10).

Candidate genes for OA may be selected based on the knowledge of joint biology and by being guided by hypotheses of the possible pathogenesis of OA. The genetic influence may involve one or any combination of the following: a structural defect such as that which occurs in collagen, alterations in the metabolism of cartilage and bone, an enhanced inflammatory component in the disease process, or a genetic factor capable of influencing a known risk factor for OA such as obesity (11, 12).

Articular cartilage mainly consists of collagens with COL2A1 being the most common (90% of the total) (13). Several studies have claimed that there are associations between *COL2A1* genotype and different joint sites affected by OA (hand, generalized, and knee) (14-21). However, other studies did not observe any association with generalized OA (22), or hip OA (17).

Tumor necrosis factor alpha (TNFα) is a pro-inflammatory cytokine and its role in inflammation is to drive the inflammatory cascade (23). The *TNFα* gene is part of the class III region of the major histocompatibility complex (MHC), the most gene-dense and polymorphic region of the entire genome (24). *TNFα* single nucleotide polymorphisms (SNPs) have been studied in association with knee and hip OA, but the results have been conflicting; overall, however, there has been a positive association reported (25).

Adipokines are proteins that are secreted by adipose tissue with pro- and anti-inflammatory properties and therefore they could be potentially metabolic risk factors in hand OA (26). Leptin (LEP) is the "satiety hormone" and cytokine that regulates adipose tissue mass and energy expenditure through the leptin receptor (LEPR). LEP has been postulated to explain nearly half of the association between elevated Body Mass Index (BMI) and knee OA (27). In addition, the polymorphism in the *LEP* gene has been associated with individual susceptibility of knee OA in a Chinese population (28).

Resistin (RETN) is an adipocytokine that has been studied in OA because its levels are elevated in synovial fluid and plasma (29). As RETN strongly up-regulates the expression of TNFα and interleukin 6 (IL6), it is considered to be pro-inflammatory (30). However, the studies on RETN serum levels and progression of OA have been contradictory, with some, but not all, claiming to detect an association (30-32).

Chemerin is encoded by the *RARRES2* gene and is an anti-inflammatory adipokine (33). *RARRES2* concentration levels have been assayed in the synovial fluid samples of the knees of OA patients and they have been shown to correlate with disease severity (34).

Several studies have shown a moderate association between weight and BMI and the incidence of hand OA (12, 35) but the aetiological mechanism behind these associations remains unknown (36).

Mechanical loading of the joint is necessary for the health of the cartilage. However, excessive loading may harm the joint and contribute to the development of OA (37-39). Forceful repeated mechanical loading causes an immediate dose-related increase in collagen denaturation in bovine articular cartilage (38). Joint loading

has been demonstrated to regulate gene expression in cartilage chondrocytes. For instance, collagen gene expression may differ and the expression of degradative enzymes such as procollagenases (matrix metalloproteinase) may be up-regulated (40).

Dentistry is one of the few occupations with an academic background that involves extensive manual work. Dentists perform arm, wrist and hand movements repeatedly, often rapidly, and for extended periods of time. Moreover, a very accurate and precise grip is required for the manipulation and handling of precision tools. This may expose the hand joints to heavy and long lasting loading forces (loadings).

Previously, our research team demonstrated that the localization of hand OA among dentists was associated with the pattern of dental work task history. The group that had spent most of their work history in restorative treatment and endodontics and thus had experienced low task variation, displayed a higher risk of OA in the most loaded fingers than the group with high task variation.(41)

Evidently, OA is a complex disorder that has been proposed to result from the mutual interplay of systemic and local factors (5). Yet, the independent and joint effects of genetic and environmental factors on OA have rarely been investigated.

Here we investigated possible genetic factors in hand OA with consideration of the putative main risk mechanisms of this disorder, i.e., physical loading, inflammation, and obesity-related factors. We also made an attempt to replicate some findings of earlier studies on hand OA by identifying potential hand OA susceptibility genes, and by investigating genetic associations that have arisen from studies on OA at other joint sites to determine whether those susceptibility genes would also be associated with hand OA.

2 REVIEW OF THE LITERATURE

2.1 Characteristics of OA

OA is a chronic and often a disabling disease of the synovial joints that leads to progressive loss of articular cartilage with subsequent joint space narrowing (42). The loss of cartilage is accompanied by changes in the synovium, periarticular ligaments, and the subchondral bone (43, 44) (see Figure 1). According to the current understanding, OA is a disease of the whole joint rather than simple loss of cartilage (45). Furthermore, OA has been described as a group of overlapping disorders with different aetiologies in different joints but similar biological, morphological and clinical outcomes (46).

OA can be diagnosed by pathological findings (mainly based on imaging), clinical features (commonly joint pain and stiffness), or a combination of these (5, 47). It is typically categorized as either primary or secondary (48). OA that manifests after trauma is defined as secondary (49), whereas in the absence of trauma or other pre-existing condition, it is referred to as primary/idiopathic OA (49, 50).

OA is an ancient disease (51) in human beings: signs of OA have been found in the remains of the "Java man" who walked on the planet around 500 000 years ago (52). When Hippocrates referred to "an arthritis which seizes the great joints, which are able to contain it, but which does not usually go beyond these", he could have been describing OA (52). Dr. William Heberden's "Commentaries on the History and Cure of Diseases"(53) first described the nodes in distal interphalangeal (DIP) joints that now carry his name. In the beginning of the 19th century, John Haygarth seems to have characterized OA in his work "A Clinical History of the Nodosity of the Joints" although the term arthritis was not yet in general use then (54). Sir Alfred Garrod differentiated chronic arthritis from gout (52), and in 1888 John Kent Spender published the first article explicitly about OA (55). The annual number of publications has increased exponentially since these and other early papers on various forms of OA were published (56-60).

2.2 Pathogenesis of OA

Although knowledge on factors involved in the pathogenesis of OA has increased recently, no root cause of it has been identified, but instead, many different hypotheses on various aspects of the OA process have been put forward (43, 44). Previously, OA was thought to be a "wear and tear"

phenomenon of the articular cartilage mainly due to aging and biomechanical factors, such as unfavourable joint morphology, injuries, and occupational loading, particularly heavy manual labour (4, 61-63). Today, OA is considered to be a complex multifactorial disease of the whole synovial joint that arises from an interplay between individual genetic susceptibility, biomechanical, inflammatory, and other biological factors (1, 43).

Hallmarks of OA are a variable degree of cartilage damage, subchondral bone sclerosis, and a synoviopathy that includes hypertrophy and inflammation (see Figure 1) (64).

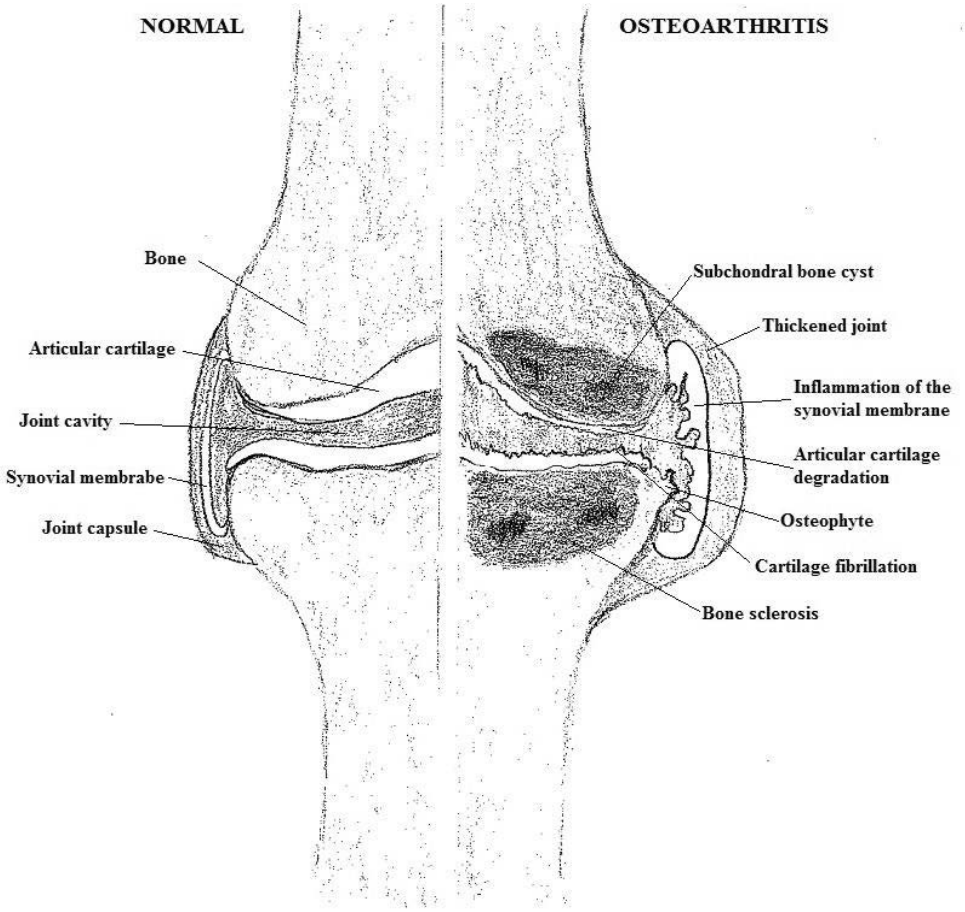

Figure 1. Normal and osteoarthritic joint
(based on figure http://www.slideshare.net/drangelosmith/osteoarthritis-35866521)

With the exception of the articular cartilage, nociceptors are present in many joint structures, including ligaments, the joint capsule, synovium, periosteum and the subchondral bone (65, 66). Inflammation may be associated with a decrease in the threshold for nociception (66, 67) Alternatively, nociceptive neurons may directly detect cytokines (68). It has also been suggested that inflammation stimulates angiogenesis in the articular cartilage accompanied by innervation (69). Nociceptive stimuli are dynamically processed in the spinal cord and brain giving rise to the perception of pain. In OA pain, the relative contribution of peripheral pathways has been estimated to be between 60% and 80% (67).

Articular cartilage

The first signs of OA appear in the articular cartilage, which is made up mostly of extracellular matrix (ECM). Two to three percent of the volume of cartilage consists of chondrocytes, which maintain the matrix by synthetizing its components (collagens, proteoglycans, and hyaluronate) and also the proteolytic enzymes responsible for their breakdown. OA is understood to result from a failure to maintain the balance between synthesis and degradation of these ECM components (45). The imbalance is due to excessive production of inflammatory cytokines (e.g., interleukins and TNFα) and matrix-degrading enzymes (MMPs), together with down regulation of anabolic signaling, finally leading to ECM destruction and cartilage degradation (67). Where the cartilage is totally destroyed, synovial fluid gets access to the bone marrow presumably leading to formation of bone cysts (44).

Subchondral bone

Subchondral bone comprises the subchondral bone plate and the underlying trabecular bone and bone marrow (45). The subchondral bone plate is separated from the articular cartilage by a zone of calcified cartilage. Bone is remodeled by bone resorption and the synthesis and formation of new bone on a previously resorbed surface. The bone remodeling process may be altered in OA, which results in structural changes in the subchondral bone (70), such as an increase in bone volume fraction and hypomineralization of the subchondral bone (71).

Synovium

The synovium that comprises a thin layer of macrophages, fibroblasts and the underlying vascularized connective tissue, is an important source of synovial fluid components (72). These components, e.g., growth factors, contribute to the properties of articular surfaces and modulate chondrocyte activity. For instance, they may induce fibrocytic and chondrometaplastic changes (72), which leads to pain and joint stiffness. Further, synoviocytes are able to secrete

matrix-degrading proteases (MMPs) and the catabolic cytokines interleukin 1(IL1) and, TNFα, which induce inflammatory signaling pathways within the chondrocytes themselves. The synovial tissues of OA patients also express and secrete transforming growth factor beta (TGFβ), mainly transforming growth factor beta 1 (TGFβ1) (44) (Figure 2). Inflammatory reactions in the synovial membrane occur to some degree in all OA joints.
Thus, it seems likely that many different factors interact in the OA process in a complex dynamic system (73). Among the challenges in studying the pathogenesis of OA is the currently held view that OA is a rather heterogeneous disorder whereby the pathway that leads to OA can vary between different joint sites and even between individuals (73, 74).

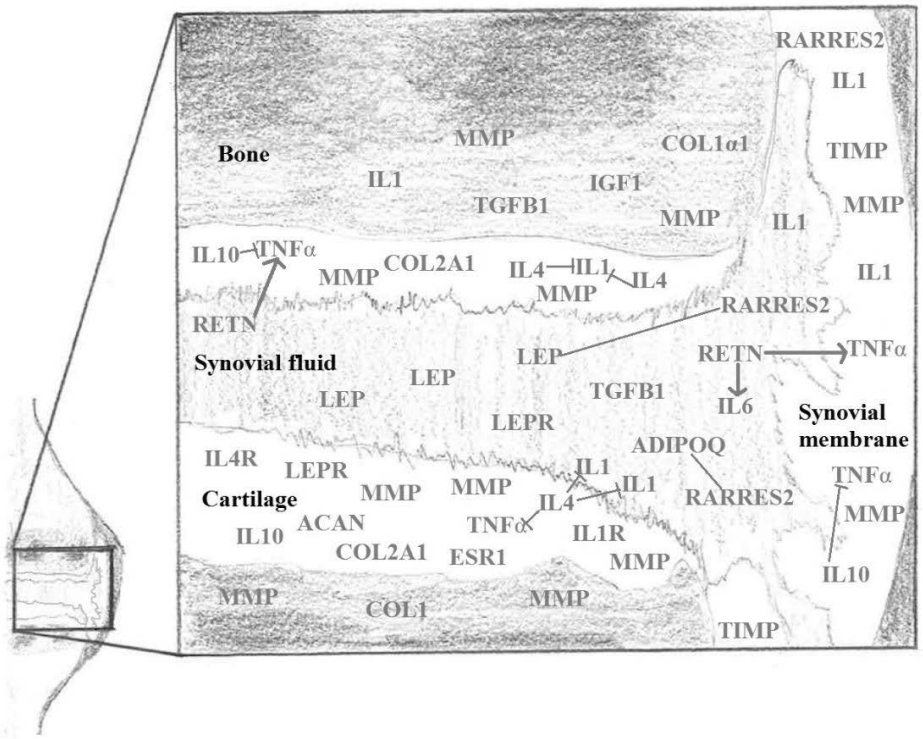

Figure 2. Illustration of the complex dynamic system of the molecules present in OA joint.

2.3 Hand osteoarthritis

2.3.1 Characteristics of hand OA

The hand is the site most frequently affected by OA (75). Hand OA is a complex entity to study because of the 30 joints (both hands, excluding the wrist joints) that may be involved in the disease process. Hand OA can also be part of generalized OA that affects many joint sites such as the hip, knee, and the spine (60).

Several different terms are used for hand OA, including: digital (76), nodal (77), finger (41), thumb (78), DIP (79), proximal interphalangeal (PIP) (80), carpometacarpal (CMC) (81), and metacarpophalangeal (MCP) (82) OA, depending on when and how it has been defined (see figure 3 which illustrates the hand joints). Moreover, an erosive/inflammatory (EOA) and generalized OA may also affect the hand (83).

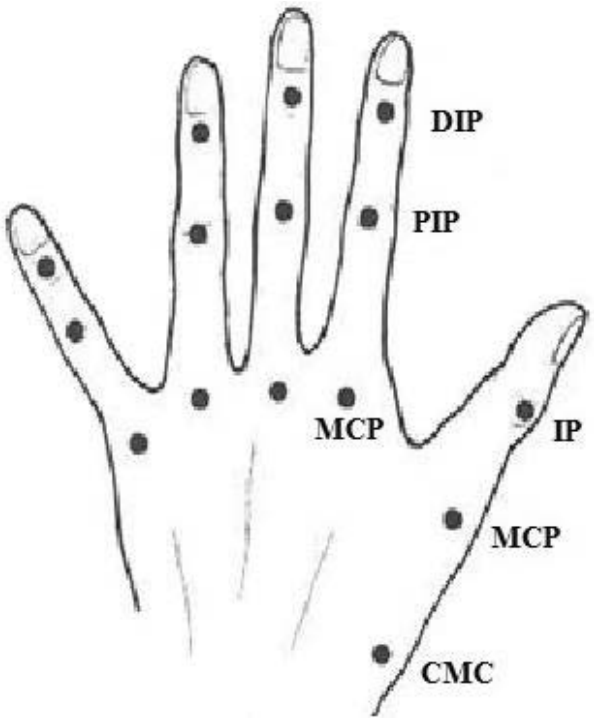

Figure 3. Locations of the hand joint site studied.

Prior to the development of radiography and other imaging modalities, hand OA was usually recognised by the appearance of Heberden's nodes that were

visually detectable and palpable on the fingers. Robert Stecher, one of the pioneers in rheumatic diseases, first described the characteristics of hand OA (84-87). Stecher used a large random population sample to map the incidence, heredity, and mechanism of inheritance of Heberden's nodes, and their relation to findings in other joints. He also described the association of Heberden's nodes with hypertension, obesity, and menopause. (84). Stecher concluded that the incidence of OA increased with age and that hereditary factors played an important role in its aetiology. He also suggested that the genetic mechanism for idiopathic Heberden's nodes would be a single autosomal gene that is dominant in women and recessive in men. Although he did not find any direct association between Heberden's nodes with obesity, he noticed an association between OA of other joints and obesity; menopause was also thought to be a contributing factor. Finally he pointed out that although Heberden's nodes are a manifestation of OA, other types of OA exhibited their own particular characteristics.

Another significant publication was that by Kellgren and Moore on "Generalized osteoarthritis and Heberden's nodes" (60). These researchers emphasized that although Heberden's nodes were often non-symptomatic, the more severe cases of hand OA appeared in rheumatism clinics. The authors studied cases of OA with multiple affected joints and suggested that Heberden's nodes were a part of what they called 'primary generalized OA'. In their seminal paper, Kellgren and Lawrence proposed a grading system for the severity of radiological findings in OA and instructions on how to evaluate the images (88). This grading system is still in use.

The Kellgren-Lawrence (K-L) grading score was used in the mid-1970's by van Saase *et al*. in the Zoetermeer Survey, that described the prevalence of OA for 22 joints in a large Dutch population sample and compared these with 10 other populations (89). Radiographs of the hands, feet, and the cervical spine were obtained from all participants aged ≥19 years, and, also the lumbar spine, pelvis, shoulders and the knees of those aged ≥45 years were radiographed. The results revealed a strong association between age and the prevalence of OA, with the highest figures in the spine, with peak prevalence values around 84% for both sexes. The occurrence of OA in the hand joints was also high, particularly in the DIP joints, with a peak prevalence of 76% in women at age 65 and 64% in men at age 80.

When van Saase *et al*. compared their findings with 10 other population studies they concluded that "osteoarthritis is a worldwide disease and that no population investigated so far has been spared" and "frequently affected joints

show signs of degeneration in all populations. It is therefore most likely that the etiology of most OA is the same in all populations" [sic].

Since the 1970's, the number of research publications on some aspect of hand OA has steadily increased, now with about 300 items appearing annually (Figure 4).

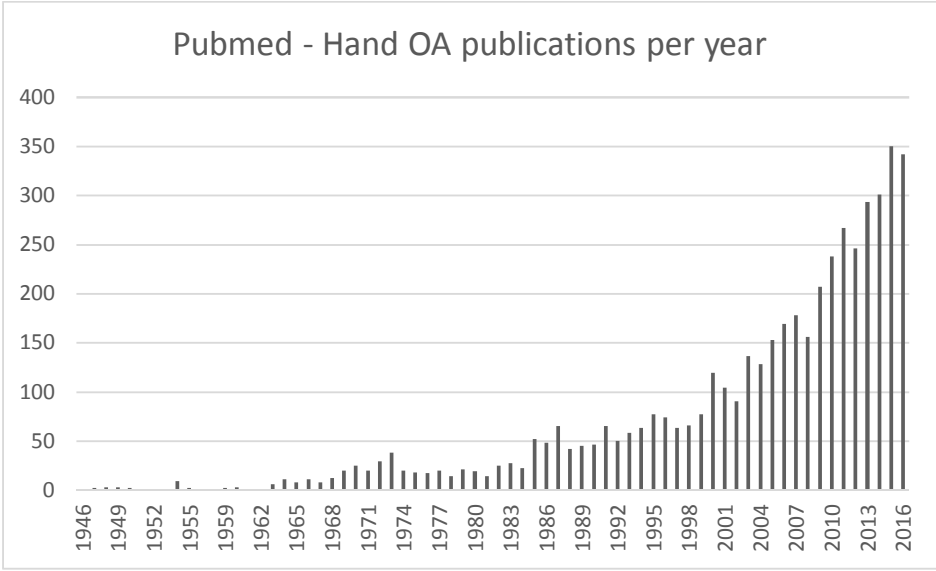

Figure 4. Hand OA publications per year collected from PubMed with the search terms "finger or hand or digital or nodal or DIP and osteoarthritis".

2.3.2 Definition of hand OA

The term radiographic hand OA (ROA) is commonly used when the diagnosis is based on imaging (90), whereas the term symptomatic hand OA usually combines imaging findings with subjective symptoms. A typical imaging sign of OA including hand OA is joint space narrowing due to the loss of cartilage from the joint surfaces. Pain, stiffness and/or swelling of hand joint(s) typically are the reasons why the patient seeks medical assistance. Pain in OA is chronic, and clinically the most disabling symptom (67). It is suggested that the pain is not only local but that central nervous system amplifies and maintains the symptoms (91). However, not all subjects develop symptoms, even if they have severe hand OA findings in their radiographs. Furthermore, some patients have only the symptoms suggestive of hand OA but without any evidence of radiographic changes (92).

Heberden's nodes in the distal DIP and Bouchard's nodes in the PIP (93) are classical signs of OA that can be seen or felt when examining the hand joints; these can also be observed in the radiographs. These nodes limit joint movement and decrease grip strength but may occur with or without symptoms (73). Today, the term osteophyte is used in all joint sites (94). However, there is some debate in the literature about whether Heberden's nodes really are osteophytes of the DIP joints or a somewhat different entity (73, 94).

2.3.3 Classification of radiographic hand OA

Classically, hand OA is defined using the K-L score (88), which includes a grading of osteophytes and, joint space narrowing (Table 1), and uses reference images.

Table 1. Kellgren-Lawrence grading scale for OA.

Grade		Criteria
0	normal	no radiographic features of OA are present
1	doubtful	doubtful joint space narrowing and possible osteophytic lipping
2	mild	definite osteophytes and possible joint space narrowing
3	moderate	multiple osteophytes, definite joint space narrowing, sclerosis, possible bony deformity
4	severe	large osteophytes, marked joint space narrowing, severe sclerosis and definite bony deformity

The detailed observations needed for scoring may lead to somewhat different results when repeated or when assessed by different readers. The reliability of readings has been studied by calculating intra-observer and inter-observer agreement values. Weighted Cohen's kappa coefficients with quadratic weights are often used as a measure of reliability and calculated for each joint between two readings by the same radiologist (intra-observer agreement) and between different radiologists (inter-observer agreement) (95). A kappa value lower than 0.20 is interpreted as poor, between 0.21 and 0.40 as fair, between 0.41 and 0.60 as moderate, between 0.61 and 0.80 as good, and between 0.81 and 1.0 as a very good agreement.

The large number of joints in the hand and different approaches to defining OA in general, make it particularly difficult to reach a consensus on the

definition of hand OA, in contrast to that of hip and knee OA (http://www.womac.org/womac/index.htm). A universal gold standard for the diagnosis of hand OA has not yet been promulgated. Each study, therefore must carefully describe the following: which joints were considered, how many joints were involved, in what manner was the occurrence of the osteophytes, the extent of joint space narrowing and also assess joint deformation; all of which must be taken into account in grading the extent of a case of hand OA. Often it is also necessary to set cut-off values for imaging findings and for the grade of symptoms or disability.

In 2007, a new atlas for grading OA was published (96). Table 2 shows the evaluated radiographic features of hand OA.

Table 2. Evaluated radiographic features of hand OA.

FINDING	**SITE**	**SCALE**
Osteophyte	DIP	0-3
	PIP	0-3
	First CMC	0-3
	Thumb (IP)	absent/present
	Naviculotrapezial joint	absent/present
Joint space narrowing	DIP	0-3
	PIP	0-3
	First CMC	0-3
	IP	absent/present
	Naviculotrapezial joint	absent/present
Malalignment	DIP	absent/present
	PIP	absent/present
	First CMC (subluxation)	absent/present
Erosion	DIP	absent/present
	DIP central erosion	absent/present
	DIP pseudo widening	absent/present
	PIP	absent/present
	First CMC	absent/present
Subchondral sclerosis	DIP	absent/present
	PIP	absent/present
	First CMC	absent/present
Subchondral cyst	PIP	absent/present
	First CMC	absent/present

Recently, a protocol was proposed for diagnosing and scoring magnetic resonance images (MRI) of hand OA patients (97). The Oslo hand OA, MRI score includes the assessment of synovitis, flexor tenosynovitis, erosions, osteophytes, joint space narrowing, and the bone marrow lesions on a 0–3 scale, and the absence/presence of cysts, malalignment (frontal/ sagittal plane), collateral ligaments and bone marrow lesions at collateral ligaments' insertion sites.

2.3.4 Phenotypes of hand OA

The use of well characterized disease phenotypes in genetic studies is preferred for genetic studies. However, a wide range of phenotypes have been used in the different studies on OA. Details of data collection obviously have an impact on which phenotypes can be formulated. The differences in phenotypes naturally complicate comparisons of the results between studies. For example, if only self-reported data are available, only the subjective manifestation of symptomatic hand OA can usually be used (98). On the other hand, if only radiographic images are available, then the classification is constructed according to their outcome (99).

In some cases, information is available on both pain and other symptoms and radiographic findings, which makes it possible to define more detailed phenotypes by combining the symptoms with radiographic findings (100). For instance, the following phenotype has been used: "the presence of both radiographic findings of grade 2 or more and symptoms in at least two DIP joints" [sic] (101).

Some studies used an overall summary score of hand OA according to the K-L grading as the outcome (102). Such a score can be calculated, by summing K-L scores of each joint and then dividing it by the number of joints assessed or using it as an overall continuous variable. The latter could theoretically vary between 0 (where all the joints are healthy) and 128 (where all the joints have severe OA) with 15 joints per hand and wrist considered as one joint.

Some other studies classified hand OA by using certain cut-off values with, the K-L score being at least mild (2) in at least *n* joints (103). Moreover, some studies focused only on certain joints, such as the DIP joints (101, 104) or the thumb joints (105), whereas other studies combined symmetrical (same joint in both hands) involvement and a certain joint group. For instance, symmetrical DIP OA (103) can refer to a phenotype where hand OA is defined

by the presence of radiographic findings in at least one symmetrical pair of DIP joints.

Phenotypes that describe the severity of the disease, such as erosive (106) or nodal (73) OA, have been used in addition to symptomatic and radiographic hand OA. The grading of the severity of erosive OA is based on a specific scoring method (107).

It would be most desirable, if some consensus could be achieved because the different scoring methods in current use lead to major differences in outcome measures (108). Some recommendations for standardization and phenotype definitions, including hand OA, have been proposed (109). At the very least it seems necessary that studies on hand OA should include a detailed description of the phenotype used.

2.3.5 Prevalence of hand OA

Hand OA is highly prevalent and displays many different clinical presentations (110). The prevalence of hand OA depends on the classification and definition used and also on the distribution of various risk factors, e.g., age, gender, and race in the study material. Overall, the prevalence of OA is higher in men until the age of 50, and higher in women older than 50 years (110).

The age-adjusted prevalence of hand OA in any finger joint (K-L 2 to 4) in the Finnish population aged ≥30 years was found to be 44% in men and 48% in women (103). The prevalence was less than 10 % in under 44 years rising to over 80% in men and over 90% in women among ≥75 years. In the same study, the prevalence of symmetrical DIP OA classified as at least two DIP joints (K-L 2 to 4) was 10 in men and 21% in women.

The Zoetermeer survey reported Dutch inhabitants age specific prevalence of each hand joint group (DIP, PIP, MCP, CMC-1) ROA. The prevalence was couple of % in under 30 years rising to highest 64% for men (age 75-79) and 76% for women (age 65-69) in DIP OA (K-L ≥2) group (89).

ROA in at least one hand joint reported by the Rotterdam study was 67% in women and 55% in men over 55 years (111). The same study found that DIP joints were affected in 47% of participants, the thumb base in 36%, PIP joints in 18%, and MCP joints in 8% (right or left hand). The prevalence of erosive OA in the Rotterdam study was 2.8% in the general population and 10% in individuals with symptomatic osteoarthritis (SOA) of the hand (112).

The age-standardised prevalence of hand ROA (≥1 joints with K-L ≥2) in the Framingham OA study was 44% in women and 38% in men. The prevalence was around 5% among <44 years men and women rising to 95% in men and 100% in women >80 years of age (106). When the Framingham study results were compared to a Chinese cohort, it was found that in China the prevalence of hand ROA (≥1 joints with K-L ≥2) was much lower: despite the older mean age of the studied group (59 years in Framingham OA study and 69 years in Chinese cohort), only 45% of men and 47% of women (30% in 60-64 years and over 85% in ≥ 80 years) had hand ROA in Beijing (113). Conversely, a population-based sample study from Turkmen (age range: 19-89) reported 62% of the men and 57% of the women had ROA (K-L ≥2) in at least at one hand joint. The prevalence was 14% in <36 years and after the age of 65, every individual in the Turkmen sample had at least one hand joint affected by ROA (114).

2.3.6 Heritability of hand OA

A British study on elderly female twins estimated the heritability of hand OA (osteophytes and joint space narrowing (JSN) score) to be 65% (9) whereas the heritability reported by the Framingham study was 34% (sum of K-L score) (7). A genome linkage scan among women, with 269 monozygous twin pairs and 628 dizygous pairs found a heritability of 48% for DIP-OA and 67% for an overall K-L score for both hands (115). In the Rotterdam study, the heritability estimate for hand OA (score of joint groups with K-L ≥2) was 56% (116).

However, after years and a plethora of studies that examined polymorphisms of candidate genes and later genome-wide association studies (GWAS), the nature of the heritable factors still remains largely unknown (117).

2.4 Risk factors of hand OA

Hand OA is a multifactorial disease (118), i.e., inherited susceptibility factors interact with environmental factors to determine the disease prognosis (5). The risk factors can be divided into systemic and local factors (5). The systemic factors comprise age, gender, race, bone density, oestrogen replacement therapy, nutritional, and genetic factors, whereas overweight/obesity, joint loading, joint injury, joint deformity, sports participation, and muscle weakness are local factors in hand OA.

Susceptibility to OA is modified by systemic factors, whereas the local factors affect the site and severity of the OA (5). The most important risk factors for OA will be reviewed in detail below.

2.4.1 Age

Age is the most evident risk factor for OA. Its effect is clear-cut, i.e., the prevalence of hand ROA (≥1 joints with K-L ≥2) increases from less than 10% in those <44 years to over 80% in individuals >75 years of age (103). However, some people never display any signs of hand OA at any age.

Age increases the risk of developing hand OA after 25 years, first in the metatarsophalangeal joints, and after the age of 45, in the IP and the MCP joints (119). Age-related changes in the musculoskeletal system increase the propensity to OA, but the joints that are affected and the severity of disease are most closely related to other OA risk factors such as joint injury, obesity, genetics, and anatomical factors that affect joint mechanics (120).

Joints undergo remodeling over the lifetime of humans and cartilage becomes vulnerable to mechanical forces, which might expose subjects to OA (44). It is now thought that aging does not have any direct causal influence on OA but rather it affects the ability of the joint tissues to maintain homeostasis when stressed, resulting in breakdown and loss of the articular cartilage (121). This phenomenon is supported by the finding that the pattern of hand ROA varies between populations with different degrees of longevity. Generally, the first joints with OA seem to appear at an older age in populations with high longevity, and the progression of hand OA is slower than in populations with a shorter life-spans (122). Age-related sarcopenia, increased bone turnover, and the so-called senescent secretory phenotype of cartilage cells, e.g., characterized by increased secretion of pro-inflammatory cytokines and MMPs, may also contribute to the development of OA (63).

2.4.2 Gender

Men have more hand OA than women before the age of 55 but the prevalence changes to become higher in women older than 55 years (4, 123). Females also have more severe hand OA than men (123). A Mediterranean area study found that women had higher scores in the DIP joints, whereas the MCP joints were more involved in men (124). The definite increase of OA in women around the time of menopause has led investigators to hypothesize that hormonal factors may play a role in the development of OA (5). Current evidence claims

that menopause hormone replacement therapy (HRT) may have some effect on ameliorating disease symptoms or severity whilst not influencing disease incidence (125).

Another possible explanation for the female gender association with hand OA has been thought to be attributable to the adipose tissue is that females normally have a higher percentage of fat than men. However, the central body fat pattern was not found to be associated with the risk of hand OA (126).

The sex chromosomes could be a possible modifying factor from a genetic point of view. In support for this speculation, many chromosomal (autosomes including 7, and the sex chromosomes X and Y) abnormalities have been found to be associated with knee and hip OA when studying OA cartilage tissue (127).

2.4.3 Overweight/obesity

In a load bearing joint such as the knee or hip, overweight is a significant risk factor as the heavier load puts more load on the joint (128). In the case of the hand, there is also a moderate association between OA and overweight/obesity, even though joints of the hand do not bear the weight load (35, 79).

The risk for OA per kg increase in body weight in a twin study was 10% in hand and 13% in the knee. Thus, overweight/obesity may influence OA in some other way than simply a direct loading on the joint; e.g., adipokines secreted by adipose tissue have been speculated to play a pro-inflammatory role in arthritis (129). These metabolic factors are known to possess catabolic and pro-inflammatory properties and to orchestrate the pathophysiological processes in OA (130).

Adipose tissue-associated inflammation is present both in the development of metabolic syndrome (MetS) and OA, which suggest that OA might be a metabolic disease (131). Interestingly, weight loss has been found to decrease the risk for OA independently from the start weight, indicating that the risk due to excess weight could be reversed and even prevented (132).

2.4.4 Joint loading

Since there is no blood circulation in joint tissues, a moderate mechanical loading of the joint is necessary to keep the tissue healthy; this loading acts by pumping the nutrients to the cells of the joint (133). Joint tissues are sensitive to mechanical loading and disuse or overuse leads to an unbalanced maintenance of the joint tissues and thus to cartilage degradation, the development of which is the hallmark of OA (134). Overloading of the joint is a risk factor, in certain occupations, especially heavy manual labour (135). An animal study shows that unloading of the joint, combined with poor muscular control and weakness, might constitute risks for the onset of joint degeneration as the articular cartilage becomes thicker, softer and more permeable (136). Furthermore, joint incongruity, laxity, impaired proprioception, trauma and heavy physical load weaken the joint (44).

2.4.5 Genetic factors

Kellgren concluded in 1963 that "more than one common inherited factor may give rise to multiple osteo-arthrosis." (137). Subsequently, two twin studies (9, 138) showed that the heritability is 65% for hand OA, which explains why genetic factors have been one of the most widely studied risk factors for hand OA.

The Framingham study found a significant genetic contribution to generalized OA, with evidence for a major recessive gene and a multifactorial component that representing either polygenic or environmental factors (139). The hand OA specific variations of the genome not only have to be identified but the environmental factors also must be taken into account. This task has proved to be extremely challenging.

Humans have 3.2 billion bases in their haploid genome. Of these, about 85 million exhibit SNPs that can vary between individuals (140). However, about 4-5 million sites differ from the reference genome in a typical genome. The majority of variants are rare (64 million <0.5%; 12 million 0.5 – 5%), and only 8 million have a frequency >5%. When observing a single genome, the majority of the variants are common, only 1-4% of the variants have a frequency <0.5%. (140) There are also several other genetic variation types including, insertions, deletions, and structural variants but they are rare compared to SNPs (140).

Some SNPs located in genes occur 1) in a coding area where they can change the amino acid sequence of the expressed protein i.e. non-synonymous:

missense (change of the amino acid) or nonsense (create a stop codon), 2) silent (synonymous) their occurrence does not change the amino acid sequence. Other SNPs can also be 3) intergenic, i.e., they occur between the genes on the non-coding part of the genome and they may alter gene splicing, transcription factor binding, messenger RNA degradation or the sequence of the non-coding RNA. Thus, depending on the location of the SNP, its effect may vary from severe, producing a totally useless protein product to be degraded, to mild, i.e., changing only the nucleotide sequence that may alter some binding site in the DNA strand.

Although initially the uncovering of hand OA susceptibility variants seemed like searching for a 'needle in a haystack', fortunately there were some hypotheses that could be used as rational starting points for these trials.

The obvious characteristics of OA led the candidate gene research to start its search from three different perspectives: the cartilage structure factors as cartilage was known to be degraded; overweight-related factors as overweight subjects have a higher prevalence of OA; and inflammation-related factors as the OA joint displays signs of low grade inflammation. However, these SNPs in the selected candidate genes can also interact with environmental factors. For example, mechanical loading of the joints can regulate the expression of the genes (141). On the other hand, the SNPs may also cause their effect independently: e.g. by altering the expression of an inflammation gene variant and thus altering the inflammation status (142).

An overview of the risk factors in the osteoarthritis is presented in Figure 5.

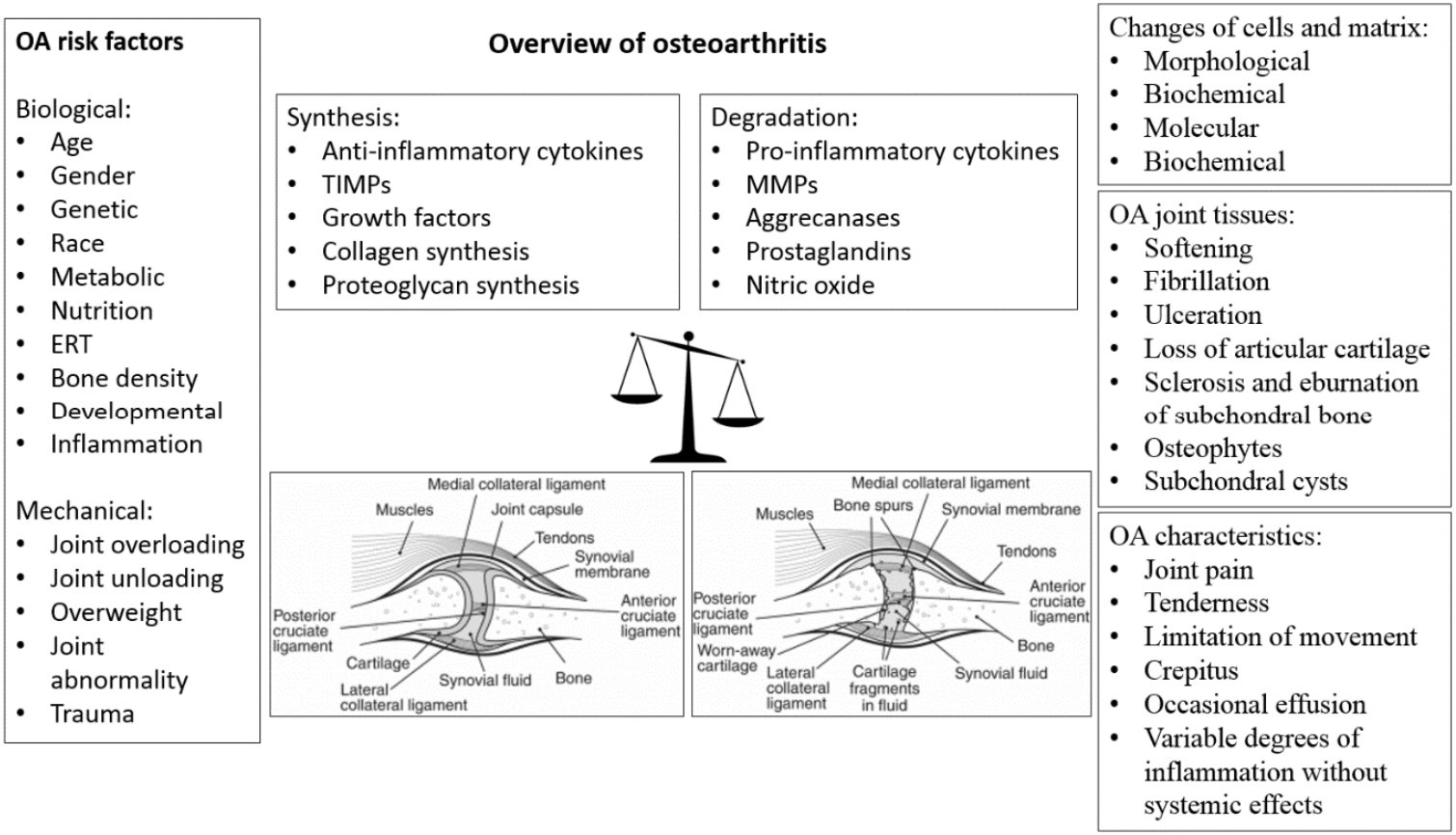

Figure 5. Overview of osteoarthritis.

2.5 Genetic studies of hand OA

The first genetic studies on hand OA involved genome wide linkage scans using family data with a large sample size. The goal of these scans was to locate the genetic loci of the possible association with the disease. This approach was based on the observation that genes that reside physically close to each other on a chromosome remain linked during meiosis. Markers closely flanking a disease gene can be used to track the mutation in a pedigree. When the linked chromosome regions were found, it was possible to initiate the search for the exact locus.

Candidate gene studies focused on the potential susceptibility loci found in the linkage scans and attempted to find the actual SNPs inside the linked region. The diseased and healthy phenotypes were compared in these studies to determine whether their genotypes differ in certain genetic loci. Gene mapping is straightforward in single gene/SNP diseases for which only one alteration affects the disease but they are more complicated with complex traits for which several genetic loci interact with environmental factors to affect the aetiology of the disease. In such complex traits, the estimated risk can be calculated as a risk ratio (RR) or an odds ratio (OR). However, a limitation is that these studies were sometimes relatively small in size and lacked the statistical power to detect any associations. In addition, the knowledge about all of the possible SNPs were not complete at the time when the first candidate gene studies were conducted.

The next approach in gene studies followed technological innovations whereby, a genome wide association study (GWAS) was conducted. Hundreds of thousands of SNPs were simultaneously analysed in large populations in GWAS (143). These studies were more expensive and were therefore first mostly done to elucidate diseases such as cardiovascular diseases that due to their high morbidity and mortality are actively researched and attract funding. Such studies had relatively abundant funding. Subsequently, the approach has been applied to several different diseases including OA. However, despite the high expectations, these studies mostly failed to locate any OA susceptibility loci. Attempts were made to overcome one issue related to many of these trials, i.e., the lack of statistical power after correction for multiple testing. This involved a retrospective approach by conducting meta-analyses of the candidate gene and by undertaking GWAS studies and pooled analyses of large sample sets (144). Finally, replication analyses have been done in different populations in attempts to identify the true disease genes and SNPs (145).

A total of more than 780 studies have been published on the genetics of OA so far (see Figure 6).

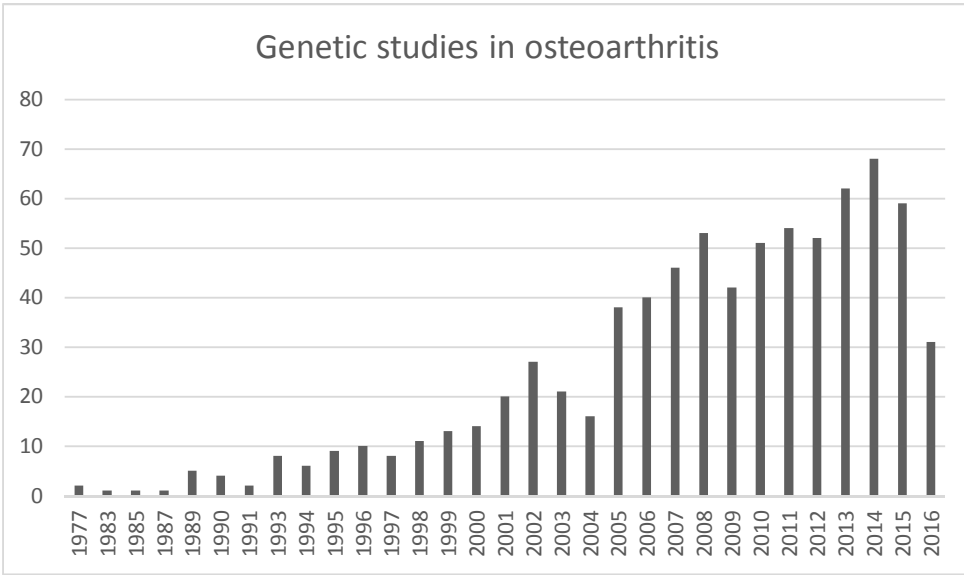

Figure 6. Genetic studies on OA, publications per year collected from PubMed with the search terms "(osteoarthritis-and-polymorphism) or (osteoarthritis-and-linkage) or (osteoarthritis-and-GWA)".

2.5.1 Linkage studies

The first linkage study to investigate hand OA was conducted in 1996 by Wright *et al.* (146), who used sibling pairs in nodal OA (NOA), and found linkage in chromosome 2q (long arm).

The next linkage study was published by Leppävuori *et al.,* in 1990 who analyzed 302 microsatellite markers in 64 study subjects and found that a locus on 2q12-q14 harboring the *IL1* gene cluster was linked to severe DIP OA. They also found three other potential chromosomal regions: 4q26-q27, 7p15-p21, and Xcen (147).

Some previous studies had suggested associations of OA with both *COL2A1* and *VDR* loci in 12q. However, Baldwin and co-workers conducted a linkage study in the FOS and concluded that mutations at the *COL2A1*/*VDR* locus did not play an important role as a cause of common (hand and knee) OA in the general population (148). The vitamin D receptor (*VDR*) gene is located adjacent to *COL2A1*, and is known to be associated with bone mineral density (149).

Demissie *et al.* reported several (chromosomes 1, 2, 7, 9, 11–13, and 19) associations for hand ROA in a linkage study from Framingham Heart Study (684 original cohort members and 793 offspring in 296 pedigrees) (7).

Stefansson *et al.* reported genome wide linkage analysis of patients with idiopathic hand OA who were phenotyped for the involvement of either or both the DIP joints and the first CMC joints (150). That group found a linkage in chromosome 2, 3 and 4, the best peak of which includes the locus *MATN3* gene, which encodes the non-collagenous cartilage extracellular matrix protein, matrilin-3. A novel missense mutation that changed the coding for amino acid threonine to that of methionine in the epidermal growth factor-like domain in matrilin-3 co-segregated with hand OA in several families. The mutation frequency was found in slightly more than 2% of patients with hand OA in the Icelandic population and it was estimated to pose a RR of 2.1.

Hunter and co-workers conducted a genome wide linkage study where they tested the hypothesis that sub-phenotypes of hand OA may exhibit stronger linkage than had been found for overall hand OA (151). A total of 16 sites (from chromosomes 1, 2, 3, 7, 8, 10, 12, 13, 14, 15, 17) were found to be in linkage with different hand OA phenotypes. The authors speculated that several chromosomes contain hand OA susceptibility genes and that a joint-specific approach may be more rewarding than a global approach to the genetics of hand OA.

Greig *et al*. conducted a linkage scan for NOA and found linkages to loci on chromosome 3 (for joint space narrowing and osteophytes), chromosome 4 (for joint space narrowing), chromosome 8 (for DIP), chromosome 11 (for ROA) and chromosome 16 (for joint space narrowing) (77).

The most recent, in 2007, and one of the largest linkage studies was conducted in twins by Livshits *et al.* (115), who examined 538 individuals (269 monzygous female twins) and 1256 individuals comprising 628 dizygous female twins and identified a linkage for DIP-OA on chromosome 2 at 90 cM (logarithm (base 10) of odds (LOD) = 2.90) and for K–L score for both hands on chromosome 19 at 65 cM (LOD = 4.26). Although several other significant linkage peaks were observed; e.g., on chromosome 1 at 250 cM and on chromosome 3 at 30 cM, these were less significant linkage peaks. These results reported by Livshist *et al,* were robust and also later replicated in smaller studies, thus the fine mapping of these regions was postulated to be the logical next step to pinpointing potential susceptibility gene(s) of interest. This was exactly the approach applied by Näkki *et al.* when they performed targeted linkage scan for 2q11.2 and found four SNPs in the *IL1R1* gene that

mapped to a 125 kb LD block, which provided evidence for an association with hand OA in family-based and case-control analysis. The strongest association found in that analysis was with rs2287047 SNP (p-value = 9 x 10^{-4}) (152).

In conclusion, eight genome wide linkage scans with different hand OA phenotypes have been undertaken and they have indicated that autosomal chromosomes 1-4, 7-17, 19, and sex chromosome X may harbor susceptibility genes (7, 77, 115, 146-148, 150, 151) (see Figure 7).

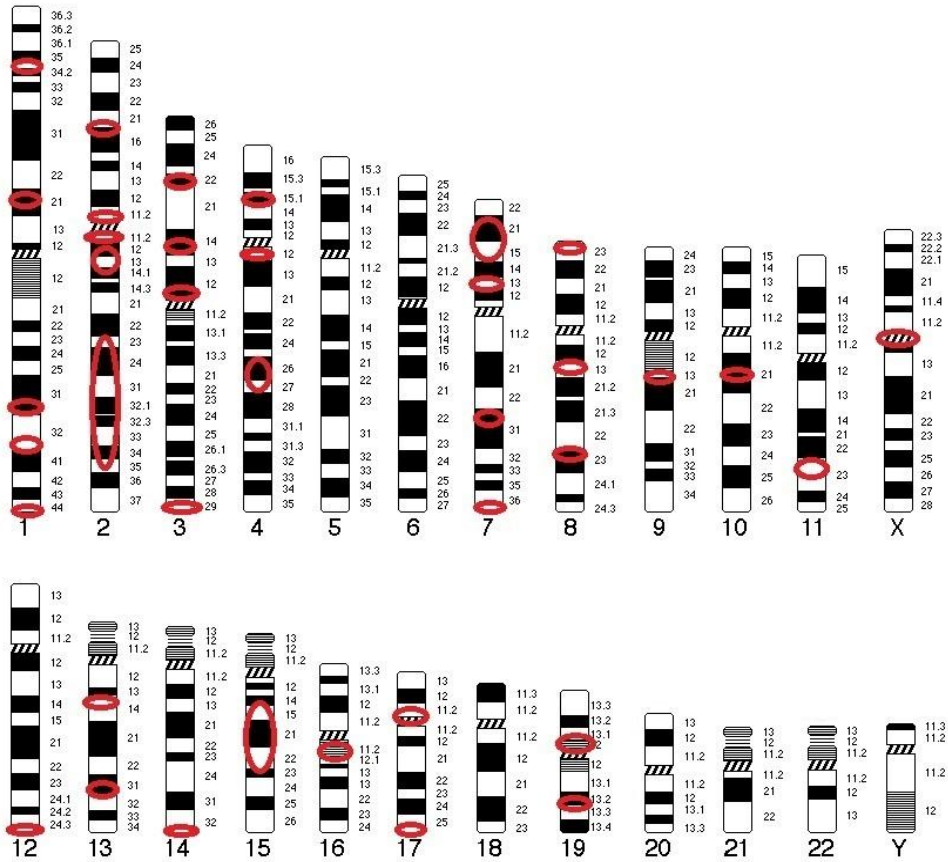

Figure 7. Potential OA susceptibility loci according to linkage studies (Modified from http://www.biologia.uniba.it/rmc/0-internal-images/z-ideograms/ideograms.html)

As a consequence of these linkage studies numerous candidate gene studies have been carried out to assess the association of a particular variant with hand OA.

2.5.2 Candidate gene studies

Only the candidate gene studies for OA that include hand joints will be reviewed in the following text. These studies are divided into five hypothesis groups, thus: cartilage structure, vitamin D receptor, growth factors, female sex associated, and cytokines.

Cartilage structure

The first investigation of hand OA was the Finnish study conducted by Vikkula and co-workers, who initially did not find any association between *COL2A1* and generalized OA or finger OA (153), but in a continuation of their study reported significant linkage. They did, however, point out that the linkage was probably not in an exon region but rather in a promoter or the intron area (14).

Meulenbelt *et al.* also studied *COL2A1* in the Rotterdam study population (99). The phenotype studied was generalized OA, that included the hand, and the genetic factors studied were allele frequencies of three dimorphisms (HaeIII, HindIII, MaeII) and a variable number tandem repeat (VNTR) polymorphism of the *COL2A1* gene. The VNTR allele 14R2 and the HindIII polymorphism displayed a significant association. Haplotype analysis of the HaeIII, HindIII and VNTR polymorphisms revealed that a specific haplotype (1-2-14R2) was strongly associated with generalized ROA in three or more joint groups OR 5.3 (95% CI; 2.3-12.7).

The Baltimore Longitudinal Study of Aging (BLSA) found an association between a human aggrecan (*ACAN*) gene polymorphic allele and hand OA (154). An Australian study also found an association between ACAN and hand OA but this time, in a VNTR polymorphism (155).

The *ACAN* gene was also studied in our dentist and teachers cross-sectional study by Kämäräinen *et al.* (156). The findings indicated that VNTR allele A27 provides protection from hand OA and that alleles shorter or longer than this may predispose subjects to the disease. Furthermore, they proposed that a certain number of tandem repeats would provide optimal functioning of the ACAN protein molecule and that the contribution of genetic factors to the development of hand OA may be even more important than that of environmental factors (156).

More structure gene associated studies have subsequently been undertaken, some reported an association but others did not. The general conclusion from these results is that one can only exclude these genes as major susceptibility loci for OA (22).

The association between the *MATN3* gene and first CMC hand OA was studied in the Rotterdam study and the Genetics, Arthrosis, and Progression (GARP) study (157). Stefansson *et al.* (150) stated that the previously described association of *MATN3* T303M with the hand OA phenotype could not be observed in their populations. However, the GARP study found that carriers of the A allele of *MATN3* SNP6 (G>A) were more likely to display OA of the first carpometacarpal joint (CMC1). In addition, Pullig *et al.* investigated 50 consecutive Caucasian patients with radiographic and symptomatic hand OA of the CMC1 (late-stage arthritis, EATON stage II–IV); they found that the *MATN3* gene was associated with CMC1 OA but not with knee OA (158).

Gu and co-workers studied the *MATN3* SNP6 (rs8176070) in 420 Chinese patients with OA (216 women and 204 men), including hand OA (28 patients), and 312 healthy controls (159). Radiographic findings of OA were classified into mild (K-L grade 1 or 2) and severe (K-L grade 3 or 4). The functional or symptomatic status of the OA patients were classified as functionally or symptomatically good (Lequesne's functional index = 10) and poor (Lequesne's functional index > 10). They found that the heterozygous rs8176070 genotype increased the risk of hand OA in their Chinese Han population.

In 2008, Rodriguez-Lopez and co-workers examined the gene that encodes a disintegrin and metalloprotease with thrombospondin motif (*ADAMTS*); this gene expresses the main aggrecanase that causes cartilage destruction in mouse models (160). The research group had samples obtained from four European Caucasian collections, comprising a total of 2715 patients with knee, hip, or hand OA and 1185 OA-free control subjects. They found a trend towards a decreased frequency of the putative deleterious allele of *ADAMTS5* rs226794 SNP among patients with severe knee OA. However, results in patients with knee OA from two additional sample collections (n = 360 and n = 265) did not confirm this trend. Moreover, no association was found with hip OA or hand OA.

Rodriguez-Lopez and co-workers also published another study on this topic (161), in which non-synonymous SNPs (nsSNP) in 18 *ADAMTS* and 31 *ADAM* genes were analyzed. Four putative damaging nsSNP were found in *ADAMTS2*, *ADAMTS14*, *ADAMTS16*, and *ADAM12*, respectively. These

nsSNPs were analyzed in case-control sample collections with a variety of phenotypes in a total of 3217 OA patients and 2214 healthy controls, all of them were Caucasians. However, no statistically significant differences were found for *ADAMTS2*, *ADAMTS16,* and *ADAM12* nsSNPs. In contrast, the rare allele of the rs4747096 nsSNP in *ADAMTS14* was over-represented in patients with symptomatic hand OA, with an OR = 1.37 (95%CI; 1.0-1.9: P=0.047). The research group concluded that *ADAMTS14* involvement, if confirmed, would open a new area of interest in OA pathogenesis because of its role in the maturation of collagen fibres.

ASPN (asporin) is an important regulator of cartilage homeostasis and other ECM molecules of cartilage, including collagens. It is expressed at low levels in normal cartilage but is expressed abundantly in OA articular cartilage. Atif and co-workers studied 10 SNPs within the *ASPN* gene in 775 affected siblings with radiographically confirmed hand or knee OA (162). The study subjects were classified as having hand OA when they met the following criteria: (1) involvement of ≥3 joints (K-L grade ≥2), including at least one DIP joint of digits 2–5; (2) two of the three involved joints within the same joint group (DIP, PIP or CMC); and (163) evidence of OA observed in both hands (bilateral hand involvement). One *ASPN* variant allele in rs7033979 showed nominal evidence of association with both hands OA (P=0.042), which suggested that polymorphisms within *ASPN* do not exert any major influence on the susceptibility to hand OA in US Caucasians.

Bijsterbisch *et al.* conducted a study to elucidate polymorphisms in *ASPN*, bone morphogenetic protein 5 (*BMP5*), and the growth differentiation factor 5 (*GDF5*) gene in relation to the progression of hand OA. Their study population consisted of 251 hand OA patients and 725 controls from the GARP study (164). The hand OA progression was based on any change in osteophytes or joint space narrowing, above the smallest detectable change. The minor allele of *ASPN* rs13301537 SNP was associated with hand OA progression over six years. An association was also found between *ASPN* rs13301537 SNP and the 2-year progression. The mean change in osteophytes and joint space narrowing was significantly higher in the homozygotes.

Suk *et al.* reported a hand OA study with the specific hypothesis that the ectonucleotide pyrophosphatase/phosphodiesterase 1 (*ENPP1*) gene, that encodes an enzyme that regulates soft tissue calcification, would be involved with periarticular calcification (165). The study population consisted of 574 Chuvashians who are Caucasians in southern Russia. Alleles of the upstream microsatellite marker and several SNP haplotypes were consistently associated with the risk of developing hand OA.

Misra *et al.* reported an interesting experiment about a polymorphism in the *MGP* gene and hand ROA (166). The *MGP* gene encodes the Matrix Gla protein, which is a mineralization inhibitor. MGP is present in bone, cartilage, and vascular smooth muscle, but in animal models, the absence of functional MGP evokes similar changes as encountered in human OA. Genetic polymorphisms (rs1800802, rs1800801, and rs4236 SNPs) and hand ROA in ≥ 1 joint of 376 participants were studied. Homozygosity of the *MGP* rs1800802 minor allele was associated with 0.56 time lower prevalence of hand OA compared with having ≥1 major allele at this locus. However, no significant association was found between serum MGP concentrations and hand ROA.

Liying *et al.* reported a case-control study of SMAD family member (SMAD3) gene rs12901499 SNP in a Northeast Chinese population of 121 hand OA patients and 236 controls (167). There was a significant association for the carriage of the G-allele rs12901499 SNP with hand OA OR = 3.60 (95% CI; 2.01-6.44; $p < 0.001$).

Growth factors

Insulin-like growth factor 1 (IGF1) is another molecule that is implicated in regulating cartilage structure. IGF1 stimulates chondrocytes to synthesize extracellular matrix (ECM) components and its locus was also found to be associated with hand OA (168).

Zhai *et al.* also studied *IGF1* and ROA in the Rotterdam study and found interactions with the *COL2A1* gene (169). The presence of ROA was defined as a K-L score of ≥2 in at least one of four joints (knee, hip, hand, and spine). Overall, no association was found between the *IGF1* polymorphism and ROA. There was, however, some evidence for an interaction between the *IGF1* and *COL2A1* genes because individuals with the risk genotype of both genes had an increased prevalence of ROA. Although *IGF1* has been studied extensively, the results are still inconsistent and no overall association can be found with ROA, perhaps because in some studies there was an absence of BMI information (170).

GDF5 is another structure associated gene (171), which was originally associated with knee and hip OA in Asia (172). Subsequently Vaes *et al.* reported an association in the Rotterdam study comprising 3050 participants with hand OA data (171). In their study the hand OA was defined as the presence of a K-L score >2 in two out of three hand joint groups (DIP, PIP, CMC1) of one or both hands. Women who were homozygotic for the *GDF5*

rs143383 C allele had a 37% lower risk for developing hand OA ($p = 8 \times 10^{-6}$).

Vitamin D receptor

Associations between *VDR* gene polymorphisms and hand OA were first studied in Japan by Huang *et al.* (173). The BsmI, ApaI, and TaqI restriction fragment length polymorphisms (RFLPs) of the *VDR* gene were analyzed in 270 Japanese female patients with ROA of the hand, hip, tibiofemoral (TF) joint, and patellofemoral (PF) joint, and compared them with Japanese female controls. No significant association was detected between the *VDR* gene RFLPs and OA phenotypes. Since subsequently both Baldwin *et al.* and our group failed to find an association between *VDR* and hand ROA (148) (174), there does not seem to be any association between single SNPs in the *VDR* gene and susceptibility to hand ROA. However, when the haplotypes were analyzed, the carriers of the *VDR* t-allele or the At haplotype had only half of the risk of displaying hand OA compared with the carriers of the T-allele and of the non-At haplotype (174). An increased risk for hand OA was observed for women with two copies of the *VDR* a-allele compared with women with the AA genotype. Conversely, the *VDR* a-allele carriage was associated with a tendency towards lowered odds of developing osteophytes. A novel finding was a combined effect of a low calcium intake and *VDR* polymorphisms, which was observed for symmetrical OA (174).

Recently, two meta-analyses have been published about *VDR* and OA, with one confirming the modest but statistically significant association (175), but not the other (176).

Gender related factors

Matkovic *et al.* examined the relationship between LEP and menarche and concluded that as LEP was a humoral link between adipose tissue and the gonads and since it mediated the effect of obesity on bone mass, it may have implications for the development of OA (177).

The fact that OA is more prevalent in women than in men led to investigations that focussed on the sex hormone, oestrogen. Ushiyama *et al.* observed that PvuII and XbaI polymorphisms in the gene that encodes the oestrogen receptor (ESR) gene were associated with generalized OA (178). This led to further studies on *ESR* gene polymorphisms and OA. These studies did not detect any association between the *ESR* gene and hand OA (81, 179).

A recent meta-analysis of 32 previous studies on OA and genetic polymorphisms of *ESR* supported an association between two SNPs (rs9340799 and rs2228480) and OA (180). However, this was contradicted by another meta-analysis that analyzed 10 case-control studies, which found no evidence for any correlation but rather suggested that the putative association could have been attributable to small-study bias (181). A third meta-analysis indicated that there may be a weak relationship between the *ERα* XbaI (rs9340799) polymorphism and OA in Europeans but not in Asians, and that the *ERα* PvuII (rs2228480) polymorphism was not associated with OA in either ethic group (182).

Cytokines

Cytokines are another area of interest as these agents are known to be present in the OA joint and they are more abundantly present in rheumatoid joints (183). Stern *et al.* studied the association between seven different *IL* polymorphisms (*IL1A, IL1B,* and *IL1* receptor antagonist (*IL1RN)*, and *IL1RN* VNTR) and severe hand OA (184). They found an association between erosive hand OA and a genomic region that contained the *IL1B 5810* SNP in a US Caucasian population.

Riyazi *et al.* also studied the *IL10* promoter polymorphism and DIP OA in the Early Arthritis Clinic (EAC) cohort (104) and found the *IL10 -2849A* polymorphism was associated with decreased IL10 expression. The research group had earlier found an association between a low innate expression of IL10 and increased risk of familial OA, and decided to expand the study to examine the association between seven novel SNPs located downstream of the *IL10* transcription start site (*-2849,-2763, -1330, -1082, -819*, and *-592)* constituting the four ancient haplotypes, and DIP OA. However, no significant association could be found between DIP OA and the *IL10* gene.

Moxley and co-workers studied 64 European-descent cases with ROA and 48 controls for *IL1B* haplotype and *IL1A-IL1B-IL1RN* extended haplotypes (185). They genotyped nine SNPs, one VNTR, and one microsatellite marker that extended across loci for *IL1A*, *IL1B*, and *IL1RN*. Their main conclusion was that a more detailed examination of *IL1* loci and haplotypes could reveal some supporting evidence that a single extended haplotype defined by the *IL1* region markers elevated the risk for hand OA. Their data suggested one *IL1B* risk haplotype and a recessive genetic model.

Our group has also examined the association of the *IL6* and *IL1* gene cluster with DIP OA in our study population (101, 186). The variant alleles at the *IL6* polymorphic promoter loci were associated with the more severe DIP OA

outcomes, i.e., the symmetrical and symptomatic forms of the disease (101). In addition, two *IL1B* SNPs (rs1143634 and rs1143633) were found to be associated with bilateral DIP OA (186).

Vargiolu *et al.* evaluated the interleukin 4 receptor that encodes the (*IL4R*) gene and hand OA in 403 Caucasian patients and 322 controls from Bologna, Italy (187). They studied a total of 18 SNPs (nine in *IL4R*, five in *IL4* and four in *IL13*). Two SNPs (rs1805013 and rs1805015) that mapped to the *IL4R* gene, were associated with hand OA with p-values of 0.01 and 0.03, respectively, in the whole sample.

None of the *IL13* SNPs analysed revealed any association with hand OA, whereas some of the analysed SNPs within the *IL4* gene showed a significant association with hand OA but only in certain subgroups of patients.

Two SNPs in *IL4* (rs2243250 and rs2243274) displayed significant association with OA (p=0.027 and p=0.018 respectively) with CMC1. However, none of these associations remained after correction for multiple testing.

Blumenfeld *et al.* studied *IL6* polymorphisms in hand ROA in the TwinsUK (1440) and Chuvash pedigree (1499) samples (188). The summary K-L grade for each of the 14 joints on both hands, and in total, were evaluated as ranging from 0 to 4 according to the original atlas. These workers found that *IL6* polymorphisms were significantly associated with hand ROA in the two samples that have different ethnicities and lifestyles. They concluded that the age–environment–genes interaction was an important factor in the progression and manifestation of hand ROA.

Inflammation has also been studied for other agents in addition to the cytokines, in particular the C-reactive protein (CRP) gene (*CRP*) in the GARP study (189). CRP is a sensitive marker of low grade and acute phase systemic inflammation that may affect susceptibility to the onset of OA. These investigators measured serum CRP levels and genotyped five tagging SNPs in the *CRP* gene of 353 individuals. A significant and consistent relationship was identified between serum high sensitive C-reactive protein (S-HsCRP) levels and observed haplotypes. Additionally, a *CRP* haplotype, which also appeared with a significantly higher than expected phenotypic mean S-HsCRP concentration, was associated with severe hand OA, this haplotype was tagged by an rs3091244 SNP. It was stated that carriers of this allele had an increased risk for the presence of severe hand OA with an OR of 2.3.

Kerkhof and co-workers also studied the *CRP* gene and hand ROA in the Rotterdam study (190). The association between CRP levels and genetic

variation in the *CRP* gene and ROA was examined in 861 patients with hand ROA, 718 with knee ROA, 349 with hip ROA and 2806 controls. No association was found between serum CRP levels or genetic variation in the *CRP* gene with the prevalence, incidence or progression of ROA independent of BMI.

Other hypotheses

OA also shares symptoms with other diseases and thus the genes associated with these diseases are worthy of examination when regarding hand OA. For example, hereditary hemochromatosis (HH) is a disease in which over 80% of the patients suffer from arthritis. The hemochromatosis gene (*HFE*) is associated with HH and its association with primary OA (including hand, elbow and ankle) has been examined (191). Both of the studied SNPs, i.e., C282Y (rs1800562) and H63D (rs1799945) were found to be associated with hand and ankle joint OA.

In conclusion, based on previous studies, *COL2A1, ACAN, MATN3, ADAMTS, IGFI, ASPN, GDF5, ENPPI, MGP, SMAD3, VDR, ESR, IL1B, IL10, IL6, IL4R, CRP* and *HFE* (positions in Figure 8) genes are potential candidates as modifiers of the risk of developing hand OA.

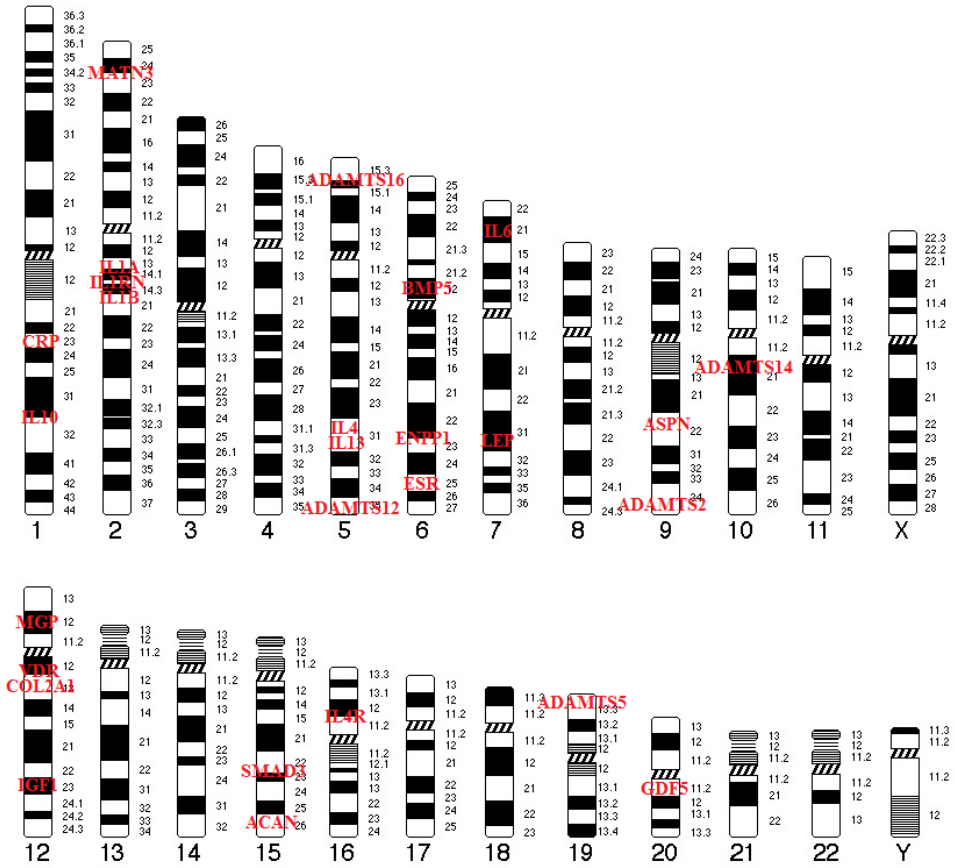

Figure 8. OA susceptibility loci according to candidate gene studies cited in this literature review (Modified from http://www.biologia.uniba.it/rmc/0-internal-images/z-ideograms/ideograms.html)

2.5.3 Genome wide association studies

In the newest studies, the focus has moved away from the traditional hypothesis-based search, i.e. the candidate gene approach to the new genome wide association approach. A huge number of polymorphisms are analysed in arrays and the other variations that are not possible to analyse in the array are calculated with computer based imputing, based on LD. In this way, theoretically, one can cover all of the variations associated with the disease with the *proviso* that the sample size guarantees enough statistical power. The GWASs have been implemented by several consortia, however, these were mostly for studying other common (and better funded) diseases (e.g., cancer

and heart diseases) and only recently extended to cover relatively less researched diseases such as OA.

After the completion of the Human Genome Project (HGP) in 2003 and the International HapMap Project in 2005, researchers have had access to extensive SNP information that has greatly helped them in their efforts to assess the genetic contributions to common diseases (192).

The first OA specified GWA studies were conducted for knee OA (193, 194) and it reported an association between *DVWA* on chromosome 3p24.3, and *PTGS2/COX2* and knee OA, respectively.

The first hand OA specified GWAS was reported by the TREAT-OA consortium (145), which found five SNPs that had a likelihood of association with hand OA in the discovery stage. These findings were based on the TwinsUK cohort and the Rotterdam discovery subset, which comprised a total of 1804 subjects. One SNP (rs716508) was successfully confirmed in the replication stage (the Chingford Study, the Chuvasha Skeletal Aging Study, the Rotterdam replication subset and the GARP study; a total of 3266 people) (meta-analysis $p=1.8x10^{-5}$). The minor allele conferred a reduced risk of 33% to 41%. This gene variation is located in intron 1 of the *A2BP1* gene and it was also found to be associated with reduced bone density at both the hip and the spine ($p<0.01$). This finding was interpreted as evidence that the potential mode of action of the gene in hand OA might be via effects on the subchondral bone.

The next GWAS, the Rotterdam study, included the knee, hip and hand, and was conducted in a study population comprising Dutch Caucasian cases and controls (195). The study also included a very large replication population (deCODE, the TwinsUK, the FOS, the Chingford Study, Oxford cohorts, the Nottingham Case–Control Study, a Greek total joint replacement study, a Spanish total joint replacement and hand OA study, the GARP study, the Study of Osteoporotic Fractures (SOF), and the Osteoporotic Fractures in Men (MrOS) study). The Rotterdam study revealed a novel common variant, in 7q22 in the intron 12 of the *COG5* gene (rs3815148) was identified, which increased the risk of developing hand OA by 14%. This SNP was one of the 18 SNPs in 12 loci identified from the original GWAS and this finding could be verified in the replication populations included in the study.

Although great hopes were initially put on GWAS as the ultimate means to identify the OA risk loci, those hopes now seem to be badly forlorn. This is supported by the observations by Panoutsopoulou *et al.*, who reported insights from the stage 1 of the arcOGEN study, a consortium that aimed at performing

GWAS on more than 7500 OA cases (knee and hip) (196). The Panoutsopoulou group found that none of the association signals that the authors identified reached genome-wide levels of statistical significance, which therefore highlighted the need for collaborations capable of achieving even larger size sample sets. The application of analytical approaches to examine the allelic architecture of disease from the stage 1 genome-wide association scan data has indicated that OA is a highly polygenic disease with multiple risk variants that confer small effects.

A recent GWAS by Styrkarsdottir *et al.* on severe hand OA in 2230 Icelanders (197) found two significantly associated loci the 15q22 in the *ALDH1A2* gene and the 1p31. The variants within the *ALDH1A2* gene were confirmed to be associated with OA in replication sets from the Netherlands and the UK.

The latest GWAS was reported by Moon *et al.* in a Korean sample, which investigated wrist and knee OA (198). Their GWAS was based on copy number variations (CNV) in 371 OA patients and 467 healthy controls. The study identified genomic regions in six genes (*TNKS, CA10, POSTN, MAMDC2, KCND3*, and *ME3*) associated with OA that encompassed the CNV loci. None of the six loci had previously been reported in GWASs for OA.

In conclusion, four GWAS have been published including hand OA phenotypes. These GWAS have postulated that *A2BP1, COG5, ALDH1A2*, chromosome *1p31*, *TNKS, CA10, POSTN, MAMDC2, KCND3*, and *ME3* may confer risk for hand OA (positions in figure 9).

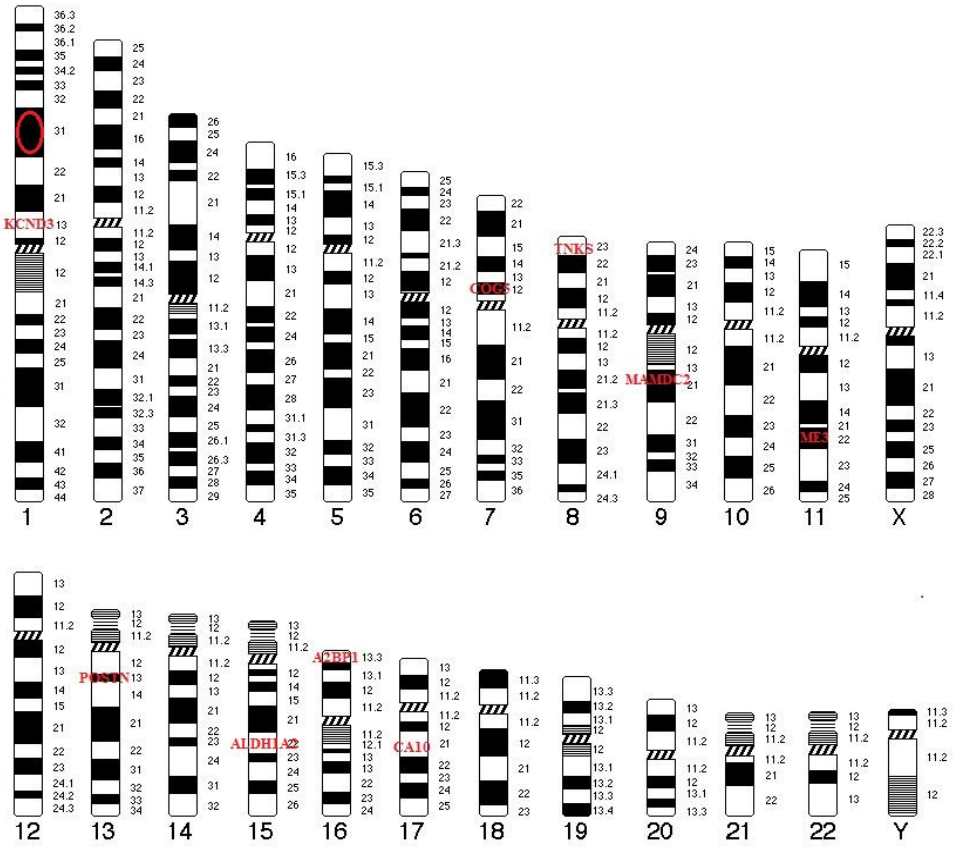

Figure 9. Hand OA loci according to the GWA studies cited in this literature review (Modified from http://www.biologia.uniba.it/rmc/0-internal-images/z-ideograms/ideograms.html)

2.5.4 Meta-analyses and replication studies

One reason for conducting replication studies is to narrow down the genetic loci found in linkage studies in order to find the exact polymorphism that would be associated with hand OA. Jakowlev *et al.* examined whether the hand ROA observed in a demographically homogeneous population (764) of European origin could be linked to several DNA polymorphisms in this chromosomal area (199). Hand ROA was characterized by two traits: (1) the total individual ROA score and (2) the osteophyte score, that were obtained from the principal components analysis of sums of the K-L grades and of the osteophyte grades, respectively, for 14 joints on each hand. Jakowlev *et al.* selected nine highly polymorphic loci in a 4.5-cM interval (6p12.3–p12.1) positioned in the middle of the 10.4-cM chromosome region as indicated by

Loughlin *et al*. (200) for hip OA. The additive genetic effects for the total individual ROA score and the osteophytes score were estimated to be 43% and 37.9%, respectively. A statistically significant association was found between the osteophytes score and rs1508632 SNP, which lies in close proximity to the *TINAG* gene, which implicates it as a possible hand OA susceptibility gene.

Meta-analyses combine several studies and thus have more power to detect associations as long as the study details and methodologies are well described and strictly comparable. The first meta-analysis including hand OA was reported by Kerkhoff *et al.* and was based on the Rotterdam Study and the Chingford Study (201). Hand ROA was defined as the presence of ROA (K/L > or = 2) in two out of three hand joint groups (DIPs, PIPs, CMC1/trapezio-scaphoid joint [TS]) of each hand. No association was seen between *FRZB, LRP5* and *LRP6* variants with ROA outcomes (knee, hip, and hand) for the two population-based cohorts. Kerkhoff and co-workers strongly recommended that future studies should have increased power and standardization of OA-phenotypes.

In line with this, Evangelou *et al.* published the second meta-analysis including hand OA (144) one year later. The second meta-analysis concentrated on the rs143383 SNP of the *GDF5* gene and the rs7775 and rs288326 SNPs of the *FRZB* gene. Fourteen teams contributed data to that study; for rs143383 SNP examination, the total number of cases and controls was 4040 and 4792 for hand OA; whereas the corresponding sample sizes were 4010 and 5151 for the rs7775 SNP analysis, and 3982 and 5152 for rs288326, for hand ROA. However, no statistically significant associations were found for hand OA in this meta-analysis.

Wise and co-workers also conducted a meta-analysis, which focussed on hand OA and *ESR1* (PvuII-rs2234693, XbaI-rs9340799, rs2077647, and rs1801132) and *ESR2* (rs1256031, rs1256034, rs1256059, rs944460) polymorphisms (81). Wise *et al*. used 539 FOS participants, with joint-specific hand ROA as defined by K–L scores ≥2 in the CMC1, DIP, IP1, or PIP joints. Hand ROA was identified in at least one investigated joint of DIP (39%), PIP (33%), and CMC1 (40%). There was no evidence of any association between ROA and genotype at any polymorphism. However, the study could not fully exclude associations of hand ROA with rs2234693, rs9340799, or rs944460 SNPs.

Moxley *et al.* published an *IL1* meta-analysis including hand OA in European-descent cases and controls (202). Their results showed little heterogeneity for hand ROA and only a modest trend toward positive association (summary OR

1.34, 95%CI; 0.83-2.17 p=0.23). No significant effect on any of the examined OA phenotypes (hip, knee, and hand) was found.

Kerkhof *et al*. published a meta-analysis concentrating on *ESR*, which also included hand OA data selected from the Rotterdam study (203). The hand OA discovery study included 874 cases and 2184 controls, and replication stage 557 cases and 1699 controls. The meta-analysis was done for 1431 hand OA cases and 3883 controls. Hand OA was defined as the presence of at least one definite osteophyte in two out of three hand joint groups (DIPs, PIPs, CMC1/TS) of each or both hands. There were no statistical significant associations detected in the Rotterdam Study-I between common genetic variation in the *ESR2* gene and OA in a dominant or recessive model or at the meta-analysis.

Cai *et al.* conducted a meta-analysis to examine the association between interleukins and OA, including hand OA (204). Seventeen independent case-control studies were included in the meta-analysis with a total number of 8022 subjects, consisting of 3293 OA patients and 4729 healthy controls. The results indicated that *IL6, IL1A*, and *IL1B* polymorphisms were statistically correlated with an increased risk of OA under the allele and dominant models. A subgroup analysis based on which form of the disease was present found a higher frequency of *IL6* polymorphisms among knee OA and hand OA patients, but not among hip OA and DIP OA patients. A higher frequency of *IL1A* polymorphisms were detected among hand OA, hip OA and DIP OA patients. Furthermore, there was a higher prevalence of the *IL1B* variant alleles among knee OA and hip OA patients, but not among hand OA patients.

The most recent meta-analysis was that published by Näkki *et al*. in 2015, which focused on the *MMP8* gene and included hand OA (205). Even though *MMP8* is a good biological candidate for OA, the Näkki group's study did not find common variants with significant association in the gene, even though the initial analysis of the *MMP8* gene pointed to a suggestive association between *MMP8* rs1940475 and knee OA (205). Although the finding could not be replicated in the other study cohorts, a trend was seen in all five cohorts that there was some predisposing allele (205).

In conclusion, seven replication studies with meta-analysis have been completed, which have included hand OA phenotypes. Two of the meta-analyses have indicated *IL1B-IL1RN, IL6* and *IL1A* as susceptibility genes for hand OA, whereas the other five studies failed to confirm the presence of any associations.

2.5.5 Genetic studies on other joint sites

Genetic studies in OA are usually first conducted to investigate the large joints such as the knee and hip, in large cohorts or in extensive consortia. Different joint sites sometimes share and sometimes do not share a susceptibility gene. Moreover, different ethnic groups have differences in OA susceptibility genes. Thus, the putative locus should be investigated in all the joint sites and for many different ethnic groups before one can conclude whether it is a risk factor in one specific joint site or in one population. Tables of other joint sites OA studies are shown in appendix (V)

3 AIMS OF THE STUDY

The overall aim of the study was to investigate possible genetic factors in the aetiology of hand OA among Finnish women. We chose the gene variants to be examined on the basis of the concurrently available information of their intragenic variation and previously proposed mechanisms that lead to the development of hand OA, namely: physical loading of cartilage structure, inflammation, and systemic factors related to overweight or obesity. Furthermore, we made an attempt to replicate genetic associations detected in hand OA studies and investigated possible associations of candidate genes from studies of OA at other joint sites. The specific aims of this study were:

1. To study the associations of the main cartilage collagen COL2A1 gene variants that had been previously reported in generalized OA including hand OA, with hand ROA. The possible modifying effect of physical occupational loading on the association between the *COL2A1* gene variants and OA were also taken into account.

2. To examine the role of *TNF*α and interleukin gene variants, i.e. genes associated with inflammation, in the aetiology of radiographic and symptomatic hand OA: and also to examine whether the possible association was independent of the polymorphisms of *IL6* and *IL1* that had been previously reported to associate with hand OA.

3. To evaluate the possible associations of adipokine gene polymorphisms with hand OA, and to examine whether overweight/obesity modified these associations.

4. To analyse in our material the findings of earlier publications that reported associations of gene variants with OA in joint sites other than the hand, and to replicate the previously found associations between hand OA and the potential susceptibility genes.

4 MATERIALS AND METHODS

4.1 Study material and data collection

The participants in the study were middle-aged women that represented two occupations, dentistry and teaching. Potential study subjects were identified through the registers of the Finnish Dental Association and the Finnish Teachers' Trade Union. Similar-sized samples of women aged 45-63 years in 2002 were randomly selected from each occupational group, using the place of residence (Helsinki metropolitan region) as a geographical restriction. Questionnaires were sent to 436 dentists and 436 teachers. Of those who received the questionnaires, 294 (67.4%) dentists and 248 (56.9%) teachers participated in a clinical examination at the Finnish Institute of Occupational Health at the turn of 2002 and 2003 (Figure 10).

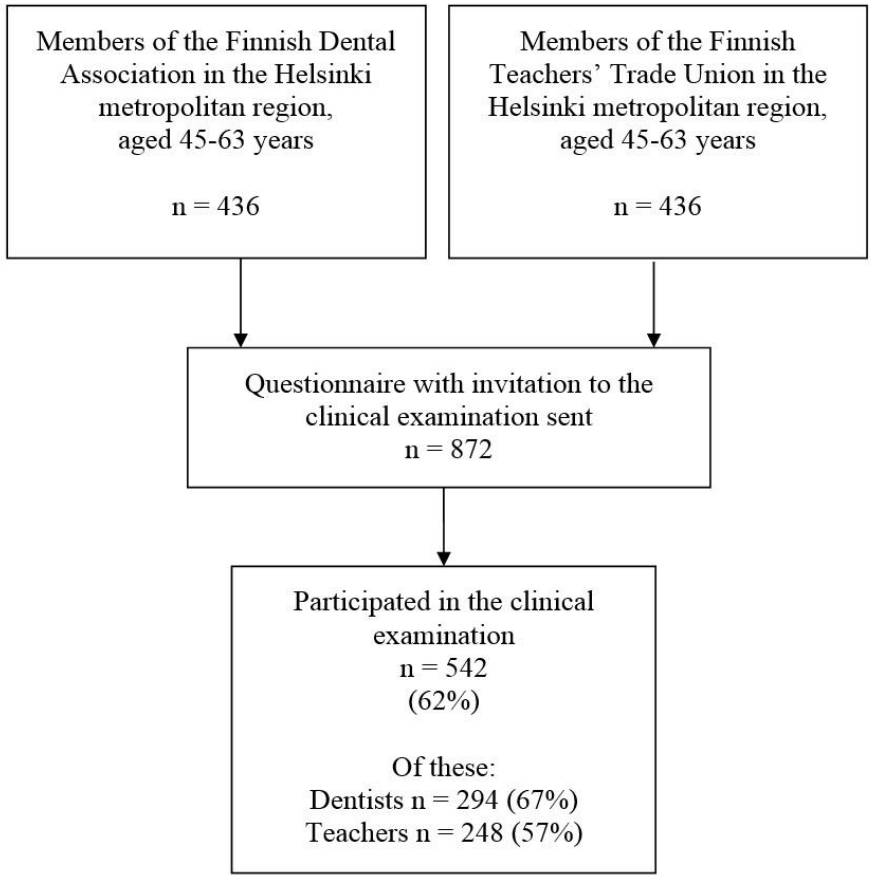

Figure 10. Flow-chart of the study.

Participation in the study was voluntary and the subjects' informed written consent was mandatory for inclusion into the study. The Hospital District of Helsinki and Uusimaa Ethics Committee for Research in Occupational Health and Safety approved the study proposal.

4.2 Hand radiography and image analysis

Both hands of the participants were exposed onto Kodak X-ray films with Siemens X-ray equipment (48kV, 10 mA, focus film distance 115 cm; Siemens, Munich, Germany). The evaluation of the analogue radiographs was made by an experienced radiologist who was blinded to the occupation, age, and all health data of the subjects. Each joint DIP, PIP, and thumb interphalangeal (IP) of both hands was graded separately and classified for the presence of OA by using a modified K-L scoring system (88) (see Table 3). The description of reference images used in the classification has been given in our earlier publication (206).

Table 3. The modified K-L scoring system for hand ROA grading used in this study

Grade	Classification	Description
0	no OA	normal finding
1	doubtful OA	finding possibly slightly abnormal
2	mild OA	a single radiographic sign indicative of OA, slight to moderate lowering of the joint space, sometimes subluxation, minimal osteophytes, degeneration cysts or slight marginal sclerosis, each of the latter signs without a clear narrowing of joint space but little, if any, additional pathology
3	moderate OA	considerable narrowing of joint space with additional degenerative pathology as indicated in grade 2, no destruction of the joint
4	severe OA	joint space destroyed or poorly visible with various advanced degenerative changes

Reliability measures were conducted by randomly choosing 46 radiographs, which were read by a second experienced radiologist and the primary radiologist independently. The reliability of the readings was estimated by

measuring intra-observer and inter-observer agreements (intraclass correlation) using the weighted Cohen's kappa coefficient with quadratic weights (95). The inter-observer agreement for OA ranged depending of the joint site: good (0.67 – 0.85) for DIP joints, moderate (0.39 – 0.61) for PIP joints, and poor to good (0.18 – 0.69) for MCP joints. The intra-observer agreement for OA ranged from good to very good (0.73 – 0.88) for DIP joints, (0.67 – 0.92) PIP joints, and (0.59 – 1.0) for MCP joints (206).

4.3 Questionnaires and interviews

All study participants received a self-administered questionnaire (that was the same except for specific questions related to either the teaching profession or dentistry), which participants took with them to the clinical assessment session at the Finnish Institute of Occupational Health. Missing data were completed in an interview by a researcher. The questionnaire included items on body height, weekly hours of hand-loading leisure-time activities (household chores, hobbies and other physical activity), smoking, and work history.

Assessment of hand joint pain.

The subjects were also asked to mark in which joints, if any, they had experienced pain or sensitivity to movement during the past month, and to give the intensity of the symptom(s) on a scale 1-3 (1=mild, 2=moderate, 3=severe) (figure 11).

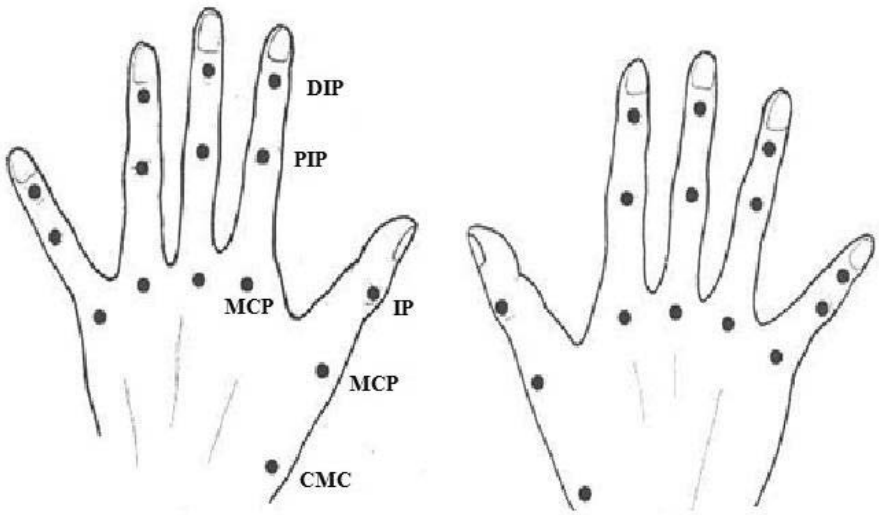

Figure 11. Hand joint sites as marked on a figure of the hands used for the collection of hand joint pain data.

Assessment of the dentists' work histories.
Six main tasks in dental work were identified prior to the study. The subjects were asked to recall their work history in 10-year periods (at the age of 25-34 years, 35-44 years and 45-54 years) in terms of mean number of working hours per week, and the proportion of time (percentage) performing each task during a typical working day. The weekly hours of the work tasks were then used to define empirically the dental task variation by using cluster analysis with the K-means algorithm (41).

Hand-loading leisure-time activities.

Similar to the definition of dental task variation, the hand-loading leisure time activities were empirically categorized into two groups using cluster analysis with the K-means algorithm. A classification procedure was performed based on the weekly hours of hand-loading household chores, hobbies, and physical activities.

Pinch grip strength

A researcher who had been trained for the measurement of pinch grip conducted measure pinch grip strength of both hands of subjects during the clinical examination. The strength of the pinch between the pad of the thumb in opposition to the pads of the index and middle fingers was measured using the Martin Vigorimeter®. The unit of measurement was kilopascal (kPa). The higher reading of the two maximal contractions was taken to represent the subject's pinch strength. A different distribution of pinch strength was found between the occupations, therefore the cut-off points of low strength (the lower 25th percentile) were defined separately for the two groups. The low pinch strength of either hand was set at 552 kPa, for the dentists and at 550 kPa for the teachers. Most subjects (96.3%) were right-handed.

Overweight/obesity

Body mass index (BMI = weight (kg)/ height $(m)^2$) was calculated based on self-reported height and weight measured during the clinical examination. One subject refused to take part in the weight measurement. BMI index was categorised as normal weight (<25) and overweight or obesity ($\geq$25).

Hand OA outcome
Several outcomes were generated including radiographic, symptomatic, and symmetrical and various combinations of the foregoing. Participants who had the selected hand OA criteria were classified as having hand OA, for example at least three finger joints with raiographic OA of grade 2 to 4 was classified

as ROA (cases). All the other participants who did not meet the criteria were classified as not having hand OA (controls).

4.4 Genetic analyses

Each study subject gave a blood sample at the clinical examination for genetic analyses. Samples were stored at +4°C until DNA was extracted from the lymphocytes by a DNA extraction kit (PUREGENE ®DNA Purification Kit; Gentra Systems, Plymouth, MN, USA). The extracted DNA was stored at -20°C until analyzed. The DNA was analysed in stock tubes and also in 96 well plate and diluted to specific concentrations depending on the analysis method being used (10-50 ng/µl).

A total of 43 SNPs in 27 genes were analyzed in this study (Table 4 and Figure 11). The genotypes were determined by using four different polymerase chain reaction (PCR) -based methods: PCR-RFLP, TaqMan® SNP Genotyping Assays (Applied Biosystems), TaqMan® OpenArray platform and Pyrosequencing®**.** One hundred ng of DNA was used in the PCR-reactions for RFLP and OpenArray® analyses, 50 ng for PCR, 30 ng for pyrosequencing analyses, and 10 ng for Taqman® analyses.

Table 4. Details of the studied genetic variants

Gene	Location	SNP	Chr.	Genotyping method	Reference
A2BP1/ RBFOX1	intron	rs716508	16	OpenArray	C__1224093_10
ADIPOQ	intron	rs1501299	3	TaqMan	C_7497299_10
ADIPOQ	nc transcript variant, synonymous	rs2241766	3	TaqMan	C_26426077_10
ADIPOQ	intron	rs182052	3	TaqMan	C_2412785_10
ADIPOQ	upstream variant 2KB	rs17300539	3	TaqMan	C_33187774_19
APLN	intron	rs3115757	X	OpenArray	C__27458731_10
BCAP29	intron	rs10953541	7	OpenArray	C__2618842_20
COG5	intron	rs3757713	7	OpenArray	C_27475119_10
COG5	intron	rs3815148	7	OpenArray	C_25994114_10
COL2A1	synonymous codon	rs3737548	12	TaqMan	C_25606536_10
COL2A1	intron	rs2276455	12	TaqMan	C_15881616_10
COL6A4P1/ DVWA	ncRNA	rs7639618	3	OpenArray	C__1176713_10
DIO2	missense	rs225014	14	OpenArray	C_15819951_10
DUS4L	intron	rs4730250	7	OpenArray	C_32373604_10
ESR1	intron	rs2234693	6	RFLP	(207)
ESR1	intron	rs9340799	6	RFLP	(207)
GDF5	other	rs143383	20	OpenArray	C__1270479_1
HFE	missense	rs1799945	6	OpenArray	C__1085600_10
HLA/BTNL2	intron	rs10947262	6	OpenArray	C_32201424_10
IL4R	downstream variant 500B, missense	rs1805015	16	TaqMan	C_234284_1
IL4R	missense	rs1805016	16	TaqMan	C_8903091_10

Table 4. Details of the studied genetic variants continues…

Gene	Location	SNP	Chr.	Genotyping method	Reference
IL10	upstream variant 2KB	rs1800896	1	RFLP	(208)
ITLN	intron	rs2274906	1	OpenArray	C__16183117_10
LEP	NA	rs7799039	7	TaqMan	C_1328079_10
LEP	utr variant 5 prime	rs2167270	7	TaqMan	C_15966471_20
LEPR	missense	rs1137100	1	OpenArray	C____518168_20
LEPR	missense	rs1137101	1	OpenArray	C___8722581_10
LEPR	missense	rs1805094/rs8179183	1	OpenArray	C___8722378_10
NAMPT	intron	rs3801266	7	OpenArray	C____340124_10
PARD3B	intron	rs1207421	2	OpenArray	C__8807483_10
PTGS2/ COX2	intergenic	rs4140564	1	OpenArray	C_31274663_20
RARRES2	utr variant 3 prime	rs4721	7	OpenArray	C___1248939_10
RETN	NA	rs4804765	19	OpenArray	C___1394116_10
RETN	NA	rs1423096	19	OpenArray	C___1394117_20
RETN	intron	rs10401670	19	OpenArray	C___1394125_10
RETN	intron	rs3745367	19	OpenArray	C___1394113_10
SERPINA12	intron	rs2236242	14	OpenArray	C___2786211_1_
TNFα	upstream variant 2KB	rs1799964	6	TaqMan	C__7514871_10
TNFα	upstream variant 2KB	rs1800630	6	Pyroseq.	Self designed
TNFα	upstream variant 2KB	rs1799724	6	TaqMan/ Pyroseq.	C___11918223_10
TNFα	upstream variant 2KB	rs1800629	6	RFLP	(209)
TRIB1	intergenic	rs4512391	8	OpenArray	C___310264_20
TGFB1	missense	rs1800470 /rs1982073	19	TaqMan	(210)

Chromosome locations of the studied 27 genes are represented in Figure 12.

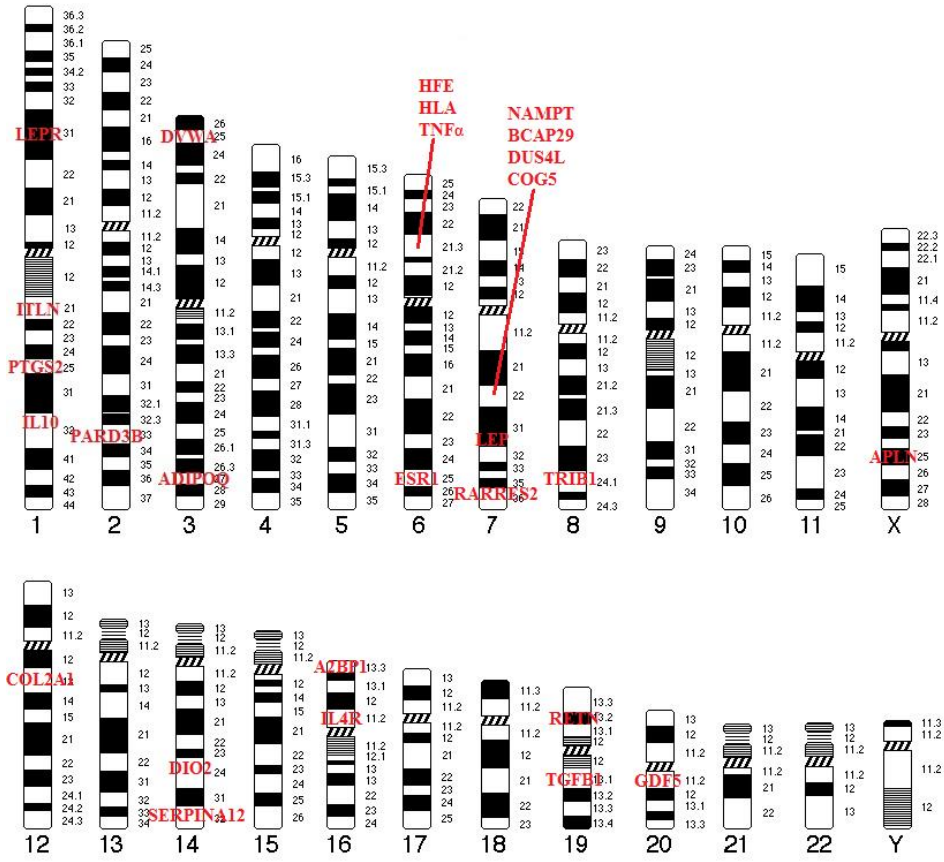

Figure 12. Studied gene locations in the chromosomes (Modified from http://www.biologia.uniba.it/rmc/0-internal-images/z-ideograms/ideograms.html)

4.4.1 Restriction fragment length polymorphism

In the standard PCR-RFLP method, the desired DNA locus is first amplified and then subsequently digested with a specific digestion enzyme. Specific primers are selected in the forward and reverse strands to multiply the target area where the SNP is located in the genomic DNA. The cut DNA fragments are then analyzed in agarose gel and the genotypes read from the stained gel.

4.4.2 TaqMan

TaqMan is commercially available analytical kit with ready-made and tested primers and probe sets. The PCR run and end point genotype read is done with a Real-Time PCR Instrument, e.g., TaqMan 7500.

One SNP can be analyzed at a time in up to 96/384 samples in the plate. This method is more costly than PCR-RFLP but it is very fast as one instrument can run up to eight plates in one day.

Primers in the TaqMan analysis are used in the same way as with PCR-RFLP. However, instead of restriction enzyme digestion and running the digested products on agarose gels, the multiplied DNA probes are used for the genotype determination, thus: one fluorescent probe is for the wild type allele and other for the variant allele. During the annealing step the probe pairs with the complementary DNA strand. In the elongation step, the polymerase cuts the probe and releases its signal molecule. In the end point read, light is focused onto the sample and the probes emit light at a certain frequency, which is the signal for each allele that can be detected by a camera. Genotypes are read automatically by the analysis program. Results are available in an electronic format that is ready to be transferred to the database.

4.4.3 Automated pipetting system

A Hamilton STARlet pipetting robot was used for pipetting samples to the 384-plates. The robot can transfer samples accurately in small volumes from tubes to 96-plates and from 96-plates to 384-plates. It also easily makes copies of the 96-plates used in the PCR and TaqMan based methods.

Part of the thesis project was that the PhD student created pipetting programs for the pipetting robot including easy user interface (see figure 12). At first, the samples were normalized from stocks to the 96-plates with the Normalization_OA -program. The deck is loaded according to the user interface with sample stock tubes, water tubes and an empty 96-plate. The programs were custom made, on site, to match the working protocols in the laboratory with the help of a programming specialist from Hamilton Robotics (see figure 13).

Figure 13. Hamilton Starlet: OpenArray96to384 program deck layout.

The robot performed all of the liquid transfers. First, the master mix (MMIX) and controls were transferred to the 384 plates. Next, the samples were transferred from the 96-storage plates to the 384 plate by single pipetting channels.

4.4.4 OpenArray

The OpenArray® system is based on TaqMan® chemistry and it is a semi-high-throughput approach. The samples were first transferred from 96-storage plate to 384-plate by Starlet pipetting robot. The Accufill pipetting robot was then used to transfer samples from the 384-plate to 3072 through hole chip.

Semi-high throughput chips were pierced-through with 3072 holes, onto walls of which the TaqMan assays were spotted. Each sample drop remained in the 300 µm hole due to the hydrophobic forces when the chips were inserted into the cases filled with immersion fluid and sealed with a UV-sensitive glue.

The sealed chips were moved to a flat PCR where a special program multiplied all assays simultaneously. The Allelic Discrimination was read at the end point in OpenArray NT Imager or with the more recent version "QuantStudio" OpenArray Reader.

At the same time, the operator had several options at his/her disposal. These options were to examine 16 SNPs from 144 samples, 32 SNPs from 72 samples, 64 SNPs from 36 samples, 128 SNPs from 18 samples or 265 SNPs from 9 samples.

The critical point in this method is that the researcher must use high quality DNA and that the DNA must be at same concentration in each sample to ensure that the end point reading will be clear.

The plate format of 16 SNPs and 144 samples per array were used. The allele calling analysis was performed using OpenArray® SNP Genotyping Analysis software (BioTrove Inc.) and the Applied Biosystems® TaqMan® Genotyper Software.

4.4.5 Pyrosequencing

Pyrosequencing was also based on PCR. The PCR-primers were similar to those used in normal PCR but one of the primers (forward or reverse) is biotinylated so that the single strand DNA can be separated and collected for the sequencing part of the run. Normal PCR cycling conditions were used (95°C for 5 minutes, 35 cycles at 95°C for 30 seconds, 54°C for 30 seconds, and 72°C for 30 seconds followed by a final extension of 72°C for 5 minutes).

The pyrosequencing was performed with PSQ™96MA (Qiagen) in this study by using Pyromark Gold Q96 Reagents (Qiagen) according to the manufacturer's recommendations. Briefly, 40 µl of the PCR product was mixed with 37 µl of binding buffer at pH 7.6 and 3 µl of Streptavidin Sepharose High Performance beads (GE Healthcare, Uppsala, Sweden).

The samples were subsequently processed in a pyrosequencing washing station. PCR products with the biotin bound to the Streptavidin beads were collected and denatured to be single-stranded by treatment with 70% ethanol, denaturation buffer, washing buffer, and mQ water in the Pyrosequencing Washing Station. The sequencing primer was attached to a single strand template (biotinylated primer with the extended DNA strand) by incubating it at 80°C in annealing buffer for 2 minutes. The pyrosequencing run was then

conducted with the dispensation order specifically designed for the previously known nucleotide order as a way to separate the SNPs alleles.

Four nucleotides (dATP, dTTP, dCTP, dGTP) were added stepwise to the template hybridized to a primer in the pyrosequencing reaction itself. The pyrophosphate released in the DNA polymerase-catalyzed reaction was detected by the ATP sulfurylase and luciferase in a coupled reaction. The added nucleotides are continuously degraded by a nucleotide-degrading enzyme. After the first added nucleotide had been degraded, the next nucleotide could be added. As this procedure was repeated, longer stretches of the template sequence were formed (211).

The pyrograms were generated and analyzed with PSQ 96 SNP Software 1.1 (Qiagen). The relative peak high of the pyrogram revealed the sequence and the genotype of the samples.

4.4.6 Quality control

For quality control, two independent readers interpreted the results and a random selection of 10% of all samples was re-tested.

4.5 Statistical analyses

The SPSS Statistical Package (SPSS, Chicago, IL, USA) and the SNPstats web tool (212) was used in the statistical analyses. The potential deviation of the allele frequencies from the Hardy-Weinberg equilibrium (HWE) was tested with the Chi-squared test. The allele and genotype frequencies were compared between individuals with and without OA by using Fisher's exact probability test or the Chi-squared test. Carriage rates for the alleles were calculated as the proportion of individuals with at least one copy of the allele. Each gene locus was also examined for an allele dosage effect by comparing the numbers of individuals who were heterozygous and homozygous for the test allele among those with and without OA.

Power calculations

The studies had variable powers to detect associations, depending on the minor allele frequency (MAF) and study size (n = 542): Study I had 80% power to detect ORs from 1.71 to 1.89 (MAF 18 – 35%), Study II had 80% power to detect ORs from 1.70 to 2.57 (MAF 6 – 44%), Study III had 80% power to

detect ORs from 1.70 to 3.02 (MAF 4 – 49%), and Study IV had 80% power to detect ORs from 1.70 to 2.44 (MAF 7 – 43%). The power calculations were performed by using standard methods, based on a two-sided alpha values of 0.05.

Linkage disequilibrium

The degree of pair-wise LD was calculated for each pair of SNPs by using the Haploview software (213) or the SNPStats web tool (212).

Haplotypes

The haplotypes were statistically reconstructed from the population genotype data by using the PHASE (214) program with the Markov chain method for haplotype assignments or with the Haploview (213) program or the SNPstats web tool (212).

Association analyses

Logistic regression analyses were performed to examine the association between the genotypes/haplotypes and hand OA phenotypes. The ORs and their 95% confidence intervals (95% CI) were calculated, both crude and adjusted. The necessary covariates for adjusting were selected according to the needs of the study. The statistical significance of the p-value was defined as the <0.8% (exploratory analyses), 1%, or <5%.

Covariates

The used covariates for adjusting were: age (continuous), occupation (dentists vs. teachers), and BMI (continuous), and in some analysis also leisure time physical activity (high vs. low), pinch grip strength (low vs. high), hormone replacement therapy (yes vs. no), and smoking history (ever vs. never).
The generalized linear model was used to examine the association between the haplotype and number of affected joints (NOAJ).

Inheritance models

Either a log additive model of inheritance or a dominant model of inheritance, with the homozygous genotype of the major allele as the reference, was fitted for the analyses between each SNP and hand OA phenotypes.

Interaction analyses

To evaluate whether the observed associations between the SNP/haplotype and hand OA phenotype was modified by other SNP or covariate, gene-gene

interactions and gene-covariate interactions were tested. Interactions were tested 1) by stratified (occupation or BMI) logistic regression analyses, or 2) by a logistic regression model with a dummy variable(s) (0, 1), or 3) by the inclusion of a product term in the model.

Stratifications

We carried out stratified analyses for the dentists and teachers separately to examine the possible modification effect of occupation (i.e., according to the loading of the hands). Within the group of dentists, the workload was also classified as either a low or a high task variation. These occupation stratifications were conducted in the first and fourth studies. However, since the groups were too small to permit meaningful analyses, we did not use the stratification approach in the studies in which the minor allele frequencies were found to be low.
We also carried out stratified analysis for the normal weight (BMI <25 km/m^2) and overweight (BMI 25 km/m^2) women separately to examine the possible modification effect of BMI in the third study.

Correction for multiple testing

P-values were adjusted for multiple testing by using the Šidák's method (215) in part of the analysis and studies. The Šidák's method control the familywise error rate to counteract the problem of multiple testing. The adjusted p-value, according to Šidák's method, is equal to 1-(1-unadjusted p-value)^k , where k is the number of comparisons in the family. The Šidák's method is similar to Bonferroni method, thought has a higher statistical power and gives slightly smaller adjusted p-values than Bonferroni.

5 RESULTS

Four studies were conducted for this thesis. The candidate genes to be studied in these four studies were selected with regard to the previously proposed aetiological mechanisms of hand OA. The first study focused on cartilage structure gene variants and the physical loading association with hand OA. The second study investigated inflammation-associated genes. The third study examined, if there were any adipokine genes that could be involved in the association between adipose tissue and hand OA. The fourth study aimed at replicating previously detected candidate genes for hand OA and also to determine whether the candidate genes associating with OA in other joint sites would have association with hand OA.

5.1 Overview of hand OA phenotypes

Several different hand OA phenotypes can be constructed based on the concurrent radiological and symptom information available. By taking the possible symmetrical occurrence of radiological findings, joint site, and symptoms into account, we initially identified 13 phenotypes as described in Table 5. These can be characterized as radiological, symmetrical (the same joint affected in both hands), symptomatic (both radiologic OA and symptoms occur in a joint), and symptomatic symmetrical. The prevalence figures vary according to the definition as shown in Table 5.

We selected phenotype 3 (Table 5) as the main outcome for our analyses so that it would be possible to conduct comparisons with earlier hand OA studies. Phenotype 3 is at least mild (K-L score 2-4) ROA in at least three finger joints. In addition, symptomatic DIP OA (phenotype 12) were used in the fourth study (216). Phenotype 12 is both radiographic findings (K-L score 2-4) and symptoms (at least grade 1) in at least two DIP joints.

Table 5. Prevalence of hand OA phenotypes

		OA		no OA	
	OA phenotype	**(n)**	**%**	**(n)**	**%**
1	OA (2+) in at least one joint	288	53.1	254	469
	Dentists	143	48.5	152	51.1
	Teachers	145	58.5	102	41.1
2	OA (2+) in at least two joints	230	42.4	313	57.6
	Dentists	105	35.6	190	64.4
	Teachers	125	50.4	123	49.6
3	OA (2+) in at least three joints	160	29.5	383	70.5
	Dentists	72	24.4	223	75.6
	Teachers	88	35.5	160	64.5
4	DIP OA (2+) in at least one joint	284	52.3	259	47.7
	Dentists	140	47.5	155	52.5
	Teachers	144	58.1	104	41.9
5	DIP OA (2+) in at least two joints	226	41.6	317	58
	Dentists	103	34.9	190	64.4
	Teachers	123	49.6	125	504
6	Symmetrical OA (2+) in any joint	213	39.3	329	60.7
	Dentists	94	31.9	200	67.8
	Teachers	119	48	129	52
7	Symmetrical OA (2+), at least two pairs	110	20.3	431	79.7
	Dentists	48	16.3	245	83.1
	Teachers	62	25	186	75
8	Symmetrical DIP OA (2+) in at least one joint pair	207	38.1	336	61.7
	Dentists	92	31.2	202	68.5
	Teachers	115	46.4	133	53.6
9	Symmetrical DIP OA (2+) in at least two pairs of joints	102	18.8	439	80.8
	Dentists	43	14.6	250	84.7
	Teachers	59	23.8	189	76.2
10	Symptomatic OA (2+) in at least 2 joints	34	6.3	509	93.7
	Dentists	12	4.1	283	95.9
	Teachers	22	8.9	226	91.1
11	Symptomatic DIP OA (2+) in at least 1 joint	96	17.7	447	82.3
	Dentists	47	15.9	248	84.1
	Teachers	49	19.8	199	80.2
12	Symptomatic DIP OA (2+) in at least 2 joints	49	9	494	91
	Dentists	20	6.8	275	93.2
	Teachers	29	11.7	219	88.3
13	Symptomatic symmetrical OA (2+) in at least 2 pairs of joints	20	3.7	522	96.1
	Dentists	6	2	288	97.6
	Teachers	14	5.6	234	94.4

5.2 Characteristics of the study participants

Selected characteristics of the study participants used for adjusting in the four studies are presented in Table 6.

Table 6. Description of the samples of female dentists and teachers aged 45-63, living in the metropolitan area of Helsinki, Finland

	All	**Dentists**	**Teachers**
n (%)	542 (100)	294 (54)	248 (46)
Age [mean ± SD]	54.0 ± 5.3	53.7 ± 5.9	54.3 ± 4.4
BMI [mean ± SD]	24.5 ± 3.6	23.9 ± 3.2	25.1 ± 3.9
Smoking status [n (%)]			
Ever smoker [n (%)]	144 (26.6)	76 (14.0)	68 (12.5)
Never smoker [n (%)]	398 (73.4)	218 (40.2)	180 (33.2)
Leisure time hand activities			
High level of activity [n (%)]	151 (28.0)	79 (14.6)	72 (13.3)
Low level of activity [n (%)]	389 (72.0)	213 (39.4)	176 (32.6)
Pinch grip strength [mean ±SD]	54.8 ± 8.1	55.4 ± 8.4	54.1 ± 7.8

5.3 Polymorphisms of the *COL2A1* gene and hand OA (I)

We examined two SNPs (rs2276455 and rs3737548) in the *COL2A1* gene. Carrying the rs2276455 SNP minor allele was associated with a 1.5-fold risk of hand ROA (OR 1.58, 95% CI; 1.05-2.36). Occupational stratification revealed that the increased risk was mainly attributable to the dentists having a doubled risk (OR 2.18, 95% CI; 1.18-4.06) of developing hand ROA.

We further analysed the haplotypes and interactions by occupation and with the extent of joint loading. Haplotype 2-1 (2 being the minor allele from rs3737548 and 1 being the major allele from rs2276455) was associated with hand ROA with more than a 3-fold risk (OR 3.21, 95% CI; 1.08-9.55). The occupational stratification revealed that in dentists there were 2.5 times (4.9 vs 1.9) more affected joints in comparison with the teachers. Finally, dentists with low task variation (repetitive load) and the rs2276455 minor allele had an almost 3-fold (OR 2.87, 95% CI; 1.05-7.89) risk compared to those with no

risk allele and high task variation. These results indicate that the *COL2A1* gene with a specific risk allele is associated with increased susceptibility to hand ROA and it is the environmental loading of the joint that increases the risk. The haplotype analysis also revealed a strong association but the number of the subjects in the risk haplotype was very small because of the minor allele included and its overall frequency.

These results indicate that those SNPs that were earlier found to be associated with generalized OA are also associated with hand ROA. In addition, the interaction between the SNPs and work task variation emphasizes that environmental stimuli are important factors in regulating the balance of the homeostasis of the joint.

5.4 Polymorphisms in genes associated with inflammation and hand OA (II)

We also investigated the associations of four common variants in the *TNFα* gene promoter area and their interactions with variants in the anti-inflammatory *IL4R* and *IL10* genes in relation to hand ROA in at least three joints, grade 2-4, among Finnish women. We observed that two of the *TNFα* SNPs (rs1799964 and rs1800630) minor alleles were associated with an increased risk (OR 1.45, 95% CI; 1.01-2.07 and OR 1.55, 95% CI; 1.06-2.25, respectively) of experiencing hand ROA. The association was independent when taking into consideration our group's earlier findings that *IL1β* and *IL6* SNPs were also associated with hand ROA. However, the anti-inflammatory *IL4R* and *IL10* SNPs interacted with these two *TNFα* hand ROA associated SNPs. This interaction, caused the risk to increase from 1.5-fold to 2-fold (OR 2.01, 95% CI; 1.26-3.22) with *IL4R* and to 2.5-fold (OR 2.54, 95% CI; 1.45-4.47) with *IL10*, thus indicating that the *IL4R* and *IL10* SNPs were functioning as effect modifiers.

We were able to confirm that the association of *TNFα* gene variants with OA could be extended to hand ROA. The *IL4R* and *IL10* SNPs were not found to be singly associated SNPs to hand ROA in our population. Furthermore, our findings suggest that the effect of *TNFα* polymorphisms on hand ROA was modified by the variants within the *IL4R* and *IL10* genes, as interactions were found between *TNFα* and the *IL4R* and *IL10* polymorphisms. This reveals the complex picture of the aetiology of hand ROA.

5.5 Polymorphisms in genes associated with overweight/obesity and hand OA (III)

We investigated the association of 18 SNPs from nine adipokine and adipokine receptor genes (*LEP, LEPR, ADIPOQ, RETN, NAMPT, SERPINA12, ITLN, RARRES2,* and *APLN*) with hand ROA in our dentists and teachers cross-sectional study **(III)**.

LEP and *LEPR*

The *LEP* SNPs (rs7799039 and rs2167270) did not have any association with hand ROA in our study using the log-additive model of inheritance and adjusting for age and occupation, but their GG-haplotype (minor-major) lowered the risk (OR 0.45, 95% CI; 0.22-0.96, p=0.04) for hand ROA in the overweight group but not of the normal weight group.

Further, we found *LEPR* SNP (rs1805094) minor allele C to be borderline associated (OR 1.45, 95% CI; 0.98-2.15) with hand ROA. BMI stratification revealed that the increased risk was mainly attributable to the overweight subjects having an almost doubled risk (OR 1.90, 95% CI; 1.03.3.52, p=0.04) of developing hand ROA. Moreover, the *LEPR* rs1805094 SNP together with rs1137101 SNP were in LD, and the AC-haplotype (rs1137101 minor and rs1805094 minor) was associated with hand ROA with an increased risk (OR 1.54, 95% CI; 1.01-2.35, p=0.05). BMI stratification revealed the same trend that the increased risk was mainly attributable to the overweight subjects having a doubled risk (OR 2.02, 95% CI; 0.98-4.15, p=0.06) of developing hand ROA. These results indicate that BMI is an effect modifier for the SNPs association with hand ROA.

RETN

We found that when using the log-additive model of inheritance and adjusting for age and occupation, the minor alleles of the *RETN* rs1423096 and rs10401670 SNPs and a GGTT-haplotype constructed from the SNP data about these minor alleles, displayed associations with hand ROA by reducing the risk (OR 0.66, 95% CI; 0.43-1.02, p=0.05 for rs1423096, OR 0.73, 95% CI; 0.55-0.97, p=0.03 for rs10401670, and OR 0.58, 95% CI; 0.37-0.93, p=0.02 for GGTT-haplotype, respectively). BMI stratification revealed that the decreased risk was mainly attributable to the overweight subjects having even lower risk (OR 0.53, 95% CI; 0.27-1.03, p=0.05 for rs1423096, OR 0.70, 95% CI; 0.46-1.07, p=0.09 for rs10401670, and OR 0.42, 95% CI; 0.20-0.86, p=0.02 for GGTT-haplotype, respectively) of developing hand ROA. These

results indicate that BMI is an effect modifier for the SNPs association with hand ROA.

The direction of the association is opposite to what was expected, as the minor alleles are linked with elevating the RETN levels, which explains 1.5% of the variance.

RARRES2

We sought out one *RARRES2* tagging (in LD with surrounding area SNPs) SNP (rs4721, which was previously called rs10278590) and found its minor allele to be associated with an increased risk of hand ROA (OR 1.41, 95% CI; 1.07-1.87, p=0.01) in a log additive model of inheritance. However, BMI stratification revealed that the *RARRES2* rs4721 minor allele decreased the risk of hand ROA (OR 0.67, 95% CI; 0.46-0.96, p=0.03) in normal weight subjects but had a trend for increased the risk in overweight subjects (OR 1.36, 95% CI; 0.87-2.11, p=0.18). These results indicate that BMI is an effect modifier for the SNP's association with hand ROA as the direction of the effect was reversed among the normal weight.

This SNP was predicted to have a functional influence in the regulation of gene splicing. In view of the anti-inflammatory nature of RARRES2, more functional studies will be needed to explain why this SNP elevated instead decreased the risk for hand ROA. Unfortunately, we did not have the possibility to perform functional studies in our study sample.

In summary, our results suggest that the *LEP, LEPR, RARRES2*, and *RETN* gene variants may have a minor role in the aetiology of hand ROA in Finnish women, and that the associations are modified by BMI. However, the associations found in the present study lost their statistical significance after adjusting for multiple testing. The other SNPs were not associated with ROA in our study sample.

5.6 Replication study (IV)

We attempted to replicate the previous findings on variants in five genes (*A2BP1, COG5, GDF5, HFE* and *ESR1*) that had been found to be associated with hand OA. We also investigated the susceptibility genes for other OA sites and selected variants from nine of them (*PTGS2, PARD3B, DVWA, HLA, BCAP29, DUS4L, TRIB1, DIO2,* and *TGFB1)* to determine if they displayed any association with the hand OA phenotypes (ROA and symptomatic DIP OA) in Finnish women.

We were able to replicate only one hand OA susceptibility gene variant, i.e., the *A2BP1* rs716508 variant allele and that SNP was associated with a reduced risk of ROA (OR 0.68, 95% CI; 0.50-0.93).

The *TGFB1* rs1800470 was the only gene variant belonging to the other site OA susceptibility list that was found to be associated with symptomatic DIP OA, the minor allele almost doubled the risk (OR 1.84, 95% CI; 1.16-2.91). However, the *ESR1* gene variant was associated with ROA but only in teachers, i.e., the minor allele of rs9340799 almost tripled the risk (OR 2.84, 95% CI; 1.25-6.48) of symptomatic DIP OA.

Gene-gene interactions were also detected; carrying the *COG5* rs3757713 C-allele had a 2.6-fold risk (OR 2.57, 95% CI; 1.08-6.10) of ROA only among those women with the *BCAP29* rs10953541 CC genotype, and carrying the minor allele of either of the *HFE* rs179945 or the *ESR1* rs9340799 was associated with a doubled risk (OR 2.12, 95% CI; 1.28-3.50) of symptomatic DIP OA.

In summary, the *A2BP1* gene variant, that had earlier been found to be associated with hand OA, was confirmed in the current study to be associated with hand OA, which led to a reduced risk. The *TGFB1* that was originally found to be associated with spinal OA (minor C-allele) increased the risk of developing hand OA. The interactions observed between *ESR1* and occupation, *COG5* and *BCAP29*, and *HFE* and *ESR1* highlight the complicated nature of the aetiology of hand OA and the need to take many genes and environmental factors into consideration when conducting genetic analyses.

6 DISCUSSION

In the presents study eight genes (*COL2A1, TNFa, LEP, LEPR, RETN, RARRES2, A2BP1,* and *TGFB1*) had SNPs which were found to have a direct association with hand OA and six genes (*IL4R, IL10, ESR1, COG5, BCAP29*, and *HFE*) displayed interactive effects with other gene variants, BMI or occupation in their association with hand OA.

Attempts to resolve the factors that are associated with OA have been on-going for decades since the disease was first identified (51). The multifactorial and complex nature of hand OA adds to the difficulty of pinpointing the susceptibility factors when one considers both the ethnic and gender differences that interact with the plethora of environmental factors (217). In addition, the large variety of different hand OA phenotypes makes it challenging to compare and replicate the studies (76).

Twin studies have estimated that the genetic contribution to hand OA may be as high as 65% (8). The novel analytical methods gave rise to the hope that finally the missing pieces from the heritability jigsaw could be found. Yet despite the entire range of approaches used: from linkage studies and candidate gene analysis to GWAS, from the small specified sample sets to the huge multi-centre international co-operation studies, from the single discoveries to the pooled and meta-analysis, this hope has not been realized and the genetic component of OA still remains mostly a mystery (218). Some of the OA susceptibility genes have been found and confirmed by replication studies in specific OA sites such as the hand (*A2BP1*) (145) but the major part of the aetiology of hand OA still remains unclear and remains to be discovered (219).

The independent and joint or interactive effects of genetic and environmental factors on hand OA have rarely been investigated. This present study, therefore examined some possible genetic factors in the aetiology of hand OA among Finnish Caucasian women. The established cross-sectional study material comprising random samples of dentists and teachers was used as the approach. The study design enabled controlling for such potential confounding factors as gender (all subjects were women), age (a rather narrow age-range was used in sampling and age was further adjusted for in the analyses), level of education (all subjects had academic education), occupational loading, overweight/obesity, and pinch grip strength, in addition to studying interactions.

6.1 The main findings of the current study

A total of 43 SNPs from 27 genes were evaluated for their association with hand OA in this thesis by using four different experimental designs. The overall goal was to clarify the missing pieces regarding the heritability of hand OA. The candidate genes were chosen with consideration of the previously proposed risk mechanisms for hand OA, which were: (I) mechanical loading of the cartilage structure, (II) inflammation, and (III) overweight. Furthermore, we made an attempt (IV) to replicate some hand OA susceptibility genes findings and investigated if there were any genetic associations at other joint sites with those detected in our hand OA subjects.

Of the 27 carefully selected candidate genes, SNPs of 14 genes were found to have some association with hand OA (*COL2A1, TNFa, IL4R, IL10, LEP, LEPR, RETN, RARRES2, A2BP1, TGFB1, ESR1, COG5, BCAP29*, and *HFE*). From these, only eight genes (*COL2A1, TNFa, LEP, LEPR, RETN, RARRES2, A2BP1,* and *TGFB1*) had SNPs which were found to have a direct association with hand OA in this study and the other genes displayed interactive effects with other gene variants, BMI or occupation.

We found some novel SNPs as modifiers for hand OA in *TNFa, LEPR, RETN, RARRES2*, and *TGFB1* genes. From these, *TNFa* do not share a location with the sites proposed in either the linkage or GWAS studies.

Replication of the previously found associations of *COL2A1* (99) and *A2BP1* (145) polymorphisms with hand OA can be considered as somewhat remarkable considering our study size.

6.2 Comparison of the current findings with the literature

Mechanical loading of cartilage structure

We aimed to partly replicate the findings obtained by Meulenbelt *et al.* (99), though we used a different OA phenotype than their generalised OA patients. The study by Meulenbelt and co-workers, studied a haplotype that consisted of the same two *COL2A1* SNPs that were chosen in the current study as being the most prominent (exon 5 B, rs3737548 and intron 33, rs2276455), together with an additional SNP and VNTR.

We noted that carrying the intron 33 (rs2276455) SNP minor allele was associated with hand ROA with a 1.5-fold (1.58, 95% CI; 1.05-2.36) elevated risk, similar to the results of Meulenbelt *et al.* (99). This finding is also supported by the study by Vikkula *et al.*, which postulates an association between *COL2A1* and hand OA that would not be located in the exon area but in some intron region (14).

Recent review concluded that more research is needed to study associations between occupational factors and OA, especially replication studies across different populations and joint sites (220). Our finding about interaction between occupation and the two *COL2A1* SNPs, and their haplotype, in the relation to hand OA were sited in their review.

This present study highlights the benefits of a detailed and well planned study design but also reveals the potential for a loss of statistical power to detect the associations if the MAF is too low and/or the groups' sizes after stratification become too small.

Inflammation

We were able to confirm that the association of *TNFα* gene variants (rs1799964 and rs1800630) with OA (25) could be extended to hand ROA. The *IL4R* and *IL10* SNPs were not found to be associated as single SNPs to hand ROA in our study. However, the anti-inflammatory *IL4R* and *IL10* SNPs interacted with these two *TNFα* hand OA associated SNPs. Consequently, the risk increased from 1.5-fold to 2-fold with *IL4R* and to 2.5-fold with *IL10*, indicating that the *IL4R* and *IL10* SNPs were functioning as effect modifiers. The increased risk with the anti-inflammatory genes was opposite to that expected, as they could be anticipated to lower the risk. Support for this unexpected finding comes from an Italian study, which found the same *IL4R* SNP minor allele has been associated with hand OA (187). Furthermore, the *IL10* SNP major allele has been linked with higher protein production (221), which possibly explains how the minor allele raised the risk. In addition, our female study participants were younger than the subjects of the Italian study who were both male and female by a mean of 10 years. Moreover, our hand ROA prevalence was also less (29.5% and 55.6%) than of the Italian study.

Overweight

Our adipokine approach was novel and the genes had hitherto not been studied with respect to hand ROA with the exception of the *LEP, LEPR* and *ADIPOQ*. Those genes have already been studied in patients with knee OA. Expressed

protein level associations to OA severity and progression, however, were revealed for some of the adipokines.

A haplotype of three tag SNPs, different from our study SNPs, within the *LEP* gene was associated with knee OA in Chinese individuals (28). A recent study in a South Indian population reported a positive dose-response association of the *LEP* rs2167270 minor allele (A) with both BMI and LEP levels and higher BMI values among the rs7799039 major AA-genotype (222). We did not find these SNPs to be associated with BMI in our study but our finding suggests that the least frequent *LEP* haplotype GG (rs7799039 minor allele (G) and rs2167270 major allele (G)) may have a protective role in hand ROA among overweight or obese women only. However, we did find that BMI is modifying the association between the haplotype and hand ROA.

Two Chinese studies reported that *LEPR* rs1137101 major G-allele was associated with mild knee OA (223, 224) with increased risk in dominant model of inheritance. The rs1137101 SNP was not associated with hand ROA in our sample but the *LEPR* AC-haplotype including the rs1137101 SNP together with rs1805094 SNP was associated with an increased risk of developing hand ROA. In addition to this, we found that the *LEPR* rs1805094 was marginally associated with an almost 1.5-fold risk of hand ROA in the total sample. After the stratification by overweight status, the risk estimate decreased in the normal weight subjects (not statistically significant result), and increased in the overweigh subjects to indicate an almost 2-fold risk of hand ROA.

We examined the *RETN* SNPs rs4804765, rs1423096, and rs10401670 SNPs that have been associated with higher plasma RETN levels (225). Our finding of the rs1423096 and rs10401670 SNPs and the all four *RETN* SNP containing haplotype displaying an association with hand ROA by reducing the risk and that the decreased risk was even lower in the overweight subjects, was an unexpected outcome. These minor alleles associated with a reduced risk to hand ROA were linked with elevated pro-inflammatory RETN levels, which explained 1.5% of the variance in the other study.

We sought one *RARRES2* tagging (in linkage with surrounding area SNPs) SNP (rs4721, previously called also rs10278590) and found the minor allele to increase the risk of hand ROA. Interestingly, after the stratification by overweight status the risk of hand OA was reduced in normal weight subjects and increased in overweight subjects. These findings suggests that BMI is an effect modifier as the direction of the effect was reversed among the normal

weight. According to the F-SNP online service (226), this SNP is predicted to have a functional influence in the regulation of gene splicing. In view of the anti-inflammatory nature of RARRES2, more functional studies must be done to explain why this SNP was found to elevate instead of decrease the risk for OA. Unfortunately, we did not have the possibility to perform functional studies in our study sample.

Replication

The *A2BP1* gene variant that was earlier found to be associated with a lower risk for hand OA (145), was confirmed to be associated with a reduced risk for hand OA. The *TGFB1* that was originally found to be associated with more than a doubled risk of spinal OA (minor C-allele) was found to increase the risk of developing hand OA to a similar extent in the present study (OR 1.84, 95% CI; 1.16-2.91). The interactions observed between *ESR1* and occupation, *COG5* and *BCAP29*, and *HFE* and *ESR1* highlight the complicated nature of the aetiology of hand OA and the need to take many genes and environmental factors into consideration when conducting genetic analyses.

The overall finding of our replication study was that we were not able to confirm most of the earlier findings (IV). Although the *A2BP1* (145) polymorphism was successfully replicated and SNPs in *COG5* (195), *HFE* (191), and *ESR1* (81, 227) genes partly replicated in the interaction analysis, the anticipated association of *GDF5* (171) gene with hand OA was not confirmed.

The association of OA susceptibility genes (*BCAP29, DIO2, DUS4L, DVWA, HLA, PTGS2, PARD3B, TGFB1* and *TRIB1*) found in other joint sites (193, 194, 196, 228-231) was considered to be less likely with hand OA. The main reason was that the previous study designs were so different to that of the current study and the joint loading characteristics of different sites are very different compared to the hand. However, as described above, we did find some associations with hand OA from these susceptibility genes.

A2BP1

The association between *A2BP1* rs716508 SNP with hand OA was first found in the GWAS conducted in 2009 (145). That study reported that the minor (C-allele) reduced the risk of hand OA by 33-41%. The *A2BP1* gene is also expressed in skeletal muscle, which led to the hypothesis that the association

with hand OA could be mediated through reduction in muscle strength (232). Although we found the *A2BP1* rs716508 C-allele to be associated with hand OA with a reduced risk (OR 0.68, 95% CI; 0.50-0.93, p=0.01), we did not find *A2BP1* rs716508 to be associated with pinch grip strength and the association with hand OA. Adjusting for the pinch grip strength did not alter this result either. In conclusion, we successfully replicated the finding that *A2BP1* is indeed a hand OA associated gene even though the hand OA phenotype in the hand OA GWAS was reported as a summary score.

COG5 and *BCAP29*

The *COG5* rs3815148 was originally found to have an association with hand OA in a large GWAS with populations in a multi-centre replication in 2010 (195). A subsequent GWAS in 2011 also detected an association with *COG5* rs4730250 SNP but with knee OA (228). However, no association was observed between the disease and *COG5* rs4730250 in another knee OA study conducted in 2012 (233).

The *BCAP29* rs10953541 SNP was found to be a candidate susceptibility gene in 2011 in a meta-analysis of GWA studies that detected a 500 kb LD block association with knee OA (228). The other susceptibility genes in this block were *PRKAR2B, HPB1, GPR22*, and *DUS4L*. The *COG5* rs3757713 and rs3815148 SNPs, in our replication study, were not found to be associated with hand OA. However, when we studied gene–gene interactions, we found evidence that there was an interaction between *COG5* rs3757713 C-allele and *BCAP29* rs10953541 CC-genotype when a combination of the at-risk genotypes of these genes increased the risk for hand OA 2.5-fold. This indicates that even though we did not have sufficient statistical power to detect a direct association in single SNP analysis with hand OA, it was possible to reveal a combined effect of these SNPs. Furthermore, the original phenotype studied for OA in the *COG5* rs3757713 and *BCAP29* rs10953541 was knee, which is rather different from our hand OA phenotypes.

ESR1 and *HFE*

The *ESR1* SNPs were studied in association with generalized OA by Ushiyama as early as 1998 (178), when it was found that the combined genotype PpXx of rs2234693 and rs9340799 SNPs increased the risk of experiencing OA. In 2009, four *ESR1* SNPs (*ESR1* PvuII-rs2234693, XbaI-rs9340799, rs2077647, and rs1801132) were examined in association with

hand OA (81). The Ushiyama study defined hand OA as being present in at least one investigated joint of K-L score ≥2 in the first CMC, DIP, first-digit IP, or PIP. However, no association was found with hand OA. Subsequently, when a meta-analysis was performed including the Ushiyama study, the same association was detected between *ESR1* and generalized and severe generalized OA.

Knee OA studies conducted in 2006, reported that the haplotype TAGA of four SNPs increased the risk (234). Later in 2014 (235) the rs2234693 T-allele and rs9340799 A-allele were reported to increase the risk. A report from 2010, stated that the CC genotype at rs2234693 reduced the risk to knee OA (227). Subsequently in 2012, it was reported that the haplotype CG from PvuII (T/C; rs2234693), and XbaI (A/G; rs9340799) reduced the risk (236).

A study published in 2014 reported that the rs9340799 AA genotype appeared to increase the risk of knee OA but the rs2234693 TT was not associated (237). Finally in 2015, it was reported that the rs9340799 G-allele increased the risk (238). There are also some knee OA studies that did not find any association with OA: these include a Korean study published in 2004 (207), and a study from Thailand published in 2009 (239). In 2015, a meta-analysis reported that there was no overall association between rs2234693 SNP and OA but in the sub-group analysis, carrying the variant allele rs2234693 (C/C + T/C vs. T/T) elevated the risk for knee OA (240).

A hip OA study (241) found that the *ESR1* rs2234693 CC genotype reduced the risk to hip OA, whereas rs9340799 was not associated with this form of OA.

The hemochromatosis gene *HFE* rs179945 SNP has previously been associated with primary OA (including hand) (191). However, no association was found between the *ESR1* (rs2234693, rs9340799 and 2228480) or *HFE* (rs1799945 and rs1800562) SNPs and hand OA in the single SNP analysis in the present study. There was, however, a suggestive interaction; the carriage of the minor allele of either of *HFE* rs179945 and the *ESR1* rs9340799 was associated with a doubling of the risk of experiencing symptomatic DIP OA.

Our hand OA phenotype was similar, but not exactly the same, as that evaluated in the discovery samples. This may account for our failure to be able to replicate the original association. False positive findings from the original report(s) may also explain why we were not able to replicate the findings in our study population. However, considering the complex nature of the aetiology of hand OA, this suggestive interaction may provide a hint of an underlying association. In order to be able to reveal any association, the power

of the study must be larger and all the possible contributing factors must be taken into consideration.

TGFB1

The *TGFB1* rs1800470 (Leu10Pro or ^{29}T->C) C-allele has been associated with elevated circulating TGFB1 levels in Chinese subjects but with decreased levels in Europeans (242). It was reported in 2015 that there was no association of the *TGFB1* rs1800470 SNP with knee OA (243). The potential association of rs1800470 SNP with spinal OA had been originally examined in 2000 (231) and the C allele was found to be a risk for spinal OA. The association with hip OA, on the other hand, was studied in 2011 (244) and carrying the C allele was claimed to increase the risk for hip OA. We also found that the variant allele (C) increased the risk for hand OA. This is in line with the earlier reports but it represents a novel finding with respect to a hand OA risk locus.

6.3 Challenges in the study of hand OA susceptibility genes

It is clear that both genetics and environmental factors play important and interacting roles in the development of OA (217). The knowledge of the aetiology of OA is still rather fragmentary (73). Many studies have been conducted using linkage scans, candidate gene analyses, recently large GWAS and pooled and meta-analyses (see figure 4, page 29). Despite this only a few gene variants have been found to be associated with OA in each joint site. It does seem evident that different ethnic groups differ in their genetic burden (113, 245, 246) as do men and women (123). In addition, there are a number of potential environmental factors that affect this complex disease (247-249).

The study designs vary a lot between the published reports and thus makes reliable comparisons of their outcomes difficult (250). The multiple aetiology of OA requires that both systemic and local factors are taken into consideration in the study design stage (251). The published reports also vary considerably in how other factors than the genetics were taken into consideration; some studies had adjusted for up to 20 confounders, whereas some did not apply any adjusting at all. Although potential confounders should be adjusted for in statistical analyses, care must be taken not to over adjust (252).

The variation in the definition of the phenotype was the greatest challenge encountered in comparing our results with other published studies on hand OA. It is not always evident from the description of the phenotype how the classification was made: thus a more detailed and standardised description is

necessary (108). Our aim was to choose a comparatively strong phenotype (at least mild, K-L 2 to 4) with a relatively severe (at least 3 finger joints) outcome. Some studies have investigated considerably milder phenotypes, e.g., K-L 2-4 in at least one finger joint (114, 253). One solution is to use a summary score of imaging findings that may enable the use of a linear outcome measure to avoid the problems introduced by cut-off values for hand OA (254). Such a choice may also be paralleled by considerations regarding whether symptoms or other clinical features of OA should be included in the outcome.

6.4 Strengths and weaknesses of the study

A major strength of our study was that it consisted of random samples of the relatively ethnically homogenous Finnish population. The Finnish population is one of the best-studied genetic isolates, originating from a small founder population. Therefore, the Finnish population has a relatively homogenous gene pool (255) offering very good material for the association studies. Consequently, we did not need to consider ethnic differences in genetic or environmental background in our statistical analyses.

We examined only women, thus it was not necessary to stratify according to gender either, and the occupational limitation to dentists and teachers meant that the level of education was similar for both groups. Moreover, the age range (45–63 years) of the participants was selected to cover the age of occupationally active women, but in whom the prevalence of hand OA would be expected to be rapidly increasing, thus ensuring that there would be a sufficient number of participants with hand OA in each comparison group.

Other strengths of our study are that we studied only SNPs with a strong hypothesis and, when possible, only functional SNPs in our studies. Thus, it was not necessary to invariably correct for multiple testing.

We also analyzed haplotypes, in addition to single SNP analyses, and thus ensured both stronger and more reliable results. This is because the grouping of SNPs into haplotypes generally leads to a stronger association with the phenotype than can be achieved with individual polymorphisms.

A strong point is also that we included either genes with environmental factors or several genes that potentially interact and would therefore exert combined effects on the disease aetiology. This makes it possible to gather a wider range of information than can be acquired from single SNP analysis that is more common in OA genetic studies.

Finally, as we had questionnaire information on a range of background factors, we were able to control for some potential confounders of the studied associations.

Our study also has some weaknesses. For instance, the relatively small number of study participants reduced the power of the study, i.e. it had low power to detect small effects, especially in some subgroup analyses. This situation was especially demanding when the studied SNPs had a small MAF. Obviously, since our findings are limited to cover women, any results that might be restricted to men remained undetectable.

When considering the possibility of selection bias in our study, it should be observed that the participants were occupationally active persons whom were already in middle-age, with no seriously disabling disease. Subjects with more severe hand OA may have taken early retirement or might not even have entered these occupations in the first place which would lead to the 'healthy worker effect'. This particularly relevant to dentistry and might have led to an underestimation of associations between occupations or occupational loading among dentists and hand OA, especially for symptomatic OA. Whether this potential underestimation could have had an effect on associations between genetic variation and hand OA is less obvious. However, any such effect has probably not been strong, as the prevalence of the hand OA phenotypes were similar to those observed in other studies in the general population (89, 98). In contrast to dentists, teachers with symptomatic hand OA may remain active in their profession for longer. Indeed, the occupation-stratified analyses revealed that most of the studied SNPs exerted different effects on hand OA between the two occupational groups. We also found that although other than occupational exposures related to hand use were similar between the occupational groups, certain lifestyle factors such as obesity, differed between the groups.

7 SUMMARY AND CONCLUSIONS

We found several associations between hand OA and the gene variants selected with regard to different aetiological hypotheses in this study. First, the cartilage structure gene *COL2A1* was found to be associated with hand ROA with increase the risk. The haplotype analysis revealed that the risk for hand ROA was even more pronounced. Furthermore, carriage of the risk allele together with low task variation in dental work more than tripled the risk for hand ROA.

Second, we investigated inflammation associated gene variants and their associations with hand ROA. We found two SNPs in the pro-inflammatory cytokine *TNFα* and their haplotype to be associated with an increased risk of hand ROA. The association was independent of the variants in the previously found hand ROA susceptibility genes *IL1β* and *IL6*. Instead, the effect of *TNFα* SNPs on hand ROA was modified by the variants within the *IL4R* and *IL10* genes.

Third, we examined adipokine genes in this context and revealed an association between variations for four of the nine studied genes with risk of hand ROA, and that the BMI modified the associations. The *LEP* haplotype decreased the risk for hand ROA only in overweight group. The *LEPR* SNP almost doubled the risk of hand ROA on overweight group. The *LEPR* haplotype increased the risk of hand ROA in total sample. However, BMI stratifications revealed trend towards higher risk in overweigh group. The *RETN* SNPs and their haplotype reduced the risk of hand ROA. BMI stratification revealed that the decreased risk was mainly attributable to the overweight subjects having even lower risk. The *RARRES2* tagging SNP increased the risk of hand ROA. However, BMI stratification revealed that the tagging SNP decreased the risk of hand ROA in normal weight subjects but had a trend for increased the risk in overweight subjects.

Fourth, we sought to verify the previously identified gene variants as risk factors for hand ROA and conducted an exploratory analysis of nine OA candidate genes associated with other joint sites. Only two associations with risk of developing hand OA were observed in these studies. The hand OA susceptibility gene *A2BP1* was associated with a reduced risk of hand ROA, and the hip OA candidate gene *TGFB1* almost doubled the risk of developing symptomatic DIP OA. However, occupation-gene interaction and gene-gene interaction were also found and their association with symptomatic DIP OA and hand ROA.

Thus, our findings show that it is worthwhile to take into account both environmental and other genetic factors in the analysis of the contribution of certain gene variants to the risk of hand OA. In our estimation these results add some substantial information to the existing knowledge about susceptibility genes for hand OA. It has to be admitted, however, that there is still a long way to go before all of the multitude of factors contributing to its heritability will be clarified.

8 FUTURE PERSPECTIVES

Although even whole genome wide scans of hand OA are available nowadays, the results emerging from genetic association studies have only been able to explain a modest part of the heritability of hand OA. For this reason, new approaches must be undertaken for elucidating the inherited factors that lead to hand OA. Epigenetics might give such new viewpoints. The epigenetic regulation of the genes exhibiting variations that have already been putatively implicated as potential risk modifiers in hand OA are of great interest in this context.

Epigenetics incorporates many different regulation mechanisms including DNA methylation, histone modifications (acetylation, phosphorylation, ubiquitylation, sumolyation), and non-coding RNAs.(256) These epigenetic mechanisms regulate DNA that has been thought to be the only inherited factor. DNA will remain as the stable blueprint of genetic plan but now it is known that the regulation of the DNA is also partly inherited. Some epigenetic regulation in OA has already been reported, such as the *GDF5* gene, which is modulated by methylation (257).

Analysis of histone modifications requires much more complex laboratory analytical procedures than the analysis of DNA methylation. There are more modification options and they undergo cross-talk to allow fine-tuning of gene expression. At present, the histone modification studies in OA have concentrated on the histone acetyltransferases that weaken the connection between DNA, and on the histone and histone deacetylase enzymes that close the structure and make it inaccessible.(258)

Non-coding RNAs, in turn, are small, 20-32 bp cytoplasmic RNAs that participate in the post-transcriptional regulation by pairing with messenger RNA and thereby lead to its silencing by suppression or total degradation. Currently, about 1000 miRNAs have been identified in the human genome. Recently, some patterns in how certain miRNAs are expressed in healthy cartilage versus OA cartilage have been found (259, 260).

My future plan is to continue the research into hand OA by conducting epigenetic studies starting from analysing DNA methylation in the promoter areas of some of the hand OA susceptibility genes.

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I

COL2A1 gene polymorphisms and susceptibility to osteoarthritis of the hand in Finnish women

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ABSTRACT

Objectives: To study the role of two *COL2A1* single nucleotide polymorphisms (rs3737548 and rs2276455) and their haplotypes in individual susceptibility to osteoarthritis (OA) of the hand in Finnish women.

Methods: Bilateral hand radiographs of 543 Finnish female dentists and teachers aged 45–63 years were examined and classified for the presence of OA by using reference images. The *COL2A1* genotypes were determined by PCR-based methods. Data regarding other risk factors were collected by questionnaire. The haplotypes were statistically reconstructed from the genotype data by the PHASE program. Associations between the genotypes/diplotypes and hand OA were studied by logistic regression.

Results: Allowing for age and occupation, the carriage of at least one *COL2A1* intron 33 minor allele was associated with an increased risk of hand OA (odds ratio (OR) 1.58, 95% CI 1.05 to 2.36) and the number of affected joints. When stratified by occupation, the increased risk associated with the intron 33 minor allele carriage appeared to be mainly attributable to the dentists (OR 2.18, 95% CI 1.18 to 4.06). The 2-1 haplotype (exon 5B minor allele-intron 33 major allele) posed a significantly higher risk of hand OA (OR 3.21, 95% CI 1.08 to 9.55) compared with non-carriers. Moreover, an interaction was observed between intron 33 minor allele carriage and low task variation history in dental work (OR 2.87, 95% CI 1.05 to 7.89 for their joint effect).

Conclusions: The results suggest that the studied *COL2A1* gene polymorphisms may play a role in the aetiology of hand OA and that this effect may be enhanced by repetitive loading work tasks.

Osteoarthritis (OA) is the most common joint disease and a frequent cause of disability in developed countries. The multifactorial aetiology of OA is not fully understood. Among the suspected risk factors are age, injury, repetitive joint loading and obesity.[1]

Current evidence suggests a genetic component to OA.[1] The genetic influence may involve either a structural defect (eg, in collagen), alterations in the metabolism of cartilage and bone, an enhanced inflammatory component in the disease process or a genetic influence on a known risk factor for OA such as obesity.[2]

Collagen is the main component of the articular cartilage and plays an important role in the maintenance of its biomechanical properties. The cartilage collagen consists mainly (90%) of the widely studied COL2A1, the structure of which is based on three precollagen fibrils that add strength to the cartilage tissue.[3 4]

Several studies have demonstrated associations between polymorphisms in the *COL2A1* gene, located in the chromosome 12q13.11, in relation to hip and knee OA, generalised OA and some rare OA phenotypes connected with chondrodysplasia.[5 6]

In two Finnish families, a LOD (logarithm of the odds) score of 2.81 for the linkage between the *COL2A1* gene and OA has been reported.[7] Pairwise linkage disequilibrium (LD) analysis of the *COL2A1* gene showed that two polymorphisms (the exon 5B G->T (rs3737548) and intron 33 G->A (rs2276455)) define the most important *COL2A1* haplotypes.[8] Meulenbelt *et al* reported an association between the three-marker (the exon 5B G->T, intron 33 G->A and VNTR polymorphisms) *COL2A1* haplotype and generalised OA.[9]

Mechanical loading of the joint is beneficial and necessary for the health of the cartilage. However, too heavy load may harm the joint and contribute to the development of OA.[10–12] Forceful repeated mechanical loading causes an immediate dose-related increase in collagen denaturation in bovine articular cartilage.[11] Joint loading has been shown to regulate gene expression in cartilage chondrocytes; for instance, collagen gene expression may differ and the expression of degradative enzymes such as procollagenases (matrix metalloproteinase) may be upregulated.[13]

Dentistry is one of the few occupations with an academic background that involves extensive bimanual work. Dentists perform arm movements repeatedly, often rapidly, and for extended periods of time. A very accurate grip is used in the handling of precision tools. This may expose hand joints to heavy and long lasting load. Previously, our group showed that the localisation of hand OA is associated with the pattern of dental work task history. The less variation there was in the dental work tasks, the higher was the risk of OA in the most loaded fingers.[14]

We investigated the possible role of individual susceptibility and work-related factors in the aetiology of hand OA. For this, we examined whether the *COL2A1* exon 5B G->T and intron 33 G->A polymorphisms and their haplotypes were associated with hand OA in 543 Finnish female dentists and teachers. We also examined the possible effect of the interaction between these polymorphisms with stereotyped high loading repetitive tasks for prolonged periods of time in dentists' work.

METHODS

Subject selection

The study subjects were identified through the register of the Finnish Dental Association and the

Finnish Teachers Trade Union. Four hundred and thirty-six women aged 45–63 years were randomly selected from both occupational groups by using the place of residence (Helsinki or its neighbouring cities) as an inclusion criterion for participation in the study. Of those who received the questionnaires in 2002, 295 (68%) dentists and 248 (57%) teachers participated in a clinical examination between October 2002 and March 2003. Participation in the study was voluntary and based on informed consent.

Hand radiography and image analysis

Both hands of the study participants were radiographed by exposing Kodak *x* ray films with Siemens *x* ray equipment (48 kV, 10 mA, focus film distance 115 cm; Siemens, Munich, Germany). The analogue radiographs were evaluated by an experienced radiologist who was blinded to the occupation, age and all health data of the subjects. Each distal interphalangeal (DIP), proximal interphalangeal (PIP) and thumb interphalangeal (IP) joint of both hands was graded separately and classified for the presence of OA using a modified Kellgren and Lawrence system;[15] the classification criteria were: grade 0 = no OA, grade 1 = doubtful OA, grade 2 = mild OA, grade 3 = moderate OA, grade 4 = severe OA. The description of reference images used in the classification has been given elsewhere.[16] The reliability of the readings was estimated by measuring intra-observer and inter-observer agreements (intraclass correlation). The inter-observer agreement for OA ranged from 0.67 to 0.85 for DIP joints and from 0.39 to 0.61 for PIP joints. The intra-observer agreement for OA ranged from 0.73 to 0.88 for DIP joints and from 0.67 to 0.92 for PIP joints.

If the subject had at least three finger joints with radiographic OA of grade 2–4, she was classified as having hand OA. We also calculated the number of interphalangeal (DIP, PIP and IP) joints affected by OA per individual.

Questionnaires and interviews

All study subjects received a self-administered questionnaire that included questions on body height, weekly hours of hand-loading leisure time activities (household chores, hobbies and other physical activity) and smoking history.

Six main tasks in dental work were identified prior to the study: (1) restorative treatment and endodontics; (2) orthodontics; (3) periodontics; (4) prosthodontics; (5) surgical treatment; and (6) other non-treatment activities (eg, dental examination, consulting and administrative tasks). The subjects were asked to recall their work history in 10-year periods (at the age of 25–34 years, 35–44 years and 45–54 years) in terms of average number of working hours per week and the proportion of time (percentage) performing each task during an average working day. Based on the weekly hours of the work tasks, dental task variation was empirically defined using cluster analysis with the K-means algorithm.[14]

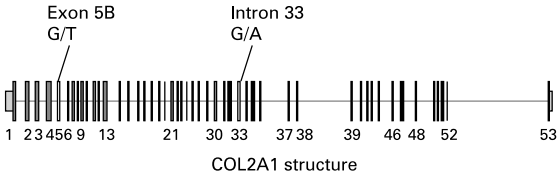

Figure 1 *COL2A1* gene structure and the studied polymorphisms.

Based on their smoking history, subjects were classified into never daily smokers or daily (current or previous) smokers. Similar to the definition of dental task variation, the hand-loading leisure time activities were empirically categorised into two groups using cluster analysis with the K-means algorithm. A classification procedure was performed based on the weekly hours of hand-loading household chores, hobbies and physical activities. Most of the subjects (n = 390, 72%) were classified into the low-level hand-loading leisure time activity group while only 28% of the women had high-level hand-loading leisure time activity.

Body mass index (BMI, weight (kg)/height $(m)^2$) was calculated based on self-reported height and weight measured during the clinical examination and put into tertiles for logistic regression analysis (low, <22.5 kg/m^2; medium, 22.5–25.5 kg/m^2; and high, >25.5 kg/m^2).

Genotyping analysis

Blood samples were taken from each study subject at the clinical examination and stored at +4°C until DNA was extracted by a DNA extraction kit (Puregene DNA Purification Kit; Gentra Systems, Plymouth, Minnesota, USA). The *COL2A1* exon 5B G->T (rs3737548) and intron 33 G->A (rs2276455) polymorphisms were genotyped by PCR-based TaqMan SNP genotyping assays (Applied Biosystems, C_25606536_10 and C_15881616_10, respectively). In the polymorphic exon 5B and intron 33 loci the G-alleles were denoted as the wild-type alleles and the T-allele and A-allele as variant alleles. The structure of the *COL2A1* gene and the above polymorphic sites are shown in fig 1.

Statistical analysis

The potential deviation of the allele frequencies from the Hardy-Weinberg equilibrium was tested with the χ^2 test. The allele and genotype frequencies were compared between individuals with and without OA using the Fisher exact probability test or the χ^2 test. Carriage rates for the alleles were calculated as the proportion of individuals with at least one copy of the allele. Each gene locus was also examined for an allele dosage effect by comparing the numbers of individuals heterozygous and homozygous for the test allele among those with and without OA. The degree of pairwise linkage disequilibrium (LD) was calculated for each pair of SNPs using

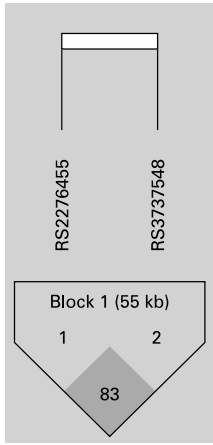

Figure 2 Haploview linkage disequilibrium plot of the *COL2A1* polymorphisms rs2276455 and rs3737548.

Table 1 Characteristics of the study population according to the presence or absence of osteoarthritis (OA) of the hand

	No OA	OA
n	383 (71%)	160 (29%)
Mean (SD) age (years)	53.0 (5.2)	56.3 (4.7)
Mean (SD) BMI (kg/m²)	24.3 (3.5)	25.0 (3.8)
Occupation		
Dentists (n)	223 (76%)	72 (24%)
Teachers (n)	160 (65%)	88 (35%)
Smoking status		
Ever smoker (n)	88 (72%)	33 (28%)
Never smoker (n)	297 (70%)	127 (30%)
Leisure time hand activities	381 (70%)	160 (30%)
High level of activity	110 (20%)	41 (8%)
Low level of activity	271 (50%)	119 (22%)

BMI, body mass index.

the Haploview software.[17] An LD plot for the SNPs studied here is presented in fig 2.

The *COL2A1* haplotypes were statistically reconstructed from population genotype data by using the PHASE program with the Markov chain method for haplotype assignments.[18] The wild-type and variant alleles of the polymorphic locus were denoted as 1 and 2, respectively.

Logistic regression analyses were performed to examine the association between the *COL2A1* genotypes/diplotypes and hand OA. To evaluate the interaction between *COL2A1* SNPs and the variation in dental tasks, the risk of OA was calculated as a function of variation in dental tasks (low task variation or high variation of dental tasks), of the presence of a risk allele, and of their interaction. The absence of the risk allele and high variation of tasks was used as the reference group. ORs and their 95% confidence intervals (CIs) were calculated by adjusting for age, occupation, BMI, leisure time physical activity and smoking history.

The Generalised Linear Model was used to examine associations between the haplotype and the number of affected joints. Interaction between haplotype and occupation was tested with the inclusion of a product term in the model. Age was used as a covariate.

All analyses were performed with the SPSS statistical package Version 14.0 (SPSS, Chicago, Illinois, USA).

RESULTS

The overall prevalence of hand OA was 29% (24% in dentists and 35% in teachers). The mean number of affected joints per individual was 3 (range 0–19). Some background characteristics of the study population are presented in table 1.

The genotype frequencies were in Hardy-Weinberg equilibrium in both of the studied polymorphic loci and there were no statistically significant differences in the frequency of genotypes and carriage rates between the occupational groups (table 2).

The frequencies of the *COL2A1* genotypes in women with and without hand OA are presented in table 3. The frequency of the intron 33 A-allele was higher among women with OA than in those without (0.39 vs 0.33, $p = 0.05$).

Allowing for age and occupation, carriers of the intron 33 A-allele had an increased risk of OA (OR 1.58, 95% CI 1.05 to 2.36, $p = 0.03$) compared with non-carriers (table 3). Stratification by occupation with respect to the intron 33 A-allele showed a difference in the risk of OA between dentists and teachers (dentists: OR 2.18, 95% CI 1.18 to 4.06, $p = 0.01$; teachers: OR 1.19, 95% CI 0.68 to 2.08, $p = 0.55$).

A significant LD was observed between the studied polymorphisms ($D' = 0.838$, $r^2 = 0.28$, $p<0.001$). The LD plot is shown in fig 2.

Four different haplotypes were identified. The most common of these was the 1-1 (major-major, 64%), followed by the 1-2 (major-minor, 19%), the 2-2 (minor-minor, 16%) and the 2-1 (minor-major, 0.9%) haplotypes. No statistically significant differences were observed in the haplotype frequencies between the occupational groups.

The frequency of the 1-1 haplotype was lower (58% vs 66%, $p = 0.01$) and that of the 2-1 haplotype higher (3% vs 1%, $p = 0.02$) in women with hand OA than in those without OA (table 3). Allowing for age and occupation, the carriers of the 2-1 haplotype had a more than threefold risk of hand OA (OR 3.21, 95% CI 1.08 to 9.55, $p = 0.02$) compared with non-carriers.

There were more affected joints in the 2-1 haplotype carriers than among the non-carriers (3.8 vs 2.2, $p = 0.002$). We observed an interaction between the 2-1 haplotype and occupation ($p = 0.003$). Dentists with the 2-1 haplotype had 2.5 times more affected joints than those without the haplotype (4.9 vs 1.9) but, among the teachers, there was no difference in the number of affected joints between carriers and non-carriers (2.5 vs 2.6).

Table 2 Genotype and allele distribution of the *COL2A1* polymorphisms

	All (n = 543)	p Value*	Dentists (n = 295)	p Value	Teachers (n = 248)	p Value
Exon 5B		0.53		0.81		0.60
GG	365 (67%)		207 (70%)		158 (64%)	
GT	165 (30%)		82 (28%)		83 (34%)	
TT	13 (2%)		6 (2%)		7 (3%)	
G-allele carriage	530 (98%)		289 (98%)		241 (97%)	
T-allele carriage	178 (33%)		88 (30%)		90 (36%)	
T-allele frequency	191 (18%)		94 (16%)		97 (20%)	
Intron 33		0.80		0.70		1.00
GG	226 (42%)		125 (42%)		101 (41%)	
GA	254 (47%)		139 (47%)		115 (46%)	
AA	63 (12%)		31 (11%)		32 (13%)	
G-allele carriage	480 (88%)		264 (90%)		216 (87%)	
A-allele carriage	317 (58%)		170 (58%)		147 (59%)	
A-allele frequency	380 (35%)		201 (34%)		179 (36%)	

*p Value is the probability of the χ^2 test for Hardy-Weinberg equilibrium of genotype frequencies.

Table 3 Association between the *COL2A1* polymorphisms and hand OA and frequency of the *COL2A1* haplotypes in women with and without OA

	No OA (n = 383)	OA (n = 160)	OR (95% CI)
Exon 5B			
GG	266 (70%)	99 (62%)	1.00
GT	109 (29%)	56 (35%)	1.27 (0.84 to 1.92)
TT	8 (2%)	5 (3%)	1.88 (0.54 to 6.57)
GT+TT	117 (31%)	61 (38%)	1.30 (0.87 to 1.96)
T-allele frequency	125 (16%)†	66 (20%)†	
Intron 33			
GG	174 (45%)	52 (33%)	1.00
GA	164 (43%)	90 (56%)	1.70 (1.11 to 2.59)
AA	45 (12%)	18 (11%)	1.15 (0.60 to 2.23)
GA+AA	209 (55%)*	108 (68%)*	1.58 (1.05 to 2.36)
A-allele frequency	254 (33%)†	126 (39%)†	
Number of 1-1 haplotypes (GG)			
0	45 (12%)	21 (13%)	
1	171 (45%)	93 (58%)	
2	167 (44%)	46 (29%)	
1-1 carriage	338 (88%)	139 (87%)	
1-1 frequency	505 (66%)	185 (58%)‡	
Number of 1-2 haplotypes (GA)			
0	261 (68%)	97 (61%)	
1	108 (28%)	57 (36%)	
2	14 (4%)	6 (4%)	
1-2 carriage	122 (32%)	63 (39%)	
1-2 frequency	136 (18%)	69 (22%)	
Number of 2-1 haplotypes (TG)			
0	376 (96%)	151 (94%)	
1	7 (2%)	9 (6%)	
2-1 frequency	7 (1%)	9 (3%)§	3.21 (1.08 to 9.55)
Number of 2-2 haplotypes (TA)			
0	273 (71%)	105 (66%)	
1	102 (27%)	53 (33%)	
2	8 (2%)	2 (1%)	
2-2 carriage	110 (29%)	55 (34%)	
2-2 frequency	118 (15%)	57 (18%)	

Odds ratios (ORs) and 95% confidence intervals (CIs) were adjusted for age and occupation.
*$p = 0.005$.
†$p = 0.05$, $p_{corr} = 0.10$.
‡$p = 0.011$, permutation $p = 0.04$.
§OR = 3.21 (95% CI 1.08 to 9.55), $p = 0.018$, permutation $p = 0.06$.

Finally, we studied the possible interaction between individual susceptibility to hand OA related to the *COL2A1* polymorphisms and the type of work history among the dentists. In those dentists who had a work history of high task variation, no association was observed between the carriage of the intron 33 minor allele and OA (table 4) whereas a statistically significant joint effect was seen among dentists with a history of low task variation (adjusted OR 2.87, 95% CI 1.05 to 7.89, $p = 0.04$). Dentists who carried the intron 33 minor allele and had low variation in tasks had a larger number of joints affected by OA than those with high task variation and without the allele (3.0 vs 1.3, $p<0.001$).

Table 4 Osteoarthritis (OA) of the hand in relation to the joint effect of the *COL2A1* intron 33 polymorphism and variation in dental tasks among the dentists

Variant allele carriage	Low variation	n (%)	OR (95% CI)*
No	No	41 (6)	1.0
No	Yes	81 (14)	1.25 (0.41 to 3.80)
Yes	No	55 (10)	1.73 (0.53 to 5.60)
Yes	Yes	114 (42)	2.87 (1.05 to 7.89)

CI, confidence interval; OR, odds ratio.
*Adjusted for age (years), body mass index, level of leisure time hand-loading physical activity and smoking history.

Similar analyses were not feasible using the haplotypes due to too small numbers of subjects.

DISCUSSION

This study investigated whether the *COL2A1* exon 5B and intron 33 polymorphisms are associated with hand OA in Finnish female dentists and teachers. The intron 33 minor allele was found to be more common in women with OA. Carriers of this minor allele also had a larger number of joints affected by OA.

Hand OA has not been examined before as a discrete disease entity in relation to variations within the *COL2A1* gene. Our results support the previously observed association between the *COL2A1* intron 33 polymorphism and generalised OA, the definition of which included the hand joints.[7] We observed that

the frequency of haplotypes with at least one minor allele (either 1-2 or 2-1 or 2-2 haplotypes) was higher in women with hand OA than in those without OA. However, only the association between the rarest haplotype (2-1) and hand OA was statistically discernible. In the Rotterdam study of elderly men and women, the haplotype 1-2-14R2 of the exon 5B, intron 33 and VNTR polymorphisms was linked to generalised OA. In that study the 2-1 haplotype was pooled with other rare haplotypes.

Furthermore, we found that the increase in risk of hand OA connected with the intron 33 minor allele was confined to the dentists (ie, an occupation with considerably high hand-loading). Among the dentists an interaction between the *COL2A1* intron 33 polymorphism and the level of variation in dental work tasks was observed, such that in subjects with low variation and carriage of the intron 33 minor allele the risk of hand OA exceeded the additive effects of the two factors. The dentists with low variation performed mainly restorative treatment and endodontics, tasks which were assessed to have the highest hand-loading according to an expert panel. These findings suggest that the intron 33 minor allele may be a risk factor for hand OA among women with monotonous and loading repetitive work tasks involving the hand. The load on the joint regulates the gene expression in cartilage chondrocytes that maintain the cartilage matrix.[13] The *COL2A1* intron 33 polymorphism may affect the gene regulation together with the load pressure and thereby promote OA formation. To our knowledge, this is the first report of such an interactive effect of mechanical loading and specific genetic susceptibility in the genesis of hand OA.

Our study partially replicates the findings by Meulenbelt *et al*[9] using a different OA outcome. Instances where gene-OA associations have been replicated in independent studies are still rare. A recent review found only two such replications in hand OA.[6]

Various factors may have contributed to the discordant results among studies, including differences in the studied phenotypes and the genetic environment and, more specifically, failure to take into account factors that modulate the effect of a gene on the risk of OA. One explanation for the divergent findings may also be that, since different joints are under dissimilar loading, they may also have different causes for development of OA. For instance, the hip and knee are weight-bearing joints whereas hand joints are under very different usage and load. The loading conditions may further diverge significantly depending on, for example, occupational demands.

In summary, our results suggest that *COL2A1* gene polymorphisms play a role in the aetiology of hand OA. In addition, the findings suggest the possibility of an interactive effect of the *COL2A1* polymorphisms and joint loading in the genesis of hand OA. However, the possibility remains that the studied polymorphisms do not directly affect the individual susceptibility to hand OA but are in linkage disequilibrium with an unknown nearby susceptibility locus.

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Variations in the *TNFa* gene and their interactions with the *IL4R* and *IL10* genes in relation to hand osteoarthritis

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Abstract

Background: The development of osteoarthritis (OA) involves inflammation, but the evidence for participation of genes propagating or inhibiting inflammation in the OA process is inconsistent. We investigated the associations of common variants in the *TNFa* gene, and their interactions with other cytokine genes, with hand OA among Finnish women.

Methods: This cross-sectional study was based on bilateral hand radiographs of 542 female dentists and teachers which were classified according to the presence of OA (radiographic K-L score ≥ 2 in ≥ 3 joints) using reference images. The genotypes were determined by PCR-based methods. The degree of pairwise linkage disequilibrium (LD) and haplotypes were constructed and analyzed by the SNPStats software. The associations between four TNF*a* SNPs and hand OA were tested using logistic regression adjusting for age, occupation, and BMI, and fitting a log-additive model of inheritance. Gene-gene interactions of *TNFa* SNPs with *IL4R* and *IL10* SNPs were examined by stratified logistic regression analyses. Possible interactions of the *TNFa* SNPs with variants in the previously reported *IL1β* and *IL6* genes in influencing hand OA were also explored.

Results: Two *TNFa* polymorphisms (*"-1031"* and *"-863"*) were associated with hand OA (OR = 1.45, 95% CI 1.01-2.07 and 1.55, 1.06-2.25, respectively). These associations retained when adjusting further for *IL1β* "3954" and *IL6* "174". The *TNFa G-A-G* haplotype was associated with an increased risk of hand OA (1.61, 1.10-2.37, $p = 0.01$). Interactions were observed between *TNFa "-1031"* and *IL4R* Ser503Pro, *TNFa "-1031"* and *IL10 "-1082"*, and *TNFa "-863"* and *IL10 "-1082"* SNPs with regard to hand OA ($p = 0.012$, $p = 0.0068$, and $p = 0.02$, respectively). The carriage of the *TNFa "-1031"* minor allele doubled the risk (2.01, 1.26 - 3.22) only in women with the *IL4R* Ser/Ser genotype. Similarly, the *TNFa "-1031"* and *"-863"* minor alleles were associated with an increased risk of hand OA only in *IL10* G/G or A/A homozygotes (2.54, 1.45-4.47 and 2.60, 1.46-4.62, respectively) but not in heterozygotes (G/A).

Conclusions: Our results suggest that the *TNF*α gene variants play a role in the etiology of hand OA. In addition, the findings are suggestive of a gene-gene interaction of the *TNF*α with *IL4R* and *IL10* genes.

Keywords: Tumor necrosis factor alpha, Gene polymorphism, Individual susceptibility, Hand osteoarthritis, Inflammation

Background

Osteoarthritis (OA) is the most common joint disorder worldwide and rapidly increasing with ageing populations. OA is a dynamic process involving all the structures within the joint, *i.e.*, cartilage, synovial membrane and subchondral bone. It shows clinical heterogeneity in joint numbers and regions involved. Some patients may have only one site affected (hip, knee, or hand) affected (local OA), while others have clustered joint regions affected in a characteristic distribution (generalized OA) [1].

The hand is among the most frequently affected site in OA [2]. The prevalence of hand OA is higher in women than in men over the age of 50 [3]. Although the pathogenesis of hand OA is largely unknown, familial aggregation and heritability studies indicate a significant genetic role in addition to the involvement of

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mechanical (repetitive joint loading) and lifestyle related factors (*e.g.* obesity) [3,4].

The development and progression of OA are nowadays believed to involve inflammation [5-8]. Chondrocytes, as well as synovial cells, of OA patients produce increased levels of pro-inflammatory cytokines, which affect metabolism and enhance the catabolism of all joint tissues affected in OA [5]. Among pro-inflammatory cytokines, interleukin-1β (IL1β) and tumor necrosis factor alpha (TNFα) seem prominent and of major importance to cartilage destruction as they are synthesized during the OA process [9-11]. *In vivo* studies have shown that these cytokines can act independently or in concert with other cytokines (e.g. IL6) in the induction and propagation of inflammation [12]. Synthesis of the IL1β and TNFα is inhibited by anti-inflammatory cytokines such as IL4, IL10 and IL13 [13]. On the other hand, expression of cytokine genes like *IL1* and *TNFα* is up-regulated in OA [14].

The gene encoding TNFα is located in the class III region of the major histocompatibility complex (MHC) which is the most gene-dense and polymorphic region of the entire genome [15]. TNFα is driving the inflammatory cascade [5]. IL4 in turn is a cytokine produced by T cells, which plays a major role in immunoglobulin E (IgE) production. Its signals are conferred to effector cells through binding to the alpha chain of IL4 receptor (IL4R). IL4 and IL4R are expressed by human articular chondrocytes; data suggest that mechanical stimulation induces the release of IL4 by human chondrocytes after the recognition and transduction of the mechanical signal by integrin [16,17]. Therefore, the IL4R is an active autocrine or paracrine signaling molecule in a regulatory pathway in the maintenance of human articular cartilage structure and function [16]. Regulation of the structure and function of human articular cartilage occurs by mediating other biochemical responses to mechanical strain, proteoglycan synthesis, or altering the expressions of other extra cellular matrix (ECM) proteins involved in the pathogenesis of OA [16,17].

So far, the evidence for involvement of genes propagating or inhibiting inflammation in the development or progression of OA is inconsistent, and the observed associations were not replicated in an independent population [18]. Most of the previous studies examined the role of a single gene in OA without taking into consideration the interaction of the genes participating in the regulation of balance between pro- and anti-inflammatory processes. Our group has reported the associations of the *IL1* extended haplotype and common *IL6* promoter variants with symmetrical DIP OA [19,20].

The aim of the current study was to investigate the associations of common variants in the *TNFα* gene and their interactions with variants in the *IL4R* and *IL10* genes in relation to hand OA among middle-aged Finnish women, representing two occupations: dentists and teachers. The possible interactions of the *TNFα* with variants in the *IL1β* and *IL6* in influencing hand OA were also explored.

Methods

Study design and participants' selection

This was a cross-sectional study, samples of which were taken randomly from two occupational groups. The study participants were identified from the registers of the Finnish Dental Association and the Finnish Teachers Trade Union. Four hundred and thirty-six women aged 45 to 63 were randomly selected from both occupational groups (altogether 872 subjects) by using the place of residence (Helsinki or its neighboring cities) as an inclusion criterion. Of those subjects who received the questionnaires in 2002, 542 (62% of the invited) participated in a clinical examination between October 2002 and March 2003. Of these, 294 (67% of the invited) were dentists and 248 (57% of the invited) teachers. Participation in the study was voluntary and based on informed consent. The study was approved by the Hospital District of Helsinki and Uusimaa Ethics Committee for Research in Occupational Health and Safety.

Hand radiography and image analysis

Both hands of the study participants were radiographed by exposing Kodak X-ray films with Siemens X-ray equipment (48 kV, 10 mA, focus film distance = 115 cm; Siemens, Munich, Germany). The analogue radiographs were evaluated by an experienced radiologist who was blinded to the occupation, age, and all health data of the participants. Each distal interphalangeal (DIP), proximal interphalangeal (PIP), and thumb interphalangeal (IP) joint of both hands was graded separately, and classified for the presence of OA using a modified Kellgren and Lawrence (K-L) system [21]; the classification criteria were: grade 0 = no OA, grade 1 = doubtful OA, grade 2 = mild OA, grade 3 = moderate OA, and grade 4 = severe OA. The description of reference images used in the classification is given elsewhere [22]. The reliability of the readings was estimated by measuring intra-observer and inter-observer agreements (intraclass correlation) within a limited sample of radiographs and a second participating radiologist. The inter-observer agreement for OA ranged from 0.67 to 0.85 for DIP joints and from 0.39 to 0.61 for PIP joints. The intra-observer agreement for OA ranged from 0.73 to 0.88 for DIP joints and from 0.67 to 0.92 for PIP joints [22].

Participants who had at least three finger joints with radiographic OA of grade 2 to 4 were classified as having hand OA. Otherwise, the participants were classified as not having hand OA.

Covariates

Weight was measured without shoes to the accuracy of 0.1 kg. Body mass index [BMI = weight (kg)/height $(m)^2$] was calculated based on weight and self-reported height. BMI data was missing from one participant. Age, occupation, and BMI were considered as possible confounders in the analyses. The variants in the *IL1β* and *IL6* that was previously shown to influence hand OA were also included among the covariates.

Genotyping analysis

Blood samples were taken from each study participant in the clinical examination and stored at +4°C until DNA extraction using a DNA extraction kit (PUREGENE ®DNA Purification Kit; Gentra Systems, Plymouth, MN, USA).

The *TNFα "-1031"* (rs1799964) and the *"-857"* (rs1799724) genotypes were determined by the TaqMan® SNP Genotyping Assay (Applied Biosystems, C___7514871_10 and C__11918223_10, respectively).

The *TNFα "-863"* (rs1800630) genotype was determined and the *"-857"* genotype re-determined by the Pyrosequencing® PSQ 96MA SNP/SQA system with PyroMark Assay Design self-designed protocol.

The *TNFα "-308"* (rs1800629) genotype was determined by PCR-RFLP method with *Nco*I (New England BioLabs (NEB) 10 U/μL) restriction enzyme. The primers were from Ozen *et al.* [23].

In the *TNFα "-1031"* locus the T-allele was denoted as the wild type allele and the C-allele as the variant allele, in the *"-863"* and *"-857"* loci the C-alleles were denoted as the wild type alleles and the A- and T-allele as the variant alleles, respectively, and in the *"-308"* locus the G-allele was denoted as the wild type allele and the A-allele as the variant allele.

The *IL4R* Ser503Pro (1507 T > C, rs1805015) and Ser752Ala (2254 T > G, rs1805016) polymorphisms were genotyped by PCR-based TaqMan® SNP Genotyping Assays (Applied Biosystems, C_234284_1 and C_8903091_10 respectively).

The *IL10 "-1082"* (rs1800896) SNP was genotyped with primers from Koch *et al.* [24].

An additional file has detailed description about the genotyping (see Additional file 1).

For quality control 10% of the genotyped samples were blindly repeated with 100% concordant results. Genotype data was available from all participants.

The earlier published *IL1β* "3954" (rs1143634), and *IL6 "174"* (rs1800795) genotyping has been described elsewhere [19,20].

Statistical analysis

The potential deviation of the allele frequencies from the Hardy-Weinberg equilibrium (HWE) was tested from controls using the chi-square test. The degree of pairwise linkage disequilibrium (LD) for four *TNFα* SNPs and two *IL4R* SNPs were calculated using SNPStats software [25]. Haplotypes were constructed and analyzed by the same software.

Logistic regression analysis was used to test the associations between SNPs and hand OA. For each SNP, a log-additive model of inheritance was fitted. To evaluate whether the observed association between the *TNFα* and OA was modified by variants in other cytokine genes, gene-gene interactions were tested for all *TNFα* SNPs by stratified logistic regression analyses.

Both crude and adjusted odds ratios (ORs) and their 95% confidence intervals (CIs) were calculated. The ORs were adjusted for the potential confounding factors, *i.e.*, age (continuous), occupation (dentists vs. teachers), and BMI (continuous). Since the crude and adjusted ORs did not differ significantly, only the adjusted ORs are shown in the results. In addition, the OR's were further adjusted for genetic variants in the *IL1β* and *IL6*.

In addition to exploring whether the effect of the *TNFα* SNPs on hand OA were independent of the genetic variants in the *IL1β* [19] and *IL6* [20], we estimated the individual and joint effects of the *TNFα* (*"-863"*), *IL1β* and *IL6* polymorphisms using the combinations of two dummy (0, 1) variables. First, we calculated the sum of the minor alleles of *IL1β* and *IL6*, by summing up the number of minor alleles of two SNPs. This was dichotomized (first dummy variable): 0 = non-carriers of any minor allele of the *IL1β* and *IL6* and 1 = carriers of at least one minor allele. For the *TNFα "-863"* SNP we used the dominant model, with the homozygous genotype of the major allele as the reference (second dummy variable).

All analyses were hypothesis driven. The statistical significance of the p-value was defined as the 1% level. P-values were adjusted for multiple testing using Sidák's method [26]. We used SNPStats software [25] and SPSS 20.0 for the analyses.

Results

The prevalence of hand OA with at least three affected finger joints was 29.5%, being higher among teachers (35.5%) than dentists (24.5%) (Table 1). Participants with

Table 1 Description of the samples of female dentists and teachers aged 45–63, living in the metropolitan area of Helsinki, Finland

	All	Dentists	Teachers
n (%)	542 (100)	294 (54)	248 (46)
Mean (SD) age (years)	54.0 (5.3)	53.7 (5.9)	54.3 (4.4)
Mean (SD) BMI (kg/m^2)	24.5 (3.6)	23.9 (3.2)	25.1 (3.9)
Hand OA cases (%)	160 (29.5)	72 (24.5)	88 (35.5)

hand OA were significantly older and had higher BMI than those without OA.

The genotype frequencies were in HWE in all of the studied polymorphic loci (Table 2). When adjusted for age, occupation and BMI, two *TNFα* SNPs (*"-1031"* and *"-863"*) were associated with hand OA (OR = 1.45, 95% CI 1.01-2.07, $p = 0.04$ and 1.55, 1.06-2.25, $p = 0.02$, respectively) (Table 3). Further adjustment for the *IL1β* and *IL6* SNPs had a negligible effect on the observed point estimates, though improving the estimate's precision ($p = 0.03$ and $p = 0.01$, respectively). No statistically significant associations were found between the other two *TNFα* SNPs and hand OA. Neither were there associations between the SNPs in the *IL4R* or *IL10* and hand OA.

Statistically significant interactions were found between the *TNFα "-1031"* and *IL4R* Ser503Pro SNPs, *TNFα "-1031"* and *IL10 "-1082"* SNPs, and *TNFα "-863"* and *IL10 "-1082"* SNPs and hand OA ($p = 0.012$, $p = 0.0068$, and $p = 0.02$, respectively). The carriage of the *TNFα* (*"-1031"*) minor allele was associated with a double risk of hand OA (2.01, 1.26 - 3.22) in women with the *IL4R* Ser/Ser genotype (Table 4). Similarly, the *TNFα "-1031"* and *"-863"* minor alleles were associated with an increased risk of hand OA only in *IL10* G/G or A/A homozygotes (2.54, 1.45-4.47 and 2.60, 1.46-4.62, respectively) but not in heterozygotes (G/A).

We also examined the individual and joint effects of the *TNFα "-863"*, *IL1β*, and *IL6* polymorphisms on hand OA. The risk of hand OA was the highest in the carriers of the minor alleles in all three genes (4.37, 1.84-10.38, $p = 0.001$). Somewhat lower risks were observed for carriers of the *TNFα "-863"* minor allele (3.73, 1.28-10.85, $p = 0.016$) and carriers of minor alleles of *IL1β* and *IL6* SNPs (2.89, 1.29-6.48, $p = 0.010$).

The degree of pairwise LD between three *TNFα* SNPs (*"-1031"*, *"-863"*, and *"-308"*) was high; the *"-1031"* and *"-863"* SNPs were in complete linkage (D' = 1, $r^2 = 0.93$, $p < 0.0001$), and the *IL4R* Ser503Pro and Ser752Ala polymorphisms were also in a strong LD (D' = 0.998, $r^2 = 0.48$, $p < 0.0001$). The three *TNFα* promoter polymorphisms composed a total of four haplotypes. The most common of these haplotypes was T-C-G (0.69), followed by G-A-G (0.16), T-C-A (0.13), and G-C-G (0.02). The two *IL4R* polymorphisms, on the other hand, composed three haplotypes, *i.e.*, Ser-Ser (0.87), Ser-Pro (0.10) and Ala-Pro (0.03).

The *TNFα G-A-G* haplotype was associated with an increased risk of hand OA when adjusted for age, occupation, and BMI (1.61, 1.10-2.37, $p = 0.01$) (Table 5). There was no difference between participants with and without hand OA in the *IL4R* haplotype distribution.

Discussion

We investigated whether the *TNF*α promoter polymorphisms are associated with hand OA among Finnish women. The minor alleles of the *TNFα "-1031"* and *"-863"* loci, as well as their haplotype, were found to be associated with an increased risk of hand OA. The observed associations were independent of the variants in the *IL1β* and *IL6* genes. Furthermore, our findings suggest that the effect of *TNFα* polymorphisms on hand OA is modified by the variants within the *IL4R* and *IL10* genes.

A traditional paradigm of OA as a "wear and tear" disease leading to the loss of cartilage has been revised.

Table 2 Description of studied SNPs

Genes	Localization	SNP ID	Chro.	Position	MAF										HWE
					Total (n = 1084)		OA Cases (n = 320)		OA Controls (n = 764)		1000 Genomes Finland# (n = 186)		HapMap¤ (n = 226)		p-value in controls
					%	n	%	n	%	n	%	n	%	n	
TNFa	*−1031*	rs1799964	6	31542308	18	191	21	66	16	125	19	35	21	48	0.41
TNFa	*−863*	rs1800630	6	31542476	16	168	19	60	14	108	15	27	15	34	0.79
TNFa	*−857*	rs1799724	6	31542482	6.4	69	5.6	18	6.7	51	6.5	12	NA/5.9*	10	0.81
TNFa	*−308*	rs1800629	6	31543031	13	144	15	47	13	97	13	24	17	39	0.59
IL4R	*Ser752Ala*	rs1805016	16	27374927	3.2	35	4.1	13	2.9	22	5.4	10	6.3	14	0.56
IL4R	*Ser503Pro*	rs1805015	16	27374180	13	139	14	44	12	95	15	27	15	34	0.17
IL10	*−1082*	rs1800896	1	206946897	42	453	42	133	42	320	39	73	47	120	0.84
IL1β	*3954*	rs1143634	2	113590390	27	294	31	98	26	196	24	44	21	47	0.97
IL6	*174*	rs1800795	7	22766645	44	475	48	153	42	322	44	82	47	105	0.88

#1000 genomes European sub-population, Finnish in Finland.
¤HapMap population is CEU: Utah residents with Northern and Western European ancestry from the CEPH collection.
*1000 genomes, population is CEU: Utah residents (CEPH) with Northern and Western European ancestry, n = 170.
SNP single nucleotide polymorphism, *MAF* minor allele frequency, *HWE* Hardy-Weinberg equilibrium, *OA* osteoarthritis.

Table 3 Association of the variants in the cytokine genes with hand OA

			Hand OA n = 160 (542)			
Genes	**Localization**	**SNP ID**	**OR (95% CI)[1]**	**p-value**	**OR (95% CI)[2]**	**p-value**
TNFα	*−1031*	rs1799964	1.45 (1.01-2.07)	0.04	1.47 (1.02-2.13)	0.03
TNFα	*−863*	rs1800630	1.55 (1.06-2.25)	0.02	1.61 (1.10-2.36)	0.01
TNFα	*−857*	rs1799724	0.77 (0.44-1.35)	0.35	0.77 (0.44-1.35)	0.36
TNFα	*−308*	rs1800629	1.25 (0.83-1.86)	0.29	1.19 (0.79-1.79)	0.39
IL4R	*Ser752Ala*	rs1805016	1.41 (0.67-2.99)	0.37	1.47 (0.69-2.32)	0.33
IL4R	*Ser503Pro*	rs1805015	1.17 (0.77-1.77)	0.47	1.17 (0.76-1.78)	0.48
IL10	*−1082*	rs1800896	0.96 (0.73-1.27)	0.80	0.95 (0.71-1.25)	0.70
IL1β	*3954*	rs1143634	1.39 (1.02-1.88)	0.03		
IL6	*174*	rs1800795	1.21 (0.92-1.59)	0.18		

[1]ORs and their 95% CIs were adjusted for age, occupation and BMI.
[2]ORs and their 95% CIs were adjusted for age, occupation, BMI and carriage of the minor allele of the *IL1β* (rs1143634) or/and *IL6* (rs1800795) SNPs.
OA osteoarthritis, *SNP* single nucleotide polymorphism, *OR* odds ratio, *CI* confidence interval, *BMI* body mass index.

Nowadays, OA is considered a complex disease with inflammatory mediators released by cartilage, bone and synovium [8]. TNFα is one of the most typical pro-inflammatory cytokines that along with IL1β is connected with cartilage destruction. These two cytokines, which are produced by chondrocytes, mononuclear cells, osteoblasts and synovial tissues, induce the production of a number of inflammatory and catabolic factors [5]. Among the four polymorphic loci studied here, only the *TNFα "-308"* locus has been shown to affect the TNFα protein levels, the minor allele of the SNP was associated with increased TNFα production in response to various stimuli [27,28]. However, also opposite observations, *e.g.*, no effect on the protein levels or lowered protein levels, have been reported [29,30]. When studying the above SNPs with F-SNP-program that is freely available on the internet (http://compbio.cs.queensu.ca/F-SNP/) connected to the main databases, and computationally predicting functional SNPs, all four SNPs are predicted to be functional as they seem to be in the transcription factor binding site [31]. However, the protein level alteration by the studied SNPs still remains unsolved and needs to be further studied.

The few studies that examined associations between *TNFα* polymorphisms and knee or hip OA have given

Table 4 Interaction of the *TNFα* SNPs with the *IL4R* and *IL10* SNPs in their effect on hand OA

		n	OR	95% CI	p-value
IL4R	**TNFα** *"-1031"*				0.01
Ser/Ser	T/T	67/270	1.00		
	T/C – C/C	52/138	2.01	1.26-3.22	
Ser/Pro-Pro/Pro	T/T	33/94	1.00		
	T/C – C/C	8/39	0.55	0.22-1.40	
IL4R	**TNFα** *"-863"*				0.05
Ser/Ser	C/C	71/285	1.00		
	C/A – A/A	33/99	2.09	1.29-3.27	
Ser/Pro-Pro/Pro	C/C	48/123	1.00		
	C/A – A/A	8/34	0.72	0.28-1.87	
IL10	**TNFα** *"-1031"*				0.007
G/A	T/T	55/175	1.00		
	T/C – C/C	20/83	0.80	0.43-1.51	
A/A-G/G	T/T	29/120	1.00		
	T/C – C/C	27/66	2.54	1.45-4.47	
IL10	**TNFα** *"-863"*				0.02
G/A	C/C	56/186	1.00		
	C/A – A/A	19/72	0.94	0.49-1.80	
A/A-G/G	C/C	48/198	1.00		
	C/A – A/A	37/85	2.60	1.46-4.62	

Odds ratios (ORs) and 95% confidence intervals (CIs) are adjusted for age, occupation and body mass index (BMI).
SNP single nucleotide polymorphism, *OA* osteoarthritis, *OR* odds ratio, *CI* confidence interval.

Table 5 Association of haplotypes with hand OA

	OA- (n = 382)	OA + (n = 160)	OR (95% CI)	p-value
TNFα-1031-863 -308				0.07*
T-C-G	0.71	0.65	1.00	
C-A-G	0.14	0.19	1.61 (1.10-2.37)	0.01
T-C-A	0.13	0.15	1.35 (0.89-2.03)	0.16
C-C-G	0.02	0.02	0.93 (0.33-2.58)	0.88
IL4R				0.65*
Ser-Ser	0.88	0.86	1.00	
Ser-Pro	0.10	0.10	1.08 (0.67-1.74)	0.73
Ala-Pro	0.02	0.04	1.43 (0.67-3.03)	0.37

ORs and their 95% CIs were adjusted for age, occupation and BMI.
*Global haplotype association p-value.

conflicting results [32]. To our knowledge, this is the first study to report an association between the *TNFα* variant alleles and hand OA. TNFα can act independently or in concert with other cytokines (*e.g.*, IL1β, IL4, IL6, and Il10) to initiate and expand inflammation [5]. Ignorance of the complex interrelationships between pro- and anti-inflammatory cytokines might be the reason for failure to detect an association between the *TNFα* polymorphisms and OA. It has been suggested that the combined use of information from multiple markers may be more effective to reveal the association between a genomic region and a trait than a single marker analysis [33].

Previously our group reported the associations of the *IL1* and *IL6* gene polymorphisms with hand OA [19,20]. The current findings suggest that *TNFα* promoter polymorphisms may increase the risk of hand OA independently of the polymorphisms in the *IL1* and *IL6* genes, and that the effect attributed to combination of variants in all three genes is larger, but less than additive.

When we examined the association of the *TNFα* polymorphisms with hand OA taking into consideration the variants of other cytokine genes, the *IL4R* and *IL10* polymorphisms appeared to act as effect modifiers.

Vargiolu *et al.* [34] reported an association of genetic variants in the coding region of the *IL4R* gene with hand OA among men and women in the age range of 41 to 84 years. However, we failed to replicate this association among our participants. Differences between the study populations, OA phenotypes, and minor allele frequencies might be the reasons for discrepancies in the findings. Our participants were younger (mean age 53 years) than in the study by Vargiolu and coworkers, which may partly explain the difference of the prevalence of hand OA between their and our study (55.6% and 29.5%, respectively).

Naturally occurring anti-inflammatory cytokines such as IL10 inhibit the synthesis of IL1 and TNFα [13]. The *IL10 "-1082"* polymorphism has been shown to affect the level of the protein production: the A-allele is connected with a significantly higher protein production than the G-allele [35]. This polymorphism was also associated with rheumatoid arthritis in a meta-analysis [36]. As to its role in DIP OA, no association was found in the Dutch population [37]. Similarly, the *IL10 "-1082"* did not associate with hand OA in our study.

A major strength of the study was that all study participants were of the ethnically relatively homogenous Finnish origin. Each ethnic group has its own set of environmental and genetic factors that contribute to the disease risk, and differences in allelic frequency often affect our ability to detect a susceptibility allele. The Finnish population is known to be a genetic isolate, which originated from a small founder population some 2000 years ago. Therefore, the Finnish population with the relatively homogenous gene pool [38] offers an optimal material for association studies.

Another strength of our study is that we analyzed haplotypes in addition to SNPs. Grouping of SNPs in haplotypes generally leads to a stronger association with the phenotype than individual polymorphisms.

Further, the prevalence of hand OA was similar to that seen in other studies [39-41], and major potential confounders were controlled for in the statistical analyses.

A limitation of our study is the relatively small number of participants, leading to reduced power to detect small effects and an increased likelihood of spurious findings. This needs to be considered while interpreting the observed associations. Another obvious limitation is the fact that our study participants were all women and consequently the results cannot be generalized to men.

Conclusions

Our results suggest that variants in the *TNFα* gene play a role in the etiology of hand OA in Finnish women. In addition, the findings are suggestive of a gene-gene interaction of the *TNFα* with the *IL4R* and *IL10* genes. However, these findings should be considered with caution until replicated in other study population.

Additional file

Additional file 1: Detailed protocols for genotyping.

Abbreviations

TNFα: Tumor necrosis factor alpha; IL6: Interleukine 6; IL1: Interleukine 1; OA: Osteoarthritis; BMI: Body mass index; PCR: Polymerase chain reaction; SNP: Single nucleotide polymorphism; DIP: Distal interphalangeal; PIP: Proximal interphalangeal; IP: Interphalangeal; IL1B: Interleukin 1, beta; K-L: Kellgren and Lawrence; RFLP: Restriction fragment length polymorphism; HWE: Hardy-Weinberg equilibrium; LD: Linkage disequilibrium; OR: Odds ratio; CI: Confidence interval; MHC: Major histocompatibility complex.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

SH carried the main responsibility of the design and performance of the genotyping and data analyses, and preparation of the manuscript; SS participated in the study design, data collection, the design of the data analyses, the interpretation of the results, and preparation of the manuscript; TV participated in the data collection, radiological examinations, data analysis, and preparation of the manuscript; PL-A carried the main responsibility of the overall study design, and participated in the data collection and preparation of the manuscript; AH participated in the conception and design of the study, the interpretation of the data, and preparation of the manuscript. All authors have read and approved the final version of the manuscript.

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III

ADIPOKINE GENES AND RADIOGRAPHIC HAND OSTEOARTHRITIS AMONG FINNISH WOMEN, A CROSS-SECTIONAL STUDY

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Key words: genetic polymorphism, adipokines, hand osteoarthritis, women

ABSTRACT

Objectives: Available evidence suggests that genetic factors and overweight play a major role in the etiology of osteoarthritis (OA). We analyzed the association of 18 single nucleotide polymorphisms (SNPs) from nine adipokine and adipokine receptor genes (*LEP, LEPR, ADIPOQ, RETN, NAMPT, SERPINA12, ITLN, RARRES2,* and *APLN*) with radiographic hand OA.

Methods: The study design was cross-sectional. Bilateral hand radiographs of 542 occupationally active Finnish female dentists and teachers aged 45-63 years were examined and classified for the presence of hand OA using reference images. Hand OA was defined as at least three finger joints with radiographic OA of grade 2–4. The genotypes were determined using PCR-based methods. BMI was calculated based on self-reported height and measured weight. Associations of the individual SNPs and their haplotypes with hand OA were tested using logistic regression analysis.

Results: The minor allele of *RETN* rs10401670 decreased (OR=0.73, 95% CI 0.55-0.97, p-value=0.03), and *RARRES2* rs4721 increased (1.41, 1.07-1.87, p=0.01) hand OA risk. Also, *LEPR* AC and *RETN* GGTT haplotypes were associated with hand OA (1.54, 1.01 - 2.35, p=0.05, and 0.58, 0.37-0.93, p=0.02, respectively). These associations were modified by BMI when comparing normal and overweight women. However, the associations lost their statistical significance after adjusting for multiple testing.

Conclusions: Our results suggest that there may be a weak association between the studied variations in *LEPR, RARRES2,* and *RETN* genes and hand OA in Finnish women, and that the associations are modified by BMI. However, these associations could not be verified in the current study.

INTRODUCTION

The multifactorial etiology of hand osteoarthritis (OA) is not yet fully understood [1]. However, it is known that age, obesity, gender and repetitive joint activity may play a role in this context [2]. In addition, genetic factors are estimated to explain a large part of hand OA [3] although only small effects have been found so far [4]

Obesity has become apparent as one of the strongest risk factors for the development of OA in weight-bearing joints such as the knee and foot [5, 6, 7], as well as in non-weight-bearing joints such as those in the hand [8, 9, 10]. Emerging evidence on a high prevalence of OA among obese people suggests that metabolic factors released mainly by the white adipose tissue may also be of importance [11, 12]. Over the past decade, adipocyte-derived molecules, or adipokines, that are known to play a role in cartilage and bone homeostasis, have been investigated for their possible significance in OA pathophysiology [13, 14]. Moreover, the association of adipokines with obesity may provide a metabolic link between obesity and OA [11, 15].

Adipocytes secrete a series of adipokines such as leptin, adiponectin, resistin, apelin, chemerin, vaspin, and visfatin [16] with pro- and anti-inflammatory activities. If dysregulated, these adipokines may lead to a chronic low-grade inflammation and further to systemic metabolic dysfunction [17]. In OA patients, leptin, visfatin and resistin were found to be distinctly elevated [18] whereas adiponectin showed decreased production [14], suggesting a catabolic and anabolic role for these adipokines.

The adiponectin, leptin, resistin, apelin, chemerin, vaspin, and visfatin are encoded by the *ADIPOQ, LEP*, *RETN*, *APLN*, *RARRES2*, *NAMPT,* and *SERPINA1* genes, respectively. The role of adipokine genes in obesity remains controversial. Although several meta-analyses have confirmed the association of single nucleotide polymorphisms (SNP) of the *ADIPOQ* gene with obesity [19, 20, 21], SNPs in the *LEP*, *LEPR*, and *RETN* have not been associated with such susceptibility [19, 22, 23]. Similarly, despite of the existing evidence on the role of adipokines in OA pathophysiology the association of adipokine genes with OA is largely unknown. So far, an association between haplotypes of the *LEP* [24] and *LEPR* [25] gene and knee OA has been reported. In contrast, no association between the *ADIPOQ* gene polymorphism and knee OA was found [26]. The *RETN*, *APLN*, *RARRES2*, *NAMPT*, *SERPINA12*, and *ITLN* genes are more novel discoveries in the adipokine family [27] and have not yet been examined as candidate genes for OA.

The aim of the present study was to investigate the possible role of variations in selected adipokine genes in the etiology of hand OA. We chose to analyze associations of 18 SNPs from nine adipokine and adipokine receptor genes (*ADIPOQ, LEP, LEPR, RETN, APLN, RARRES2, NAMPT, SERPINA12,* and *ITLN*) with radiographic hand OA among 542 Finnish female dentists and teachers. The potential effect of relative body weight on the association of these SNPs with radiographic hand OA was also examined.

SUBJECTS AND METHODS

Study design and selection of participants

The study design was cross-sectional. The study base comprised of all occupationally active female dentists and teachers in the secondary level schools in the Helsinki metropolitan region.

The participants were identified from the registers of the Finnish Dental Association and the Finnish Teachers' Trade Union. At the beginning of 2002, 436 women aged 45 - 63 were randomly selected from both occupational groups using the place of residence (Helsinki or its neighboring cities) as an inclusion criterion. Of the 872 women who received the questionnaires, 542 (62 %) participated in a clinical examination between October 2002 and March 2003. Of them, 294 (67% of the invited) were dentists and 248 (57% of the invited) teachers.

Participation in the study was voluntary and based on informed consent. The study was approved by the Hospital District of Helsinki and Uusimaa Ethics Committee for Research in Occupational Health and Safety.

Hand radiography and image analysis

Both hands of the study participants were radiographed by exposing Kodak X-ray films with the Siemens X-ray equipment (48 kV, 10 mA, focus film distance = 115 cm; Siemens, Munich, Germany). The analogue radiographs were evaluated by an experienced radiologist who was blinded to the occupation, age, and all health data of the subjects. Each distal interphalangeal (DIP), proximal interphalangeal (PIP), and thumb interphalangeal (IP) joint of both hands was graded separately, and classified for the presence of hand OA by using a modified Kellgren and Lawrence system [28];

the classification criteria were: grade 0 = no, grade 1 = doubtful, grade 2 = mild, grade 3 = moderate, and grade 4 = severe. The description of reference images used in the classification has been given elsewhere.[29]

The intra-observer agreement for hand OA ranged from 0.73 to 0.88 for DIP joints and from 0.67 to 0.92 for PIP joints [29]. The inter-observer agreement (a second radiologist classified a subset of images) for hand OA ranged from 0.67 to 0.85 for DIP joints and from 0.39 to 0.61 for PIP joints.

If the subject had at least three finger joints with radiographic OA of grade 2–4 (OA 2+), she was classified as having radiographic hand OA (cases). Otherwise, the subject was classified as not having radiographic hand OA (controls).

Questionnaires and interviews

Weight was measured without shoes to the accuracy of 0.1 kg, whereas height was inquired by questionnaire. Body mass index (BMI = weight (kg)/ height $(m)^2$) was calculated and overweight/obesity was defined as BMI $\geq$ 25 kg/m^2. One woman refused to measure her weight and was excluded from the analysis.

SNPs selection

The *ADIPOQ* and *LEP* SNPs included in this study were chosen based on their well-known functional effects. In case of the novel adipokine genes, tag SNPs were chosen to be able to cover a larger area of the studied gene and, if available, the functional tag

SNPs were preferred. More detailed information about the localization and functions of the selected SNPs is presented in Table 1.

Genotyping analysis

Blood samples were collected from each study participant at the clinical examination and stored at +4 °C until DNA was extracted by a DNA extraction kit (PUREGENE ®DNA Purification Kit; Gentra Systems, Plymouth, MN, USA). The *LEP* and *ADIPOQ* SNPs (rs7799039 and rs2167270 in *LEP*, and rs1501299, rs2241766, rs182052, and rs17300539 in *ADIPOQ*) were genotyped by polymerase chain reaction (PCR) -based TaqMan® SNP Genotyping Assays (Applied Biosystems, C_1328079_10 and C_15966471_20 in *LEP* and C_7497299_10, C_26426077_10, C_2412785_10 and C_33187774_19 in *ADIPOQ* respectively). The rest of the studied polymorphisms in *LEPR* (rs1137100, rs1137101, and rs1805094/rs8179183), *RETN* (rs4804765, rs1423096, rs10401670, and rs3745367), *NAMPT* (rs3801266), *SERPINA12* (rs2236242), *ITLN1* (rs2274906), *RARRES2* (rs4721/ rs10278590), and *APLN* (rs3115757) we analyzed simultaneously using OpenArray® equipment and TaqMan® SNP Genotyping Assays (Applied Biosystems, C____518168_20, C___8722581_10, C___8722378_10, C___1394116_10, C___1394117_20, C___1394125_10, C___1394113_10, C____340124_10, C___2786211_1_, C__16183117_10, C___1248939_10, C__27458731_10, respectively).

For quality control 10% of the genotyped samples were blindly repeated with 100% concordant results. Genotype data were available from all participants.

Statistical analysis

The potential deviation of the allele frequencies from the Hardy-Weinberg equilibrium (HWE) in the controls was tested from controls using the chi-square test. The degree of pair-wise linkage disequilibrium (LD) was calculated for each pair of SNPs by using the SNPStats web tool [30]. Logistic regression analysis was used to test the associations of the SNPs and the haplotypes with hand OA. For each SNP a log-additive model of inheritance was fitted. Both crude and adjusted odds ratios (ORs) and their 95% confidence intervals (CI) were calculated. The ORs were adjusted for age (continuous) and occupation (dentists vs. teachers). Since the crude and adjusted ORs did not differ significantly, only the adjusted ORs are shown in the results.

To study the role of BMI as possible effect modifier of associations between SNPs/haplotypes and hand OA, stratified analyses were made among overweight/obese women and among normal weight women.

All analyses were hypothesis driven. P-values were adjusted for multiple testing using Sidák's method [31]. Šidák method is used to counteract the problem of multiple testing by controlling the familywise error rate. According to this method, the adjusted p-value is equal to $1-(1-\text{unadjusted p-value})^k$, where k is the number of comparisons in the family. The method is similar to Bonferroni method, thought has a higher statistical power and gives slightly smaller adjusted p-values than Bonferroni. SNPStats web tool [30] were used in the above analyses.

The power calculations, based on a two-sided alpha values of 0.05, were performed using standard methods.

RESULTS

The overall prevalence of hand OA was 29.5%, (24.5% in dentists and 35.5% in teachers). The mean age was 54.0±5.3 years (53.7±5.9 in dentists and 54.3±4.4 in teachers) and mean BMI 24.5±3.6 kg/m^2 (23.9±3.2 in dentists and 25.1±3.9 in teachers).[32] The number of subjects with BMI < 25 was 342 (63.1%) and >25 was 200 (36.9%).

Participants with radiographic hand OA were older (56.3±4.7 vs. 53.0±5.2 years, p< 0.001) and had a higher BMI (25.0±3.8 vs. 24.3±3.5, p= 0.04) than those without radiographic hand OA.

The minor allele frequencies of the studied SNPs and their comparison to HapMap and 1000 genomes corresponding frequencies are presented in Table 2. Most of the genotype distributions were in the HWE in controls.

The study had the power of 80% to detect ORs from 1.70 to 3.02 (MAF 4 – 49 %).

SNP analysis

In single SNP analysis using the log-additive model of inheritance and adjusting for age and occupation, the *RETN* rs10401670 minor allele was inversely associated with hand OA risk (OR=0.73 95% CI 0.55-0.97, p-value=0.03), whereas the *RARRES2* rs4721 minor allele increased the risk (1.41, 1.07 – 1.87, p=0.01) (Table 2). Adjustment for BMI did not change the results (data not shown). However, when stratified by overweight status, the *RARRES2* rs4721 minor allele decreased the risk of hand OA (0.67, 0.46-0.96, p=0.03) in normal weight women but not in overweight

women (1.36, 0.87-2.11, p=0.18) (Table 2). Further, the *LEPR* rs1805094 minor allele almost doubled the risk of hand OA (1.90, 1.03-3.52, p=0.04) only in the overweight women.

Haplotype analysis

Haplotype analyses showed significant LD between SNPs of *ADIPOQ, LEP, LEPR* and *RETN*: in the *ADIPOQ* gene the LD was between rs17300539 and rs182052 (D'= 1.0, r^2= 0.045), and between rs2241766 and rs1501299 (D'= 1.0, r^2= 0.031); in the *LEP* gene the LD was between rs7799039 and rs2167270 (D'= 0.99, r^2= 0.59); in the *LEPR* gene the LD was between rs1137101 and rs1805094 (D'= 0.84, r^2= 0.17); in *RETN* the LD was between rs3745367, rs4804765, rs1423096, and rs10401670 .

When adjusted for age and occupation, the *LEPR* AC-haplotype was associated with increased risk of hand OA (1.54, 1.01 - 2.35, p=0.05), whereas the *RETN* GGTT-haplotype was associated with decreased risk of hand OA (0.58, 0.37-0.93, p=0.02) (Table 3). Furthermore, the *LEP* GG haplotype and the *RETN* GGTT-haplotype were associated with hand OA lowering the risk among the overweight but not among the normal weight women (0.45, 0.22-0.96, p=0.04, and 0.42, 0.20-0.86, p=0.02 respectively) (Table 3).

DISCUSSION

We investigated whether 18 SNPs from nine adipokine genes were associated with hand OA in Finnish women. The *RETN* minor allele and haplotype were found to reduce the risk of hand OA, while the *RARRES2* minor allele and *LEPR* haplotype increased the risk. Furthermore, when considering the results stratified by overweight

status, the *LEP* GG haplotype and the *RETN* GGTT-haplotype were associated with a decreased risk of hand OA among the overweight but not among the normal weight women. However, none of the associations remained statistically significant after adjustment for multiple testing.

LEP, an adipocyte produced hormone and cytokine, regulates adipose tissue mass and energy expenditure through the LEPR. *LEP* and *LEPR* expression have been shown to be elevated in the synovial fluid of OA joints and the expression was correlated with the BMI [33, 34].

Recently, it was found that almost half of the association between elevated BMI and knee OA is due to LEP, suggesting that it might be a mediator to OA [35]. A haplotype of three tag SNPs, different from our study, within the *LEP* gene was associated with knee OA in the normal weight and overweight Chinese individuals [24]. A recent study in a South Indian population reported a positive dose-response association of the *LEP* rs2167270 minor allele (A) with both BMI and LEP levels and higher BMI values among the rs7799039 major AA-genotype [36]. We did not find these SNPs to be associated with BMI in our study but our finding suggests that the least frequent *LEP* haplotype GG (rs7799039 minor allele (G) and rs2167270 major allele (G)) may have a protective role in hand OA among overweight or obese women only.

We found that the *LEPR* rs1805094 was marginally associated with an almost 1.5-fold risk of hand OA in the total sample, but when stratified by overweight status, the risk estimate decreased in the normal weight subjects and increased in the overweigh subjects to indicate an almost 2-fold risk of hand OA. We also found that the *LEPR* AC haplotype was associated with 1.5-fold risk of hand OA in the total sample and

that in the stratified analysis there was a similar trend of an increasing risk in the overweight women. This haplotype includes the rs1137101 and rs1805094 SNPs. These missense SNPs change the amino acid in the LEPR protein and have been associated with obesity and body fat levels. F-SNP predicts many functions for these two *LEPR* SNPs in addition to a change in protein coding, a changed splicing regulation, and a post translational function, giving the high functional scores of 0.29 and 0.53 [37]. These predictions suggests that these SNPs may alter the structure of the protein expressed, its expression itself, and even the proteins' function. These changes may expose the tissues for dysregulation and in the end to a disease such as OA.

RETN is present in knee OA joints and is released from knee OA cartilage [38]. The level of RETN strongly upregulates the expression of tumor necrosis factor (TNF) and interleukin 6 (IL6) and thus is pro-inflammatory in nature [39]. A recent systematic review and meta-analysis confirmed that high expression of *RETN* is a significant marker of poor progression in patients with knee, hip, and spinal OA, especially in males [40]. However, the role of RETN in hand OA is contradictory. The presence of radiographic changes in hand OA was shown to be dependent on serum RETN levels [39], while a more recent study did not find any association of the serum RETN level with the progression of hand OA [41].

The minor alleles of the studied *RETN* rs4804765, rs1423096, and rs10401670 SNPs have been associated with higher plasma RETN levels explaining 1.5% of the variance at RETN levels [42]. Moreover, the F-SNP predicts that rs1423096 functions in transcriptional regulation [37].

To our knowledge no published studies have examined associations of the variations within the *RETN* gene and hand OA. In our study, the minor alleles in *RETN* rs1423096 and rs10401670 SNPs (T and T) and haplotype containing these minor alleles (GGTT) were associated with a reduced risk of hand OA. This is opposite to the expected result, RETN being pro-inflammatory in nature. The explanation for this could be that the outcome was hampered by the fact that the rs1041670 SNP was not in HWE in the controls (Table 2).

There is evidence on a protective effect of the ADIPOQ level on the progression of OA [41, 43]. However, we did not find an association between the studied *ADIPOQ* SNPs and hand OA. Similarly, no association of the *ADIPOQ* +276G/T (rs1501299) SNP with knee OA was recently found [26]. No other reports of negative nor null associations between OA and the other *ADIPOQ* variants were found.

RARRES2 is a newly found adipokine expressed in adipose tissue and plays an anti-inflammatory role [44]. RARRES2 has been shown to modulate the expression of *ADIPOQ* and *LEP* [45], and its level in the synovial fluid of knee OA patients has been shown to correlate with the disease severity [46]. Since an increase of RARRES2 in fat tissue and serum of obese patients has been reported, the RARRES2 may represent a functional link between obesity and OA [47].

To date, no studies examining the association of the variations within the *RARRES2* gene with hand OA have been published. We found that the minor allele of the rs4721 SNP posed a 1.4-fold risk of hand OA in a log additive model of inheritance. Stratification by BMI status suggested that BMI is an effect modifier as the direction

of the effect was reversed among the normal weight. This SNP has been predicted to have a function in splicing regulation by the F-SNP (Table 1) [37].

To our knowledge no studies have yet examined associations of the *NAMPT, SERPINA12, ITLN,* and *APLN* genes variations with hand OA. We did not find associations of the *APLN* rs3115757, the *NAMPT* rs3801266, *SERPINA12* rs2236242 and *ITLN1* rs2274906 SNPs with hand OA.

Since each ethnic group has its own set of environmental and genetic factors that contribute to the disease risk, and differences in allelic frequency often affect our ability to detect a susceptibility allele, an evident strength of our study was that all study participants were of ethnically relatively homogenous Finnish origin. The Finnish population is known to be a genetic isolate, which originated from a small founder population some 2000 years ago. Therefore, the Finnish population with the relatively homogenous gene pool [48] offers an optimal material for association studies, while also limiting the generalizability of the results to other populations.
Another strength of our study is that we analyzed haplotypes in addition to SNPs. A grouping of SNPs in haplotypes generally leads to a stronger association with the phenotype than individual polymorphisms. Further, the prevalence of hand OA was similar to that seen in other studies [49, 50, 51].

Our study material included a relatively small number of participants, leading to reduced power (80% power to detect ORs from 1.70 to 3.02) to detect small effects and an increased likelihood of null associations. This needs to be considered while interpreting the findings.

Another obvious limitation of our study is the fact that the study participants were all women and therefore the results cannot be generalized to men. The cross-sectional study design also limits our study as it only represents a snapshot of the situation and causal interference is not possible to obtain.

Lastly, we had only radiographic hand OA outcome in our use and not inflammatory data that could have been more interesting to study according to adipokine genotypes.

CONCLUSIONS

Our results suggest that there may be a weak association between the studied *LEP*, *LEPR*, *RARRES2,* and *RETN* gene variants and radiographic hand OA in Finnish women, and that the associations are modified by BMI. However, these findings should be considered with caution until replicated in other studies.

LIST OF ABBREVIATIONS

OA: osteoarthritis; ADIPOQ: adiponectin; LEP: leptin; LEPR: leptin receptor; RETN: resistin; APLN: apelin; RARRES2: retinoic acid receptor responder 2 / chemerin; NAMPT: nicotinamide phosphoribosyltransferase / visfatin; SERPINA12: serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 12 / vaspin; ITLN: intelectin/omentin; SNP: single nucleotide polymorphism; DIP: distal interphalangeal; PIP: proximal interphalangeal; IP: interphalangeal; BMI: body mass index; PCR: polymerase chain reaction; HWE: Hardy-Weinberg equilibrium; LD: linkage disequilibrium; OR: odds ratio; CI: confidence interval; MAF: minor allele frequency; TNF: tumor necrosis factor; IL6: interleukin 6

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

SH carried the main responsibility of the design and performance of the genotyping and data analyses, and preparation of the manuscript; SS participated in the study design, data collection, the design of the data analyses, the interpretation of the results, and preparation of the manuscript; TV participated in the data collection, radiological examinations, data analysis, and preparation of the manuscript; PL-A carried the main responsibility of the overall study design, and participated in the data collection and preparation of the manuscript; AH participated in the conception and design of the study, and preparation of the manuscript. All authors have read and approved the final version of the manuscript.

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Table 1. Location and function of the selected SNPs.

Gene	SNP ID	Chro.	Position	Location	Minor allele	Function	F-SNP predicted function #	score (0-1)
ADIPOQ	rs17300539	3	186559460	upstream 2KB	A	(G) insulin resistance, metabolic syndrome	1	0.50
ADIPOQ	rs182052	3	186560782	intron	G	(G) adiponectin levels	1	0.11
ADIPOQ	rs2241766	3	186570892	nc transcript, synonymous	G	adiponectin plasma levels	1,2,3,4	0.33
ADIPOQ	rs1501299	3	186571123	intron	T	type II diabetes, adiponectin plasma levels	1	0.27
LEP	rs7799039	7	127878783	upstream gene	G	obesity, BMI (AA-genotype)	NA	NA
LEP	rs2167270	7	127881349	UTR 5 prime	A	higher plasma leptin level (A)	1	0.18
LEPR	rs1137100	1	66036441	missense K109R	G	glucose tolerance and insulin response, obesity	1,2,3,4	0.53
LEPR	rs1137101	1	66058513	missense G223R	A	obesity and type II diabetes	2,3,4	0.29
LEPR	rs1805094	1	65610269	missense K656N	C	low body fat levels, elevated high-density cholesterol	2,3,4	0.53
RETN	rs3745367	19	7734511	intron 2 +298G>A	A	adiposity, insulin resistance	1	0
RETN	rs4804765	19	7737840	promoter, 3' UTR	T	higher plasma resistin level (T)	1	0.5
RETN	rs1423096	19	7739177	promoter, 3' UTR	T	higher plasma resistin level (T)	1	0.5
RETN	rs10401670	19	7742802	promoter, 3' UTR	T	higher plasma resistin level (T), plasma glucose	NA	NA
NAMPT	rs3801266	7	105924250	intron 1	C	obesity, cardiovascular disease	1	0.21
SERPINA12	rs2236242	14	94960052	intron	A	metabolic syndrome	NA	NA
ITLN1	rs2274906	1	160849420	intron 6	A	Crohn's disease	NA	NA
RARRES2	rs4721	7	150035526	downstream 500B, intron, UTR variant 3 prime	G	minor allele G associated with lower visceral adipose tissue mass	3	NA
APLN	rs3115757	X	128782412	intron	G	obesity	NA	NA

SNP: single nucleotide polymorphism; F-SNP: http://compbio.cs.queensu.ca/F-SNP/

coding: 1 transcriptional regulation; 2 protein coding; 3 splicing regulation, changed; 4 post translation; 5 conserved

Table 2. MAF and HWE of the studied SNPs and association with hand OA.

		MAF						HWE	Hand OA					
Gene	**Minor**	**Total**		**Cases**		**Controls**		**in**	**All (n=542)**		**BMI < 25 kg/m^2**		**BMI ≥ 25 kg/m^2**	
SNP ID	**allele**	**(n=1084)**		**(n=320)**		**(n=764)**		**controls**			**(n=342)**		**(n=200)**	
ADIPOQ		**%**	**n**	**%**	**n**	**%**	**n**	**p**	**OR* (95% CI)**	**p**	**OR* (95% CI)**	**p**	**OR* (95% CI)**	**p**
rs17300539	A	4	41	3	8	4	33	1.00	0.77 (0.33-1.79)	0.54	1.17 (0.39-3.49)	0.78	0.46 (0.12-1.74)	0.23
rs182052	G	46	503	47	150	46	353	0.92	1.06 (0.80-1.39)	0.70	1.26 (0.87-1.83)	0.22	0.78 (0.51-1.20)	0.26
rs2241766	G	6	62	5	16	6	46	0.15	0.76 (0.42-1.40)	0.38	0.71 (0.29-1.72)	0.44	0.78 (0.34-1.81)	0.55
rs1501299	T	34	365	34	108	34	257	0.42	1.01 (0.76-1.34)	0.94	1.19 (0.82-1.74)	0.36	0.84 (0.54-1.31)	0.44
LEP														
rs7799039	G	45	490	42	136	46	354	0.35	0.87 (0.66-1.15)	0.33	1.00 (0.69-1.44)	0.98	0.75 (0.48-1.16)	0.20
rs2167270	A	33	361	32	104	34	257	0.49	0.98 (0.73-1.31)	0.89	1.01 (0.69-1.48)	0.96	0.96 (0.60-1.54)	0.87
LEPR														
rs1137100	G	39	418	40	127	38	291	0.39	1.04 (0.78-1.39)	0.78	0.90 (0.62-1.32)	0.59	1.32 (0.83-2.09)	0.24
rs1137101	A	38	415	40	129	37	286	0.83	1.13 (0.84-1.50)	0.42	1.18 (0.81-1.71)	0.39	1.08 (0.68-1.72)	0.74
rs1805094	C	13	141	17	53	12	88	0.20	1.45 (0.98-2.15)	0.06	1.21 (0.72-2.04)	0.48	1.90 (1.03-3.52)	0.04
RETN														
rs3745367	A	21	229	20	64	22	165	0.36	0.89 (0.64-1.25)	0.50	0.92 (0.60-1.41)	0.69	0.85 (0.49-1.47)	0.56
rs4804765	T	25	272	25	79	25	193	0.18	1.00 (0.72-1.38)	0.99	1.03 (0.65-1.65)	0.89	0.94 (0.59-1.49)	0.78
rs1423096	T	13	144	9	30	15	114	0.84	0.66 (0.43-1.02)	0.05	0.75 (0.42-1.35)	0.33	0.53 (0.27-1.03)	0.05
rs10401670	T	36	394	31	99	39	295	0.01	0.73 (0.55-0.97)	0.03	0.74 (0.50-1.07)	0.11	0.70 (0.46-1.07)	0.09
NAMPT														
rs3801266	C	17	187	19	61	16	126	0.58	1.16 (0.82-1.65)	0.40	1.04 (0.66-1.66)	0.86	1.32 (0.75-2.30)	0.34
SERPINA12														
rs2236242	A	45	491	45	145	45	346	0.04	1.01 (0.76-1.35)	0.93	0.99 (0.68-1.44)	0.95	1.07 (0.69-1.66)	0.77
ITLN1														
rs2274906	A	38	408	38	121	38	287	0.66	1.02 (0.77-1.34)	0.91	0.86 (0.59-1.26)	0.43	1.23 (0.81-1.87)	0.33
RARRES2														
rs4721	G	49	533	54	174	47	359	0.22	1.41 (1.07-1.87)	0.01	0.67 (0.46-0.96)	0.03	1.36 (0.87-2.11)	0.18
APLN														
rs3115757	G	5	51	2	8	6	43	1.00	0.58 (0.26-1.29)	0.16	0.47 (0.15-1.43)	0.15	0.70 (0.22-2.19)	0.53

SNP: single nucleotide polymorphism; MAF: minor allele frequency; HWE: Hardy-Weinberg equilibrium; OA: osteoarthritis; BMI: body mass index

*Adjusted for age and occupation, log-additive model of inheritance

Table 3. Association of the haplotypes with hand OA and BMI.

			Hand OA					
			All		**BMI < 25 kg/m^2**		**BMI ≥ 25 kg/m^2**	
Gene	**Haplotype**	**Frequency**	**OR* (95% CI)**	**p**	**OR* (95% CI)**	**p**	**OR* (95% CI)**	**p**
ADIPOQ	GA	0.54	1.00		1.00		1.00	
rs17300539	GG	0.43	1.08 (0.81-1.43)	0.60	1.26 (0.86 - 1.83)	0.23	0.83 (0.53 - 1.29)	0.41
rs182052	AG	0.04	0.80 (0.34-1.86)	0.60	1.33 (0.44 – 4.04)	0.62	0.44 (0.11 - 1.66)	0.22
ADIPOQ	TG	0.61	1.00		1.00		1.00	
rs2241766	TT	0.34	0.99 (0.74-1.32)	0.92	1.17 (0.80 - 1.70)	0.42	0.80 (0.51 - 1.27)	0.35
rs1501299	GG	0.06	0.76 (0.41-1.41)	0.38	0.75 (0.30 - 1.84)	0.53	0.70 (0.29 - 1.68)	0.42
LEP	AG	0.55	1.00		1.00		1.00	
rs7799039	GA	0.33	0.93 (0.69-1.26)	0.65	1.01 (0.68 - 1.50)	0.98	0.88 (0.54 - 1.43)	0.60
rs2167270	GG	0.12	0.70 (0.44-1.12)	0.13	0.95 (0.53 - 1.72)	0.92	0.45 (0.22 - 0.96)	0.04
LEPR	GG	0.60	1.00		1.00		1.00	
rs1137101	AG	0.27	0.94 (0.67-1.32)	0.73	1.06 (0.69-1.63)	0.80	0.81 (0.47-1.41)	0.47
rs1805094	AC	0.12	1.54 (1.01 - 2.35)	0.05	1.33 (0.77-2.28)	0.31	2.02 (0.98-4.15)	0.06
	GC	0.01	0.67 (0.16-2.81)	0.59	0.00 (-Inf-Inf)	1.00	0.98 (0.19-5.01)	0.98
RETN	GGCC	0.45	1.00		1.00		1.00	
rs3745367	GTCT	0.15	0.82 (0.53-1.27)	0.37	0.81 (0.42 - 1.54)	0.51	0.77 (0.41 - 1.45)	0.42
rs4804765	GGTT	0.13	0.58 (0.37-0.93)	0.02	0.70 (0.37 - 1.32)	0.27	0.42 (0.20 - 0.86)	0.02
rs1423096	AGCC	0.12	0.67 (0.41-1.11)	0.12	0.72 (0.36 - 1.44)	0.35	0.57 (0.27 - 1.23)	0.15
rs10401670	rare comb.	0.15	0.76 (0.50-1.17)	0.21	0.88 (0.52 - 1.49)	0.63	0.54 (0.24 - 1.18)	0.12

*Adjusted for age and occupation

IV

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Genetic Influences on Hand Osteoarthritis in Finnish Women – A Replication Study of Candidate Genes

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Abstract

Objectives: Our aims were to replicate some previously reported associations of single nucleotide polymorphisms (SNPs) in five genes (A2BP1, COG5, GDF5, HFE, ESR1) with hand osteoarthritis (OA), and to examine whether genes (BCAP29, DIO2, DUS4L, DVWA, HLA, PTGS2, PARD3B, TGFB1 and TRIB1) associated with OA at other joint sites were associated with hand OA among Finnish women.

Design: We examined the bilateral hand radiographs of 542 occupationally active Finnish female dentists and teachers aged 45 to 63 and classified them according to the presence of OA by using reference images. Data regarding finger joint pain and other risk factors were collected using a questionnaire. We defined two hand OA phenotypes: radiographic OA in at least three joints (ROA) and symptomatic DIP OA. The genotypes were determined by PCR-based methods. In statistical analysis, we used SNPStats software, the chi-square test and logistic regression.

Results: Of the SNPs, rs716508 in *A2BP1* was associated with ROA (OR = 0.7, 95% CI 0.5–0.9) and rs1800470 in *TGFB1* with symptomatic DIP OA (1.8, 1.2–2.9). We found an interaction between ESR1 (rs9340799) and occupation: teachers with the minor allele were at an increased risk of symptomatic DIP OA (2.8, 1.3–6.5). We saw no association among the dentists. We also found that the carriage of the *COG5* rs3757713 C allele increased the risk of ROA only among women with the *BCAP29* rs10953541 CC genotype (2.6; 1.1–6.1). There was also a suggestive interaction between the *HFE* rs179945 and the *ESR1* rs9340799, and the carriage of the minor allele of either of these SNPs was associated with an increased risk of symptomatic DIP OA (2.1, 1.3–2.5).

Conclusions: Our results support the earlier findings of *A2BP1 and TBGF1* being OA susceptibility genes and provide evidence of a possible gene-gene interaction in the genetic influence on hand OA predisposition.

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Introduction

Osteoarthritis (OA) shows clinical heterogeneity in localization and progression [1]. Patients may have only one affected joint (monoarthritis) at the time of diagnosis, several affected joints within a single region (*e.g.* in hand OA), or several involved joints at various sites, *e.g.* the hip, knee, and hand (polyarticular OA, generalized OA). Twin and family studies have demonstrated a significant contribution of genetic factors that account for up to half of the risk of developing OA [2,3].

The hand is among the most prevalent sites affected by OA, especially among women over the age of 50 [4]. The simultaneous involvement of multiple hand joints makes hand OA a heterogeneous disorder that is complex to study [5]. Hand OA, largely mirroring the generalized OA variant, is thought to be more heritable than hip or knee OA [3,6]. It is generally accepted that as a complex disorder, the development of hand OA is modulated by many genes with small effects and gene-environment interaction. The genetic influence may involve either a structural defect (*e.g.*, in collagen), alterations in the structural extracellular matrix (ECM) proteins of cartilage and bone, an enhanced inflammatory component in the disease process, or a genetic influence on a known risk factor for OA, such as obesity [7].

Genome-wide scans with different hand OA phenotypes suggested that chromosomes 1, 2, 3, 4, 7, 8, 9, 11, 13, 15, 16, 19, 20 may harbor susceptibility genes [8–13]. Numerous candidate gene studies have been carried out to assess the association of a particular variant with hand OA. However, according to a systematic review, specific associations between a gene and hand OA have rarely been analyzed by more than one study, and only for two genes (*AGC1* and *HFE)* have significant associations been replicated by at least two independent studies [14].

A genome-wide association study (GWAS) is a promising tool for discovering the genetic basis of common diseases [15]. GWAS

were successful in the identification of 11 loci associated with different OA phenotypes, in particular knee and hip OA [16]. Nevertheless, under the strict criteria of replication and a known functional role, the growth and differentiation factor 5 (GDF5) is the only truly OA-associated gene at present [17]. To our knowledge only two GWAS of hand OA have been published so far [18,19].

The first GWAS study detected and replicated an association with an SNP (rs716508) in the first intron of the ataxin 2-binding protein 1 gene (A2BP1) [18]. In the second GWAS, a novel common variant (rs3815148) in intron 12 of the component of the oligomeric Golgi complex 5 gene (COG5) was associated with knee and/or hand OA in both discovery and replicated samples. This SNP is in almost complete linkage disequilibrium with rs3757713 (68 kb upstream of GPR22), which could be the link to OA association. In the same GWAS, three loci in the *GDF5* gene (rs4911494, rs6088813, rs6087705) were associated with hand OA in the discovery sample, but were not replicated [19].

In order to devise future prevention and treatment, strategies for OA replication studies that verify positive findings from GWAS are needed. Such studies will also enable us to determine whether the effect is specific to a certain OA phenotype, and/or to a particular population. Furthermore, GWAS have made it evident that most of the genetic risk of complex diseases will be attributed to numerous genes with small to moderate effect sizes [16]. In order to understand the manner in which the individual genes that are implicated in OA exert their effect, gene-gene combination effect and interaction need to be explored.

Our aims were to replicate some previously reported associations with hand OA for single nucleotide polymorphisms (SNPs) in five genes (A2BP1, COG5, GDF5, HFE, ESR1) and to examine whether variants in nine genes (BCAP29, DIO2, DUS4L, DVWA, HLA, PTGS2, PARD3B, TGFB1 and TRIB1) are associated with hand OA among Finnish middle-aged women. All selected SNPs were located on genes with prior evidence from GWAS or candidate gene association studies of an association with different OA phenotypes.

Participants and Methods

Study Population

The study participants were identified from the registers of the Finnish Dental Association and the Finnish Teachers Trade Union, and randomly selected from both occupational groups by using the place of residence (Helsinki or its neighboring cities) as an inclusion criterion. The samples were restricted to women aged 45 to 63. Of those who received the questionnaires in 2002, 295 (68%) dentists and 248 (57%) teachers participated in a clinical examination.

Ethics Statement

Participation in the study was voluntary and based on written informed consent. The study proposal was approved by the Hospital District of Helsinki and Uusimaa Ethics Committee for Research in Occupational Health and Safety.

Hand Radiography and Image Analysis

Both hands of the study participants were radiographed by exposing Kodak X-ray films with Siemens X-ray equipment (48 kV, 10 mA, focus film distance = 115 cm; Siemens, Munich, Germany). The analogue radiographs were evaluated by an experienced radiologist who was blinded to the occupation, age, and all health data of the participants. Each distal interphalangeal (DIP), proximal interphalangeal (PIP), thumb interphalangeal, and metacarpophalangeal (MCP) joint of both hands was graded separately, and classified for the presence of OA using a modified Kellgren and Lawrence system [20]. The classification criteria were: grade 0 = no OA, grade 1 = doubtful OA, grade 2 = mild OA, grade 3 = moderate OA, and grade 4 = severe OA. Reference images were used; their description is given elsewhere [21]. A second experienced radiologist interpreted 46 randomly chosen radiographs. A second reading of these 46 radiographs was independently performed by the primary radiologist (TV). The reliability of the OA classification was estimated by measuring intra-observer and inter-observer agreements using the weighted Cohen's kappa coefficient with quadratic weights [22]. The inter-observer agreement for OA ranged from 0.67 to 0.85 (good) for DIP joints, from 0.39 to 0.61 (moderate) for PIP joints, and from 0.18 to 0.69 (poor to good) for MCP joints. The intra-observer agreement for OA ranged from good to very good, 0.73 to 0.88 for DIP joints, from 0.67 to 0.92 for PIP joints, and from 0.59 to 1.0 for MCP joints. [21].

Questionnaires and Interviews

All study participants received a self-administered questionnaire that included questions on anthropometric measures. Information on symptoms (pain, tenderness) in each joint studied was collected with the prompt: 'Please point out on the picture below in which finger joint you have felt pain or tenderness during the past 30 days.' The participants were also asked to mark the intensity of the pain: 0 = no pain, 1 = mild pain, 2 = moderate pain, 3 = severe pain. Weight was measured without shoes to the accuracy of 0.1 kg. Body mass index (BMI) (weight (kg) per height squared (m^2)) was calculated on the basis of self-reported height and measured weight.

Hand OA Phenotypes

Two hand OA phenotypes were used. If the participant had radiographic findings (grade $\geq$2) in at least three finger joints she was classified as having radiographic OA (ROA). Otherwise, the participant was classified as not having ROA. If the participant had both radiographic findings (grade $\geq$2) and symptoms (grade $\geq$1) in at least two DIP joints, she was classified as having symptomatic DIP OA. Otherwise, the subject was classified as not having symptomatic DIP OA.

SNP Selection

We aimed primarily to replicate the associations for the candidate genes that have been identified by two recent GWAS of hand OA [18,19] and genes with a known association with hand OA pathology. We chose two SNPs (rs716508 and rs3815148) that reached a genome-wide level of significance for association with hand OA [18,19], and variants in the GDF5 (rs143383) [23], HFE (rs1799945) [24], and ESR1 (rs2234693, rs9340799) [25,26] genes. The latter genes were chosen on the basis of the rapidly increasing prevalence of hand OA in women over the age of 45. In addition, we searched PubMed for studies reporting an association between any OA phenotype and the candidate genes located on the chromosomes identified by genome-wide linkage studies as harboring susceptibility genes for hand OA [8–13] (Leppävuori et al. 1999, Demissie et al. 2002, Stefánsson et al. 2003, Hunter et al. 2004, Greig et al. 2006, Livshits et al. 2007). Studies published by 10.11.2010 were reviewed in order to select relevant SNPs. Whenever at least one significant functional SNP was reported for a given candidate gene in hand OA, the SNP was selected for analyses in our samples. Finally, we selected variants from nine candidate genes (BCAP29, DIO2, DUS4L, DVWA, HLA, PTGS2, PARD3B, TGFB1 and TRIB1) from seven

publications [27–33] for exploratory analysis in our study. Several predicted functional effects were found in the F-SNP database for the studied polymorphisms [34]. The description of SNPs selected for replication is given in Table S1.

Genotyping Analysis

Blood samples were taken from each study participant at the clinical examination and stored at +4°C until we extracted DNA using a DNA extraction kit (PUREGENE DNA Purification Kit; Gentra Systems, Plymouth, MN, USA).

The ESR1 *PvuII*, and *Xbal* polymorphisms (rs2234693, and rs9340799 respectively) were genotyped using the RFLP method as essentially described in [35]. The *TGFB1* Leu10Pro (29T>C, rs1800470 formerly known as rs1982073) polymorphism was genotyped by the TaqMan PCR method as described in [36] with somewhat altered PCR conditions (2 min +50°C, 10 min +95°C and 40 cycles 15 s +95°C, 1 min +62°C).

We analyzed the rest of the studied polymorphisms in A2BP1 (rs716508), BCAP29 (rs10953541), COG5 (rs3757713 and rs3815148), PTGS2 (rs4140564), DIO2 (rs225014), DUS4L (rs4730250), DVWA (rs7639618), GDF5 (rs143383), HFE (rs1799945), HLA (rs10947262), PARD3B (rs1207421) and TRIB1 (rs4512391) simultaneously using OpenArray equipment and the TaqMan SNP Genotyping Assays (Applied Biosystems, C__122 4093_10, C__2618842_20, C_27475119_10, C_25994114_10, C_31274663_20, C_15819951_10, C_32373604_10, C__11767 13_10, C__1270479_1, C__1085600_10, C_32201424_10, C__88 07483_10 and C___310264_20, respectively).

Genotyping completion rate was from 99.8 to 100%.

Statistical Analysis

The potential deviation of the allele frequencies from the Hardy-Weinberg equilibrium (HWE) was tested from total population using the chi-square test. The degree of pairwise linkage disequilibrium (LD) for two SNPs in the *COG5* gene and two SNPs in the *ESR1* gene were calculated using SNPStats software [37]. Each SNP was analyzed in turn. Haplotypes were constructed and analyzed by the SNPStats program. Logistic regression analysis was used to test the associations between selected SNPs and two hand OA phenotypes. For each SNP, a log additive model of inheritance was fitted. Gene-occupation interaction was tested for all SNPs, to evaluate whether the association between the SNPs and OA was modified by occupation. In addition, gene-gene interactions and gene-gene combination effects were evaluated by a logistic regression model with a dummy variable (0, 1) for the SNPs to compare the magnitude of their odds ratios (ORs). From each gene, we selected a representative SNP that showed the lowest p-value in the replication analysis. We used the dominant model, with the homozygous genotype of the major allele as the reference. Both crude and adjusted ORs and their 95% confidence intervals were calculated. We adjusted the ORs for the potential confounding effects of age (continuous), occupation (dentists vs. teachers) and BMI (continuous). Since the crude and adjusted ORs did not differ significantly, only the adjusted ORs are shown.

All analyses were hypothesis driven. We have used $p<0,05$and $p<0.008$ as significance level in replication and exploration analyses, respectively. In exploratory analyses p-values were adjusted for multiple testing using Sidák's method [38]. We used SNPStats software [37] for the analyses.

Results

The prevalence of ROA and symptomatic DIP OA were 29.5%, and 9.0%, respectively. The ROA prevalence was statistically significantly higher among the teachers than the dentists (Table 1). The genotype distributions of the selected SNPs did not deviate from the HWE. There was no difference between the minor allele frequencies for any SNPs of the two occupations.

Replication Analyses of Hand OA-associated SNPs

Of the two SNPs identified in GWAS, only the rs716508 located in the *A2BP1* gene was associated with ROA (OR = 0.68, 95% CI 0.50–0.93) (Table 2). However it was not associated with symptomatic DIP OA (Table 3). The risk of symptomatic DIP OA was marginally, though not statistically significantly, elevated with the occurrence of the minor alleles of two SNPs (rs3757713, rs3815148) in the *COG5* gene.

We found no statistically significant associations between the SNPs in the *GDF5*, *HFE* and *ESR1* genes and hand OA. Linkage disequilibrium was very strong between the two *COG5* SNPs (rs3757713 and rs3815148) as well as the two *ESR1* SNPs (*PvuII* rs2234693 and *XbaI* rs9340799) (D' = 0.95, $p<0.0001$ and D' = 0.99, $p<0.0001$, respectively). Haplotypes comprised of the two *COG5* SNPs or of the two *ESR1* SNPs were not associated with hand OA.

We found an interaction between the *ESR1* rs9340799 SNP and occupation ($p = 0.0064$) in relation to symptomatic DIP OA. The teachers with the minor allele were at an almost three-fold increased risk of symptomatic DIP OA (OR = 2.84, 95% CI 1.25–6.48) while the dentists carrying the allele were at a lower risk (OR = 0.50, 95% CI 0.19–1.33) than those with the major allele. Adjustment for the use of hormone therapy did not affect the observed associations.

Exploratory Analyses of Nine OA-associated SNPs

Of nine SNPs associated with different OA phenotypes (knee, hip, spine and multiple joints) in previous studies, only the *TGFB1 Leu10Pro* (rs1800470) SNP was associated with symptomatic DIP OA (Table 3, OR = 1.84, 95% CI 1.16–2.91). We observed a suggestive ($p = 0.047$) interactive effect between the *PTGS2* rs4140564 SNP and occupation in relation to ROA. The teachers with the minor allele were at a 1.44-fold (95% CI 0.69–3.02) increased risk, and the dentists were at a lower risk (OR = 0.40, 95% CI 0.13–1.21) (Table 2).

Gene-gene Combination Effects and Interaction

To evaluate possible gene-gene combination effects and interactions, we selected two SNPs from the genes located on chromosome 7 (*COG5* rs3757713 and *BCAP29* rs10953541) and two SNPs from the genes located on chromosome 6 (*HFE* rs179945 and *ESR1* rs9340799) that showed relatively stronger associations with our hand OA phenotypes. We found a statistically significant interaction ($p = 0.034$) between the *COG5* rs3757713 and *BCAP29* rs10953541 SNPs and a suggestive interaction ($p = 0.096$) between the *HFE* rs179945 and *ESR1* rs9340799 SNPs (Table 4). Carriage of the *COG5* rs3757713 C allele increased the risk of ROA only in women with the *BCAP29* rs10953541 CC genotype. However, the likelihood of ROA in the carriers of the minor allele of either SNPs (rs3757713 or rs10953541) was 1.49 (95% CI 1.07–2.06) times higher than that in the non-carriers of these alleles. The carriage of the *HFE* rs179945 G allele increased the risk of symptomatic DIP OA in women homozygous for the *ESR1* rs9340799 major allele (AA genotype). Carriage of the minor allele of either SNP (rs179945 or

Table 1. Selected characteristics of study population.

	Radiographic OA*		Symptomatic DIP OA**	
	No	**Yes**	**No**	**Yes**
All (n = 542)	382 (70.5%)	160 (29.5%)	493 (91.0%)	49 (9.0%)
Age (mean ± SD)	53.0±5.2	56.3±4.7	53.6±5.2	57.7±4.2
BMI (mean ± SD)	24.3±3.5	25.0±3.8	24.4±3.6	25.1±3.3
Pinch grip strength(mean ± SD)	55.9±7.4	52.3±9.2	55.2±8.1	51.5±7.0
Dentists (n = 294)	222 (75.5%)	72 (24.5%)	274 (93.2%)	20 (6.8%)
Age (mean ± SD)	52.6±5.6	57.1±5.6	53.3±5.8	59.4±4.8
BMI (mean ± SD)	23.8±3.2	24.5±3.3	23.9±3.3	23.9±3.0
Pinch grip strength(mean ± SD)	56.3±7.5	52.7±10.1	55.8±8.2	50.4±8.4
Teachers (n = 248)	160 (64.5%)	88 (35.5%)	219 (88.3%)	29 (11.7%)
Age (mean ± SD)	53.5±4.5	55.7±3.8	53.9±4.5	56.5±3.3
BMI (mean ± SD)	25.0±3.8	25.4±4.1	25.0±4.0	25.9±3.3
Pinch grip strength(mean ± SD)	55.3±7.1	52.0±8.4	54.4±8.0	52.2±5.8

*p = 0.006 for difference between prevalence among dentists and among teachers.
**p = 0.05 for difference between the prevalence among dentists and among teachers.
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rs9340799) was associated with an approximately two-fold increased risk of symptomatic DIP OA (OR = 2.12, 95% CI 1.28–2.50).

Discussion

In this study we attempted to replicate some previously reported genetic associations with hand OA. Of the two SNPs identified in GWAS, we found a statistically significant association between the *A2BP1* rs716508 SNP and radiographic hand OA. In addition, we observed evidence of an interactive effect between the *COG5* rs3757713 and the *BCAP29* rs10953541, and radiographic hand OA, and between the *HFE* rs179945 and the ESR1 rs9340799 and symptomatic DIP OA. Moreover, our exploratory analysis of the SNPs in nine selected genes that have a hypothetical association with hand OA provided evidence of an association between the *TGFB1 Leu10Pro* (rs1800470) SNP and symptomatic DIP OA.

In order to succeed in the replication of genotype-phenotype associations, both the initial and replication studies should preferably have the same gene variants, disease phenotypes, genetic models, and population segment (*e.g.* the same gender), and should be adjusted for the same confounding factors. We selected two hand OA phenotypes (ROA in at least three finger joints and symptomatic DIP OA), that are alike or very similar to those in the discovery studies. Both our phenotypes were defined on the basis of a K–L score of grade 2 or more (at least mild OA), which has been commonly used for classification in other studies [24,26,39]. Radiographic hand OA in our study was based solely on radiographic evidence of joint destruction, while symptomatic DIP OA was defined on the basis of the simultaneous presence of radiographic changes and pain in the most commonly affected joint (DIP).

GWAS with two discovery and four replication samples reported an association between the rs716508 SNP located in the first intron of the *A2BP1* gene and hand OA in the population of European ancestry [18]. Meta-analysis suggested that the minor allele of this SNP diminished the risk of hand OA by 33–41%, with the protective effect larger for the severe hand OA phenotype (at least three joints affected). In line with the initial study, we observed a 32% reduction in the risk of radiographic OA per minor allele. However, we found no association between the rs716508 SNP and symptomatic DIP OA.

The *A2BP1* gene is known to be associated with several human diseases, such as autism [40], cancer [41] and obesity [42], though its role in the development of hand OA needs further exploration. Since the *A2BP1* gene is abundantly expressed in the skeletal muscle [43], it is possible that the association between the rs716508 SNP and hand OA could be via muscle strength. We have earlier reported an association between pinch grip strength and symptomatic ROA, but radiographic findings or pain were not associated with pinch grip strength [44]. In the present study, the rs716508 SNP was not associated with pinch grip strength and the odds of ROA remained unchanged after adjustment for pinch grip strength (data not shown).

Although we failed, in the sample of Finnish female dentists and teachers, to replicate the associations of the other SNPs with reported effects on hand OA, the effect sizes of the risk alleles on ROA and symptomatic DIP OA were similar to those found in the initial studies. Reasons for the lack of replication could be due to differences in phenotypes, in minor allele frequencies, and the high likelihood of false negative findings, because our study was not large enough to detect very small effects.

Multiple susceptibility variants with small effect sizes may also interact with each other or with environmental factors in their influence on hand OA predisposition.

In agreement with this, our result suggests that the effect of the rs3757713 SNP (that is in complete linkage with rs3815148) on hand ROA might be diluted by the carriage of the *BCAP29* rs10953541 minor allele. The *COG5* and *BCAP29* genes together with the other four genes (*PRKAR2B*, *HBP1*, *GPR22* and *DUS4L*) are located on chromosome 7q22 within a block with high-linkage disequilibrium. The GWAS meta-analyses of knee OA showed that this LD block contains an OA susceptibility locus [27]. Both genes were expressed in OA joint tissue but were down-regulated in OA cartilage compared to normal control cartilage [45].

In addition, we found that the effect of the *HFE* rs1799945 on symptomatic DIP OA was diluted by the carriage of the *ESR1* rs9340799 minor allele. Both genes may play a role in hand OA,

Table 2. Association of studied polymorphisms with ROA in the total material and by occupation.

Gene	Chr	SNP	MAF (%)		Group	MAF (%) Our study			HWE	ROA		
			NCBI	HapMap		Total	Case	Control	(p-value)	OR	95% CI	p-value
Replication analyses												
A2BP1	16	rs716508	39	28	All	35	31	36	0.09	**0.68**	**0.50–0.93**	**0.01**
					Dentists	35	33	36		0.69	0.39–1.23	0.19
					Teachers	34	28	37		0.58	0.34–1.01	0.05
COG5	7	rs3757713	14	21	All	19	20	18	0.07	1.16	0.83–1.63	0.38
					Dentists	19	23	18		1.60	0.90–2.86	0.09
					Teachers	18	18	18		0.97	0.54–1.75	0.92
COG5	7	rs3815148	19	22	All	19	20	18	0.12	1.14	0.81–1.59	0.45
					Dentists	19	24	18		1.51	0.84–2.71	0.11
					Teachers	18	17	18		0.90	0.50–1.62	0.74
GDF5	20	rs143383	29	32	All	43	41	43	0.55	0.93	0.70–1.22	0.58
					Dentists	43	40	44		0.80	0.44–1.43	0.49
					Teachers	42	42	42		1.21	0.68–2.14	0.51
HFE	6	rs1799945	8	18	All	13	13	12	0.53	1.15	0.76–1.74	0.52
					Dentists	12	10	12		0.86	0.43–1.74	0.68
					Teachers	14	15	13		1.55	0.84–2.88	0.19
ESR1	6	rs2234693	44	41	All	40	39	41	0.60	0.94	0.71–1.24	0.67
					Dentists	41	40	41		0.80	0.45–1.43	0.45
					Teachers	40	39	40		1.08	0.62–1.89	0.82
ESR1	6	rs9340799	26	30	All	25	26	25	0.20	1.05	0.76–1.45	0.78
					Dentists	27	26	27		0.83	0.47–1.45	0.51
					Teachers	24	26	23		1.62	0.94–2.79	0.08
Exploratory analyses												
PTGS2	1	rs4140564	4	8	All	7	6	7	0.35	0.90	0.50–1.61	0.72
					Dentists	6	3	7		0.40	0.13–1.21	0.08
					Teachers	8	9	7		1.44	0.69–3.02	0.33
PARD3B	2	rs1207421	13	9	All	10	11	9	1.00	1.04	0.66–1.64	0.87
					Dentists	10	12	9		1.17	0.59–2.33	0.67
					Teachers	10	10	9		0.90	0.45–1.82	0.80
DVWA	3	rs7639618	26	18	All	16	16	16	0.95	1.11	0.76–1.63	0.58
					Dentists	18	19	17		1.52	0.84–2.76	0.16
					Teachers	14	14	14		0.82	0.44–1.52	0.57
HLA	6	rs10947262	17	6	All	14	15	14	0.68	1.06	0.72–1.56	0.78
					Dentists	15	16	14		1.10	0.59–2.05	0.82

Table 2. Cont.

Gene	Chr	SNP	MAF (%)		Group	MAF (%) Our study			HWE	ROA		
			NCBI	HapMap		Total	Case	Control	(p-value)	OR	95% CI	p-value
					Teachers	14	14	14		1.01	0.54–1.89	0.99
BCAP29	7	rs10953541	14	27	All	26	27	25	0.18	1.09	0.81–1.48	0.58
					Dentists	27	29	26		1.43	0.81–2.51	0.18
					Teachers	24	25	24		1.06	0.61–1.83	0.78
DUS4L	7	rs4730250	12	17	All	15	17	15	0.23	1.15	0.80–1.66	0.46
					Dentists	16	20	14		1.63	0.89–2.97	0.10
					Teachers	14	14	15		0.80	0.43–1.49	0.49
TRIB1	8	rs4512391	38	41	All	26	25	27	0.95	0.87	0.64–1.20	0.40
					Dentists	27	28	27		1.17	0.66–2.04	0.58
					Teachers	25	22	26		0.75	0.44–1.31	0.32
DIO2	14	rs225014	42	39	All	28	27	29	0.10	0.85	0.62–1.17	0.32
					Dentists	28	30	27		1.05	0.60–1.85	0.88
					Teachers	28	24	31		0.72	0.42–1.23	0.24
TGFB1	19	rs1800470/rs1982073	44	NA	All	29	31	29	0.58	1.15	0.85–1.57	0.36
					Dentists	31	29	31		1.10	0.63–1.93	0.71
					Teachers	28	32	25		1.29	0.75–2.23	0.36

Logistic regression analysis with log-additive model in group "All", and dominant model in groups "Dentists" and "Teachers".
ORs and 95% CIs are adjusted for age, occupation and body mass index (BMI) in group "All" and for age and BMI in groups "Dentists" and "Teachers".
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Table 3. Association of studied polymorphisms with symptomatic DIP OA in the total material and by occupation.

Gene	Chr	SNP	MAF (%)		Group	MAF (%)This study			HWE	Symptomatic DIP OA		
			NCBI	HapMap		Total	Case	Control	(p-value)	OR	95% CI	p-value
Replication analyses												
A2BP1	16	rs716508	39	28	All	35	35	35	0.09	0.94	0.58–1.52	0.80
					Dentists	35	38	35		0.69	0.26–1.81	0.38
					Teachers	34	33	34		1.06	0.47–2.38	0.94
COG5	7	rs3757713	14	21	All	19	24	18	0.07	1.46	0.90–2.37	0.13
					Dentists	19	23	19		1.35	0.51–3.54	0.62
					Teachers	18	24	17		1.81	0.80–4.12	0.16
COG5	7	rs3815148	19	22	All	19	25	18	0.12	1.35	0.83–2.19	0.24
					Dentists	19	23	19		1.37	0.52–3.59	0.60
					Teachers	18	26	17		1.41	0.61–3.23	0.42
GDF5	20	rs143383	29	32	All	43	39	43	0.55	0.83	0.54–1.29	0.41
					Dentists	43	38	43		0.76	0.29–2.00	0.50
					Teachers	42	40	43		0.74	0.32–1.69	0.50
HFE	6	rs1799945	8	18	All	13	15	12	0.53	1.49	0.80–2.76	0.22
					Dentists	12	18	11		2.29	0.83–6.29	0.11
					Teachers	14	14	14		1.41	0.57–3.49	0.48
ESR1	6	rs2234693	44	41	All	40	45	40	0.60	1.27	0.83–1.94	0.28
					Dentists	41	45	40		1.33	0.48–3.70	0.56
					Teachers	40	45	39		1.47	0.62–3.46	0.38
ESR1	6	rs9340799	26	30	All	25	31	25	0.20	1.39	0.86–2.24	0.19
					Dentists	27	23	27		0.50	0.19–1.33	0.15
					Teachers	24	36	22		**2.84**	**1.25–6.48**	**0.01**
Exploratory analyses												
PTGS2	1	rs4140564	4	8	All	7	4	7	0.35	0.53	0.18–1.56	0.22
					Dentists	6	0	6		–	–	–
					Teachers	8	7	8		0.83	0.26–2.64	0.73
PARD3B	2	rs1207421	13	9	All	10	10	10	1.00	0.91	0.45–1.87	0.80
					Dentists	10	10	10		0.90	0.28–2.93	0.84
					Teachers	10	10	10		0.79	0.27–2.25	0.68
DVWA	3	rs7639618	26	18	All	16	14	16	0.95	0.93	0.50–1.71	0.81
					Dentists	18	13	18		0.81	0.27–2.36	0.70
					Teachers	14	16	14		1.02	0.42–2.52	0.97
HLA	6	rs10947262	17	6	All	14	13	14	0.68	0.90	0.48–1.68	0.74
					Dentists	15	20	14		1.78	0.67–4.71	0.25

Table 3. Cont.

Gene	Chr	SNP	MAF (%)		Group	MAF (%)This study			HWE	Symptomatic DIP OA		
			NCBI	HapMap		Total	Case	Control	(p-value)	OR	95% CI	p-value
					Teachers	14	9	14		0.59	0.21–1.64	0.32
BCAP29	7	rs10953541	14	27	All	26	32	25	0.18	1.36	0.86–2.15	0.19
					Dentists	27	28	27		1.07	0.42–2.77	0.99
					Teachers	24	35	23		1.64	0.73–3.65	0.21
DUS4L	7	rs4730250	12	17	All	15	20	15	0.23	1.44	0.84–2.45	0.19
					Dentists	16	20	16		1.76	0.66–4.65	0.31
					Teachers	14	21	14		1.49	0.63–3.50	0.34
TRIB1	8	rs4512391	38	41	All	26	20	27	0.95	0.67	0.39–1.15	0.14
					Dentists	27	25	28		0.99	0.39–2.53	0.96
					Teachers	25	17	26		0.53	0.22–1.23	0.13
DIO2	14	rs225014	42	39	All	28	31	28	0.10	1.13	0.69–1.85	0.63
					Dentists	28	35	27		1.76	0.66–4.68	0.28
					Teachers	28	28	29		0.92	0.41–2.04	0.79
TGFB1	19	rs1800470/rs1982073	44	NA	All	29	40	28	0.58	**1.84**	**1.16–2.91**	**0.01**
					Dentists	31	40	30		1.57	0.60–4.09	0.36
					Teachers	28	40	26		**2.72**	**1.16–6.39**	**0.03**

Logistic regression analysis with log-additive model in group "All", and dominant model in groups "Dentists" and "Teachers".
ORs and 95% CIs are adjusted for age, occupation and body mass index (BMI) in group "All" and for age and BMI in groups "Dentists" and "Teachers".
NCBI-population is 1000 genomes.
HapMap population is CEU: Utah residents with Northern and Western European ancestry from the CEPH collection.
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Table 4. Individual and joint effect of selected SNPs on ROA and symptomatic DIP OA.

		ROA				Symptomatic DIP OA			
		N	OR	95% CI	p-value*	N	OR	95% CI	p-value*
COG5-BCAP29	*COG5* rs3757713 C-allele carriage	*BCAP29* rs10953541 T-allele carriage			0.034				0.54
	No	74/277	1.00			21/277	1.00		
	Yes	11/27	**2.57**	**1.08–6.10**		3/27	2.20	0.57–6.40	
	No	30/88	1.53	0.89–2.65		8/88	1.24	0.51–3.00	
	Yes	45/149	1.24	0.78–1.97		17/149	1.62	0.80 –3.26	
Carriage of COG5 C or BCAP29 T allele		86/264	**1.48**	**1.07–2.06**		28/264	1.55	0.93–2.58	
HFE–ESR1	*HFE* rs179945 G–allele carriage	*ESR1* rs9340799 C–allele carriage			0.19				0.096
	No	63/237	1.00			15/237	1.00		
	Yes	21/60	1.60	0.84–3.03		9/60	**3.01**	**1.19–7.58**	
	No	58/180	1.34	0.86–2.11		19/180	1.86	0.89–3.87	
	Yes	18/65	1.16	0.61–2.21		6/65	1.77	0.63 –4.95	
Carriage of HFE G or ESR1 C allele		97/305	1.35	0.98–1.86		34/305	**2.12**	**1.28–3.50**	

*p-value for interaction; ORs and 95% CIs are adjusted for age, occupation and body mass index.

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which is particularly prevalent among women, especially post menopause. The rs1799945 variant in the *HFE* (hemochromatosis Fe) gene is the most common genetic factor in hereditary hemochromatosis. A high risk of developing OA has been observed in iron overload patients, who carry an *HFE* mutation [46]. Increasing evidence shows that a link between defective iron metabolism and tissue responses may drive the OA phenotype [47]. A significant increase occurs in women's iron levels during menopausal transition [48]. Therefore, iron increase could be a risk factor in age-related OA. Candidate gene association studies consistently showed associations between mutations within the *HFE* gene and different hand OA phenotypes [49]. Inverse changes have been observed between iron and estrogen levels in healthy women during menopausal transition [50].

The estrogen receptor 1 alpha (ESR1) gene has been investigated in several genetic studies of knee OA, but very little in relation to hand OA. Valdes *et al.* [51] reported an association between the gene and clinical knee OA in women but not in men. Bergink *et al.* [52] found an association between the *ESR1* haplotype and radiographic knee OA in elderly men and women. One study of Japanese women that investigated the association of *PvuII* (rs2234693) and *XbaI* (rs9340799) SNPs in the *ESR1* gene with generalized hand OA observed an approximately two-fold increase in the risk of severe generalized OA among participants carrying heterozygous genotypes for both rs2234693 and rs9340799 loci [53]. However, these associations have not been replicated in European descent for hand OA [26]; in European descent the *PvuII* variant allele has been associated with a reduced risk of hip OA in elderly women [54].

The SNPs selected for exploratory analysis in our study are located in potential candidate genes for OA identified by GWAS. We found that the variants in the *BCAP29*, *DIO2*, *DUS4L*, *DVWA*, *HLA*, *PARD3B*, *PTGS2*, *TGFB1* and *TRIB1* genes were associated with OA across multiple sites [27–33]. We also discovered that the *TGFB1* (rs1800470, formerly known as rs1982073) variant allele (*Leu10*) was associated with a substantially increased risk of symptomatic DIP OA, but not radiographic OA. Transforming growth factor-β (TGFB) is a multifunctional growth factor with widespread effects on multiple tissues, including an important role in cartilage matrix metabolism. Elevated TGFB1 levels have been identified in the synovial fluid of patients with OA [55], and levels after meniscectomy are reflective of future bone and cartilage changes [56]. The functional role of the *Leu10Pro* polymorphism remains largely unknown, but some evidence exists that it affects TGFB1 secretion and functions in hepatic cells [57]. Earlier, this polymorphism was believed to be associated with a radiographic spinal OA among Japanese women [32].

Our study had both strengths and limitations. The main limitation was the relatively small sample size, due to which the study had low power to detect small effects. One major strength was that it consisted of random samples of ethnically relatively homogenous Finnish origin. The Finnish population is one of the best-studied genetic isolates, which originated from a small founder population some 2000 years ago. Therefore, the Finnish population has a relatively homogenous gene pool [58] offering optimal material for association studies. We also chose to have only women in our study, thus findings that might be restricted to men were left undetected. Moreover, the age range (45–63 years) of the participants was selected to cover the age of occupationally active women, whose prevalence of hand OA is rapidly increasing. Finally, dentists and teachers have a similar level of education, although occupational exposures related to hand use, and lifestyle factors such as obesity, differed.

The current findings were also unlikely to have been influenced by selection bias. The prevalence of the studied hand OA phenotypes was similar to those observed in other studies [59,60]. Yet, the 'healthy worker effect' may have led to an underestimation of associations between SNPs and hand OA, especially for symptomatic DIP OA. Dentists suffering from severe pain in the hand joints may not be able to continue in their profession and therefore were outside the occupationally active group that was our target population. In contrast, teachers with symptomatic hand OA may remain active in their profession. Indeed, occupation-stratified analyses revealed that most of the studied SNPs had a different effect on hand OA in the two occupational groups.

To summarize, by replicating and partly confirming earlier studies of OA susceptibility polymorphisms, our results further support the theory that the *A2BP1* and *TBGF1* genes are hand OA susceptibility genes. OA is a globally significant medical, social, and economic problem and therefore merits attention in order to develop better prevention methods and therapies that can be applied worldwide. The identification of susceptibility genes is a promising basis for OA prevention and treatment.

Supporting Information

Table S1 Description of SNPs selected for replication and exploratory analyses. References for Table S1 [61,62]. (DOC)

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Author Contributions

Conceived and designed the experiments: SH SS TV KL PL-A AH. Performed the experiments: SH SS TV KL. Analyzed the data: SH SS TV KL. Contributed reagents/materials/analysis tools: AH. Wrote the paper: SH SS TV KL PL-A AH.

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V

Appendix V Genetic studies on OA at other joint sites.

Table 1. Genetic studies in generalized OA

Reference	Gene	SNP	n	Ethnicity	OR (95 % CI) p / gene
Sakkas et al. 1990 (1)	A1AT A1ACT	SstI TaqI TaqI	NA	NA	NS NS 2.9, 0.01
Ala-Kokko et al. 1990 (2)	COL2A1	Arg519Cys	66	Caucasian, Finnish	SNP in one family
Pun et al. 1994 (3)	COL2A1	Arg519Cys	79	NA	SNP in 2 of 7 families
Loughlin et al. 1994 (4)	COL2A1, CRTL1 CRTM		76	NA	NS NS
Ushiyama et al. 1998 (5)	ESR	PvuII XbaI	383	Japanese	combined: 1.86 (1.03-3.24) 0.039
Bleasel et al. 1998 (6)	COL2A1	Arg519Cys	5 families +62	International	found three different founders
Meulenbelt et al. 2008 (7)	DIO2	rs225014 rs12885300	3430	International	combined: 1.79 (1.37-2.34) 0.00002

Table 2. Genotype studies in knee OA.

Reference	Gene	SNP	n	Ethnicity	OR (95 % CI) p / gene
Uitterlinden et al. 1997 (8)	VDR	BsmI, ApaI, TaqI	846	Caucasian, Rotterdam	haplotype 2.27 (1.46-3.52)
Keen *et al.* 1997 (9)	VDR	TaqI	351	Caucasian, Chingford	2.82 (1.16-6.85) 0.02
Uitterlinden et al. 2000 (10)	COL2A1 VDR	VNTR BsmI, ApaI, TaqI	851	Caucasian, Rotterdam	0.89 (0.34–2.33) 1.78 (1.02–3.12)
Loughlin et al. 2002 (11)	IL1A IL1B IL1RN	-889 +3954 9589	1114	Caucasian, Oxford	1.5 (1.0–2.1) 0.67 (0.5–1.0) 1.6 (1.0–2.4)
Hong et al. 2003 (12)	ACE	insertion	277	Korean	NS
Bergink et al. 2003 (13)	ESR1	haplotype (Pvu II and Xba I)	1483	Caucasian, Rotterdam	Px 1.3 (0.9-1.7) PX 2.2 (1.5-3.4)
Fytili et al. 2005 (14)	ESR1 & 2	repeat	351	Greek	$p<0.001$ & $p<0.0001$
Smith et al. 2004 (15)	IL1R1 IL1A IL1B IL1RN	haplotype	304	Caucasian, UK	2-fold risk haplotype 5-fold reduced risk haplotype
Jin et al. 2004 (16)	ESR	haplotype	548	Korean	NS

Table 2. Genotype studies in knee OA continues…

Reference	Gene	SNP	n	Ethnicity	OR (95 % CI) p / gene
Jakkula et al. 2005 (17)	COL2A1, COL9A1, COL9A2, COL9A3, COL11A1, COL11A2	72 SNPs	175	Caucasian, Finnish	linkage found
Smith et al. 2005 (18)	LRP5	haplotype	455	Caucasian, UK	1.6, 0.021
Mustafa et al. 2005 (19)	ASPN	D repeat	1995	Caucasian, UK	1.48 (1.09-2.01) 0.016
Spector et al. 2006 (20)	GWAS	25 000 SNPs	2026	Caucasian	LRCH1
Valdes et al. 2006 (21)	AACT, ADAM12, BMP2, CD36, CILP, COX2, ESR1, NCOR2, OPG, TNA, TNFAIP6, VDR	25 SNPs	1199	Caucasian, Nottingham	ADAM12 haplotype 7.1 (3.3-33.8) ESR1 haplotype 3.6 (1.18-10.98) CILP haplotype 0.38 (0.23-0.62)
Snelling et al 2007 (22)	LRCH1	intron 1	1521	Caucasian, UK	NS
Jiang et al. 2006 (23)	ASPN	D repeat	672	Chinese	2.04 (1.32-3.15) 0.0013

Table 2. Genotype studies in knee OA continues…

Reference	Gene	SNP	n	Ethnicity	OR (95 % CI) p / gene
Valdes et al. 2007 (24)	ASPN, CALM1, COL2A1, COMP, FRZB	12 SNPs	1202	Caucasian, Nottingham	FRZB 2.87, <0.04 COL2A1 0.68, <0.005 COMP <0.014 ASPN <0.02
Tsezou et al. 2008 (25)	GDF5	rs143383	519	Greek	NS
Nakamura et al. 2007 (26)	ASPN	D repeat	5446	Meta	D14:1.46, 0.003 D13: 0.84, 0.026
Park et al. 2007 (27)	CCL2	rs1024611 rs4586	423	Korean	1.45 (1.07-1.96) NS
Chapman et al 2008 (28)	GDF5	rs143383	1100 0	European and Asian	1.48, <0.0001
Poulou et al. 2008 (29)	CALM1	rs12885713	351	Caucasian, Greek	NS
Tsezou et al. 2008 (30)	KLOTHO	G395A G1110C C1818T C2298T	752	Caucasian, Greek	0.039 NS NS 0.013
Barlas et al. 2009 (31)	MMP1 MMP2 MMP9	-1607 -1306 -1562	241	Turkish	0.001 NS NS

Table 2. Genotype studies in knee OA continues…

Reference	Gene	SNP	n	Ethnicity	OR (95 % CI) p / gene
Valdes et al. 2009 (32)	DVWA GDF5	rs11718863 rs7639618 rs143383	3008	Caucasian, UK	NS NS 1.29 (1.14-1.47) $8x10^{-5}$
Meulenbelt et al. 2009 (33)	DVWA	rs7639618 rs11718863 rs9864422	4749	Caucasian. Dutch, UK, Spain, Greece	1.29 (1.15-1.45) $2.7x10^{-5}$ (global incl. Asia)
Kerna et al. 2009 (34)	ADAM12	rs3740199 rs1871054	189	Caucasian,Estonian	0.014 NS
Magana et al. 2010 (35)	CT	CA repeat	199	Mexican	2.62 (1.30-5.27)
Tawonsawatruk et al. 2009 (36)	ESR1	rs2228480	208	Thai	NS
Nakajima et al. 2010 (37)	HLA	rs7775228 rs10947262	4302	Japanese	1.34 (1.21-1.49) 1.32 (1.19-1.46)
Schneider et al. 2011 (38)	COX2/ PTGS2	rs20417	931	Caucasian, German	0.57 (0.43-0.75) 0.0001
Riancho et al. 2010 (39)	CYP19A1 ESR1	rs1062033 rs2234693	5528	Caucasian, Spain, UK	1.23, 0.04 0.76, 0.028
Valdes et al. 2010 (40)	SMAD3	rs12901499	7454	Caucasian, UK	1.22 (1.12-1.34) $7.5x10^{-6}$
Qin et al. 2010 (41)	LEP	3 Tag SNP haplotype	1396	Chinese	0.015
Shi et al. 2010 (42)	HLA	rs7775228 rs10947262	2527	Chinese, Australian	NS 1.26 (1.08-1.27) $3x10^{-8}$

Table 2. Genotype studies in knee OA continues…

Reference	Gene	SNP	n	Ethnicity	OR (95 % CI) p / gene
Meulenbelt et al 2011 (43)	DIO3	rs945006	5384	Caucasian	0.81 (0.70-0.93) 0.039
Valdes et al. 2011 (44)	GDF5	rs143383	6306	Caucasian	meta 1.17 (1.12-1.23) $6.2x10^{-11}$
Evangelou et al. 2011 (45)	GWAS meta	rs4730250	51148	Caucasian	DUS4L $9.2x10^{-9}$
Kerkhof et al. 2011 (46)	IL1B, IL1RN		14145	Meta	NS
Borgonio-Cuadra et al. 2012 (47)	ESR1	rs2234693 rs9340799	232	Mexican	haplotype 0.5 (0.3-0.9) 0.04
Jotanovic et al. 2012 (48)	IL1B IL1RN	rs16944 VNTR	733	Caucasian, Croatia	NS NS
Su et al. 2012 (49)	TLR2 TLR4 TLR9	G2408A 2 SNPs 3 SNPs	931	Chinese	NS NS -1486: 2.29 (1.39-3.75) <0.001
Valdes et al. 2011 (50)	HLA/BTNL2	rs10947262 rs7775228	42157 34660	Caucasian	NS NS
Honsawek et al. 2011 (51)	IL6	-174	215	Thai	3.3 (1.6-6.9) 0.001
Day-Williams et a. 2011 (52)	MCF2L	rs11842874	8071	1000 genomes	1.17 (1.11-1.23) $2.1x10^{-8}$
Muraki et al. 2011 (53)	VDR	Fok1, Cdx3, Apa1	787	Caucasian, UK	NS

Table 2. Genotype studies in knee OA continues…

Reference	Gene	SNP	n	Ethnicity	OR (95 % CI) p / gene
Tawonsawatruk et al. 2011 (54)	GDF5	rs143383	193	Thai	2.41 (1.02-5.67) 0.04
Lv et al. 2012 (55)	IL6 ICAM1 IL10	-634 496 -1082, -819, -592	1917	Chinese	0.017 NS NS
Hulin-Curtis et al. 2012 (56)	IL18 IL18R1	haplotype haplotype	358	Caucasian	1.36 (1.01-1.85) 0.04 NS
Bos et al. 2012 (57)	DIO2	rs225014	31	Caucasian	1.3
Valdes et al. 2012 (58)	GDF5, COG5, MCF2L	rs143383 rs4730250 rs11842874	3474	Caucasian, UK	0.05 (0.02-0.08) 0.0011 NS 0.027
Chen et al. 2012 (59)	BDKRB2	-58 +9/-9	509	Chinese	NS 2.356, <0.001
Hao et al. 2013 (60)	GDF5	+104T/C	7262	Caucasian Asian	0.72 (0.61-0.86)
Hulin-Curtis et al. 2013 (61)	NFKB1A	8 SNPs	386	Caucasian	NS
Hulin-Curtis et al. 2013 (62)	CCL2	5 SNPs	386	Caucasian	NS
Yang et al. 2013 (63)	TLR3	rs3775296 rs3775290 rs3775291 rs5743312	1417	Chinese	1.32 (1.10–1.58) 0.002 NS 0.85 (0.72–0.99) 0.041 NS

Table 2. Genotype studies in knee OA continues…

Reference	Gene	SNP	n	Ethnicity	OR (95 % CI) p / gene
Jiang et al. 2013 (64)	OPN	-156GG/G, -443C/T, -66T/G	1544	Chinese	NS 0.69 (0.58-0.90) < 0.001 0.88 (0.77-0.88) 0.011
Lee et al. 2014 (65)	ESRB	rs1256049	580	Korean	<0.0001
Ma et al. 2013 (66)	LEPR	A668G	303	Ningxia Hui	0.008
Garcia-Ibarbia et a. 2013 (67)	WNT1, NT10A, WNT16, DVL2, FZD5, BCL9, SFRP1, TCF7L1, SFRP4	87 SNPs	2062	Caucasian	Associations were found but were not able to be replicated
Panoutsopoulou et al. 2014 (68)	FTO	rs8044769	10771	Caucasian, Australian	Associated to BMI, not OA
Lepetsos et al. 2013 (69)	NADPH	C242T A640G -A930G	294	Caucasian, Greek	NS NS NS
Xing et al. 2013 (70)	ASPN	D repeat	5515	Caucasian, Asian	NS
Jazayeri et al. 2013 (71)	ASPN	D repeat	200	Iran	1.73 (1.01-2.94) 0.045

Table 2. Genotype studies in knee OA continues…

Reference	Gene	SNP	n	Ethnicity	OR (95 % CI) p / gene
Ruedel et al. 2014 (72)	CDH2	rs11083255 rs11564299 rs11083271	571	Slovak	0.48 (0.23–0.83) 0.63 (0.46–0.87) 1.44 (1.05–1.96)
Poonpet et al. 2013 (73)	ADAMTS14	rs4747096	227	Thai	2.72 (1.10-6.87)
Song et al. 20014 (74)	ASPN	D repeat	12577	European, Asian	NS
Qiu et al. 2014 (75)	SREBP2	rs2228314 rs2267443	812	Chinese	1.36 (1.36-1.70) <0.001 NS
Zhan et al. 2014 (76)	ADIPOQ	rs1501299	200	Thai	NS
Rodriguez-Fontenla et al. 2014 (77)	199 genes	27 501 SNPs	22608	Caucasian	COL11A1 rs4908291 hip VEGF rs833058 hip
Dai et al. 2014 (78)	ESR1	rs2234693 rs9340799	991	Chinese	0.025 0.031
Lepetsos et al. 2014 (79)	MMP1	rs1799750	294	Greek	0.25 (0.07-0.91) 0.035
Yang et al. 2014 (80)	FN	rs940739	1621	Chinese	1.44 (1.16-1.80)
Ai et al. 2014 (81)	IL6	rs1800795	6464	Meta	NS
Zhou et al. 2014 (82)	DOT1L	rs12982744 rs12459350	1220	Chinese	C associated NS
Liang et al. 2014 (83)	ASPN BMP5	rs13301537 rs373444	1030	Chinese	C associated NS

Table 2. Genotype studies in knee OA continues…

Reference	Gene	SNP	n	Ethnicity	OR (95 % CI) p / gene
Poornima et al. 2014 (84)	ACE	I/D	200	Indian	DD 2.14 (1.10-4.15) 0.02 D 2.08(1.39-3.10) 0.0003
Lou et al. 2014 (85)	ADAM12	rs3740199, rs1871054, rs1278279, rs1044122	331	Chinese	NS 1.80 (1.31-2.48)<0.0001 NS NS
Etokebe et al. 2015 (86)	FAM46A BAG6	VNTR rs3117582	1042	Croatian	4&7 repeats higher risk C higher risk
Yin et al. 2015 (87)	ESR1	rs9340799	8792	Meta	G 1.21 (1.03-1.43) 0.02
Kou et al. 2014 (88)	TNFA	-308	2338	Meta	11.08(4.75-25.86) <0.001
Zheru et al. 2014 (89)	PPARgamma	rs1801282, rs12629751, rs2292101, rs4135275, rs1175543	200	Chinese	NS 0.34 (0.17-0.67) 0.002 NS 0.39 (0.19-0.79) 0.01 NS
Liu et al. 2014 (90)	ESR1	XbaI PvuII	294	Chinese	1.98 (1.13-4.20) 0.036 NS

Table 3. Genetic studies in hip OA.

Reference	Gene	SNP	n	Ethnicity	OR (95 % CI), p
Aerssens et al. 1998 (91)	VDR COL1A1 COL2A1	BsmI Acc B7I PvuII	314	Belgian	NS NS NS
Forster et al. 2004 (92)	IL4R	rs3024571 rs1805013 rs1805016 + 6 SNPs	855	Caucasian, UK	1.5 (1.1-2.1) 0.03 2.0 (1.2-3.3) 0.01 2.1 (1.3-3.5) 0.004 NS
Loughlin et al. 2004 (93)	TNFAIP6 ITGA6 FRZB3	rs1046668 rs2737085 rs10209072 rs288326 rs7775	1696	Caucasian, UK	NS NS NS NS 0.04 (women)
Kawahara et al. 2005 (94)	IGFBP1 ADAMTS3 IL8	14 SNPs	675	Caucasian, UK	NS
Mototani et al. 2005 (95)	CALM1	IVs-293 C>T	94	Japanese	9.8×10^{-7}
Lian et al. 2005 (96)	COL1A1	Sp1	4746	USA	0.36 (0.13-0.99) 0.048
Pola et al. 2005 (97)	IL6	-174	182	Italy	0.4 (0.1-0.9) 0.04
Loughlin et al. 2006 (98)	CALM1	IVs-293 C>T	1672	Caucasian, UK	NS
Lian et al. 2007 (99)	ESR1	PvuII XbaI	4746	Caucasian	0.71 (0.54-0.94) 0.01 NS

Table 3. Genetic studies in hip OA continues…

Reference	Gene	SNP	n	Ethnicity	OR (95 % CI), p
Nakamura et al. 2007 (26)	ASPN	D13	5446	Meta	NS
van Meurs et al. 2009 (100)	COMT	Val158Met	3033	Caucasian, Rotterdam	hip pain in women 4.9 (1.6-14.8) 0.005
Valdes et al. 2009 (101)	ANP23A	rs7164503	5019	Caucasian	0.67 (0.53-0.84) 0.00038
Wilkins et al. 2009 (102)	BMP5	18 SNPs, microsa-tellites and INDEL	1546	Caucasian, UK	D6S1276 p=0.018 rs921126 p=0.013
Mototani et al. 2010 (103)	CALM1 CALM2 CALM3	18 SNPs	732	Japanese	CALM2 intron 1: rs10153674 p=0.036, novel SNP p= 0.031
Näkki et al. 2011 (104)	COL9A2	99 SNPs	345	Caucasian	rs7533552 p=0.0025 rs568725 p=0.002
Kolundzic et al. 2011 (105)	IL6 TGFB1	-572T>C 29T>C	48	Caucasian	6.2, p=0.024 13.4, p=0.016
Jotanovic et al. 2011 (106)	IL1B IL1RN	-511 G>A VNTR	777	Caucasian, Croatian	0.72 (0.52-0.99) 0.036 NS
Evangelou et al. 2013 (107)	DOT1L	rs12982744	41662	Caucasian	1.10 (1.06-1.14) $8.1x10^{-8}$
Garcia-Ibarbia et al. 2013 (67)	Wnt-related genes (9)	78 SNPs	2062	Caucasian, Spanish	combined 3.13 (1.34-7.28) 0.009
Evangelou et al. 2014 (108)	Meta	GWAS	78000	European	NCOA3 rs6094710 1.28 (1.18-1.39) $7.9x10^{-9}$

Table 4. Genetic studies in spinal OA.

Reference	**Gene**	**SNP**	**n**	**Ethnicity**	**OR (95 % CI), p**
Yamada et al. 2000 (109)	TGFB1	Leu10Pro	540	Japanese	2.3, 0.04
Jordan et al. 2005 (110)	VDR	BsmI	392	Caucasian, UK	0.04
Urano et al. 2007 (111)	LRP5	Q89R	357	Japanese	0.0019
Urano et al. 2007 (112)	WISP1	3'-UTR	304	Japanese	2.91 (1.34-6.30) 0.007
Urano et al. 2011 (113)	HAPLN1	rs179851	622	Japanese	2.12 (1.45-3.11) 0.0001

Appendix V references

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