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## Generalised Osteoarthritis: from Mendelian Disorder to Complex Disease

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**6****6.1 Summary and discussion of results**

Osteoarthritis (OA) is a prevalent and complex disorder with a high hereditary probability. Despite the major socioeconomic burden imposed by OA, the precise aetiology of this condition remains unclear. Previous genetic research into OA has revealed multiple positive linkage areas in genome scans and susceptibility loci for specific joint sites in association studies<sup>1,2</sup>. These studies have yielded several common gene variants contributing to several OA phenotypes. Generalised phenotypes, however, received little attention so far. Only few studies allowed an examination of multiple joint sites in the patients included. In this thesis, we have focused, on the identification and investigation of OA susceptibility of rare and common GOA in family based studies and common OA in the population. We investigated previously reported relationships between two candidate genes (*FRZB* and *MATN3*) with OA in a random sample from the population-based Rotterdam study and in siblings from the GARP study (Chapter 3). A functional variant in *FRZB* indeed associates to OA but this seems not confined to hip only (Chapter 3.1). Associations of *MATN3* variants suggest that genetic variation in this gene determines susceptibility to spinal disc degeneration (DD) and OA of the first carpometacarpal joint (CMC1) (Chapter 3.2). Different linkage areas were identified for early and late onset GOA (Chapter 4 and 5). We mapped a major locus for OA at multiple joint sites on 14q32.11 in middle aged siblings from the GARP study (Chapter 4) and on 2q33.3 in seven early onset families (Chapter 5). From our studies so far, *DIO2* (Chapter 4), *IDH1* and *NRP2* (Chapter 5) may be new OA loci.

**6.1.1 Classification of OA**

In all studies of OA, including genetic research, classification of OA remains a problem which has yet to be solved. It has become clear that OA is a heterogeneous and complex group of overlapping entities. The extent of overlap in the aetiology for these entities is unknown. There is a yet little understanding to what extent clinical heterogeneity reflect genetic heterogeneity.

Most of the current genetic studies focus on OA of one specific joint site such as hip, hand or knee to reduce the clinical heterogeneity. These studies assume that underlying genetic variation is associated only to OA at a specific joint site. This hypothesis relies on the assumption that functional effects of a variant may be highly dependent on the interplay of systemic with local factors. The interaction with local environmental factors increases OA susceptibility of a specific joint<sup>3,4</sup>. Many individuals with OA at a specific joint site are however also affected at other joint sites at different stages of development<sup>5</sup>. It remains unclear whether the involvement of multiple joint sites in time may reflect an age-related or progressive feature of the OA process or an independent systemic disease entity with specific underlying causes. In a complex disease as OA, it seems likely that each concept holds true. Selection and screening of a specific joint site may obscure distinct subtypes with different underlying aetiologies involving different genes. Genetic studies should therefore reduce clinical heterogeneity by more extensive phenotyping i.e. scoring the most prevalent joint sites for the presence of various features of OA. Phenotypes based on osteophytes for example may represent bone formation pathways whereas phenotype comprising joint space narrowing characterises pathways in cartilage loss. Genetic OA studies may profit from identical scoring systems that allow meta-analysis and comparisons between studies.

### ***6.1.2 Osteoarthritis phenotypes and true replication***

The only way to understand the heterogeneous complex nature of OA and to assess validity of reported genetic associations is to confirm these findings in several large sized studies. Genetic effects observed were often weak and most observations were not consistently reproduced with the exception of the interleukin-1 cluster and *FRZB*<sup>6-11</sup>. Other compelling findings (*ASPN* and *CALM1*) were not confirmed in studies of different ethnic origin<sup>12-15</sup>. The variation in OA definition between different studies contributed undoubtedly to the lack of replication although a considerable rate of false positive findings is also expected considering the numerous statistical tests that have been performed. Finally, OA is likely to be caused by multiple overlapping genes each with a small overall contribution and relative risk<sup>16</sup>. In this case, true replication of true findings is very difficult.

DNA collection (even by mouth swabs) and large-scale genotyping is no longer a technical problem in such studies. Our optimised mouth swab procedure is suitable for large population-based genetic studies because samples can be easily collected by participants themselves, stored for months prior to DNA extraction and results in high yield and high quality DNA appropriate for large-scale genotyping (Chapter 2).

Especially elaborate and costly is the collection of imaging data (X-ray, MRI) of different joint sites in a single patient and the objective interpretation of such data. Development of biomarkers in serum, plasma or urine samples representing systemic or joint specific OA processes would improve our studies by reducing clinical heterogeneity.

## 6.2 Confirmation of involvement of *FRZB* and *MATN3* with OA

In chapter 3, we tried to confirm previously reported associations of variants in *FRZB* (chapter 3.1) and *MATN3* (chapter 3.2) in specific joint sites and we investigated whether these loci also associated to other heritable phenotypes such as OA at multiple joint sites.

For these investigations, we used two different study approaches. First, we used case and control groups from the population-based Rotterdam study in which all subjects have been scored for the presence of ROA in four different joint sites. Such a well-designed case-control study should generally aid the detection of common alleles with low effect sizes<sup>17</sup>. In the Rotterdam sample ( $n = 809$ ), hand ROA at one or two hand joint groups (56% and 37%, respectively) or spinal DD at one level (61%) is already very prevalent and likely to be a part of the normal “wear and tear” process. We considered subjects only in the highest quartile of the sumscore of affected hand joint groups or affected spinal disc levels as affected, respectively. In this way, a generalised ROA case group was composed by individuals with two or more affected joint sites which did not rely on individuals with the combination of hand OA or spinal DD alone. Second, as case group we used siblings with symptomatic OA at multiple joint sites from the GARP study. These siblings were selected for a positive family history of OA to enrich this population for genetically predisposed individuals. Such an ascertainment scheme may increase the relative frequency and, therefore, aid the detection of rarer alleles<sup>17</sup>. The whole population-based sample ( $n = 809$ ) from the Rotterdam study was used as control group. Such a population-based control group might be more robust to examine associations than the rare group that is completely negative for OA. Together, these two studies enable a complimentary and distinct approach to investigate previously identified causal variants.

### 6.2.1 Association of *FRZB* variant with generalised OA phenotypes

The functional R324G variant within the *FRZB* gene is associated with female hip replacement. Its variant protein has diminished ability to antagonise Wnt signalling via nuclear translocation of  $\beta$ -catenin *in vitro*<sup>10</sup>. We observed two moderate associations with the G allele of R324G with generalised ROA in the Rotterdam study and with familial symptomatic OA at multiple joint sites in the GARP study. In these study populations, the variant did not associate with hip OA specifically (Chapter 3.1).

Recently, the same variant was associated with severe joint space narrowing (JSN) of the hip in women in a large US study<sup>9</sup>. In both the US and UK hip OA studies, however, the presence of OA at multiple joint sites other than hip are not available. Our result raises the question whether ascertainment of hip OA patients in these studies may have accumulated patients with OA also at other sites. The variant may be sufficient, however not restricted to the development of hip OA.

In our study, we did not observe association of *FRZB* variants with radiographic hip OA cases in the Rotterdam sample which may reflect the poor correlation between radiographic and symptomatic hip OA. The absence of association is unlikely to be due to the use of a phenotype other than joint replacement, however, Lane et al<sup>9</sup> did find association of *FRZB* variants with radiographic hip OA in women in the Study of Osteoporotic Fractures, which recorded no symptomatic data and excluded women with joint replacement.

*FRZB* encodes secreted frizzled-related protein 3 and its product may antagonise Wnt signalling in developing chondrocytes. These developing cells may maintained in a healthy and functional state in which the cells would be able to establish the position, shape and number of the skeletal elements<sup>18;19</sup>. In this state, activation of the Wnt signalling pathway may lead to terminal chondrocyte differentiation and transition from hypertrophic cartilage to bone. During embryonic development, carriers of a *FRZB* variant might have developed skeletal abnormalities leading to a disturbed integrity of the cartilage matrix which predisposes individuals to OA in later life.

Moreover, osteoarthritic cartilage expresses *FRZB* and displays signs of activation of the Wnt/ $\beta$ -catenin pathway<sup>10;20;21</sup>. Overexpression of  $\beta$ -catenin in mature chondrocytes stimulates expression of matrix degradation enzymes<sup>19</sup>. Strong Wnt signalling may therefore activate cartilage matrix catabolism and may have roles in cartilage destruction under pathological conditions<sup>19</sup>.

### 6.2.2 Association of *MATN3* variants with joint-specific OA phenotypes

The conserved T303M variant in the *MATN3* gene was previously associated to idiopathic hand OA in the Icelandic population<sup>22</sup>. We found, however, that carriers of the T allele of this variant showed increased susceptibility to have spinal DD in the Rotterdam sample (Chapter 3.2). We did not find any significant association of SNP5 with hand phenotypes in the GARP study and the Rotterdam sample.

Mutations in the genes encoding matrilin-3, COMP and collagen type IX cause a wide spectrum of chondrodysplasias<sup>23;24</sup>. Mutations in these genes cause retention and accumulation of their products within the endoplasmatic reticulum and might involve a general pathogenic mechanism for chondrodysplasias<sup>23;25;26</sup>. In contrast, the T303M has been synthesised, processed, secreted and deposited in a way indistinguishable from the wild type matrilin-3<sup>25</sup>. OA-associated variants with small effect sizes, such as T303M, may therefore result in only mild structural and functional changes and may cause OA by other biological mechanisms. Variants may impair interactions with other matrix proteins which affect extracellular matrix assembly causing a disturbed cartilage metabolism. Investigation of genetic variation in binding sites of matrilin-3 domains would be of great interest<sup>27</sup>.

In addition, SNP6 and the corresponding haplotype of the *MATN3* gene indicated a small but specific effect of CMC1 OA in GARP patients (Chapter 3.2) and might represent a different subset of generalised OA patients. A small but insignificant effect for this haplotype was also observed in the Icelandic study, but it is unknown whether these carriers also have OA at other joint sites<sup>22</sup>. The causal variant itself might be difficult to identify since the risk haplotype was not further delineated with SNPs from the HapMap or the detected SNPs of the Icelandic study<sup>22</sup>. Extensive resequencing of the intronic regions might be required to determine the total population risk attributable to matrilin-3 variants.

The stability of the cartilage extracellular matrix (ECM) and normal tissue function critically depend on strong interactions between the collagen and aggrecan network. Matrilins and cartilage oligomeric matrix protein (COMP) mediate these interactions suggesting a function as adaptor proteins within the ECM<sup>27</sup>. The expression of matrilin-3 is enhanced in knee osteoarthritic cartilage with a strong correlation between enhanced protein expression and the extent of tissue damage<sup>28;29</sup>. This might be a response of the chondrocyte to an underlying process of OA.

### 6.2.3 Study limitations

Our studies confirmed two previously reported joint specific associations with other OA related phenotypes. As expected for a complex disease, the effect sizes were small for the genetic variants studied. Our studies concerning *FRZB* and *MATN3* did not allow for true replication of the previous studies due to the lack of information on symptomatic OA characteristics in the Rotterdam study and/or the selection criteria for OA at multiple joint sites in the GARP study.

Furthermore, confirmation of previously reported findings should be rather gene based, covering total genetic variation of a gene than allele based taking into account LD differences<sup>30;31</sup>. For our *FRZB* investigations, only variants with possible biologic plausibility were tested. For the *MATN3* gene, the risk allele of SNP6 was not delineated with tagging SNPs available from the HapMap. Using the selected SNPs, we covered one out of three blocks for *FRZB* and one out of two blocks for *MATN3* based on the HapMap data. As previously demonstrated with the findings of the interleukin-1 cluster<sup>6;8</sup>, the accuracy of these associations may be enhanced with extended haplotype analysis.

Another limitation of our studies is that two distinct studies have been used which may therefore be susceptible for population stratification. Population stratification occurs when both allele frequencies and disease prevalence differ between cases and controls as a result of undetected population structure<sup>32</sup>. Although the Rotterdam study is a distinct study, it is not likely that population stratification occurs: both studies consist of Caucasian participants of Dutch ancestry with a mean age of 60.3 years and show similar allele frequencies. However, the GARP study contains a significant higher frequency of women as compared to the Rotterdam sample and therefore we stratified for women only.

## 6.3 Genome-wide scan in Dutch sibling pairs from the GARP study

In the OA affected siblings (the GARP study), a major locus on chromosome 14q32.11 was identified that was mainly attributable to OA at multiple joint sites (chapter 4). The location of the linkage peak coincides with three candidate genes: *CALM1* previously associated with hip OA<sup>15</sup>, *FLRT2* encoding a fibronectin fragment which is structural component of cartilage, and *DIO2* which regulates thyroid hormone metabolism during skeletal development<sup>33</sup>. Analysis of these three loci using tagging SNPs taken from the HapMap and the previously associated functional *CALM1* variant (rs12885713) revealed two findings: first, the observed linkage signal could

not be explained by SNPs in the *CALM1* or *FLRT2* gene. Second, in a combined linkage and association analysis, the C allele of rs225014, the C allele of rs12885300 in the *DIO2* gene and the corresponding haplotype, were associated with OA at multiple joint sites. In addition, siblings sharing two alleles identical by descent showed again a significant increased frequency of the C allele of rs225014 and the corresponding haplotype as compared to the Rotterdam sample. Together, these approaches provided the first modest evidence for the *DIO2* gene to be involved in OA susceptibility at multiple joint sites.

The gene *DIO2* encodes, iodothyronine deiodinase type 2 (D2). This enzyme is important in the regulation of local thyroid hormone bioactivity i.e. the availability of T3 in the growth plate<sup>33</sup>. Thyroid hormone itself has been found to play an essential role in stimulation of chondrocyte differentiation and maturation that is required for bone formation<sup>33-35</sup>. Previously, the rare T allele of rs12885300 but not the C allele of rs225014 showed an association with decreased plasma levels of iodothyronine or thyroid hormone ratios indicating a higher activity of D2<sup>36;37</sup>. It is therefore not likely that the C allele of rs225014 itself is functional<sup>37</sup>. Remarkably, carriers of the risk haplotype in our study, encompassing the common allele of rs12885300 showed an association with OA at multiple joint sites. Therefore, lower D2 activity might be associated with OA at multiple joint sites resembling lower *DIO2* expression in chicken with chondrodysplasia<sup>38</sup>. Confirmation of the involvement of this gene in other OA populations and elucidating of its function in relation to OA is required to obtain further evidence.

Notably, a large fraction of our siblings (32%) contributed to the linkage at 14q32.11 and 23% of those siblings were carrier of at least one risk allele in *DIO2*. However, this allele (35%) and its corresponding haplotype (32%) were very common in the random population. Most likely, this allele may be in LD with another causal variant with a lower frequency.

More genetic variation in the *DIO2* gene but also in other genes at this locus may attribute to OA at multiple joint sites. An attractive candidate gene at this locus for future study is the thyroid stimulating hormone receptor (*TSHR*). TSH stimulates the secretion of thyroid hormone and also regulates the turnover of bone<sup>39</sup>. Other candidate genes in this region are *PTPN21*, *TTC7B* and *K6A5* which might play a role in respectively, chondrocyte differentiation, proteolysis and TGF $\beta$  signalling.

Tagging SNPs and the functional promoter SNP within the *CALM1* gene discovered in a Japanese population did not explain the linkage signal. Lack of association of the functional promoter SNP was also observed for hip replacement in the UK population<sup>40</sup>. Ethnic specific differences in environmental or genetic risk factors might account for these differences between Japanese and Caucasian populations.



We conclude that we have no evidence that the promoter SNP marks a major risk allele for GOA in our population. We cannot exclude, however, that allelic variation of rare alleles at *CALM1* contribute to the linkage at the 14q32.11 locus. Extensive resequencing in this region is probably required to explain the linkage signal.

This genome scan revealed suggestive evidence for linkage which result did not reach genome-wide significance. The lack of significant linkage signals could be explained by the limited statistical power for mapping a complex trait with 183 informative sibling pairs. A more powerful analysis might be provided by genotyping available additional siblings or a dense map of SNPs in the linkage region. In this way, maximal genetic information for estimating the IBD probabilities, which is restricted in sibling pairs, is extracted<sup>41-43</sup>. These days, it is recognised that a 5 cM sparsed scan by microsatellites or a few hundred thousand well-chosen SNPs should be optimal<sup>43,44</sup>.

We did not observe any positive linkage signal at 2q33.3, the locus we identified in families with Mendelian inheritance and early onset (chapter 5.1). This observation suggests that the locus at 2q33.3 is a major, most likely rare locus with a large effect. The functional allele of the *FRZB* variant, investigated in chapter 3.1, was associated with generalised phenotypes in both the population-based Rotterdam sample and the GARP sibling pairs. However, no evidence for linkage was observed at the *FRZB* or *MATN3* loci illustrating the differences in abilities of linkage and association studies to detect genetic variation with a small effect size. The genetic component and causal genes may be easier to be detected in relatively severe phenotypes such as generalised OA in the GARP study or hip OA with hip replacements but might be not representative for the population at large<sup>45</sup>.

## **6.4 Genome-wide scan in seven families with familial early onset OA**

### ***6.4.1 Linkage analysis***

Rare Mendelian OA occurring in families may be caused by rare, highly penetrant mutations with severe impact and early ages of onset. Although impressive progress has been made for chondrodysplasias, a fundamental understanding of the biological cause of Mendelian OA without dysplasia is still lacking, warranting further research in this area. In a genome-wide scan of seven families with familial early onset OA (FOA) without dysplasia, we mapped a locus on 2q33.3 with a maximum LOD score of 6.05 (Chapter 4.1). This locus is not overlapping with previous mapped loci of either OA or chondrodysplasia genome searches<sup>1;24;46;47</sup>.

Research in families with chondrodysplasia have demonstrated that locus, allelic and clinical heterogeneity frequently occur<sup>23;24</sup>. These effects hamper the possibilities of obtaining robust evidence for linkage. In our analysis, six out of seven FOA families showed maximal positive linkage signals at 2q33.3 and no other loci emerged suggesting locus heterogeneity for only one family. However, linkage analysis was performed in earlier days of linkage analysis with a relatively sparsely spaced scan (18 cM) and therefore additional loci could have been missed. An analysis of haplotypes in the FOA families contributing to the linkage showed allelic heterogeneity: different families had different haplotypes at which the disease mutation is expected. The ultimate evidence for which gene in this area is the causal gene may come from identification of significant mutations in a single gene segregating in these unrelated but clinically similar families<sup>48;49</sup>.

#### 6.4.2 Mutation analysis of the 2q33 linkage region

The minimal positional gene area, defined by recombinant haplotypes in the families that contributed most to the linkage, harbours 27 candidate genes. Initially, two candidate genes (*FZD5* and *PTHR2*) were investigated based on the biological and physiological relevance of their products to OA. *FZD5* encodes the frizzled 5 receptor which has relevant activity in Wnt signalling<sup>9-11</sup>. *PTHR2* encodes parathyroid hormone receptor 2 which might be involved in calmodulin (CaM) mediated signalling<sup>15;50</sup>. Mutations in the *PTHR1* gene, strong homologue to *PTHR2*, cause chondrodysplasia<sup>51;52</sup>. Although a nonsynonymous *PTHR2* variant, was weakly associated with generalised ROA in the population, it is unlikely, based on the *in silico* analysis, that this variant is deleterious. Even a nonsense variant abolishing the receptor function at one allele does not lead to OA since we found such a variant in unaffected family members. Since loss of this receptor by a nonsense variant might be compensated by another gene or the other allele, a potential OA causing mutation might have a dominant negative effect on the protein which results in for example diminishing interactions or altering of binding sites rather than influencing gene expression.

The enormous wealth of biological databases makes prioritising candidate genes extremely hard often resulting in a possible biological hypothesis for each gene. Generally, a systemic mutation approach of all positional candidates may be the only proper way to find causal variants when linkage has been found and finemapped. Such an approach is, however, time consuming and expensive. Including predicted genes as positional candidates, even if the biological function is unknown is very important as illustrated by the unknown *LRCH1* gene recently associated in several populations with knee OA<sup>53</sup>. We performed a mutation analysis of the coding and untranslated regions of 20 out of 27 positional candidate genes and the *FRZB* as a

biological candidate located at the periphery of the linkage peak (Chapter 5.2). Our analyses revealed nine novel cosegregating variants in six different genes: three missense variants, three UTR variants and three intronic variants. Although some potential OA causing variants were found, we could not detect mutations in a single gene in the different families contributing to the linkage.

The *IDH1* Y183C variant, cosegregating in one family, was the most promising one since it showed predicted functional impact on the structure of the protein *in silico*. In addition, carriers of this variant in the general population showed an increased but insignificant risk to have GOA. A second intronic variant located in *NRP2*, might be also involved in OA susceptibility. Despite the functional effect of this variant is unknown, it cosegregated in three families and was weakly associated with generalised ROA in the population. The relation between *IDH1* and OA is speculative; it may make chondrocytes more susceptible to cell death which might contribute to the onset of OA<sup>54;55</sup>. *NRP2*, encoding the co-receptor of vascular endothelial growth factor<sub>165</sub> (VEGF<sub>165</sub>) is expected to play a role in cartilage degradation<sup>56;57</sup> and endochondral ossification<sup>58;59</sup>.

Both variants can only be causal when reduced penetrance is allowed in the segregation pattern which may indicate that additional genetic factors or modifier genes may be involved. Identifying such modifier genes may be similar as identifying multiple causal alleles in non Mendelian diseases for which no large effect exist<sup>48</sup>. The variants found in the *NRP2* gene may be neutral polymorphisms or in LD with a causal variant that may even be present in a neighbouring gene. Such causal variants, especially when located in regulatory elements, may have escaped in our search for mutations. Functional and tissue expression studies are necessary to prove that any of the variants are causal to OA. The weak association of the *NRP2* variant in the general population and the cosegregation of this variant in three families with FOA might indicate that variation in this gene contributes to common OA in the population which should be further investigated.

For future studies, at least two issues need to be taken into account. First, in two families, two haplotypes segregated with OA instead of one allowing one or two phenocopies. Allowing variable penetrance makes exclusion of novel variants that do not segregate with OA difficult. As illustrated in our study, of the ten novel variants found, only two variants segregated on the most likely haplotypes assuming dominant transmittance. Adding more distant related family members in the linkage analysis could give more insights in the segregation pattern and the penetrance, although this could also introduce more genetic heterogeneity.

Another important consideration is that The Human Genome Mutation Database (April 2005) comprises approximately 51,000 mutations (<http://www.hgmd.cf.ac.uk/>) predominately missense/nonsense mutations (58%), small insertions/deletions (24%), splicing mutations (10%), gross deletions/duplications/insertions/rearrangements (7%) and regulatory mutations (1%). These data provide support that Mendelian phenotypes are associated primarily with changes in the coding sequences whereas regulatory changes are underrepresented because these mutations are difficult to identify<sup>48</sup>. Mutation screening of the remaining coding regions and detection of insertions/deletions may therefore have more priority for a follow-up study than a search for regulatory changes. Detection of apparent Mendelian inconsistencies or comparative genome hybridisation may reveal possible (heterozygous) deletions.

## 6.5 Aetiology of osteoarthritis

OA results from the repeated failure of the tissues to respond adequately to injury or mechanical stress. In OA, a loss of homeostasis in the anabolic and catabolic activity of joint tissue in combination with decreased matrix integrity leads to the pathological degeneration of articular cartilage in OA<sup>60,61</sup>.

This thesis underscores the importance of genes and pathways that play a role in development and maintenance of joint tissue and this thesis highlights the relevance of genes such as *FRZB* and possibly *DIO2* encoding products that play a role in endochondral ossification which is essential for skeletal development<sup>18,19,33-35,62</sup>. During endochondral ossification, development of long bones occurs from the cartilage anlagen. This process involves terminal differentiation of chondrocytes to the hypertrophic phenotype, cartilage matrix calcification, vascular invasion and ossification<sup>63</sup>. Interestingly, *NRP2* encodes the co-receptor of VEGF<sub>165</sub> which is an essential coordinator during the process of endochondral ossification by modulating ECM remodelling, angiogenesis and bone formation<sup>58,64</sup>.

In osteoarthritic cartilage, chondrocytes attempt to repair the damaged matrix and activate their anabolic activity by increasing gene expression. Finally, chondrocytes undergo phenotypic dedifferentiation implicating an overall severely altered gene expression profile. Indeed, when OA progresses chondrocyte proliferation, chondrocyte hypertrophy, and chondrocyte activation occur. These regenerative features are similar to those seen in the hypertrophic zone of growth plate cartilage during endochondral ossification<sup>65</sup>. In addition, OA is characterised by osteophyte formation which also shows similar features as those observed in endochondral ossification<sup>66</sup>.

Polymorphisms in genes such as *FRZB*, *NRP2* and *DIO2* might result in mild skeletal abnormalities during embryonic development, leading to decreased cartilage matrix integrity and predispose to OA in later life. Therefore, these variants might play a role in initiation of OA. In contrast, frizzled-related protein and VEGF and its receptors are both expressed in OA cartilage which is not known for *DIO2* yet, although it is synthesised by chondrocytes<sup>10;20;21;33;56</sup>. During the OA process, these variant proteins may have a detrimental effect especially when the chondrocytes are dedifferentiated.

These genes might therefore also be involved in advanced OA processes such as in attempts to repair by the chondrocytes and osteophyte formation. Although both hypotheses (involvement in initiation or progression of OA) might be true, the latter seems more likely since the observed associations with *FRZB* and *DIO2* are found in populations with severe OA (Chapter 3.1 and 4). The involvement of the *FRZB* polymorphisms is confirmed in several studies yet<sup>9;10</sup>. Involvement of *DIO2*, *NRP2* and their pathways should be further investigated in other OA populations.

## 6.6 Future perspectives

OA is a major cause of disability in elderly and has a substantial economic impact. To date, there is an urgent need to improve the treatment of OA. The currently available treatments for OA focus on symptom relief and improving function and has been based on analgesics and nonsteroidal anti-inflammatory drugs or arthroplasty<sup>67;68</sup>. However, there is a lack of an effective disease modifying treatment in which degradation of joint tissue is stabilised, retarded or halted and at the same time an improvement of symptoms is reached. A main problem in the development of drugs is that the biological changes that occur in all OA joint tissues are extremely diverse and vary with the cause and the phase of disease<sup>68;69</sup>. Results of genetic studies will contribute to the clarification of the complex genetic background of OA. Furthermore, identification of genetic variation associated with OA will improve our understanding of the pathogenesis and may elucidate new molecular targets for the development of new treatment.

The major problem in genetic OA studies seems the classification of appropriate phenotypes in sufficiently large and comparable studies. Although a careful selection of the GOA phenotype has been made in our studies, still each person has a variable expression represented by varying combination of joint sites affected at varying severities. During the OA process multiple joints may become affected in time, even

as an age-related background in patients with joint specific (local) disease (such as early hip OA and replacement). Genetic causes of OA may lead to a systemic propensity to OA development at a higher rate than the average age-related increase of OA incidence in all joint sites<sup>70</sup>.

In most of the genetic OA studies, including ours, OA is defined as a qualitative trait. If systemic factors play an active role in OA, the disease may be regarded as a quantitative trait which is only possible when OA status has been assessed in multiple joints. Quantitative phenotypes with high heritabilities may divide OA patients into distinct classes of entities and may be more powerful<sup>65;71</sup>. Possible quantitative traits that may be used are: a proportionate sumscore of the number of affected joints as a severity measure<sup>72;73</sup> or factor scores representing subsets of patients based on clusters of joints<sup>74</sup>. Different quantitative measures may highlight distinct biological pathways. Specific heritable clusters may enhance genetic effects whereas sumscores may enhance a systemic effect. A prerequisite, to apply quantitative measures is to continue collecting carefully graded data and improve current scoring methods reflecting the different changes occurring within the joint. Alternatively, clustering of phenotypic data based on heritable traits as genetic expression profiles could also provide new subsets of OA<sup>75;76</sup>. In addition, a selection of siblings either concordant for high or low trait values or extremely discordant for high or low level trait values will be even more powerful in linkage analysis<sup>49</sup>.

The identification of clinical measures that monitors the structural integrity of the joint and its changes during OA is therefore of key importance<sup>77</sup>. A biomarker, however, that is useful for early OA detection, that reflects the course of joint destruction, and that predicts long-term outcome is currently lacking<sup>77</sup>. Development of classical molecular biomarkers based on the presence of bone or cartilage turnover products in serum, urine or joint fluid are thus far not sufficiently sensitive or specific<sup>68</sup>. However, combining several biomarkers has been shown to be more discriminative<sup>77</sup>. In addition, the recent developments in the field of profiling metabolites and proteins by nuclear magnetic resonance spectrometry and mass spectrometry have provided promising tools to identify biomarker profiles that could distinguish in early stages unaffected from OA affected individuals<sup>78;79</sup>. Ultimately, this information will lead to early and more accurate prediction and diagnosis of the disease and differentiation of subsets of OA<sup>80</sup>.

Furthermore, some data shows that the rate of progression varies between individuals. However, relatively little is known about risk factors for progression<sup>81</sup>. Future research should focus on the identification of those patients that will have a rapid progression of disease and those who do not or slowly progress. If a strong

genetic basis exists for progression, elucidating the relevant genetic factors may help us understanding the aetiology of affection of multiple joint sites or detect early OA markers. Patients that progress rapidly may be a more suitable and informative population in clinical trials for disease modifying drugs to inhibit or stop joint destruction<sup>82</sup>.

Despite the current developments of novel genetic tools, identification of a gene for OA susceptibility has remained a significant challenge. Ultimately, a complete description of OA will require finding all variants, common and rare, and understanding their interactions with one another, with environmental exposures<sup>83</sup>. Identification of genetic risk factors in combination with other genomic tools may improve therapeutic efficiency and identification of new drugs targets. Furthermore, genetic variants could be used to identify individuals that are at high risk of OA and together with appropriate management their risk might be reduced<sup>80</sup>. It is exciting to live in a time when the necessary tools are becoming available and genes related to OA emerge.

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