CHAPTER 10

SUMMARY

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Juvenile idiopathic arthritis (JIA) is a non-common disease in children that can persist into adulthood. JIA consist of a heterogeneous group of disorders characterized by chronic arthritis. Following the ILAR criteria several subtypes can be distinguished based on the number of joints that are affected at presentation and during the course of disease, and on some blood values. The JIA subtypes oligoarthritis (persistent and extended) and rheumatoid factor (RF) negative polyarthritis are the most common within the group of JIA. These subtypes share phenotypic features and have a similar course of disease. In all subtypes the course of disease follows an unpredictable pattern of episodes with active disease alternating with episode of inactive disease. Prolonged episodes of active disease will lead to (irreversible) damage of the joints. At this point, no reliable prediction can be made about the course that the disease will follow in the individual patient.

The goal of treatment is to bring the ongoing arthritis to a halt shortly after disease onset to avoid damage to the joints. In recent years the treatment of JIA has shifted towards a more aggressive treatment with the use of potent anti-rheumatic medication in earlier stages of the disease. The level of response to medication differs between patients. No reliable prediction regarding the response to medication can be made, although this would be essential for choosing the optimal treatment for the individual patient.

JIA is a complex disease

JIA is considered to be an auto-immune disease, although the precise pathophysiology is not clear. The immune reaction against an unknown agent causes an ongoing inflammation of the joint, leading towards destruction of cartilage and bone. Several factors are believed to be involved in the pathogenesis of JIA. Besides environmental factors, also genetic factors seem to be important. Twin and family studies show that siblings of patients with JIA have a higher risk of developing (the same type of) JIA. In recent years several genetic studies have been performed trying to reveal an association between genetic variants (for example single nucleotide polymorphisms (SNPs)) and JIA by comparing the frequency of the variant in patient to the frequency in healthy persons. If the frequency of the genetic variant varies significantly between patients and controls, the genetic variant is considered associated with the disease. Replication of an association in an independent JIA cohort makes an association stronger and is essential. Multiple associations have been replicated in independent JIA cohorts, like HLA, PTPN22, STAT4, TNFAIP3, IL2RA. It is estimated that 30% of the JIA risk can be explained by (common) genetic variations.

Aim of this thesis

The goal of this research project is to discover genetic associations with JIA. Genetic factors that are associated with the development of JIA can give more insight in the pathogenesis and might indicate lead points for new treatment strategies. Of more clinical relevance are the genetic factors that are associated with the course of disease or with the response to certain medication. These genetic associations might serve as a predictive tool, leading to a better understanding of the individual disease and more optimal individualized treatment.

In order to perform genetic association studies, a new and independent JIA cohort has been created including 639 patients from North-West Europe (the Netherlands, Belgium, Germany and Switzerland) of Caucasian origin. The focus has been on patients with oligoarthritis (persistent and extended) and RF-negative polyarthritis, because of their similar clinical phenotype. Several genetic polymorphisms (SNPs) have been studied in this JIA cohort. The frequencies of SNPs have been compared between JIA patients and healthy Caucasian (Dutch) individuals in order to discover association with the susceptibility to JIA.

Additionally, detailed clinical information regarding the progression of disease and the use of medication has been collected for studying the association of genetic polymorphisms with the course of disease or the response to medication. The percentage of active disease in the first two years has been selected as outcome measure regarding course of disease. Patient with a mild (remitting) disease (< 35% of the time with active disease in the first two years) were compared to patients with an intermediate (35-65% active disease) and with a severe (non-remitting) disease (>65% active disease) to study association with the course of disease. Genetic association with response to methotrexate (MTX) was studied by comparing the frequency of selected genetic polymorphisms between MTX responders and MTX non-responders. The response to MTX was based on the improvement of the (subjective) physician score combined with the decrease in number of affected joints after 6 months of treatment.

For these genetic studies multiple genes and loci have been selected. Genes/loci have been selected based on a known association with JIA in order to replicate this association in a new and independent cohort. Also genes/loci have been selected that have been associated with other autoimmune diseases, especially with rheumatoid arthritis that shares many features with JIA, in order to strengthen the hypothesis of a shared common auto-immune susceptibility. Furthermore, genes have been selected that are involved in immune-regulation following an educated guess.

Genetic associations with the susceptibility to JIA

In this cohort we have discovered new associations in JIA in the genes/loci TRAF1/C5 (chapter 2), 4q27 (chapter 3), CD226 and CD28 (chapter 4). These genes have already been associated with other auto-immune diseases and might be part of a shared common auto-immune susceptibility. Also known JIA associations were replicated in our JIA cohort; VTCN1, 4q27, TNFA, PTPN22 and ANKRD55. By performing a meta-analysis involving our results and already published results, additional positive (overall) associations were revealed with PTPRC, AFF3, CCR5, TNFAIP3, TNPO3, IL2RA and CLEC16A.

TRAF1/C5 (chapter 2)

The genetic locus TRAF1/C5 includes both genes encoding TRAF-1 (TNF-receptor-associated factor-1) and complement 5 (C5). Genetic information of this locus is transmitted in total to the next generation without recombination with its sister chromosome. This phenomenon is called linkage disequilibrium (LD). The positive association of JIA with this locus could reflect an association with TRAF1 as well as with C5 (or non-coding DNA). Both genes are likely to play a role in the pathogenesis of JIA. TRAF-1 is part of the TNF-pathway that seems to be of high importance in JIA, considering the effect of anti-TNF treatment in JIA. Complement 5 has a role in attracting neutrophils and dysregulation could be involved in the development of JIA. Following our result, the association of TRAF-1/C5 and JIA has been studied in other cohorts with conflicting results. Additional (more detailed) research of this locus is needed.

4q27 (chapter 3)

The 4q27 locus has already been associated with multiple auto-immune diseases, but for the first time an association with JIA has been discovered in our cohort. The 4q27 locus contains several genes of which two genes are highly likely to be involved in the pathogenesis of JIA. These genes encode interleukin 2 (IL2) and interleukin 21 (IL21) and are inherited together (LD). Interleukin 2 is involved in the proliferation and differentiation of T-cells and in particular the regulatory T-cells. These regulatory T-cells have a role in regulating or inhibiting the inflammatory response and dysfunction can lead to an excessive (auto)immune-response. Genetic variation in the gene coding the IL2-receptor (*IL2RA*) is associated with multiple autoimmune diseases, including JIA, underlining the importance of the IL2 pathway in autoimmunity. IL21 is involved in the differentiation and expansion of T-helper-17 cells. These T-helper-17 cells seem to play a central role in JIA and have a pro-inflammatory effect. In several autoimmune diseases the association with IL21-receptor has been described. In recent years the association of 4q27 and JIA has been replicated in several cohorts.

*CD226 (DNAM-1)(*chapter 4*)*

For the first time an association of CD226 and JIA is described in chapter 6. However CD226 has already been associated with other auto-immune diseases. CD226 (known as DNAX accessory molecule 1 or DNAM-1) is involved in the co-stimulation of T-cells and NK cells. Therefore, CD226 could have a role in the regulation of the balance between the pro- and anti-inflammatory immune response. In other JIA cohorts this association has been studied, but no association was found. However, performing a meta-analysis with our results and all published data, an overall positive association is revealed.

Genetic association with the course of JIA (chapter 5 and 6)

For the first time genetic association with the course of disease, defined by the percentage of active disease in the first two years, has been studied in this cohort. The same polymorphisms that were selected for studying the association with the susceptibility to JIA were used for discovering association with the course of disease. Detailed clinical data necessary for determining the clinical course of disease were available for 272 patient with oligoarthritis (persistent and extended) and RF-negative polyarthritis (out of the 639 patient). First the predictive value of several clinical parameters was determined (chapter 5). This showed that the JIA subtypes have a different course of disease; patients with persistent oligoarthritis have a favorable course with a low percentage of active disease in the first two years, whereas patients with an extended oligoarthritis have the highest percentage of active disease. Because the percentage of active disease in the first 2 years corresponded with the percentage of active disease in the three following years, this parameter was regarded a suitable outcome measure reflecting course of disease.

When analyzing the association with course of disease, both clinical parameters (subtype) and genetic factors were included. Multivariate analysis showed that VTCN1 (rs10923223) is associated with the course of disease, regardless of JIA subtype (chapter 6).

VTCN1 has already been associated with susceptibility to JIA and other auto-immune diseases (with varying results), but seems to have a prominent role in the progression of the inflammatory response in JIA. *VTCN1* encodes B7-H4 that is involved in the co-stimulation of T-cells and inhibits the immune-response. Our data show that the minor allele is associated with a lower percentage of active disease in the first two years (remitting disease). This allele might enhance the function of VTCN1 leading towards a stronger inhibition of the immune response. Additional studies concerning the functional consequence of this VTCN1 variation will be essential. However until the precise role of VTCN1 will be clarified, the genetic association might be of

use in predicting the course of disease and helping the physician in choosing the optimal treatment strategy. Further prospective study should be conducted to verify the predictive value of VTCN1.

Genetic association and the response to methotrexate (chapter 7)

Both clinical and genetic factors have been studied in relation to the response to MTX in a cohort of 55 MTX non-responders and 73 MTX-responders. Several polymorphisms in genes involved in the folate and adenosine pathway have been selected. A multivariate analysis showed that the time to start MTX (the time from diagnosis to start MTX) is associated with response to MTX; treatment in more early stages of the disease has a better response. No association with genetic factors was found. Because of the small group size the lack of association with genetic factors might be due to low power to detect smaller effects.

These first genetic studies in this new (Caucasian) JIA cohort, including patients with similar phenotypic features, have led to discovery of new association (TRAF1/C5, 4q27, CD226 and CD28) and replication of known associations. Of clinical importance is the association of VTCN1 with the course of disease that might already be useful as factor predicting the course of disease and guiding the physician in the choice of treatment. Future plans following the results of this thesis will be to perform functional studies to elucidate the role of the VTCN1 polymorphism and its inhibitory effect on the immune-response. Possibly, this might have therapeutic consequences. Furthermore the size of the cohort should be enlarged to enhance the power of our research. Especially increasing the cohort of patients with a known detailed course of disease would enable discovery of clinical useful associations.