Selected genetic factors contributing to susceptibility to multiple sclerosis as well as to disease outcome

Multiple sclerosis (MS) is a chronic disease of the central nervous system leading to demyelination and axonal damage in the brain and the spinal cord\(^1\). It is estimated that the disease affects around 2.5 million people worldwide. In Poland over 40 000 people suffer from MS. Multiple sclerosis most commonly starts in young adults, between 20 and 40 years of age, and women are affected twice as often as men, which is characteristic of many autoimmune diseases\(^2\).

The pathomechanism of MS is complex and its course and progression can be various. Most patients, about 85%, experience relapsing–remitting (RR) course of the disease characterized by relapse followed by recovery period. Only approximately 20% of these patients have benign form of MS and remain clinically stable within 15-20 years after the onset of the disease, while most of them evolve into a secondary progressive (SP) phase characterized by a steady increase in disability within 25 years. About 10-15% of patients experience primary progressive (PP) form defined by the accumulation of disability from the onset of the disease\(^3\). Expanded disability status scale (EDSS) is most often used to rate the multiple sclerosis progression\(^4\). However, multiple sclerosis severity scale (MSSS), which takes into account disease duration, is also used\(^5\).

Having taken into consideration high incidence of MS, early age of onset and usually progressive course of the disease, multiple sclerosis seems to be not only the medical but also the social problem. Therefore studies aimed to explain still not well establish etiology and pathogenesis of MS are of great importance.

Susceptibility to MS is determined by a combination of environmental and genetic factors. In the 1970s, an association of 6p21 region (containing \textit{HLA} genes) with MS was revealed and for almost three decades it comprised the only indisputable susceptibility region. In recent years, genome wide association studies (GWAS) have identified a large number of new genetic risk \textit{loci} for MS\(^6\). However, it is important to note that SNPs analyzed in GWAS may not necessarily represent the causal disease variants themselves, but they may merely be markers for them. Thus, the results of GWAS need to be confirmed and investigated in independent studies. Associations of some of numerous \textit{loci}, selected in GWAS (\textit{IL7R},...
IL2RA, CLEC16A, CD58, EVI5, TYK2) have been already verified in independent populations but most of them need to be replicated. In this project two independent approaches, i.e. GWAS and the candidate gene study, which are used to identify the genetic risk factors, were combined by incorporation of prior knowledge of pathways involved in the pathogenesis of the disease into published GWAS data. Taking the above into consideration, the following genes encoding molecules associated with regulation of T and B cells activation were chosen:

- ALCAM, CD6,
- CD28, CTLA-4, CD80, CD86,
- CD40 and CD40L.

24 polymorphisms in the above-mentioned genes were investigated in the first stage of this study. SNPs were selected on the basis of in silico analysis, and their association with MS risk and progression of the disease was studied. Furthermore, the interactions between polymorphic variants and their influence on the MS susceptibility and the course of the disease were examined. As part of the project, the presence of HLA-DRB1*15:01 allele was analyzed and stratification for this known MS risk factor was conducted. The first stage of this project was performed on a group of 336 MS patients and 322 healthy individuals. (On the basis of) Based on the conducted study, ALCAM, CD6, CD40 and CD40L genes were selected for further analysis. For these genes, mRNA levels in blood samples from 39 RR MS patients and 40 healthy controls were compared. What is more, the correlation between genotypes at studied polymorphic sites of selected genes and their mRNA expression was analyzed.

**ALCAM, CD6**

One of the putative susceptibility regions for MS identified by GWAS was 3q13, containing, inter alia, the gene for activated leukocyte cell adhesion molecule (ALCAM). ALCAM, by interacting with its receptor CD6, plays an important role in the regulation of T cell activation. Moreover, it was shown, that ALCAM-CD6 interactions are involved in the transmigration of leukocytes across the blood-brain barrier. In this project six ALCAM polymorphisms (rs6437585C>T, rs1044240A>G, rs579565G>A, rs1044243C>T, rs34926152G>T, rs11559013G>A) and two CD6 SNPs (rs17824933C>G, rs12360861G>A) were investigated and their possible association with MS risk and progression was analyzed.
The results of this study revealed that rs6437585CT individuals (homozygotes for minor allele were not identified) had about 1.7 higher risk of MS, lower values of MSSS and earlier age of onset in comparison to CC individuals. The mean value of age of onset was also lower for carriers of A allele in rs579565G>A. Due to the interaction between these variants, their effects were not additive.

In this study, similarly to the data from other European populations, the carriers of HLA-DRB1*15:01 allele had almost 3-fold higher risk of MS in comparison to HLA-DRB1*15:01 individuals. However, two ALCAM polymorphisms were able to modify this effect. HLA-DRB1*15:01 positive individuals who were heterozygotes in rs34926152G>T or rs11559013G>A (homozygotes for minor allele were not identified) did not have higher risk of MS caused by the presence of HLA-DRB1*15:01 allele.

The current study demonstrated also that SNP rs12360861G>A in CD6 gene was associated with susceptibility to MS. In details, the rs12360861AA individuals (the recessive model) had lower risk of MS in comparison to GG homozygotes. The other examined polymorphism of CD6 gene - rs17824933C>G, although not associated with susceptibility to the disease, was able to modify its progression. The results showed that carriers of G allele at this polymorphic site had lower values on the scales of disability – EDSS and MSSS than CC individuals.

Next, the level of mRNA expression of ALCAM and CD6 in MS patients and healthy individuals was determined. The results indicated no difference in the ALCAM mRNA level. However, lower expression of CD6 mRNA in patients than in controls was noticed.

CD28, CTLA-4, CD80, CD86

As part of the presented PhD project, CD28, CTLA-4, CD80 and CD86 genes, encoding molecules which play an important role in regulation of T cell activation were analyzed. Two polymorphisms of CD28 gene (rs35593994, rs3116496T>C) and three SNPs of CTLA-4 (rs5742909C>T, rs231775A>G, rs11571302G>T), CD80 (rs6641T>G, rs1599795T>A, rs16829980T>C) and CD86 (rs2715267T>G, rs1129055G>A, rs17281995G>C) were investigated.

The analysis revealed that in HLA-DRB1*15:01 negative individuals, G allele in rs231775A>G of CTLA-4 gene was associated with higher risk of multiple sclerosis. Additionally, the association of rs2715267T>G of CD86 gene with MS susceptibility was detected. In details, carriers of G allele at this polymorphic site possessed higher risk of MS in
comparison to TT homozygotes. On the other hand, the lower risk of MS was observed in individuals carrying A allele at the rs1599795T>A polymorphic site of CD80 gene. Furthermore, the analysis revealed an interaction between three polymorphisms: rs3116496T>C (CD28), rs6641T>G (CD80) and rs17281995G>C (CD86), associated with the age of MS onset.

\[ CD40, CD40L \]

Having taken into consideration the data from GWA studies as well as the crucial role of CD40-CD40L pathway in both humoral and cellular immunity, CD40 and CD40L genes were also included in this project. Of the five polymorphisms tested in this study (three of CD40 rs752118C>T, rs1883832C>T and rs11569343C>G and two of CD40L rs3092923T>C and rs3092952A>G) the association with MS risk and progression was observed only for rs1883832C>T.

The carriers of the T allele at this polymorphic site had higher susceptibility to MS as well as higher age of onset and later transition into secondary progressive phase of MS. No difference in CD40 expression between patients and controls was found. However, further analysis revealed that rs1883832CT and rs1883832TT individuals had lower level of CD40 mRNA in comparison to those with rs1883832CC genotype.

Additionally, in the control group, women had higher CD40L mRNA level in comparison to men. Furthermore, lower CD40L mRNA expression was observed in women with MS than in healthy ones. The analysis of the correlation between mRNA expression level of CD40 and CD40L showed that in the control group individuals having higher mRNA level of CD40 had also higher expression of CD40L. Such a correlation was not observed in MS patients.

To summarize:

- The risk of MS may be modulated by polymorphic variants of ALCAM, CD6, CTLA-4, CD80, CD86 and CD40 genes.
- The susceptibility to MS and disease progression may be modified by gene-gene interaction: CTLA-4 and HLA-DRB1*15:01, ALCAM and HLA-DRB1*15:01 as well as CD28, CD80 and CD86.
- The polymorphisms of ALCAM, CD28, CD80, CD86 and CD40 genes are associated with the age of MS onset.
• The polymorphic variants of *ALCAM* and *CD6* genes may have an influence on the degree of disability, while SNP of *CD40* gene may modify the duration of the RR course of the disease.

• The lower mRNA level of *CD6* gene may contribute to the disease development in both genders, while in women MS development may be also modified by the *CD40L* mRNA level.

• Polymorphism rs1883832C>T of *CD40* gene may have an effect on its mRNA level.

The results of presented study on *ALCAM* gene were published in *Journal of Neuroimmunology*\textsuperscript{10} and it was the first report to demonstrate the association of this gene with susceptibility to MS and with disease progression. The follow-up study on *ALCAM* and *CD6* genes in the context of MS was also printed in *Journal of Neuroimmunology*\textsuperscript{11}. It was the first study of *CD6* gene and its association with MS conducted on well-defined Polish population.

This project is the first, in which the association of *CD40* and *CD40L* polymorphisms and their interactions with MS risk and progression was studied on Polish population. The results of the study were published in *Human Immunology*\textsuperscript{12}. Similarly to others, we found association between rs1883832 and susceptibility to MS. Moreover, for the first time, we observed the association of *CD40* gene with disease progression.

To date, relatively low number of studies has been concerned with the association of *CD28, CD80* and *CD86* genes with MS risk and disease progression. The results of studies on polymorphisms of *CTLA-4* are not consistent. Therefore, the association of *CD80* and *CD86*, with MS, described in this thesis, may produce the new knowledge about the genetic background of multiple sclerosis. The results obtained in this project may also serve as a source of new ideas for future functional studies.