SUMMARY

Alzheimer's disease (AD) is a neurodegenerative, multifactorial and polygenic disease which can be influenced not only by the genetic polymorphisms but also by the epigenetic factors such as methylation within CpG dinucleotides. The environmental factors as well as a person's lifestyle also play a role in the development of AD.

The most common form of AD is the late-onset one occuring in people at the age of or over 65 and it is called sporadic Alzheimer's disease (SAD) or late-onset AD (LOAD). The genetic factors are responsible for the development of the early form of the disease EOAD – (early onset Alzheimer's disease) when the symptoms appear before the age of 60. It occurs familially and is inherited in an autosomal dominant manner. The Alzheimer's disease continuum is divided into the following stages: MCI - mild cognitive impairment (predementia), AD I - mild dementia, AD II - moderate dementia and AD III - severe dementia. Undoubtedly, the strongest risk factor for AD is the age, that is being over 65.

The purpose of this dissertation was to design and conduct molecular studies that would provide answers to the hypothesis that the DNA methylation levels and the presence of genetic polymorphisms influence the development of AD.

The study group consisted of 223 individuals whereas the control group included 62 people without symptoms and diagnosis of AD matched on the basis of age of people in the former. The analyzed study group embraced 161 people with confirmed dementia. This included 53 people with diagnosis of MCI and 108 people in different stages of dementia AD: AD I - 50 people, AD II - 48 people, AD III - 10 people.

The genomic DNA was isolated from peripheral blood leukocytes and was used for molecular studies. The classical polymerase chain reaction (PCR) or the restriction fragment length polymorphism (RFLP) technique was used to amplify the fragments with genetic polymorphisms in the study genes.

The dissertation conducts the analysis of the frequency of selected genetic polymorphisms located in the genes: *APOE, APOC1, TOMM40,* which are located in chromosome 19 as well as the frequency of the polymorphism of the *ACE* gene located in chromosome 17. In the study group of individuals (n=223), there was a predominance of the percentage of the ε3/ε3 genotype in the analysis of the rs429358 and rs7412 polymorphisms of the *APOE* gene. Among the people with the ε3/ε4 genotype, there was an increased odds ratio of 3.539 for the disease.

The major contributor to the development of dementia in the sporadic form of AD was the presence of the ε4 allele of the *APOE* gene in the patient's genotype. Allel ε4 increased the odds ratio for dementia by about 4.3 times. The highest percentage occurrence of this allele was found in AD I and AD II patients. In another polymorphism analyzed, the insertion allele (I) in rs11568822 of the *APOC1* gene was found to increase the odds ratio of AD by 4.2 times. The heterozygous genotype showed an increased odds ratio OR=3.14 times. The prevalence of the rs10524523 polymorphism of the *TOMM40* gene in the study group was also analyzed. The obtained results show that the presence of the S allele in the genotype probably has a protective effect against the onset of Alzheimer's disease but only in the homozygotes: S/S. The presence of the L allele of this gene in the genotype of the group of individuals increased the probability of developing Alzheimer's dementia by 3 times. In the subject group, this allele was mainly present in AD I and AD II in the 3 types of genotypes: S/L, L/L and L/VL. The difference in the length of the poly-T homopolymer between homologous alleles also played an important role in the polymorphic variant of the *TOMM40* gene studied. The greater the difference between the number of nucleotides in homologous chromosomes in the genotype studied, the more severe the form of the disease. In the analysis of the rs1799752 polymorphism of the *ACE* gene, it was found that the presence of an insertion allele (I) increased the risk of AD by 1.66 times. However, based on the studies performed, it was not possible to indicate in which genotypes this risk occurred. On the other hand, based on the results obtained, it was shown that the largest number of people with dementia had the ID genotype among the possible genotypes of the *ACE* gene polymorphism.

The analyses conducted on a separate subgroup of young people diagnosed with dementia at ≤ 60 years of age showed similar results as above. For the rs429358 and rs7412 polymorphisms of the *APOE* gene, there was also a predominance of the ε3/ε3 genotype percentage. Among people with the ε3/ε4 genotype, there was an increased odds ratio almost 5 times for AD. The strongest risk factor for EOAD was the presence of the ε4 *APOE* allele in genotypes. The ε4 *APOE* allele increased the odds ratio for an early dementia almost 6 times. When the rs11568822 polymorphism of the *APOC1* gene was analyzed, the presence of the I allele increased the odds ratio 2.88 times for development of AD in the young group of individuals. L allel was a risk factor for AD for study polymorphism rs10524523 of the *TOMM40* gene. An increased odds ratio of more than 3 times of AD occurrence was obtained in the case of presence of the L allele in the genotype of the young subjects. Moreover, in the statistical analysis of the L/L homozygous genotype of the rs10524523 polymorphism, the risk

of AD onset in the young people was OR = 5.9 times. In the analysis of the rs1799752 polymorphism of the *ACE* gene, there was an increased odds ratio equal 4.8 times for AD development for the presence the I allele. Yet, no information was obtained in which type of genotype (II, ID) this risk might occur.

In the analyses of the relationships between the genes which considered only the genotypes or alleles for which an increased odds ratio for dementia was found, no arrangement of the expected "p" result of a statistically significant importance was shown.

The analysis of the relative methylation levels in the promoters of genes: *APOE, TOMM40* and *ACE* was the next stage of the dissertation. The experiments were performed by qMSP-PCR (Quantitative Methylation-Specific Polymerase Chain Reaction) with the use of genomic DNA from the blood leukocytes.

The aim of the analyses was to demonstrate the existing differences in the relative methylation levels between a group of healthy people and the study group with dementia. Based on the results of the relative methylation analysis in the promoter of the *APOE* gene, it was found that the level of methylation decreases in the early stages of AD, while with the advancement of the disease there is an increase in the methylation up to the same value as in the control group. The results of analyzing the methylation level in the *TOMM40* gene promoter shown there was no occurring relationship between the relative methylation value in the *TOMM40* gene promoter and the presence or absence of dementia in the individuals. The statistical analysis of possible stages of dementia presented only a possible tendency to distinguish the group of people with AD II from other forms of dementia. It was also shown that the relative methylation value in the promoter of this gene increased with age. No correlation was found between the level of methylation in the promoter of the *ACE* gene and the stage of AD. However, a negative correlation was observed between the age of a patient and the relative methylation. The value of relative methylation in the promoter of the *ACE* gene decreases with the age of a patient.

As a result of the analyses of possible correlations between the relative methylation in the promoters of selected genes (*APOE, TOMM40* and *ACE*) and the presence of the polymorphisms of the genes in question (*APOE, APOC1*, *TOMM40* and *ACE*) in various possible combinations, no concurrent correlations were obtained. There have been no correlations between the relative methylation and the genotype and their possible influence on the onset of dementia. However, it has been found that the diseases associated with old age such

as hypertension and hyperlipidemia may influence the development or progression of an Alzheimer-type dementia. In people with dementia under the age of 60 such an effect has been noted in case of the early diagnosed depression and hyperlipidemia whereas in case of present hypertension at a young age, no effect on the development of AD in the form of EOAD was found.

In conclusion, based on the obtained results, we can say that the occurrence of certain genetic polymorphisms and changes in the level of DNA methylation may play a significant role in the development of AD. Further studies are needed on a larger group of patients to confirm the influence of genetic and epigenetic mechanisms on the development and the course of dementia.