

# Genetic background of neurological diseases

Ph.D. Thesis

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## I. INTRODUCTION

### *1.1. Pathogenesis of the ischemic demyelination*

The ischemic demyelinating disorder, also known as age-related white matter lesion of the brain, is a common clinical phenomenon. It is the most frequently seen lesion in neuroimaging scans. Ischemic demyelination is called Leukoaraiosis (LA) in neuroimaging terms. Here, LA refers to diffuse areas of hyperintensity in T2-weighted magnetic resonance imaging (MRI) scans and it involves white matter. One quarter of people over the age of 65 are affected to some degree by white matter changes. Moreover, LA is definitely associated with cognitive decline, slowness of mental processing (dementia) and a poor quality of life. It is a slowly developing illness, associated with various clinical risk factors and endogenous genetic factors. Two major mechanisms have been proposed for it. The first is the chronic endothelial dysfunction. The increased levels of circulating markers of endothelial cell activation or dysfunction such as ICAM-1 and thrombomodulin were found in leukoaraiosis, and ICAM-1 levels correlated with the progression of LA. The second is the elevated blood–brain barrier permeability, which results in the leakage of plasma components into the vessel wall and surrounding brain parenchyma. Another hypothesis states that cerebral hypoperfusion may cause an energy deficit in the glia cells and neurons. Chronic hypoxia may be presumed to primarily damage the function of the mitochondria.

Aging, hypertension, diabetes mellitus, a prior stroke event, and cardiac diseases are the main vascular risk factors for LA. The temporal and occipital border zones are the most frequent regions of LA, because the superficial and deep perforans are end arterioles. The two systems do not connect, but only meet in a junctional zone around the lateral ventricles.

The key genetic factors of LA pathogenesis are the methylene tetrahydrofolate reductase (MTHFR) C677T variant, the angiotensin-converting enzyme (ACE) I/D and the apolipoprotein E (APOE) 2 or 4 polymorphisms. The homozygous MTHFR 677TT variant is associated with an elevated serum homocysteine level, which has an unfavourable effect on vasoregulation. The increased plasma homocysteine level will result in endothelial dysfunction. Homocysteine directly damages the vascular matrix because it affects the biosynthetic and biochemical functions of the vascular cell. Endothelial-derived nitric oxide production is also caused by homocysteine. LA is positively correlated with the plasma homocysteine level, so it is inversely correlated with the plasma folate level. The MTHFR A1298C polymorphism is also a common genetic variant and it is thought to be a vascular risk factor. The ACE I/D polymorphism is an important genetic factor in the renin-angiotensin system, and it has regulatory roles in the cardiovascular system. Alongside this, the homozygous ACE D/D polymorphism has an unfavourable effect on the vasoregulatory system. Also, the incidence of the ACE D/D genotype might adversely affect vasoconstriction, vascular walls undermine thickening, reducing the proliferation of smooth muscle cells and cause a stroke. The

APOE 2 or 4 allele is also shown to give the rise to LA. The APOE 4 allele leads to a reduced neuroregeneration ability, and the cytoskeleton can become unstable and the glia cells may become more vulnerable. Despite this, the APOE 4 genotype is supposed to be a risk factor for ischemic brain injury or stroke. In the presence of the APOE 2 allele, the cytoskeleton might become too rigid and this may be harmful during the prolonged hypoperfusion of the brain, and it may decrease the range of mechanical and chemical flexibility of the glial cytoskeleton. The homozygous MTHFR 677TT mutation in combination with the homozygous ACE D/D genotype has been associated with a higher risk of LA. Furthermore, the MTHFR 677TT variant and the ACE D/D polymorphism contribute to small-vessel disease or vasoregulation impairment. These two genotypes in combination with the APOE 4 or 2 allele have also been reported to give rise to LA. A new finding is the role of the aquaporin-4 (AQP-4) genotypes in LA. AQP-4 is the major water channel in the brain and the spinal cord, and it aids the movement of water between the blood and brain, and between the brain and the cerebrospinal fluid. AQP-4 is alleged to be involved in the etiology of neuromyelitis optica, cerebral oedema, sudden infant death syndrome and migraine. The CT/TT genotypes of the AQP-4 *rs2075575* polymorphism could be associated with cerebral small-vessel disease and these genotypes may be a significant promoting factor for LA.

The CADASIL study, which investigated the cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy, is caused by mutations in the *notch-3* gene. The deposition of the extracellular portion of notch 3 in the arterial wall of both cerebral and systemic arteries may be identified adjacent to granular osmiophilic material, a deposition of unknown composition. This should be investigated further.

### *1.2. The pathogenesis of the Multiple sclerosis (MS)*

MS is a chronic immune-mediated inflammatory disease of the central nervous system (CNS) that typically commences in the third or fourth decade of life. Demyelination and subsequent axonal damage is characteristic in MS. The main pathological symptoms of the disease are the destruction of the myelin sheaths of nerve fibres, the relative sparsity of the axons, and the infiltration of inflammatory cells in a perivascular surrounding. The prevalence of MS in different regions of the world ranges from 15/100,000 to 250/100,000. In 1996, the US National Multiple Sclerosis Society (NMSS) Advisory Committee provided standardized definitions for MS clinical courses; namely relapsing-remitting (RR), secondary progressive (SP), primary progressive (PP) and progressive relapsing (PR). In 2013, the clinically isolated syndrome (CIS) form was added and the PR form has been eliminated by the Committee. CIS is a well-defined syndrome that includes optic neuritis, cerebellar dysfunction and partial myelitis, and it forms a part of the RR MS spectrum.

The original diagnostic criteria for MS were based on clinical features suggestive of CNS demyelination. However, there are other different criteria used for the diagnosis of MS as well. The

oldest is the Schumacker committee criteria (1965) and the most recent is the modified McDonald criteria (2010).

The Expanded Disability Status Scale (EDSS) is a method for quantifying disability in MS patients (0.0: Normal neurological status and 10.0: Death due to MS). EDSS steps 1.0 to 4.5 refer to patients with MS who are fully ambulatory, while EDSS steps 5.0 to 9.5 are defined in the terms of ambulation impairment.

The pathogenesis in MS is not clear. It is a multifactorial disease, where environmental, genetic and infectious factors play important roles in its pathogenesis. The inflammatory process is anticipated in MS pathogenesis, which is propagated by an autoimmune cascade. Four types of immunopathological mechanisms have been identified in MS lesions. Type I is T-cell mediated. In this way, demyelination is induced by macrophages either directly or by macrophage toxins. The most common pathology of MS lesions is type II, where demyelination is caused by specific antibodies and intense activity of the complement system. Type III is known as distal oligodendropathy, where degenerative changes occur in distal processes and they are followed by apoptosis. Type IV involves primary oligodendrocyte damage followed by secondary demyelination.

The geographical situation (high risk in northern Europe, in northern US, Canada, in southern Australia and in New-Zealand), vitamin D deficiency, smoking habits and infections (Epstein-Barr virus) are the most important environmental risk factors in MS.

There are many genetic polymorphisms that can influence the pathogenesis of MS. The cytokines and their receptor genes (IL-1 $\beta$ , IL-2, IL-10, TNF- $\alpha$ , - $\beta$ ), chemokines and their receptor genes (CCL-5, -3, -4), the cytotoxic T lymphocyte associated-4 (CTLA-4) gene, APOE, genes associated with apoptosis, MHC classes I and II genes (HLA-DRB1\* 1501-DQA1\* 0102-DQB1\* 0602 (DR15)) and the T-cell receptor (TCR) gene polymorphisms are the most serious, and these things were described in genome-wide association studies (GWAS). Viral infections (such as the Epstein-Barr virus and human herpes virus 6) are suspected of being a trigger for MS.

### *1.3 Alzheimer's disease*

Alzheimer's disease (AD) is a progressive neurodegenerative disease and it is the leading cause of cognitive and behavioural impairment in our society. AD is pathologically characterised by the observed accumulation and deposition of amyloid  $\beta$  (A  $\beta$ ) peptides and the appearance of neuronal inclusion of abnormally phosphorylated tau in the brain, especially in the hippocampus and cerebral cortex. Gene mutations in early onset familial AD (eFAD) may cause the production of the longer A $\beta$ 42 variant that preferentially accumulates in plaques. In sporadic or late-onset AD (LOAD), this commences after the age of 65 years. Many genetical and environmental factors may contribute to the pathogenesis of LOAD, and it affects about 95 % of sufferers. Several genes are implicated as a risk factor, but it is at present not known how they alter amyloid levels. Five percent of elderly persons

over 65 years suffer from AD and this percentage for those over 85 years increases to 30 %. By 2040, it is expected that 81 million people will suffer from AD.

AD is multifactorial and heterogeneous disorder (Iqbal & Iqbal), and recent studies indicate that some epigenetic or environmental effects are risk factors to AD. It has also been postulated that bacterial or viral infection in the brain may play an initiating role in amyloid plaque formation and the development of AD. Various animal studies have demonstrated that several pathogens like *Chlamydia pneumoniae*, Herpes simplex, *Escherichia coli*, and *Cryptococcus neoformans* play a significant role in the development of amyloid plaque formation, and bacterial or viral CNS pathogens may speed up the development of AD. Amyloidogenesis and most of the changes seen in AD, such as inflammation, brain cell atrophy, immunological aberrations, altered gene expression and cognitive deficits are also known to be a result of microbial infection.

New scientific evidence appears to strengthen the view that the initiating event in AD is related to the abnormal processing of the beta-amyloid (A $\beta$ ) peptide, ultimately leading to the formation of A  $\beta$  plaques in the brain. However, the cause of the amyloid -  $\beta$  (A $\beta$ ) imbalance is still an open question in the pathogenesis of AD.

## 2. AIMS OF THE STUDY

An investigation of various genetic polymorphisms in leukoaraiosis and multiple sclerosis, which have a multifactorial origins.

This is involved examining:

- The methylene tetrahydrofolate reductase (MTHFR) A1298C genetic variant in patients with leukoaraiosis;
- The absolute number of mitochondria in patients with leukoaraiosis;
- The role of human beta-defensins-1 and -2 (HBD1, HBD2) in MS: the relevance of the SNPs of the DEFB1 gene and the copy number polymorphism of the DEFB4 genes in multiple sclerosis.
- The role of defensin  $\beta$ -2 and  $\alpha$  defensins (HNP 1-3) in Alzheimer's disease.

## 3. MATERIALS AND METHODS

### 3.1 Patients and controls

Control subjects and patients gave their informed consent to their participation in our studies and the local ethics committees (Human Investigation Review Board of Pándy Kálmán County Hospital in Gyula, Hungary, Human Investigation Review Board at the University of Szeged) approved it. All controls and patients were of Hungarian ethnic origin and resident in Hungary.

### *3.1. MTHFR A1298C variant and the absolute number of mitochondria in the patient group with leukoaraiosis and controls*

The MTHFR A1298C study population comprised 198 LA patients with a mean age of  $64.9 \pm 9.23$  years (105 women and 93 men). The patients were diagnosed and recruited from the Department of Neurology of the Pándy Kálmán County Hospital in Gyula. In contrast, the controls consisted of 235 neuroimaging alteration free subjects (127 women and 108 men, age  $54.7 \pm 12.8$  years). They were randomly selected from the Pándy Kálmán County Hospital practice register subject to the proviso of having negative brain MRI scans.

The mitochondria study group consisted of 234 LA patients (123 women and 111 men, age  $71.6 \pm 10.8$  years) and 123 MRI alteration – free controls (65 women and 58 men, age  $59.4 \pm 8.62$  years). Once again, the patients and controls were recruited from the Pándy Kálmán County Hospital in Gyula, Hungary.

### *3.2. Human beta defensins in patients group with multiple sclerosis and controls*

The study included 250 MS patients. The population in question contained 250 RR (relapsing remitting) and 53 SP (secondary progressive) persons with a mean age of  $44.23 \pm 13.02$  years. Patients were diagnosed and registered at the Department of Neurology at the University of Szeged and Department of Neurology of the Pándy Kálmán County Hospital in Gyula. Here, the criterion was applied in the diagnosis of clinically definitive MS.

### *3.3. Human $\alpha$ - and $\beta$ - defensins in Alzheimer's group and controls*

The study included 206 AD patients (69 men and 137 women, the average age and standard deviation (S.D.) was  $76.42 \pm 4.21$  years, and the average onset was at 65 years of age.

### *3.4. DNA isolation*

Genomic DNA was extracted from 200  $\mu$ l of peripheral whole blood anticoagulated with EDTA. Here, two kinds of leukocyte DNA extraction were applied. One type of DNA isolation was the desalting method devised by Miller et al.<sup>51</sup> The other method was DNA extraction with the High Pure PCR Template Preparation Kit (Roche Diagnostic GmbH, Mannheim, Germany, Cat.No: 1796828) and the manufacturer's instructions were adhered to. The DNA concentration was measured with a Qubit fluorometer (Invitrogen, Carlsbad, USA). Afterwards, the genomic DNA was then stored at  $-20$  °C until needed.

#### *3.4.1 Determination of MTHFR A1298C variant in leukoaraiosis*

The genetic variants of MTHFR A677T and A1298C were identified by using a LightCycler probe system.

#### *3.4.2 Determination of the absolute number of mitochondria per cell in leukocyte cells*

The TaqMan real-time PCR assay, specially designed for the amplification of mitochondrial (m) and deleted mitochondrial (dm) DNA, was carried out using a specific set of primers and probes.

#### *3.4.3 Determination of the DEFBI SNPs in multiple sclerosis*

Human  $\beta$ -defensin 1 (DEFB1) – Genotyping was carried out using Custom TaqMan® SNP Genotyping Assays (Applied Biosystems, Foster City, CA, USA).

#### *3.4.4 Determination of the DEFB4 gene copy number in multiple sclerosis*

The determination of the DEFB4 Gene Copy Number – A TaqMan real-time PCR assay, specifically employed for the amplification of genomic DEFB4, was performed using a specific set of amplification primers.

#### *3.4.5 The determination of the DEFB4 gene copy number in AD patients*

A TaqMan real-time PCR assay, specially designed for the amplification of genomic DEFB4.

3.5. *ELISA procedures* based on the manufacturer's recommendations.

#### *3.6. Statistical analysis*

The GraphPad Prism 5 statistical program was used to perform the statistical calculations (GraphPad Software Inc. San Diego, CA, USA).

## **4. RESULTS AND DISCUSSION**

### *4.1 The MTHFR A1298C variant in leukoaraiosis*

Previous studies demonstrated that an elevated serum homocysteine level may be associated with LA. Also, an elevated serum homocysteine level is presumed to be associated with an endothelial dysfunction or microangiopathy. The genetic studies relating to the MTHFR C677T variant suggest that, although the 677T variant is unfavourable because it raises the serum homocysteine level, it does not increase the risk of contracting LA if it is present on its own. We also demonstrated earlier that the 677T variant in combination with other genetic variants increases the risk of contracting LA.

The present study reveals that a person carrying either a heterozygous A1298C or a homozygous 1298CC variant is at a higher risk of contracting LA than one carrying neither of them. This genetic variant has not been examined so far in any LA case-control study. Our findings therefore suggest for the first time that the A1298C variant may be more important than the C677T variant in the evolution of LA. We also found that the heterozygous C677T and A1298C variants do

not pose a risk of contracting LA if they are present by themselves. However, their combination in the same person leads to a marked risk of contracting LA. Here, although the number of patients displaying the combination of the two heterozygous MTHFR variants was low, the significance level relating to the approximately tenfold increase in the unfavourable combination in the LA group compared with that in the control group suggests that there is a definite link. At present the exact cause of this synergistic effect is not known. However, two possible explanations readily emerge. They are: (1) the two variants can potentially increase the serum homocysteine level in an additive manner and (2) the co-occurrence of the two unfavourable MTHFR genetic variants may influence the regulation of the enzyme. A properly balanced regulation of the MTHFR may be a key factor that can define the daily shifts in the serum homocysteine level. The clustering of the A1298C and C677T variants might give rise to an unfavourable regulatory nature in the dynamically changing activity of the MTHFR. Then the presence of the two heterozygous variants might result in a significantly unfavourable phenotype of the conformation of the enzyme protein.

#### *4.2 The absolute number of mitochondria per cell in leukocyte cells in leukoaraiosis*

The basic contents for mDNA and dmDNA were found to be statistically the same in the LA group and control group, and the K value was significantly lower for the LA group than that for the control group. This suggested that there was a larger proportion of dmDNA present. Having dmDNA may possibly lead to a mitochondrial malfunction in the following way: a, lower energy production; b, a lower free radical scavenging capacity; c, a lower rate of adaptation to the prevailing demand for energy production; d, a narrower range in the adjustment to the prevailing energy demand; e, a lower metabolic function capacity in general; f, a greater extent of free radical production; and g, a general malfunction of the mitochondrial genetic regulation.

No genetic or biochemical data is available to suggest which of these postulated mechanisms actually exist, but a lower and narrower energy capacity appears probable as the main pathomechanism behind LA. It was demonstrated earlier at the molecular level that LA can arise from a very slight, but chronic level of hypoxia, which may be caused by various environmental and genetic susceptibility factors. Our present findings agree with the earlier ones that uncoupling protein genetic variants play a role in the development of LA. The uncoupling proteins govern the electro-chemical gradient between the inner and outer spaces of the mitochondria, this gradient being essential for the energy production of the mitochondria.

If dmDNA is associated with any kind of biochemical malfunction, an uncompensated and larger proportion of dmDNA in the cells may be unfavourable from an energetic viewpoint. Our results appear to indicate that the lower the difference between the contents of mDNA (which compensates for malfunctions of the dmDNA) and dmDNA, the larger the risk of contracting LA in a given individual.



Overall, the results of our study suggest that the ratio of the dmDNA content and mDNA content may play a significant role in the pathogenesis of LA. These results also point to the need for new approaches for the examination of mitochondrial contents in other common brain disorders.

#### Limitations of the study

1. The numbers of mitochondria in the affected brain tissues could not be examined, as this study was a clinical one in a human patient population. Brain biopsies would not have been ethical; hence we were unable to identify associations between the numbers of mitochondria in different human tissues.
2. Although we found no apparent change in the number of mitochondria in a small cohort of study subjects over several weeks (which involved several turnovers of the mitochondria in the white blood cells), insufficient scientific data is available concerning the stability of the absolute numbers of mitochondria in the different tissues. This should be clarified in future studies.
3. In this study, no investigations were carried out to identify the properties of the normal functioning of the mitochondria with deletion DNAs.

However, these limitations do not greatly affect the present results, since they are not directly associated with the findings. Moreover, they really should be viewed as open scientific questions, that should be addressed in future investigations.

4. Although the logistic regression statistical method has greatly decreased the confounding effects of the clinical factors such as age, hypertension, and diabetes mellitus, the results need to be confirmed using a larger population group.

#### 4.3 Genetic polymorphisms of human $\beta$ -defensins in patients with multiple sclerosis

In our present study, an association between human  $\beta$ -defensins and multiple sclerosis was found. By investigating three SNPs of *DEFB1*, the distributions of the C-44G genotypes were found to be different between patients with MS and those in the healthy control group, while the frequency of the GG genotype was significantly higher in the control population. This suggests that the presence of the G allele most likely leads to strengthened HBD1 antimicrobial activity, which is less frequent among patients with MS. The G allele of C-44G SNP generates a putative binding site for nuclear factor  $\kappa$ B (NF- $\kappa$ B) and in all likelihood induces overexpression. The proposed effect of this SNP could partly explain why the GG genotype was considered to be a protective genotype in atopic dermatitis and also a contributory factor in the susceptibility to *Candida* colonisation in diabetic patients. By contrast, in these studies, subjects carrying the CC genotype at the -44 locus site of the gene were at a greater risk of becoming infected. It was recently suggested that the C allele of *DEFB1* C-44G SNP probably abrogates NF- $\kappa$ B -dependent *DEFB1* up regulation.

The above findings are consistent with our present observation that the GG phenotype might also play a protective role in MS, and *vice versa*, and the higher frequency of the CC genotype might be connected with a lower expression of human defensin  $\beta$ -1. Among the 250 patients with MS, only 9

(4%) had GG homozygote and 62% of the patients had CC homozygote, in contrast with 45% of the control group that had CC homozygote. These observations appear to emphasise the importance of *DEFB1* polymorphisms in MS.

Similarly, the production of the inducible hBD-2 is lower in MS patients. It is suggested that the significantly lower frequency of the copy number of *DEFB2* might be one of the reasons for the decreased levels of circulating hBD2 in the blood samples of patients with MS.

When the association between the copy numbers and the plasma levels of hBD2 was investigated, a correlation between the ELISA results and copy number genotypes was found in the control group, but not in the groups of patients with MS. Moreover, the low hBD2 levels correlated nicely with the low frequency of copy numbers (i.e. <4 copy) in the control group, but not in the groups of patients with MS. The low hBD2 levels correlated nicely with the low frequency of copy numbers (i.e. <4 copy), but in the patients with copy numbers >4, the plasma levels of hBD2 did not seem to be elevated. We suppose that other factors not yet defined might be responsible for the low levels of hBD2 even in the case of higher copy numbers. We hypothesise that abnormalities in the production and the function of human defensin- $\beta$  might be connected with an altered microbiome in MS, as suggested in a recent study.

While it is unclear whether enteric microbiota affects human MS, a higher proportion of MS patients exhibited antibody responses against gastrointestinal antigens than those in with healthy control subjects. This might indicate an altered gut microbiome and immune status.

In addition, as  $\beta$  defensins can be produced not only by epithelial cells, but also by astrocytes, and microglia cells, their importance in the central nervous system (CNS) needs to be taken into account. Human defensin- $\beta$  might function as an initial line of defence within the CNS either as an antimicrobial, or an immunomodulator, or both. What is more, these defensins may also be neuroprotective through their ability to inhibit cellular apoptosis in the CNS.

The extreme low frequency of the GG genotype of the C-44G SNP of *DEFB1*, the high frequency of the low copy number (<4) of *DEFB2*, and the significantly decreased plasma levels of hBD2 in patients highlight the importance of human defensin- $\beta$  levels in MS patients.

Further studies are necessary to elucidate the precise way the impaired function of human defensin- $\beta$  influences the pathomechanism of multiple sclerosis.

#### *4.4 Association between human defensin $\beta$ -2 and AD, and between human defensin- $\alpha$ (HNP 1-3) and AD*

Higher concentrations of the inducible hBD-2 were found in the cerebrospinal fluid and in the sera of AD patients. It is suggested that the significantly higher frequency of the copy number of *DEFB4*, encoding hBD2, might be one of the reasons for the increased levels of circulating hBD2 in the blood samples and in the CF of patients with MS.

As  $\beta$  defensins can be produced not only by epithelial cells, but also by astrocytes, and microglia cells their importance in the CNS should be taken into consideration in future studies. Human defensin- $\beta$  might function as an initial line of defence within the CNS either as an antimicrobial, or an immunomodulator, or both.

In addition, the contribution of the microbiota to AD pathogenesis was recently investigated and it supports the hypothesis of a microbiome-brain axis. Here, microbiome means the collective genomes of total microbiota. Recent studies have also begun to clarify the degree of involvement of microbiome in AD pathogenesis. It was found that the composition of the human microbiome and exposure to pathogens varies with age, diet, lifestyle and biological environment. Studies indicate that incidence of AD and microbiome exposure and complexity vary greatly in different human populations. Here, the participation of defensins in AD should not be neglected.

An elevated hBD expression in the CF suggests that brain synthesises antimicrobial peptides and they circulate throughout the ventricular system and protect the CNS against microbial infection. Choroid plexus regulates immune functions between peripheral and brain circulation, and it is well documented in AD neuropathology (epithelial cell atrophy, impaired secretory and transporter functions, reduced amyloid  $\beta$  clearance). The dysfunction of the choroid plexus may give rise to neuropathological and inflammatory processes. *In vitro* results suggest that astrocytes may be responsible for local hBD-2 synthesis in the brain. Astrocytes and microglia are important in the cerebral neuroinflammatory response, and *in vitro* they express hBD-1 and -2; and they may modulate adaptive immunity. Williams et al. found a significant elevation of the hBD-1 mRNA level in the choroid plexus, and increased protein level in hippocampal neurons of an AD brain. The increased hBD-1 expression within an AD brain may be a protective response to inflammatory stimuli and potential modulator of the host's innate immune response within the CNS.

It has been suggested that chronic infections might be initial events in AD pathogenesis, which can lead to persistent inflammatory stimuli. The inflammatory response thereafter may indirectly lead to the upregulation of amyloid  $\beta$  production. It may well be that the induction of defensins is also involved in the amyloid development. Many antimicrobial peptides exhibit structural characteristics including  $\beta$ -sheet conformation similar to amyloid  $\beta$  that contribute to oligomerization. It should also be mentioned here that the existence of the oligomerization of monomeric hBD-2 has now been demonstrated.

Not only were the inducible hBD-2 elevated in the cerebrospinal fluid and in the sera of AD patients, but the levels of HNP1-3 were also higher both in the sera and in the CF. Our findings are in accordance with recent data by Watt et al. in 2015, who reported that peripheral  $\alpha$ -defensins are elevated in Alzheimer's disease. However, no measurements of the defensins in the CF were included in their study. While copy number polymorphism of the DEFB4 gene has been reported to influence

the production of hBD2, the secretion of HNP 1-3 however seems to be independent of the copy number of the DEFA gene. Hence we did not investigate it here.

The present study supports the view of the potential role for antimicrobial peptides like human  $\alpha$  and  $\beta$  defensins in AD pathology; they are pathogen targeting agents in brain infections involving AD. Whether the elevated levels of defensins are a consequence of inflammation, or they themselves induce neurodegeneration and amyloid formation is currently unknown. Further investigations are therefore necessary to elucidate the regulatory functions of defensins in the pathomechanism of AD.

## 5. SUMMARY AND NEW RESULTS

- Our results revealed an association between the A1298C polymorphism of the MTHFR gene and leukoaraiosis. A person carrying either a heterozygous A1298C or a homozygous 1298CC variant is at a higher risk of contracting LA than one carrying neither of them.
- Our study suggests that the ratio of the dmDNA content and mDNA content may be of great significance in the pathogenesis of LA. The findings here suggest the need for a new perspective when we investigate the mitochondrial contents in other common brain disorders.
- The extremely low frequency of the GG genotype of the C-44G SNP of DEFB1, the high frequency of the low copy number (<4) of DEFB2, and the significantly decreased plasma levels of hBD2 in patients all highlight the importance of the role of human defensin- $\beta$  in MS.
- Human  $\alpha$  and  $\beta$  defensins in AD pathology, as pathogen targeting agents, play a role in brain infections related to AD. Whether the elevated levels of defensins are a consequence of inflammation, or they themselves induce neurodegeneration and amyloid formation, is currently not known.

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