Investigations in the Hungarian Multiple Sclerosis Patient Population: New Data on the Genetic Background and Validation of the Fatigue Impact Scale

Summary of Ph.D. Thesis

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List of Abbreviations

A	adenine
APOE	apolipoprotein E gene
ApoE	apolipoprotein E glycoprotein
BDI	Beck Depression Inventory
EDSS	Expanded Disability Status Scale
FIS	Fatigue Impact Scale
G	guanine
HC	healthy control
ICC	intraclass correlation coefficient
MHC	Major histocompatibility complex
MS	multiple sclerosis
MSSS	Multiple Sclerosis Severity Score
PI	progression index
PPMS	primary progressive multiple sclerosis
RRMS	relapsing-remitting multiple sclerosis
SNP	single nucleotide polymorphism
SPMS	secondary progressive multiple sclerosis
TNF	tumour necrosis factor

I. Introduction

Multiple sclerosis (MS) is a chronic inflammatory demyelinating disease of the central nervous system. The prevalence of the disease varies with geography ranging between 2 and 150 per 100,000 [1, 2]. In the majority of MS patients, the disease begins with a relapsing course (RRMS), characterized by relapses and remissions, and followed by a progressive phase (secondary progressive MS, SPMS) [3]. In a smaller subset of patients, the relapsing phase is not observed and the disease progresses from the beginning (primary progressive form, PPMS). The appearance of the disease is determined by a combination of exogenous factors and the genetic background [4].

Two of the genes whose potential association emerged from the analyses published previously by our MS Workgroup [5] were selected for further analysis:

- Tumour necrosis factor (TNF) is a proinflammatory cytokine involved in the pathogenesis of infectious and autoimmune disorders. The human TNF gene maps to chromosome 6p21.3 in the highly polymorphic major histocompatibility complex (MHC) region. The location suggests that TNF-α single nucleotide polymorphisms (SNPs) may be involved in influencing the disease course during MHC-associated diseases such as MS. Most

studies to date have concerned the relevance of the TNF gene SNPs to MS, with conflicting results [6, 7].

- Apolipoprotein E (ApoE), an important glycoprotein in the transport, uptake and redistribution of cholesterol, is necessary in nerve tissue repair. The APOE gene (APOE) is involved in neurodegenerative diseases, the best-known association being that between the APOE ε4 allele and Alzheimer's disease [8]. The APOE gene is mapped to chromosome 19. Two SNPs within exon 4 of the APOE, at codons 112 and 158, result in three common alleles (ε2, ε3 and ε4). The literature reports on the role of APOE in MS are controversial [9-15]. Additionally, no Hungarian data are available regarding the APOE status of MS patients.

In addition to the genetic investigations, as a secondary aim we intended to better understand fatigue a very important feature of MS [16]. The Fatigue Impact Scale (FIS) [17], one of the 30 available fatigue questionnaires, is commonly applied because it evaluates multidimensional aspects of fatigue [18]. An objective questionnaire for evaluation of the impact of fatigue in Hungarian MS patients has not yet been approved.

II. Aims

Our primary aims were a multicentre assessment of the possible influence of the TNF-α -376 polymorphism and of the APOE gene on the susceptibility to PPMS in Hungary.

On the basis of our previous experience with the adaptation and validation process of the Multiple Sclerosis Quality of Life Instrument [19], we set out to test the validity, test-retest reliability and internal consistency of the Hungarian version of the FIS.

III. Patients and methods

Genetic Analysis

Polymerase chain reaction and restriction fragment length polymorphism were carried out on 45 PPMS patients, 45 age and sex-matched RRMS patients and 45 healthy controls (HCs).

Validation of the Fatigue Impact Scale

One hundred and eleven MS patients and 85 HCs completed the FIS and the Beck Depression Inventory (BDI), a large majority of them on 2 occasions, 3 months apart.

Statistical analysis

For statistical comparison between the PPMS patients, the RRMS patients and the HC group as concerns TNF-α dimorphism, we used the χ^2 test and Fischer's exact test (exact

p). As concerns the APOE, the Pearson χ^2 test was performed to study the distribution of the alleles by the investigated groups. The combined effect of the MS course and the alleles on the clinical parameters was analysed by two-way analysis of variance.

Regarding the validation of FIS, both the t-test and the Mann-Whitney U test were used to detect differences between the groups before elimination of the effect of depression. The differences in FIS scores between the MS and HC groups were investigated by covariance analysis after elimination of the effect of depression. The intraclass correlation coefficients (ICCs) were determined to assess the test-retest reliability of the FIS. Cronbach's alpha was determined to test the reliability of FIS.

IV. Results

TNF-α

For the GG genotype, a statistically significant higher level was found in the PPMS group as compared with the HCs (exact p=0.027). As regards the G allele, a significant difference was observed between the PPMS and HC groups (exact p=0.032). The GA genotype was underrepresented in the PPMS group relative to the HCs (exact p=0.027); for the A allele, the distribution was similar (exact p=0.032). No significant differences in genotype were found between the

RRMS and HC groups or between the RRMS and PPMS groups (exact p=0.144 and exact p=0.677, respectively). The distributions of the alleles in the groups were similar (RRMS-HC: exact p=0.162; RRMS-PPMS: exact p=0.682).

No association was found between the genotype status of the TNF-α -376 polymorphism and the age at onset, the disease duration, Expanded Disability Status Scale (EDSS), progression index (PI) or Multiple Sclerosis severity Score (MSSS).

APOE

The number of PPMS patients without the ε2 allele was found to be notably high (p<0.001), whilst the ε2 allele was overrepresented in the RRMS group (p<0.003). In addition, the pairwise comparisons indicated that the difference between the RRMS and HC groups was also significant (p<0.001).

The presence of the ε4 allele was typical in the PPMS and the RRMS groups. A markedly high frequency of this allele was found in the PPMS group (p<0.001) and a very low frequency in the HCs (p<0.001). The pairwise comparisons revealed that the frequency of the ε4 allele was also higher in the RRMS group than that in the HCs (p<0.05).

As concerns the clinical parameters (EDSS, PI and MSSS), significant differences were observed between the RRMS and PPMS groups (p<0.001 for all parameters). Differences were

also detected regarding the EDSS and MSSS scores when the patients were grouped by the presence or absence of the ε2 allele (p<0.004 and p<0.001, respectively). As for the ϵ4 allele, no differences were found in any of the clinical parameters at the p=0.005 decision level, but at the p=0.05 level the MSSS value differed significantly (p=0.045). All of the observed differences in the clinical parameters disappeared when we further stratified the patients by the type of MS. Two-way analysis of the combined effect of the two variables revealed that the difference in the clinical parameters can only be attributed to the type of MS.

Fatigue

Ninety-nine of the 111 MS patients (89%) and 79 of the 85 HC subjects (93%) completed the scales on both occasions. The total FIS scores were statistically higher in the MS group in both sessions (p_1<0.001; p_2<0.001), and after elimination of the BDI scores (p_1=0.001; p_2=0.024).

The ICCs between the two sessions were high in both the MS (ICC=0.857) and the HC (ICC=0.814) groups.

As concerns the internal consistency of the FIS scales, the values of Cronbach's alpha for total FIS_1 and total FIS_2 were 0.984 and 0.992 in the HCs, and 0.987 and 0.987 in the MS group. The item-specific FIS_1 statistics indicated large item-to-total correlations, most of them > 0.8.

V. Discussion

TNF-α

The results suggest that in the Hungarian population the G allele in the examined position might have a role as regards progression in MS, while the A allele is rather a probable protective factor. Four of the five papers relating to the -376 SNP did not detect any association between the SNP and MS [20-23]. However, none of them examined PPMS patients. In one article, the susceptibility to MS and the A allele were reported to be correlated [6], but the subtypes of the patients were not reported, and therefore no comparison can be made with our results on PPMS patients. Consequently, until the publication of our findings (2009), there were no available data on an association between PPMS and TNF-α gene -376 SNP. In 2010, Nada and Labib, utilizing our methods, confirmed our results in the Egyptian PPMS population [24]. To confirm our findings and to improve the statistical power, extension of the study is clearly needed, because inhibition of the TNF-α signalling pathway (e.g. TNF-α blockers) could be an attractive therapeutic strategy for the treatment not only of MS, but also of other neurodegenerative diseases.

APO-E

The literature reports on the role of APOE in MS are controversial, with claims that the presence [9, 11, 13, 25] or

absence [14, 15, 26-28] of the APOE ϵ4 allele is connected with susceptibility to the disease or its severity. Population differences in susceptibility alleles, allele heterogeneity or the detected different prevalence rate might be the reasons why the association between APOE and MS could not be confirmed unequivocally. The literature information relating to the genetic background of PPMS patients is incomplete because of the low number of such patients. Only three APOE analysis studies (from Sardinia, The Netherlands and Australia) involved a larger PPMS group than that in the present study [15, 28, 29].

Although there is no direct evidence that ApoE contributes isoform-dependently to the maintenance of blood-brain barrier integrity, ApoE isoforms may differ in protecting humans from MS. The observed differential occurrence of the ε2 allele in the PPMS and the RRMS groups leads us to suspect that the presence of this allele makes the patients susceptible to the RRMS course. The observed distribution of the ε4 allele across the groups indicated that this allele is linked with both forms of the disease but with a higher propensity to the PPMS course. Our findings suggest that the presence of the ε2 and ε4 alleles may play a role in the development of the disease. However, when any type of the disease has already developed, the alleles show no association with the clinical parameters.

Fatigue

The total FIS score and subscale scores differed statistically between the MS patients and the HCs in both FIS sessions. The results in the two sessions did not differ statistically in either group. This is an indication that the test-retest reliability of the Hungarian FIS is good, similarly as for other validation [30-32].The results of our study indicate that the FIS can be regarded as a valid and reliable scale with which to improve our understanding of the impact of fatigue on the health-related quality of life in MS patients without severe disability.

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VII. Original publications directly related to the Ph.D. thesis

1. **Losonczi E**, Bencsik K, Nagy ZF, Honti V, Szalczer E, Rajda C, Illés Z, Mátyás K, Rózsa C, Csépány T, Füvesi J, Vécsei L. (2009) Tumour necrosis factor alpha gene (TNF-alpha) -376 polymorphism in Hungarian patients with primary progressive multiple sclerosis. *J Neuroimmunol*. **208:**115-118. **IF: 2.841**
2. **Losonczi E**, Bencsik K, Fricska Nagy Z, Honti V, Szalczer E, Rajda C, Illés Z, Mátyás K, Rózsa C, Csépány T, Füvesi J, Vécsei L. (2010) APOE epsilon status in Hungarian patients with primary progressive multiple sclerosis. *Swiss Med Wkly*. **26:** 140: w13119, doi: 10.4414/smw.2010.13119. **IF: 1.681**
3. **Losonczi E**, Bencsik K, Rajda C, Lencsés G, Török M, Vécsei L. (2010) Validation of the Fatigue Impact Scale in Hungarian patients with multiple sclerosis. *Qual Life Res*. DOI: 10.1007/s11136-010-9749-7 **IF: 2.376**
   Total impact factor: 6.898

Publications not directly related to the thesis

4. Füvesi J, Bencsik K, **Losonczi E**, Fricska-Nagy Z, Mátyás K, Mészáros E, Benedek K, Rajda C, Lencsés G, Vécsei L. (2010) Factors influencing the health-related quality of life in Hungarian multiple sclerosis patients. *J Neurol Sci*. 15; 293: 59-64. **IF: 2.324**
5. Csillik B, Schwaller B, Mihaly A, Henzi T, **Losonczi E**, Knyihar-Csillik E. (2010) Upregulated expression of oncomodulin, the beta isoform of parvalbumin, in perikarya and axons in the diencephalon of parvalbumin knockout mice. *Neuroscience.* 3;165:749-757. **IF: 3.292**
6. Füvesi J, Bencsik K, Benedek K, Mátyás K, Mészáros E, Rajda C, **Losonczi E,** Fricska Nagy Zs, Vecsei L. (2008) Cross-cultural

adaptation and validation of the „Multiple Sclerosis Quality of Life Instrument" in Hungarian *Mult Scler.* 14: 391-398 **IF: 3.312**

7. Bencsik K, Füvesi J, Fricska-Nagy Z, Rajda C, **Losonczi E**, Torok M, Vecsei L (2006) Treatment of Relapsing-Remitting Multiple Sclerosis 96 Patients with IFN-beta 1b: Results of a 6-Year Follow-Up *J Interferon Cytokine Res.* 26: 96-100 **IF: 2.094**

Cumulative impact factor: 17.92

VIII. References

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