

**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
Erika Eszter Losonczy M.D.

Department of Neurology
University of Szeged

Szeged
2011

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 ϵ 4 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 (ϵ 2, ϵ 3 and ϵ 4). 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 $\epsilon 2$ allele was found to be notably high ($p<0.001$), whilst the $\epsilon 2$ 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 $\epsilon 4$ 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 $\epsilon 4$ 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 $\epsilon 2$ allele ($p < 0.004$ and $p < 0.001$, respectively). As for the $\epsilon 4$ 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 FIS₁ and total FIS₂ were 0.984 and 0.992 in the HCs, and 0.987 and 0.987 in the MS group. The item-specific FIS₁ 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 $\epsilon 4$ 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 $\epsilon 2$ 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 $\epsilon 4$ 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 $\epsilon 2$ and $\epsilon 4$ 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.

VI. Acknowledgements

I would like to express my thanks to my supervisor Krisztina Bencsik M.D., Ph.D. for her clinical scientific guidance to Viktor Honti Ph.D. for his guidance related to genetics and to all members of the MS Workgroup in Szeged (Cecília Rajda M.D., Ph.D., Judit Füvesi M.D., Zsanett Friczka Nagy M.D. and Estilla Szalczzer M.D.).

I would also like to thank Professor László Vécsei, Member of the Hungarian Academy of Sciences, Head of the Department of Neurology, University of Szeged, for the opportunity to work in his laboratory. I would like to express my special gratitude to Professor Bertalan Csillik and Professor Erzsébet Knyihár-Csillik for our fruitful collaboration.

My thanks are also due to Gyula Lencsés for his statistical support and all of my colleagues at the 4 MS Centres around Hungary (Zsolt Illés M.D., Ph.D., Pécs; Klotild Mátyás M.D., Kecskemét; Csilla

Rózsa M.D., Ph.D., Budapest; and Tünde Csépany M.D., Ph.D., Debrecen) for collecting blood samples from PPMS patients, thereby supporting my work.

I am grateful to the patients who participated in these studies and to Margit Török for collecting the blood samples and scales.

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, Csépany 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, Csépany 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

- [1]. Bencsik K, Rajda C, Füvesi J, *et al.* The prevalence of multiple sclerosis, distribution of clinical forms of the disease and functional status of patients in Csongrád County, Hungary. *Eur Neurol.* 2001 **46**: 206-209.
- [2]. Rosati G. The prevalence of multiple sclerosis in the world: an update. *Neurol Sci.* 2001 **22**: 117-139.
- [3]. Lublin FD, Reingold SC. Defining the clinical course of multiple sclerosis: results of an international survey. National Multiple Sclerosis Society (USA) Advisory Committee on Clinical Trials of New Agents in Multiple Sclerosis. *Neurology.* 1996 **46**: 907-911.
- [4]. Rejdak K, Jackson S, Giovannoni G. Multiple sclerosis: a practical overview for clinicians. *Br Med Bull.* 2010 **95**: 79-104.
- [5]. Rajda C, Bencsik K, Seres E, *et al.* A genome-wide screen for association in Hungarian multiple sclerosis. *J Neuroimmunol.* 2003 **143**: 84-87.
- [6]. Fernandez-Arquero M, Arroyo R, Rubio A, *et al.* Primary association of a TNF gene polymorphism with susceptibility to multiple sclerosis. *Neurology.* 1999 **53**: 1361-1363.
- [7]. Mihailova S, Ivanova M, Mihaylova A, Quin L, Mikova O, Naumova E. Pro- and anti-inflammatory cytokine gene polymorphism profiles in Bulgarian multiple sclerosis patients. *J Neuroimmunol.* 2005 **168**: 138-143.
- [8]. Kim J, Basak JM, Holtzman DM. The role of apolipoprotein E in Alzheimer's disease. *Neuron.* 2009 **63**: 287-303.
- [9]. Chapman J, Vinokurov S, Achiron A, *et al.* APOE genotype is a major predictor of long-term progression of disability in MS. *Neurology.* 2001 **56**: 312-316.

- [10]. Ballerini C, Campani D, Rombolà G, *et al.* Association of apolipoprotein E polymorphism to clinical heterogeneity of multiple sclerosis. *Neurosci Lett.* 2000 **296**: 174-176.
- [11]. Fazekas F, Strasser-Fuchs S, Kollegger H, *et al.* Apolipoprotein E epsilon 4 is associated with rapid progression of multiple sclerosis. *Neurology.* 2001 **57**: 853-857.
- [12]. Ferri C, Sciacca FL, Veglia F, *et al.* APOE epsilon2-4 and -491 polymorphisms are not associated with MS. *Neurology.* 1999 **53**: 888-889.
- [13]. Høgh P, Oturai A, Schreiber K, *et al.* Apolipoprotein E and multiple sclerosis: impact of the epsilon-4 allele on susceptibility, clinical type and progression rate. *Mult Scler.* 2000 **6**: 226-230.
- [14]. Burwick RM, Ramsay PP, Haines JL, *et al.* APOE epsilon variation in multiple sclerosis susceptibility and disease severity: some answers. *Neurology.* 2006 **66**: 1373-1383.
- [15]. van der Walt A, Stankovich J, Bahlo M, *et al.* Apolipoprotein genotype does not influence MS severity, cognition, or brain atrophy. *Neurology.* 2009 **73**: 1018-1025.
- [16]. Egner A, Phillips VL, Vora R, Wiggers E. Depression, fatigue, and health-related quality of life among people with advanced multiple sclerosis: results from an exploratory telerehabilitation study. *NeuroRehabilitation.* 2003 **18**: 125-133.
- [17]. Fisk JD, Ritvo PG, Ross L, Haase DA, Marrie TJ, Schlech WF. Measuring the functional impact of fatigue: initial validation of the fatigue impact scale. *Clin Infect Dis.* 1994 **18 Suppl 1**: S79-83.
- [18]. Dittner AJ, Wessely SC, Brown RG. The assessment of fatigue: a practical guide for clinicians and researchers. *J Psychosom Res.* 2004 **56**: 157-170.
- [19]. Füvesi J, Bencsik K, Benedek K, *et al.* Cross-cultural adaptation and validation of the 'Multiple Sclerosis Quality of Life Instrument' in Hungarian. *Mult Scler.* 2008 **14**: 391-398.
- [20]. de Jong BA, Huizinga TW, Zanelli E, *et al.* Evidence for additional genetic risk indicators of relapse-onset MS within the HLA region. *Neurology.* 2002 **59**: 549-555.
- [21]. Kauffman MA, Morón DG, Sandoval G, Sica RE, Garcea O, Villa AM. Is tumor necrosis factor-376A promoter polymorphism associated with susceptibility to multiple sclerosis? *Medicina (B Aires).* 2007 **67**: 436-438.

- [22]. Weinschenker BG, Hebrink DD, Atkinson E, Kantarci OH. Association of a tumor necrosis factor alpha polymorphism with MS susceptibility. *Neurology*. 2001 **57**: 1341-1342.
- [23]. Wirz SA, Morale MC, Marchetti B, *et al*. High frequency of TNF alleles -238A and -376A in individuals from northern Sardinia. *Cytokine*. 2004 **26**: 149-154.
- [24]. Nada MA, Labib DA. Tumor Necrosis Factor Alpha Gene - 376 Polymorphism and Susceptibility to Multiple Sclerosis: An Egyptian Study. *J Neuroimmune Pharmacol*. 2010.
- [25]. Evangelou N, Jackson M, Beeson D, Palace J. Association of the APOE epsilon4 allele with disease activity in multiple sclerosis. *J Neurol Neurosurg Psychiatry*. 1999 **67**: 203-205.
- [26]. Savettieri G, Andreoli V, Bonavita S, *et al*. Apolipoprotein E genotype does not influence the progression of multiple sclerosis. *J Neurol*. 2003 **250**: 1094-1098.
- [27]. Ramagopalan SV, Deluca GC, Degenhardt A, Ebers GC. The genetics of clinical outcome in multiple sclerosis. *J Neuroimmunol*. 2008 **201-202**: 183-199.
- [28]. Zwemmer JN, van Veen T, van Winsen L, *et al*. No major association of ApoE genotype with disease characteristics and MRI findings in multiple sclerosis. *Mult Scler*. 2004 **10**: 272-277.
- [29]. Cocco E, Sotgiu A, Costa G, *et al*. HLA-DR,DQ and APOE genotypes and gender influence in Sardinian primary progressive MS. *Neurology*. 2005 **64**: 564-566.
- [30]. Armutlu K, Keser I, Korkmaz N, *et al*. Psychometric study of Turkish version of Fatigue Impact Scale in multiple sclerosis patients. *J Neurol Sci*. 2007 **255**: 64-68.
- [31]. Flensner G, Ek AC, Söderhamn O. Reliability and validity of the Swedish version of the Fatigue Impact Scale (FIS). *Scand J Occup Ther*. 2005 **12**: 170-180.
- [32]. Debouverie M, Pittion-Vouyovitch S, Louis S, Guillemin F. Validity of a French version of the fatigue impact scale in multiple sclerosis. *Mult Scler*. 2007 **13**: 1026-1032.