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

Ph.D. Thesis

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APOE epsilon status in Hungarian patients with primary progressive multiple sclerosis. *Swiss Med Wkly*. **26:** 140: w13119, doi: 10.4414/smw.2010.13119.

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V. 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.

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Table of Contents

List of Abbreviations and Acronyms

A	adenine
APOE	apolipoprotein E gene
ApoE	apolipoprotein E glycoprotein
BBB	blood-brain barrier
BDI	Beck Depression Inventory
BDI_1 and BDI_2	first and second sessions of Beck Depression Inventory
BMS	benign multiple sclerosis
CD	cluster of differentiation
CI	confidence interval
C	cytosine
CSF	cerebrospinal fluid
CPMS	chronic progressive multiple sclerosis
DNA	deoxyribonucleic acid
EDSS	Expanded Disability Status Scale
EDTA	ethylene diamine tetra-acetic acid
FIS	Fatigue Impact Scale
FIS_1 and FIS_2	first and second sessions of Fatigue Impact Scale
FSS	Fatigue Severity Scale
G	guanine
GAMES	Genetic Analysis of Multiple Sclerosis in Europeans
GWAS	genome-wide association study
HC	healthy control

ICC	intraclass correlation coefficient
IFN-β	interferon-beta
IL	interleukin
MHC	Major histocompatibility complex
MRI	magnetic resonance imaging
MS	multiple sclerosis
MSSS	Multiple Sclerosis Severity Score
OR	odds ratio
PCR	polymerase chain reaction
PET	positron emission tomography
PI	progression index
PPMS	primary progressive multiple sclerosis
PRMS	progressive-relapsing multiple sclerosis
RFLP	restriction fragment length polymorphism
RRMS	relapsing-remitting multiple sclerosis
SD	standard deviation
SNP	single nucleotide polymorphism
SPECT	single photon emission computed tomography
SPMS	secondary progressive multiple sclerosis
T	thymine
TNF	tumour necrosis factor

Summary

Multiple sclerosis (MS) is a chronic inflammatory demyelinating disease of the central nervous system. The disease is heterogeneous, which results in different clinical manifestations. In the majority of MS patients, the disease begins with a relapsing course (relapsing-remitting form, RRMS), characterized by relapses and remissions, and followed by a progressive phase (secondary progressive MS, SPMS). 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.

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

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

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. 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).

In our study, the GG genotype and the guanine allele (G) in the TNF-α gene at position -376 were detected significantly more often in the PPMS group than in the HC group. As regards the APOE gene, the number of PPMS patients without the ε2 allele was found to be notably high, whilst the ε2 allele was overrepresented in the RRMS group. A markedly high frequency of the ε4 allele was found in the PPMS group and a very low frequency in the HC group. As concerns the clinical parameters, significant differences were observed between the RRMS and PPMS groups. Differences were also detected regarding the

Expanded Disability Status Scale (EDSS) and Multiple Sclerosis Severity Score (MSSS) scores when the patients were grouped according to the presence or absence of the ε2 allele. All of the observed differences in the clinical parameters disappeared when the patients were further stratified according to the type of MS.

Our findings suggest that the G allele at position -376 of the TNF-α gene may be one of the factors responsible for progression in PPMS, and 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.

In addition to the genetic investigations, as a secondary aim we intended to better understand fatigue a very important feature of MS. Fatigue is one of the most frequent complaints of patients with MS. The Fatigue Impact Scale (FIS), one of the 30 available fatigue questionnaires, is commonly applied because it evaluates multidimensional aspects of fatigue. An objective questionnaire for evaluation of the impact of fatigue in Hungarian MS patients has not yet been approved. On the basis of our previous experience with the adaptation and validation process of the Multiple Sclerosis Quality of Life Instrument, we set out to test the validity, test-retest reliability and internal consistency of the Hungarian version of the FIS. 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.

The total FIS score and subscale scores differed statistically between the MS patients and the HCs in both FIS sessions. In the test-retest reliability assessment, the ICCs were statistically high in both the MS and HC groups. Cronbach's alpha values were also notably high. Consequently, our results 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.

I. Introduction

I.1. Definition

Multiple sclerosis (MS; first described in 1868 by Jean-Martin Charcot) is an inflammatory disorder of the central nervous system in which focal lymphocytic infiltration leads to myelin and in particular cases to axonal damage. The current consensus is that MS is an autoimmune disease triggered by environmental agents acting in genetically susceptible subjects [1]. The prevalence of the disease varies with geography, racial and ethnic group, ranging between 2 and 150 per 100,000 [2-4]. The disease is especially common in Scotland, Sardinia, Scandinavia, and throughout northern Europe, while it is rare among native Siberians, Chinese, Japanese, etc. [2]. The annual incidence ranges from 2 to 10 per 100,000, making MS the most common cause of neurological disability in young adults. It is more common in women than in men (female to male ratio: 3.2:1); however, genome-wide association studies (GWAS) have failed to provide any support for any genes on the X chromosome, and thus the increased incidence in women might be related to female-specific physiology, and could be hormone-related [5]. The disease was untreatable until the 1990s, when interferon-beta (IFN-β) reached the market for the first time. However, there is still no cure for MS and existing treatments merely slow the disease progression and mitigate the symptoms. The progress, severity and specific symptoms in MS are unpredictable. The understanding of the basic causes of the disease is incomplete.

I.2. Clinical Course

The disease is heterogeneous, which results in different clinical manifestations [6, 7].

- In the majority of MS patients, the disease begins with a relapsing course (relapsing-remitting form, RRMS), characterized by relapses (periods when symptoms get worse) and remissions (periods when symptoms are better). During the relapses (also called “flares,” “exacerbations” or “attacks”), the patients perceive a loss of function or the development of new symptoms. During the remissions, these symptoms fully or partially disappear. This most common type of MS affects 80-85% of MS patients at onset. Among those aged 20-40, it is twice as common in women as in men. The pathological hallmark is primarily the inflammation, and the neurodegeneration is secondary [8].

- About 50% of all RRMS patients convert to secondary progressive MS (SPMS) within 10 years, and 90% within 25 years of the disease onset. In the early phases of SPMS, patients may still experience some relapses, but in a short time these merge into a general progression.
- Progressive-relapsing MS (PRMS) is progressive from the disease onset, but with superimposed relapses. There is a significant recovery immediately following a relapse, but between relapses there is a gradual worsening of the symptoms. As the least common type, it affects 5% of MS patients.
- In a smaller subset of patients, the relapsing phase is not observed and the disease worsens slowly but steadily from the onset (primary progressive form, PPMS), though the rate of worsening varies greatly individually. Men are as likely as women to develop it, and the initial disease activity is in the spinal cord rather than in the brain. PPMS affects about 10-15% of MS patients and is most common after the age of 40. In this subtype, the neurodegeneration is the driving force [8].

Clinical Severity Definitions

- The consensus definition of benign MS (BMS) is as follows: the patients have still not suffered any serious, enduring disability 10 years after the disease onset [9].
- Malignant MS: this is a condition with a rapid progressive course, leading to significant disability in multiple neurologic systems [6].

I.3. Etiology

The appearance of the disease is determined by a combination of exogenous factors in genetically susceptible individuals. Studies show that MS is more common in certain parts of the world. If an individual moves from an area with higher risk to one of lower risk, the risk of the new home is acquired if the move occurs prior to adolescence, but there is not a consistent increase as concerns migration in the opposite direction [10, 11]. Exposure to certain environmental agents (at a population level rather than in the familial microenvironment) before puberty may predispose a person to the disease. Additionally, MS is a disease of temperate climates: its prevalence increases with the distance from the equator in both hemispheres.

The pivotal data supporting the genetic influence on MS susceptibility emerged from early observations of the disease which demonstrated the unusual intrafamily occurrence of MS, and Eichhorst described MS as an “inherited transmissible disease”. The findings revealed

that first, second and third-degree relatives of subjects with MS are at an increased risk of developing the disease [12-14]. Twin studies have indicated that MS has a concordance rate of 14.5-26% in monozygotic twins (the fact that it is not 100% strongly implies the influence of environmental factors) and 2.3-5.4% in dizygotic twins. Siblings of an affected person have a 1.9-2.9% risk of developing MS [12, 15-17].

Taken together, the familial recurrence and twin concordance rates indicate that the MS-prone genotype is probably highly polygenic.

Early attempts to detect genes influencing the susceptibility to MS were very successful and quickly identified the now well-established relevance of the major histocompatibility complex (MHC), primarily derived from the class II region. It is now clear that the association of MS with the DRB1*1501 allele is almost ubiquitous, though the risk may vary, depending on which other MHC haplotype is carried in the heterozygous state [18]. GWAS and subsequent replication efforts have revealed other genes with modest effects in MS, including interleukin-7 receptor α, interleukin-2 receptor α, C-type lectin-domain family 16 member A, CD58, tumour necrosis factor receptor superfamily member 1A, interferon regulatory factor 8 and CD6 [19, 20].

In 2003, our MS Workgroup at the Department of Neurology at the University of Szeged published the results of a genome-wide screen for association in Hungarian MS patients [21], a study that represented one component of the Genetic Analysis of Multiple Sclerosis in Europeans (GAMES) project [22]. As regards the disease course, the recruited patients in the project had either the RRMS or the SPMS type of the disease, and none of them had the PPMS form. Two of the genes whose potential association emerged from these analyses were selected by our group for further analysis in this less investigated PPMS group:

I.3.1. Tumour Necrosis Factor Alpha (TNF-α)

TNF is a proinflammatory cytokine involved in the pathogenesis of infectious and autoimmune disorders, including MS. It is released by activated macrophages and lymphocytes and acts via receptors belonging in the TNF family of receptors. TNF receptors 1 and 2 trigger several signal transduction pathways, resulting in the activation of transcription factors such as nuclear factor kappa-B and cFos/cJun and leading to a number of responses including inflammation, proliferation, cell migration, apoptosis and necrosis [23].

The human TNF gene maps to chromosome 6p21.3 in the highly polymorphic MHC region. The chromosome location suggests that TNF-α single nucleotide polymorphisms (SNPs) may be involved in influencing the disease course during MHC-associated diseases such as MS.

The most widely investigated SNPs of the TNF-α gene are at positions -238, -308 and -376, all of which are guanine (G) to adenine (A) substitutions. These have been associated with numerous infectious and autoimmune diseases, such as malaria [24], rheumatoid arthritis [25] and MS [26]. Studies relating to MS have reported elevated TNF levels in active lesions in postmortem brain samples; moreover, cerebrospinal fluid (CSF) and serum TNF levels in individuals with MS are elevated as compared with unaffected individuals, correlating to the severity of the lesions. In addition, peripheral blood mononuclear cells from MS patients just prior to symptom exacerbation display an increased level of TNF secretion after stimulation as compared with cells from the same patients during remission. TNF-α is associated with clinical activity in RRMS and development of the progressive form of the disease [27-29]. Most studies to date have concerned the relevance of the TNF gene polymorphisms to MS, with conflicting results [26, 30-34].

I.3.2. Apolipoprotein E (ApoE)

ApoE plays important roles in the transport, uptake and redistribution of cholesterol, which is necessary in the repair of nerve tissue. While it primarily functions as a lipid transporter, it is also linked to atherosclerosis, cognitive function, immunoregulation, neurite outgrowth, brain trauma and infectious diseases [35]. ApoE functions on the immune system by suppressing T cell proliferation, neutrophil activation and regulating macrophage functions [36]. Moreover, ApoE suppresses the production of pro-inflammatory cytokines such as TNF-α in an isoform-specific manner (E2 > E3 > E4); conversely, the activation of macrophages by inflammatory stimuli (for instance, TNF-α) is simultaneously accompanied by the downregulation of ApoE production. The APOE gene (APOE) is additionally involved in neurodegenerative diseases; the best-known association is that between the APOE ε4 allele and Alzheimer's disease [37-39].

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 corresponding protein variants ApoE2, E3 and E4 are distinguishable by having different combinations of the amino acids arginine and cysteine at these positions [35, 40] (Table 1).

Table 1. The main differences among ApoE isoforms

Isoform	Aminoacid residues	
	112	158
ApoE 2	Cysteine	Cysteine
ApoE 3	Cysteine	Arginine
ApoE 4	Arginine	Arginine

ApoE is produced in various organs and tissues, predominantly in the liver (presumably accounting for 60% to 70% of the plasma ApoE), followed by the astrocytes, which are the main ApoE-producing cells in the brain, macrophages and non-myelinating Schwann cells, etc. ApoE3 seems to be the normal form, while ApoE2 and ApoE4 can each be dysfunctional. The protein is a glycoprotein that contains 299 amino acids, with a molecular weight of 34.2 kDa.

The literature reports on the role of APOE in MS are controversial (Table 2). Moreover, no Hungarian data are available regarding the APOE status of MS patients. It was our general hypothesis that APOE genotypes can influence the mechanism of maintenance and repair of the nervous system, leading to distinct clinical courses.

Table 2. Summary of studies investigating the association between APOE and MS

First author	Country	Number of MS subtype patients	Findings
Al-Shammri [41]	Kuwait	33 RRMS; 5 PRMS; 1 benign MS	no association (only a trend) between disease severity and ε4 allele
Ballerini [42]	Italy	32 CPMS; 34 stable MS	the ε2 allele has a protective role against the onset of the progression form
Bonetti [43]	Finland	459 MS trio families	no association
Chapman [44]	Israel	47 RRMS	the ε4 allele increases the rate of disease progression, but no association with an increased risk of MS or relapses
Chapman [45]	Israel	172 RRMS; 31 SPMS; 2 PPMS	the ε4 allele is associated with the faster progression of disability
Cocco [46]	Sardinia	773 RRMS; 98 PPMS	a gender-specific association between ε4 and PP
Evangelou [47]	UK	52 RRMS; 32 SPMS; 11 PPMS	the ε4 allele is associated with more rapid progression
Fazekas [48]	Austria	76 RRMS; 5 SPMS; 2 PP	more extensive tissue destruction or less efficient repair in carriers of the ε4 allele
Fazekas [49]	Austria	253 RR; 97 SP; 24 PPMS	an association of ε4 allele with a more severe course
Ferri [50]	Italy	161 RR	no association with the occurrence of MS
Høgh [51]	Denmark	104 RRMS; 29 PPMS; 105 SPMS	the ε4/ε4 genotype is a risk for the development of MS and faster disease progression
Kantarci [52]	Turkey	221 MS	a gender-specific association between APOE ε2 and lesser disease severity
Masterman [53]	Sweden	124 benign; 140 severe	no significant differences between benign and severe MS
Mustafina [54]	Russia	120 MS patients	the APOE 2/3 genotype is associated with a low risk of MS development in women
Niino [55]	Japan	95 RRMS; 40 SPMS	no association with disease progression
Pinholt [56]	Denmark	249 RRMS; 94 SPMS; 42 PPMS	no influence on the development of MS, but the ε4 allele is associated with faster progression
Portaccio [57]	Italy	75 RRMS; 40 BMS; 49 SPMS; 9 PPMS	no association with disease course and severity
Santos [58]	Portugal	34 PPMS; 184 other forms	the ε4 allele is associated with disease progression only in a subset of patients with a disease duration of <10 years
Savettieri [59]	Italy	319 RRMS; 90 SPMS; 19 PPMS	an association between the ε2 allele and longer disease duration
Schmidt [60]	USA	379 RRMS; 30 PPMS; 182 SPMS; 19 PRMS	an association between ε4 and a more severe form and between ε2 and mild disease
van der Walt [61]	Australia	663 RRMS; 343 SPMS; 102 PPMS	no association between the phenotype and APOE genotype
Weatherby [62]	UK	162 RRMS; 188 SPMS; 20 PPMS	no association with susceptibility or clinical course
Zwemmer [63]	The Netherlands	159 RRMS; 159 SPMS; 90 PPMS	“no major association” with disease characteristics and MRI findings

I.4. Fatigue

Definition

Aaronson et al. proposed the following definition of fatigue: "The awareness of a decreased capacity for physical and/or mental activity due to an imbalance in the availability, utilization, and/or restoration of resources needed to perform activity" [64]. Patients report that they are well during the first few hours of the day, but by afternoon feel completely exhausted; often having a short rest helps them to recover.

Prevalence

Fatigue is one of the most frequent complaints of patients with MS [65-70] and the majority of people with this illness often experience it chronically. Egner et al. stated that all of their examined MS patients reported symptoms of fatigue at some point after diagnosis; moreover, some of them reported that it had lasted for more than 1 year, and 80% of them consistently experienced severe fatigue [71]. Fatigue appears to be unrelated to the disability status and many patients complain of fatigue even when other symptoms are in complete remission. Patients with BMS can experience the same degree of fatigue as patients with disabling disease [72].

Etiology

The cause of MS fatigue is poorly understood; its background may include changes in neurotransmitters or cytokines (IL-6, TNF-α, etc.), and a dysfunction of premotor, limbic or basal ganglia, disturbances of the neuroendocrine axis, and changes in serotonin pathways or other neurotransmitters [73, 74]. Functional brain imaging studies using positron emission tomography (PET) and single photon emission computed tomography (SPECT) indicate that MS is associated with a widespread hypometabolism, extending to the cerebral cortex, subcortical grey matter nuclei, and periventricular white matter [75-77]. Roelcke et al. showed that hypometabolism in the bilateral prefrontal cortex and basal ganglia was associated with MS fatigue, implicating a role for cortical-subcortical pathways. Taken together, these findings suggest that MS fatigue is related to impaired interactions between functionally related cortical and subcortical areas. Fatigue can be caused by a disease process itself (primary fatigue) or by infections, depression, insomnia, etc. (secondary fatigue). Since the causes of secondary fatigue are generally treatable, it is important to distinguish the two types [78].

Diagnosis

Fatigue affects cognitive, physical and social aspects of life. The diagnosis of MS fatigue includes the presence of fatigue symptoms on at least 50% of the days for more than 6 weeks. The differential diagnosis of MS fatigue includes depression, physical disability, and side-effects of medications (e.g. disease-modifying therapy). Fatigue is a subjective experience and consequently it can be difficult to measure. Self-report questionnaires are useful to set up the diagnosis. Thirty fatigue questionnaires are available [79], but only two are most generally used: the Fatigue Impact Scale (FIS) [65, 80] and the Fatigue Severity Scale (FSS) [81]. An objective questionnaire for evaluation of the impact of fatigue in Hungarian MS patients has not yet been approved. On the basis of our previous experience with the adaptation and validation process of the Multiple Sclerosis Quality of Life Instrument [82], we set out to validate one of the two most generally used fatigue questionnaires. We selected the multidimensional FIS since the FSS is a one-dimensional 9-item scale which evaluates fatigue more briefly and appears less relevant. The FIS contains 40 questions, 10 of which relate to cognitive, 10 to physical, and 20 to social subscales (Copyright 1991, J.D. Fisk, P.G. Ritvo and C.J. Archibald). Each question is scored with from 0 (minimal degree) to 4 (severe degree) points. The FIS is a retrospective tool; it measures the impact of fatigue over the past month.

Treatment

Several medications have been studied for this indication, including the dopaminergic agent amantadine, the amphetamine-related stimulant pemoline, the wake-promoting agent modafinil, the potassium channel-blocking aminopyridines and *Prokarin*, a proprietary blend of histamine and caffeine [83].

II. Aims

Our primary aim was to investigate the possible influence of the TNF-α -376 polymorphism and the APOE gene polymorphism within exon 4 at codons 112 and 158 on the susceptibility to PPMS in Hungary in a multicentre survey and to compare the PPMS genotype with those of RRMS patients and healthy controls (HCs).

Furthermore, we set out to test the validity, including the internal consistency, test-retest reliability and construct validity, of the Hungarian version of the FIS in HC and MS populations.

III. Patients and Methods

III.1. Genetic Analysis

III.1.1. Patients

The study protocol was approved by the local ethics committee (No. 16/2006) and written in accordance with the Helsinki Declaration. Over a period of one year (between 2006 June and 2007 June), participation in the study was offered to the PPMS patients who were consecutively referred to one of the 5 involved Hungarian MS Centres at the regular check-up every 5 months. After written informed consent had been provided, blood was collected from 45 PPMS patients, 45 age- and sex-matched RRMS patients and 45 HCs. The last two groups served as control groups for the PPMS group. Thirteen of the 45 PPMS patients were followed up by the MS Outpatient Unit of the Department of Neurology at the University of Szeged, 12 patients by the Department of Neurology at the University of Pécs, 7 patients by the Department of Neurology at Ferenc Jahn Hospital in Budapest, 7 patients by the Department of Neurology at the County Hospital in Kecskemét, and 6 patients by the Department of Neurology at the University of Debrecen. The 45 RRMS patients and the HCs were from Szeged. Clinical data such as the Expanded Disability Status Scale (EDSS) [84], date of birth, year of diagnosis and onset of the disease, were collected by using the up-to-date MS register; the progression index (PI; the ratio between the EDSS and the disease duration in years) and the Multiple Sclerosis Severity Score (MSSS) [85] were also determined. The MSSS gives a hint as to the severity of the disease, expressed numerically via the level of disability and the disease duration. The RRMS patients met the McDonald diagnostic criteria [86]. The PPMS patients had undergone at least one year of disease progression and exhibited a positive brain or spinal cord magnetic resonance imaging (MRI) or positive CSF picture [87]. The patients were relapse-free and had not been taking steroids for at least 1 month before the assessment. To the best of our knowledge, a central database of healthy controls for genetic research is not available in Hungary. We therefore offered study participation to age- and sex-matched attendants and relatives of MS patients and to healthy staff at the clinic. If the health inclusion criteria were satisfied (no current acute or chronic physical or mental illness), the participant signed an informed consent form.

III.1.2. Methods

DNA isolation

Genomic DNA was isolated from 1 ml EDTA-treated blood. For extraction, the Genisol™ Maxi-Prep Kit (ABgene House, Epsom, UK) was used.

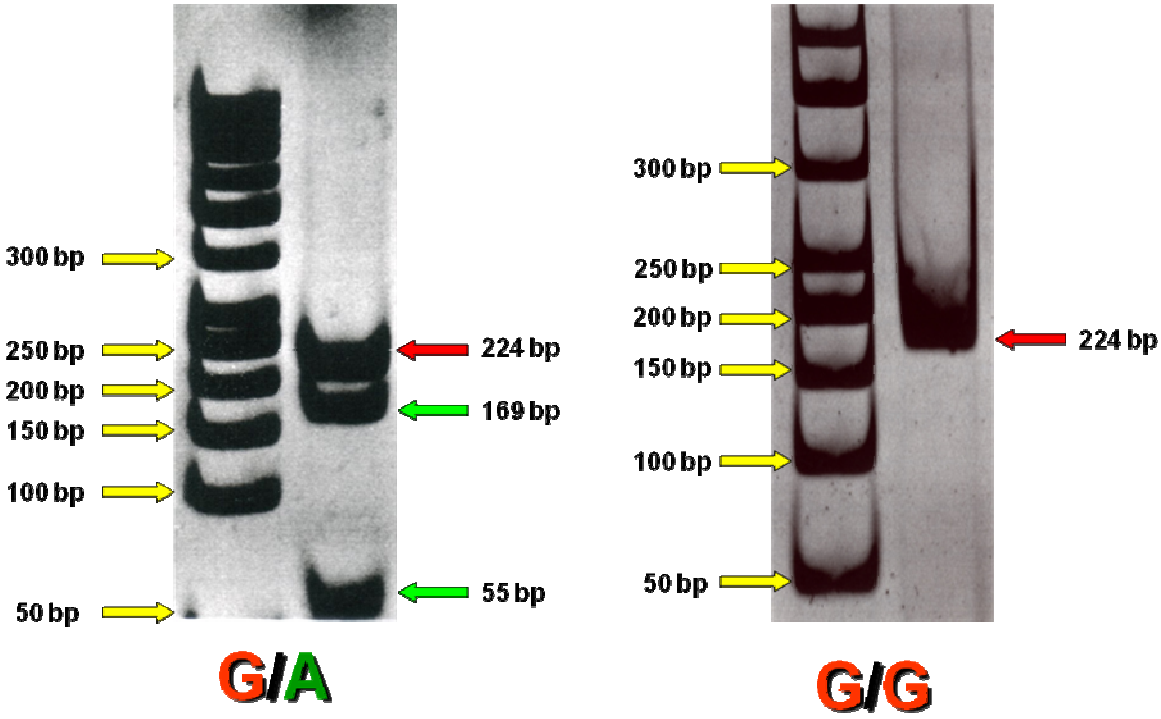
Polymerase Chain Reaction (PCR) and Allelic-Discrimination Restriction Fragment Length Polymorphism (RFLP)

The reactions were performed on a 2720 Thermal Cycler (produced by PE Applied Biosystems). The oligonucleotides were from the Biological Research Centre, Hungarian Academy of Sciences, Szeged. As an allelic imbalance can alter the findings, we usually measured the samples 3 times, but more times (4-6) in the event of need.

TNF-α

To determine the polymorphism at position -376 in the TNF-α promoter and the zygosity status of the individuals, we applied a PCR system. The relevant fragments of 224 basepairs (bp) were amplified by using the forward primer 5'-TTTCTGAAGCCCCTCCCAGTTC-3' and the reverse primer 5'-TACCCCTCACACTCCCCATCC-3'. The primers created a restriction site for the TasI enzyme (Tsp509I, Fermentas) (5'-^AATT-3'). PCR products were digested overnight at 65°C under paraffin oil in a capped vial. The digestion products were resolved on a polyacrylamide gel and detected under ultraviolet light after staining with ethidium bromide. If G was present at the -376 position, no digestion occurred and only a single 224 bp fragment could be detected on the gel; if A was at that position, the digestion yielded 2 fragments, of 169 and 55 bp (Figure 1). To test the TasI activity, a positive control was used for each run.

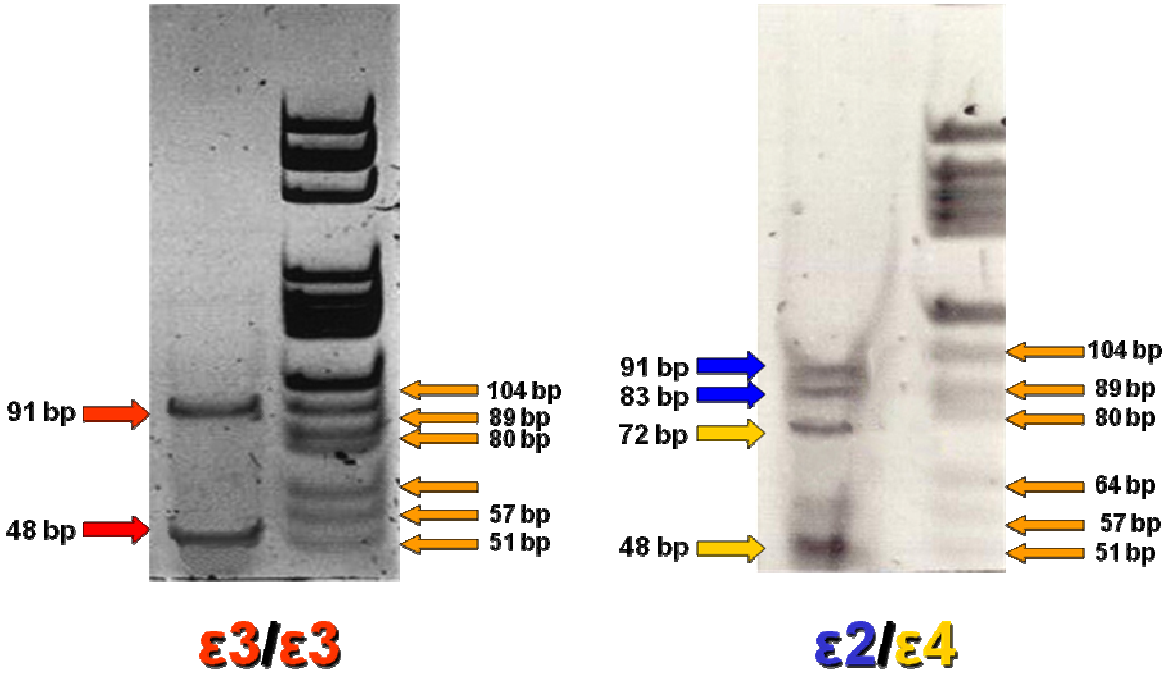
Figure 1. The products of the amplified fragment of TNF-α gene after digestion with TasI enzyme



APOE

To determine the APOE genotypes, the primers 5'-TCCAAGGAGCTGCAGGCG-3' and 5'-CCGGCCTGGTACACTGCC-3' were used. The primers created a restriction site for the Hin6I enzyme (Fermentas) (5'-G^CGC-3'). The digestion products were resolved and detected as in the case of TNF-α. The ε2 allele gave visible fragments of 91 and 83 bp, the ε3 allele gave fragments of 91 and 48 bp, and the ε4 allele gave fragments of 72 and 48 bp (Figure 2).

Figure 2. The products of the amplified fragment of APOE after digestion with Hin6I enzyme



III.1.3. 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*). We calculated the statistical power of Fischer's exact test by using the R 2.8.0 software package. The strengths of association were given as odds ratios (OR), and their 95% confidence intervals (CI) too were calculated. The level of significance was chosen to be $p<0.05$.

As concerns the APOE genotype, the t-test and variance analysis were used to detect the differences between the three groups as regards the demographic and clinical parameters. We grouped the subjects according to the presence or absence of the ε2, ε3 or ε4 alleles because the sample size did not allow making groups of all possible genotypes. The Pearson χ^2 test was performed to study the distribution of the alleles by the investigated groups. The *p* values were calculated on the basis of adjusted residual values. The combined effect of the MS course and the alleles on the clinical parameters was analysed by two-way analysis of variance. Because of the multiple comparisons, the level of significance was chosen to be $p<0.005$. For statistical analyses, the SPSS 15.0 statistical package was used.

III.2. Validation of the Fatigue Impact Scale

III.2.1. Patients and Methods

The original English scale was translated into Hungarian independently by two specialists located in Hungary, working for the MAPI Institute [88] the area of expertise is the linguistic validation of patient-reported outcome instruments; these were then back-translated to English by one other specialist. The Institute has worked in close collaboration with Professor Fisk (the developer of the FIS) to elaborate a list of concepts. The aim of this list was to clarify the notions investigated through each item of the original questionnaire in order to obtain an appropriate reflection of these in each language version produced and to enhance harmonization across all language versions and to explain the conceptual notions underlying each item in clear and plain language and to provide acceptable translation alternatives.

Some problematic items relating to the Hungarian version of the FIS resulted in translation issues, which were resolved after discussions. For instance, Item 10 (I am more clumsy and uncoordinated); the developer had confirmed that the term "uncoordinated" should refer only to movement and not to any cognitive ability. It has been agreed to use the alternative "my movements are more awkward", which was confirmed to render the intended concept in an idiomatic and easily understandable way for Hungarian lay people. Item 20 (Normal day-to-day events are stressful for me): It was confirmed by the developer that “stressful” only refers to mental stress, which would not be perceived by people who do not suffer from fatigue. It was agreed not to use the direct Hungarian equivalent of “stressful”, which would have referred to both mental and physical stress and implied stressful situations that can be perceived by anybody. The alternative “put mental strain” has been substituted instead, hence adding “mental” explicity, in order to guarantee conceptual equivalence with the English original. For further information, please contact the MAPI Research Trust. Link: http://www.mapi¬trust.org/services/questionnairelicensing/cataloguequestionnaires/123-fis.

After detailed consultations with the MAPI Trust, our MS Group obtained the right to test the FIS questionnaire in the Hungarian HC and MS population (please see the Hungarian version of the FIS attached in Appendix I). The study, conducted at the Multiple Sclerosis Outpatient Unit of the Department of Neurology in Szeged between May and November 2008, was approved by the local ethics committee (protocol no. 45/2008) and performed in

accordance with the 1964 Declaration of Helsinki. In Hungary, a central database of HCs for the completion of questionnaires is not available. We therefore offered study participation to age- and sex-matched attendants and relatives of MS patients and to healthy staff at the clinic. If the health inclusion criteria were satisfied (no current acute or chronic physical or mental illness), the participant signed an informed consent form. The inclusion criterion for patients was a diagnosis of MS in accordance with the McDonald diagnostic criteria [86]. The exclusion criteria were a relapse during the previous month; the use of medication which can induce fatigue; or the presence of secondary fatigue [78]. For both HC and MS subjects, questionnaires were completed at interviews on two occasions 3 months apart.

After checks on the inclusion and exclusion criteria, medical interviews and neurological examinations of the participants were carried out by two neurologists at the MS Outpatient Unit. Besides the FIS questionnaire, the Hungarian version of the Beck Depression Inventory (BDI; Appendix II) was completed.

III.2.2. Statistical Analysis

The Student t-test was used to test the difference between the groups as regards mean age, and χ^2 statistics as regards gender, educational status and marital status. 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. The Spearman correlation was utilized to establish the relationship between the EDSS and FIS scores.

As regards the construct validity, it was presumed that (1) the MS patients might well exhibit higher scores on each FIS subscale and the total scale than the HCs, and (2) there might be a significant positive correlation between the EDSS score and fatigue. Accordingly, the conceptual model that we set up was based on the following hypotheses: (a) for internal consistency, Cronbach's alpha and item-to-total correlations would be high; (b) for test-retest reliability, ICCs would be high, and (c) for construct validity, the MS population would give significantly higher scores than those of the HC population on each FIS subscale and the total scale (both before and after elimination of the effect of depression), and an advanced physical disability would be associated with higher FIS

scores. If these hypotheses proved to hold, the Hungarian version of the FIS would be considered valid.

IV. Results

IV.1. TNF-α

The clinical characteristics data for the three examined groups are displayed in Table 3. As the groups were age- and sex-matched, there were no differences by gender or mean age (p=0.998). The age at onset in the PPMS patients was higher and the disease duration was shorter than in the RRMS patients but not significantly so (p=0.404 and p=0.316, respectively). In the PPMS form, the EDSS score, the PI and the MSSS values were higher as compared with those for the RRMS group (p<0.001 in all three cases).

Table 3. Clinical characteristics of the three examined groups

Group	Patients number Male/Female	Age (years)	Age at onset (years)	Disease duration (years)	EDSS	PI	MSSS
PPMS	23/22	49.1 ± 10.5	38.0 ± 9.4	11.1 ± 8.9	5.7 ±1.8	0.96 ± 1.13	7.2 ± 2.0
RRMS	23/22	49.2 ± 9.8	36.3 ± 9.5	12.9 ±8.0	2.8 ± 1.9	0.32 ± 0.35	3.2 ± 2.5
HC	23/22	49.0 ± 10.6	-	-	-	-	-
p	-	0.998	0.404	0.316	<0.001	<0.001	<0.001

Values are given as means ± standard deviation (SD).

The distributions of the genotype and allele frequencies of the TNF-α -376 polymorphism in the MS patients and the HC are displayed in Table 4.

Table 4. Genotype and allele frequencies of TNF-α -376 gene polymorphism

TNF-α gene	PPMS (n=45)	RRMS (n=45)	HC (n=45)
Genotype			
GG	43 (95.6%) ♦	41 (91.1%)	35 (77.8%) ♦
AG	2 (4.4%) ♦	4 (8.9%)	10 (22.2%) ♦
AA	0	0	0
Allele			
G	88 (97.8%) ▪	86 (95.6%)	80 (88.9%) ▪
A	2 (2.2%) ▪	4 (4.4%)	10 (11.1%) ▪

♦= exact p=0.027; ▪=exact p=0.032

We could not detect the AA homozygote genotype in either the MS patients or the HCs. For the GG genotype, a statistically significant higher level was found in the PPMS group as compared with the HCs (χ^2=6.154; df=1; *p*=0.013; exact *p*=0.027; OR=6.143; CI=1.26-29.90). As regards the G allele, a significant difference was observed between the PPMS and HC groups (χ^2=5.714; df=1; *p*=0.017; exact *p*=0.032; OR=5.5; CI=1.17-25.86). The GA genotype was underrepresented in the PPMS group relative to the HCs (χ^2=6.154; df=1; *p*=0.013; exact *p*=0.027; OR=6.143; CI=1.26-29.90); for the A allele, the distribution was similar (χ^2=5.714; df=1; *p*=0.017; exact *p*=0.032; OR=5.5; CI=1.17-25.86). No significant differences in genotype were found between the RRMS and HC groups or between the RRMS and PPMS groups (χ^2=3.045; df=1; *p*=0.081; exact *p*=0.144 and χ^2=0.714; df=1; *p*=0.398; exact *p*=0.677, respectively). The distributions of the alleles in the groups were similar (RRMS-HC: χ^2=2.788; df=1; *p*=0.095; exact *p*=0.162; RRMS-PPMS: χ^2=0.690; df=1; *p*=0.406; exact *p*=0.682).

The genotype did not influence the clinical characteristics. No association was found between the genotype status of the TNF-α -376 polymorphism and the age at onset, the disease duration, EDSS, PI or MSSS (Table 5).

Table 5. Relationship between genotype status and clinical characteristics

Genotype	Age at onset (years)	Disease duration (years)	EDSS	MSSS	PI
GG (n=84)	37.3 ± 9.5	12.0 ± 8.6	4.2 ± 2.3	5.3 ± 3.0	0.7 ± 0.9
AG (n=6)	34.7 ± 10.0	12.0 ± 7.5	3.7 ± 2.0	4.5 ± 2.8	0.4±0.4
AA (n=0)	-	-	-	-	-
p	0.556	0.997	0.556	0.529	0.556

Values are given as means ± SD.

We calculated the statistical power of Fischer's exact test: the mean value was 0.64±0.09. The statistical power was also calculated for the double headcount of RRMS and HC: the value was 0.8±0.11.

IV.2. APOE

The examined groups were the same as in the case of TNF-α; consequently, the clinical characteristics data for the three examined groups can be found in Table 3. The population analysed was in Hardy-Weinberg equilibrium, because the value of the goodness-of-fit test was p=0.082 (χ^2=9.78, d.f.=5). The distribution of all APOE genotypes by the investigated groups is displayed in Table 6. Neither the ϵ2/ϵ2 nor the ϵ4/ϵ4 homozygote genotype was detected.

Table 6. The distribution of all APOE genotypes by the investigated groups

APOE gene	**MS patients**			**Total**
	PPMS (*n*=45)	**RRMS (*n*=45)**	**HC (*n*=45)**	
Genotype				
ε2/ε2	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
ε2/ε3	3 (6.7%)	17 (37.8%)	17 (37.8%)	37 (27.4)
ε2/ε4	1 (2.2%)	5 (11.1%)	0 (0.0%)	6 (4.4%)
ε3/ε3	18 (40.0%)	17 (37.8%)	24 (53.3%)	59 (43.7%)
ε3/ε4	23 (51.1%)	6 (13.3%)	4 (8.9%)	33 (24.4%)
ε4/ε4	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Total	45 (100.0%)	45 (100.0%)	45 (100.0%)	135 (100.0%)

Due to the low number of subjects with absence of the ε3 allele (5 RRMS and 1 PPMS patients), grouping patients by this allele was not possible. Tables 7 and 8 show the occurrence of the ε2 and ε4 alleles by the investigated groups.

Table 7. The occurrence of the ε2 allele by the investigated groups (Pearson χ^2 p<0.001)

		HC	**PPMS**	**RRMS**	**Total**
non ε2	**Count**	28	41	23	92
	% within group	62.2	91.1	51.1	68.1
	Adjusted residual (*p*)	-1.0 (0.317)	4.0 (<0.001)	-3.0 (0.003)	
ε2	**Count**	17	4	22	43
	% within group	37.8	8.9	48.9	31.9
	Adjusted residual (*p*)	1.0 (0.317)	-4.0 (<0.001)	3.0 (0.003)	
Total	**Count**	45	45	45	135
	% within group	100.0	100.0	100.0	100.0

Table 8. The occurrence of the ε4 allele by the investigated groups

		HC	PPMS	RRMS	Total
non ε4	Count	41	21	34	96
	% within group	91.1	46.7	75.6	71.1
	Adjusted residual (*p*)	3.6 (<0.001)	-4.4 (<0.001)	0.8 (0.424)	
ε4	Count	4	24	11	39
	% within group	8.9	53.3	24.4	28.9
	Adjusted residual (*p*)	-3.6 (<0.001)	4.4 (<0.001)	-0.8 (0.424)	
Total	Count	45	45	45	135
	% within group	100.0	100.0	100.0	100.0

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 ($p<0.003$) (Table 7). 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 (Table 8). 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 ($p<0.05$) and was twice as frequent in the PPMS group as in the RRMS group ($p<0.005$).

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; Table 3). 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; Table 9). 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.

Table 9. The mean values of the measured clinical parameters by MS groups depend on the presence or not of the ε2 or ε4 alleles

	PPMS				RRMS				PPMS and RRMS			
	N=41	N=4	N=45	*p*	N=23	N=22	N=45	*p*	N=64	N=26	N=90	*p*
	non ε2	ε2	Total		non ε2	ε2	Total		non ε2	ε2	Total	
Disease duration (year)	11.2±8.9	10.3±10.2	11.1±8.9	0.872	12.7±6.9	13.1±9.2	12.9±8.0	0.842	11.7±8.2	12.7±9.2	12.0±8.5	0.618
EDSS	5.6±1.8	6.3±2.1	5.7±1.8	0.586	3.0±2.1	2.6±1.7	2.8±1.9	0.429	4.7±2.2	3.2±2.2	4.2±2.3	**0.004**
PI	1.0±1.2	0.6±0.6	1.0±1.1	0.475	0.3±0.3	0.4±0.4	0.3±0.4	0.456	0.7±1.0	0.4±0.5	0.6±0.9	0.093
MSSS	7.2±2.1	7.0±0.8	7.2±2.0	0.858	3.5±2.6	3.0±2.4	3.2±2.5	0.534	5.9±2.9	3.6±2.6	5.2±3.0	**0.001**
	PPMS				**RRMS**				**PPMS and RRMS**			
	N=21	N=24	N=45	*p*	N=34	N=11	N=45	*p*	N=55	N=35	N=90	*p*
	non ε4	ε4	Total		non ε4	ε4	Total		non ε4	ε4	Total	
Disease duration (year)	10.7±8.4	11.5±9.5	11.1±8.9	0.768	14.2±8.0	8.8±6.9	12.9±8.0	0.052	12.9±8.3	10.6±8.7	12.0±8.5	0.226
EDSS	5.6±1.9	5.7±1.7	5.7±1.8	0.934	3.0±1.9	2.1±1.8	2.8±1.9	0.169	4.0±2.3	4.6±2.4	4.2±2.3	0.287
PI	0.9±0.9	1.0±1.3	1.0±1.1	0.819	0.3±0.4	0.3±0.3	0.3±0.4	0.851	0.6±0.7	0.8±1.2	0.6±0.9	0.242
MSSS	7.0±2.2	7.4±1.8	7.2±2.0	0.530	3.3±2.5	3.0±2.7	3.2±2.5	0.759	4.7±3.0	6.0±2.9	5.2±3.0	0.045

Values are given as means ± SD.

Two-way analysis of the combined effect of the two variables (the presence or absence of the alleles and the type of MS) revealed that the difference in the clinical parameters can only be attributed to the type of MS (Table 10). Interaction between these variables could not be detected.

Table 10. The results of two-way analysis of variance

		p
Dependent variable: EDSS		**-**
Grouping variables	**Type of MS**	**<0.001**
	ε2	0.866
	Type of MS* ε2	0.327
Dependent variable: MSSS		**-**
Grouping variables	**Type of MS**	**<0.001**
	ε2	0.631
	Type of MS* ε2	0.838

IV.3. Fatigue

Ninety-nine of the 111 MS patients (89%) and 79 of the 85 HC subjects (93%) completed the scales on both occasions. Demographic and clinical data are presented in Table 11.

Table 11. Demographic and clinical data on MS patients and HCs

		MS patients (N=111)	**HCs (N=85)**
Age (mean±SD), years		43.82±11.62	41.59±12.42
Gender	Female	83 (75%)	59 (69%)
	Male	28 (25%)	26 (31%)
Education status	Elementary school	8 (7%)	7 (8%)
	Middle school	26 (23%)	16 (19%)
	High school	50 (45%)	36 (42%)
	University	26 (24%)	26 (31%)
	Not indicated	1 (1%)	0 (0%)
Marital status	Single	25 (23%)	27 (32%)
	Married	66 (59%)	47 (55%)
	Divorced	13 (12%)	10 (12%)
	Widowed	6 (5%)	1 (1%)
	Not indicated	1 (1%)	0 (0%)
Disease duration (mean±SD), years		11.12±8.29	-
EDSS (mean±SD)		1.94±1.37	-

There were no statistically significant differences between the MS and HC groups as regards mean age (t=1.293, p=0.259), gender (χ^2=0.694, p=0.424), educational status (χ^2=1.547, p=0.671) or marital status (χ^2=4.097, p=0.251). Because of the relatively high standard deviations, t-tests and non-parametric Mann-Whitney tests were also performed when the differences in the total and subscale scores were examined. As no significant differences were seen, only covariance analysis was used to investigate the differences after elimination of the effect of depression (Table 12).

Table 12. Validity of the FIS before and after elimination of the effect of depression with parametric and non-parametric statistical methods

FIRST SESSION					
	MS patients (N=111)	**HC subjects (N=85)**	***p*$_1$ value with t-test (with depression)**	***p*$_1$' value with Mann-Whitney U test (with depression)**	***p*$_1$'' value with covariance analysis (after eliminating depression)**
Cognitive subscale	12.2 ± 10.4	8.1 ± 8.5	0.004	0.01	**0.531**
Physical subscale	18.0 ± 11.7	6.5 ± 7.3	< 0.001	< 0.001	<0.001
Social subscale	27.3 ± 20.9	13.6 ± 15.9	< 0.001	< 0.001	0.017
Total	55.8 ± 41.2	25.7 ± 28.1	< 0.001	< 0.001	0.001
BDI$_1$	11.9 ± 8.8	7.0 ± 7.0	< 0.001	< 0.001	-
SECOND SESSION					
	MS patients (N=99)	**HC subjects (N=79)**	***p*$_2$ value with t-test (with depression)**	***p*$_2$' value with Mann-Whitney U test (with depression)**	***p*$_2$'' value with covariance analysis (after eliminating depression)**
FIS$_2$	**-**	**-**	**-**	**-**	
Cognitive subscale	13.6 ± 11.1	8.2 ± 9.7	0.001	0.001	**0.639**
Physical subscale	18.4 ± 12.1	8.1 ± 9.2	< 0.001	< 0.001	<0.001
Social subscale	28.9 ± 22.8	14.8 ± 18.0	< 0.001	< 0.001	**0.054**
Total	60.9 ± 44.6	29.5 ± 35.8	< 0.001	< 0.001	0.024
BDI$_2$	12.2 ± 9.5	6.8 ± 7.5	< 0.001	< 0.001	-

Values are given as means ± SD.

The total FIS scores were statistically higher in the MS group in both sessions (p_1<0.001; p_2<0.001; p_1'<0.001; p_2'<0.001), and after elimination of the BDI scores (p_1''=0.001; p_2''=0.024). The cognitive subscale scores were statistically higher in the MS group than in the HCs in both FIS sessions (p_1=0.004; p_2=0.001; p_1'=0.01; p_2'=0.001), but this disappeared after elimination of the effect of depression (p_1''=0.531; p_2''=0.639). Significantly higher physical subscale scores were found in the MS group in both sessions (p_1<0.001; p_2<0.001; p_1'<0.001; p_2'<0.001). The relationship remained significant after elimination of the BDI scores (p_1''<0.001; p_2''<0.001). Significantly higher social subscale scores were observed in the MS group (p1<0.001; p_2<0.001; p_1'<0.001; p_2'<0.001). After elimination of the effect of depression, the difference between the groups remained statistically different only in the first session (p_1''=0.017; p_2''=0.054).

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 (Table 13).

The EDSS score showed statistically significant associations with the total-, all sub- and BDI scores at both assessments. The Spearman correlation coefficients ranged from 0.309 to 0.502 and their pertinent p values were 0.003 or lower.

Table 13. FIS_1 item-specific statistics for patients and healthy subjects

Subscales		MS group			HCs		
	Item number	Mean	SD	Corrected item-total correlation	Mean	SD	Corrected item-total correlation
Cognitive subscale	1	1.52	1.11	0.766	0.92	0.91	0.623
	5	1.40	1.23	0.775	0.68	0.92	0.730
	6	1.07	1.15	0.703	0.62	0.93	0.770
	11	1.38	1.26	0.776	0.80	0.92	0.772
	18	1.12	1.08	0.825	0.46	0.81	0.904
	21	1.01	1.07	0.831	0.61	0.87	0.818
	26	1.01	1.13	0.829	0.62	0.85	0.812
	30	1.08	1.19	0.817	0.59	0.77	0.791
	34	1.17	1.26	0.761	0.76	0.96	0.866
	35	1.22	1.24	0.811	0.83	0.88	0.810
Physical subscale	10	1.64	1.32	0.839	0.49	0.91	0.785
	13	1.76	1.21	0.818	0.59	0.86	0.850
	14	1.73	1.25	0.888	0.68	0.89	0.819
	17	1.97	1.33	0.846	0.61	0.92	0.799
	23	1.84	1.37	0.819	0.65	0.80	0.740
	24	1.67	1.38	0.898	0.68	0.89	0.771
	31	1.89	1.32	0.888	0.56	0.81	0.803
	32	1.11	1.21	0.754	0.32	0.58	0.521
	37	1.84	1.36	0.836	0.52	0.81	0.884
	38	1.86	1.29	0.820	0.75	0.81	0.750
Social subscale	2	1.15	1.17	0.780	0.65	0.90	0.718
	3	1.79	1.30	0.867	0.76	1.05	0.703
	4	1.37	1.02	0.589	0.92	1.08	0.708
	7	1.70	1.36	0.903	0.77	1.00	0.711
	8	1.40	1.28	0.812	0.51	0.98	0.819
	9	1.38	1.27	0.837	0.63	0.98	0.797
	12	1.50	1.13	0.714	1.07	1.07	0.768
	15	1.38	1.28	0.818	0.76	0.95	0.658
	16	1.25	1.25	0.853	0.51	1.00	0.809
	19	1.09	1.22	0.769	0.51	0.83	0.731
	20	1.11	1.16	0.824	0.63	0.80	0.846
	22	0.95	1.09	0.819	0.48	0.71	0.773
	25	1.32	1.25	0.874	0.63	0.91	0.791
	27	1.29	1.39	0.876	0.55	0.89	0.747
	28	1.55	1.56	0.859	0.58	1.02	0.801
	29	1.33	1.34	0.604	0.61	0.78	0.713
	33	0.90	1.16	0.756	0.62	0.82	0.717
	36	1.36	1.31	0.905	0.56	0.92	0.862
	39	1.13	1.36	0.767	0.59	0.95	0.788
	40	1.60	1.41	0.885	0.65	0.96	0.854

V. Discussion

V.1. TNF-α

The possible association between MS and TNF-α polymorphisms has already been analysed in several studies, with conflicting results [26, 31, 33, 34, 89-104]. Only four studies found any association between TNF-α -308 G/A and MS, depending on the race: one of them indicated an increased risk in an Asian population [99] and three studies showed a reduced risk in the Caucasian population, with a potentially protective effect of the rare A allele [31, 91, 98]. The last two studies were from countries neighbouring Hungary: Serbia and (jointly) Croatia and Slovenia. In our study, the PPMS group displayed a lower A allele frequency than in the HCs, indicating that carriage of this less common allele may decrease the risk of the development of this progressive subtype, though our results related to the position -376 and not to -308. As regards the TNF-α -238 G/A polymorphism, no association has been identified in the literature [26, 92, 94, 100].

Our findings demonstrated that the GG genotype and the G allele at position -376 were more frequent in the PPMS group, suggesting a possible genetic predisposition to this more progressive disease form. Four of the five papers relating to the -376 SNP did not detect any association between the SNP and MS [30, 96, 101, 105]. However, none of them examined PPMS patients. In one article, the susceptibility to MS and the A allele were reported to be correlated [26], 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 [106]. However, the sample size was lower and additionally a significant association was detected not only between the PPMS and HC groups, but also between the RRMS and HC groups, which was not confirmed in our study.

In our study, the frequency of the rare allele ranged from 2% to 11%, which is in line with international findings of 2% to 8% [24, 26]. It differs from the data on the Sardinian population, where the frequency of the A allele is markedly high [105].

This paper did not reveal significant differences between the genotypes and the EDSS, PI and MSSS scores. Thus, the protective role of the A allele was not reflected in the clinical outcome measurement scores. This is consistent with the results of certain international studies relating to EDSS [95, 97, 98, 107]; we have not found any publication in which the relationship between genotype and MSSS was examined.

The present study was based on a relatively large number of a subpopulation with a rare disease, and the patients were well classified. The prevalence of MS in the Hungarian county of Csongrád is 65 per 100,000 inhabitants, ~ 11% of the MS patients here exhibiting PPMS; on this basis, the

estimated number of PPMS patients in the country (the population of Hungary is ~ 10 million) is therefore ~ 700 [3]. Accordingly, the 45 patients in this study comprise 7% of the Hungarian PPMS population, a fairly high proportion considering the prevalence of PPMS.

We did not measure the TNF-α level in the blood or CSF. -376 GG donors are known to produce more TNF-α than do -376 GA donors [94], and patients with progressive MS have high levels of TNF-α in the CSF [29]. The patients with the G allele in this study are more likely to undergo progression, and might therefore produce more TNF-α, which can induce degradation of the myelin sheath; this may contribute to oligodendroglial cell death and demyelinization, a potential feature from the start in PPMS patients [8]. However, conflicting results have been reported, with suggestions that neither the -308 nor the -376 region is of functional relevance for the TNF-α level [108].

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. 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 [109].

V.2. APOE

APOE is associated with the prevention of neurotoxicity and repair processes in a variety of neurological disorders. The literature reports on the role of APOE in MS are controversial, with claims that the presence [44-49, 51, 54, 56, 58, 60] or absence [41, 43, 53, 55, 57, 59, 61-63, 110-112] of the APOE ϵ4 allele is connected with susceptibility to the disease or its severity. The literature information relating to the genetic background of PPMS patients is incomplete because of the low number of such patients [45, 47-49, 51, 58-60, 62]. Only three APOE analysis studies (from Sardinia, The Netherlands and Australia) involved a larger PPMS group than that in the present study (Table 2) [46, 61, 63]. 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 prevalence of MS in The Netherlands is 76 per 100,000 inhabitants [2], while Australia demonstrates considerable geographical differences in MS prevalence rates, ranging from 11 to 59 per 100,000. In Sardinia, the rate is approximately 2.3-fold higher than that in Hungary [3, 4]; the study from this area revealed that the ε4 allele increases the risk of PPMS, but only in women. The studies from The Netherlands and Australia did not confirm any association. This could arise if different disease-causing alleles are predominant in different study populations. In the Israeli patients, the APOE ε4 allele was found to be associated with faster progression, especially among those with the primary progressive form [45]. An analysis of 614 patients with MS from 379 families indicated that APOE

ε4 carriers were more likely to be involved in severe diseases [60]. Kantarci et al. found that APOE polymorphisms were associated with the disease severity in MS only in females [52], while the sex stratification in the Australian cohort failed to identify any association of the APOE ε2 or ε4 carrier status with gender [61]. The role of the APOE gene in MS has been extensively studied, but the debate remains open (Table 2). Studies on the APOE status in the Hungarian MS population have not been published so far, though the role of chromosome 19 was raised by Rajda et al. [21].

A recent meta-analysis by Burwick et al. of the results from a pooled analysis (353 PPMS cases) did not furnish any evidence of an association between the ε2 or ε4 carrier status in PPMS [110]. The pooled analysis was performed on the results of 11 published (from 10 different countries) and one unpublished article. On the other hand, this meta-analysis did not include the results from Sardinia with 98 PPMS patients [46] and from Denmark with 42 PPMS patients [56]. Cocco et al. detected a gender-specific association between the ε4 allele and the PPMS course in Sardinia, whilst Pinholt et al. found that in Denmark the ε4 allele is associated with faster progression (Table 2), independently of the gender. A study from a country geographically adjacent to Hungary (Austria) also detected an association between the ε4 allele and rapid progression (24 PPMS subjects) [49].

The present study did not consider the genotype-phenotype relationship, but in the series in the study by Masterman et al., the APOE ε3/ε4 genotype was more common in severe MS than in benign MS [53]. Fazekas et al. found that patients carrying the ε3/ε4 genotype exhibited a significantly higher black hole ratio, demonstrating the disabling effect of the ϵ4 allele [48]. The black hole ratio indicates the proportion of more severe tissue destruction among MS lesions. A study from Denmark indicated that the ϵ4/ϵ4 homozygote genotype is a risk factor for MS and determines the clinical progression [51]. However, attempts by other studies to confirm this finding failed [41, 56, 113]. One neuroimaging study also detected a negative correlation between the APOE ε allele and the disease severity [63]. An Italian study on MS patients with MRI found lower total brain volumes in ε4 allele carriers as compared with non-carriers [114], a finding which provides new evidence linking APOE-ε4-related impaired restoration with severe tissue destruction in MS. Decreased ApoE levels in MS patients relative to those in healthy subjects were detected not only in the CSF but also in the serum [115, 116]. However, other studies did not reveal such a decrease of ApoE in CSF [117], or detected the completely opposite [118]. We did not measure the ApoE levels either in the CSF or in the peripheral blood, but an extension of the study in this direction would be interesting.

Similarly as in other studies, we could not identify patients homozygous for the ϵ4 allele [45, 46, 50, 55], most probably because of the lower number of the overall examined population, and additionally the prevalence of the ε4/ε4 genotype is rare (in most cases 2.3-2.8%) [36, 61]. In contrast, 2.1-7.7% of the patients were genotyped as ϵ4/ϵ4 in studies from Denmark [51, 56],

Sweden [53] and Kuwait [41]. In general, the low frequency of ε4 homozygotes limits the analyses as concerns an independent effect of this genotype.

The frequencies of the APOE alleles vary worldwide. The predominant allele for most populations is the ε3 allele (70-80%), which is often considered to be the ancestral allele. We also found this to be the most frequent allele in our population (68.9% in the PPMS group, 63.3% in the RRMS group and 76.7% in the HCs). While the proportion of ε4 carriers increases steadily from 10-15% in southern Europe to 40-50% in the north, the proportion of ε2 allele carriers is slightly higher in central Europe than in the south or the north [35]. The distributions of the ϵ4 allele in this study exhibited a wide range, from 4.4% to 26.7%, and its frequency was outstandingly high in the PPMS group. The ϵ2 allele frequency was underrepresented in the PPMS group (only 4.4%).

As concerns the relation between the age at onset and the APOE polymorphisms, a previous study concluded that the ϵ4 allele is associated with an earlier age of onset in MS patients [45]. In addition, Huang et al. reported that female African-Americans MS patients with the APOE ε4 allele had an earlier age of onset than female Caucasian MS patients [113]. Most of the other studies did not strengthen such a positive connection [42, 47, 49]; nor did we observe any significant association between either the ϵ4 or the ϵ2 allele and the disease duration. Huang et al. also found that African American MS patients have a significantly higher progression index and a higher (though not significantly so), MSSS score than that of Caucasian.

Fewer studies have investigated the association of the ε2 allele in MS. Some authors reported evidence of a protective effect of the ε2 allele [42, 60], and in one study this appeared to be limited to women [52], but the meta-analysis by Burwick et al. disputed this [110]. However, neuroimaging results too support the possible protective effect of ε2 allele, as a decreased annual brain volume loss was observed by MRI in MS patients with the APOE ε2 allele [119]. Our results provide support for an association between carrying of the ϵ2 allele and more favourable disease parameters. This is in line with the findings of Savettieri et al. [59], Kantarci et al. [52] and a study which demonstrated that patients with the ϵ3/ϵ2 genotype had a significantly reduced and delayed risk of chronic progressive MS [42]. The potential association of the APOE ϵ3/ϵ2 genotype with a low risk of disease development has been reported, but only in Russian women [54]. The presence of the ϵ2 allele may possibly exert a protective effect against progression.

The limitations of this study are the low number of subjects of control groups; consequently, increase of the numbers of RRMS patients and HCs is clearly needed. On the other hand, further SNP assessments related to disease progression or association, and longitudinal follow-up supplemented with magnetic resonance findings are suggested for a more reliable result.

Achievement of a definitive conclusion is difficult because of the variations in the findings from the different studies, without any apparent explanation for the differences. To the best of our knowledge, clear evidence of an association of APOE polymorphism with a particular clinical subtype is not available to date. Most of the studies failed to demonstrate an association between a

particular APOE ε allele or genotype and MS subgroups, but the relatively small sample size often limited the statistical power of the research [56, 58]. A possible reason for the inconsistency of the results between studies could be haplotypic heterogeneity in the APOE gene region due to varying linkage disequilibrium with neighbouring areas in different populations. The use of different MS severity and progression measures could also contribute to the variability of results between studies.

Data from animal experiments suggest that a blood-brain barrier (BBB) dysfunction resulting from an ApoE deficiency may lead to a greater susceptibility to experimental autoimmune encephalomyelitis. Although there is no direct evidence that ApoE contributes isoform-dependently to the in maintenance of BBB 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.

V.3. Fatigue

Fatigue is a common and disabling feature of MS that may exacerbate disability and reduce the quality of life by limiting daily activities. To measure the effect of fatigue on patients with MS, we set out to validate the FIS questionnaire. This is a multidimensional questionnaire which has been validated in countries with different cultural backgrounds, e.g. Germany, Turkey, Sweden and France [120-123]; it assesses the impact of fatigue on different areas of functioning (cognitive, physical and psychosocial) rather than the severity of this complaint.

The overall score for the Hungarian sample (Table 12) was notably higher than that for the Turkish study participants [121], which may be attributed to differences in the samples as well as cultural and inevitable linguistic differences between the two languages. On the other hand, it could imply that in Hungary people generally associate fatigue with tiredness. All in all, the difference in the scores between the two national populations did not stem from depression, because the BDI scores were similar in the two studies.

Statistically significant differences were found between the MS patients and the HC volunteers in both sessions. The most noteworthy differences were detected in the physical and social subscales, while they differed least in the cognitive subscale. Similar findings were observed in the initial validation and in the Turkish version too. After elimination of the effect of depression, the significant differences in the cognitive subscales disappeared, as we expected on the basis of the Turkish study [121]. An unexpected result of this study was that the significant difference in the

social subscale disappeared in the second session after elimination of the effect of depression. The above-mentioned findings might mean that depression is associated with the cognitive aspect and possibly the social components of fatigue. Other investigators have pointed out a significant relationship between fatigue and depression in MS [67, 80, 124].

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 validations [121-123].

We chose the 3-month interval in the present study because the MS patients are followed up at our MS Outpatient Unit every 3 months. Accordingly, in order not to impose an extra burden on the patients (e.g. travel, time and costs), but to achieve a high responder rate not only on the first, but also on the second occasion, we chose the second time point as the next regular visit date. The authors of the Turkish validation of the FIS similarly used a 3-month period in their publication, which makes our data comparable with their literature data. The disadvantages of using a 3-month period could include changes in the subjects' health (a relapse in MS status, the appearance of new diseases or the introduction of medication) or a change in the intention to participate.

The evaluation of fatigue in patients with various levels of EDSS revealed a strong correlation. Most previous studies likewise demonstrated a positive association between the fatigue severity and the EDSS score [66, 68, 70, 123-126]. However, some investigators found no such relationship [80, 127].

The present study is not a multinational or multicentre study; it has a relatively low number of patients with no severe disability, and generalization of this study therefore has limitations. Furthermore, we did not examine the impact of the disease form on fatigue because of the low numbers of patients with the clinically isolated syndrome (15 patients), or benign MS (6 patients) or secondary progressive MS (1 patient). No patients with the primary progressive form were involved at all. International studies have concluded that patients affected by progressive MS (both primary and secondary) have a significantly higher risk of fatigue [68, 70, 126], and analysis of covariance has revealed that this difference is attributable to the difference in the level of disability [124]. One study indicated that not all of the subscales are dependent on the form of MS, but only the physical and social dimensions [125]. Fisk et al. and Ford et al. reported that there was no difference in the level of fatigue between patients with different subtypes [67, 80].

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 patients with MS without severe disability.

VI. Conclusions

First of all, our findings 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. This is the first investigation of the SNP of TNF-α at position -376 in PPMS.

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 further 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.

The FIS seems to be a good tool for evaluation of the impact of MS-related fatigue in Hungarian clinical studies and clinical practice. This is the first fatigue scale to be introduced in Hungary which has been translated and culturally adapted.

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VIII. References

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IX. Appendices

Appendix I. Fáradtság hatásának mértéke

Beteg száma: Dátum:

Az alább található lista állításai leírják, milyen problémákat okozhat a fáradtság az ember életében. Minden állítást olvasson el figyelmesen. Azt a számot karikázza be, amely legjobban jellemzi, hogy milyen mértékű probléma volt Önnek a fáradtság **az elmúlt négy (4) hétben**, a mai napot is beleértve. Kérjük, hogy minden állításnál csak egy számot karikázzon be, és egyetlen állítást se hagyjon ki.

Mindegyik sorban egy számot karikázzon be	Nem probléma	Kis probléma	Mérsékelt probléma	Nagy probléma	Óriási probléma
1. *A fáradtságom miatt* **úgy érzem, hogy figyelmetlenebb vagyok.**	0	1	2	3	4
2. *A fáradtságom miatt* **úgy érzem, hogy jobban el vagyok szigetelve a társas kapcsolatoktól.**	0	1	2	3	4
3. *A fáradtságom miatt* **csökkentenem kell a munkám mennyiségét vagy a kötelezettségeimet.**	0	1	2	3	4
4. *A fáradtságom miatt* **szeszélyesebb vagyok.**	0	1	2	3	4
5. *A fáradtságom miatt* **nehezen tudok huzamos időn keresztül odafigyelni valamire.**	0	1	2	3	4
6. *A fáradtságom miatt* **úgy érzem, hogy nem tudok tisztán gondolkodni.**	0	1	2	3	4
7. *A fáradtságom miatt* **kevésbé hatékonyan dolgozom. (Ez az otthoni és a nem otthoni munkára is érvényes.)**	0	1	2	3	4
8. *A fáradtságom miatt* **nagyobb mértékben kell másokra támaszkodnom, hogy segítsenek vagy végezzenek el helyettem teendőket.**	0	1	2	3	4
9. *A fáradtságom miatt* **nehezen tudok elfoglaltságokat előre eltervezni, mert a fáradtságom befolyásolhatja az elvégzésüket.**	0	1	2	3	4
10. *A fáradtságom miatt* **ügyetlenebb vagyok és a mozgásom esetlenebb.**	0	1	2	3	4
11. *A fáradtságom miatt* **úgy érzem, hogy feledékenyebb vagyok.**	0	1	2	3	4
12. *A fáradtságom miatt* **ingerlékenyebb vagyok, és könnyebben haragra gerjedek.**	0	1	2	3	4
13. *A fáradtságom miatt* **óvatosabbnak kell lennem fizikai tevékenységeim gyakoriságával és időtartamával.**	0	1	2	3	4

Mindegyik sorban egy számot karikázzon be	Nem problém a	Kis problém a	Mérsékel t problém a	Nagy problém a	Óriási problém a
14. ***A fáradtságom miatt*** kevésbé vagyok motivált, hogy fizikai megterhelést igénylő tevékenységet végezzek.	0	1	2	3	4
15. ***A fáradtságom miatt*** kevésbé vagyok motivált, hogy társasági tevékenységekben vegyek részt.	0	1	2	3	4
16. ***A fáradtságom miatt*** korlátozott mértékben vagyok képes elhagyni az otthonom.	0	1	2	3	4
17. ***A fáradtságom miatt*** nehezen tudok fizikai erőkifejtést huzamos időn át fenntartani.	0	1	2	3	4
18. ***A fáradtságom miatt*** úgy érzem, hogy nehezen hozok döntéseket.	0	1	2	3	4
19. ***A fáradtságom miatt*** az otthonomon kívül kevés társasági kapcsolatom van.	0	1	2	3	4
20. ***A fáradtságom miatt*** a szokványos napi események szellemileg megterhelőek számomra.	0	1	2	3	4
21. ***A fáradtságom miatt*** kevésbé vagyok motivált arra, hogy bármilyen gondolkodást igénylő dolgot tegyek.	0	1	2	3	4
22. ***A fáradtságom miatt*** kerülöm a számomra szellemileg megterhelő helyzeteket.	0	1	2	3	4
23. ***A fáradtságom miatt*** úgy érzem, hogy az izmaim sokkal gyengébbek a kelleténél.	0	1	2	3	4
24. ***A fáradtságom miatt*** a fizikai rossz közérzetem erősödött.	0	1	2	3	4
25. ***A fáradtságom miatt*** nehezen tudok bármilyen új dologgal foglalkozni.	0	1	2	3	4
26. ***A fáradtságom miatt*** kevésbé vagyok képes gondolkodást igénylő feladatokat elvégezni.	0	1	2	3	4
27. ***A fáradtságom miatt*** úgy érzem, nem tudok megfelelni az emberek velem szembeni elvárásainak.	0	1	2	3	4
28. ***A fáradtságom miatt*** úgy érzem, hogy kevésbé tudom pénzügyileg támogatni magamat és a családomat.	0	1	2	3	4
29. ***A fáradtságom miatt*** szexuálisan kevésbé vagyok aktív.	0	1	2	3	4
30. ***A fáradtságom miatt*** úgy érzem, hogy nehezen rendezem a gondolataimat, amikor otthon vagy a munkában csinálok valamit.	0	1	2	3	4
31. ***A fáradtságom miatt*** kevésbé vagyok képes fizikai megterhelést követelő feladatokat elvégezni.	0	1	2	3	4
32. ***A fáradtságom miatt*** aggódom, hogy a külső megjelenésemről mások mit gondolnak.	0	1	2	3	4
33. ***A fáradtságom miatt*** kevésbé vagyok képes érzelmi kérdésekkel foglalkozni.	0	1	2	3	4
34. ***A fáradtságom miatt*** úgy érzem, hogy lelassul a gondolkodásom.	0	1	2	3	4

Mindegyik sorban egy számot karikázzon be	Nem problém a	Kis problém a	Mérsékel t problém a	Nagy problém a	Óriási problém a
35. ***A fáradtságom miatt*** nehezemre esik koncentrálni.	0	1	2	3	4
36. ***A fáradtságom miatt*** nehezen tudok teljes emberként részt venni családi tevékenységekben.	0	1	2	3	4
37. ***A fáradtságom miatt*** korlátoznom kell a fizikai tevékenységeimet.	0	1	2	3	4
38. ***A fáradtságom miatt*** gyakoribb vagy hosszabb pihenésekre van szükségem.	0	1	2	3	4
39. ***A fáradtságom miatt*** a kelleténél kevesebb érzelmi támogatást tudok nyújtani a családomnak.	0	1	2	3	4
40. ***A fáradtságom miatt*** kis nehézségek nagyoknak tűnnek.	0	1	2	3	4

Appendix II

Beck Depression Inventory [1]

Útmutató

A kérdőív csoportosított állításokat tartalmaz.
Kérjük, gondosan olvasson át minden állításcsoportot.
Válassza ki a csoport tagjai közül azt az egy állítást, amelyik a legjobban írja le az Ön érzéseit az elmúlt héttől egészen a mai napig.
Karikázza be a kiválasztott állítás betűjelét. Ha az adott csoportból több állítást is választana, akkor az ABC-sorrendben a legkésőbb következő betűt karikázza csak be.
Bízunk benn, hogy az adott csoporton belül minden egyes állítást el fog olvasni, mielőtt a kiválasztást megtenné.

Példa

.P. a) Ajándékozni épp úgy szeretek, mint régen.
b) Mostanában már nem szeretem az ajándékozást.
c) Az ajándékozás kellemetlen dolog a számomra.
d) Kínos élmény nekem az ajándékozás.

Kérdések:

1. a) Nem érzek szomorúságot.
b) Szomorúságot érzek.
c) Mindig szomorú vagyok, és nem tudok kivergődni belőle.
d) Annyira szomorú és boldogtalan vagyok, hogy már nem bírom ki.

2. a) Nem félek különösebben a jövőtől.
b) Félek a jövőtől.
c) Úgy érzem, semmi kilátásom sincs a jövőre nézve.
d) Úgy látom, hogy a jövőm reménytelen és a dolgok nem fognak megváltozni.

3. a) Nem érzem magam sikertelennek.
b) Úgy érzem, több kudarc ér, mint másokat.
c) Visszatekintve életemre, kudarcok sorát látom.
d) Úgy érzem, mint ember teljesen kudarcot vallottam.

4. a) A dolgok ugyanolyan elégedettséggel töltenek el, mint máskor.
b) Nem örülök a dolgoknak annyira, mint máskor szoktam.
c) Valójában többé semmi sem okoz elégedettséget nekem.
d) Mindennel elégedetlen vagyok, vagy unok mindent.

5. a) Nem hibáztatom különösebben magam.
b) Gyakran hibáztatom magam.
c) Majdnem mindig hibáztatom magam valami miatt.
d) Állandóan hibáztatom magam.

6. a) Nem érzem, hogy büntetnének.
b) Úgy érzem, hogy megbüntethetnek.
c) Azt várom, hogy büntessenek.
d) Úgy érzem, hogy büntetnek engem.

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[1] Hungarian translation: Pálfi Tibor pszichológus, SZOTE – dr. Stadinger Zsuzsanna pszichiáter, K.Á.K 1986
Szakmai lektorok: dr. Fodor Andrásné klin. pszichológus, SZOTE, dr. Janka Zoltán pszichiáter, kandidátus, SZOTE, dr. Kurimay Tamás pszichiáter, K.Á.K
Forrás: A.T. Beck M.D.: Depression:causes and treatment (pp. 333-335)
Ed: University of Pennsylvania Press, 1982

7. a) Nem érzek csalódottságot magammal szemben.
b) Csalódtam magamban.
c) Ki nem állhatom magam.
d) Gyűlölöm magam.

8. a) Nem érzem, hogy rosszabb lennék, mint bárki más.
b) Kritikus vagyok önmagammal, hibáimmal, tévedéseimmel szemben.
c) Mindig vádolom magam a hibáim miatt.
d) Minden rosszért magamat vádolom.

9. a) Nincs semmi öngyilkossággal kapcsolatos gondolatom.
b) Van öngyilkossággal kapcsolatos gondolatom, de nem valósítom meg.
c) Öngyilkos szeretnék lenni.
d) Ha alkalmam lenne rá, öngyilkos lennék.

10. a) Semmivel sem sírok többet, mint általában.
b) Mostanában többet sírok, mint korábban.
c) Mostanában mindig sírok.
d) Korábban tudtam sírni, mostanában hiába is akarok, nem megy.

11. a) Nem vagyok ingerlékenyebb, mint máskor.
b) Könnyebben leszek ingerült vagy mérges, mint szoktam.
c) Mostanában állandóan ingerült vagyok.
d) Már nem izgatnak fel olyan dolgok, amik korábban ingerültté tettek.

12. a) Nem vesztettem el érdeklődésemet más emberek iránt.
b) A korábbiakhoz képest kevésbé érdeklődöm más emberek iránt.
c) Jelentősen csökkent mások iránti érdeklődésem.
d) Minden érdeklődésem elvesztettem mások iránt.

13. a) Éppen olyan jól döntök, mint korábban.
b) Gyakrabban halogatom a döntést, mint korábban.
c) Nagyobb nehézséget okoz, ha döntenem kell, mint azelőtt.
d) Semmiben sem tudok dönteni többé.

14. a) Nem érzem, hogy valamivel is rosszabbul néznék ki, mint korábban.
b) Aggaszt, hogy nem vagyok vonzó többé, vagy öregnek látszom.
c) Úgy érzem, hogy hátrányomra változtam, és kevésbé vagyok vonzó.
d) Azt hiszem, csúnya vagyok.

15. a) Éppen olyan jól tudok dolgozni, mint máskor.
b) Erőfeszítésre van szükségem belefogni valamibe, vagy megcsinálni valamit.
c) Nagy erőfeszítésre van szükségem ahhoz, hogy megcsináljak valamit is.
d) Nem tudok már semmit sem elvégezni.

16. a) Ugyanolyan jól tudok aludni, mint korábban.
b) Nem alszom olyan jól, mint máskor.
c) A szokottnál 1-2 órával korábban ébredek, és nehezen tudok újra elaludni.
d) Több órával korábban ébredek, mint szoktam, és nem tudok újra elaludni.

17. a) Nem fáradok jobban, vagy könnyebben, mint máskor.
b) Jóval könnyebben fáradok el, mint szoktam.
c) Majdnem minden, amit csinálok, fáraszt.
d) Túlságosan fárasztó csinálnom bármit is.

18. a) Az étvágyam nem rosszabb, mint általában.
b) Az étvágyam nem olyan jó, mint lenni szokott.
c) Mostanában az étvágyam sokkal rosszabb.
d) Egyáltalán nincs étvágyam többé semmihez.

19a. a) Semmivel sem vesztettem többet a súlyomból, mint máskor.
b) Többet vesztettem, mint 2 kg.
c) Többet vesztettem, mint 5 kg.
d) Többet vesztettem, mint 8 kg.

19b. Kevesebb evéssel tudatosan igyekszem lefogyni: igen / nem

20. a) Az átlagosnál nem aggódom jobban az egészségemért.
b) Aggódom olyan testi-fizikai problémák miatt, mint a fájdalom, a székrekedés és a gyomorpanasz.
c) Nagyon aggódom testi-fizikai panaszaim miatt, és nehéz valami másra gondolnom.
d) Annyira aggódom testi-fizikai panaszok miatt, hogy másra nem tudok gondolni.

21. a) Nem vettem észre semmi lényeges változást szexuális érdeklődésemben.
b) A szokottnál kevésbé érdeklődöm a szex iránt.
c) Mostanában jóval kevésbé érdeklődöm a szex iránt.
d) Teljesen elvesztettem a szex iránti érdeklődésem.

Név: Neme: férfi / nő Születési év:

Település név (lakóhely):

Családi állapota:	Nőtlen/hajadon	1., nőtlen, hajadon, nincs élettársa
		2., nőtlen, hajadon, élettárssal él
	Házas	3., házas, házastárssal él
		4., házas, élettárssal él
		5., házas, egyedül él
	Elvált	6., elvált, nincs élettársa
		7., elvált, élettárssal él
	Özvegy	8., özvegy, nincs élettársa
		9., özvegy, élettárssal él

Gyermekeinek száma és kora:

Egy helyen töltött leghosszabb munkaviszonya: év

Eddigi munkahelyeinek száma:

Jelenlegi munkaviszonyának időtartama: év

Jelenlegi munkaviszonya:
1 - tanuló
2 - nyugdíjas
3 - van munkaviszonya
4 - nincs munkaviszonya

Iskolai végzettsége:
1 - legfeljebb általános iskola
2 - szakmunkásképző
3 - érettségi
4 - diploma

A múltévi betegállományban töltött idő: kb…….nap

Volt-e a közelmúltban valamilyen, a családot megrázó esemény? igen / nem

Kezelték-e valaha is lelki bajokkal, idegi problémákkal? igen / nem

Hozzátartozóinál volt-e ilyen jellegű probléma valaha is? igen / nem

Milyen az Ön közérzete, hangulata általában?

1 - Kitűnő
2 - Jó
3 - Közepes
4 - Elfogadható
5 - Lehangolt
6 - Nyomasztó
7 – Kínos