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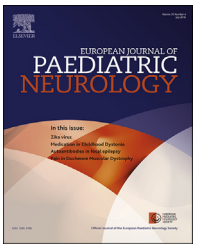

Original article

TNFRSF1A and *MEFV* mutations in childhood onset multiple sclerosis

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ABSTRACT

To investigate frequency and phenotype of *TNFRSF1A* and *MEFV* mutations in childhood-onset multiple sclerosis (MS). Twenty-nine clinically well characterized patients were investigated for mutations in exons 2, 3, 4, and 6 of the *TNFRSF1A* gene and in exons 2, 3, 9, 10 of the *MEFV* gene. Standardized morbidity ratio (SMR) was used to assess whether the number of observed mutations was higher than expected. Eleven out of 29 patients tested positive for mutations. Heterozygosity for the *TNFRSF1A* R92Q (rs4149584) variant was found in 6/11 mutation-positive patients. The SMR for R92Q in our pediatric MS population was 4.6 (95% CI 1.7–10.0), 7.0 (95% CI 2.6–15.2), and 13.6 (95% CI 5.0–29.7), depending on reference population. Six patients carried at least one heterozygous *MEFV* mutation with SMRs of 21.4 (95% CI 7.9–46.6) and 14.6 (95% CI 5.4–31.9). Clinical characteristics of childhood MS patients with or without mutations did not differ significantly. *Conclusion* One third of our childhood MS patients had a heterozygous mutation in the *TNFRSF1A* and/or *MEFV* gene. This proportion by far exceeds the number of mutations expected and was higher than in adult MS patients, suggesting that these mutations might contribute to the pathogenesis of childhood MS.

Abbreviations: EDSS, expanded disability status scale; FMF, familial Mediterranean fever; IFNβ, interferon beta; MEFV, Mediterranean fever gene; SMR, standardized morbidity rate; TNFRSF1A, tumor necrosis factor receptor superfamily 1A; TRAPS, tumor necrosis factor receptor 1–associated periodic syndrome.

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1. Introduction

Tumor necrosis factor receptor 1–associated periodic syndrome (TRAPS, MIM 142680) and familial Mediterranean fever (FMF, MIM 249100) belong to the group of hereditary autoinflammatory syndromes characterized by recurrent episodes of systemic inflammation associated with fever, abdominal and thoracic pain, arthralgias, myalgias, rashes, and headache. Cases of central nervous system (CNS) involvement have been reported in association with TRAPS[1,2] and FMF.[3]

In addition, adult patients with MS carrying mutations in the tumor necrosis factor receptor superfamily 1A gene (*TNFRSF1A*) were described, showing symptoms compatible with TRAPS.[4,5] In 2009, a genome wide association study identified the *TNFRSF1A* gene as a new susceptibility locus for MS,[6] which has been confirmed since by several other groups.[7,8]

Adults with concomitant MS and FMF have been reported mainly from Turkey and Israel.[3,9,10] We have investigated the prevalence of Mediterranean fever gene mutations (*MEFV*) in adult MS patients and controls in Germany and found mutations in a significant number of patients not only with Mediterranean ancestry and the E140Q exchange co-segregated with MS in three families.[11] The K695R substitution seemed to be associated with a more progressive course of MS.[11] Another study from Belgium did not show an association between different rare variants in the *MEFV* gene and MS but detected variants were associated with increased side effects during interferon-beta (IFN-β) therapy.[12] Recently, Terzi et al. assessed the influence of *MEFV* mutations on the radiological and clinical course of Turkish MS patients but found no association between *MEFV* mutation carrier status and MS severity.[13] More recently we described in more detail a case of childhood-onset MS with a concomitant *TNFRSF1A* R92Q and pyrin E230K mutation.[14]

The impact of mutations in the *TNFRSF1A* and *MEFV* gene on MS might best be assessed in a childhood population, as their exposure period to environmental factors is short. We therefore screened 29 consecutive patients with childhood onset MS for mutations in exons 2, 3, 4, and 6 of the *TNFRSF1A* gene and for mutations in exons 2, 3, 9, 10 of the *MEFV* gene. These exons represent loci, in which almost all deleterious sequence variants have been found.[15] The detailed clinical phenotype between mutation-positive and -negative patients was compared.

2. Patients and methods

2.1. Patients

Twenty-nine consecutive pediatric patients with MS [20 female/9 male; mean age at first manifestation 13.1 ± 2.5 years, age range 7.6–16.8 years] were included in the study between 2009 and 2014. Inclusion criteria were an age below 18 years and a definite diagnosis of MS according to the 2010 Revised McDonald Diagnostic criteria, in accordance with published criteria for pediatric multiple sclerosis by the International Pediatric Multiple Sclerosis Study Group.[16]

All patients were clinically evaluated in a standardized manner prior to genetic testing and during follow-up based on the Kurtzke Expanded Disability Status Scale (EDSS). Patients were seen for follow-up every 6 months.

Clinical characteristics including MS therapies and magnetic resonance imaging (MRI) and cerebrospinal fluid (CSF) data were evaluated in all patients. A detailed medical and family history including symptoms suggestive of an autoinflammatory syndrome could be obtained from 27/29 patients.

Patients and parents gave their written informed consent prior to genetic testing. The study was approved by the local institutional review board (Project number UE Nr. 137-14).

2.2. DNA sequence analysis

Genomic DNA was isolated from 200 μl of EDTA blood with the DNA blood mini kit from QIAGEN (Hilden, Germany).

Exons 2, 3, 4, and 6 of the *TNFRSF1A* gene and exons 2, 3, 9, 10 of the *MEFV* gene were amplified by PCR. Amplification products were purified with the QIAquick PCR purification kit (QIAGEN, Hilden, Germany) and sequenced with the ABI PRISM Big Dye Terminator v3.1 Ready Reaction Cycle Sequencing kit (Applied Biosystems, Foster City, CA, USA). Capillary electrophoresis was performed on an ABI 3130 and 3500 Genetic Analyzer (Applied Biosystems).

2.3. Statistical analysis

Demographic data and clinical characteristics are given as means ± standard deviation (SD) and were compared with the two-tailed Fisher's exact test. In order to assess whether the number of observed mutations was higher in childhood MS patients than to be expected (based on prevalence of these mutations in the general population), the standardized morbidity rate (SMR) was calculated. Since not all of our patients were of German descent (Table 4 for details), we considered different European reference populations for calculation of SMR (http://web1.sph.emory.edu/cdckms/exact-midP-SMR.html).

3. Results

3.1. Frequency of TNFRSF1A and MEFV mutations

Overall, 11 patients with childhood MS (37.9%) carried at least one heterozygous mutation in the *TNFRSF1A* and/or *MEFV* gene (Table 1).

Six patients (20%) were heterozygotes for an arginine$_{92}$ (CGG) → glutamine (CAG)/R92Q substitution (rs 4149584) encoded by exon 4 of the *TNFRSF1A* gene. Irrespective of the reference population used,[5,17,18] the lower limit of the SMR clearly exceeds one, indicating that the number of observed mutations was higher than the number expected (Table 2). Compared with available adult MS data, the 95% confidence intervals do not overlap.

Four patients (13.3%) were heterozygous carriers of a single *MEFV* mutation in exons 2 and 10. Two patients (6.7%) carried a complex allele with two (P369S/R408Q) or three sequence alterations (E148Q/P369S/R408Q) on one chromosome. One patient who has been previously described by us in detail,

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