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Genetic variants of homocysteine metabolism and multiple sclerosis: A case–control study

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HIGHLIGHTS

- Enzymatic variants of methionine metabolism are associated with development of MS.
- Two genetic variants of methionine pathway were associated with MS age of onset.
- Methionine metabolism can be manipulated by supplementation of vitamins.
- We propose novel preventive and therapeutic strategies for MS.

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ABSTRACT

Methylenetetrahydrofolate reductase (MTHFR) is necessary for the synthesis of methionine and S-adenosylmethionine, which is necessary for CNS (re-)myelination. The MTHFR variant c.1298A>C was associated with the development of relapsing remitting multiple sclerosis (RRMS) in a German population. This study aimed at analyzing whether further genetic variants of methionine metabolism are associated with the development or the clinical course of RRMS. Therefore, genomic DNA of 147 serial German RRMS patients and 147 matched healthy controls was genotyped for five polymorphic variants of methionine metabolism. Statistical analyses were performed using multivariate binary and linear regression analyses. We show that the insertion allele of cystathionine beta-synthase (CBS) c.844_855ins68bp and the G-allele of reduced folate carrier 1 (RFC1) c.80G>A were associated with an earlier age of onset of MS, suggesting gene-dose effects (median age of onset in years: 25-26-32; standardized regression coefficient beta: 0.216; $p = 0.030$, and 29-31-35 years; beta: 0.282; $p = 0.005$, respectively). Conclusively, mutant variants of CBS and RFC1 may be associated with the age of RRMS onset. Since methionine metabolism can be manipulated by supplementation of vitamins and amino acids, our data provide a rationale for novel ideas of preventive and therapeutic strategies in RRMS.

1. Introduction

Multiple sclerosis (MS) is a complex neurological disease that affects the central nervous system (CNS) and is characterized by inflammation, demyelination and neurodegeneration. Despite intensive research, both the etiology and the pathogenesis of MS are not completely understood. The current concepts suggest a multifactorial etiology with interplay between immunological, environmental and genetic factors. Furthermore, MS has been explored with regard to methionine-homocysteine metabolism as an alliance of genetics and environment. For example, homocysteine plasma levels are increased in relapsing remitting MS (RRMS) patients [1]. In addition, vitamin B12 deficiency may be associated with RRMS, and severe vitamin B12 deficiency shares some neurodegenerative and inflammatory pathophysiological characteristics with RRMS including central demyelination [2]. Vitamin B12 is necessary for the remethylation of neurotoxic homocysteine to methionine. Methionine is a semi-essential amino acid that can be activated to S-adenosylmethionine (SAM) by methionine-adenosyltransferase (MAT, EC 2.5.1.6; Fig. 1). S-adenosylmethionine serves as ubiquitous methyl donor and is essential for several components of the myelin sheet, such as myelin basic protein or phospholipids. In experimental and

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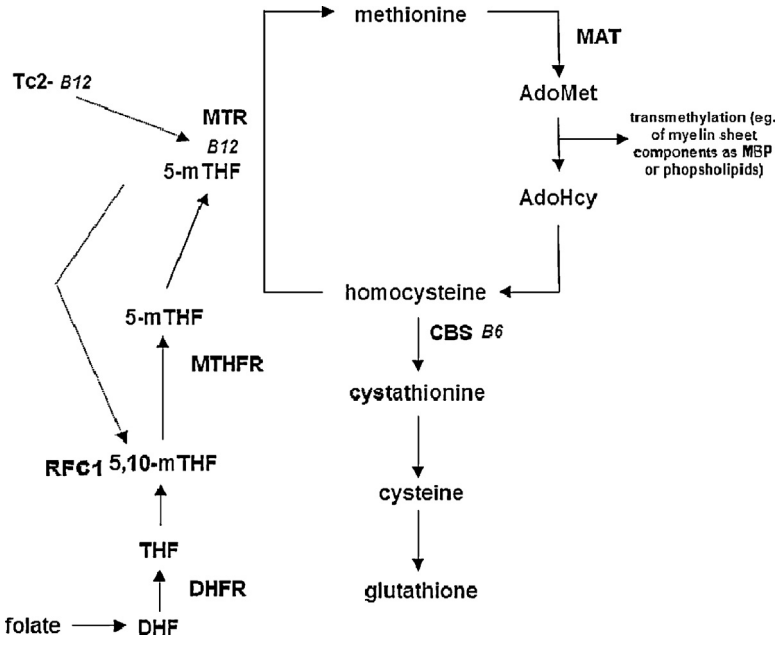

Fig. 1. The sulfur-containing amino acid methionine can be activated by methionine-adenosyltransferase (MAT) to S-adenosylmethionine (AdoMet), which is a ubiquitous methyl-group donor, e.g. for myelin sheet components like phospholipids or myelin basic protein. The degradation product of AdoMet is S-adenosylhomocysteine (AdoHcy), which becomes hydrolyzed to homocysteine in a reversible reaction. Homocysteine can either be transsulfurated by vitamin B6-dependent CBS or remethylated to methionine and AdoMet via methyltetrahydrofolate-homocysteine S-methyltransferase (MTR), which depends on vitamin B12 and 5-methyltetrahydrofolate (5-mTHF) as cofactors. The reduced folate carrier 1 (RFC1) acts as a transmembrane transport protein for 5-mTHF and is essential for folate uptake into the CNS. Dihydrofolate reductase (DHFR) reduces the precursor dihydrofolic acid (DHF) to the active tetrahydrofolate (THF), which is further converted into 5-methyltetrahydrofolate by 5,10-methylenetetrahydrofolate reductase (MTHFR). Tc2 is as a transport protein for vitamin B12.

clinical studies, anti-inflammatory and analgesic effects of SAM have been shown [3]. Transmethylation of S-adenosylmethionine results in S-adenosylhomocysteine, which becomes hydrolyzed to homocysteine, a reactive neuro- and vasculotoxic amino acid [4,5]. Homocysteine can be metabolized in two different pathways: First, cystathionine beta-synthase (CBS, EC 4.2.1.22), which depends on pyridoxal-phosphate (vitamin B6), can convert homocysteine to cystathionine. In the other pathway, methionine can be regenerated from homocysteine in the CNS via methyltetrahydrofolate-homocysteine S-methyltransferase (MTR, EC 2.1.1.13), which depends on cobalamin (vitamin B12) and 5-methyltetrahydrofolate. Transcobalamin 2 (Tc2) acts as a transporter protein of cobalamin. Reduced folate carrier 1 (RFC1) is a transmembrane transporter protein mainly expressed in the brain [6]. Dihydrofolate reductase (DHFR, EC 1.5.1.3) reduces the precursor dihydrofolic acid (DHF) to the active component tetrahydrofolate and is thereby essential for the availability of folates in the CNS [7].

Interestingly, this metabolism shows high inter-individual variety depending on renal function, individual vitamin and methionine uptake and on the genetic profile of methionine-homocysteine metabolism, i.e. on the expression of allelic variants with functional consequences for the encoded enzymes, transporter proteins or carriers. Severe mutations affecting the enzymes of methionine-homocysteine metabolism can lead to CNS demyelination [8]. Thus, we hypothesize that variants with functional consequences impact chronic inflammatory demyelinating diseases. Previously we observed that the allelic variant 5,10-methylenetetrahydrofolate reductase (MTHFR) c.1298A>C was associated with the incidence of RRMS in a German cohort [9]. In the present study we extended the analysis of that cohort by testing whether five further functionally relevant variants of methionine-homocysteine metabolism are associated with the development or the age of onset of RRMS (Table 1).

2. Materials and methods

2.1. Study population

The study population consisted of 147 serial German patients of Caucasian origin with RRMS according to McDonald criteria [10] (106 female; mean age ± standard deviation (SD): 31.5 ± 8.7 years). The patients were recruited from the Department of Neurology, University of Bonn, Germany. In addition, we analyzed 147 German age- and gender-matched healthy local controls of Caucasian origin without apparent signs or a history of neurological or immunological disease (106 female; 30.5 ± 7.3 years). All individuals of the present study were unrelated. The local ethics committee approved the study and informed written consent was received from all subjects.

2.2. Genotyping

DNA was extracted from peripheral leukocytes using QIAquick DNA extracting Kit (Qiagen, Hilden, Germany). Genomic DNA was amplified by PCR (Thermocycler T Professional, Biometra, Göttingen, Germany; Taq Polymerase and Polymerase buffer, Roche Diagnostics, Basel, Switzerland; Primers, Microsynth, Balgach, Switzerland) followed by restriction enzyme digestion (New England Biolabs, Ipswich, USA) and agarose gel electrophoresis. The PCR and restriction analysis conditions were described previously [11–15].

2.3. Statistical analysis

Data were analyzed with the Statistical Package for the Social Sciences (SPSS statistics, Version 16). To test the independent association of the genetic variants on development of RRMS, all variants were simultaneously analyzed together with age and gender as covariables in multivariate binary regression analysis. Accordingly,