Association of a Null Mutation in the CNTF Gene With Early Onset of Multiple Sclerosis

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Background: Immune-mediated demyelination and axonal damage lead to early functional impairment in multiple sclerosis (MS). Ciliary neurotrophic factor (CNTF) is a potent survival factor for neurons and oligodendrocytes and may be relevant in reducing tissue destruction during inflammatory attacks.

Subjects and Methods: We screened 288 unselected patients with multiple sclerosis (MS) (mean age, 40.2 ± 10.2 years; range, 18-71 years) for a previously described homozygous null mutation within the CNTF gene leading to a truncated, biologically inactive protein. The G-to-A CNTF null mutation at position −6 of the second exon was identified by a HaeIII polymorphism of the polymerase chain reaction–amplified genomic DNA.

Results: The homozygous CNTF null mutation (CNTF−/−) was found in 7 (2.4%) of the 288 randomly selected patients with MS. Patients with the CNTF−/− genotype had a significantly earlier onset of disease (17 vs 27 years; Mann-Whitney test, \( P = .007 \)) with predominant motor symptoms.

Conclusions: These results suggest that CNTF contributes to time and site of early clinical manifestation. The frequency of patients with MS with a homozygous CNTF null mutation in this population was not higher than in control groups, indicating that the CNTF null mutation is not a risk factor for development of MS.

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MULTIPLE sclerosis (MS) is a chronic inflammatory demyelinating disease of the central nervous system with presumed autoimmune origin. In addition to exogenous factors, genetically determined dispositions that cause deviations in the regulatory balance of proinflammatory and anti-inflammatory cytokines1 are regarded as relevant for disease susceptibility and expression. As has been demonstrated for the HLA system,2 functional relevant polymorphisms in candidate genes could modify both the onset and course of the disease.

However, in light of the ongoing discussion about mechanisms leading to axonal damage in MS, not only genes encoding for immunoregulatory molecules are of particular interest, but also genes that control oligodendrocyte and neuronal survival. In this context, the gene-encoding ciliary neurotrophic factor (CNTF) is an interesting candidate to reduce immune-mediated structural damage in MS. The CNTF was identified as a survival and differentiation factor for a variety of neuronal cell types, including motoneurons and sensory and sympathetic neurons.3 In the central nervous system, CNTF is expressed mainly by astrocytes and promotes mitosis and maturation of oligodendrocyte precursor cells in vitro and in vivo.4 Moreover, CNTF protects oligodendrocytes from tumor necrosis factor–induced apoptotic cell death.5 Therefore, CNTF may be involved in repair mechanisms in MS lesions in the course of the disease.

In this study, we screened patients with MS for a previously described homozygous null mutation within the CNTF gene leading to a truncated, biologically inactive protein6 and correlated the CNTF−/− genotype with disease activity.

RESULTS

The homozygous CNTF null mutation (CNTF−/−) was found in 7 (2.4%) of the 288 randomly selected patients with MS. This frequency corresponds to numbers previously reported for a Japanese (2.3%) and a German (2%) population.6,8 Four of the 7 patients with MS and the CNTF−/− genotype (mean age, 36.3 ± 15.5 years; Expanded Disability Sta-
PATIENTS AND METHODS

The study was approved by the local ethics committee. After written informed consent was obtained, blood was collected from 288 unselected adult Germans (mean [SD] age, 40.2±10.2 years; range, 18-71 years) with clinically definite MS. The patients were tracked in our MS outpatient clinic under highly standardized follow-up conditions.

DNA was isolated from 1 mL of EDTA-treated blood. For extraction of chromosomal DNA, a commercially available kit (QIAamp Blood Midi Kit; QIAGEN GmbH, Hilden, Germany) was used according to the manufacturer’s instructions.

The G-to-A CNTF null mutation at position −6 of the second exon of the CNTF gene, which leads to a frameshift in the derived CNTF messenger RNA coding for a truncated, biologically inactive protein, was identified by a Hae III polymorphism of the polymerase chain reaction (PCR)–amplified genomic DNA as recently described. The mutation eliminates a Hae III restriction site. Therefore, the PCR product with a length of 134 base pairs (bp) including the intron 1–exon 2 boundary remains undigested, whereas the PCR product of the wild-type allele is cleaved by Hae III, resulting in 2 fragments of 94 and 40 bp (Figure 1). For confirmation, PCR products that showed the digestion pattern for the homozygous CNTF null mutation were sequenced on an automated DNA sequencer (Perkin-Elmer 373 A; Applied Biosystems, Foster City, Calif). All PCRs were performed at least twice with patient DNA to avoid PCR errors.

Magnetic resonance (MR) imaging and MR spectroscopy were performed in patients with the CNTF+/− genotype and age- and sex-matched patients with the CNTF +/+ allele with a 1.5-T scanner (Magnetom Vision, standard head coil; Siemens, Erlangen, Germany). The T1-weighted (repetition time, 532 milliseconds; echo time, 17 milliseconds) and T2-weighted (repetition time, 2000 milliseconds; echo time, 20/80 milliseconds) images were obtained by the nonanagulated conventional double spin-echo technique (thickness, 6 mm). The MR spectroscopy was performed according to standard protocols (SVS [single-voxel] PRESS [point resolved spectroscopy in steady state] technique, 2-dimensional–CSI [chemical shift imaging]–spectroscopic imaging). Clinical data for patients with the CNTF+/− genotype and those with the CNTF +/+ allele were compared by χ² test, and statistical analysis of age at disease onset in relation to the homozygous CNTF null mutation was performed with the Mann-Whitney test (GraphPad Prism; GraphPad Software, San Diego, Calif).

In this study, the CNTF+/− mutation was associated with a significantly earlier onset of disease and motor disability in patients with MS. The frequency of patients with MS with a homozygous CNTF null mutation in this population was not higher than in control groups, indicating that the CNTF null mutation is not a risk factor for development of MS, but that this genotype may rather predispose to earlier disease onset and early motor involvement. Our data suggest that trophic support of neurons and oligodendrocytes as provided by CNTF may be critical to reduce early damage in the inflammatory lesions typical of MS. The lack of significant data from MR spec-
trophic studies might be due to our heterogeneous study group, with patients having varying disease length up to 25 years. On the other hand, measurements that represent the net effect of tissue destruction, such as atrophy measurements, show greater values in patients with the CNTF−/− genotype.

The homozygous CNTF null mutation is very rare. Therefore, the observation that patients carrying the CNTF−/− genotype have an earlier disease onset is based on findings in 7 patients. However, our results are in complete accordance with the observations made in experimental allergic encephalomyelitis in CNTF knock-out mice. After induction of myelin oligodendrocyte glycoprotein, CNTF−/− mice with experimental autoimmune encephalomyelitis showed a significantly earlier disease onset and a delayed recovery from relapses.

A number of recent studies established that patients with a first attack may already have signs of previous central nervous system lesions by MR imaging. Moreover, numerous lesions remain latent without overt clinical bouts, and the brain may even show mild atrophy early in the disease. Against this background, failure to produce neurotrophic factors like CNTF could be responsible for a more extended structural damage during an inflammatory attack (eg, due to proinflammatory cytokines) and may lead to a shorter preclinical interval between silent lesion development and first clinical symptoms of MS. This hypothesis is also supported by findings in experimental autoimmune encephalomyelitis in CNTF−/−, since these mice histopathologically showed a pronounced vesicular demyelination, resembling tumor necrosis factor α-mediated oligodendrogliopathy.

Our study clearly demonstrates that, in addition to evaluation of the immunoregulatory mechanism, it is necessary to evaluate the role of neurotrophic factors in MS. Moreover, this study supports the view of MS as an inflammatory neurodegenerative disease. This change of paradigm could also widen the therapeutic approaches in MS. Administration of CNTF or other trophic factors, by adequate pharmacologic approaches, could be useful to enhance regeneration, particularly in patients lacking endogenous CNTF.

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