TPP2 mutation associated with sterile brain inflammation mimicking MS

Eva M. Reinthaler, PhD, Elisabeth Graf, PhD, Tobias Zrzavy, MD, Thomas Wieland, PhD, Christoph Hotzy, BSc, Chantal Kopecky, PhD, Sandra Pfersch, Christiane Schmied, MD, Fritz Leutmezer, MD, Mohammad Keilani, MD, Christina M. Lil, PhD, Sabine Hofjan, MD, Jörg T. Epplen, MD, Uwe K. Zettl, MD, Michael Hecker, Angela Deutschländer, MD, Sven G. Meuth, PhD, Mamoun Ahram, MD, Baha Mustafa, PhD, Mohammed El-Khateeb, PhD, Carles Vilariño-Guell, PhD, A. Dessia Sadovnick, MD, Fritz Zimprich, MD, PhD, Birgitta Tomkinson, PhD, Tim Strom, PhD, Wolfgang Kristoferitsch, PhD, Hans Lassmann, PhD, and Alexander Zimprich, PhD

Neurol Genet 2018;4:e285. doi:10.1212/NXG.0000000000000285

Abstract

Objective
To ascertain the genetic cause of a consanguineous family from Syria suffering from a sterile brain inflammation mimicking a mild nonprogressive form of MS.

Methods
We used homozygosity mapping and next-generation sequencing to detect the disease-causing gene in the affected siblings. In addition, we performed RNA and protein expression studies, enzymatic activity assays, immunohistochemistry, and targeted sequencing of further MS cases from Austria, Germany, Canada and Jordan.

Results
In this study, we describe the identification of a homozygous missense mutation (c.82T>G, p.Cys28Gly) in the tripeptidyl peptidase II (TPP2) gene in all 3 affected siblings of the family. Sequencing of all TPP2-coding exons in 826 MS cases identified one further homozygous missense variant (c.2027C>T, p.Thr676Ile) in a Jordanian MS patient. TPP2 protein expression in whole blood was reduced in the affected siblings. In contrast, TPP2 protein expression in postmortem brain tissue from MS patients without TPP2 mutations was highly upregulated.

Conclusions
The homozygous TPP2 mutation (p.Cys28Gly) is likely responsible for the inflammation phenotype in this family. TPP2 is an ubiquitously expressed serine peptidase that removes tripeptides from the N-terminal end of longer peptides. TPP2 is involved in various biological processes including the destruction of major histocompatibility complex Class I epitopes. Recessive loss-of-function mutations in TPP2 were described in patients with Evans syndrome, a rare autoimmune disease affecting the hematopoietic system. Based on the gene expression results in our MS autopsy brain samples, we further suggest that TPP2 may play a broader role in the inflammatory process in MS.

From the Department of Neurology (E.M.R., S.P., C.S., F.L., F.Z., A.Z.), Medical University of Vienna, Austria; Institut für Humangenetik (E.G., T.W., T.S.), Helmholtz Zentrum München, Germany; Center for Brain Research (T.Z., H.L.), Medical University of Vienna; Division of Nephrology and Dialysis (C.K.), Department of Internal Medicine III, Medical University of Vienna; Department of Physical Medicine (M.K.), Rehabilitation and Occupational Medicine, Medical University of Vienna, Austria; Lübeck Interdisciplinary Platform for Genome Analytics (C.M.L.), Institutes of Neurogenetics and for Cardiogenetics, University of Lübeck; Department of Neurology and Neuroimaging Center (NIC) (C.M.L.), Focus Program Translational Neuroscience (FTN), University Medical Center of the Johannes Gutenberg University Mainz; Department of Human Genetics (S.H., J.T.E.), Ruhr-University Bochum; Herdecke (J.T.E.); ZBAF, Faculty of Health, University Witten; Department of Neurology (U.K.Z., M.H.), Neuroimmunological Section, University of Rostock; Department of Neurology (A.D.), Department of Clinical Genomics (A.D.), Department of Neuroscience (A.D.), Jeweils Mayo Clinic, Jacksonville, FL; Department of Neurology (S.G.M.), University of Muenster, Germany; Department of Physiology and Biochemistry (M.A., B.M.), School of Medicine, the University of Jordan; The National Center (Institute) for Diabetes (M.E.-K.), Endocrinology and Genetics (NCDEG), Amman, Jordan; Department of Medical Genetics (C.V.-G., A.D.S.), University of British Columbia, Vancouver, Canada; Department of Medical Biochemistry and Microbiology (B.T.), Uppsala University, Sweden; Karl Landsteiner Institute for Neuroimmunological and Neurodegenerative Disorders (W.K.), SM2-Ost-Donauspital, Vienna, Austria; and Institute for Neuromolecular and Neurodegenerative Disorders (W.K.), SM2-Ost-Donauspital, Vienna, Austria.

Funding information and disclosures are provided at the end of the article. Full disclosure form information provided by the authors is available with the full text of this article at Neurology.org/NG.

Correspondence
Dr. A. Zimprich alexander.zimprich@meduniwien.ac.at

The Article Processing Charge was funded by the authors.

This is an open access article distributed under the terms of the Creative Commons Attribution-NonCommercial-NoDerivatives License 4.0 (CC BY-NC-ND), which permits downloading and sharing the work provided it is properly cited. The work cannot be changed in any way or used commercially without permission from the journal.
Glossary

ERCC5 = excision repair cross-complementation group 5; ExAC = exome aggregation consortium; LoF = loss of function; MAF = minor allele frequency; MHC = major histocompatibility complex; NAWM = normal-appearing white matter; PBMC = peripheral blood mononuclear cell; TPP2 = tripeptidyl peptidase II; TRIANGLE = TPP2-related immunodeficiency, autoimmunity and neurodevelopmental delay with impaired glycolysis and lysosomal expansion.

Evans syndrome (ES) is a rare autoimmune disorder, which is defined by a combination of direct Coombs test positive hemolytic anemia and immune thrombocytopenia. Although most cases have no obvious underlying etiology, rare monogenic forms have been identified in the last years. ES can affect different organs, manifesting with hepatomegaly, splenomegaly,3–5 and lymphocytic infiltration of nonlymphoid organs, including the brain. A case report from 2013 described a patient who had ES in addition to a sterile brain inflammation, which, from a clinical perspective, was indistinguishable from MS.

Recessive loss-of-function (LoF) mutations in the tripeptidyl peptidase II (TPP2) gene were found to cause a specific form of ES in patients, manifesting with neurodevelopmental delay and impaired glycolysis. TPP2 is a cytosolic protease; its main activity is the removal of tripeptides from the N-terminus of longer peptides, to generate free amino acids for protein synthesis and energy production. This TPP2-linked phenotype was proposed to be designated as “TPP2-related immunodeficiency, autoimmunity and neurodevelopmental delay with impaired glycolysis and lysosomal expansion” (TRIANGLE) disease. Here, we report on a family with 3 affected siblings initially diagnosed with a mild and nonprogressive form of MS. We identified a homozygous missense mutation in TPP2 as a likely cause for the disease in this family.

Methods

Study participants
We clinically evaluated a consanguineous family from Syria with 3 siblings diagnosed with MS and a suggestive pattern of autosomal recessive inheritance. In brief, the disease course observed in the siblings is consistent with a benign relapsing-remitting MS even 27 (II.1) and 24 (II.2) years after the onset of clinical symptoms. The female sibling (II.3) had a single neurologic episode of demyelination and no further clinical relapses over the following 14 years. The Expanded Disability Status Scale was not more than 1.5 in any of the siblings. All of them reported to have had frequent infections of the upper respiratory tract during their childhood and adulthood. All 3 siblings showed low but normal lymphocyte count between 1 and 1.3 × 10^3/µL (normal range is between 1.0 and 4 × 10^3/µL) as tested several times between 2008 and 2017. However, they have never experienced other clinical signs typical for ES, such as thrombocytopenia or hemolytic anemia. A detailed clinical description in form of a timetable and magnetic resonance tomography images are provided in figure e-1 (links.lww.com/NXG/A108), figure e-2 (links.lww.com/NXG/A109; legends links.lww.com/NXG/A130), and in the supplementary information (links.lww.com/NXG/A110).

For the targeted sequence analysis, we ascertained 382 MS patients from Europe (Austria and Germany) and 183 MS cases from Jordan. In addition, we surveyed exome data from 261 MS patients from Canada for mutations in the TPP2 gene, which were collected through the longitudinal Canadian Collaborative Project on the Genetic Susceptibility to MS12 (table 1). All patients were diagnosed with MS according to published criteria.13–15

Standard protocol approvals, registrations, and patient consents
Written informed consent was obtained from all study participants; the study was approved by the local ethics committee (EK.535/2004/20179 for the neuropathologic part and EK Nr.2195/2016 for MS patients).

Homozygosity mapping and exome sequencing
Homozygous regions shared between all 3 siblings were mapped using Affymetrix GenomeWideSNP 6 data from the affected siblings and their healthy parents with the online homozygosity Mapper tool (homozygositymapper.org).

Whole exome data were generated from individuals II.1 and II.2. Exomes were enriched with SureSelect Human All Exon 50 Mb kit (AgilentTechnologies, Santa Clara, CA). Sequencing of postenrichment libraries was carried out on the Illumina HiSeq 2000 sequencing instrument (Illumina, San Diego, CA) as 2 × 100 bp paired-end runs. Variants were filtered for homozygosity and a minor allele frequency (MAF) smaller than 2% in our in-house data set of approximately 10,000 control exomes from patients with other unrelated diseases and exomes and in public available databases (exome aggregation consortium [ExAC] database and 1000 Genomes).

Exome sequencing of the Canadian patients was done on an Ion Proton sequencer (Life Technologies, Carlsbad, CA). Exome data were analyzed as previously described.16

Targeted capture sequencing, single-nucleotide polymorphism genotyping, and in silico prediction
Illumina TruSeq Custom Amplicon Kit was used to target all exonic and flanking intrinsic regions of TPP2 and excision repair cross-complementation group 5 (ERCC5) in 382
European and 183 Jordanian MS patients. Fast-QC files were subsequently analyzed with an in-house pipeline. All identified variants were subsequently validated with Sanger sequencing. Homozygous variants in 
\(\text{TPP2}\) (p.Cys28Gly, p.Thr676Ile) and \(\text{ERCC5}\) (p.S1078A) were genotyped with custom TaqMan single-nucleotide polymorphism genotyping assays.

**TPP2 protein expression in MS and control brain tissue**
Expression of TPP2 was assessed in formaldehyde-fixed and paraffin-embedded autopsy tissues from 13 MS patients and 16 controls archived at the Center for Brain Research of the Medical University of Vienna (detailed clinical characteristics in supplementary information, links.lww.com/NXG/A110). Expression of TPP2 was analyzed in the normal-appearing white matter (NAWM); initial, early active, late active, and inactive lesion sites in the white matter of MS patients; and in the normal white matter of controls. Lesion stages were defined as described in detail before.\(^{17}\)

**TPP2 RNA Western blot analysis**
Using a TaqMan gene expression assay, TPP2 whole blood mRNA expression was quantitatively assessed for all individuals of the index family, 7 randomly chosen individuals affected with MS and 3 healthy controls (GAPDH, Hs03929097_g1). Immunoblotting was performed with peripheral blood mononuclear cell (PBMC) lysates from the index patient (II.1), his affected brother (II.2), and 3 control individuals. Primary antibodies for TPP2 (1:500, 14981S; Cell Signaling) and endogenous control beta-actin were used.

**TPP2 enzymatic activity**
Blood samples from index patient II.1 and 2 healthy controls were prepared according to the partial lysis method (wch.sa.gov.au/services/az/divisions/labs/geneticmed/nrl_methods.html). Enzymatic activity was measured as described previously.\(^{18}\)

### Results

**Identification of a homozygous mutation in the TPP2 gene as a likely cause for a sterile brain inflammation in a consanguineous Syrian family**
To identify the responsible gene of a benign brain inflammation, mimicking a nonprogressive form of MS, in a consanguineous family with 3 affected siblings, we performed homozygosity mapping and whole exome sequencing. After filtering variants for allele frequency (MAF < 2%) and homozygosity, only 2 novel autosomal homozygous missense variants were left to be shared by all siblings. We identified a c.82T>G, p.Cys28Gly variation in the \(\text{TPP2}\) gene, a serine peptidase that removes tripeptides from the N-terminus of longer peptides and a c.3232T>G, p.Ser1078Ala variation in the \(\text{ERCC5}\) gene, known to be involved in DNA repair. These 2 variants were confirmed in heterozygous state in the healthy parents. None of the 2 variants is present in any publicly available databases. The 2 genes are in close vicinity, located on chromosome 13q33.1 and map to the only homozygous region shared by all 3 affected siblings (figure 1, A–C).

**Identification of a second rare homozygous variant in the TPP2 gene in a Jordanian MS patient**
As the phenotype of the family was almost indistinguishable from a benign form of MS, we wondered whether other patients diagnosed with MS might carry biallelic variants in either of the 2 genes. Sequencing of both genes in additional 382 unrelated MS cases from Europe and 183 MS patients from Jordan identified one further homozygous variation in TPP2 (c.2027C>G, p.Thr676Ile, rs760347832), in a Jordanian patient (table 2). This variant was also present in heterozygous state in 2 additional MS patients from Jordan (figure 1D, clinical details in supplementary information, links.lww.com/NXG/A110). We did not find any rare

| Site       | Cases | Male | Female | Aao | Positive family history/recessive a |
|------------|-------|------|--------|-----|-----------------------------------|
| Austria    | 352   | 150  | 202    | 29  | 56/9                              |
| Germany    | 30    | 10   | 20     | 30  | 20/20                             |
| Jordan     | 183   | 63   | 120    | 29  | 14/8                              |
| Canada     | 261   | 78   | 183    | 32  | 173/18                            |

Abbreviations: Aao = age at onset; ERCC5 = excision repair cross-complementation group 5; TPP2 = tripeptidyl peptidase II.

a Recessive: the number of cases who have a recessive mode of inheritance, which is here defined by at least one other affected sibling with both parents being healthy or with no second-degree relative affected.

---

**Table 1 Additional MS cases and controls for targeted sequence and genotyping analysis**

| Site       | Cases | Male | Female | Aao | Positive family history/recessive a |
|------------|-------|------|--------|-----|-----------------------------------|
| Austria    | 352   | 150  | 202    | 29  | 56/9                              |
| Germany    | 30    | 10   | 20     | 30  | 20/20                             |
| Jordan     | 183   | 63   | 120    | 29  | 14/8                              |
| Canada     | 261   | 78   | 183    | 32  | 173/18                            |
| Jordan MS cases | 233 | 82  | 151    | 29  | 10/3                              |
| Jordan controls | 452 | 289 | 163    | 40  |                                   |

Abbreviations: Aao = age at onset; ERCC5 = excision repair cross-complementation group 5; TPP2 = tripeptidyl peptidase II.
homozygous or compound heterozygous variants in the ERCC5 gene in the additionally sequenced MS cases. Inspection of the ExAC database reveals TPP2 as highly constraint (missense: \(Z\) score = 3.07, LoF: pLI = 1.00) and less tolerant to variation than ERCC5 (missense: \(Z\) score = -0.61, pLI = 0.00). In fact, considering the evidence for both genes, TPP2 appeared to be the stronger candidate because of the greater inherent biological plausibility.

In total, we identified 4 novel or known rare missense variants (MAF < 2%) in TPP2. Apart from p.Thr676Ile, all other variations (p.Ile551Val, p.Glu1012Gly, p.Gln1141Pro) occurred in heterozygous form (table 2). We further assessed exome data of 261 MS cases from Canada but did not identify any rare homozygous or compound heterozygous variants in either gene. Subsequently, we genotyped all 3 homozygous variations (TPP2: p.Gly28Cys, p.Thr676Ile and ERCC5: p.S1078A) in an additional cohort of 233 Jordanian MS cases and 452 ethnically matched Jordanian controls and identified one further Jordanian MS patient carrying the TPP2-p.Thr676Ile variant in heterozygous state. Of note, the variant was not found in any of the 452 Jordanian controls. However, TPP2-p.Thr676Ile is present in heterozygous form in 6 individuals in the ExAC database. Although, there is no definite evidence for TPP2 as a putative high-penetrant variant in other MS patients, the TPP2-Thr676Ile is an interesting candidate variant, worth to be followed up in more probands.

**TPP2 protein levels are reduced in patients of the Syrian MS family**

Next, we investigated whether TPP2 mRNA and protein levels differed in patients and controls. Whole blood mRNA levels did not show any difference between TPP2 homozygous mutation carriers, heterozygous parents, and controls (data not shown). However, Western blot analysis showed a marked reduction in TPP2 protein amount in PBMCs from the 2 affected brothers, when compared with sex- and age-matched control individuals (figure 1, E). Unfortunately, no blood was available from the affected sister for further analysis.
complementation group 5; het = heterozygote; hom = homozygote; TPP2 = tripeptidyl peptidase II.

Abbreviations: CADD = Combined Annotation Dependent Depletion; dbSNP = database of Single Nucleotide Polymorphisms; ERCC5 = excision repair cross-complementation group 5; het = heterozygote; hom = homozygote; TPP2 = tripeptidyl peptidase II.

Table 2 Missense variants identified in the index family and follow-up cases

| Gene | Position (hg19) | Nucleotide change | Amino acid change | CADD | dbSNP ID | Unrelated European/Canadian MS cases | Unrelated Jordanian MS cases | Unrelated Jordanian controls | ExAc counts ethnicity mixed |
|------|----------------|-------------------|-------------------|------|----------|--------------------------------------|----------------------------|----------------------------|---------------------------|
| TPP2 | chr13:103249470 | c.82T>G           | p.C28G            | 18.4 |          | 0/643                                | 0/416                      | 0/452                      | Not present               |
| TPP2 | chr13:103288715 | c.1651A>G         | p.I551V           | 21.4 |          | 1/643                                | 0/183                      | n.a.                       | Not present               |
| TPP2 | chr13:103295578 | c.2027C>T         | p. T676I          | 23.3 | rs760347832 | 0/643                                | 1x hom, 3x het/416         | 0/452                      | 6 x het/ 59.292           |
| TPP2 | chr13:103298652 | c.2402G>A         | p.S801N           | 23.5 | rs140329690 | 1/643                                | 0/183                      | n.a.                       | 8 x het/ 58.580           |
| TPP2 | chr13:103309488 | c.3035A>G         | p.E1012G          | 23.4 | rs199569052 | 1/643                                | 0/183                      | n.a.                       | 15 x het/ 60.493          |
| TPP2 | chr13:103326722 | c.3422A>C         | p.Q1141P          | 5.4  | rs199702252 | 1/643                                | 0/183                      | n.a.                       | 14 x het/ 66.692          |
| ERCC5 | chr13:103527924 | c.3232T>G         | p.S1078A          | 11   |          | 0/643                                | 0/416                      | 0/452                      | 14 x het/ 60.629          |

Abbreviations: CADD = Combined Annotation Dependent Depletion; dbSNP = database of Single Nucleotide Polymorphisms; ERCC5 = excision repair cross-complementation group 5; het = heterozygote; hom = homozygote; TPP2 = tripeptidyl peptidase II.

To assess a possible effect of the mutation on TPP2 enzymatic activity, we used lysates from erythrocytes and leukocytes from patient II.1 and 2 healthy control individuals. The activity in erythrocytes from 3 samples per person was 0·42 ± 0·10 and 0·50 ± 0·15 nmol min⁻¹ mg⁻¹ protein for the patient and the controls, respectively. In leukocyte lysates, the activity was 8·8 ± 1·3 and 7·1 ± 1·1 nmol min⁻¹ mg⁻¹ for the patient and the controls, respectively. The measured activity is inhibited to >95% with the specific TPP2 inhibitor buta-bin-dide,19 thus demonstrating that the activity is indeed dependent on TPP2 and not because of a contaminating or compensatory activity. TPP2 in lysates from erythrocytes from both, the patient and the controls, form the normal size complex, as determined by size exclusion chromatography (data not shown). In conclusion, these data show that, although the mutation goes along with reduced protein levels, no significant reduction in enzyme activity is observed in the patients at least in peripheral blood erythrocytes and leukocytes.

**TPP2 protein and mRNA levels are increased at sites of active inflammatory demyelination**

To find out whether *TPP2* also plays a role in MS disease process in nonmutation carriers, we performed immunohistochemistry analysis in brain autopsy samples of 13 MS patients and 16 controls. We found a constitutive expression of TPP2 in neurons and astrocytes. Expression in these cells was similar between controls and MS patients. However, we found a highly selective expression of TPP2 in microglia within and around active MS lesions. While expression was sparse or absent in the NAWM of MS and control brain tissue, increased expression was seen in the periplaque white matter (figure 2, A–C). In particular, microglia nodules, which are abundant in this area close to active lesions, showed pronounced staining. The highest expression of TPP2 was seen in initial stages of MS lesions, characterized by profound microglia activation, oligodendrocyte apoptosis, and initial myelin damage. In the demyelinated portion of the lesions, the expression of TPP2 in microglia decreased from the lesion edge toward the lesion center and was largely absent in microglia in the inactive lesion center. The pattern of microglia TPP2 expression was similar in classic active lesions seen in acute and relapsing MS and in slowly expanding lesions, which are the dominant active lesions in progressive MS. Double staining confirmed that TPP2 expression was present in macrophages/microglia, expressing the phagocytosis-associated marker CD68 and major histocompatibility complex (MHC) Class I and Class II molecules (figure 2, B). In addition, some lymphocytes in perivascular inflammatory infiltrates also expressed TPP2.

In addition, we surveyed the gene expression omnibus profile (GEO Profile) database for *TPP2* expression linked to MS. In line with our experiment, a microarray analysis performed in brain samples of MS patients revealed a significant upregulation of *TPP2* mRNA in MS brain lesions compared with control individuals (figure 2, D).20

**Discussion**

Our study shows the identification of a causative mutation in a consanguineous family with a suggestive recessive inheritance pattern presenting with a sterile brain inflammation, mimicking MS. The family members presented clinically characteristic attacks of MS and typical MRI lesions fulfilling diagnostic criteria for MS. Two siblings were positive for oligoclonal bands in their CSF. However, the exceptionally
benign disease course, with almost no progression in over 20 years, even in the absence of any therapy in one of the siblings let us doubt on the diagnosis of typical MS.

In line with our anticipated inheritance pattern, we identified the homozygous missense mutation (p.Cys28Gly) in the TPP2 gene in all 3 siblings. Homozygosity mapping and exome sequence filtering identified the genomic location around TPP2 and ERCC5 as the only region within in the entire genome shared by all 3 affected siblings. Moreover, the TPP2 p.Cys28Gly mutation is absent in all publicly available databases (including >60,000 individuals from the ExAC database and 10,000 in house controls). TPP2 LoF mutations were found in patients with ES; those patients present among other severe symptoms, respiratory tract infections.9 Notably, all 3 siblings suffered from frequent upper respiratory tract infections in their childhood and adulthood. In addition, they all showed marginally low lymphocytic counts. We think, this

Figure 2 TPP2 expressions in MS

(A) No expression in NAWM, while increased expression was seen in the PPWM with staining of some microglia nodules. The highest expression of TPP2 was seen in initial stages (initial) of MS lesions, where most of the activated microglia expressed TPP2. TPP2 expression decreased from the lesion edge toward the lesion center. (B) Double staining confirmed that TPP2 expression was present in macrophages/microglia, expressing the phagocytosis-associated marker CD68 and MHC Class I and Class II molecules. (C) Quantification of TPP2-positive cell counts. Box plots displaying the number of microglia per mm² in regions of interest. Significantly more TPP2 expression was found in MS NAWM, inactive MS lesions, PPWM, initial lesions and in EA or LA lesions of MS patients compared with control white matter. ***p < 0.001, **p < 0.01, Wilcoxon test and Mann-Whitney U test. (D) TPP2 mRNA expression in brain lesions of MS patients and controls. Figure is derived from the data set GDS4218 deposited in the GEO Profile database. EA = early active; GEO Profile = gene expression omnibus profile; LA = late active; MHC = major histocompatibility complex; NAWM = normal-appearing white matter; PPWM = periplaque white matter; TPP2 = tripeptidyl peptidase II.
possibly reflects subthreshold signs for ES and supports that the TPP2 variant is phenotypically effective.

As we assume recessively inherited TPP2 variations to be linked with the disease, we were interested in how frequently control individuals carry biallelic rare variants. We surveyed our in-house exome database, including more than 10,000 control individuals for TPP2 variants. Notably, no single individual was found to carry 2 rare TPP2 variants (MAF < 2%). These data indicate that rare variants in the TPP2 gene, particularly when occurring on both alleles, are not well tolerated in healthy individuals.

Because of the close phenotypic resemblance of our family to MS, we wondered whether recessive TPP2 mutations might also be found in typical MS cases. We sequenced a large MS cohort from Austria, Germany, Canada, and Jordan and identified 1 MS case from Jordan, with a homozygous TPP2 variant, p.Thr676Ile. However, as this variant also occurs in heterozygous form in other MS cases and in 6 ExAC control individuals, it remains questionable whether this variant is pathogenic.

It was found that TPP2 has a major role in maintaining the cellular homeostasis of amino acids. An increase in lysosomal function and, as a consequence, an inability of immune cells to mobilize aerobic glycolysis was seen in patients with TPP2 LoF mutation. Unlike other cytosolic peptidases, TPP2 influences the MHC Class I metabolism, usually through the destruction of MHC Class I epitopes. Increased MHC Class I and Class II expression has been shown in TPP2-deficient mice. The familial mutation, p.Cys28Gly, results in reduced protein expression in the patients, however, it does not seem to drastically affect the activity of TPP2 in the patients at least in peripheral blood leukocytes. One can only speculate on possible reasons. TPP2 is a low abundant protein involved in immunologic processes; any circumstances affecting the immune status of a person might also affect protein levels. It might also be that the sensitivity of the enzymatic test is not sufficient to capture small differences. The reduced protein expression in the patients, however, might reflect instability in the complex formed by active TPP2. For example, dissociated TPP2 is more sensitive to proteolytic degradation.

Our work shows a marked upregulation of TPP2 at sites of active inflammatory demyelination in MS autopsy brain samples without TPP2 mutations and a reduced TPP2 blood expression in mutation carriers. Why TPP2 is upregulated in MS brains and downregulated in TPP2 mutation carriers with an MS phenotype cannot be answered here. Is TPP2 upregulation in brain secondary to MS onset and follows as a kind of compensatory mechanism to “defend” the inflammation process? Such a mechanism seems not unreasonable, regarding its role in aerobic glycolysis for immune cell function. This speculation attributes TPP2 a rather protective role in the MS process, which in TPP2 mutation carriers is no longer fulfilled but instead leads to long-lasting impairment of aerobic glycolysis and consequently to MS.

The pronounced and highly selective expression of TPP2 in cells with high MHC Class I and Class II expression (figure 2, B) suggests an alternative disease mechanism. As mentioned before, TPP2 appears to play a role in peptide trimming and in the destruction of MHC Class I epitopes. Notably, the few known TPP2 processed epitopes include the Epstein-Barr virus–derived antigen latent membrane protein 1. In case of a reduced efficiency or changed specificity of this process because of the TPP2 mutation, one can expect the escape of self-peptides, which may then be recognized by autoreactive T cells in a process that amplifies the inflammatory process.

We argue that the homozygous p.Cys28Gly mutation in the TPP2 gene is likely responsible for the MS-like phenotype in the present family broadening the phenotypic spectrum of TRIANGLE disease. We therefore consider it as an intriguing possibility that other cases of TPP2-linked brain inflammations might be covered under a diagnosis of MS. The strong increase of TPP2 expression in MS brain lesions in non-mutation carriers suggests a broader role in MS physiopathology. The mechanisms by which TPP2 mutations contribute to disease pathogenesis cannot be answered in our work. Previous studies claim aerobic glycolysis, lymphocytic immunosenescence, or MHC peptide trimming as candidate mechanisms in disease development. Further functional studies on MS patients with TPP2 mutations in these particular directions will help to elucidate this question.

Author contributions

E.M. Reinthaler and A. Zimprich designed the study, analyzed exome data, performed “Truseq” resequencing experiments, and Affymetrix GenomeWideSNP 6 array experiments for homozygosity mapping as shown in figure 1, A and B. T. Zrzavy and H. Lassmann designed, executed, and analyzed experiments shown in figures 2, A–C. C. Kopecky and S. Pferschy performed Western blot experiments as shown in figure 1, E. C. Hotzy helped and participated in Western blot and array experiments and performed Taqman analyses. B. Tomkinson executed and analyzed TPP2 enzymatic activity assays. W. Kristoferitsch recruited and clinically investigated patients from family MS01 and helped to generate data for supplementary figure e-1 (links.lww.com/NXG/A108) and

Neurology.org/NG Neurology Genetics | Volume 4, Number 6 | December 2018 7
provided and interpreted data for supplementary figure e-2 (links.lww.com/NXG/A109). E. Graf, T. Wieland, and T. Strom performed Illumina exome sequencing and provided the bioinformatic pipeline for exome data analysis and supported with the Truseq resequencing experiments. C. Schmied, F. Leutmezer, M. Keilani, C.M. Lill, S. Hoffjan, J.T. Epplen, U.K. Zettl, M. Hecker, A. Deutschländler, S.G. Meuth, M. Ahram, B. Mustafa, and M. El-Khateeb recruited and clinically investigated patients and control individuals for replication analyses as shown in tables 1 and 2. C. Vilarino-Güell and A.D. Sadovnick recruited and clinically investigated patients, performed and analyzed exome data from Canadian patients as shown in table 1. E.M. Reinthaler, F. Zimprich, W. Kristoferitsch, H. Lassmann, and A. Zimprich had important intellectual content and wrote the paper.

Acknowledgment
The authors thank all patients and family members for their participation in this study.

Study funding
The study was partly funded by the Austrian Science Foundation (FWF; Project I 2114 Meltra-BBB), Canada Research Chair program (950-228408), Michael Smith Foundation for Health Research (16827), Canadian Institute of Health Research (MOP-137051), Vancouver Coastal Health Research Institute, the Milan & Maureen Ilich Foundation (11-3209500), and the Vancouver Foundation (ADV14-1597).

Disclosure
E.M. Reinthaler, E. Graf, and T. Zrzavy report no disclosures. T. Wieland is an employee of Foundation Medicine Inc. C. Hotzy and C. Kopecky report no disclosures. S. Pferschy is an employee of IQVIA. C. Schmied has received travel funding/speaker honoraria from Roche Austria and Sanofi-Aventis Österreich. F. Leutmetzer and M. Keilani report no disclosures. C.M. Lill serves on the editorial boards of Gene and MetaGene. S. Hoffjan serves on the editorial board of the journal MCP. J.T. Epplen serves on the editorial board of the journal MCP and is an employee of Amedes Genetics (Hannover, Germany). U.K. Zettl has received speaker honoraria and travel funding from Bayer Pharma, Aventis Pharma, TEVA Pharma, Merck-Serono Pharma, and Biogen-Idec Pharma. M. Hecker has received speaker honoraria and travel funding from Bayer Health Care, Biogen, Novartis, and Teva. A. Deutschländler is supported by a gift from Carl Edward Bolch Jr and Susan Bass Bolch, and by the Sol Goldman Charitable Trust; and has received research support from Allergan. S.G. Meuth receives speaker honoraria and travel funding from Almirall, Amicus Therapeutics Germany, Bayer Health Care, Biogen, Celgene, Diamed, Genzyme, MedDay Pharmaceuticals, Merck Serono, Novartis, Nordisk, ONO Pharma, Roche, Sanofi-Aventis, Chugai Pharma, QuintilesIMS, and Teva; receives research support from the German Ministry for Education and Research (BMBF), Deutsche Forschungsgesellschaft (DFG), Else Kröner-Fresenius Foundation, German Academic Exchange Service, Hertie Foundation, Interdisciplinary Center for Clinical Studies (IZKF) Muenster, German Foundation Neurology and Almirall, Amicus Therapeutics Germany, Biogen, Diamed, Fresenius Medical Care, Genzyme, Merck Serono, Novartis, ONO Pharma, Roche, and Teva; serves on the editorial board of PLoS One; and holds patents for effectivity of specific FXII/FXIIa inhibitors (particularly rHA-Infestin 4 used to treat neuro-inflammatory diseases) (WO 2013/113774 A1 and EP 2 263 110 A1), and for diagnosis of a novel autoimmune disease (European patent; 15001186.4—1402). M. Ahram has received research support from the Ministry of Higher Education and Scientific Research, the University of Jordan, the King Abdullah II Fund for Development (KAFD), the Abdul Hameed Shoman Fund for Supporting Scientific Research, and the Hashemite University. B. Mustafa, M. El-Khateeb, and C. Vilarino-Güell report no disclosures. A.D. Sadovnick has received travel funding from Biogen; has served on the speakers’ bureau of the Consortium of Multiple Sclerosis Centers (CMSC); and has received research support from Biogen MA Inc, Novartis Pharmaceuticals Canada Inc, Genzyme Canada Inc, and Biogen Idec Inc (Canada). F. Zimprich serves on an editorial board (unspecified). B. Tomkinson reports no disclosures. T. Strom has received research support from the European Union; and receives license fee payments from FGF23, Kirin Brewery. W. Kristoferitsch has served on scientific advisory boards for Biogen Austria and Novartis; and serves on the editorial board of Journal für Neurologie, Neurochirurgie und Psychiatrie. H. Lassmann has received speaker and travel honoraria from Biogen Idec, Novartis, Roche, and Teva; serves on the editorial boards of several journals in the fields of neurology and neuroscience (unspecified); has served as a consultant for Medday and Roche; and has received research support from the Austrian Science Foundation and the European Union. A. Zimprich reports no disclosures. Full disclosure form information provided by the authors is available with the full text of this article at Neurology.org/NG.

Received March 28, 2018. Accepted in final form September 26, 2018.

References
1. Evans RS, Takahashi K, Duane RT, Payne R, Liu C. Primary thrombocytopenic purpura and acquired hemolytic anemia: evidence for a common etiology. Arch Intern Med 1951;87:48–65.
2. Besnard C, Levy E, Aladjidi N, et al. Pediatric-onset Evans syndrome: heterogeneous presentation and high frequency of monogenic disorders including LRBA and CTLA4 mutations. Clin Immunol 2018;188:52–57.
3. Michel M, Chanet V, Dechartres A, et al. The spectrum of Evans syndrome in adults: new insight into the disease based on the analysis of 68 cases. Blood 2009;114:3167–3172.
4. Wang W, Herrold H, Pui CH, Presbury G, Wilimas J. Immunoregulatory abnormalities in Evans syndrome. Am J Hematol 1983;15:381–390.
5. Aladjidi N, Fernandes H, Leblanc T, et al. Evans syndrome in children: long-term outcome in a prospective French national observational cohort. Front Pediatr 2015;3:79.
6. Schubert D, Bode C, Kenebeck R, et al. Autosomal dominant immune dysregulation syndrome in humans with CTLA4 mutations. Clin Immunol 2018;188:52–57.
7. Kuemn HS, Ouyang W, Lo B, et al. Immune dysregulation in human subjects with heterozygous germline mutations in CTLA4. Science 2014;345:1623–1627.
8. Simon DJ, Kuhlmann T, Bittner S, et al. Evans syndrome associated with sterile inflammation of the central nervous system: a case report. J Med Case Rep 2017:3:626.
9. Lu W, Zhang Y, McDonald DO, et al. Dual proteolytic pathways govern glycosylation and immune competence. Cell 2014;159:1578–1590.
10. Steepensky P, Rensing-Eld A, Gather R, et al. Early-onset Evans syndrome, immunodeficiency, and premature immunosenescence associated with tripeptidyl-peptidase II deficiency. Blood 2015;125:753–761.
11. Tomkinson B, Lindas AC. Tripeptidyl-peptidase II: a multi-purpose peptidase. Int J Biochem Cell Biol 2005;37:1933–1937.
12. Sadovnick AD, Risch NJ, Ebers GC. Canadian collaborative project on genetic susceptibility to MS, phase 2: rationale and method. Canadian Collaborative Study Group. Can J Neurol Sci 1998;25:216–221.
13. Poser CM, Paty DW, Scheinberg L, et al. New diagnostic criteria for multiple sclerosis: guidelines for research protocols. Ann Neurol 1983;13:227–231.
14. McDonald WI, Compston A, Edan G, et al. Recommended diagnostic criteria for multiple sclerosis: guidelines from the International Panel on the diagnosis of multiple sclerosis. Ann Neurol 2001;50:121–127.
15. Polman CH, Reingold SC, Banwell B, et al. Diagnostic criteria for multiple sclerosis: 2010 revisions to the McDonald criteria. Ann Neurol 2011;69:292–302.
16. Wang Z, Sadovnick AD, Traboulsee AL, et al. Nuclear receptor NR1H3 in familial multiple sclerosis. Neuron 2016;90:948–954.
17. Lassmann H. Review: the architecture of inflammatory demyelinating lesions: implications for studies on pathogenesis. Neuropathol Appl Neurobiol 2011;37:698–710.
18. Tomkinson B. Association and dissociation of the tripeptidyl-peptidase II complex as a way of regulating the enzyme activity. Arch Biochem Biophys 2000;376:275–280.
19. Rose C, Vargas F, Facchinetti P, et al. Characterization and inhibition of a cholecystokinin-inactivating serine peptidase. Nature 1996;380:403–409.
20. Han MH, Lundgren DH, Jassows S, et al. Janus-like opposing roles of CD47 in autoimmunity. Proc Natl Acad Sci U S A 2008;105:5177–5182.
21. Endert P. Role of tripeptidyl peptidase II in MHC class I antigen processing—the end of controversies? Eur J Immunol 2008;38:609–613.
22. Kawahara M, York IA, Hearn A, Farfan D, Rock KL. Analysis of the role of tripeptidyl peptidase II in MHC class I antigen presentation in vivo. J Immunol 2009;183:6069–6077.
23. Huai J, Firat E, Nil A, et al. Activation of cellular death programs associated with immunosenescence-like phenotype in TPPII knockout mice. Proc Natl Acad Sci U S A 2008;105:5177–5182.
24. Firat E, Huai J, Saveana L, et al. Analysis of direct and cross-presentation of antigens in TPPII knockout mice. J Immunol 2007;179:8137–8145.
25. Tomkinson B, Zetterqvist O. Immunological cross-reactivity between human tripeptidyl peptidase II and fibronectin. Biochem J 1990;267:149–154.
26. Palsson-McDermott EM, O’Neill LA. The Warburg effect then and now: from cancer to inflammatory diseases. Bioessays 2013;35:965–973.
27. Carbone F, De Rosa V, Carri rer PB, et al. Regulatory T cell proliferative potential is impaired in human autoimmune disease. Nat Med 2014;20:69–74.
28. Viglietta V, Baecker-Allan C, Weiner HL, Hafler DA. Loss of functional suppression by CD4+CD25+ regulatory T cells in patients with multiple sclerosis. J Exp Med 2004;199:971–979.
29. De Rosa V, Galgani M, Porcellini A, et al. Glycolysis controls the induction of human regulatory T cells by modulating the expression of FOXP3 exon 2 splicing variants. Nat Immunol 2015;16:1174–1184.
30. Chang CH, Curtis JD, Maggi LB, Jr, et al. Posttranscriptional control of T cell effector function by aerobic glycolysis. Cell 2013;153:1239–1251.
31. Diekmann J, Adamopoulos E, Beck O, et al. Processing of two latent membrane protein 1 MHC class I epitopes requires tripeptidyl peptidase II involvement. J Immunol 2009;183:1587–1597.