

# 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 Pferschy, Christiane Schmied, MD, Fritz Leutmezer, MD, Mohammad Keilani, MD, Christina M. Lill, PhD, Sabine Hoffjan, MD, Jörg T. Epplen, MD, Uwe K. Zettl, MD, Michael Hecker, Angela Deutschländer, MD, Sven G. Meuth, PhD, Mamoun Ahran, MD, Baha Mustafa, PhD, Mohammed El-Khateeb, PhD, Carles Vilariño-Güell, PhD, A. Dessa Sadovnick, MD, Fritz Zimprich, MD, PhD, Birgitta Tomkinson, PhD, Tim Strom, PhD, Wolfgang Kristoferitsch, PhD, Hans Lassmann, PhD, and Alexander Zimprich, PhD

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

*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.), Jewells 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.), SMZ-Ost-Donauspital, Vienna, Austria; and Institute for Neuroimmunological and Neurodegenerative Disorders (W.K.), SMZ-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](http://Neurology.org/NG).

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.<sup>1</sup> Although most cases have no obvious underlying etiology, rare monogenic forms have been identified in the last years.<sup>2</sup> ES can affect different organs, manifesting with hepatomegaly, splenomegaly,<sup>3–5</sup> and lymphocytic infiltration of nonlymphoid organs, including the brain.<sup>6,7</sup> 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.<sup>8</sup>

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.<sup>9,10</sup> *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.<sup>11</sup> 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.<sup>9</sup> 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  $1.3 \times 10^3/\mu\text{L}$  (normal range is between 1.0 and  $4 \times 10^3/\mu\text{L}$ ) 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](https://links.lww.com/NXG/A108)), figure e-2 ([links.lww.com/NXG/A109](https://links.lww.com/NXG/A109)); legends [links.lww.com/NXG/A130](https://links.lww.com/NXG/A130), and in the supplementary information ([links.lww.com/NXG/A110](https://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 MS<sup>12</sup> (table 1). All patients were diagnosed with MS according to published criteria.<sup>13–15</sup>

### 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](http://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 (Agilent Technologies, Santa Clara, CA). Sequencing of postenrichment libraries was carried out on the Illumina HiSeq 2000 sequencing instrument (Illumina, San Diego, CA) as  $2 \times 100$  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.<sup>16</sup>

### Targeted capture sequencing, single-nucleotide polymorphism genotyping, and in silico prediction

Illumina TruSeq Custom Amplicon Kit was used to target all exonic and flanking intronic regions of *TPP2* and excision repair cross-complementation group 5 (*ERCC5*) in 382

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

Site	Cases	Male	Female	Aao	Positive family history/ recessive <sup>a</sup>
<b>Targeted sequence analysis (TruSeq) of the <i>TPP2</i> and <i>ERCC5</i> gene in MS cases</b>					
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
<b>Targeted mutation screening of <i>TPP2</i>, <i>Cys28Gly</i>; and <i>TPP2</i>, <i>Thr676Ile</i> in Jordanian cases and controls</b>					
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.

<sup>a</sup> 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.

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 *TPP2* (p.Cys28Gly, p.Thr676Ile) and *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](https://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.<sup>17</sup>

### 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](http://wch.sa.gov.au/services/az/divisions/labs/geneticmed/nrl_methods.html)). Enzymatic activity was measured as described previously.<sup>18</sup>

## Results

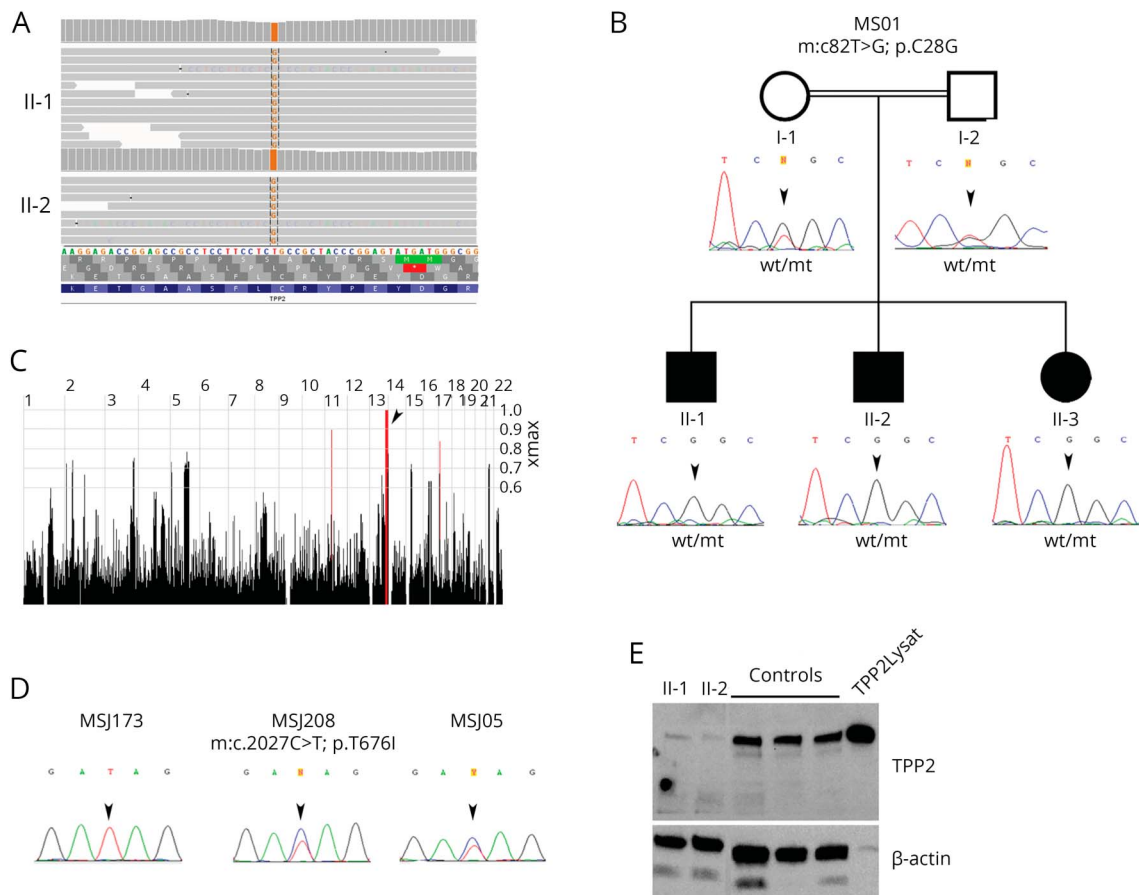
### Identification of a homozygous mutation in the *TPP2* gene as a likely cause for a sterile brain inflammation in a consanguineous Syrian family

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 *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 *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](https://links.lww.com/NXG/A110)). We did not find any rare

**Figure 1** Mutation identification and Western blot



(A) Exome sequencing alignment data of the homozygous *TPP2* mutation in II.1 and II.2 visualized with the IGV tool. (B) Pedigree of consanguineous Syrian family (MS01) affected with MS. Sanger chromatograms of heterozygous parents and homozygous siblings are depicted below their respective symbol. Arrow, position of mutation. (C) Homozygosity plot from Syrian family. Red bars, regions of homozygosity; arrow, homozygous block shared by all 3 siblings containing *TPP2* and *ERCC5*. (D) Chromatograms of homozygous and heterozygous p. Thr676Ile *TPP2* mutation carrier of confirmatory sequencing cohort. (E) Western blot of *TPP2* in PBMCs from II.1, II.2, and 3 healthy control individuals. Black filled symbols = affected; ERCC5 = excision repair cross-complementation group 5; IGV = integrated genome viewer; mt/mt = homozygous for the mutation; open symbols = unaffected; PBMC = peripheral blood mononuclear cell; *TPP2* = tripeptidyl peptidase II; wt/mt = heterozygous for the mutation.

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.

**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
<i>TPP2</i>	chr13:103249470	c.82T>G	p.C28G	18.4		0/643	0/416	0/452	Not present
<i>TPP2</i>	chr13:103288715	c.1651A>G	p.I551V	21.4		1/643	0/183	n.a.	Not present
<i>TPP2</i>	chr13:103295578	c.2027C>T	p.T676I	23.3	rs760347832	0/643	1x hom, 3x het/416	0/452	6 x het/59.292
<i>TPP2</i>	chr13:103298652	c.2402G>A	p.S801N	23.5	rs140329690	1/643	0/183	n.a.	8 x het/58.580
<i>TPP2</i>	chr13:103309488	c.3035A>G	p.E1012G	23.4	rs199569052	1/643	0/183	n.a.	15 x het/60.493
<i>TPP2</i>	chr13:103326722	c.3422A>C	p.Q1141P	5.4	rs199702252	1/643	0/183	n.a.	14 x het/66.692
<i>ERCC5</i>	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  $0.42 \pm 0.10$  and  $0.50 \pm 0.15$   $\text{nmol} \cdot \text{min}^{-1} \cdot \text{mg}^{-1}$  protein for the patient and the controls, respectively. In leukocyte lysates, the activity was  $8.8 \pm 1.3$  and  $7.1 \pm 1.1$   $\text{nmol} \cdot \text{min}^{-1} \cdot \text{mg}^{-1}$  for the patient and the controls, respectively. The measured activity is inhibited to >95% with the specific TPP2 inhibitor butabindide,<sup>19</sup> 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

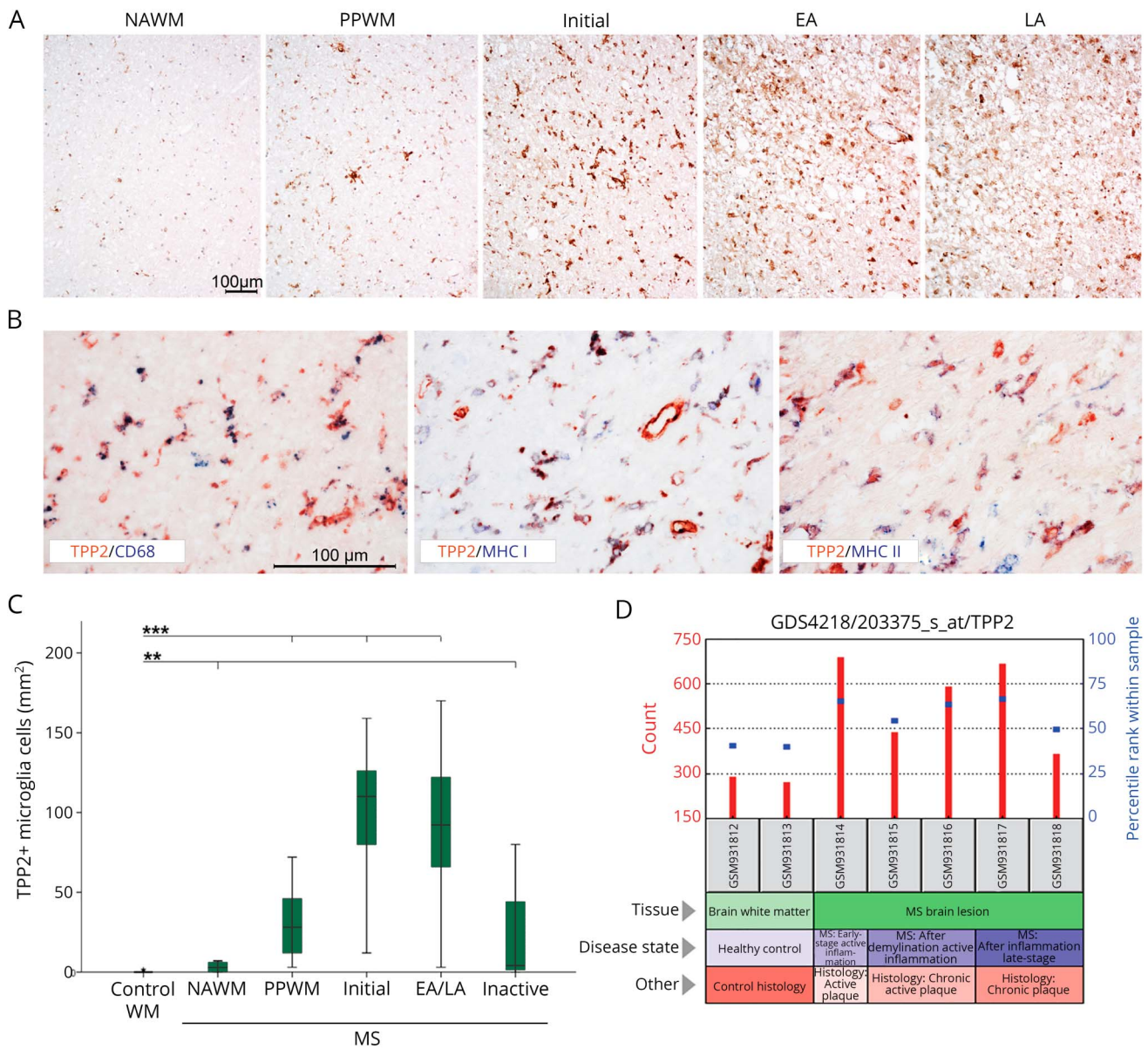
pronounced staining. The highest expression of TPP2 was seen in initial stages of MS lesions, characterized by profound microglia activation, oligodendrocyte apoptosis, and initial myelin damage. In the demyelinated portion of the lesions, the expression of TPP2 in microglia decreased from the lesion 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).<sup>20</sup>

## 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

**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 mm<sup>2</sup> 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.

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.<sup>9</sup> 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

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.<sup>9</sup> Like other cytosolic peptidases, *TPP2* influences the MHC Class I metabolism, usually through the destruction of MHC Class I epitopes.<sup>21,22</sup> Furthermore, increased MHC Class I and Class II expression has been shown in *TPP2*-deficient mice.<sup>23,24</sup> The familial mutation, p.Cys28Gly, results in reduced protein expression in the patients, as seen in the Western blot experiments. 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*.<sup>18</sup> For example, dissociated *TPP2* is more sensitive to proteolytic degradation.<sup>25</sup>

Given the previously reported defect in aerobic glycolysis because of impairment of lysosomal function in *TPP2*-deficient patients, one could anticipate a similar pathogenic mechanism in our *TPP2*-mutant patients.<sup>9</sup> The aerobic glycolysis pathway is used in situations of high-energy demand. Specifically, tumor cells and immune cells when activated are known to switch their metabolism towards aerobic glycolysis.<sup>26</sup> Particularly, induction and suppressive function of regulatory T cells have been shown to be critically dependent on this pathway.<sup>27–30</sup> Intriguingly, it was shown that regulatory T cells from relapsing-remitting MS patients have an impaired capacity to induce aerobic glycolysis.<sup>29</sup>

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.<sup>31</sup> 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 pathophysiology. 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](https://links.lww.com/NXG/A108)) and

provided and interpreted data for supplementary figure e-2 ([links.lww.com/NXG/A109](https://links.lww.com/NXG/A109)). E. Graf, T. Wieland, and T. Strom performed Illumina exome sequencing and provided the bioinformatical 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. Deutschländer, 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. Vilariño-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 (11-32095000), 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. Leutmezer 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. Deutschländer 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, Novo 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 (KAJD), the Abdul Hameed Shoman Fund for Supporting Scientific Research, and the Hashemite University. B. Mustafa, M. El-Khateeb, and C. Vilarino-Guell 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 Fund 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](https://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. *AMA 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, Herrod 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, Kenefack R, et al. Autosomal dominant immune dysregulation syndrome in humans with CTLA4 mutations. *Nat Med* 2014;20:1410–1416.
7. Kuehn 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 OJ, Kuhlmann T, Bittner S, et al. Evans syndrome associated with sterile inflammation of the central nervous system: a case report. *J Med Case Rep* 2013;7:262.
9. Lu W, Zhang Y, McDonald DO, et al. Dual proteolytic pathways govern glycolysis and immune competence. *Cell* 2014;159:1578–1590.
10. Stepensky P, Rensing-Ehl 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, Linds 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, Jaiswal S, et al. Janus-like opposing roles of CD47 in autoimmune brain inflammation in humans and mice. *J Exp Med* 2012;209:1325–1334.
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, Saveanu 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, Carrieri PB, et al. Regulatory T cell proliferative potential is impaired in human autoimmune disease. *Nat Med* 2014;20:69–74.
28. Viglietta V, Baecher-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, Adamopoulou 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.

# Neurology<sup>®</sup> Genetics

## TPP2 mutation associated with sterile brain inflammation mimicking MS

Eva M. Reinthaler, Elisabeth Graf, Tobias Zrzavy, et al.

*Neurol Genet* 2018;4;

DOI 10.1212/NXG.0000000000000285

**This information is current as of November 13, 2018**

<b>Updated Information &amp; Services</b>	including high resolution figures, can be found at: <a href="http://ng.neurology.org/content/4/6/e285.full.html">http://ng.neurology.org/content/4/6/e285.full.html</a>
<b>References</b>	This article cites 31 articles, 10 of which you can access for free at: <a href="http://ng.neurology.org/content/4/6/e285.full.html##ref-list-1">http://ng.neurology.org/content/4/6/e285.full.html##ref-list-1</a>
<b>Subspecialty Collections</b>	This article, along with others on similar topics, appears in the following collection(s): <b>Autoimmune diseases</b> <a href="http://ng.neurology.org/cgi/collection/autoimmune_diseases">http://ng.neurology.org/cgi/collection/autoimmune_diseases</a> <b>Multiple sclerosis</b> <a href="http://ng.neurology.org/cgi/collection/multiple_sclerosis">http://ng.neurology.org/cgi/collection/multiple_sclerosis</a>
<b>Permissions &amp; Licensing</b>	Information about reproducing this article in parts (figures, tables) or in its entirety can be found online at: <a href="http://ng.neurology.org/misc/about.xhtml#permissions">http://ng.neurology.org/misc/about.xhtml#permissions</a>
<b>Reprints</b>	Information about ordering reprints can be found online: <a href="http://ng.neurology.org/misc/addir.xhtml#reprintsus">http://ng.neurology.org/misc/addir.xhtml#reprintsus</a>

*Neurol Genet* is an official journal of the American Academy of Neurology. Published since April 2015, it is an open-access, online-only, continuous publication journal. Copyright © 2018 The Author(s). Published by Wolters Kluwer Health, Inc. on behalf of the American Academy of Neurology. All rights reserved. Online ISSN: 2376-7839.

