Tubers are neither static nor discrete

Evidence from serial diffusion tensor imaging

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ABSTRACT

Objective: To assess the extent and evolution of tissue abnormality of tubers, perituber tissue, and normal-appearing white matter (NAWM) in patients with tuberous sclerosis complex using serial diffusion tensor imaging.

Methods: We applied automatic segmentation based on a combined global-local intensity mixture model of 3T structural and 35 direction diffusion tensor MRIs (diffusion tensor imaging) to define 3 regions: tuber tissue, an equal volume perituber rim, and the remaining NAWM. For each patient, scan, lobe, and tissue type, we analyzed the averages of mean diffusivity (MD) and fractional anisotropy (FA) in a generalized additive mixed model.

Results: Twenty-five patients (mean age 5.9 years; range 0.5–24.5 years) underwent 2 to 6 scans each, totaling 70 scans. Average time between scans was 1.2 years (range 0.4–2.9). Patient scans were compared with those of 73 healthy controls. FA values were lowest, and MD values were highest in tubers, next in perituber tissue, then in NAWM. Longitudinal analysis showed a positive (FA) and negative (MD) correlation with age in tubers, perituber tissue, and NAWM. All 3 tissue types followed a biexponential developmental trajectory, similar to the white matter of controls. An additional qualitative analysis showed a gradual transition of diffusion values across the tissue type boundaries.

Conclusions: Similar to NAWM, tuber and perituber tissues in tuberous sclerosis complex undergo microstructural evolution with age. The extent of diffusion abnormality decreases with distance to the tuber, in line with known extension of histologic, immunohistochemical, and molecular abnormalities beyond tuber pathology. *Neurology*® 2015;85:1536–1545

GLOSSARY

DTI = diffusion tensor imaging; **FA** = fractional anisotropy; **FLAIR** = fluid-attenuated inversion recovery; **FOV** = field of view; **GAMM** = generalized additive mixed model; **MD** = mean diffusivity; **MPRAGE** = magnetization-prepared rapid-acquisition gradient echo; **NAWM** = normal-appearing white matter; **TE** = echo time; **TR** = repetition time; **TSC** = tuberous sclerosis complex.

Tuberous sclerosis complex (TSC) is a genetic, multisystem disorder characterized by hamartoma formations in various organs, including the brain, where they are referred to as tubers. Cerebral cortical tubers are present in more than 80% of patients with TSC and arise due to abnormal cellular differentiation, migration, and proliferation.¹

Although TSC traditionally has been considered a disorder of discrete, multifocal abnormalities, a growing body of evidence suggests that TSC neuropathology exists far beyond tuber borders visible on conventional MRI. Tuber-like pathology has recently been identified in the direct vicinity of tubers, as well as diffusely throughout the white matter.^{2,3} Studies using diffusion tensor imaging (DTI) are in agreement and describe decreased fractional anisotropy (FA) or increased mean diffusivity (MD) in tubers,⁴ in perituber tissue,⁵ and in otherwise normal-appearing white matter (NAWM).^{6–8}

In addition, changes in tissue contrast, gadolinium enhancement, and cyst-like degeneration over time on conventional imaging have changed the view of tubers from static to dynamic.^{9–11}

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While cross-sectional studies have linked DTI measures of tubers to epilepsy localization and severity,¹² and DTI measures of NAWM to neurodevelopmental disorders,^{13,14} the longitudinal evolution of tissue diffusion in TSC has not been investigated, to date.

In TSC, therefore, it is unclear when and where such tissue abnormalities occur in the developmental trajectory. We sought to describe maturational changes of DTI measures in young patients with TSC and assess the extent of diffusion abnormality across tuber, perituber, and NAWM tissue types.

METHODS Participants. Twenty-five children and young adults followed in the Multidisciplinary TSC Program at Boston Children's Hospital with a definite diagnosis of TSC¹⁵ underwent 2 to 6 MRI scans. Patients who underwent surgery for epilepsy or for resection of subependymal giant cell astrocytoma were excluded. There were no age cutoff criteria. Medical record review provided clinical and genetic data. Autism spectrum disorder was diagnosed clinically by *DSM-IV* criteria, and supplemented by ADOS (Autism Diagnostic Observation Schedule)¹⁶ in most patients. Intractability was defined as ongoing seizures in the presence of 2 or more adequate antiepileptic drugs. Seventy-three control participants, not age-matched, were recruited as part of this research study, and each underwent a single scan with normal MRI results per review by a pediatric neuroradiologist (S.P.P.).

Standard protocol approvals, registrations, and patient consents. Recruitment and data acquisition of patients and controls were conducted using a protocol approved by the institutional review board.

Image acquisition. Imaging was performed on a Siemens Trio 3T MRI system. Acquisition parameters were unchanged with repeat imaging for each participant, and images acquired with a different protocol were excluded from the study. Sedation was used only in participants undergoing clinical imaging if necessary to prevent significant motion.

The imaging protocol included the following: (1) a T1-weighted high-resolution magnetization-prepared rapid-acquisition gradient echo (MPRAGE) sequence (voxel size [mm] $0.5 \times 0.5 \times 1$ to $1 \times 1 \times 1$, field of view [FOV] 19.2–25.6 cm, echo time [TE] 1.66–3.39 milliseconds [ms], repetition time [TR] 1,130–2,530 ms, flip angle 7° – 9°); (2) a T2-weighted turbo spin echo sequence; (3) sagittal 3-dimensional isotropic T2 fluid-attenuated inversion recovery (FLAIR) (voxel size [mm] = $0.9 \times 0.9 \times 1$, number of excitations 1, TR 5,000 ms, TE 390–400 ms, echo train length 141, FOV 19–26 cm, flip angle 20° , acquisition matrix 256×256); and (4) diffusion imaging with single-shot spin echo acquisition in the axial plane, with twice refocused gradients to minimize Eddy currents, using 30 images with $b = 1,000$ s/mm² and 5 images with $b = 0$ s/mm² (voxel size [mm] $1.72 \times 1.72 \times 2.2$, FOV 22 cm, slice thickness 2.2 mm, TE 88 ms, TR 10 seconds, acquisition matrix 128×128 , number of excitations 1, iPAT 2, TR 1,000 ms, flip angle 90° , in-plane GRAPPA, modified as necessary to facilitate completion of the scan).

Compensation for residual distortion and patient motion was achieved by rigid registration of the diffusion images to the T1-weighted MPRAGE scan, with appropriate reorientation of the gradient directions.¹⁷ Tensors were estimated using robust least squares.¹⁸

Longitudinal alignment. For each participant, to ensure longitudinal analysis of the same structural regions, segmentation of the 3 tissue types (tuber, perituber, and NAWM) was only done once on a *reference scan* of highest-quality structural imaging. Given maturational increases in tissue contrast, this was typically the latest image series acquired. For each scan, the diffusion tensors were resampled and aligned to the T1 MPRAGE space. The other T1-weighted MRIs underwent rigid then nonrigid registration to the T1 reference scan, and this transform was applied to the diffusion images.¹⁹

Tissue segmentation. Segmentation of 3 tissue types was based on a combined global-local intensity mixture model²⁰ on the FLAIR image. We used the expectation–maximization algorithm to estimate the parameters that maximize the tissue maximum a posteriori probabilities. Cortical tubers were modeled as outliers²¹ and specifically validated for use in TSC²² (figure 1).

The tuber segmentation was expanded in 3 dimensions to the adjacent perituber white matter, until a volume equal to the tuber was defined, and truncated in case of overlap with gray matter or other tubers. In patients, white matter that was neither tuber nor perituber was designated as NAWM. Radial migration lines were included in the tuber and perituber tissues, depending on local density values. Parcellation of lobes was done using a local MAP PSTAPLE algorithm.²³

Empirically, small volumes of tissue classified as tuber with a volume of less than 500 voxels were excluded from analysis as the false detection rate approached zero at that cutoff, and so was the associated perituber rim in such cases. Calcified and cyst-like tubers were omitted because calcium deposits and free water, respectively, alter tensor estimates (figure e-1 on the *Neurology*[®] Web site at Neurology.org).

Diffusion measures. For each participant, in each scan, in each lobe, the average MD and average FA of the tissue types were calculated. In each region, the median and skew were also calculated to assess distribution of MD and FA values.

As a qualitative analysis, the boundaries between different tissue types were examined by measuring neighboring voxels from one tissue type to the next in 6 random participants. In the axial plane, the FA values were acquired with regular intervals along a trajectory from the periphery (tuber), via perituber tissue, to deep NAWM. The plots were smoothed with a moving average of 3 data points to correct for jitter.

Histopathology and immunohistochemistry. A left occipital lobe resection from a 6-year-old patient with TSC (consented for research, not part of the imaging cohort), as part of an epilepsy surgery, was studied histopathologically. Sections were stained with hematoxylin & eosin and Luxol fast blue, and immunohistochemical staining was performed for glial fibrillary acidic protein, neuronal nuclear antigen, phosphorylated neurofilament (SMI 31), neurofilament protein, and synaptophysin. Standard clinical laboratory protocols were used for each staining.

Statistical analysis. Normal distribution of variables was tested using the Shapiro-Wilk test, and when applicable, nonparametric testing was applied. Associations were tested by Kendall tau rank correlation coefficient. Distributions were compared by nonparametric Wilcoxon rank sum or Kruskal-Wallis testing, and the influence of tissue types (tuber, perituber, NAWM, control white matter), confounding for all other factors in the dataset as a block variable, was tested using the nonparametric Friedman test. If distributions differed, Tukey or Dunnnett multiple comparisons were made for further analysis.

Figure 1 Segmentation algorithm and distribution histograms of FA values of tuber, perituber, and NAWM tissues

(A–D) Manual and automated tuber segmentation. (A) Axial fluid-attenuated inversion recovery image, with tubers appearing as bright areas of T2 prolongation. (B) Manual segmentation; areas indicating tuber tissue are marked red. (C) Intensity likelihood of outlier voxels with color spectrum from blue (least likely) to red (most likely), given the global-local intensity mixture model. (D) Automated tuber segmentation, tuber areas indicated in red and perituber tissue in salmon, after removal of false-positive areas (e.g., CSF pulsation artifacts). (E) Example of distribution histograms of 3 tissue types in 3 scans of one patient. On the right, 3 axial FA maps are aligned, each acquired at a different time point. The superimposed automated segmentation reveals 3 different tissue types of the right frontal lobe (dark blue cortex not included): red (tuber), salmon (perituber), light blue (NAWM). On the left, the corresponding distribution histograms of FA values are displayed for each tissue type. For clarity, only the histograms of the right frontal lobe of the *first* acquired image are shown. The mean (dotted line) and median (vertical line) reflect the skew in each histogram. FA = fractional anisotropy; NAWM = normal-appearing white matter.

The generalized additive mixed model (GAMM) was used for longitudinal analysis of the data. This extension of the linear model is suited whenever the outcome variable cannot be explanatory variables in a nonlinear fashion (figure e-2), with no known explicit parametrization.²⁴

In addition, the mixed component of the GAMM allows for the introduction of random effects that, in conjunction with repeated-measure data, enables separation of inter- and intrasubject variability from the random error component, which ultimately yields greater statistical power to reveal the most important mechanisms affecting the outcome. The interested

reader is invited to explore both the technical and layman introduction to GAMM (appendix e-1).

RESULTS Patient characteristics. Patients were younger than controls (age at first scan 5.9 years, range 0.5–24.6 vs 13.0, range 1.1–25.2, $p < 0.0001$). Average time between 2 consecutive scans in patients was 1.2 years (range 0.4–2.9). Sex was not significantly different (17 [68%] patients vs 37 [51%] controls were male). Seventeen patients

(68%) had *TSC2* mutations, 5 (20%) had *TSC1* mutations, and 3 had negative genetic testing. Eleven (44%) had autism spectrum disorder. All patients had epilepsy (15 [60%] intractable) and were on antiepileptic drugs; 10 (40%) had a history of infantile spasms.

DTI evolution. The mean and median in each tissue type confirmed unimodal distribution of FA and MD values, and tail values of 2.5% on each end of the distribution histograms were omitted to ensure robust estimates of averages (figure 1). Univariate analyses revealed that side and sex were poorly

Figure 2 Diffusion measures over time in tuberous sclerosis complex

FA (A) and MD (B) measures are fitted with the GAMM, and evolution with age in years is shown for every lobe. Three different tissue types are color-coded: red (tuber), salmon (perituber), and light blue (normal-appearing white matter). White matter of controls is represented in black. Note the early, steep, linear increase before age 5 to 6 years and the slower increase thereafter. FA = fractional anisotropy; GAMM = generalized additive mixed model; MD = mean diffusivity.

associated with both FA ($p = 0.28$ and 0.44) and MD ($p = 0.11$ and 0.41) and were thus discarded. The dependency of FA/MD with age in our regression model was described by 5 regressors (see detailed methods in appendix e-1), and after GAMM modeling, all regression coefficient estimates were statistically non-null (data not shown).

The selected GAMM model describes an evolution of FA and MD over time common to all lobes and tissue types, an evolution of these measures with a volume specific for each lobe, and a different value of FA and MD at the intersect in each tissue type and in each lobe (figure 2). The biexponential trajectory of FA and MD changes with age, is seen not only for the control participant and in the NAWM of patients, but also for tuber and perituber tissues. The tuber tissue type trajectory reaches a plateau at a lower FA and higher MD value, and the perituber tissue type displays characteristics intermediate to tuber and NAWM tissues.

Tuber and perituber tissues were different than the NAWM of controls, both globally and in each lobe. There was no consistent pattern of differences in the

comparison of NAWM to control white matter. For the FA only, and only in temporal and parietal regions, the NAWM tissue evolution differed significantly from the white matter of controls. Confidence intervals of these coefficients approached but did not cross zero, suggesting that this may be a spurious finding. With the analysis of tissue types independently from lobes, the NAWM DTI trajectory with age did not differ significantly from controls (figure e-3).

DTI across tissue boundaries. To qualitatively evaluate whether boundaries between the tissue types were discrete, we plotted DTI measures along a trajectory of adjacent voxels in the axial plane from tuber to deep white matter. For each of the 6 random examined patients, we found a gradual transition across tissue-type boundaries, rather than abrupt changes. The change from tuber tissue to perituber tissue was smooth, and so was the change from perituber tissue to NAWM (figure 3).

Neuropathologic examination of illustrative case. Histopathology and immunohistochemistry of an occipital resection specimen of a 6-year-old patient with TSC

Figure 3 Gradual transition between tissue types from tuber to deep NAWM

(A) Changes in FA measured along trajectories from tuber to deep NAWM in each lobe of 5 patients reveal a gradual transition between tissue types (example trajectories in black above plots). The boundaries between the tuber (red), perituber (salmon), and NAWM (light blue) tissue types are not discrete, and thus segmentation is dependent on factors such as imaging contrast, resolution, and arbitrary thresholding. Note the deep "dips" in the curves of the NAWM, reflecting areas with more complex white matter,²⁷ for example, the crossing of pathways. (B) The trajectories of 5 healthy controls, also with some increase of FA values in deeper white matter pathways. FA = fractional anisotropy; NAWM = normal-appearing white matter.

Figure 4 Histopathology and immunohistochemistry of tuber tissue (A-D), perituber white matter (E-H), a microtuber area (I-L), and NAWM (M-P)

The gross specimen (top left), low-magnification H&E/LFB (top middle), and pocket of tuber pathology (microtuber;² top right) indicate the studied areas. Tuber tissue (A) H&E/LFB staining demonstrates absence of cortical lamination and presence of cells of "ambiguous phenotype" in tubers, variably positive for glial (B) and neuronal markers (C). SMI 31 (phosphorylated neurofilament) positivity in the perikarya is a feature of dysplastic neurons (D). In the white matter of perituber tissue (E), scattered balloon cells are also present, as well as occurring in small confluent clusters in the microtuber (I). In more remote white matter (M), apparently normal myelination is seen. Reactive astrocytes, indicating gliosis likely related to seizure activity, are seen in tubers (B), as well as remote from the tuber, in (F) and (J), and in the NAWM (N). Scattered heterotopic neurons are seen in perituber tissue (G), in the microtuber (K), and are easy to appreciate at lower magnification in the NAWM (O). NFP stain of perituber white matter marks some dysplastic neurons (H), as does the synaptophysin stain in the microtuber area (L). (P) A normal NFP stain shows white matter, with axons taking a radial turn into the cortex. GFAP = glial fibrillary acidic protein; H&E/LFB = hematoxylin & eosin/Luxol fast blue; NAWM = normal-appearing white matter; NeuN = neuronal nuclear antigen; NFP = neurofilament protein; SMI 31 = phosphorylated neurofilament.

revealed a tuber characterized by a loss of lamination, abundant large dysplastic neurons, mixed with balloon cells of “ambiguous phenotype” and dense gliosis (glial fibrillary acidic protein). Surrounding this were a variety of pathologic features including multiple small, less-compact tubers composed of balloon cells in both gray and white matter and abnormal neocortex with disorganized architecture and lamination, scattered large neurons in the superficial cortex, and extensive reactive gliosis. There were scattered focal disruptions of the myelination (Luxol fast blue) by small clusters of balloon cells in the adjacent white matter, and more remotely in the NAWM, referred to as microtubers² (figure 4).

DISCUSSION This study presents a novel characterization of longitudinal diffusion changes of various tissue types in TSC and establishes a natural evolution of maturing NAWM, perituber tissue, and even tuber tissue. In addition, we show a gradient of abnormality between these tissue types, with a smooth transition across boundaries, confirming that tuber-like pathology is widespread and decreases with distance to the tuber itself.

Because tuber pathology is not discrete, the perituber rim is arbitrarily defined and may microscopically

include parts of both the NAWM and tuber tissue types. Thus, the finding of DTI measures of intermediate value between tuber and NAWM tissue types is not surprising. Likewise, small amounts of normal white matter could have been included in the tuber, and more so in perituber image segmentation, which may be responsible for the DTI changes resembling the NAWM developmental trajectory.

The smooth transition, however, between the 3 segmented tissue types, and the similar evolution, are consistent in every lobe, across all ages, and corrected for volumetric differences. Indeed, the perituber cortex contains similar but milder histologic, immunohistochemical, and molecular abnormalities, suggesting dysplasia and aberrant mTOR (mammalian target of rapamycin) signaling beyond tubers into perituber tissue.³ Our neuropathologic example illustrates that dysmorphic and heterotopic cells with a hybrid glial-neuronal differentiation and abnormal cell size are found far beyond tubers, throughout the white matter.²

Several potential explanations exist for the differences between lobes. These differences, however, are minor and not consistent for FA and MD, and may simply reflect measurement variance. Regional differences in the timing and rate of myelination are

Figure 5 The changing view of TSC pathology

(A) Extent of neuropathology is indicated by the density of black dots. With a segmentation based on imaging density values, an arbitrary cutoff is established to obtain the borders of 3 tissue types: tuber area in red, perituber in salmon, and the remainder of NAWM in light blue. Tubers (and other macroscopic abnormalities) represent the “tip of the iceberg,” and in the direct vicinity but also more remotely, areas of tuber-like pathology are found as well. With distance from the tuber, the extent of pathology diminishes. (B) Studies using depth electrodes (diagonal black lines) to elucidate whether seizures start in or next to a tuber,^{30–33} may be subject to measuring abnormal activity of microscopic collections of tuber-like pathology next to the tuber. (C) Reported pockets of normal white matter diffusion in patients with TSC compared with controls³⁷ may in fact describe regions (green circles) in which the pathologic burden is below imaging resolution. (D) Studies suggesting tuber volume changes over time⁹ may be biased by different segmentation thresholds because the border of tuber pathology is not discrete. From top to bottom, thresholding may be subject to subtle differences in acquisition (1.5T, 3T, MRI from different vendor), in maturation (myelin), and in partial voluming effects or angulation. NAWM = normal-appearing white matter; TSC = tuberous sclerosis complex.

insufficient as an explanation because myelination is largely completed by age 5.²⁵ Alternatively, the single tensor model falls short, as voxels containing crossing fiber pathways can be falsely low in FA because there is not a single preferential diffusion direction,²⁶ and the model does not account for more intricate, complex, multifascicle pathways. Finally, if white matter is more normal as a function of distance from the tuber, smaller lobes will have more white matter in the proximity of a tuber—even if our model accounts for volume.

In both healthy individuals and in patients with TSC, DTI maturational changes of white matter follow a biexponential time evolution, with the most dramatic DTI changes occurring in the first few years, and a slower rate in childhood and thereafter.²⁷ The steepest DTI maturational changes co-occur in time with the first clinical appearance of autism and epilepsy, reflecting the importance of medical and behavioral interventions during this critical period of development.²⁸ If indeed DTI changes are amenable to intervention with mTOR inhibitors,²⁹ and parallel clinical outcome, then the greatest impact could be made in the first 5 years of maturational change.

In TSC epilepsy surgery, the apparently contradicting reports of intratuber^{30,31} vs perituber^{32,33} epileptogenic activity may reflect a continuous spectrum of neuropathology. Multistage invasive monitoring approaches,³⁴ and better outcomes with wide resection margin and even postoperative deficits,³⁵ again suggest widespread pathology beyond the tuber. Our longitudinal imaging data confirm nondiscrete borders of tuber pathology, which may in turn affect surgical planning and approach. Indeed, the various degrees of neuropathology beyond the tuber in the resection specimen (figure 4) illustrate why a depth electrode could potentially register activity from perituber and more remote sites with tuber-like pathology.

Images obtained through advances in high-resolution MRI suggest that tubers and their ill-defined borders now represent the “tip of the iceberg” (figure 5). Using structural imaging, both subtle increases and decreases in tuber volume have been reported.^{9,10,36} The current view, however, suggests that these volumetric differences may be attributed to inconsistent imaging acquisition schemes, operator dependence, and maturational changes in contrast. Using diffusion imaging, one study sampled multiple small regions of interest in lobes without any structural white matter abnormalities and found no difference of FA and MD between patients with TSC and controls.³⁷ We suggest that residual NAWM, remote from tubers or associated transmantle structural white matter abnormalities, has in fact subtle pathology below imaging resolution.

This study had a highly consistent imaging acquisition scheme, but its retrospective nature and

insufficient power limited the assessment of correlations with clinical and neurobehavioral phenotype, which requires a dedicated study with prospective data collection. Patients who underwent surgery were excluded, which may bias the study toward patients with less severe seizures. In addition, controls were not age-matched. Radial migration lines were not analyzed separately, and because of effects of free water and calcium on diffusion, calcified and cyst-like tubers were omitted.

Although inaccuracies in registration and segmentation may account for some of the observed DTI changes, all changes consistently had the same sign, which would not occur if extra-axial space, deep gray matter, or CSF were included in the analysis. Tuber segmentation performed on an older individual, and projected onto a scan acquired at a younger age, may lead to under- or overestimation of the tuber volume if tissue contrast has improved over time. However, as outlined in the discussion, tuber boundaries are not discrete at any age and the arbitrary nature of the segmentation cutoff reflects the continuous spectrum of underlying neuropathologic abnormality.

DTI and the single-shell HARDI acquisition scheme are limited by the inability to solve for multiple fascicle orientations, do not account for isotropic diffusion, and are susceptible to partial voluming effects.²⁶ These limitations may lead to underestimation of anisotropy and can hamper the detection of group differences. Novel acquisition schemes allow for the collection of multiple nonzero b values while maintaining a good signal-to-noise ratio,³⁸ which is critical to estimate a multifascicle model. High-resolution ex vivo tissue imaging and correlation with histopathology will yield further insight into underlying neuropathology.

AUTHOR CONTRIBUTIONS

Jurriaan Peters participated in drafting and revising the manuscript for content, including medical writing for content, in study concept and design, in analysis and interpretation of data, acquisition and postprocessing of data, and study supervision or coordination. Anna Prohl participated in revising the manuscript for content, including medical writing for content, in analysis and interpretation of data, acquisition and postprocessing of data. Xavier Tomas-Fernandez participated in drafting and revising the manuscript for content, including medical writing for content, and in analysis and postprocessing of data. Maxime Taquet participated in drafting and revising the manuscript for content, including medical writing for content, and design of the figures. Benoit Scherrer participated in drafting and revising the manuscript for content, including medical writing for content, in analysis and interpretation of data, acquisition and postprocessing of data, and study supervision or coordination. Sanjay Prabhu participated in drafting and revising the manuscript for content, including medical writing for content, in review of radiology images, and in analysis and interpretation of radiologic data. Hart Lidov participated in drafting and revising the manuscript for content, including medical writing for content, in study concept and design, and in analysis, interpretation, and visualization of neuropathology data. Jolene Singh participated in drafting and revising the manuscript for content, including medical writing for content, and in interpretation and visualization of neuropathology data. Floor Jansen participated in drafting

and revising the manuscript for content, including medical writing for content, in study concept and design, and in analysis and interpretation of data. Kees Braun participated in drafting and revising the manuscript for content, including medical writing for content, in analysis and interpretation of data, and in study concept and design. Mustafa Sahin participated in drafting and revising the manuscript for content, including medical writing for content, in analysis and interpretation of data, and study supervision or coordination. Simon Warfield participated in drafting and revising the manuscript for content, including medical writing for content, in study concept and design, in analysis and interpretation of data, acquisition and postprocessing of data, and study supervision or coordination. Aymeric Stamm participated in drafting and revising the manuscript for content, including medical writing for content, in study concept and design, in statistical analysis and interpretation of data, and generation of figures and tables.

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This Week's *Neurology*[®] Podcast

Rituximab in treatment-resistant CIDP with antibodies against paranodal proteins (see the October 2015 issue of *Neurology*[®] Neuroimmunology & Neuroinflammation)

This podcast begins and closes with Dr. Robert Gross, Editor-in-Chief, briefly discussing highlighted articles from the November 3, 2015, issue of *Neurology*. In the second segment, Dr. Kelly Gwathmey talks with Dr. Isabel Illa about her *Neurology* Neuroimmunology & Neuroinflammation paper on rituximab in treatment-resistant CIDP with antibodies against paranodal proteins. In our “What’s Trending” feature of the week, Dr. Ted Burns interviews Dr. Murray Grossman about his paper on the classification of primary progressive aphasia and its variants. In the next part of the podcast, Dr. Stacey Clardy focuses her interview with Dr. Jeff Bennett on the latest developments in neuromyelitis optica using his antibody library.

Disclosures can be found at Neurology.org.

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