

Peroxisomal D-bifunctional protein deficiency

Three adults diagnosed by whole-exome sequencing

Matthew A. Lines, MD
Rebekah Jobling, MD
Lauren Brady, MSc
Christian R. Marshall,
PhD
Stephen W. Scherer, PhD
Amadeo R. Rodriguez,
MD
Liesly Lee, MD
Anthony E. Lang, MD
Tiago A. Mestre, MD
Ronald J.A. Wanders,
PhD
Sacha Ferdinandusse,
PhD
Mark A. Tarnopolsky,
MD, PhD
On behalf of the
Canadian Pediatric
Genetic Disorders
Sequencing
Consortium (FORGE
Canada)

ABSTRACT

Objective: To determine the causative genetic lesion in 3 adult siblings with a slowly progressive, juvenile-onset phenotype comprising cerebellar atrophy and ataxia, intellectual decline, hearing loss, hypogonadism, hyperreflexia, a demyelinating sensorimotor neuropathy, and (in 2 of 3 probands) supratentorial white matter changes, in whom numerous prior investigations were nondiagnostic.

Methods: The patients' initial clinical assessment included history and physical examination, cranial MRI, and nerve conduction studies. We performed whole-exome sequencing of all 3 probands, followed by variant annotation and selection of rare, shared, recessive coding changes to identify the gene responsible. We next performed a panel of peroxisomal investigations in blood and cultured fibroblasts, including assessment of D-bifunctional protein (DBP) stability and activity by immunoblot and enzymologic methods, respectively.

Results: Exome sequencing identified compound heterozygous mutations in *HSD17B4*, encoding peroxisomal DBP, in all 3 probands. Both identified mutations alter a conserved residue within the active site of DBP's enoyl-CoA hydratase domain. Routine peroxisomal screening tests, including very long-chain fatty acids and phytanic acid, were normal. DBP enzymatic activity was markedly reduced.

Conclusion: Exome sequencing provides a powerful and elegant tool in the specific diagnosis of "mild" or "atypical" neurometabolic disorders. Given the broad differential diagnosis and the absence of detectable biochemical abnormalities in blood, molecular testing of *HSD17B4* should be considered as a first-line investigation in patients with compatible features. **Neurology® 2014;82:963-968**

GLOSSARY

CoA = coenzyme A; **DBP** = D-bifunctional protein; **OMIM** = Online Mendelian Inheritance in Man.

Peroxisomal D-bifunctional protein (DBP) deficiency (OMIM 261515) is classically described as a Zellweger-like syndrome comprising neonatal encephalopathy, polyneuropathy, retinopathy, hearing loss, hepatomegaly, and dysmorphic features, and death in infancy. Autosomal recessive mutations of the gene *HSD17B4* are responsible.¹⁻³ Patients lack either or both of DBP's 2 enzymatic activities (enoyl-CoA hydratase and D-3-hydroxyacyl-CoA dehydrogenase), which are essential for the oxidation of a wide range of peroxisomal substrates (very long-chain acyl-CoAs, branched-chain acyl-CoAs including pristanoyl-CoA, and bile acid precursors).⁴⁻⁷ Recently, *HSD17B4* mutations have been identified in 4 persons with a relatively indolent, juvenile-onset condition comprising cerebellar ataxia, hearing loss, peripheral neuropathy, and premature ovarian failure; this disorder, termed type IV DBP deficiency, is allelic with some cases of Perrault syndrome (OMIM 233400).^{8,9}

Correspondence to
Dr. Tarnopolsky:
tarnopol@mcmaster.ca

Supplemental data
at Neurology.org

From Metabolics and Newborn Screening (M.A.L.), University of Ottawa, Children's Hospital of Eastern Ontario; Clinical and Metabolic Genetics (R.J.), and The Centre for Applied Genomics and Program in Genetics and Genome Biology (C.R.M., S.W.S.), The Hospital for Sick Children, Toronto; Neuromuscular and Neurometabolic Disorders (L.B., M.A.T.), and Department of Ophthalmology (A.R.R.), McMaster University, Hamilton; Department of Molecular Genetics, McLaughlin Centre (C.R.M., S.W.S.), and Division of Neurology, Sunnybrook Health Sciences Centre (L.L.), University of Toronto; Morton and Gloria Shulman Movement Disorders Centre and the Edmond J. Safra Program in Parkinson's Disease (A.E.L., T.A.M.), Toronto Western Hospital, Canada; and Laboratory Genetic Metabolic Diseases (R.J.A.W., S.F.), Academic Medical Center, University of Amsterdam, the Netherlands.

Canadian Pediatric Genetic Disorders Sequencing Consortium (FORGE Canada) coinvestigators are listed on the *Neurology®* Web site at Neurology.org.

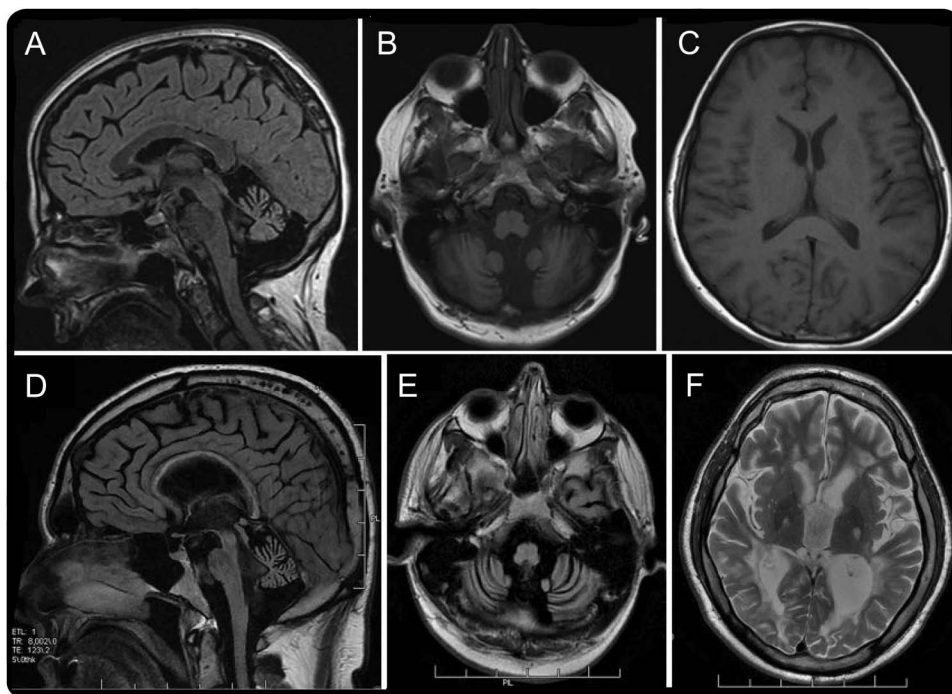
Go to Neurology.org for full disclosures. Funding information and disclosures deemed relevant by the authors, if any, are provided at the end of the article.

METHODS Standard protocol approvals, registrations, and patient consents. This study was approved by the McMaster University Research Ethics Board. Each participant (or legal guardian) signed an informed consent document before enrollment. Written consent to disclose health information was obtained from each individual.

Whole-exome sequencing and variant annotation. Total leukocyte DNA was enriched using the Agilent SureSelect Human All Exon 50 Mb capture kit (Agilent Technologies, Santa Clara, CA), and subjected to bidirectional 100-bp paired-end sequencing (Illumina Hi-Seq 2000; Illumina, Inc., San Diego, CA). Mean exon coverage was 134–162×, with >98% of target base positions having at least 10× coverage. Sequence reads were aligned to the hg19 reference human genome using Burrows-Wheeler Aligner (<http://bio-bwa.sourceforge.net>), and duplicate reads were removed with MarkDuplicates (Picard tools version 1.35; <http://picard.sourceforge.net>). Alignments were refined using local realignment in Short Read Micro re-Aligner version 0.1.15 (<http://sourceforge.net/projects/srma>). Single nucleotide variants and insertion/deletions were detected using Genome Analysis Toolkit version 1.1.28 (<http://www.broadinstitute.org/gatk>) with default parameters. Single nucleotide variants and insertion/deletions were annotated with SnpEff (<http://snpeff.sourceforge.net/>) and filtered to remove common (frequency >0.01) polymorphisms by screening against the following databases: dbSNP (<http://www.ncbi.nlm.nih.gov/projects/SNP/>); 1000 Genomes (www.1000genomes.org); the NHLBI Exome Variant Server (<http://evs.gs.washington.edu/EVS/>); and data from 130 local control exomes. Filtered variants were annotated with SIFT (<http://sift-dna.org>) and PolyPhen-2 (<http://genetics.bwh.harvard.edu/pph2/>). Mutations were confirmed by Sanger sequencing according to standard protocols.

RESULTS Clinical description. The patients were 3 adult siblings (2 women and 1 man) of nonconsanguineous Italian background, presenting with sensorineural hearing loss in middle childhood, followed by ataxia and progressive cognitive decline from adolescence onward. Clinical findings in these 3 individuals are summarized in table 1. Cranial MRI revealed marked cerebellar atrophy (figure 1); this was accompanied by a progressive peripheral neuropathy, with diminished deep tendon reflexes, reduced vibration sensation, and a demyelinating motor polyneuropathy on nerve conduction testing. Neuro-ophthalmic assessment, including dilated fundus examination, was normal. Symptoms were gradually progressive in all cases; however, the course of patient 2 was further complicated by episodes of left-sided weakness with abnormal eye movements and the appearance of multiple, gadolinium-enhancing white-matter lesions. These occurred in both infra- and supratentorial compartments, and in a pericallosal/periventricular distribution, including the presence of Dawson fingers. Oligoclonal bands were present in CSF; pulse steroids were given with a good initial response, and the patient was maintained on immunosuppressive therapy (initially with azathioprine, and later with fingolimod as newer agents became available). On follow-up imaging, the individual lesions exhibited interval evolution and contraction; however, there was a gradual overall

Figure Cranial MRI findings in D-bifunctional protein deficiency



(A–C) Cranial MRI of patient 1 shows marked, diffuse cerebellar atrophy without cerebral atrophy. (D–F) MRI of patient 2 (clinically more severe) shows an even greater degree of cerebellar atrophy, with additional cystic white matter lesions, thinned corpus callosum, and diffuse cerebral volume loss.

Table 1 Clinical findings in juvenile DBP deficiency

	Patient 1	Patient 2	Patient 3	"MK" (Ref 8)	"LK" (Ref 8)	"Patient 1" (Ref 9)	"Patient 2" (Ref 9)
Sex, age at last report, y	F, 39	M, 38	F, 35	F, 27	F, 16	M, 16	M, 14
Age at chief symptom onset, y							
Hearing loss	5	8	10	8	4	3.5	2
Ataxia	12	10	14	5-10	–	11	–
Head tremor	–	–	26	+	NR	NR	NR
Headaches	37	35	–	NR	NR	NR	NR
Intellectual disability	Mild (IQ = 81)	Severe	Moderate	LD	NR	None (11 y)	None (14 y)
Cerebellum							
Ataxia	+	+	+	+	–	+	–
Mobility aids (age required)	Walker	Wheelchair (32 y)	None	Wheelchair (≤ 23 y)	None	Wheelchair for long distances	None
Scanning dysarthria	+	+	+	+	–	NR	NR
Nystagmus	+	+	+	+	NR	NR	NR
Oculomotor apraxia	+	–	+	"Limited EOM"	NR	NR	NR
Internuclear ophthalmoplegia	–	+ (bilateral)	–	"Limited EOM"	NR	NR	NR
Motor							
Weakness	Mild, distal LE	–	Mild, distal LE	+	+	Mild, distal	NR
LE spasticity	+	+	+	+	–	NR	NR
Pes cavus	+	+	+	+	+	+	NR
Hyperkinesia	UE: mild posturing, distal chorea	–	Dystonic head tremor latero/retrocollis	NR	NR	NR	NR
Deep tendon reflexes	Absent LE; reduced UE	Increased LE; normal UE	Reduced LE; absent/reduced UE	Present	Present	Absent	NR
Plantar responses	Extensor	Extensor	Flexor	NR	NR	Flexor	NR
Sensory							
Pinprick	Mildly reduced	PF	Normal	Reduced	Normal	Reduced	NR
Vibration	Moderately reduced	Moderately reduced	Mildly reduced	Reduced	Reduced	Normal	NR
Distribution	Peripheral	Peripheral	Peripheral	Stocking/glove	"Peripheral"	Stocking	NR
Cranial MRI							
Cerebellar atrophy	+ (21 y)	+ (25 y)	+ (24 y)	+ (21 y)	– (≤ 16 y)	+ (12 y)	+
White matter lesions	+ (39 y)	+ (25 y)	– (24 y)	– (23 y)	– (≤ 16 y)	– (12 y)	–
Pallidal mineral deposition	+ (39 y)	–	– (24 y)	– (23 y)	– (≤ 16 y)	– (12 y)	–
Nerve conduction studies							
Neuropathy, type	Demyelinating, motor	Demyelinating, motor	Demyelinating, motor	Demyelinating, sensorimotor	Axonal, sensorimotor	Demyelinating, sensorimotor	Demyelinating, sensorimotor
NCV, m/s (site)	37.9 (tibial)	23.9 (tibial)	23.3 (tibial)	23-34 (various)	NR	15-27 (various)	26-43 (various)
Peripheral retinopathy	Normal	Normal	Normal	NR	NR	+ (15.5 y)	+ (14 y)
Endocrine							
Amenorrhea	Primary	NA	Secondary	Primary	NA	NA	NA
FSH, IU/L	$\uparrow 31.8$ (3.0-8.1)	$\uparrow 12.3$ (1.0-12.0)	$\uparrow 38.9$ (3.8-8.8)	81, 89 (NRI)	NR	NR	NR
LH, IU/L	$\uparrow 17.9$ (1.8-11.8)	8.5 (0.6-12.1)	$\uparrow 17.6$ (2.1-10.9)	111 (NRI)	NR	NR	NR

Continued

Table 1 Continued

	Patient 1	Patient 2	Patient 3	"MK" (Ref 8)	"LK" (Ref 8)	"Patient 1" (Ref 9)	"Patient 2" (Ref 9)
Estradiol, pmol/L	210 (77-921)	NA	↓<73 (88-418)	NR	"Low"	NR	NR
Testosterone, free, pmol/L	NA	↓23 (30-87)	NA	NR	NR	NR	NR

Abbreviations: DBP = D-bifunctional protein; EOM = extraocular movements; FSH = follicle-stimulating hormone; LD = learning difficulties; LE = lower extremity; LH = luteinizing hormone; NA = not applicable; NCV = nerve conduction velocities; NR = not reported; NRI = no reference interval quoted by author; PF = item not assessed because of patient factors; UE = upper extremity.

progression of the supratentorial lesions toward confluence (figure 1). From a functional standpoint, the patient became wheelchair-bound at age 32 years, and at 38 years is now dependent for all aspects of his care. In comparison, both of his sisters have remained able to volunteer in the community. The eldest sister (patient 1; 39 years) had recently developed nonspecific T2 signal changes in her deep white matter, along with bilateral pallidal mineral deposition. The youngest (patient 3; 35 years) had not been imaged since 2001.

Before this study, the patients underwent a panoply of diagnostic and laboratory investigations, none of which revealed a specific diagnosis. These included very long-chain fatty acids and phytanic acid in

plasma (normal, genomic microarray, muscle biopsy) with full mitochondrial disease workup, and genetic testing for each of the following: spinocerebellar ataxia types 1, 2, 3, 6, 7, 8, and 17, Friedreich ataxia, autosomal recessive hereditary spastic paraplegias (*CYP7B1*, *SPG11*, *SPG7*, *SPG15*, *PNPLA6*, *SPG20*, *SPG21*, *CCT5*, and *SPG44*), and autosomal recessive spastic ataxia of Charlevoix-Saguenay.

Mutation identification. To identify the causative gene in this family, we conducted whole-exome sequencing at The Centre for Applied Genomics (see methods), revealing the presence of 2 rare, nonsynonymous coding variants in *HSD17B4* in all 3 affected individuals. The specific mutations identified were

Table 2 Peroxisomal investigations in blood and cultured fibroblasts

	Patient 1	Patient 2	Patient 3	Reference
Peripheral blood				
Phytanic acid (μmol/L)	1.6	2.3	0.8	0-10.0
Total bile acids (μmol/L)	↑15.5	↑12.1	↑11.7	<6.7
Very long-chain fatty acids (μmol/L)				
C26:1	0.386	0.364	0.320	0.247-1.095
C26:0	0.550	0.665	0.379	0.269-0.923
C26/C22	↓0.004	0.007	0.006	0.005-0.017
C24/C22	↓0.301	0.583	↓0.529	0.543-0.941
Cultured skin fibroblasts				
Very long-chain fatty acids (μmol/g protein)				
C22:0	4.13	4.08	↓3.74	3.84-10.20
C24:0	10.02	10.93	↓7.74	7.76-17.66
C26:0	0.30	0.34	0.23	0.18-0.38
C24:0/C22:0	↑2.43	↑2.68	2.07	1.55-2.30
C26:0/C22:0	0.07	↑0.08	0.06	0.03-0.07
Peroxisomal substrate oxidation (pmol/h/mg protein)				
C26:0 β-oxidation	1,096	949	763	800-2,040
Pristanic acid β-oxidation	↓427	↓349	↓186	790-1,690
Phytanic acid α-oxidation	70	72	62	28-95
DBP enoyl-CoA hydratase activity (pmol/min/mg protein)	↓33	↓32	↓32	115-600
Peroxisome morphology (anti-catalase immunofluorescence)	Normal	Normal	Normal	Normal

Abbreviations: CoA = coenzyme A; DBP = D-bifunctional protein.

NM_000414.3:c.1537C>A (p.Pro513Thr; maternally inherited) and c.1628G>C (p.Arg543Pro; paternally inherited). Both of these mutations alter a highly conserved residue within the active site of DBP's enoyl-CoA hydratase domain (figure e-1 on the *Neurology*[®] Web site at Neurology.org).

Biochemical features. In light of the molecular findings, a more detailed assessment of peroxisomal function in both plasma and skin fibroblasts (table 2) was undertaken. Of note, total serum bile acids were increased (approximately 2-fold) in all cases; phytanic and very long-chain fatty acids were normal. DBP enoyl-CoA hydratase activity was markedly reduced, confirming the diagnosis. The normal 45-kDa fragment containing DBP's (posttranslationally cleaved) hydratase domain was absent by immunoblot in patient fibroblasts (figure e-2).

DISCUSSION This report increases to 7 the total number of reported individuals with juvenile-onset DBP deficiency; our study participants are the oldest reported patients to date. All reported diagnoses of juvenile DBP deficiency to date have been accomplished by whole-exome sequencing, rather than by conventional clinical means. The difficulty in clinically recognizing this condition stems from (1) its extreme rarity, (2) its poor resemblance to the classic neonatal form, and (3) the presence of many of its key findings in other, relatively common, conditions. For instance, compatible symptoms may be seen in many of the spinocerebellar ataxias and in the complicated hereditary spastic paraplegias *SPG7* (OMIM 607259), *SPG20* (Troyer syndrome, OMIM 275900), and *SPG21* (Mast syndrome, OMIM 248900), and in mitochondrial diseases. Given the low pretest probability for each of these disorders, exome- or panel-based molecular testing may indeed be the most practical initial test in patients with overlapping clinical presentations.

White matter lesions, a variable feature in our patients, are a known sequela of classic DBP deficiency, with periventricular cerebral demyelination and callosal thinning reported to occur in 17% and 55% of cases, respectively.² As far as we are aware, white matter disease has not previously been reported in later (juvenile)-onset patients. The presence of CSF oligoclonal bands in patient 2 is interesting, especially considering the recent report of severe, chronic cerebral inflammation in knockout mice lacking DBP.¹⁰ Whether this represents simple coincidence vs an autoimmune component to the disease process itself is unclear.

No proven treatments exist to address the underlying biochemical defect in this disorder, although dietary phytanic acid restriction and/or bile acid supplementation may warrant further study. Although

our probands do not demonstrate phytanic acid accumulation in peripheral blood, concentrations in other tissues (e.g., CSF and brain tissue) are unknown. It is reasonable to suppose that juvenile-onset patients may possess a degree of residual enzyme activity *in vivo*; enhancing this (e.g., via chaperone therapy) is another possible approach. Although the number of reported patients is currently too few to permit clinical trials, the recognized prevalence of this disorder is likely to increase as exome sequencing enters the clinical mainstream.

AUTHOR CONTRIBUTIONS

Dr. Lines and Dr. Jobling: analysis and interpretation, drafting and critical revision of the manuscript. L. Brady: acquisition of data, analysis and interpretation, critical revision of the manuscript. Dr. Marshall: acquisition of data, analysis and interpretation, drafting and critical revision of the manuscript. Dr. Scherer: analysis and interpretation, critical revision of the manuscript. Dr. Rodriguez, Dr. Lee, Dr. Mestre, and Dr. Lang: acquisition of data, critical revision of the manuscript. Dr. Wanders: analysis and interpretation, critical revision of the manuscript. Dr. Ferdinandusse: acquisition of data, analysis and interpretation, critical revision of the manuscript. Dr. Tarnopolsky: study concept and design, acquisition of data, analysis and interpretation, critical revision of the manuscript, study supervision.

ACKNOWLEDGMENT

The authors gratefully acknowledge the participation of the patients and family, without whom this work could not be undertaken. This work was selected for study by the FORGE Canada Steering Committee, consisting of K. Boycott (University of Ottawa), A. Mackenzie (University of Ottawa), M. Samuels (University of Montreal), F. Bernier (University of Calgary), and M. Innes (University of Calgary).

STUDY FUNDING

Supported by Genome Canada, the Canadian Institutes of Health Research, and the Ontario Genomics Institute (OGI-049), with additional funding from Genome Québec, Genome British Columbia, The University of Toronto McLaughlin Centre, and The Centre for Applied Genomics.

DISCLOSURE

The authors report no disclosures relevant to the manuscript. Go to Neurology.org for full disclosures.

Received September 5, 2013. Accepted in final form December 4, 2013.

REFERENCES

1. Clayton PT, Lake BD, Hjelm M, et al. Bile acid analyses in "pseudo-Zellweger" syndrome: clues to the defect in peroxisomal beta-oxidation. *J Inher Metab Dis* 1988; 11:165–168.
2. Ferdinandusse S, Denis S, Mooyer PA, et al. Clinical and biochemical spectrum of D-bifunctional protein deficiency. *Ann Neurol* 2006;59:92–104.
3. Suzuki Y, Jiang LL, Sourri M, et al. D-3-hydroxyacyl-CoA dehydratase/D-3-hydroxyacyl-CoA dehydrogenase bifunctional protein deficiency: a newly identified peroxisomal disorder. *Am J Hum Genet* 1997;61:1153–1162.
4. Adamski JF, Normand T, Leenders F, et al. Molecular cloning of a novel widely expressed human 80 kDa 17 beta-hydroxysteroid dehydrogenase IV. *Biochem J* 1995; 311:437–443.

5. Möller G, van Grunsven EG, Wanders RJ, Adamski J. Molecular basis of D-bifunctional protein deficiency. *Mol Cell Endocrinol* 2001;171:61–70.
6. Adamski JF, Leenders F, Carstensen JF, et al. Steroids, fatty acyl-CoA, and sterols are substrates of 80-kDa multifunctional protein. *Steroids* 1997;62:159–163.
7. Ferdinandusse S, Ylianttila MS, Gloerich J, et al. Mutational spectrum of D-bifunctional protein deficiency and structure-based genotype-phenotype analysis. *Am J Hum Genet* 2006;78:112–124.
8. Pierce SB, Walsh T, Chisholm KM, et al. Mutations in the DBP-deficiency protein HSD17B4 cause ovarian dysgenesis, hearing loss, and ataxia of Perrault syndrome. *Am J Hum Genet* 2010;87:282–288.
9. McMillan HJ, Worthylake T, Schwartzentruber J, et al. Specific combination of compound heterozygous mutations in 17beta-hydroxysteroid dehydrogenase type 4 (HSD17B4) defines a new subtype of D-bifunctional protein deficiency. *Orphanet J Rare Dis* 2012;7:90.
10. Verheijden S, Böttelbergs A, Krysko O, et al. Peroxisomal multifunctional protein-2 deficiency causes neuroinflammation and degeneration of Purkinje cells independent of very long chain fatty acid accumulation. *Neurobiol Dis* 2013;58:258–269.

Register for the 2014 AAN Annual Meeting!

Connecting All of Neurology with Unparalleled Science, Education, and Networking

Register today for the upcoming AAN Annual Meeting, coming to Philadelphia, PA, April 26–May 3, 2014, and featuring breakthrough scientific research, premier education programming, and unparalleled networking opportunities.

- **Early registration discount deadline: April 3, 2014**
- **Hotel deadline: March 26, 2014**

Visit AAN.com/view/AM14 now!

Visit the *Neurology*[®] Web Site at www.neurology.org

- Enhanced navigation format
- Increased search capability
- Highlighted articles
- Detailed podcast descriptions
- RSS Feeds of current issue and podcasts
- Personal folders for articles and searches
- Mobile device download link
- AAN Web page links
- Links to Neurology Now[®], Neurology Today[®], and Continuum[®]
- Resident & Fellow subsite

 Find *Neurology*[®] on Facebook: <http://tinyurl.com/neurologyfan>

 Follow *Neurology*[®] on Twitter: <https://twitter.com/GreenJournal>