Clinical/Scientific Notes

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Supplemental Data

PHOSPHORYLATION PREVENTS POLYGLUCOSAN TRANSPORT IN LAFORA DISEASE

Neuronal distal axons have limited access to glucose, their myelin sheaths preventing direct interface with blood, and their axoplasms being at great distances from their cell bodies. Glycogen, the mammalian glucose store, is characterized by extreme branching, which allows packing 55,000 glucoses/glycogen, and maintaining solubility. In neurons, glycogen phosphorylase, the glycogen-digesting enzyme, is located in distal axons where it can generate large amounts of glucose from glycogen.[1]

Two neurologic diseases, adult polyglucosan body disease (APBD) and Lafora disease (LD), are associated with neuronal formation of poorly branched glycogen, termed polyglucosan, which precipitate and accumulate into large masses called polyglucosan or Lafora bodies. APBD is caused by mutations in the *GBE1* gene encoding the glycogen branching enzyme. APBD polyglucosans appear to be subject to transport from cell body to axons, accumulating exclusively in axons and axon hillocks, with no accumulation in the somatodendritic compartment. Subcortical and spinal cord fiber tracts and peripheral nerves are replete with polyglucosans and often obstructed. Expectedly, the disease is an axonopathy (onset ~age 50) with progressive upper and lower motor, sensory, and bladder control deficits. MRI shows diffuse subcortical signal abnormality, and nerve conduction studies and EMG axonal sensorimotor peripheral neuropathy.[2] There is no epilepsy.

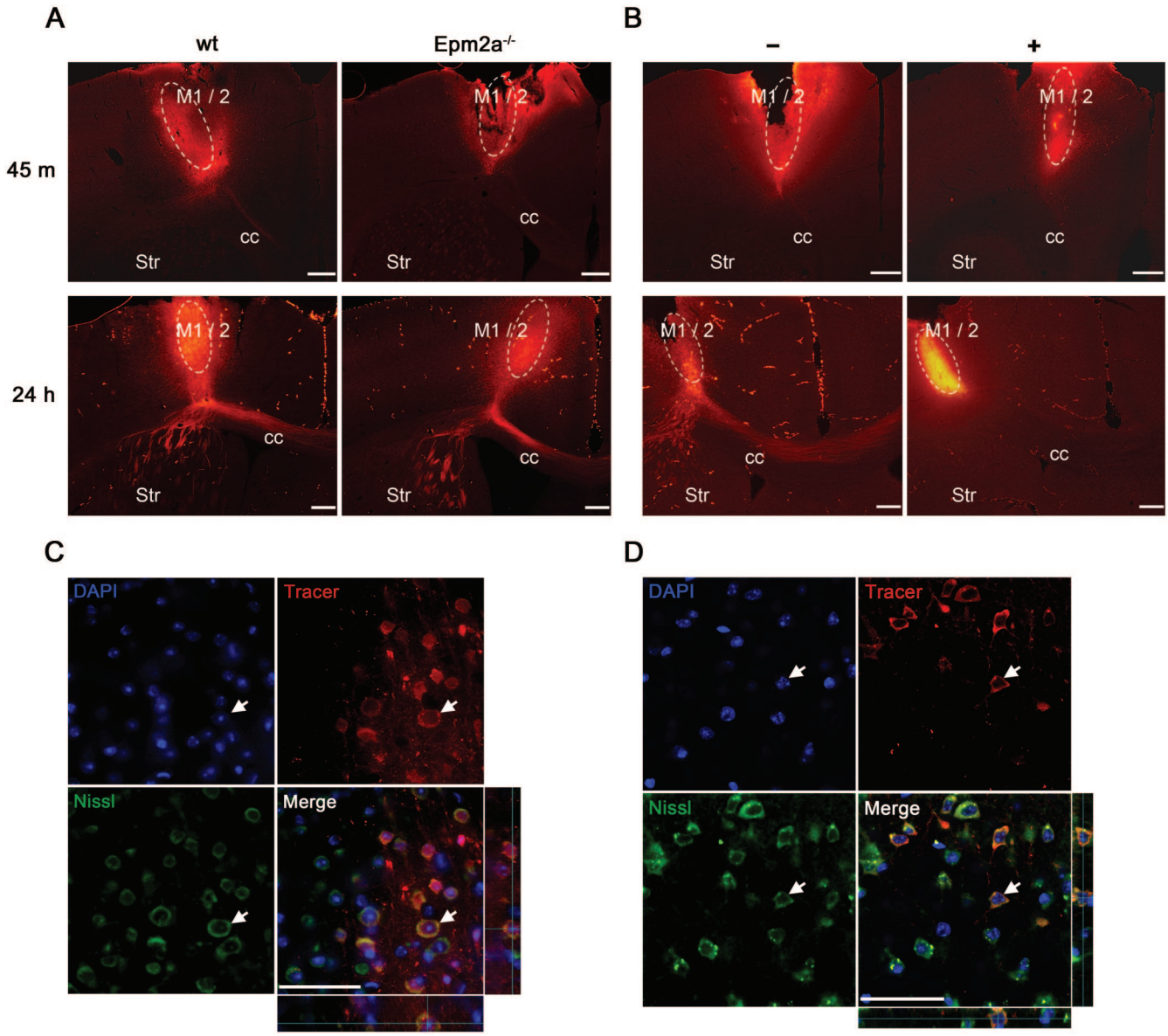
LD is a fatal progressive myoclonus epilepsy (onset ~age 15),[3] with no axonopathy, caused by mutations of genes *EPM2A* (laforin) or *EPM2B* (malin).[3,4] Laforin is a phosphatase that prevents accumulation of phosphate on glycogen.[5] Malin is an E3 ubiquitin ligase which regulates laforin.[4] Phosphate accumulation on glycogen leads glycogen to unfold and precipitate.[5] Glycogen synthase (GS), the enzyme that elongates glycogen, remains bound to the precipitating glycogen, while branching enzyme does not.[5] Elongation by GS without branching may explain subsequent conversion of precipitated glycogen to polyglucosan. LD polyglucosans are identical to APBD polyglucosans, except that they are phosphorylated.[3–5,e5] They also differ in the neuronal compartment in which they accumulate, namely cell body and dendrites, gradually replacing the cytoplasms of countless dendrites. Axons are rarely affected. Subcortical MRI signal and nerve conduction studies are normal.[3] Likely, progressive overtaking of dendritic cytoplasms underlies the progressive epilepsy of LD as does the accumulation in axons the axonopathy of APBD.[3]

The standard neuronal tracer dextran is a poorly branched nonphosphorylated polyglucosan produced by fermenting bacteria. It is structurally similar to APBD and LD polyglucosans, differing in having α1–6 instead of α1–4 interglucosidic linkages. When injected in the brain, it is taken up by neuronal cell bodies and transported via active transport (not in vesicles) to axons and then distal axons.[6]

Results. In this study we use dextran to explore why LD polyglucosans accumulate in the somatodendritic compartment. First, we asked whether LD neurons have a defect in their ability to transport polyglucosans to axons. We injected fluorescently labeled dextran into M1/M2 primary motor cortex of 6 3-month-old epm2a−/− and 6 wild-type mice and studied brain sections after 45 minutes and 24 hours. At 45 minutes, dextran was present at the injection site (figure, A) within neurons (figure, C). At 24 hours, it had been transported to the distal axons of M1/M2 neurons in the corpus callosum and striatum, equally in wild-type and epm2a−/− mice (figure, A), indicating that polyglucosan transport mechanisms are intact. Next, we asked whether phosphorylation, which characterizes LD polyglucosans, inhibits transport. We phosphorylated the dextran (20% of glucoses phosphorylated) (e-Methods in appendix e-1 on the *Neurology*® Web site at www.neurology.org) and injected 6 wild-type mice, 3 with phosphorylated and 3 with nonphosphorylated dextran (figure, B). Nonphosphorylated dextran traveled normally as above. Phosphorylated dextran entered neurons normally (figure, D), but was not transported (figure, B), indicating that polyglucosan phosphorylation prevents transport.

Discussion. The origin of the phosphorylation which initiates LD pathogenesis was recently discovered: GS, while attaching glucoses to glycogen, epi-

Figure Phosphorylation inhibits dextran transport in brain



(A) Transport of nonphosphorylated dextran (10 kDa fluoro-ruby dextran) is identical in wt and epm2a−/− mice. Red fluorescence, dextran at the injection site; cc = corpus callosum; str = striatum; bars, 100 μm. (B) Phosphorylated dextran is not transported in wt mice. (–), nonphosphorylated dextran; (+), phosphorylated dextran. (C, D) Lack of phosphorylated dextran transport is not due to lack of neuronal entry. Confocal imaging at 45 minutes shows that phosphorylated dextran (D) is within neuronal cytoplasms, similar to nonphosphorylated dextran (C). Arrowheads, examples of neurons containing dendrites; blue, nuclei; red, dextran; green, Nissl; bars, 50 μm. Methods for brain injections and dextran phosphorylation are detailed in appendix e-1.

sodically introduces phosphates by enzymatic error, normally corrected by laforin.[5] The present study indicates that the phosphorylation may also underlie the somatodendritic localization of polyglucosans, by preventing their removal into axons. Two caveats of our study are that dextran is not exactly identical to LD and APBD polyglucosans, and its phosphorylation in our experiments (~20%) exceeds that of LD polyglucosans (~1.26%).[e5]

LD includes defects in autophagy and protein ubiquitination/clearance in addition to polyglucosan formation. In a recent study, preventing polyglucosan formation by downregulating GS in LD mice prevented myoclonus and neurodegeneration, and cured the disease, highlighting the role of polyglucosans.[7] The present study suggests that the epileptogenesis initiated by accumulating polyglucosans occurs in the somatodendritic domain of affected neurons. Downregulating GS would prevent this accumulation, with important therapeutic significance.

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Author contributions: Dr. Girard, Dr. Lohi, and Dr. Minassian conceived the study. Dr. Girard, Dr. Blaszykowski, and A. Draginov prepared and analyzed the phosphorylated dextran. Dr. Stone performed the mouse injections and fluorescence analyses with contributions by Dr. Teixeira and A. Wang and under the supervision of Dr. Frankland. Dr. Turnbull, Dr. P. Wang, and Dr. Ackerley provided important conceptual insights. X.C. Zhao took care of the animals and their genotyping. Dr. Girard and Dr. Minassian wrote the paper. Dr. Minassian supervised the overall work.

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1. Pfeiffer-Guglielmi B, Francke M, Reichenbach A, Hamprecht B. Glycogen phosphorylase isozymes and energy metabolism in the rat peripheral nervous system: an immunocytochemical study. Brain Res 2007;1136:20–27.
2. Lossos A, Meiner Z, Barash V, et al. Adult polyglucosan body disease in Ashkenazi Jewish patients carrying the Tyr329Ser mutation in the glycogen-branching enzyme gene. Ann Neurol 1998;44:867–872.
3. Minassian BA. Lafora's disease: towards a clinical, pathologic, and molecular synthesis. Pediatr Neurol 2001;25:21–29.
4. Turnbull J, Wang P, Girard JM, et al. Glycogen hyperphosphorylation underlies Lafora body formation. Ann Neurol 2010;68:925–933.
5. Tagliabracci VS, Heiss C, Karthik C, et al. Phosphate incorporation during glycogen synthesis and Lafora Disease. Cell Metab 2011;13:274–282.
6. Vercelli A, Repici M, Garbossa D, Grimaldi A. Recent techniques for tracing pathways in the central nervous system of developing and adult mammals. Brain Res Bull 2000;51:11–28.
7. Turnbull J, Depaoli-Roach AA, Zhao X, et al. PTG depletion removes Lafora bodies and rescues the fatal epilepsy of Lafora disease. PLoS Genet 2011;7:e1002037.

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EARLY IVIg TREATMENT HAS NO EFFECT ON POST-H1N1 NARCOLEPSY PHENOTYPE OR HYPOCRETIN DEFICIENCY

We previously suggested clinical effects of early IV immunoglobulin (IVIg) treatment in sporadic narcolepsy with cataplexy (NC).[1] Below we present the first post-H1N1 vaccination NC case treated with IVIg, treated 19 days after the clear and abrupt onset of the disease.

Level of evidence. This is a single observational study without controls (evidence level IV).

Case report. On October 14, 2010, a 21-year-old Danish/Japanese man received H1N1vaccination (Influvac). He had previously received Pandemrix vaccination in autumn 2009. Apart from chronic asthma, he was generally healthy. On January 22, 2011, he developed extreme sleepiness, characterized by 14–16 hours sleep/night and 5 naps/day. His sleep was disrupted by awakenings and dream-enacting behavior. On January 26, the first cataplectic attacks emerged, triggered by laughter, jokes, witty replies, excitement, and exercise.

We examined him on February 8. He presented with multiple sleep attacks and intermittent status cataplecticus (series of partial attacks of the face/knees/hands mixed with falls/near-falls). Sleep paralysis and hypnagogic hallucinations were absent. Neurologic examination, brain MRI, EEG, and routine blood and CSF parameters were normal. His score on the Epworth Sleepiness Scale (ESS) was 18/24. Polysomnography (PSG) revealed short sleep latency, sleep onset REM periods (SOREMPs), and increased REM sleep muscle activations. The multiple sleep latency test (MSLT) mean score was 2.1 minutes with SOREMPs in 4/5 naps. CSF hypocretin-1 was very low (<10 pg/mL) and HLA-type was DQB1*06:01;03:01. Serum was streptolysin O (ASO) positive (200 IU).

On February 14, we initiated IVIg 1 g/kg/day for 2 days after obtaining informed consent. The treatment was temporarily complicated by headache and a moderate rise in CSF leukocytes. Immediately after IVIg treatment he reported a reduction of cataplexy (3–8 attacks/day) and sleepiness (ESS 15–17). Objectively, however, there was no obvious change. For example, he was still wheelchair-bound by cataplexy. Moreover, hypnagogic hallucinations and sleep paralysis appeared on February 16 and 28, respectively.

On February 22, post-IVIg CSF hypocretin-1 remained <10 pg/mL. Likewise, PSG parameters were unchanged and a MSLT mean sleep latency of 36 s with SOREMPs in 5/5 naps. We refrained from administering further IVIg due to the adverse reaction to the treatment and lack of apparent efficacy.

Discussion. This first attempt to immunomodulate a post-H1N1 narcoleptic phenotype with