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The wmN1 Enhancer Region in Intron 1 Is Required for Expression of Human *PLP1*

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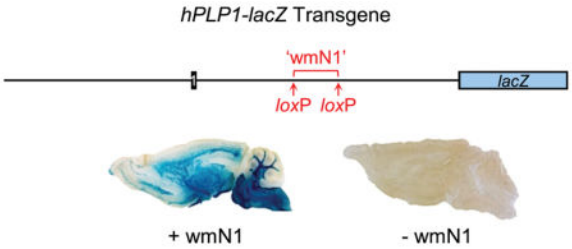
Abstract

The myelin proteolipid protein gene (*PLP1*) encodes the most abundant protein present in myelin from the central nervous system (CNS). Its expression must be tightly controlled as evidenced by mutations that alter *PLP1* dosage; both overexpression (elevated *PLP1* copy number) and lack thereof (*PLP1* deletion) result in X-linked genetic disorders in man. However, not much is known about the mechanisms that govern expression of the human gene. To address this, transgenic mice were generated which utilize human *PLP1* (*hPLP1*) sequences [proximal 6.2 kb of 5′-flanking DNA to the first 38 bp of exon 2] to drive expression of a *lacZ* reporter cassette. *Lox*P sites were incorporated around a 1.5-kb section of *hPLP1* intron 1 since it contains sequence orthologous to the wmN1 region from mouse which, previously, was shown to augment expression of a minimally-promoted transgene coincident with the active myelination period of CNS development. Eight transgenic lines were generated with the parental, 6.2hPLP(+)Z/FL, transgene. All lines expressed the transgene appropriately in brain as evidenced by staining with X-gal in white matter regions and olfactory bulb. Removal of the 'wmN1' region from 6.2hPLP(+)Z/FL with a ubiquitously expressed Cre-driver caused a dramatic reduction in transgene activity. These results demonstrate for the first time that the wmN1 enhancer region: (i) is functional in *hPLP1*; (ii) works in collaboration with its native promoter – not just a basal heterologous promoter; (iii) is required for high levels of *hPLP1* gene activity; (iv) has a broader effect, both spatially and temporally, than originally projected with *mPlp1*.

Graphical Abstract



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Keywords

gene regulation; myelin proteolipid protein gene; transgenic mouse; *lacZ*; Cre/*lox*P

Introduction

The *PLP1* gene encodes the most abundant protein in CNS myelin, accounting for nearly 20% of the myelin proteome (Jahn et al., 2009). The gene is highly expressed in oligodendrocytes and olfactory ensheathing cells (OECs) (Griffiths et al., 1995; Dickinson et al., 1997), and to a lesser extent in select populations of neurons and additional cell types in the periphery (reviewed in Wight and Dobretsova, 2004). The gene is located on the X chromosome with seven major exons distributed across nearly 16 kb of DNA in human. *PLP1*-linked disorders in man include Pelizaeus-Merzbacher disease (PMD) and spastic paraplegia type 2 (SPG2) which can be traced to alterations in *hPLP1* gene dosage, point mutations, and indels (for reviews see Hobson and Garbern, 2012; Hobson and Kamholz, 2013). Mice with related *Plp1* mutations are similarly affected, and have been used as model systems to understand the consequences of these alterations (Simons et al., 2002; Krämer-Albers et al., 2006; McLaughlin et al., 2006; Rosenbluth et al., 2006; Karim et al., 2007, 2010; Tatar et al., 2010; Clark et al., 2013; Werner et al., 2013; Appikatla et al., 2014; Lüders et al., 2017). More recently, *hPLP1* mutations that alter the ratio of PLP1 to DM20 have been shown to cause the X-linked disorder, Hypomyelination of Early Myelinating Structures (HEMS; Kevelam et al., 2015); the DM20 isoform is generated by alternative splicing through utilization of an internal splice donor site in exon 3 resulting in a protein similar to PLP, but lacks 35 innermost residues (Nave et al., 1987). Because PMD/SPG2 can result from either too much expression (elevated *hPLP1* copy number), or lack of expression (*hPLP1* deletion), the gene must be under tight control. However the pertinent mechanisms that govern expression of *hPLP1*, specifically, are poorly understood.

Previously we demonstrated with *Plp1-lacZ* transgenic mice that the first intron of the mouse *Plp1* (*mPlp1*) gene is required in order for the *mPlp1* promoter to drive significant levels of *lacZ* expression in brain (Li et al., 2002). The intron is moderately conserved between human and mouse (60% identity) being a bit larger in *hPLP1* (8579 bp; Hamdan et al., 2015) than *mPlp1* (8140 bp; Wight and Dobretsova, 1997). Overall it comprises more than half of the gene (beginning of exon 1 to the end of exon 7). In addition to seven major exons, there are a couple of minor (supplementary) exons located within the first intron of *PLP1* in human (Sarret et al., 2010) and mouse (Bongarzone et al., 1999; Li et al., 2009), although the alternatively spliced exons are altogether distinct between human and mouse, and largely (solely for *mPlp1*) confined to these species. One of the supplementary exons (exon C) from *hPLP1* intron 1 lies within sequence orthologous to the mouse wmN1 enhancer region. The mouse wmN1 region consists of 1171 bp. Its inclusion in a minimally promoted *eGFP/lacZ* transgene driven by the heat shock protein (*hsp*) basal promoter conferred high level expression to the superficial layer of the olfactory bulb as early as postnatal day 0 (P0), and to white matter tracts in brain and spinal cord, coincident with the onset of CNS myelination (Tuason et al., 2008). However, whether the wmN1 region is capable of acting as an enhancer in conjunction with its native promoter (not just a basal

heterologous promoter) was unknown. To address this matter, and to test the importance of intron 1 sequences in general on the regulation of *hPLP1* expression, transgenic mice were generated which utilize *hPLP1* sequences spanning the proximal 6.2 kb of 5′-flanking DNA to the first 38 bp of exon 2 to drive expression of a *lacZ* reporter gene cassette. A 1.5-kb segment within *hPLP1* intron 1, which includes sequence orthologous to the mouse wmN1 region, was flanked by *lox*P sites for its eventual removal by crossing the parental [6.2hPLP(+)Z/FL] transgenic line with Cre-deleter mice. Results presented here suggest that the 'wmN1' region in man plays a pivotal role in regulating *hPLP1* expression throughout development.

Material and Methods

Transgenic Mice

The 6.2hPLP(+)Z/FL transgene is essentially the same as the 6.2hPLP(+)Z plasmid (Hamdan et al., 2015) except for the addition of paired sets of *Frt* and *lox*P sites in *hPLP1* intron 1. It utilizes *hPLP1* genomic DNA spanning the proximal 6.2 kb of 5′-flanking DNA to the first 38 bp of exon 2 to drive expression of a *lacZ* expression cassette. Bacterial recombination sites were incorporated at opportune restriction enzyme sites (PmlI and BspEI for *Frt*; XbaI and EcoRI for *lox*P) in *hPLP1* intron 1 through the use of intermediary *hPLP1*-containing plasmids described in Hamdan et al. (2015). Plasmid BA8.7, which contains *hPLP1* genomic DNA including all of intron 1, was digested with BspEI and ligated to a 5′ phosphorylated BspEI adapter that contains an internal *Frt* site [sense: 5′-CCGGAAGTTCCTATTCTCTAGAAAGTATAGGAACTT-3′; antisense: 5′-CCGGAAGTTCCTATACTTTCTAGAGAATAGGAACTT-3′] to yield BA8.7(BspEI-*Frt*). Subsequently, plasmid BA8.7(BspEI-*Frt*) was digested with SfiI and PmlI, and ligated to a 5′ phosphorylated SfiI/PmlI adapter which likewise contains an internal *Frt* site [sense: 5′-CACGTGAAGTTCCTATTCTCTAGAAAGTATAGGAACTTC-3′; antisense: 5′-GAAGTTCCTATACTTTCTAGAGAATAGGAACTTCACGTGACT-3′]. The resulting plasmid was named BA8.7(2-*Frt*) since it contains two direct repeats of *Frt* that are located near the beginning (position 214) and end (position 8467) of *hPLP1* intron 1; the endogenous intron contains a total of 8579 bp (Hamdan et al., 2015). *Lox*P sites were also incorporated at convenient restriction enzyme sites within *hPLP1* intron 1 that encompass sequence orthologous to the mouse wmN1 region, including an additional 330 bp of sequence immediately upstream of the 'wmN1' region. At first, a *lox*P site was added at the XbaI site of plasmid BA8.7ΔSphI through the use of an XbaI/*lox*P adapter [sense: 5′-CTAGATAACTTCGTATAGCATACATTATACGAAGT-TAT-3′; antisense: 5′-CTAGATAACTTCGTATAATGTATGCTATACGAAGTTAT-3′]. The resulting plasmid (BA8.7ΔSphI-*lox*P), as well as BA8.7(2-*Frt*), were digested with EcoRI and BstEII, and appropriate fragments ligated together to generate the BA8.7(2-*Frt/lox*P) plasmid. Subsequently, BA8.7(2-*Frt/lox*P) was digested with EcoRI, dephosphorylated by treatment with Antarctic Phosphatase (New England BioLabs, Ipswich, MA), and ligated to the EcoRI/*lox*P adapter [sense: 5′-AATTATAACTTCGTA-TAGCATACATTATACGAAGTTAT-3′; antisense: 5′-AATTATAACTTCGTATAAT-GTATGCTATACGAAGTTAT-3′] to generate the BA8.7(2-*Frt*/2-*lox*P) plasmid, with both *lox*P sites sharing the same orientation (i.e. direct repeats). BA8.7(2-*Frt*/2-*lox*P) was

digested with PmlI and AgeI and the fragment containing the two pairs of recombination sites exchanged for the analogous region in 6.2hPLP(+)Z to produce the 6.2hPLP(+)Z/FL transgene.

Mice that harbor the 6.2hPLP(+)Z/FL transgene were generated onsite at the Transgenic Mouse Core Facility by microinjection of an 18.7 kb XhoI-NotI fragment from 6.2hPLP(+)Z/FL into fertilized C57BL/6 mouse eggs. All procedures involving the use of mice were approved by the Institutional Animal Care and Use Committee at the University of Arkansas for Medical Science in compliance with the Public Health Service Policy on Humane Care and Use of Laboratory Animals and the National Research Council's Guide for the Care and Use of Laboratory Animals, and adhered to ARRIVE guidelines (Kilkenny et al., 2010). Transgenic mice were identified through PCR analysis of genomic DNA isolated from tail biopsies according to the methods of Truett et al. (2000) using the *lacZ* primer pair described by Stratman et al. (2003). In addition, Southern blot analysis was performed to estimate the transgene copy number within the various 6.2hPLP(+)Z/FL lines using a ^{32}P-labeled *lacZ* probe (XhoI-NotI fragment from pGALΔK), as previously described by Wight et al. (1993).

Transgenic sublines that are missing *hPLP1* intron positions 3174–4660 (6.2hPLPΔwmN1) were derived by crossing 6.2hPLP(+)Z/FL hemizygous mice from Lines 776 and 777 with B6.FVB-Tg(EIIa-Cre)C5379Lmgd/J homozygous mice (The Jackson Laboratory; Bar Harbor, ME), and then backcrossing the resulting *lacZ*-positive progeny with the Cre-deleter strain for two more generations to ensure that the targeted 'wmN1' region had been completely removed from all copies of the transgene. The Cre transgene is under the control of the adenovirus EIIa promoter, which targets expression of Cre recombinase to the early mouse embryo in a wide range of tissues, including germ cells. PCR analysis was performed using a pair of 'external' primers which flank the targeted region [sense: 5′-CCTGCTTTCAGAGCCTACTCAGTGCCAAAC-3′; antisense: 5′-CAAGAAGGCTGGAAGAGATTCTAGGGGAG-3′], as well as an 'internal' pair directed against human-specific sequences [sense: 5′-CCCAGAGACTTCGGGACTGTTTTCCAGCAC-3′; antisense: 5′-ACTCCAACTTTAAGCCCTTCTCACCAGCGC-3′]. Once full excision was established by complete loss of an amplicon with the internal wmN1 primer pair, the resulting 6.2hPLPΔwmN1 sublines were maintained in the hemizygous state by breeding to wild-type C57BL/6 mice.

Reverse Transcription-Quantitative PCR (qRT-PCR) Analysis

6.2hPLP(+)Z/FL transgenic mice at postnatal day 21 (P21) of age from Line 777 were decapitated, and tissues rapidly dissected, snap-frozen in liquid nitrogen, and stored at −70°C. Total RNA was extracted using the TRIzol Reagent (Invitrogen) according to the manufacturer's specifications. The concentration of RNA was calculated from the optical density obtained at 260 nm in a NanoDrop 2000c Spectrophotometer (Thermo Scientific, Wilmington, DE). Subsequently, cDNA synthesis was performed with the iScript cDNA Synthesis Kit (Bio-Rad, Hercules, CA) per the supplier's instructions using 1.0 μg of total RNA in a final reaction volume of 20 μl. The reaction was run in a PCR machine under the

following conditions: single step of 25°C for 5 min, followed by a single step of 42°C for 30 min, and a final step of 85°C for 5 min. cDNAs were utilized right away or stored at −70°C until needed. Gene expression analysis was performed using the TaqMan Gene Expression Assay (Applied Biosystems, Foster City, CA). All primer/probe sets were commercially available from Applied Biosystems (#4351372 for *lacZ*; #4331182E for β-actin; #4319413E for 18S). Quantitative PCR (qPCR) was carried out in a final reaction volume of 20 μl containing Ssofast Probes Supermix (Bio-Rad), 2 μl of the RT reaction mixture (single-stranded cDNA), 900 nM of primers, and 250 nM of probe. qPCR was run for 40 cycles (95°C for 15 sec, 60°C for 1 min) in a CFX96 Real-Time PCR Detection System (Bio-Rad) following a hot start (95°C for 30 sec). Each run consisted of (in triplicate) a 'no template' negative control and cDNAs prepared from the assorted tissues. CFX manager software from Bio-Rad $\Delta\Delta^{cq}$ method was used to quantify the amount of *lacZ* expression relative to that produced from reference genes (*18S* and *β-actin*).

β-Galactosidase Histochemistry

hPLP1-lacZ transgenic mice were anesthetized with isoflurane and perfused intracardially with cold Phosphate-buffered saline (PBS) pH 7.3, followed by fixative (1.0% glutaraldehyde in PBS, pH 7.3), and then fixative containing 10% sucrose. Perfused animals were left undisturbed for 1 h, after which the brain was removed and immersed in fixative containing 10% sucrose. After sinking, brains were transferred to the same fixative containing 25% sucrose (overnight at 4°C) and subsequently embedded in Tissue-Tek CRYO-OCT Compound (Andwin Scientific/Thermo Fisher Scientific, Pittsburg, PA) and stored at −70°C until cryostat sectioning. Tissue sections (30 μm) were incubated with the chromogenic substrate 5-bromo-4-chloro-3-β-D-galactopyranoside (X-gal; Research Organics, Cleveland, OH) as previously described (Wight et al., 1993). Brain slices from 6.2hPLP(+)Z/FL mice at P21 of age were incubated in X-gal stain for 30 min except for slices obtained from Lines 776 and 778, which were stained for 6 h. For developmental studies of 6.2hPLP(+)Z/FL and 6.2hPLPΔwmN1 expression in brain (Line 777), sections were incubated with X-gal stain for 6 h (P2–P9), 1 h (P12–15) or 30 min (P18–P21).

Embryos were harvested at embryonic day 14.5 (E14.5) of gestation and fixed for 90 min at 4°C in fixative [1% formaldehyde, 0.2% glutaraldehyde, 5 mM EGTA, 2 mM $MgCl_2$, 0.02% NP-40, and 0.01% sodium deoxycholate in PBS (pH 7.3)]. Embryos were soaked three times (10 min each) in ice-cold wash solution [0.02% NP-40 and 2 mM $MgCl_2$ in PBS] and then permeabilized for 30 min in a detergent solution [0.1% Triton X-100, 0.01% sodium deoxycholate, and 2 mM $MgCl_2$ in PBS] at room temperature. Embryos were stained at 37°C for 3 h in the same X-gal stain used for tissue sections, washed three times (10 min each) in room temperature PBS, and post-fixed overnight at 4°C in PBS with 10% formalin. Subsequently, the embryos were dehydrate in ethanol [overnight at 4°C in 70% ethanol, followed by a 15 min incubation (each) in 95% and 100% ethanol at room temperature], cleared in xylene (10 min at room temperature), incubated in methyl salicylate (5 min at room temperature), and then photographed.

β-Galactosidase Enzyme Assay

Mice were decapitated, brains rapidly dissected and homogenized individually in Lysis Solution [0.2% Triton X-100 and freshly added phenylmethylsulfonyl fluoride (0.2 mM) and leupeptin (5 μg/ml) in 100 mM potassium phosphate, pH 7.8]. The homogenate was centrifuged at 12,500 *g* for 10 min at 4°C and the resulting supernatant incubated at 48°C for 1 h to inactivate any endogenous β-galactosidase (β-gal) activity according to the methods of Young et al. (1993). The lysate was then centrifuged at 12,500 *g* for 5 min at 4°C and the supernatant (10 μl) assayed for β-gal activity in triplicate using the Galacto-Light Plus Kit as previously described (Pereira et al., 2013). Lysate protein concentrations were determined using the BCA Protein Assay Kit (Pierce, Rockford, IL). Results are presented as the mean ± SD of β-gal activity (relative light units; RLU) per μg of total protein from three or more animals for each line/genotype.

In addition to *hPLP1-lacZ* mouse lines generated here, the relative level of β-gal activity was determined for Line 26H (Wight et al., 1993), which harbors a related transgene [PLP(+)Z] that utilizes *Plp1* sequences from mouse instead of human. The transgene is referred to here as mPLP(+)Z to indicate the source (species) of *Plp1* DNA.

Combined X-Gal Staining with Immunohistochemistry

6.2hPLP(+)Z/FL mice (Line 777) at P9 of age were anesthetized with isoflurane and perfused intracardially first with cold PBS (pH 7.3), and then with fixative (2% paraformaldehyde-1% glutaraldehyde in PBS, pH 7.3) for 20 min. Animals were left undisturbed for 40 min, after which the brain, heart, kidneys, liver, lungs, and sciatic nerves were removed and submerged in fixative for 1 h at 4°C. Subsequently, the tissues were incubated in PBS (pH 7.3) containing 10% sucrose at 4°C, allowed to sink, transferred to PBS (pH 7.3) containing 25% sucrose overnight at 4°C, and then were immersed in CRYO-OCT compound and frozen at −70°C until sectioning on a cryostat. Brain slices (16 μm) or intact sciatic nerve were subjected to staining with X-gal, followed by immunofluorescence. The X-gal stained sciatic nerve was sectioned longitudinally into 16 μm slices prior to immunofluorescence. The incubation period for staining with X-gal varied between tissues (40 min for brain sections, 30 min for heart, kidney, liver and lung sections, and 20 min for intact sciatic nerve) and was performed at 40°C in a water bath. Subsequently, the slides were rinsed three times in PBS (5 min per wash). Antigen retrieval with brain slices was achieved by incubating the sections in 10 mM citrate buffer (pH 6) for 20 min in a 75°C water bath, and then allowing the solution to come to room temperature at the bench. The slices were then washed three times with 1% Triton X-100 in PBS (5 min per wash) and incubated in blocking buffer (10% goat serum in PBS) for 2 h at 25°C. After which, sections were reacted overnight at 4°C with one of the following (primary) monoclonal antibodies: mouse anti-claudin-11 (#sc-271232; Santa Cruz Biotechnology, Inc., Santa Cruz, CA; 1:500 dilution in PBS); mouse anti-NG2 (#ab50009; Abcam, Cambridge, MA; 1:1000 dilution in PBS); mouse anti-glial fibrillary acidic protein (anti-GFAP; #sc-33673; Santa Cruz Biotechnology, Inc.; 1:500 dilution in PBS). The sections were then incubated with goat anti-mouse IgG secondary antibody conjugated to Alexa Fluor 568 (#ab150106; Abcam; 1:500 dilution in PBS except for a 1:300 dilution following treatment with the anti-GFAP primary antibody). Longitudinal slices of X-gal stained sciatic nerve were incubated first

with a mouse monoclonal (primary) antibody directed against S-100 protein (#NCL-S100; Leica Biosystems, Buffalo Grove, IL; 1:500 dilution in PBS) overnight at 4°C, and then with goat anti-mouse IgG secondary antibody conjugated to CY3 (#111-165-003; Jackson ImmunoResearch, Laboratories, Inc.; West Grove, PA; 1:500 dilution in PBS). Incubation with secondary antibodies was for 2 h at room temperature. Images were captured with a CoolSNAP fx camera (Photometrics, Tucson, AZ) mounted on a BX-51 microscope (Olympus, Center Valley, PA) and processed with ImageJ software (Schneider et al. 2012).

Results

All of the 6.2hPLP(+)Z/FL Mouse Lines Express the Transgene

To test the importance of intron 1 in regulating *hPLP1* gene expression, *in vivo*, transgenic mice were generated which utilize *hPLP1* genomic sequences that span the proximal 6.2 kb of 5′-flanking DNA to the first 38 bp of exon 2 to drive expression of a *lacZ* reporter gene cassette. Two pairs of short bacterial recombination sites were integrated in the 6.2hPLP(+)Z/FL transgene for the eventual removal of portions of *hPLP1* intron 1 (Fig. 1). *Lox*P sites were incorporated at intron 1 positions 3174 and 4660, embracing sequences orthologous to the wmN1 region, and *Frt* sites incorporated at positions 214 and 8467, which envelop most of the 8579 bp intron. Inclusion of these recombination sites did not significantly alter the expression capacity, as assessed by transfection analysis in Oli-neu cells (Supp. Info. Fig. 1). Crossing 6.2hPLP(+)Z/FL transgenic mice with mice that express Cre recombinase led to a transgenic subline missing intron 1 positions 3174–4660 (designated 6.2hPLPΔwmN1), which maintains the same genomic integration site as the parental line from which it was derived.

A total of thirty-five pups (C57BL/6 genetic background) were born from transplanted embryos which had previously been microinjected with the 6.2hPLP(+)Z/FL transgene. Of these, eight harbored the transgene for a 23% yield in transgenesis. Transgenic lines were established from all of the founders, and numbered consecutively from 772 to 779. Transgene copy number was estimated by Southern blot analysis (data not shown) and is reported in Table 1. All lines expressed the 6.2hPLP(+)Z/FL transgene to varying degrees as determined by the relative amount of β-gal activity present in brain homogenates from mice at P2 or P21 of age, which were consistently higher than that from wild-type (non-transgenic) littermates (Table 1). Surprisingly, most of the lines showed comparable or slightly higher amounts of β-gal activity at P2 relative to P21 (Table 1) unlike Line 26H (Wight et al., 1993), which harbors a similar transgene [mPLP(+)Z] except that it utilizes *mPlp1* genomic DNA (spans proximal 2.4 kb of 5′-flanking DNA to the first 37 bp of exon 2) to drive the *lacZ* reporter; β-gal activity for Line 26H was 20-fold higher at P21 than P2 (Table 1).

The 6.2hPLP(+)Z/FL transgene appeared to be expressed appropriately in all lines as evidenced by X-gal staining in white matter areas of brain from mice at P21 (Fig. 2); brain slices from Lines 776 and 778 were incubated in X-gal stain longer (6 h instead of 30 min) due to modest levels of β-gal activity in these lines (see Table 1). Because Line 777 expresses 6.2hPLP(+)Z/FL to a relatively high degree in brain, and has a fairly low transgene copy number, this line was selected for subsequent in-depth analysis.

The 6.2PLP(+)Z/FL Transgene Is Spatially Regulated in Line 777

Spatial expression of the 6.2PLP(+)Z/FL transgene was assessed by qRT-PCR analysis and X-gal staining with mice from Line 777. As shown in Fig. 3, transgene expression at P21 was highest in the CNS (brain and spinal cord), moderate in the peripheral nervous system (PNS; e.g. sciatic nerve), compared to the other tissues tested (heart, kidney, liver, lung, spleen), which is consistent with endogenous *mPlp1* expression (for review see Wight and Dobretsova, 2004). The negligible amount of transgene expression in heart, kidney, liver, and lung was also confirmed by X-gal staining with tissue sections obtained from mice at P9 (Supp. Info. Fig. 2) and P21 (data not shown), which demonstrated low to nonexistent levels of staining that was equivalent between 6.2PLP(+)Z/FL mice and wild-type littermates. Conversely, there was ample staining with X-gal in sciatic nerve from P9 transgenic mice, which was absent in wild-type littermates (Supp. Info. Fig. 3). Taken together, these data suggest that the regulatory element(s) responsible for directing the spatial expression of *hPLP1* are present in the 6.2PLP(+)Z/FL transgene.

Expression of 6.2hPLP(+)Z/FL in Brain Is Developmentally Regulated in Line 777

To characterize the spatiotemporal expression of 6.2hPLP(+)Z/FL in brain, prior to and during the active myelination period, brains were isolated from mice at P2-P21 of age (Line 777). Mid-sagittal brain slices were subjected to staining with X-gal. As shown in Fig. 4, expression of the 6.2PLP(+)Z/FL transgene was quite evident at P2, most prominently in the olfactory bulb, neostriatum, corpus callosum (splenium), subventricular zone (SVZ), and developing cerebellum. Expression in the olfactory bulb appeared to lessen over the course of development (P2-P21), while expression in white matter areas occurred primarily in a caudal to rostral direction similar to the pattern previously observed for mPLP(+)Z (Line 26H; Wight et al., 1993), only expedited.

The wmN1 Region within hPLP1 Intron 1 Is Essential for Robust Expression of 6.2hPLP(+)Z/FL in Brain

Because Line 777 expresses 6.2hPLP(+)Z/FL strongly in brain and contains only 2–3 copies of the transgene, this line was crossed with Cre-deleter mice to generate the 6.2hPLPΔwmN1 subline. As well, 6.2hPLP(+)Z/FL mice from Line 776 were used to derive another 6.2hPLPΔwmN1 subline; although Line 776 is a relatively low expressor, it contains only a single copy of 6.2hPLP(+)Z/FL, thus eliminating the possibility for any differences between transgene copy number in the parental line and subline. Removal of the wmN1 region from Line 777 greatly diminished transgene activity as shown by the minimal/lack of X-gal staining in brain with 6.2hPLPΔwmN1 mice at P2 to P21 of age (Fig. 5). In fact, expression of the 6.2hPLPΔwmN1 was highest at P2 in the vicinity of the corpus callosum and SVZ, which decreased at P6, and was no longer evident at later ages (P9-P21). This is consistent with the study by Ivanova et al. (2003) where *mPlp1*gene activity was detected around the lateral ventricle at early postnatal ages. A slight amount of staining was also evident in the olfactory bulb at P6. A developmental decline in the residual amount of β-gal activity present in 6.2hPLPΔwmN1 mice was also observed with whole brain lysates; 345 RLU/μg at P2 compared with only 185 RLU/μg at P21 (Table 1). In fact, the level of β-gal activity at P21 with the 6.2hPLPΔwmN1 subline derived from Line 777 was not much above

background, as determined with non-transgenic (wild-type) littermates (Table 1). The importance of the wmN1 region was confirmed likewise with Line 776 (Supp. Info. Fig. 4), signifying that the decrease in activity with the 6.2hPLPΔwmN1 subline derived from Line 777 is not due, merely, to a (potential) decrease in transgene copy number.

Similar to brain, X-gal staining was greatly diminished (absent) in sciatic nerve when the 'wmN1' region was removed from the transgene; compare X-gal staining in intact sciatic nerve from 6.2hPLP(+)Z/FL mice (Supp. Info. Fig. 3) with that of 6.2hPLPΔwmN1 mice (Supp. Info. Fig. 5) at P9 of age (Line 777). Thus, the effect of the wmN1 enhancer is not restricted solely to the CNS.

The wmN1 Region Is Required for Embryonic hPLP1-lacZ Expression

Line 777 embryos were incubated in X-gal stain to assess the level of transgene activity during fetal development. β-gal activity was readily apparent at E14.5 in the developing brain, dorsal root ganglia, as well as other areas (e.g. tail and hind limb), in embryos hemizygous for the 6.2PLP(+)Z/FL transgene, while there was no discernable background (i.e. staining with X-gal) in non-transgenic littermates (Fig. 6). Lack of the wmN1 region in 6.2hPLPΔwmN1 embryos derived from Line 777 resulted in a complete loss of staining with X-gal at E14.5 (Fig. 6) and E17 (data not shown). Thus the wmN1 region appears to be required for *hPLP1* gene activity as early as fetal development. Moreover, effects mediated by this regulatory element are not limited solely to the developing nervous system.

Colocalization of β-gal Activity from 6.2hPLP(+)Z/FL with Cell Type-Specific Markers

To determine the cell types that express the 6.2hPLP(+)Z/FL transgene, immunohistochemistry was performed following staining with X-gal. Mice from Line 777 at P9 of age were used for these studies. As shown in Fig. 7, the transgene is expressed in oligodendrocyte precursor cells (OPCs) as well as mature oligodendrocytes as evidenced by colocalization of X-gal staining with immunolabeling for NG2 and claudin-11, respectively, in white matter tracks of cerebellum. Similarly, X-gal staining overlapped immunolabeling for GFAP in the superficial layer of the olfactory bulb indicating that the 6.2hPLP(+)Z/FL transgene is expressed in OECs (Fig. 7); besides astrocytes, GFAP is a cell type-specific marker of OECs (Theofilas et al., 2017). In sciatic nerve, there was good overlap of X-gal staining with immunolabeling for S-100 protein indicating that the transgene is also expressed in Schwann cells in the PNS (Fig. 7).

Discussion

To elucidate the mechanisms that regulate expression of the *hPLP1* gene during development, transgenic mice were generated that harbor an *hPLP1-lacZ* transgene termed 6.2hPLP(+)Z/FL since it utilizes *hPLP1* genomic DNA spanning the proximal 6.2 kb of 5′-flanking DNA to the first 38 bp of exon 2 to drive expression of a *lacZ* reporter gene cassette. The transgene encodes a fusion protein with the first 13 amino acids of classic PLP/DM20 attached to the N-terminus of β-gal (PLP- and DM20-related products cannot be differentiated as the transgene does not contain *hPLP1* exon 3). Because *Plp1* intron 1 was shown to be required for expression of a related transgene which contains *mPlp1* DNA in

lieu of *hPLP1* sequences (Li et al., 2002), bacterial recombination sites were incorporated in 6.2hPLP(+)Z/FL for subsequent removal of portions of *hPLP1* intron 1 by mating mice carrying the parental transgene to appropriate deleter strains. We also elected to include more 5′-flanking *hPLP1* DNA relative to the mouse-based mPLP(+)Z transgene (which contains only 2.4 kb of 5′-flanking *mPlp1* DNA) since a previous study (Nadon et al., 1994) showed that the proximal 4.2 kb of *hPLP1* 5′-flanking DNA drove tissue-specific expression of a PLP cDNA transgene.

The 6.2hPLP(+)Z/FL transgene was expressed as early as fetal development in the developing CNS and PNS (Line 777; Fig. 6) in accordance with an earlier study (Wight et al., 1993) which detected β-gal activity in olfactory bulb and dorsal root ganglia in mPLP(+)Z mice (Line 26H) at E14.5. At P21 of age, all of the 6.2hPLP(+)Z/FL lines generated (Lines 772–779) expressed the transgene appropriately in brain (Fig. 2). β-gal activity was highest in white matter areas and the superficial layer of the olfactory bulb. In fact, expression in the olfactory bulb appeared highest at P2 and seemed to decrease thereafter (out to P21) for Line 777 (Fig. 4). In rodents, OECs express the *Plp1* gene to high degree (Griffiths et al., 1995; Dickinson et al., 1997). Immunohistochemistry for GFAP coupled with X-gal staining confirmed that the 6.2hPLP(+)Z/FL transgene is expressed in OECs in P9 mice from Line 777 (Fig. 7). Interestingly, transplantation of OECs in the CNS has been shown to remyelinate lesions produced in experimental models of CNS demyelination (Sasaki et al., 2011; Coutts et al., 2013). The 6.2hPLP(+)Z/FL transgene also displayed the expected pattern of expression, with the highest amount being in CNS, a moderate level in PNS (sciatic nerve), and a negligible quantity in the other tissues tested (Fig. 3; Supp. Info. Figs. 2 & 3). X-gal staining coupled with immunohistochemistry showed that the parental transgene is expressed in oligodendrocytes as well as in OPCs (Fig. 7). Taken together, these data indicate that the *hPLP1* sequences in 6.2hPLP(+)Z/FL are sufficient to direct appropriate spatial expression of the transgene.

While expression of 6.2hPLP(+)Z/FL in brain was fairly consistent to that of the previously reported mouse-based transgene, mPLP(+)Z (Wight et al., 1993), the transgene bearing *hPLP1* DNA appeared to be more broadly expressed during fetal and early postnatal development as determined by X-gal staining (Figs. 4 and 6). This is consistent with the rather substantial level of β-gal activity present in whole brain lysates prepared from P2 mice harboring the 6.2hPLP(+)Z/FL transgene, compared to the relatively low level observed with Line 26H (Table 1), which contains the mPLP(+)Z transgene. It is possible that some of the extra activity observed for 6.2hPLP(+)Z/FL at P2 originates from neuronal sources due to inclusion of a larger amount of upstream sequence; 6.2hPLP(+)Z/FL contains roughly 3.5 kb more of *PLP1* 5′-flanking DNA than does mPLP(+)Z. In the same study (Tuason et al., 2008) that identified wmN1, another evolutionarily conserved region termed 4250-Opo (positions −4170 to −2232, upstream of the *mPlp1* transcription start site) was shown to direct expression of a minimally promoted *eGFPlacZ* reporter gene in discrete neuronal populations including spinal cord gray matter, hippocampus, striatum, and numerous ventral neuronal nuclei in brain, but not in glia. The orthologous 4250-Opo region is present in the 6.2hPLP(+)Z/FL transgene, but primarily missing from mPLP(+)Z. Therefore it is possible that some of the early postnatal activity observed with 6.2hPLP(+)Z/FL at P2 may be mediated through regulatory elements within the *PLP1* 5′-

flanking DNA exclusive to 6.2hPLP(+)Z/FL. Alternatively, some of the early developmental activity observed for 6.2hPLP(+)Z/FL may be due to expression of additional isoforms generated through alternative splicing. The first intron of *hPLP1* contains two supplementary exons (AB and C) that are incorporated, separately, in splice variants (Sarret et al., 2010). Utilization of these exons is restricted for the most part to the human species; these isoforms are not expressed in mouse. The 'human-specific' splice variants are expressed primarily in neurons and have been detected as early as fetal development (Sarret et al., 2010). Due to internal splice donor and acceptor sites within exon AB, two additional splice variants are formed that contain sequence limited to the A-specific region of exon AB, and encode a PLP product having 9 amino acids of additional sequence at the N-terminus. Thus it is possible that the relatively early developmental activity in brain with 6.2hPLP(+)Z/FL (Table 1) may be due, in part, to expression of splice variants that contain sequence limited to A-specific portions of exon AB, which are not generated by mPLP(+)Z. The longest open reading frame (ORF) for transcripts that contain exon AB in its entirety, or exon C, begins in *hPLP1* exon 4, leading to a predicted product that corresponds to the last 72 amino acids (residues 205–276) of PLP (Sarret et al., 2010). Exon AB- or exon C-containing splice variants derived from 6.2hPLP(+)Z/FL are not expected to contribute to the overall level of β-gal activity due to the introduction of multiple (in-frame) stop codons downstream of the classic translation start site near the end of *hPLP1* exon 1; the predicted truncation product lacks the N-terminal domain of β-gal which is necessary for enzymatic activity (Mohler and Blau, 1996). Previously we have shown that these splice variants are expressed in a mouse-derived oligodendroglial cell line transfected with a related [2.7hPLP(+)Z] plasmid (Hamdan et al., 2015).

Excision of the wmN1 region from 6.2hPLP(+)Z/FL in Line 777 caused a dramatic reduction in the level of β-gal activity in whole brain homogenates prepared from mice at P2 and P21 [compare 6.2hPLP(+)Z/FL to 6.2hPLPΔwmN1 for Line 777 in Table 1]. Similarly, X-gal staining of mid-sagittal brain slices was diminished in the 6.2hPLPΔwmN1 subline at all postnatal ages tested (Fig. 5), as well as embryonically (Fig. 6). An earlier study showed that the wmN1 region from mouse was able to direct intense expression of a minimally promoted *eGFPlacZ* transgene to white matter tracts in the brain and spinal cord, and to the superficial layer of the olfactory bulb (Tuason et al., 2008). Activity of the *eGFPlacZ* transgene containing wmN1 was detected as early as E15.5 in some of the cells of the emerging olfactory bulb by X-gal staining. A few labeled cells were identified in the cervical spinal cord at E19 and P0, while weak diffuse labeling was detected in most regions of future spinal cord white matter. However, it was unclear from that study whether the wmN1 enhancer region is capable of augmenting expression in conjunction with the native (*mPlp1*) promoter. Moreover, mice that possess the rudimentary *eGFPlacZ* transgene that lacks any *mPlp1* sequence inexplicably showed widespread straining with X-gal (Tuason et al., 2008). Results presented here with the 6.2hPLPΔwmN1 subline demonstrate that the wmN1 region is a critical regulatory element that works in association with the *hPLP1* promoter to significantly augment expression. While transgene activity in brain was decreased at all postnatal ages examined with the 6.2hPLPΔwmN1 subline derived from Line 777, a slight amount of β-gal activity was detected in animals at P2, but essentially lost by P21 (Table 1 and Fig. 5). The decrease in activity from loss of the wmN1 region in Line 777 is not merely

the result of a (potential) reduction in transgene copy number between the parental line [estimated to contains 2–3 copies of 6.2hPLP(+)Z/FL] and the derivative subline (6.2hPLPΔwmN1) since similar results were obtained with Line 776 (Supp. Info. Fig. 4); both parental Line 776 and its rearranged subline contain only a single copy of the transgene. Thus, the wmN1 region appears to play a pivotal role in regulating *hPLP1* gene expression. It is conceivable that the Sox10 transcription factor may be involved in the process, at least in oligodendrocytes. Sox10 directly controls the expression of several myelin genes, including *PLP1* (for review see Stolt and Wenger, 2010) and was recently shown by ChIP-seq analysis to bind preferentially to the wmN1 region of spinal cord (but not sciatic nerve) chromatin isolated from Sprague-Dawley rats at P15 (Lopez-Anido et al., 2015). It is important to note that excision of the wmN1 region from 6.2hPLP(+)Z/FL results in partial (B-specific segment of exon AB) and complete (exon C) loss of *hPLP1* supplementary exons. However loss of exon AB- and C-containing splice variants, on their own, are not expected to alter the level of β-gal activity since the predicted truncation product from these variants is missing a critical domain required for its activity, as discussed earlier.

Because loss of the wmN1 region from the parental transgene significantly diminished β-gal activity in 6.2hPLPΔwmN1 animals throughout development, it is unlikely that further deletion of *hPLP1* intron 1 sequences from 6.2hPLP(+)Z/FL would significantly change the outcome. Thus far we have been unable to completely excise the intronic sequence flanked by *Frt* sites from all copies of the transgene in Line 777, after crossing to FLPeR mice (The Jackson Laboratory) for 6 generations. This may be due to the relatively large expanse (intron 1 positions 214–8467) between *Frt* sites. Ringrose et al. (1999) noted efficiency with the FLP/*Frt* system was optimal when *Frt* sites were separated by 200 bp, and decreased progressively with increasing distance. Not only does the targeted *Frt*-flanked fragment in 6.2hPLP(+)Z/FL embrace wmN1, but it also contains another putative enhancer region in *hPLP1* intron 1 called wmN2. The wmN2 region was identified in the same study as wmN1, and is believed to mediate high level expression in Schwann cells and their progenitors, dorsal root ganglion (DRG) satellite cells and OECs, and weak expression in oligodendrocyte lineage cells (Tuason et al., 2008). Results presented here suggest that activity of the wmN1 enhancer extends beyond the cell types initially identified by Tuason and coworkers (2008), and includes those ascribed as being regulated by the wmN2 enhancer. It is possible that there is partial redundancy in the cell types affected by the two enhancers, or alternatively, species-specific differences may exist between the enhancers in human and mouse. Future studies are needed to test whether loss of the wmN2 region can single-handedly diminish expression in conjunction with the native (*PLP1*) promoter. Curiously, deletion-transfection analysis (Hamdan et al., 2015) of *hPLP1-lacZ* constructs in Oli-neu cells revealed that loss of the wmN1 region had no effect *in vitro* (i.e. β-gal activity did not decrease), unlike the wmN2 region which was required for maximal expression. However this apparent discrepancy, at least for the wmN1 enhancer region, could be explained if its mechanism of action was mediated through an effect (*in vivo*) on chromatin remodeling, which would be of no consequence in transient transfection assays that utilize naked DNA.

It has been known for a long time that inclusion of an intron in a transgene generally has a positive effect on expression levels (Brinster et al., 1988; Palmiter et al., 1991). Increased output may be due to augmentation at the level of transcription or, alternatively, at some post-transcriptional step. Many of the steps regulating gene expression have been found to be functionally, and sometimes physically, connected (for review see Orphanides and Reinberg, 2002). Thus, transgenes that contain intron(s) may have increased output due to a beneficial effect on nuclear export or stability of their mRNA products by virtue of RNA splicing. Splicing signals located near the promoter can enhance gene transcription, directly (Furger et al., 2002). While exon C and a portion of exon AB are absent from 6.2hPLPΔwmN1, the transgene still contains the promoter proximal-most 5′ splice site adjoining *hPLP1* exon 1. Thus it appears that the most likely explanation for the decrease in activity with the 6.2hPLPΔwmN1 subline is due to a loss of a transcriptional enhancer (i.e. wmN1) from the parental transgene; excision of the wmN1 region thwarts the formation of splice variants that do not give rise to β-gal activity (those that contains all of exon AB or C) while preserving those that do (variants that contain only A-specific segments of exon AB).

In summary, the results presented here demonstrate the importance of the wmN1 region in regulating *hPLP1* gene activity, at least in context of the 6.2hPLP(+)Z/FL transgene. Its presence is absolutely required to attain maximal expression in oligodendrocytes, OPCs, OECs and Schwann cells. Our results definitively demonstrate that wmN1 can function in collaboration with the native *hPLP1* promoter, not just a basal heterologous promoter as shown previously by Tuason and coworkers (2008). It is conceivable that mutations which disrupt wmN1 function in humans may result in Pelizaeus-Merzbacher disease (PMD) or spastic paraplegia type 2 (SPG2) due to a shortage of *hPLP1* expression in males having standard *hPLP1* gene dosage and a normal (unmutated) coding sequence. Future studies should be directed at identifying the factors that mediate wmN1 function, and determining whether the enhancer is required for *hPLP1* gene activity during times of remyelination. Differences in the composition of transcription factors present in the developing nervous system, versus those in adults, may partially help to explain the relatively poor levels of remyelination achieved in persons with multiple sclerosis. Along these lines, Glasgow and et al. (2014) showed that nuclear factor I-A (NFIA) can directly antagonize Sox10 regulation of *Plp1* gene expression in chick and mouse spinal cord. Whatever the factors may be, it is clear that the wmN1 enhancer appears to be central to the regulation of *hPLP1* expression.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Main Points

- An *hPLP1-lacZ* transgene contains sequences sufficient for correct spatiotemporal expression.
- The wmN1 region within *hPLP1* intron 1 is necessary robust expression of *hPLP1-lacZ*.
- The wmN1 enhancer can work in association with its native promoter.

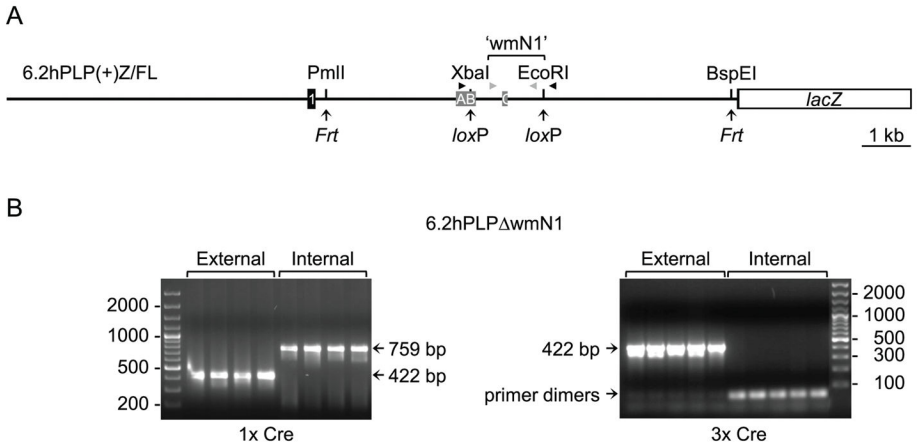

FIGURE 1.

Schematic of the parental 6.2hPLP(+)Z/FL transgene and PCR screening for the ensuing 6.2hPLPΔwmN1 subline. (**A**) The 6.2hPLP(+)Z/FL transgene utilizes *hPLP1* DNA spanning the proximal 6.2 kb of 5′-flanking DNA to first 38 bp of exon 2 to drive expression of the *lacZ* reporter gene. Black boxes indicate *hPLP1* exon 1 and the beginning of exon 2, while light gray boxes depict the minor (alternatively spliced/incorporated) exons AB and C. Due to the miniscule amount of *hPLP1* exon 2 sequence that directly precedes the *lacZ* expression cassette, it is not numbered, as the transgene is drawn to scale. Black lines represent *hPLP1* 5′-flanking DNA and intron 1. *Frt* and *lox*P sites were introduced at the designated restriction enzyme sites in *hPLP1* intron 1; only pertinent restriction enzyme sites are indicated. The *lox*P sites encase DNA orthologous to the mouse wmN1 enhancer region ('wmN1') and the adjoining 330 bp, upstream. Arrowheads indicate the relative position and orientation of external (black) and internal (gray) primers used to determine whether the 'wmN1' region was effectively removed from the transgene after mating 6.2hPLP(+)Z/FL mice with Cre-deleter mice. (**B**) PCR analysis for production of the 6.2hPLPΔwmN1 subline, which was generated by mating 6.2hPLP(+)Z/FL mice (Line 777) to a Cre-deleter strain for several generations to remove the region flanked by *lox*P sites from all transgene copies. The external primer pair borders the floxed region and results in an amplicon of 422 bp if the targeted sequence is missing (the predicted 1952 bp product that still retains the floxed sequence is not generated under the conditions utilized for PCR). The internal primer pair amplifies a 759 bp segment within the wmN1 region and is used to assess whether some copies of the transgene still retain the sequence targeted for removal. As shown for Line 777, a single cross with the Cre-deleter removed the targeted wmN1 region from some, but not all, copies of the transgene (1× Cre). However, backcrossing the resulting progeny for two more generations with the Cre-deleter resulted in full excision of the wmN1 region from all transgene copies (3× Cre).

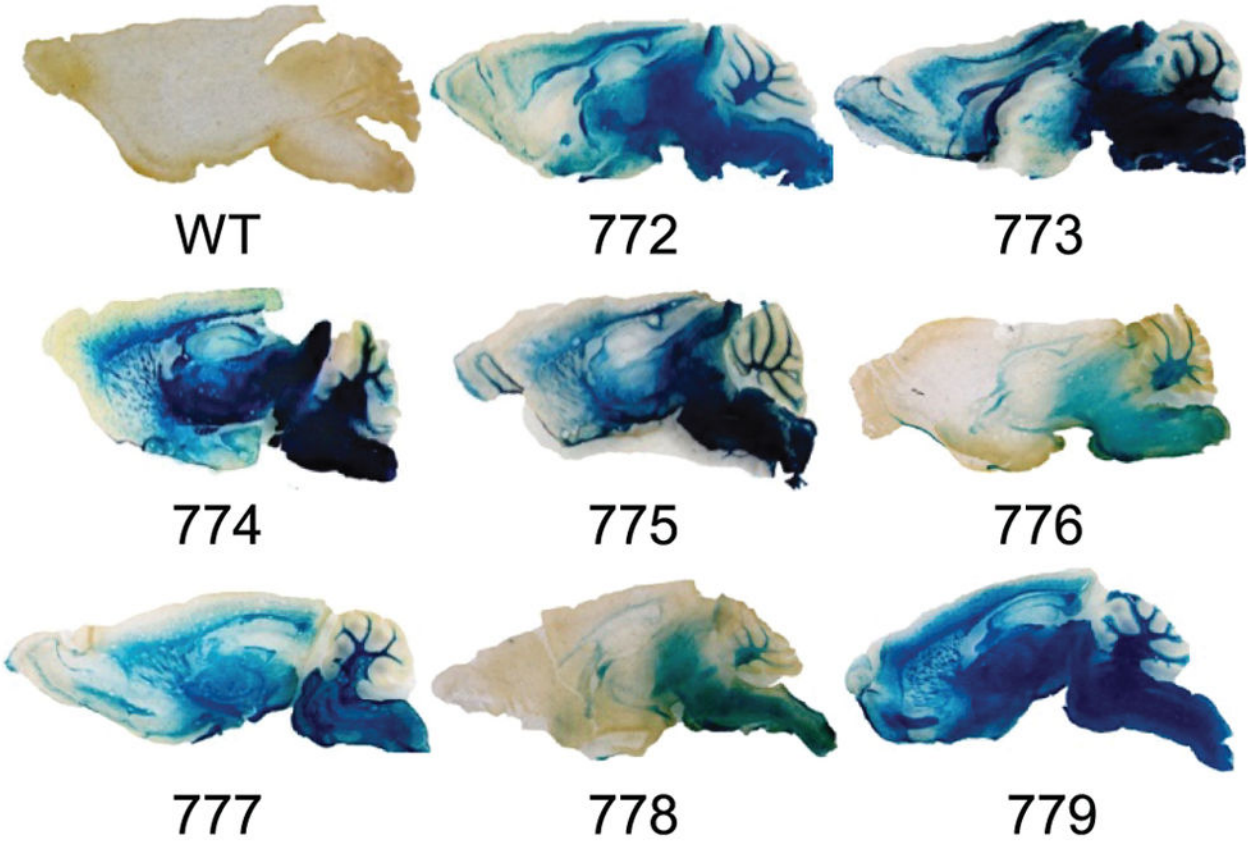

FIGURE 2.

β-gal activity in brain of 6.2hPLP(+)Z/FL transgenic mice at P21 as determined by X-gal staining. Mid-sagittal brain sections (30 μm) were stained with X-gal for 30 min (Lines 772, 773, 774, 775, 777 and 779) or 6 h (Lines 776 and 778). Staining was evident in white matter areas of brain for all of the transgenic lines, but absent in a wild-type (WT) non-transgenic littermate.

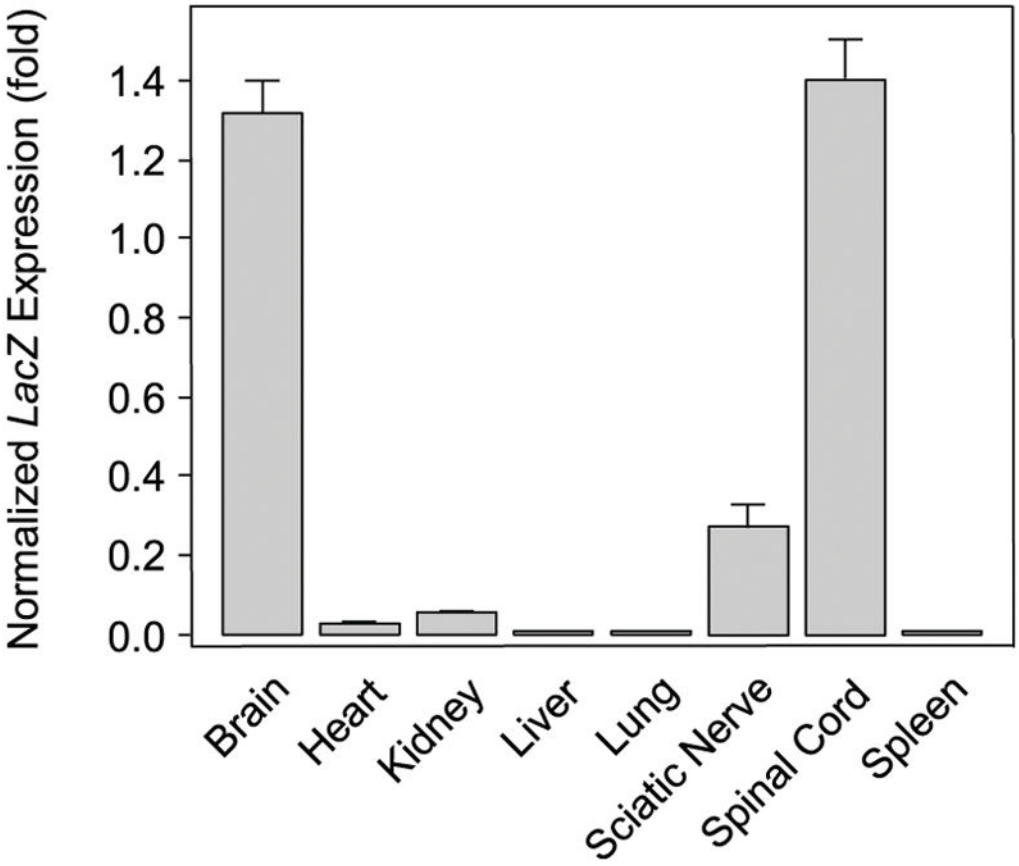

FIGURE 3.

Spatial expression of the 6.2hPLP(+)Z/FL transgene for Line 777. Total RNA was isolated separately from the indicated tissues of three animals at P21 and subjected to qRT-PCR analysis. Results are reported as the fold mean ± SE of *lacZ* expression (n = 3) relative to that from the 18S and β-actin reference genes. The plotted amounts are relative to that generated using a pooled sample of brain RNA from all three animals, which was arbitrarily set at 1 (data not shown).

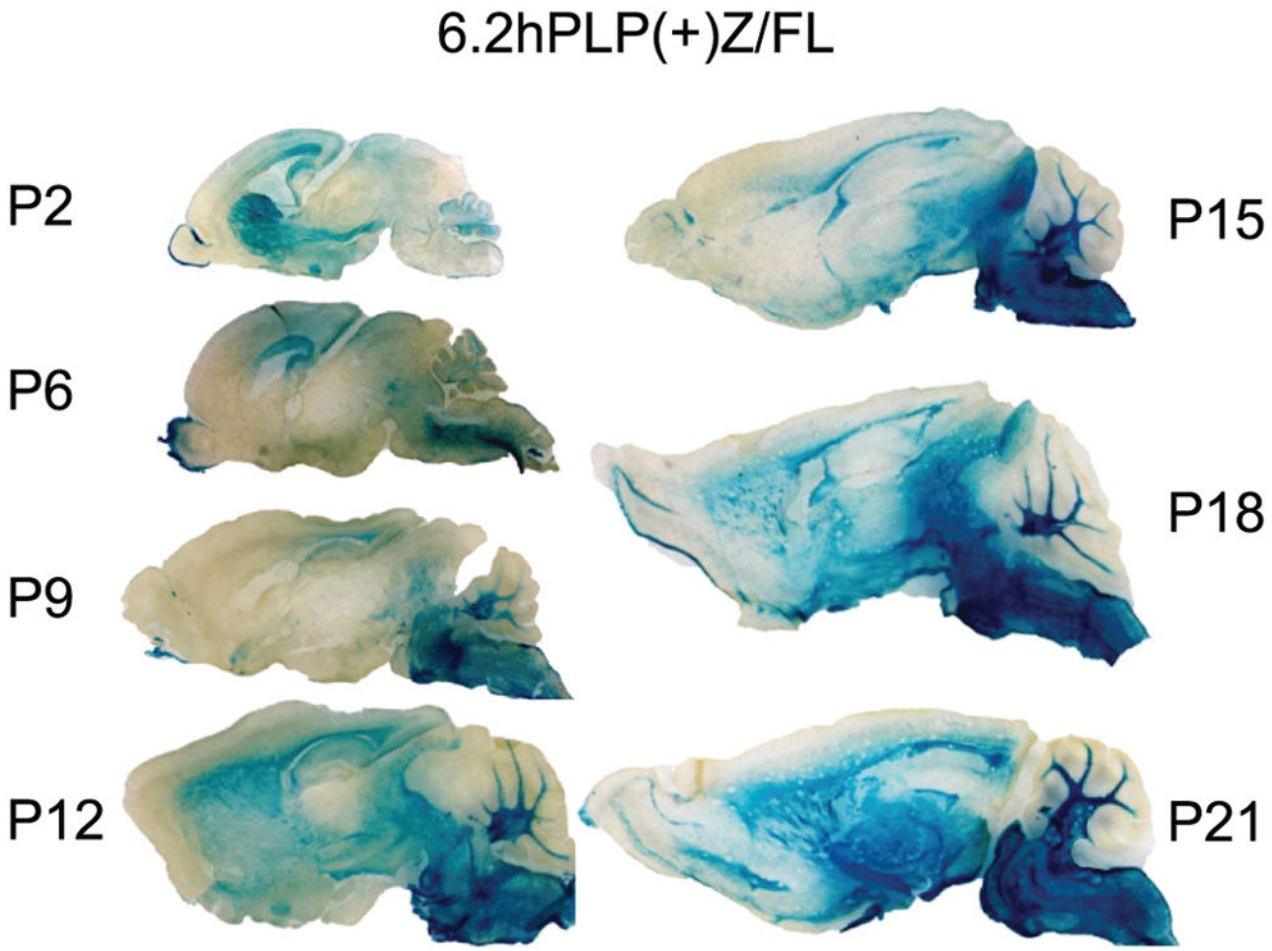

FIGURE 4.

Developmental expression of the 6.2hPLP(+)Z/FL transgene in brain with Line 777. Mid-sagittal sections (30 μm) from brains of mice at the indicated ages (postnatal days) were subjected to X-gal staining to identify areas of β-gal activity. Sections from animals at P2–P9 were stained for 6 h, while sections from animals at P12 and P15 were stained for 1 h, and those from animals at P18 and P21 were stained for only 30 min. Robust staining was detected in the olfactory bulb (far left side of each section) as early as P2. Staining was also evident in white matter areas during development. For instance, note the staining in white matter tracks of the developing cerebellum (cauliflower-shaped region towards the upper right side of each section).

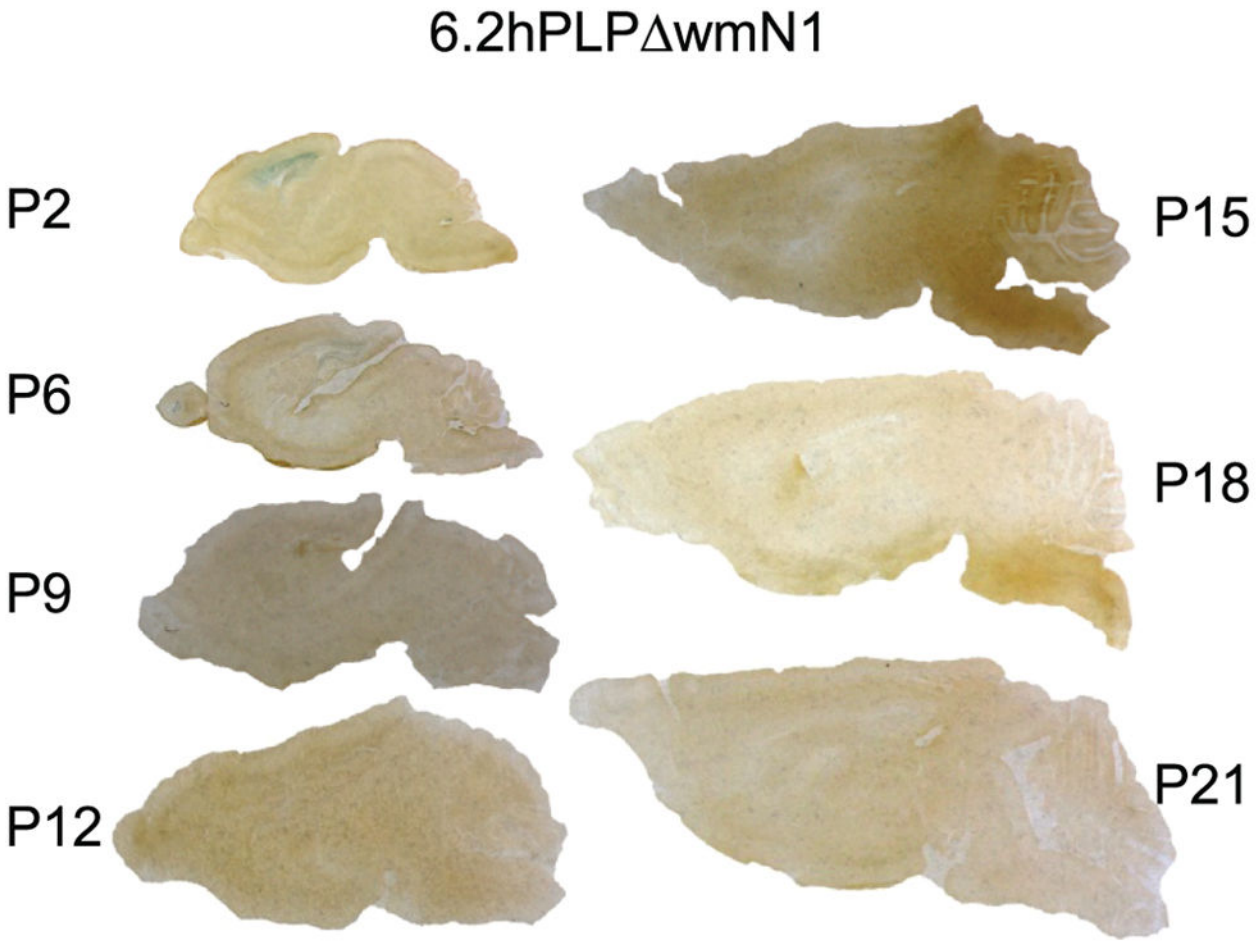

FIGURE 5.

Developmental expression of the 6.2hPLPΔwmN1 transgene in brain with the 777 subline. Mid-sagittal sections (30 μm) from brains of mice at the indicated ages (postnatal days) were subjected to X-gal staining to identify regions positive for β-gal activity. Sections from animals at P2-P9 were stained for 6 h, while sections from animals at P12 and P15 were stained for 1 h, and those from animals at P18 and P21 were stained for 30 min to be consistent with the staining of brain sections from 6.2hPLP(+)Z/FL mice (Fig. 4). Slight staining was detected in the corpus callosum and SVZ at P2, less so at P6, and virtually undetectable at latter ages. A little bit of staining was also present in the developing olfactory bulb of mice at P2 and P6.

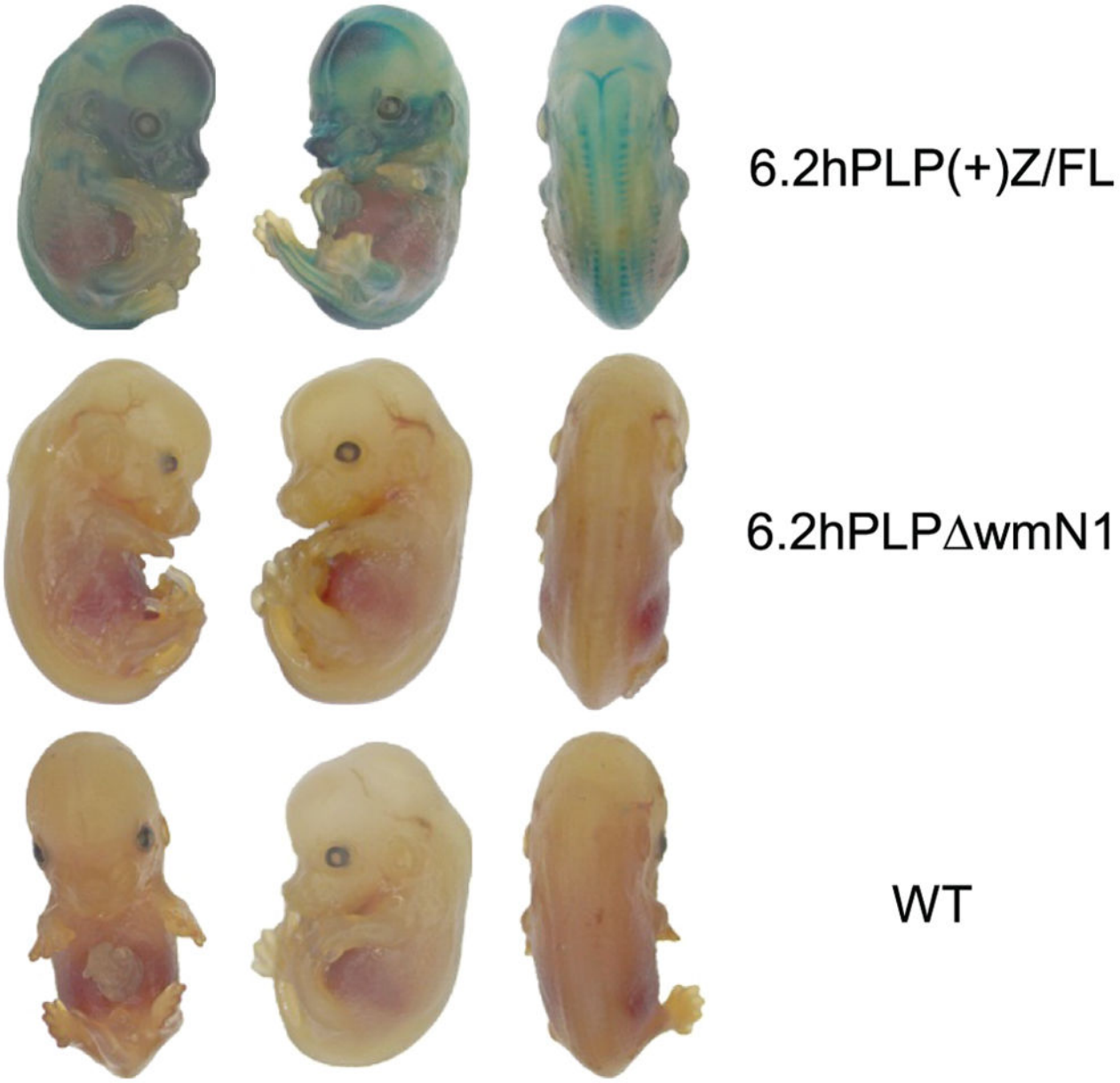

FIGURE 6.

hPLP1-lacZ transgene activity in embryos at E14.5 as determined with X-gal staining. All transgenic embryos were derived from Line 777 and are hemizygous for the parental [6.2PLP(+)Z/FL] or rearranged [6.2hPLPΔwmN1] transgene. Non-transgenic wild-type (WT) embryos are littermates to those carrying the parental transgene shown at the top.

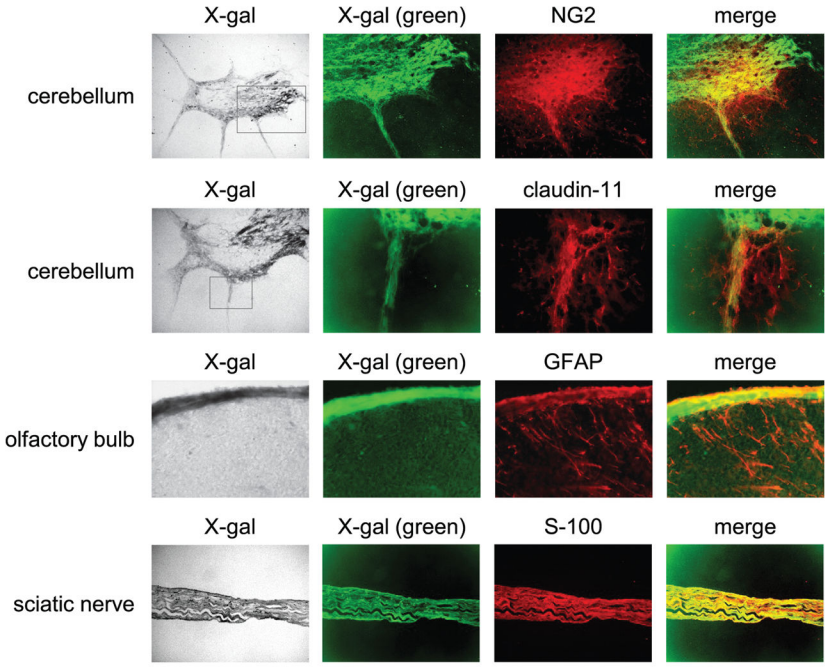

FIGURE 7.

Colocalization of β-gal activity with immunofluorescence for cell type-specific markers in brain and sciatic nerve. Tissues were obtained from a 6.2hPLP(+)Z/FL mouse (Line 777) at P9 of age. Brain slices (16 μm) and intact sciatic nerve were stained with X-gal followed by immunofluorescence staining with the indicated markers (red color); the X-gal stained sciatic nerve shown in Supp. Fig. 3 was sectioned longitudinally (16 μm) prior to immunofluorescence. The bright-field microscopic images for X-gal staining shown in black and white were converted to a green color in ImageJ for application with the merged pictures. Boxes represent the area magnified (40×) for immunofluorescence with cerebellum. Magnification: 20× for olfactory bulb; 10× for sciatic nerve.

TABLE 1

Characterization of *hPLP1-lacZ* transgenic mouse lines.*

Line	Transgene	Copy #	β-gal activity (P2)	β-gal activity (P21)
772	6.2hPLP(+)Z/FL	4–5	10887 ± 1711	5909 ± 525
773	6.2hPLP(+)Z/FL	>20	11942 ± 1223	16283 ± 4594
774	6.2hPLP(+)Z/FL	4–5	16758 ± 1104	4641 ± 821
775	6.2hPLP(+)Z/FL	3–4	3895 ± 981	3096 ± 1296
776	6.2hPLP(+)Z/FL	1	438 ± 120	613 ± 22
777	6.2hPLP(+)Z/FL	2–3	8440 ± 492	3267 ± 233
778	6.2hPLP(+)Z/FL	1–2	769 ± 62	822 ± 67
779	6.2hPLP(+)Z/FL	>50	10402 ± 1574	9098 ± 982
26H	mPLP(+)Z	>100	238 ± 50	4837 ± 576
WT	non-tg	0	107 ± 3	119 ± 5
Subline	Transgene	Copy #	β-gal activity (P2)	β-gal activity (P21)
777	6.2hPLPΔwmN1	N.D.	345 ± 51	185 ± 54
WT	non-tg	0	64 ± 2	154 ± 10

*Transgene copy number was estimated by Southern blot analysis in hemizygous animals. Transgene copy number was not determined (N.D.) for the 6.2hPLPΔwmN1 subline (Line 777). The mPLP(+)Z transgene is similar to 6.2hPLP(+)Z/FL except it uses *mPlp1* genomic DNA (lesser amount) to drive *lacZ* expression; see Wight et al. (1993) for characterization of Line 26H. β-gal activity is reported as the mean RLU/μg protein ± SD (n ≥ 3) from whole brain homogenates prepared from mice at the indicated postnatal days (P) of age.