A zinc finger protein that regulates oligodendrocyte specification, migration and myelination in zebrafish

Harwin Sidik and William S. Talbot*

ABSTRACT

Precise control of oligodendrocyte migration and development is crucial for myelination of axons in the central nervous system (CNS), but important questions remain unanswered about the mechanisms controlling these processes. In a zebrafish screen for myelination mutants, we identified a mutation in zinc finger protein 16-like (znf16l). znf16l mutant larvae have reduced myelin basic protein (mbp) expression and reduced CNS myelin. Marker, time-lapse and ultrastructural studies indicated that oligodendrocyte specification, migration and myelination are disrupted in znf16l mutants. Transgenic studies indicated that znf16l acts autonomously in oligodendrocytes. Expression of Zfp488 from mouse rescued mbp expression in znf16l mutants, indicating that these homologs have overlapping functions. Our results defined the function of a new zinc finger protein with specific function in oligodendrocyte specification, migration and myelination in the developing CNS.

KEYWORDS: Myelin, Oligodendrocyte, Zebrafish

INTRODUCTION

Precise temporal control of cell fate specification and migration is essential to coordinate the development of different cell types. In the nervous system, for example, newly differentiated neurons extend axons over long distances to form connections with their postsynaptic targets (Araújo and Tear, 2003). Oligodendrocytes, the myelinating cells of the central nervous system (CNS), provide another example. During development, oligodendrocyte precursor cells (OPCs) migrate throughout the CNS. After completing their migration, some OPCs differentiate as oligodendrocytes and myelinate their associated axons immediately, whereas other OPCs delay differentiation until much later stages (Young et al., 2013). Formation of new myelin from OPCs in the adult is essential for motor learning, underscoring the importance of the precise temporal control of differentiation of myelinating oligodendrocytes in the healthy CNS (Scholz et al., 2009; McKenzie et al., 2014). In some demyelinated lesions in individuals with multiple sclerosis, OPCs are present but apparently unable to form myelin (Koller et al., 2006; Boyd et al., 2013), highlighting the need to understand the temporal control of oligodendrocyte maturation in health and disease.

OPCs are derived from a group of Olig2-expressing progenitors in the motoneuron progenitor (pMN) domain in the ventral CNS. Cells of the pMN domain generate motoneurons at early stages, when they express Olig2 and the neurogenic factor Ngn2 (Mizuguchi et al., 2001; Novitch et al., 2001), and they form OPCs at later stages, when they express Olig2 and the gliogenic factor Sox10 (Stolt et al., 2002; Pozniak et al., 2010). This transition between neurogenic fate and gliogenic fate is mediated by Notch receptors and their ligands (Wang et al., 1998; Park and Appel, 2003), organizing the pMN domain into temporal compartments. Diverse signals, including Pdgf and Netrin-1, direct OPC migration away from the midline and towards the axons that they eventually myelinate (de Castro and Bribian, 2005; Tsai et al., 2006; Miyamoto et al., 2008). After contacting their target axons and initiating differentiation, maturing oligodendrocytes express transcription factors, such as Zfp191 and Myrf, that initiate and maintain the expression of the genes essential for myelination (Emery et al., 2009; Howng et al., 2010). Despite much progress in identifying essential regulators of oligodendrocyte development and myelination, many aspects of the developmental program are not well understood. In particular, little is known about the factors that specify OPCs within the pMN domain and initiate their migration.

To define new genes that regulate oligodendrocyte specification and differentiation, we have conducted a genetic screen for mutations that disrupt myelin gene expression in zebrafish. In this study, we report that the zinc finger protein Znf16-like (Znf16l) regulates the onset of OPC migration and differentiation. In late embryonic stages when OPCs normally begin their migration, znf16l mutants had Olig2-expressing progenitors at the CNS midline but lacked OPCs in most regions of the CNS. Time-lapse imaging revealed that although OPCs were present in the mutants at these early stages, their migration was delayed. At later stages, OPC migration recovered in the mutants, but CNS myelin remained significantly reduced. Through transgenic analyses, we determined that Znf16l acts autonomously in the oligodendrocyte lineage. We also discovered that the normal OPC development could be rescued in znf16l mutants by transgenic expression of the mammalian zinc finger protein Zfp488, which was previously shown to cooperate with Notch signaling to specify OPC fate in the chicken (Wang et al., 2006). Lastly, we showed that despite similar myelination defects in both znf16l mutants and previously studied notch3 mutants (Zaucker et al., 2013), the two genes have different roles in regulating development of the oligodendrocyte lineage. These findings indicate that Znf16l is an essential regulator of specification, migration and myelination in the oligodendrocyte lineage.

RESULTS

Znf16-like is essential for normal myelination in the CNS

In a genetic screen for zebrafish mutants with abnormal myelination, we identified st78 as a recessive mutation that reduced expression of myelin basic protein (mbp) mRNA expression in the CNS at 5 days postfertilization (dpf; Fig. 1A). Homozygous st78 mutants exhibited normal mbp expression in the peripheral nervous system (PNS) and normal gross morphology at 5 dpf (Fig. 1A,B). High-resolution meiotic mapping localized the
st78 mutation to a 0.9 Mb region of linkage group 7 (LG7; Fig. 1C). Sequence analyses of genomic DNA from st78 mutants revealed a G-to-T transversion in the previously uncharacterized gene XM_694039, which is predicted to encode a zinc finger protein, Zinc finger 16-like (Znf16l, also known as Zinc finger 697-like/Znf697l). This mutation changed a cysteine in one of the six predicted Cys2His2 zinc fingers of Znf16l to a phenylalanine (C428F; Fig. 1D,E).

To construct a null mutation in znf16l, we generated a second mutant allele using transcription activator-like effector nucleases (TALEN; Sanjana et al., 2012). We identified an allele, st97, with a deletion of coding nucleotides 133-136 of znf16l (Fig. 1E,F). This 4 bp deletion caused a frameshift in the open reading frame, resulting in a premature stop codon upstream of all six predicted zinc fingers in the Znf16-like protein. Like homozygotes for the st78 point mutation, homozygous st97 mutants lacked mbp expression in the CNS at 5 dpf and exhibited normal gross morphology (Fig. 1G,H). Furthermore, the st97 frameshift mutation failed to complement the st78 point mutation: st78/st97 transheterozygotes also lacked mbp expression at 5 dpf (Fig. 1G). These results confirmed that Znf16l is essential for normal mbp expression in the larval CNS and further suggested that the disruption of one zinc finger module in st78 mutants abolished the function of Znf16-like.

Specific defects in oligodendrocyte lineage in znf16l mutants

In the developing spinal cord, most oligodendrocytes are derived from a ventral region known as the pMN domain, which also gives rise to motoneurons (Rowitch, 2004). At 24 hours postfertilization (hpf), we detected no difference in the expression of pMN markers between znf16l mutants and wild-type siblings (Fig. 2A,B), indicating that the oligodendrocyte defects did not arise from a delay in the formation of the pMN domain. Likewise, motoneuron development appeared similar in the mutants and wild-type siblings, based on the comparable expression patterns of the motoneuron marker islet1 at 52 hpf (Fig. 2C). By contrast, at this same stage (52 hpf), we detected differences between the mutants and wild-type siblings in the expression of sox10 and olig2 in oligodendrocytes (Fig. 2D,E). olig2-expressing cells had migrated widely in the brain and the spinal cord of the wild-type siblings at this stage, whereas olig2 expression in the mutant was restricted to the ventral midline (Fig. 2D). We also examined expression of sox10, which is restricted to cells in the oligodendrocyte lineage in the CNS. At 52 hpf, sox10-expressing oligodendrocyte-lineage cells were evident in the ventral spinal cord of wild-type embryos but not in mutants (Fig. 2E). These results support the possibility that znf16l functions specifically in the development of cells in the oligodendrocyte lineage.

Loss of znf16l function delays the onset of oligodendrocyte migration

To define the role of znf16l in oligodendrocyte lineage development, we examined znf16l mutants bearing the Tg(olig2:GFP) transgene in timecourse and time-lapse studies. This transgene is expressed in OPCs and throughout differentiation and maturation of myelinating oligodendrocyte-lineage cells (Shin et al., 2003; Zannino and Appel, 2009), allowing observation of different stages of the oligodendrocyte lineage. At 60 and 84 hpf, we observed significantly fewer olig2-expressing cells in the forebrain, midbrain and hindbrain of the mutants compared with their wild-type siblings (Fig. 3A,B,F). By 108 hpf, however, the number of...
olig2-expressing cells in the mutants was comparable to that of the wild-type siblings (Fig. 3C,F).

Time-lapse studies showed that olig2-GFP-expressing OPCs migrated from the midline of wild-type embryos between 48 and 52 hpf, but few if any OPCs migrated from the midline in znf16l mutants at these stages (Movies 1, 2; Fig. 3D). In the mutants, OPCs were observed migrating from the midline, starting at about 60 hpf (Movies 3, 4; Fig. 3E). After OPCs began migrating in znf16l mutants, they appeared to move over longer distances and over longer periods of time than their wild-type counterparts. To quantify this, we tracked migration of 30 OPCs in mutants over longer periods of time than their wild-type counterparts. To assess whether the abnormal expression of oligodendrocyte markers reflects any defects in myelination at later stages, we analyzed the expression of mbp at 9 dpf (Fig. 4C). Despite the strong reduction of mbp expression at 5 dpf (Fig. 1A), znf16l mutants had detectable mbp expression at 9 dpf, although the level of expression was less than in their wild-type siblings. These results indicated that oligodendrocyte maturation is delayed in the mutants, despite the recovery in OPC number that occurred at earlier stages.

Myelin is reduced in znf16l mutants
To assess whether the abnormal expression of oligodendrocyte markers reflects any defects in myelination in znf16l mutants, we examined the ultrastructure of the myelinated axons in the ventral spinal cord. In accordance with the marker analyses, the number of myelinated axons in the mutants was significantly lower than in their wild-type siblings at 5 dpf (1.7±0.8 per hemisegment of the spinal cord in mutants, n=6, compared with 18.7±0.9 in wild type, n=6; Fig. 4D,E,H). At 9 dpf, the number of myelinated axons increased in mutants (7.7±0.7, n=3), but the number remained significantly reduced compared with the wild-type siblings at the same stage (27.3±0.3, n=3; Fig. 4F-H). These results indicated that znf16l is required for myelination for at least several days after OPC migration is complete. Although myelin was reduced in znf16l mutants, some myelin did form and some homozygous mutants survived to become fertile adults (Fig. 4I; in the progeny of heterozygous intercrosses: 6.8% homozygous st78 mutants at 90 dpf, n=177; 7.2% homozygous st97 mutants at 90 dpf, n=111).
Znf16l functions autonomously in oligodendrocytes for proper CNS myelination

To determine which cell types in the CNS require Znf16l function for myelination, we performed transgenic rescue experiments using full-length Znf16l expressed under the control of different tissue-specific regulatory elements. Control uninjected mutants were raised and stained alongside to ensure that rescued mbp expression was not a result of the weak expression of mbp seen in older mutant embryos. In transient transgenic assays, we analyzed constructs that expressed Znf16l in neurons (huC, mnx1), oligodendrocytes (cldnk, sox10) and macrophages or microglia (mpeg1; Fig. 5A,B). The cldnk::znf16l and sox10::znf16l transgenes rescued mbp expression in the CNS of mutants at 5 dpf (cldnk: 45% of the mutants, n=77; sox10: 57% of the mutants, n=47; Fig. 5B,C). No rescue was detected with the mnx1::znf16l construct (Fig. 5B). The huC::znf16l construct rescued at a low frequency (17% of the mutants, n=59; Fig. 5B,C), perhaps because of expression of the huC regulatory sequences in some cells of the oligodendrocyte lineage (Fig. S1). The mpeg1::znf16l construct did not rescue mbp expression in the mutants at 5 dpf (Fig. 5B). These results provide evidence that znf16l acts autonomously in OPCs and early oligodendrocytes to promote CNS myelination.

Mouse Zfp488, but not Zfp191, can rescue znf16l mutants

Sequence analysis did not identify a clear ortholog of Znf16l in mammals. Previous studies have identified several zinc finger proteins that have specific functions in the oligodendrocyte lineage in mammals, including Zfp191, which is required for mature oligodendrocyte-lineage cells to myelinate (Howg et al., 2010), and Zfp488, which can promote oligodendrocyte lineage differentiation and maturation in combination with Notch signaling (Wang et al., 2006). The zebrafish genome contains an ortholog of Zfp191, but no gene clearly orthologous to Zfp488. Thus, we analyzed the previously characterized mouse genes in...
these functional studies. To test whether either of these zinc finger proteins shares any functional overlap with Znf16l, we performed a transgenic rescue experiment with full-length mZfp191 or mZfp488 under the control of \textit{sox10} promoter (Fig. 5A). Zfp488 rescued \textit{mbp} expression in the CNS of \textit{znf16l} mutants (47% of the mutants, \(n=30\)), whereas expression of Zfp191 had little or no effect (2.9% of the mutants, \(n=35\); Fig. 5B,C). These data suggest that mammalian Zfp488 and zebrafish Znf16l have a shared function in promoting oligodendrocyte-lineage cell migration and differentiation despite the divergent sequences of these zinc finger proteins.

Different requirements for \textit{znf16l} and \textit{notch3} in oligodendrocyte development

Previous analysis showed that \textit{notch3} mutants share phenotypic similarities with \textit{znf16l} mutants (Zaucker et al., 2013). In \textit{notch3} mutants, an early reduction in \textit{olig2}-expressing OPCs underlies a reduction in CNS \textit{mbp} expression (Zaucker et al., 2013). In \textit{notch3} mutants, \textit{mbp} expression begins to recover by 7 dpf, and some homozygous \textit{notch3} mutants survive to adulthood (Zaucker et al., 2013), similar to \textit{znf16l} mutants. In addition, previous work in the chicken indicates that Zfp488, which can compensate for loss of Znf16l in our \textit{in vivo} rescue assays, cooperates with Notch signaling, because overexpression of Zfp488 promotes ectopic oligodendrocyte precursor formation only when Notch signaling is activated (Wang et al., 2006).

To investigate the possible relationship of Znf16l and Notch3 in oligodendrocyte development, we examined the expression of early markers in both mutants in parallel. As previously described (Zaucker et al., 2013), \textit{notch3} mutants showed marked reductions in expression of \textit{deltaD} and \textit{notch3}, whereas heterozygous siblings had increased expression of both genes compared with wild-type siblings (Fig. 6B,C,E,F). By contrast, \textit{znf16l} mutants expressed both \textit{deltaD} and \textit{notch3} at the same level as their wild-type siblings (Fig. 6A,D), suggesting that Znf16l and Notch3 have distinct functions, despite the similar timecourse of \textit{mbp} reduction and recovery in the mutants. In addition, we observed that \textit{znf16l} mutants lacked a ventral population of \textit{olig2}-expressing progenitors in the hindbrain (Fig. 2D′; Fig. 6G″), whereas \textit{notch3} mutants lack a more dorsal population (Fig. 6H″,I″). Furthermore, mutants in these two genes displayed differences in the lateral migration of \textit{olig2}-expressing OPCs (Fig. 2D‴; Fig. 6G′-I′). These results indicate that Znf16l and Notch3 have different effects on OPCs in the developing embryo.

Severe oligodendrocyte defects in \textit{znf16l};\textit{notch3} double mutants

To investigate the relationship of \textit{znf16l} and \textit{notch3} in oligodendrocyte development further, we analyzed \textit{znf16l};\textit{notch3} double mutants. The double mutants had a greater reduction of \textit{olig2}-expressing OPCs at 52 hpf compared with either single mutant (Fig. 7A-H). The double mutants also lacked laterally migrating OPCs, similar to \textit{znf16l} mutants (Fig. 7C,D,G,H). By 4 dpf, \textit{znf16l} and \textit{notch3} single mutants significantly recovered the number and distribution of their OPCs (Fig. 7I-K, but \textit{znf16l};
DISCUSSION
Many studies indicate that Hedgehog signaling activity specifies the progenitors in the pMN domain in the ventral neural tube (Richardson et al., 2006) and that these pMN progenitors sequentially form motoneurons at early stages of embryogenesis and oligodendrocyte precursor cells at later stages (Wu et al., 2006). Notch signaling activity is important for this transition (Wang et al., 1998; Genoud et al., 2002; Park and Appel, 2003; Kim et al., 2008; Zaucker et al., 2013), but the other factors that direct pMN progenitors to become migratory OPCs are not well understood. Our study demonstrates that Znf16l is essential for neural progenitors to become migratory OPCs. In znf16l mutants, early patterning of the pMN domain is normal, but oligodendrocyte precursors are specifically delayed in initiating their migration from the midline of the CNS. Moreover, CNS myelin is greatly diminished in znf16l mutants. Despite the severe disruption of OPC migration in mutant embryos, migratory OPCs are evident in the mutant CNS at early larval stages. CNS myelin also partly recovers in larval mutants, but myelin remained significantly reduced in mutants at stages after OPCs have dispersed throughout the CNS. This reduction in myelin indicates that znf16l also regulates the onset of myelination, in addition to early events in OPC specification and migration. Expression of Znf16l in the oligodendrocyte lineage rescues mbp expression in the mutants, indicating that Znf16l acts autonomously in these cells. Our analysis identifies Znf16l as a key autonomous regulator of OPC specification, migration and myelination.

In light of the severe defects in oligodendrocyte development in znf16l mutant embryos and early larvæ, it is interesting that some CNS myelin is present at later stages. Likewise, previous work in zebrafish indicates that the notch3 gene is required for OPC specification and myelination in the early larva and that myelin partly recovers at later stages (Zaucker et al., 2013). These studies point to redundancy in the control of oligodendrocyte development in zebrafish. There are at least two levels at which this redundancy might operate. First, it is possible that there are genetically distinct populations of OPCs that are able to compensate for loss of each other. Second, it is possible that different members of the Zinc finger and Notch receptor families have overlapping functions, such that homologous genes are able to substitute for each other at some stages.

Previous studies discovered heterogeneity in oligodendrocyte populations in mammals (Tomassy and Fossati, 2014). Spatially, oligodendrocytes arise in distinct regions of the neocortex (Kessaris et al., 2006), cerebellum (Buffo and Rossi, 2013), dorsal spinal cord and ventral spinal cord (Cai et al., 2005). Temporally, multiple waves of oligodendrogenesis occur in the healthy, developing CNS (Kessaris et al., 2006). Ablation studies show that these OPC populations can compensate for the loss of each other, highlighting the redundancy in the lineage despite the apparent heterogeneity in origins of OPCs (Kessaris et al., 2006). Our study is consistent with, but does not demonstrate conclusively, the possibility that such redundancy in the oligodendroglial lineage also exists in the zebrafish. Different domains of olig2 expression are disrupted in znf16l and notch3 single mutants, and the combined loss of both Notch3 and Znf16l in the double mutants resulted in a more severe disruption of oligodendrocyte development and myelination. Thus, it is possible that znf16l and notch3 are required in distinct populations of OPCs that can compensate for loss of each other. A conclusive test of this possibility will require identification of molecular markers that distinguish different populations of OPCs.

There is also evidence for redundancy of individual gene functions in the control of oligodendrocyte development. For example, Notch receptors other than Notch3, notably Notch1, regulate neural progenitor differentiation to promote gliogenesis (Genoud et al., 2002). It is likely that Notch1 activity can partly compensate for the loss of Notch3, contributing to the recovery of CNS myelination in notch3 mutants. Likewise, our in vivo rescue experiments indicate that a mammalian homolog of znf16l, Zfp488, can substitute for znf16l despite their sequence divergence. Although we have not detected an ortholog of Zfp488 in the zebrafish genome, its characteristic structure of two zinc finger motifs flanking a nuclear localization signal is shared with another protein, PR domain containing 8 (Prdm8), that is conserved among zebrasfish, mice and humans (Wang et al., 2006; Ross et al., 2012). Prdm8 has been shown to interact with Bhlhb5, a basic helix-loop-helix protein related to Olig2, to form a repressor complex in the context of neurogenesis (Ross et al., 2012). It will be of interest to examine the role of prdm8 and the possibility that Prdm8 and Znf16l
have overlapping functions in the control of oligodendrocyte specification, migration and myelination.

Future work is required to identify Znf16l target genes, but genes controlling OPC migration are among the likely candidates. Netrin-1 in the ventral CNS repels migrating OPCs, and in Netrin-1 mutants, OPCs remain near the CNS midline rather than dispersing throughout the CNS as they do in wild type (Tsai et al., 2006). The phenotypic similarity between Netrin-1 and znf16l mutants suggested that receptors for Netrin-1 or other midline repellants might be among the genes activated by Znf16l. In preliminary analyses, however, we observed normal expression of the Netrin receptor genes ddc and unc5a in znf16l mutants (data not shown), suggesting that that Znf16l promotes OPC migration by other mechanisms.

Concluding remarks

Precise control of different steps of OPC development is critical for the development, function and repair of the CNS, but the regulatory mechanisms controlling these processes are not well understood. We have identified Znf16l-like as a novel regulator of OPC specification, migration and myelination. Our in vivo rescue assays established that Znf16l-like shows functional overlap with its mammalian homolog, Zfp488. Comparison of defects in znf16l and notch3 mutants indicates a degree of redundancy in oligodendrocyte development. Many znf16l mutants survive to adulthood, providing an opportunity to test the role of this gene in response to CNS injury and myelin repair.

MATERIALS AND METHODS

Zebrafish lines and embryos

Experiments involving zebrafish were approved by the Stanford University Institutional Animal Care and Use Committee. Embryos from wild-type (TL, AB/TU and WIK), Tg(olig2:GFP) (Park et al., 2002), znf16lst78, znf16lst97, notch3st51 and notch3ZM (Zaucker et al., 2013) strains were raised at 28.5°C and staged as described by Kimmel et al. (1995). Embryos were treated with 0.003% 1-phenyl-2-thiourea (PTU) in Methylene Blue embryo water to inhibit pigmentation.

N-ethyl-N-nitrosourea mutagenesis, genetic screen and genetic mapping

Founder P0 wild-type males were mutagenized with N-ethyl-N-nitrosourea and subsequently crossed to raise F1 and F2 families for screening as described by Pogoda et al. (2006). An F3 genetic screen was conducted to identify putative mutants with defects in PNS or CNS myelination by whole-mount in situ hybridization at 5 dpf using a riboprobe for myelin basic protein (mbp; Pogoda et al., 2006). st78 mutant embryos were phenotypically sorted from wild-type siblings by a lack of mbp expression in the CNS. Bulk segregant analysis with 480 simple sequence length polymorphisms (SSLPs; Talbot and Schier, 1999) identified markers on LG7 flanking the st78 mutation. High-resolution mapping was conducted using additional SSLPs and single nucleotide polymorphisms linked to the mutation, which were found by sequencing PCR fragments amplified from genomic DNA of mutants and wild-type siblings.

Fig. 6. znf16l and notch3 mutants lack distinct populations of OPCs. In situ hybridization of znf16l, notch3 and olig2 embryos at 54 hpf for deltaD (A-C), notch3 (D-F), and olig2 (G-I) mRNA expression. (A-F) znf16l homozygous mutants do not exhibit decreased deltaD (A) or notch3 (D) expression seen in notch3 mutants (asterisks in B,C,E). (G-I) znf16l mutants exhibit a delay in lateral migration of OPCs (red brackets in G) that is not seen in either notch3 mutant (H,I). (G′-I′) Lateral view of the hindbrain shows that znf16l mutations are mostly lacking the ventral OPC population (white arrowheads), whereas notch3 mutants are mostly lacking the dorsal OPC population (black arrowheads). Genotypes of all fish analyzed were determined by PCR assay. Scale bars: 100 μm.
TALEN-mediated genomic deletion

Transcription activator-like effector nucleases (TALEN; Sanjana et al., 2012) were employed to generate the st97 allele. Transcription activator-like effector binding sites were identified in the znf16l genomic sequence using the TALE-NT 2.0 tool (Cermak et al., 2011; Doyle et al., 2013). pCS2+ vectors expressing TALENs targeting the following sequences were cloned:

5′-TCCGAGCTCGAGCCCGAC-3′ and 5′-TCCGTGATCTCGGTCACC-GA-3′. Expression vectors were linearized with SmaI and transcribed in vitro using the mMessage mMachine T7 Ultra Kit (Ambion). A mixture containing equal amounts of each mRNA (∼400 pg each) was injected into one-cell stage zebrafish embryos. On the next day, some surviving injected embryos were lysed and genotyped following the protocols described below to measure the efficiency of inducing genomic deletions. The remaining fish were raised to adulthood, and mosaic carriers were identified by assaying for transmission of genomic deletions to their progeny. Sequencing identified st97 as a 4 bp deletion that induces a frameshift in the open reading frame of znf16l.

Genotyping

To genotype the st78 lesion, PCR was conducted (primers: 5′-CAGAG-TCCATTCCCTTGTCCACAAT-3′ and 5′-CAGTTTGACACCTTGCTT-CTTG-3′), and the amplification products were cleaved with the restriction enzyme MluCl; the st78 mutation disrupts one of the two restriction sites in the PCR product, so that the mutant allele produces fragments of 170, 110 and 20 bp. To genotype the st97 allele, PCR was conducted (primers: 5′-TGGTGCTCTATGGTGTCTGTCT-3′ and 5′-GTGATGTTCCTGCCCAGATG-3′), and the amplification products were cleaved with PvuII; the 4 bp deletion in st97 removes the restriction site in the PCR product, so that the mutant allele results in an uncleaved, full-length PCR product.

Whole-mount RNA in situ hybridization

In situ hybridization on whole zebrafish embryos and larvae from 24 hpf to 9 dpf was performed using standard methods (Thisse and Thisse, 2008). Antisense riboprobes were transcribed from the following cDNAs cloned in the pCRII-Topo vector: mbp (Pogoda et al., 2006), claudin k (Münzel et al., 2012), nkx2.2a (Barth and Wilson, 1995), olig2 (Park et al., 2002), islet1 (Inoue et al., 1994), sox10 (Dutton et al., 2001), deltaD and notch3 (Zaucker et al., 2013). Imaging was performed using a Zeiss Stemi SV 11 Apo stereomicroscope using the 1.6× and 10× objectives and images were captured using the AxioCam Hrc and AxioVision imaging software. For differential interference contrast microscopy, a Zeiss Axio Imager M2 microscope was used using the 20× objective, and images were captured on an AxioCam Mrc with AxioVision imaging software.

Transmission electron microscopy

Transmission electron microscopy was performed as described by Lyons et al. (2009). Stained copper grids were imaged on a JEOL JEM-1400 transmission electron microscope.
Expression constructs
Full-length znf16l coding sequence (XM_694039) of 1587 bp with a Kozak sequence at the 5′ end was amplified from a 3 dpf embryonic cDNA pool (using the primers: 5′-GGCCGCACTGATGCGAACGAAAGGAA-3′ and 5′-TTACCGGATTCGACACTTCTG-5′) and directionally inserted into the pCR8-GW-TOPO vector (Invitrogen). Tissue-specific expression plasmids were made by LR recombination between this plasmid, pTO2, p3′E-polyA plasmid and the following tissue-specific promoter-containing p5′E plasmids: huC promoter (neurons; Shiao et al., 2013), mpeg1 (macrophages and microglia; Shiao et al., 2013) and claudin k (oligodendrocytes; Münzel et al., 2012). ssox10 promoter flanked by Tol2 sequences was previously described by Glenn and Talbot (2013). Briefly, znf16l with adapter sequences were amplified with the following primers: 5′-GTCGACCAGAAGCTTGGACGCCGCGCCACCAAGTCTGAGATCGAAGGGA-3′ and 5′-CATGTCGTTGATCTAGATCAGTGTCTCATACCGATTACGACACGTCTGACCATGC-3′ (upper case indicates homology to the ssox10 promoter vector and lower case indicates gene-specific sequence) and cloned into the ssox10-promoter vector with CloneEZ PCR-Cloning kit (GenScript). mZfp191 and mZfp488 were amplified from mouse cDNA (a gift from Natasha O’Brown, Kingsley Lab) using the following primers: mZfp191: 5′-GTCGCCGACCA-GAACGAGTGGACCGGCCGCCACCATGCTGCTGAGTAA-3′ and 5′-CA-GTGTCGGATCACTCATGATTCCCCACGCGAGTCTAAGGGA-3′ and mZfp488: 5′-GTCGACCGAAGCTTGGACGCCGCAGCTCGTCCGAGTACTCCAC-3′ and 5′-CATGTCGTTGATCTAGATCAGTGTCTCATACCGATTACGACACGTCTGACCATGC-3′. PCR fragments were cloned into ssox10-promoter vector described above with CloneEZ PCR-Cloning kit. Another neuronal expression construct was made by inserting a 3.1 kb fragment containing previously defined regulatory sequences from mnox1/hsb9 into the p5′E donor vector (Arkhipova et al., 2012). Transgenes were transiently expressed by co-injecting −12-25 pg of Tol2 constructs and −50-100 pg of Tol2 transposable mRNA at the one-cell stage. Uninjected embryos were raised in parallel as negative controls.

Time-lapse and fluorescent imaging
Live imaging was performed by anesthetizing embryos in 0.016% (w/v) tricaine and mounting them with the dorsal side up in 1.5% low melting-point agarose. Static fluorescent images were captured using a Zeiss LSM 5 Pascal confocal microscope with the Axioplan 2 imaging system under a 63X Plan-Apochromat 1.40 NA oil objective. Live imaging was performed by anesthetizing embryos in 0.016% (w/v) tricaine and mounting them with the dorsal side up in 1.5% low melting-point agarose.

Statistical analyses
Statistical analyses were performed using the Prism6 software (GraphPad). Student’s t-test was used for all comparisons between mutants and heterozygous siblings. Oligodendrocyte displacement and activity appeared to be normally distributed, and analysis was performed with one-way ANOVA with post hoc comparisons between individual means.

Acknowledgements
We are grateful to M. Barna and A. Villeneuve for sharing equipment. We thank C. E. Shiua for help with the genetic screen and expert advice; A. M. Meireles, K. Shen and D. Lysoy for helpful discussions; T. Reyes and C. Hill for maintaining the fish facility; and T. D. Glenn and J. Perrino for help with electron microscopy.

Competing interests
The authors declare no competing or financial interests.

Author contributions
H.S. identified and mapped the st78 mutation, generated st97 and conducted all experiments on znf16l and notch3 mutants. H.S. and W.S.T. analyzed the data and wrote the manuscript.

Funding
H.S. was supported by an ASTAR fellowship (Singapore). W.S.T. is a Catherine R. Kennedy and Daniel L. Grossman Fellow in Human Biology. This work was supported by the National Institutes of Health [R01NS050232] and the National Multiple Sclerosis Society [RG-4756-A-3 to W.S.T.]. Deposited in PMC for release after 12 months.

Supplementary information
Supplementary information available online at http://dev.biologists.org/lookup/suppl/doi:10.1242/dev.128215/-/DC1

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