The astrocytic MLC1 protein is differentially expressed during development in humans compared to mice

Margreet C. Ridder,¹
Marianna Bugiani,¹,²
Emiel Polder,¹
Nienke L. Postma,¹
Johannes C. Baayen,³
Vivi M. Heine,¹
Gert C. Scheper,¹
Marjo S. van der Knaap,¹

¹Department of Child Neurology, VU University Medical Center, Neuroscience Campus Amsterdam, 1081 HV Amsterdam, The Netherlands
²Department of Neuropathology, VU University Medical Center, Neuroscience Campus Amsterdam, 1081 HV Amsterdam, The Netherlands
³Department of Neurosurgery, VU University Medical Center, Neuroscience Campus Amsterdam, 1081HV Amsterdam, Netherlands

Submitted
Abstract

MLC1 mutations result in the white matter disorder megalencephalic leukoencephalopathy with subcortical cysts. An abnormally high white matter water content causes patients to develop macrocephaly within the first year of life. After several years slowly progressive, incapacitating cerebellar ataxia and spasticity evolve. The early and rapid disease development is followed by slow progression of symptoms with normalization of the head growth rate, which suggests that MLC1 plays its most important role early in life. We investigated whether MLC1 is more abundantly expressed during early life in both mice and humans. We generated transgenic mice with an eGFP reporter gene expressing both MLC1 and GFP. Immunohistochemical studies revealed abundant GFP-positive cells throughout the brain. GFP did not colocalize with the neuronal marker NeuN, the oligodendrocyte marker Olig2 or the endothelial marker CD31. The GFP positive cells expressed the astroglial markers GFAP and S100β and were identified as astrocytes, radial glia and ependymal cells. Immunohistochemistry, RT-PCR and immunoblotting indicated that GFP and MLC1 mRNA and protein levels increase into adulthood in MLC1-eGFP mice. In humans we found that MLC1 levels are higher in infants than in adults. In conclusion, the present study shows that MLC1 expression is limited to astrocytes and ependymocytes. The highest MLC1 levels in humans are seen early in life, but in mice the highest levels are present in adulthood. Considering the differential developmental expression of MLC1 in mice as compared to humans it is questionable whether the mouse is an appropriate animal to model the human disease MLC.

Introduction

Megalencephalic leukoencephalopathy with subcortical cysts (MLC, MIM 604004) is a genetic brain disorder with onset in infancy, first described in 1995. MLC patients develop rapidly progressive macrocephaly during the first year of life. After the first year, the head growth rate returns to normal. Only after several years, slowly progressive cerebellar ataxia and spasticity ensue. Most patients become wheelchair-dependent in their teens, but mental capacities remain relatively intact. MRIs of MLC patients show diffuse signal abnormality and swelling of the cerebral white matter from early on, with evidence of highly increased white matter water content. Over the years, the white matter swelling slowly decreases. Brain biopsies reveal that the increased water content is due to increased extracellular spaces and vacuoles within myelin sheaths and to a lesser degree in astrocytic endfeet around blood vessels.

Approximately 75 percent of MLC patients have autosomal recessive MLC1 mutations. MLC1 encodes an orphan plasma membrane protein expressed almost exclusively in the brain, enriched in astrocytic endfeet. Mutations in the MLC1 protein impair cell volume
regulation and reduce volume regulated anion channel activity in vitro \(^\text{11}\). The remaining approximately 25 percent of MLC patients have mutations in \textit{HEPACAM} \(^\text{12}\), which encodes the glial cell adhesion molecule GlialCAM \(^\text{13}\). GlialCAM mutations disrupt the localization of MLC1, whereas MLC1 mutations do not affect GlialCAM localization \(^\text{14}\), indicating that MLC1 is central in the pathophysiology of MLC.

In the years following the discovery of MLC1, multiple research groups have reported strong MLC1 expression in Bergmann glia as well as in perivascular, subependymal and subpial astrocytes in both humans and mice \(^\text{8,10,15,16}\). MLC1 expression has also been reported in human and mouse ependymal cells \(^\text{8,9,16}\), whereas the expression of MLC1 by neurons has only been reported in mice \(^\text{9,16}\). While creating a transgenic MLC1-eGFP knockout mouse as a model for MLC, we decided to use the heterozygous mice, which express both MLC1 and green fluorescent protein (GFP), to study which cell types express MLC1.

The early and rapid disease onset followed by stabilization and delayed slow deterioration suggests that MLC1 may have its most important role during the first year of life. This is the period of most intense myelination. Data obtained from mice provide evidence that MLC1 expression increases during development into adulthood, suggesting the most important role in the adult brain \(^\text{8,9}\). No developmental MLC1 expression study performed in humans has been published.

In our present study we used the heterozygous MLC1-eGFP mice to study MLC1 mRNA and protein expression levels during mouse brain development. We additionally studied human MLC1 mRNA and protein expression levels during development.

**Materials and Methods**

\textit{Generation of MLC1-eGFP transgenic mice.}

The C57BL/6-\textit{MLC1}\textsuperscript{flox}\textsubscript{eGFP} transgenic mice were generated by replacing exons two and three of \textit{MLC1} by the coding region of the enhanced GFP reporter gene, which was thus placed under control of the endogenous \textit{MLC1} promoter. The C57BL/6-\textit{MLC1}\textsuperscript{flox}\textsubscript{eGFP} mice were generated by Xenogen Biosciences Corporation, a Caliper company situated in Cranbury New Jersey in the USA, using homologous recombination in mouse embryonic stem cells and subsequent blastocyte injections of the appropriate targeted embryonic stem cells. Homozygous \textit{MLC1}\textsuperscript{eGFP/eGFP} mice express GFP instead of MLC1, whereas \textit{MLC1}\textsuperscript{eGFP/+} mice express both GFP and MLC1. All experiments were approved by the Animal Ethical Committee of the VU University Amsterdam, in accordance with Dutch and European laws.
Human brain tissue

Frozen human control brain tissue was obtained from the NICHD Brain and Tissue Bank for Developmental Disorders at the University of Maryland, Baltimore, MD. The brain tissue specimens were obtained from patients without neurological disease or confounding neuropathologic abnormalities. Brain tissue of a total of 16 patients with an age range between one day and 30 years was used.

Immunohistochemistry

Postnatal and adult mice were anaesthetized and perfusion-fixated with 4% paraformaldehyde (PFA) in phosphate buffered saline (PBS). For studies on prenatal mouse brain, embryos were removed from the mother after she was perfusion-fixated with 4% PFA in PBS. Brains were dissected out for 16-day (E16) embryonic mice and older. All samples were post-fixed in the same fixative solution for 48 hours at 4°C, followed by cryoprotection with 30% sucrose in PBS. Samples were immersed in Optimal Cutting Temperature (OCT) solution and frozen with dry ice-cooled isopentane and stored at -80°C. Samples were cut with a cryostat (8 µM thick) along the sagittal plane and collected on microscope slides. Slides were fixed with methanol for 30 min and subsequently permeabilized with 1% goat serum, 0.9% NaCl and 0.1% Saponin in PBS for 10 min at room temperature and blocked in 5% normal goat serum, 0.9% NaCl and 0.05% Saponin in PBS for 30-60 minutes. Primary antibodies (listed in Table 1) were diluted in 1% goat serum, 0.9% NaCl and 0.01% Saponin in PBS and slides were incubated over-night at 4°C. After staining with the primary antibody slides were washed and incubated for 1 hour at room temperature with the appropriate secondary Alexa antibodies (Alexa488-. 568- and 594-tagged antimouse, antirabbit, and antichicken IgG; 1:400; Molecular probes, Invitrogen, Breda, the Netherlands). After completion of the staining, microscope slides were mounted in vectashield medium with 1.5 pg/ml 4’-6-Diamidino-2-phenylindole (DAPI) (Vector Laboratories Burlingame, CA, USA) and photographed using an DMI6000 B inverted DIC and fluorescent microscope (Leica Microsystems BV, Rijswijk, The Netherlands)
Table 1. Primary antibodies

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Species</th>
<th>Immonogen &amp; Supplier</th>
<th>WB dilution</th>
<th>IHC dilution</th>
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<tr>
<td>Anti-GFP</td>
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<td>Anti-GFP</td>
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<td>GFP tagged fusion protein Sigma, St. Louis, MO, USA</td>
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<td>Anti-MLC1</td>
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<td>Mouse MLC1 synthetic peptide Prof. R. Estévez (Teijido et al. 2004)</td>
<td>1:10</td>
<td>1:100</td>
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<tr>
<td>Anti-MLC1</td>
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<td>Human MLC1 synthetic peptide Prof. R. Estévez (Duarri et al. 2008)</td>
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<td>1:100</td>
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<td>Anti-GialCAM</td>
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<td>1:200</td>
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<td>Anti-GFAP</td>
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<td>Anti-NeuN</td>
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<td>Anti-CD31</td>
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<td>Polyoma T transformed EC line tEnd.1 BioLegend, San Diego, CA, USA</td>
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RNA isolation, Reverse Transcription, and Real-time Quantitative Polymerase Chain Reaction

Total RNA was extracted from whole mice brain and human frontal white matter samples using TRIzol (Invitrogen, San Diego, CA, USA). Subsequent reverse transcription to complementary DNA (cDNA) was performed with SuperScript III Reverse Transcriptase (Invitrogen, San Diego, CA, USA), and PCR was carried out on cDNA with Platinum Taq according to the manufacturer’s protocol (Invitrogen, San Diego, CA, USA). Quantitative RT-PCR was performed using a volume of 10 µl containing SYBR Green PCR mix (Roche Diagnostics, Rotkreuz, Switzerland), 3.0 µmol/L primers, and 0.03 µg of cDNA on a LightCycler 480 II (Roche Diagnostics, Rotkreuz, Switzerland). Transcript-specific primers (Table 2) were generated with Primer Express software (Applied Biosystems, Foster City, CA) and designed to overlap exon-exon boundaries to prevent genomic DNA amplification. The relative abundance of transcript expression in mice samples was calculated using the cycle of threshold value and normalized to the endogenous controls ribosomal protein S14 (RPS14) and cyclophilin B (Cyp B). The relative abundance of transcript expression in the human samples was normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and Cyp
B. In addition, relative mRNA expression levels were calculated using mRNA level of the 7-month-old MLC1-eGFP mice or adult human samples as 100%.

### Table 2. Primers

<table>
<thead>
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<th>Mouse targets</th>
<th>Forward sequences</th>
<th>Reverse sequences</th>
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<td>mRPS14</td>
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<td>ATCTTCTCCACAGAGAGAGC</td>
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<td>MLC1</td>
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<td>GFP</td>
<td>ACGACAGAGAGCGCCA</td>
<td>CGCTGAACCTTGTCGGTT</td>
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<tr>
<td>GlialCAM</td>
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<td>CAACCTCCTCTCCTTAGAC</td>
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<table>
<thead>
<tr>
<th>Human targets</th>
<th>Forward sequences</th>
<th>Reverse sequences</th>
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<tbody>
<tr>
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<tr>
<td>hGAPDH</td>
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<td>TGAGCGATGTGGCGGT</td>
</tr>
<tr>
<td>MLC1</td>
<td>AGAAGGGCTCCATGTCTGACA</td>
<td>AAGATTCCAGGCCGAGGAGC</td>
</tr>
<tr>
<td>GlialCAM</td>
<td>CGTATACAGAAGATGCCTCCT</td>
<td>GTCACCAAGGTCACAAGGA</td>
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**SDS-Page and Western blotting**

Mouse whole brain and human frontal white matter tissue samples were homogenized in 5 volumes of lysis buffer containing 20 mM Hepes (pH7.0), 1 mM EDTA, 1 mM DTT, an EDTA-free protease inhibitor cocktail (Roche Diagnostics, Rotkreuz, Switzerland), 120 mM NaCl and 0.5% Triton X100. Protein content of brain tissue samples was determined by the Bradford assay and diluted in a lithium dodecyl sulfate (LDS) sample buffer (Invitrogen, San Diego, CA, USA) to a final concentration of 5mg/ml protein. Samples were run on 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) gels and transferred onto polyvinylidene difluoride (PVDF) membranes (Millipore, Amsterdam, The Netherlands). Membranes were blocked in PBS containing 0.1% Tween-20 (PBS-T) and 5% milk. Proteins of interest were detected by incubating the membranes with the primary antibodies (see: Materials and Methods “antibody characterization”) in PBS-T containing 0.5% milk for either 3 hours at room temperature or overnight at 4°C. After washing with PBS-T, the appropriate alkaline phosphatase-conjugated secondary antibodies (1:5000, Sigma St. Louis, MO, USA) were added for 1 hour and the immunoreactive bands were detected using electrochemical fluorination (ECF) substrate (Amersham, Buckinghamshire, UK) on a fluorescent image analyzer (FLA-5000 image reader, Fujifilm, Tokyo, Japan). The GAPDH housekeeping gene was used to control equal protein loading control of human samples. Equal protein loading and equal sample transfers were achieved for mouse samples by adding 0.5% 2,2,2 trichlorethanol to all SDS-PAGE gels \(^7\), which were then visualized using a gel imaging system (DOC EZ imager, Biorad, Hercules, CA, USA). Protein expression levels were corrected for the total amount of loaded protein and relative protein expression was calculated
by using protein level of four-month-old $MLC1^{eGFP/+}$ mice as 100%. Western blots were quantified and corrected for total amount of protein using the ImageJ software.

**Antibody characterization**

Information about the primary antibodies used for Western blotting (WB) or Immunohistochemistry (IHC) or both is given in Table 1. The primary antibodies used for Western blotting revealed bands of the expected size. Omission of the primary or secondary antibody during immunohistochemical staining resulted in the absence of labeling. The characterization of different cell types was achieved with commercial primary antibodies that stained the appropriate pattern of cellular morphology. The antibodies against MLC1 were a gift from Professor R. Estévez (University of Barcelona) and have previously been characterized 9,18.

**Results**

*MLC1 and GFP expression in adult transgenic $MLC1^{eGFP/+}$ mice.*

We generated MLC1 transgenic mice expressing GFP under the endogenous MLC1 promoter. $MLC1^{eGFP/+}$ mice express both the MLC1 membrane protein and the cytosolic GFP protein. We found strong GFP and MLC1 expression in the cerebellum, and at the subpial and subventricular zones of these mice (Fig. 1A-C). In the $MLC1^{eGFP/eGFP}$ mice we found GFP expression but no MLC1 expression (Fig. 1D), whereas we found MLC1 expression in the $MLC1^{+/+}$ mice, but no GFP (Fig. 1E).
Figure 1. Adult MLC1\textsuperscript{eGFP/+} mice express both MLC1 and GFP. GFP and MLC1 expression patterns in various brain regions of 4-month-old mice. Immunofluorescent images of MLC1\textsuperscript{eGFP/+} mice show GFP expression (green) and MLC1 expression (red) in the cerebellum (A-A'), subventricular zone (B-B') and cerebral cortex (C-C'). Note the enriched GFP expression in the subpial and perivascular astrocytes. MLC1\textsuperscript{eGFP/eGFP} mice show abundant GFP (green) expression but no MLC1 expression (red) (D-D'). In contrast, MLC1 (red) expression but no GFP (green) expression was observed in the MLC1\textsuperscript{+/+} mice (E-E'). In all panels GFP visualization was enhanced using monoclonal anti-GFP antibodies coupled to an Alexa 488 secondary (green) and nuclei were stained with 4',6-diamidino-2-phenylindole (DAPI blue). Scale bars are indicated in the bottom right corner of all panels and correspond to 50 µM in panels A and B and to 25 µM in panels C to F.

In the 4-month-old, adult MLC1\textsuperscript{eGFP/+} mice we found abundant GFP positive cells throughout the brain, including the cerebellum, cerebral cortex, olfactory bulb, brainstem, hippocampus and subventricular zone (Fig. 2).

To determine which cell types express MLC1 we stained brain slices of the 4-month-old MLC1\textsuperscript{eGFP/+} mice for specific neuronal, oligodendrocytic, endothelial and astroglial cell markers. When stained with the neuron-specific nuclear protein antibody NeuN, we found high GFP and NeuN expression in the cortex. However, we did not observe any colocalization of NeuN positive cells with GFP positive cells (Fig. 3A).
Figure 2. Adult MLC1<sup>eGFP/+</sup> mice show abundant GFP positive cells throughout the brain. GFP expression patterns in various brain regions of a 4-month-old MLC1<sup>eGFP/+</sup> mouse. Immunofluorescence images show GFP expression in the cerebellar cortex and white matter cerebellum (A), cerebral cortex (B), olfactory bulb (C), brainstem (D), hippocampus (E) and subventricular zone (F). In all panels GFP visualization was enhanced using monoclonal anti-GFP antibodies coupled to an Alexa 488 secondary antibody (green) and nuclei were stained with DAPI (blue). Scale bars correspond to 100 µM and are indicated in the bottom right corner of all panels.

No colocalization of GFP and NeuN was found anywhere in the adult mouse brain, indicating that GFP positive cells are not neurons. When we immunostained with the oligodendrocyte-specific marker oligodendrocyte transcription factor 2 (Olig2) and the endothelial marker CD31, also known as Platelet Endothelial Cell Adhesion Molecule-1 (PECAM-1), we also did not observe any colocalization between in GFP-positive cells and Olig2 positive cells or CD31 positive cells (Fig. 3B and C), indicating that GFP positive cells are not oligodendrocytes or endothelial cells.
Figure 3. GFP-positive cells are not neurons, oligodendrocytes or endothelial cells. Brain slices from 4-month-old \textit{MLC1}^{eGFP/+} mice were stained with neuronal marker NeuN, oligodendrocyte marker Olig2 and endothelial cell marker CD31. Double immunofluorescence stainings show no colocalization of GFP fluorescence (green) with NeuN (red) positive cortical neurons (A-A”), with Olig2 (red) positive cerebellar white matter oligodendrocytes (B-B”) and CD31 (red) positive cortical endothelial cells (C-C”). Note the enriched GFP expression in the perivascular cells. In all panels GFP visualization was enhanced using monoclonal anti-GFP antibodies coupled to an Alexa 488 secondary antibody (green) and nuclei were stained with DAPI (blue). Scale bars correspond to 25 µM and are indicated in the bottom right corner of all panels.

In contrast, in slides immunostained with the astroglial marker GFAP we found high colocalization between GFP-positive cells and GFAP positive cells (Fig. 4). In the brainstem white matter (Fig. 4A), as well as in the corpus callosum, internal capsule, anterior commissure and cerebellar white matter (data not shown), we found that GFP/GFAP double-positive cells had the morphology of fibrous astrocytes. In the cortex we found less GFAP expressing cells and thus fewer cells that were both GFP and GFAP positive. Here the cells expressing both GFP and GFAP had the morphology of protoplasmic astrocytes (Fig. 4B). An even more mature astroglial cell marker is S100β.\textsuperscript{19} In slides immunostained with the astroglial cell marker S100β we found a high degree of colocalization of GFP and S100β-positive cells throughout the brain, including Bergmann glia in the cerebellum (Fig. 4C) and ependymal cells lining the third ventricle (Fig. 4D).
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Figure 4. GFP-positive cells express astroglial markers and are astrocytes, radial glia and ependymal cells. Brain slices from 4-month-old MLC1<sup>eGFP/+</sup> mice were stained with astroglia markers GFAP and S100β. Double immunofluorescence stainings show colocalization of GFP (green) and GFAP (red) positive fibrous astrocytes in the brainstem (A-A") and GFAP positive (red) protoplasmic astrocytes in the cerebral cortex (B-B"). Immunofluorescent colocalization is also found between GFP and S100β positive Bergman glia in the cerebellar cortex (C-C") and GFP and S100β positive ependymal cells lining the third ventricle and neighboring astrocytes (D-D"). In all panels GFP visualization was enhanced using monoclonal anti-GFP antibodies coupled to an Alexa 488 secondary (green) and nuclei were stained with DAPI (blue). Scale bars correspond to 25 µM and are indicated in the bottom right corner of all panels.

**Developmental GFP expression in transgenic MLC1<sup>eGFP/+</sup> mice**

To assess whether MLC1 expression levels change during development, we investigated the immunohistochemical GFP expression in tissue sections of transgenic MLC1<sup>eGFP/+</sup> mice from
embryonic day 18 (E18) through 4 months of age. We found increasing GFP expression in the cortex with advancing age (Fig. 5A-F). We found that subventricular astrocytes and ependymal cells were one of the first cells to become GFP positive (Fig. 5G-L).

**Figure 5. GFP expression increases during development in $MLC_1^{eGFP^+}$ mice.** Immunofluorescence images showing GFP expression in the cortex of E18 (A), P2 (B), P7 (C), P14 (D), P21 (E) and 4-month-old (F) $MLC_1^{eGFP^+}$ mice. Immunofluorescence images showing GFP expression in the subventricular zone of E18 (G), P2 (H), P7 (I), P14 (J), P21 (K) and 4-month-old (L) $MLC_1^{eGFP^+}$ mice. In all panels GFP visualization was enhanced using monoclonal anti-GFP antibodies coupled to an Alexa 488 secondary antibody (green) and nuclei were stained with DAPI (blue). Scale bars are shown in the bottom right corner of all panels. Scale bars are indicated in the bottom right corner of all panels and correspond to 50 µM in panels A through F and to 100 µM panels in G and L.
Additionally, we studied GFP mRNA and protein levels in the brains of $MLC1^{eGFP/+}$ mice from embryonic stage E13 into adulthood and found that relative GFP and MLC1 mRNA levels increase similarly over time (Fig. 6A). In young mice (P7) we found approximately half the amount of GFP and MLC1 mRNA of adult mice. GFP protein was detected in prenatal ages in E16 and older mice (Fig. 6B). We found that GlialCAM mRNA and protein levels also increase during development (Fig. 6A and 6C-H). Like MLC1, we found GlialCAM to be noticeably expressed at the glia limitans (Fig. 6C-E) and around the ventricles (Fig. 6F-H) with the highest expression in adult mice (Fig. 6 E and H).

Figure 6. GFP mRNA and protein levels increase during development into adulthood in $MLC1^{eGFP/+}$ mice. Immunofluorescence stainings, quantitative RT-PCR and western blot of mouse whole brain at different developmental stages. Relative mRNA expression levels were calculated using mRNA level of the 7-month-old $MLC1^{eGFP/+}$ mice as 100%. Relative expression of GFP (green bars), MLC1 (black bars) and GlialCAM (blue bars) mRNA in $MLC1^{eGFP/+}$ mice at ages E16, E13, E18, P2, P7, P14, P21, 4 months and 7 months (A). Western blot showing GFP (+/- 28 kDA band in top panel) protein expression levels in $MLC1^{eGFP/+}$ mice at ages; E13, E16, E18, P2, P7, P14, P21 and 4 months. Green bars show relative GFP protein expression (B). Immunofluorescence images showing GlialCAM expression in the cortex of P2 (C), P14 (D), and 4-month-old (E) $MLC1^{eGFP/+}$ mice and
GlialCAM expression in the subventricular zone of P2 (E), P14 (F), and 4-month-old (G) MLC1\(^{eGFP/+}\) mice. Scale bars are displayed in the bottom right corner of each panel and correspond to 50 µM in panels C through E and to 100 µM in panels F and G.

**MLC1 and GlialCAM mRNA and protein expression levels in humans**

To investigate whether the MLC1 and GlialCAM proteins are developmentally regulated also in the human brain, we performed mRNA and protein expression studies in white matter tissue samples obtained from healthy subjects aged 1 day to 30 years. We found that, unlike in mice, human MLC1 mRNA and protein levels are highest in the first two years of life and then decrease during adulthood (Fig. 7A, C and D). By contrast, in humans as in mice, the GlialCAM transcripts and protein levels progressively increase after birth into adulthood (7B-D).

![Figure 7](image)

**Discussion**

We have previously shown that *MLC1* mutations cause the disease in the majority of MLC patients \(^7,20\). The MLC1 protein is located mainly in the brain and was reported to be...
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abundantly expressed in astrocytes that contact the blood-brain and cerebrospinal fluid-brain barriers in both humans and mice. Expression in ependymal cells has also been reported in human subjects and mice, whereas the expression of MLC1 in neurons was only reported in mice (Teijido et al., 2007; Teijido et al., 2004).

In the present study, we have investigated which cell types express MLC1 using transgenic mice with an eGFP reporter gene under control of the MLC1 promotor. We demonstrate that GFP-positive cells in heterozygous mutant mice are astrocytes, Bergman glia and ependymal cells, indicating that in mice MLC1 is predominantly expressed by these cells. These results are in accordance with the in situ hybridization results of Schmitt et al. 2003. Additionally we stained for neuronal marker NeuN, oligodendrocytic cell marker Olig2 and endothelial cell marker CD31. We did not find any colocalization of GFP-positive cells with the oligodendrocytic cell marker Olig2 or the endothelial cell marker CD31. In contrast to the previously reported expression of MLC1 in neurons, we did not find any immunoreactivity for the neuronal marker NeuN with GFP positive cells The lack of colocalization of GFP with NeuN could be due to expression level differences. It is therefore not excluded that neurons express MLC1 at low levels that do not enable GFP detection in adult MLC1-eGFP mice. These findings in mice are in complete agreement with the findings in humans.

MLC patients are normal at birth and develop a rapidly progressive macrocephaly during the first year of life, followed by stabilization. Early MRI studies reveal abnormal and swollen cerebral white matter with highly increased water content. Over time the white matter swelling decreases. Electron microscopy of brain tissue from a MLC patient demonstrates that fluid-filled vacuoles within myelin sheaths and, to a lesser extent, astrocytic endfeet (Duarri et al., 2011a) form the anatomic substrate of the white matter edema. Cerebral white matter contains little myelin at birth and acquires most of its myelin during the first year of life. Thus, the macrocephaly and neuroradiological characteristics of MLC develop during a period of rapid myelin deposition, suggesting that MLC1 might play a pivotal role during early development and less so later in life. In this study we confirm that MLC1 levels in humans are highest in early life and lower later in life. These findings are in striking contrast with those in mice, in which MLC1 expression increases during development with highest levels in adult mice.

In conclusion, our study indicates that MLC1 is expressed by the same cells in humans and mice, but that the developmental expression is strikingly different. The difference in MLC1 levels in humans and mice points to a difference in human and mouse physiology, which may also be important in MLC disease development in human and mice. Unfortunately, the mouse might therefore not be the ideal animal to model the disease.
Acknowledgements

Human tissue was obtained from the NICHD Brain and Tissue Bank for Developmental Disorders at the University of Maryland, Baltimore, MD. The role of the NICHD Brain and Tissue Bank is to distribute tissue and does not influence the studies performed or the interpretation of results. We thank professor Raúl Estévez from the University of Barcelona for the MLC1 primary antibodies used in this study.

References