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Research Paper

Progressive leukoencephalopathy impairs neurobehavioral development in sialin-deficient mice

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ABSTRACT

Slc17a5$^{-/-}$ mice represent an animal model for the infantile form of sialic acid storage disease (SASD). We analyzed genetic and histological time-course expression of myelin and oligodendrocyte (OL) lineage markers in different parts of the CNS, and related this to postnatal neurobehavioral development in these mice. Sialin-deficient mice display a distinct spatiotemporal pattern of sialic acid storage, CNS hypomyelination and leukoencephalopathy. Whereas few genes are differentially expressed in the perinatal stage (p0), microarray analysis revealed increased differential gene expression in later postnatal stages (p10–p18). This included progressive upregulation of neuroinflammatory genes, as well as continuous down-regulation of genes that encode myelin constituents and typical OL lineage markers. Age-related histopathological analysis indicates that initial myelination occurs normally in hindbrain regions, but progression to more frontal areas is affected in *Slc17a5*$^{-/-}$ mice. This course of progressive leukoencephalopathy and CNS hypomyelination delays neurobehavioral development in sialin-deficient mice. *Slc17a5*$^{-/-}$ mice successfully achieve early neurobehavioral milestones, but exhibit progressive delay of later-stage sensory and motor milestones. The present findings may contribute to further understanding of the processes of CNS myelination as well as help to develop therapeutic strategies for SASD and other myelination disorders.

1. Introduction

Sialic acid storage disease (SASD) is an autosomal recessive lysosomal storage disorder caused by deficiency of the lysosomal membrane transporter sialin, and ensuing intralysosomal accumulation of free sialic acid and other acidic monosaccharides. Sialin is encoded by the *SLC17A5* gene, mutations of which give rise to SASD (Mancini et al., 1989; Mancini et al., 1991; Havelaar et al., 1998; Verheijen et al., 1999). Salla disease (OMIM #604369) and infantile sialic acid storage disease (ISSD; OMIM #269920) represent 2 major clinical phenotypes

Abbreviations: (4V), 4th ventricle; (AR), air righting; (aca), anterior commissure; (alv), alveus of the hippocampus; (av), arbor vitae cerebellum; (AS), auditory startle; (CA), cliff aversion; (CNS), central nervous system; (CC), corpus callosum; (cg), cingulum; (Cnp1), cyclic nucleotide phosphodiesterase 1; (dc), dorsal cochlear nucleus; (dg), dentate gyrus; (ET), ear twitch; (ec), external capsule; (fi), fimbria; (fl), flocculus; (FG), forelimb grasping; (Galc), galactosylceramidase; (HPS), hematoxylin phloxin saffrane; (icp), inferior cerebellar peduncle; (ISSD), infantile sialic acid storage disorder; (LD), locomotion development; (LAMP-1), lysosomal associated membrane protein, isoform 1; (Mpeg1), macrophage expressed gene 1; (mt), mammilothalamic tract; (MEF), mouse embryonic fibroblast; (MBP), myelin basic protein; (Mag), myelin-associated glycoprotein; (Mopb), myelin-associated oligodendrocytic basic protein; (Myrf), myelin regulatory factor; (NG), negative geotaxis; (Cspg4), NG2 chondroitin sulphate proteoglycan; (OL), oligodendrocyte; (OP), oligodendrocyte progenitors; (Omg), oligodendrocyte-myelin glycoprotein; (PNS), peripheral nervous system; (Pdgfra), platelet-derived growth factor-α; (PSA-NCAM), polysialylated neural cell adhesion molecule; (Plp1), proteolipid protein 1; (py), pyramidal tract; (RLV), rat liver lysosomal vesicles; (SASD), sialic acid storage disease; (SAM), significance analysis of microarray; (sp5), spinal trigeminal tract; (SVZ), subventricular zone; (SR), surface righting; (vcp), ventral cerebellar peduncle.

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of SASD at either end of the disease spectrum. Children suffering from ISSD present with severe neurodevelopmental defects that rapidly deteriorate, leading to early death. Salla disease does involve psychomotor and cognitive disability, but patients experience a slower deterioration than ISSD patients and survive into adulthood (Haataja et al., 1994).

Myelination defects occur in clinical SASD as well as in mice with sialin deficiency (Prolo et al., 2009), but little is known about their progression and pathological concomitants. SASD leads to progressive cerebellar atrophy as well as decreased cerebral white matter volume, which have been suggested to be caused by hypomyelination (i.e., insufficient myelin deposition and/or maturation), rather than myelin breakdown (Haataja et al., 1994; Sonninen et al., 1999; Varho et al., 2000; Parazzini et al., 2003; Morse et al., 2005; Steenweg et al., 2010). The process of myelination comprises a sequence of intricate developmental events that start by oligodendrocyte progenitors (OPs) proliferating in the ventral part of the embryonic neural tube (Baumann and Pham-Dinh, 2001; Miller, 2002; Miller, 2005; Kessaris et al., 2006; Bercury and Macklin, 2015). These OPs subsequently migrate dorsally and rostrally throughout the forebrain and spinal cord, contact axons, and differentiate into myelin-forming oligodendrocytes (OLs). The OL lineage is generally divided into four stages: early OPs, preoligodendrocytes or late OPs, immature OL and mature OL. Craig et al. (2003) studied the analogy of human and rodent OL lineage progression. Their quantification of these events in rodents revealed that cerebral white matter mainly contains late OPs at postnatal day 2 (p2), whereas immature OLs remain a minority (Craig et al., 2003). Later (around p7), immature OLs become the majority and initiate myelination.

Sialin-deficient mice were generated to study the pathophysiological mechanisms of clinical SASD (Prolo et al., 2009; Moechars et al., 2005). Sialin deficiency was indeed shown to cause hypomyelination in these mice, which was attributed to defective OL lineage maturation, leading to cell death and functional defects. Clinical SASD studies showed that functional capacities of patients were related to their levels of residual sialin activity (Wreden et al., 2005; Ruivo et al., 2008). In the present report, we further detailed the various histopathological features of postnatal progressive leukoencephalopathy in sialin-deficient mice, and assessed their functional consequences. Genetic and histological time-course expression of myelin, OL lineage and other pathogenically relevant markers was examined during postnatal brain development. Finally, we identified milestones of neurobehavioral development in mouse pups to document potential developmental disabilities caused by their sialin deficiency. Our results provide new insights on the pathophysiological mechanisms of SASD hypomyelination and could contribute to therapeutic strategies in these and more common myelination disorders.

2. Results

2.1. *Slc17a5*$^{-/-}$ mice show a progressive disease course and premature mortality

The *Slc17a5*$^{-/-}$ mice were first presented in 2005 (Moechars et al., 2005) and have been previously characterized (Prolo et al., 2009). Our qualitative observations of these mice have been consistent with their published phenotype i.e. they experience growth delays, making them smaller than equivalently aged controls, and they had a severely reduced lifespan.

2.2. *Slc17a5*$^{-/-}$ mice show free sialic acid accumulation and defective lysosomal transport

SASD results from defective clearance of free sialic acid out of the lysosome via the lysosomal transport protein sialin. To determine the level to which *Slc17a5*$^{-/-}$ mice mimic biochemical characteristics of clinical SASD, the amount of free sialic acid in embryonic fibroblasts of *Slc17a5*$^{+/+}$, *Slc17a5*$^{+/-}$ and *Slc17a5*$^{-/-}$ mice was first compared with diagnostic values of cultured fibroblasts of all clinical variants of SASD patients (ISSD, intermediate SASD and Salla disease; Fig. 1A). *Slc17a5*$^{-/-}$ mice showed an accumulation of free sialic acid (66 nmol/mg protein) in fibroblast homogenates, exceeding the most severe clinical phenotype ISSD (30 nmol/mg protein).

Next, we confirmed that these elevated levels could indeed be linked to defective sialin-mediated lysosomal clearance. Proton-gradient mediated transport, featuring tritiated glucuronic acid as a sialin substrate, was assessed using purified lysosomal membrane vesicles from livers of rat (RLV), *Slc17a5*$^{+/+}$, *Slc17a5*$^{+/-}$ and *Slc17a5*$^{-/-}$ mice. Increased

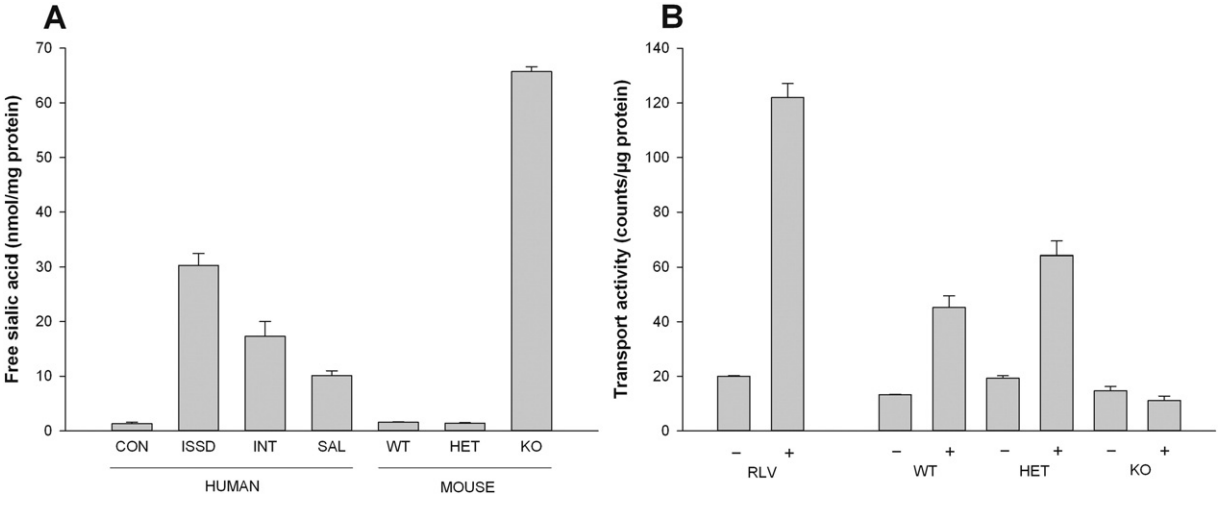

Fig. 1. Biochemical characteristics of sialin-deficient mice. (A) Concentration of free sialic acid in cultured fibroblasts of *Slc17a5*$^{+/+}$ mouse (WT), *Slc17a5*$^{+/-}$ mouse (HET) and *Slc17a5*$^{-/-}$ mouse (KO). For comparison free sialic acid was also measured in human skin fibroblasts from controls (CON) and the three clinical variants of SASD, infantile (ISSD), intermediate (INT) and Salla (SAL) type. (B) Transport activity of sialin in purified lysosomal membrane vesicles of mouse livers. Uptake of radiolabeled glucuronic acid in WT, HET and KO mice was evaluated. Activity was measured in the presence (+) and in the absence (−) of a proton gradient. As reference, activity was also measured in rat liver vesicles (RLV). Data are presented as mean + SEM.