



● INVITED REVIEW

Heterozygous carriers of galactocerebrosidase mutations that cause Krabbe disease have impaired microglial function and defective repair of myelin damage

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Funding: This research we conducted on twitcher $GALC^{+/-}$ heterozygotes was supported by the following funding sources: National Institutes of Health, F31-NS078911, <https://www.nih.gov> (NSH); New York State Department of Health, NYS-DOH-C026877, <http://www.stemcell.ny.gov> (NSH); New York State Department of Health, NYS-DOH-C029557, <http://www.stemcell.ny.gov> (MN); New York State Department of Health, NYS-DOH-C026877, <http://www.stemcell.ny.gov> (CJF); Hunter's Hope, <http://www.huntershope.org/site/PageServer> (MN); Children's Neurobiological Solutions Foundation, <http://pediatricbrainfoundation.org> (MN); and the Legacy of Angels, <http://tloaf.org> (MN).

Abstract

This review addresses two puzzling findings related to mutations in galactocerebrosidase (*GALC*) that cause Krabbe disease (KD), a severe lysosomal storage disorder characterized by extensive myelin damage in children with mutations in both *GALC* alleles. First, heterozygous carriers of KD-causing mutations, which include the biological parents of children with KD, exhibit increased risk for developing other diseases. Second, variants in the *GALC* locus increase the risk of developing multiple sclerosis (MS), another disease characterized by extensive myelin damage. What explains these correlations? In studies on cuprizone-induced myelin damage in heterozygous ($GALC^{+/-}$) mice carrying one copy of a mutation that causes KD-like disease, the extent of damage was similar in $GALC^{+/-}$ and wild-type (WT) mice. In contrast, $GALC^{+/-}$ mice had striking defects in repair of cuprizone-induced damage. We further found unexpected microglial defects in myelin debris clearance and in the ability to up-regulate the Trem2 microglial protein critical for debris uptake. These defects were rescued by exposure to a lysosomal re-acidifying drug discovered in our studies on KD, and which provides multiple clinically relevant benefits in the twitcher ($GALC^{+/-}$) mouse model of KD. Thus, heterozygous *GALC* mutations cause effects on biological function that may help to understand the increased disease risk in heterozygous carriers of such mutations and to understand why *GALC* variations increase the risk of MS. Our findings indicate that while some genetic risk factors may contribute to complex diseases by increasing the risk of tissue damage, others may do so by compromising tissue repair.

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doi: 10.4103/1673-5374.228712

Accepted: 2018-02-23

Key Words: vulnerability locus; multiple sclerosis; Krabbe disease; galactocerebrosidase; microglia; myelin repair; lysosomal storage disorders; heterozygous carriers; Trem2; demyelination

Introduction

Although it is widely recognized that being a heterozygous carrier of mutations in a wide range of genes can increase the likelihood of developing a variety of different diseases, our understanding of the biological foundations underlying such increased vulnerability is greatly lacking.

One example of this problem is seen in families of children with Krabbe disease (KD), a severe lysosomal storage disorder (LSD) caused by mutations in the enzyme galactocerebrosidase (*GALC*). Children with KD, who carry mutations in each copy of the *GALC* gene (Wenger et al., 1974, 1997, 2000), develop a severe neurodegenerative disease characterized by extensive damage to myelin (the lipid-rich insulating material critical for neuronal conduction in both the central nervous system (CNS) and the peripheral nervous system (PNS)). In severe forms of KD, nervous system damage is so extensive as to typically cause death within 2–3 years after birth (Suzuki, 2003).

Although it has long been assumed that heterozygous carriers of mutations able to cause KD (which include all parents of children with KD) are biologically normal, it is increasingly apparent that this is not correct. Although this research is in

its early stages, it is already clear that carriers of mutations that cause KD are at increased risk for development of open angle glaucoma (Liu et al., 2011) and also for pulmonary artery enlargement in association with chronic obstructive pulmonary disease (Lee et al., 2015). Such mutations also have been suggested to be of possible interest in late-onset synucleinopathies (Marshall and Bongarzone, 2016).

A similar, and more extensively studied, situation is also seen in the case of Gaucher disease (GD, another severe LSD). Heterozygosity for variants in glucocerebrosidase (*GBA*, the enzyme mutated in GD) is one of the strongest genetic risk factors for Parkinson's disease (PD), and *GBA* mutations are found with even more frequency in the PD population than mutations in genes associated with familial forms of this disease (e.g., Neumann et al., 2009; Schapira, 2015; O'Regan et al., 2017). Moreover, PD patients with *GBA* variants tend to have earlier onset of disease symptoms and more severe cognitive decline when compared to PD patients that do not harbor *GBA* variants. While the genetic link between *GBA* and PD is well established, the underlying biological mechanisms are only beginning to be understood.

The increased disease vulnerability for heterozygous

carriers of mutations that cause KD or GD affects the parents and families of afflicted children, of course, but is also more broadly relevant: Although the frequency of KD and GD is very low (1:80,000–100,000), the gene frequency for heterozygous carriers of disease-causing mutations is high enough (1:125–150) (Wenger, 1993) as to represent a significant proportion of the population. Just these two lysosomal storage disorders alone provide a predicted incidence of disease-relevant mutations in the population of ~1:60 to 1:75. Moreover, there are over 40 different lysosomal storage disorders for which information on associated disease risks has not yet been determined but which also may confer similar vulnerabilities.

One example of the potential relevance of variation in genes that cause KD to the general population comes from identification of genetic loci that increase the risk of developing multiple sclerosis (MS). MS is the most common autoimmune disorder affecting the CNS, and is associated with widespread damage to myelin and eventual failure to repair such damage, with multiple neurological sequelae (Sawcer et al., 2011, 2014; George et al., 2016). It has long been recognized that there are genetic factors that increase the likelihood of an individual developing MS. For example, it has long been known that there is a strong association with the human leukocyte antigen (HLA)-DRB1 locus (a group of genes that serve as the major histocompatibility complex (MHC) (Barcellos et al., 2006)). More recent studies have identified over 100 associated common variants that also increase the risk for development of MS (Sawcer et al., 2014). Some of the genetic loci associated with an increased risk of developing MS are known to be involved in immunological activities, and have often been associated with other autoimmune disorders (Sawcer et al., 2011, 2014). For many others, however, any potential biological linkage to MS remains obscure (Bashinskaya et al., 2015; Jokubaitis and Butzkueven, 2016).

We were particularly interested in findings that one of the genetic risk factors for MS maps to the *GALC* locus (Sawcer et al., 2011, 2014). The *GALC* SNP, rs74796499, was one of the strongest vulnerability loci identified in the genome-wide association study (GWAS) study conducted by the International Multiple Sclerosis Genetics Consortium and the Wellcome Trust Case Control Consortium, and was one of the few loci in this study to show an odds ratio > 1.2 and an allele frequency close to 1 (Sawcer et al., 2011, 2014). This SNP is predicted to lead to a splice-region variant in the *GALC* gene (Sawcer et al., 2014), and while it remains unknown if the variant identified by the MS GWAS is associated with KD, intronic variants have been described in several late-onset KD patients (Kukita et al., 1997), and variants leading to altered mRNA splicing are also known to cause disease (Tappino et al., 2010).

The observations discussed in this introduction lead to two questions: First, how does being a heterozygous carrier of KD-causing mutations in *GALC* increase the risk of any disease? Second, what are the biological mechanisms that may enable variants in the *GALC* locus to increase the risk

of developing MS? More broadly, these concerns could be phrased as asking what is the underlying biology that makes a genetic variant a risk factor?

Mice heterozygous for loss-of-function mutation in the *GALC* gene have normal levels of myelin and do not show increased myelin damage in response to cuprizone exposure

Two possible means by which mutations in *GALC* could increase the risk for developing MS would be to cause sub-clinical damage to myelin and/or to increase the amount of damage caused by demyelinating insults. To determine whether heterozygous loss of function in the *GALC* gene can cause or exacerbate myelin damage we examined unmanipulated *twitcher* (*twi*) heterozygotes (*GALC*^{+/-}) and wild-type (WT) littermates and also examined mice exposed to cuprizone, a copper-chelating compound, in their food for four weeks. Cuprizone exposure is one of the most widely studied models of myelin damage (e.g., Kipp et al., 2009; Gudi et al., 2014; Praet et al., 2014) and a four-week exposure period causes extensive myelin loss in the corpus callosum (the major myelinated tract of the rodent CNS) (Matsushima and Morell, 2001). Following removal of cuprizone from the diet, remyelination then occurs. This is characterized by an initial increase in the recruitment and proliferation of the cells that give rise to myelin-forming oligodendrocytes, the oligodendrocyte/type-2 astrocyte progenitor cells (also referred to as oligodendrocyte precursor cells, and here abbreviated as O2A/OPCs). After proliferation, these progenitor cells then differentiate into oligodendrocytes, which go on to generate new myelin. Cuprizone exposure is also accompanied by increases in neuroinflammation (Gudi et al., 2014).

We first observed that unmanipulated WT and *GALC*^{+/-} age-matched animals did not exhibit any differences in amounts of corpus callosal myelin, oligodendrocytes or proliferating O2A/OPCs, and no differences in motor behavior. There were no detectable differences in callosal myelin (as measured by FluoroMyelin™ staining), in numbers of oligodendrocytes (defined as Olig2/glutathione-S-transferaseπ double-positive cells) or in numbers of proliferating O2A/OPCs (defined as Olig2/Ki67 double-positive cells). In addition, there were no gross changes in motor function as examined by analysis of several gait parameters that are altered in *GALC*^{-/-} animals (Folts et al., 2016). Moreover, even though *GALC*^{+/-} mice only have half the *GALC* enzyme activity of WT mice, they also have normal levels of psychosine, the toxic lipid that accumulates in KD and that disrupts multiple lysosomal and cellular functions (see Folts et al., 2016 and references therein).

Due to the extensive myelin damage that occurs in *twi* homozygotes, our expectations were that *GALC*^{+/-} mice exposed to cuprizone for four weeks would show an increase in the amount of demyelination caused, but this prediction turned out to be incorrect. Two-month-old WT and *GALC*^{+/-} littermates exposed to cuprizone-containing chow for 4 weeks were similar in the extent of myelin damage observed, and also in weight loss during cuprizone exposure and in weight gain after removal from cuprizone chow.

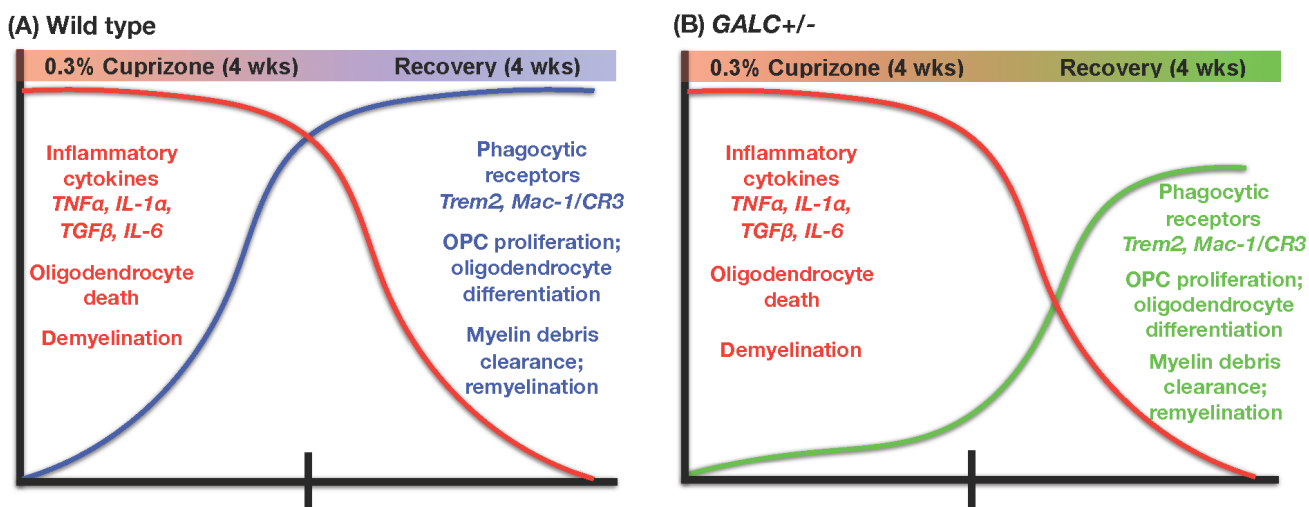


Figure 1 Summary of differences between wild-type (WT) mice (A) and unmanipulated *twitcher* (*twi*) heterozygotes ($GALC^{+/-}$) mice (B) exposed to cuprizone for four weeks.

$GALC^{+/-}$ mice show decreased myelin debris clearance, expression of microglial phagocytic receptors and remyelination. TNF α : Tumor necrosis factor alpha; IL-1 α : interleukin 1 alpha; TGF β : transforming growth factor beta; IL-6: interleukin 6; wks: weeks.

Heterozygous $GALC^{+/-}$ mice are defective in repair of cuprizone-induced myelin damage

Where $GALC^{+/-}$ mice and their WT littermates did differ, however, was in their ability to repair the damage caused by exposure to cuprizone (as summarized in **Figure 1**). When examined at 2-weeks after restoration to a normal (cuprizone-free) diet, the $GALC^{+/-}$ mice showed significantly less myelin staining when compared to recovery-matched WT littermates. This defect in remyelination was present for at least 4 weeks of recovery.

Differences in the remyelination response were also seen at the cellular level, for both oligodendrocytes and dividing progenitor cells. Although $GALC^{+/-}$ and WT mice showed similar reductions in oligodendrocyte numbers when examined immediately following 4 weeks of cuprizone exposure, the $GALC^{+/-}$ mice had significantly fewer oligodendrocytes during recovery as compared with recovery-matched WT littermates. $GALC^{+/-}$ mice also showed a significant reduction in O2A/OPC numbers when compared to recovery-matched WT littermates at 2 and 3 weeks after restoration to a cuprizone-free diet. Such a difference was not seen in mice at the completion of the cuprizone treatment, and seemed instead to be specific to the repair period.

Heterozygous loss of $GALC$ function causes an impaired microglial response to myelin damage

Alterations in the response to myelin damage also were seen in clearance of myelin debris. Such clearance is necessary for repair following a demyelinating injury, and delayed clearance of debris can prevent proper O2A/OPC differentiation and subsequent remyelination (Kotter et al., 2006; Kuhlmann, 2008; Lampron et al., 2015). When we stained brains of treated mice with an antibody that specifically recognizes damaged myelin basic protein (dMBP; Matsuo et al., 1997; Ihara et al., 2010), we found that $GALC^{+/-}$ animals showed significantly more dMBP staining than their WT counter-

parts at both 2- and 3-week recovery. This difference was seen even though staining for dMBP did not differ between $GALC^{+/-}$ and WT mice examined promptly at the end of 4 weeks of cuprizone exposure, and thus was a characteristic of the repair phase rather than the damage phase.

Our further studies suggested that an important contributor to the failure to clear myelin debris may be a compromised function of microglia, which are thought to be the primary phagocytic cell responsible for contributing to remyelination after cuprizone injury (e.g., Napoli and Neumann, 2010; Voss et al., 2012; Gudi et al., 2014; Praet et al., 2014; Lampron et al., 2015). As with our examination of myelin damage, changes in the microglial response appeared to be specific to the repair phase after cuprizone was removed from the diet. Microglial numbers and morphology were similar in WT and $GALC^{+/-}$ mice examined immediately after cuprizone exposure (as determined by staining with Iba-1, a microglia specific marker (Ito et al., 1998)), both in numbers of microglia and in their morphology. In contrast, when we examined the CNS at 4 weeks post-recovery, we saw a more rapid return to baseline for numbers of microglia in mutant mice. We also observed that the microglia in WT mice appeared more ramified, with increased process complexity, while the $GALC^{+/-}$ microglia appeared to have stunted, less complex processes.

In further studying microglial differences, we first examined expression levels of several microglia-produced cytokines known to be elevated following cuprizone exposure, including tumor necrosis factor alpha (TNF α), interleukin 6 (IL-6), interleukin 1 alpha (IL-1 α), and transforming growth factor beta (TGF β) (Arnett et al., 2003; Olah et al., 2012). Although there were some differences in expression at isolated recovery time points between WT and $GALC^{+/-}$ animals, we generally saw similar increases in expression early in recovery (0-, 1-week) that declined at later recovery time points.

Examination of the microglial relationship with myelin

debris during the recovery period, however, revealed striking differences in uptake of this debris in $GALC^{+/-}$ mice. Clearance of myelin debris is a process in which microglia play a critical role (Napoli and Neumann, 2010; Olah et al., 2012; Voss et al., 2012; Gudi et al., 2014; Lampron et al., 2015). When we analyzed the number of microglia co-localized with myelin (Iba-1/FluoroMyelin double-positive cells), we found fewer double positive microglia at 2- and 3-week post-cuprizone treatment in the $GALC^{+/-}$ animals compared to WT mice. This outcome suggests the $GALC^{+/-}$ microglia were less effective at myelin phagocytosis compared to WT microglia.

It was particularly intriguing that decreased clearance of myelin debris in the CNS of $GALC^{+/-}$ mice only became apparent as recovery was progressing. It has been shown recently that microglia can become overburdened when processing myelin debris, resulting in lysosomal inclusions and neuroimmunological dysfunction (Safaiyan et al., 2016). One possible interpretation of our observations is that $GALC^{+/-}$ microglia may be hypersensitive to overload, resulting in lower thresholds of myelin debris that can be efficiently phagocytosed and eliminated. Why this defect was not apparent in our studies during the period of cuprizone treatment itself is not known, but it may be that cuprizone modifies microglial function such that other aspects of function of these cells are dominant during the actual time of treatment.

$GALC^{+/-}$ microglia show compromised elevation of proteins important in uptake of damaged myelin

Another indication that $GALC^{+/-}$ microglia were defective in their response to myelin debris was that microglia from heterozygous mutant mice showed decreased elevation of proteins critical in this response. Microglia from $GALC^{+/-}$ mice had decreased expression of Triggering receptor expressed on myeloid cells-2 (Trem2), which is thought to play such an important role in microglial clearance of myelin debris that elevated expression of Trem2 has been used as a readout of myelin phagocytosis after cuprizone exposure (Cantoni et al., 2015; Lampron et al., 2015; Poliani et al., 2015). Trem2 in cuprizone-injured animals was significantly decreased in $GALC^{+/-}$ animals when compared to WT recovery-matched animals at the same time points when there also were significant decreases in numbers of Iba1/FluoroMyelin™ positive microglia (i.e., 2 and 3 weeks after cuprizone was removed from the diet). Trem2 was not the only microglial protein that differed in its expression, however, and we also found a significant reduction in expression of alphaM/beta2 integrin complement receptor-3 (MAC-1/CR3), another microglial receptor involved in myelin debris phagocytosis (Rotshenker, 2003), at 2 weeks of recovery.

In vitro studies on isolated microglia demonstrated that harboring a heterozygous loss-of-function at the $GALC$ locus alters the ability of these cells to engulf myelin debris and to increase Trem2 levels when exposed to such debris (as summarized in **Figure 2**). In these experiments, microglia and myelin debris were isolated from brains of 3-month-old WT and $GALC^{+/-}$ littermates ($n = 3$ animals per genotype; Lee and Tansey, 2013). The isolated myelin debris was la-

beled with the lipophilic dye Di-I, and cells were exposed to labeled myelin debris overnight. We observed that phagocytic-mediated endocytosis was impaired and significantly less myelin was engulfed by $GALC^{+/-}$ cells than by WT microglia. The $GALC^{+/-}$ microglia also showed a decreased elevation of Trem2 expression compared to WT cells when challenged with myelin debris. In WT microglia exposed to myelin debris, there was a nearly six-fold increase in Trem2 expression. In contrast, *in vitro* exposure to myelin debris did not cause an increase in Trem-2 expression in microglia isolated from $GALC^{+/-}$ animals.

Myelin phagocytosis and Trem2 expression are enhanced in $GALC^{+/-}$ microglia *in vitro* by NKH-477, a drug that corrects lysosomal dysfunction in models of KD *in vitro* and *in vivo*

While we do not know what causes the failure of myelin debris uptake in $GALC^{+/-}$ microglia, our previous studies showed that $GALC^{-/-}$ O2A/OPCs, or WT O-2A/OPCs exposed to psychosine, show multiple lysosomal-related defects. These defects include a failure of endolysosomal processing as measured by uptake of fluorescently labeled dextran beads (Folts et al., 2016).

In our previous studies on KD and other LSDs we also identified several drugs with the unexpected property of restoring normal lysosomal and cellular function in cells exposed to psychosine. We further showed that one of these drugs, NKH-477 (a.k.a. colforsin, a water-soluble derivative of forskolin that is approved for treatment of acute heart failure in Japan (No authors listed, 1999)) provided multiple benefits *in vivo* (including rescue of myelin damage and extension of lifespan) in *twi* $GALC^{-/-}$ mice. NKH-477 treatment also restored normal endolysosomal trafficking, lysosomal pH and processing of neutral triglycerides and phospholipids in WT O2A/OPCs exposed to psychosine *in vitro* (Folts et al., 2016).

When we exposed $GALC^{+/-}$ microglia to myelin debris together with NKH-477, we found that this drug increased myelin phagocytosis and Trem2 expression in the $GALC^{+/-}$ cells. Thus, one of the protective agents discovered in our studies on KD was able, at least *in vitro*, to overcome the defects in the $GALC^{+/-}$ microglia. Interestingly, this drug did not alter myelin phagocytosis or Trem2 expression in WT microglia exposed to myelin debris, and effects seemed limited to restoring normal function in defective cells rather than enhancing function generally in both $GALC^{+/-}$ and WT cells.

Implications of our findings

Does defective repair provide a new perspective on disease vulnerability?

The finding that being a heterozygous carrier of a disease-causing mutation can compromise the repair of tissue damage appears to provide a new mechanism by which vulnerability to disease could be increased. It is recognized that genetic variants, and/or environmental factors that increase the risk of disease, can cause damage directly and/or can

increase the risk that damage will occur. In addition, contributions to disease pathogenesis characterized by a failure to repair damage are recognized to occur at the molecular level; for example, compromised repair of DNA lesions is seen in individuals with diseases, such as Fanconi's anemia, in which DNA repair enzyme function is altered by mutation (e.g., van der Heijden et al., 2003; Vrouwe et al., 2007). At the cellular level, however, our studies appear to offer the first example of a vulnerability factor, and of a recessive genetic disease, in which heterozygotes show specific defects in the ability to repair tissue damage.

It seems unlikely that compromising the ability to repair lesions is unique to mutations in *GALC*. For example, recent studies reported that migration of O2A/OPCs in the CNS is inhibited in cells expressing the U94A latency-associated protein of human herpesvirus-6 (HHV6) (Campbell et al., 2017), and it is thought that insufficient migration also can contribute to poor repair of myelin damage (Boyd et al., 2013). HHV6 infections are endemic in the human population, and it is likely that most individuals harbor a latent HHV6 infection in their CNS. More importantly, HHV6 appears to be unique in its ability to integrate into human chromosomes and thus become inherited *via* germline transmission (Arbuckle and Medveczky, 2011; Kuhl et al., 2015). Such integration is not uncommon, and may affect about 1% of the population (Clark, 2016; Pantry and Medveczky, 2017).

Possible relevance of our findings to MS

The defects in myelin debris clearance and microglial function caused by *GALC* mutation offer an alteration in biological function that is attractive for its potential relevance to MS. Repair of myelin damage grows increasingly limited as MS progresses, leading to severe neurological disability. For multiple reasons, it has even been suggested that the inability to efficiently repair demyelinated lesions is what ultimately leads to the development of progressive MS (Kuhlmann, 2008; Voss et al., 2012; Dutta and Trapp, 2014; Harlow et al., 2015; Mahad et al., 2015; Kipp, 2016; Ontaneda et al., 2017). The lack of repair would itself compromise neuronal function, and the unprocessed debris would additionally provide a continued reservoir of damaged myelin that would serve as an endogenous vaccine that would expand the range of the auto-immune attack against white matter through the process of epitope spreading.

In addition, the presence of damaged myelin also can prevent the differentiation of O2A/OPCs into oligodendrocytes, which would limit the number of cells responsible for the production of new myelin. This failure of repair, with associated neuronal dysfunction, would also contribute to irreversible degeneration (Kotter et al., 2006).

Alterations in function of microglia, the CNS-resident immune cells, also could be important in multiple aspects of MS pathology (Napoli and Neumann, 2010; Voss et al., 2012; Gudi et al., 2014; Harlow et al., 2015; Lampron et al., 2015). These cells contribute to the neuroinflammation that can cause widespread tissue damage, and also play critical

roles in normal neural development, tissue surveillance and repair, and in the clearance of debris that is necessary for repair (Matsushima and Morell, 2001; Napoli and Neumann, 2010; Paolicelli et al., 2011; Franklin et al., 2012; Olah et al., 2012; Voss et al., 2012; Gudi et al., 2014; Harlow et al., 2015; Lampron et al., 2015; Hong et al., 2016; Kipp, 2016).

Defects in Trem2 elevation could be relevant to MS

The failure of *GALC*^{+/-} microglia to elevate levels of Trem2 in response to myelin debris is a finding of particular interest for multiple reasons. Trem2 appears to be critical for the response to demyelination (Cantoni et al., 2015; Poliani et al., 2015), and loss of Trem2 function leads to impaired remyelination following cuprizone-induced demyelination, much like the phenotype we observed in *GALC*^{+/-} mice. Engagement of Trem2 decreases inflammation, and blockade of Trem2 worsens disease progress in the experimental allergic encephalomyelitis (EAE) animal model of MS (Piccio et al., 2007). Conversely, transplantation of myeloid precursor cells genetically modified to express Trem2 limits tissue destruction in this same model (Takahashi et al., 2007). In humans, loss-of-function mutations in Trem2 cause the rare Nasu-Hakola disease, which is associated with demyelination of subcortical white matter damage and lethal early onset dementia (Neumann and Takahashi, 2007). Thus, a failure in Trem2 elevation may contribute to several different outcomes relevant to the MS phenotype.

Defects in Trem2 function also may have implications that extend far beyond illnesses with myelin damage as a critical contribution to disease pathology, and that will warrant attention in further studies on the effects of the heterozygous mutations in the *GALC* gene. Alterations in Trem2 function are of active interest in respect to Alzheimer's disease and potentially other neurodegenerative disorders (Jiang et al., 2013, 2014; Rohn, 2013; Jay et al., 2015; Lill et al., 2015; Lue et al., 2015; Painter et al., 2015; Poliani et al., 2015; Wang et al., 2015, 2016; Walter, 2016; Han et al., 2017). For example, in respect to microglial function, Trem2 is also critical for clearance of neuronal apoptosis (Takahashi et al., 2005) and of amyloid plaques in the presence of anti-amyloid beta antibodies (Xiang et al., 2016). Trem2 knockdown also worsens outcomes in experimental stroke models (Kawabori et al., 2015) and Trem2 loss may even generally accelerate aging processes (Poliani et al., 2015; Raha et al., 2017). Thus, the relevance of a defect in mobilization of Trem2 could have relevance to the pathology of a broad range of diseases.

It also is of interest to speculate whether our findings on microglial dysfunction are relevant to KD itself. Could failures in normal microglial function themselves contribute to the massive white matter damage in this disease?

Understanding the biology of vulnerability may lead to new treatments for neurodegenerative disorders

One of the intriguing examples of how studies on genetic contributions to disease vulnerability may have therapeutic implications is seen in studies on the relationship between GD and Parkinson's disease. Based on findings that 5–10%

Isolated microglia

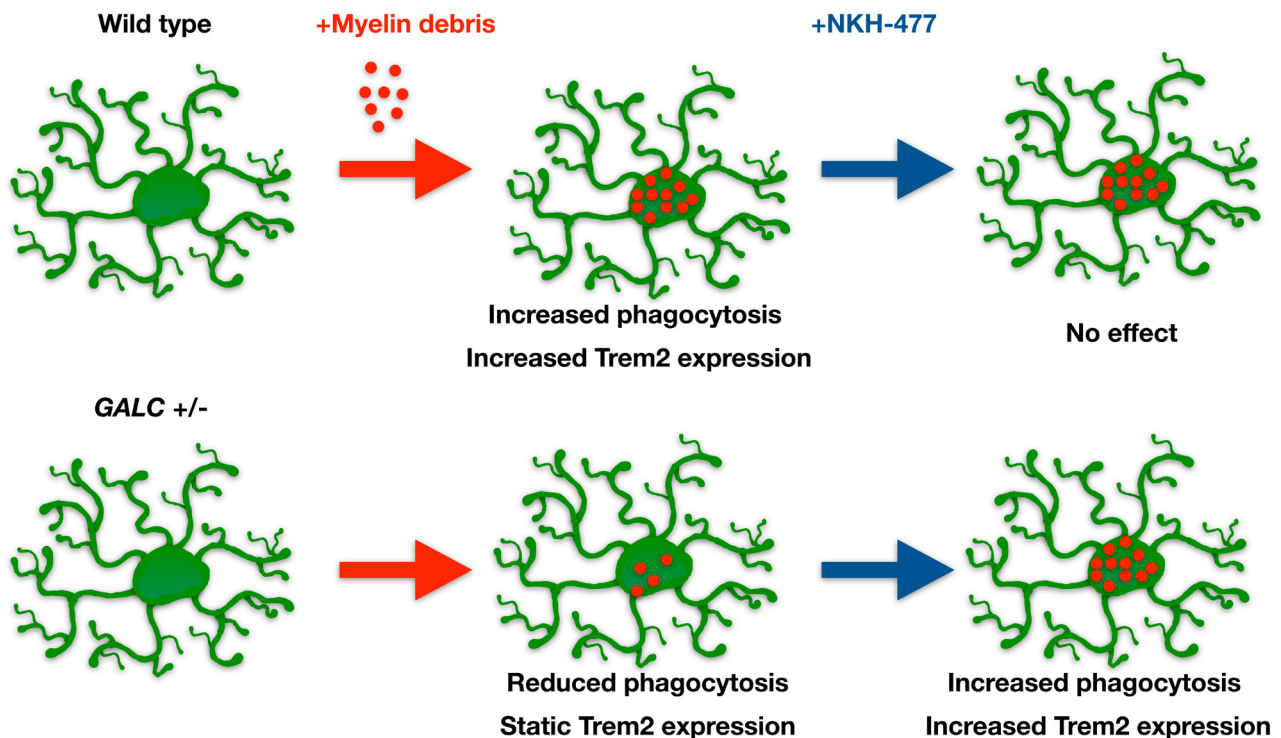


Figure 2 Summary of differences between unmanipulated *twitcher* (*twi*) heterozygotes ($GALC^{+/-}$) microglia and wild-type (WT) microglia exposed to myelin debris *in vitro*.

$GALC^{+/-}$ mice show decreased myelin debris clearance, expression of microglial phagocytic receptors and remyelination. TNF α : Tumor necrosis factor alpha; IL-1 α : interleukin 1 alpha; TGF β : transforming growth factor beta; IL-6: interleukin 6; wks: weeks.

of Parkinson's patients may have mutations in glucocerebrosidase (the enzyme mutated in GD), there is an emerging interest in using molecular chaperones to stabilize and increase the activity of this enzyme in Parkinson's patients harboring such mutations with the hope of slowing disease progression (e.g., Ambrosi et al., 2015; Migdalska-Richards et al., 2016, 2017; Ishay et al., 2018).

Analogously to studies on GD and PD, one of the most encouraging outcomes of our own experiments was the demonstration that it is possible to enhance myelin debris uptake and Trem2 expression in $GALC^{+/-}$ microglia using one of the drugs discovered in our research on KD and other LSDs (Folts et al., 2016). Impaired myelin debris clearance is thought to contribute to the inefficient remyelination that is often observed in MS patients, particularly in patients with progressive MS where the imbalance of demyelination and remyelination leads to severe neurological disability. Currently, there are no efficacious treatment options for progressive MS patients (Dutta and Trapp, 2014; Ontaneda et al., 2017). Thus, there exists a great need to better identify underlying disease mechanisms, particularly those that contribute to the impaired remyelination associated with the transition into progressive MS, and to identify better therapeutic options for these patients. While much further work needs to be done to

determine whether the approach we are studying might be of therapeutic relevance in MS, it is nonetheless a promising first step in demonstrating that it is possible to identify pharmacological agents that normalize myelin debris uptake even in mutationally altered $GALC^{+/-}$ microglia.

Additional next steps

One of the central questions to emerge from the study of effects of heterozygosity for mutations that cause KD or GD is the extent to which such findings will apply to other LSDs. For example, we recently showed that there may be general principles relevant to understanding and preventing the cellular and lysosomal abnormalities that occur in at least four of the spingolipidoses (a sub-family of LSDs that includes KD, GD, metachromatic leukodystrophy, Fabry disease, Tay-Sachs disease, Niemann-Pick disease). It will be of great interest to determine if our findings on changes in lesion repair and/or microglial function also occur in heterozygous carriers of mutations that cause other LSDs.

Two lines of investigation would greatly speed progress in answering the above question. The first of these would be for the foundations that are interested in LSDs to obtain detailed family histories that might reveal increases in the occurrence of specific other diseases. Such information may provide use-

ful clues on the types of pathological changes to study.

In addition, a targeted sequencing of genes involved in LSDs seems like it would be warranted, at a minimum in populations for which these genes are established as risk loci or that have pathologies that resemble an LSD in at least some features. For example, it may be of future interest to directly determine the prevalence of *GALC* mutations in MS patients. There has been a single report of a child with exceptionally severe KD born into a family in which several members had MS (Sahai et al., 2005). It also is intriguing that analysis of 56 MS patients for mutations causing pseudodeficiency in arylsulfatase-A (the mutation of which causes the demyelinating LSD of metachromatic leukodystrophy) revealed mutations in 13 of these patients. Moreover, patients with mutations had a greater total number of lesions and the number of hypo-intense lesions on T1-weighted images was greater in MS patients carrying such mutations (Baronica et al., 2011).

The potential relevance of *GALC* mutations and arylsulfatase-A mutations to MS also raises the question of whether therapies targeting the biological consequences of such mutations might be of relevance in MS treatment, as suggested by the outcome of our studies on NKH-477. While much further work needs to be done to determine whether this approach might be of therapeutic relevance in MS, it is nonetheless a promising first step in demonstrating that it is possible to identify pharmacological agents that normalize myelin debris uptake even in mutationally altered *GALC*^{+/-} microglia. In addition, if *GALC* mutations are as common in MS patients as suggested for Arylsulfatase-A mutations, then there might also be therapeutic utility in treatments aimed at improving *GALC* enzymatic activity in MS patients using approaches being explored as possible KD therapies (Lee et al., 2010; Berardi et al., 2014; Hill et al., 2015; Hossain et al., 2015; Graziano et al., 2016; Spratley et al., 2016).

Increasing evidence is linking genes known to cause LSDs with neurodegenerative diseases. Although we do not yet understand the functional roles of many of these associations, it is possible that changes in underlying lysosomal biology could contribute to the development of disease symptoms as well as the failure of repair mechanisms that often occurs concurrently. Thus, treatments identified for LSD patients may also be able to provide therapeutic benefits for patients with neurodegenerative diseases, a possibility that warrants further exploration.

Acknowledgments: We thank Dr. Margot Mayer-Proschel from University of Rochester for critical comments on the manuscript.

Author contributions: All authors contributed to the writing of this manuscript. The experiments discussed were conducted by NJSH and CJF. **Conflicts of interest:** The authors have declared that no conflicts of interest exist.

Financial support: This research we conducted on twitcher *GALC*^{+/-} heterozygotes was supported by the following funding sources: National Institutes of Health, F31-NS078911, <https://www.nih.gov> (NSH); New York State Department of Health, NYS-DOH-C026877, <http://www.stemcell.ny.gov> (NSH); New York State Department of Health, NYS-DOH-C029557, <http://www.stemcell.ny.gov> (MN); New York State Department of Health, NYS-DOH-C026877, <http://www.stemcell.ny.gov> (CJF); Hunter's Hope, <http://www.huntershope.org/site/PageServer>

(MN); Children's Neurobiological Solutions Foundation, <http://pediatricbrainfoundation.org> (MN); and the Legacy of Angels, <http://tloaf.org> (MN).

Plagiarism check: Checked twice by iThenticate.

Peer review: Externally peer reviewed.

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Open peer review report:

Reviewer: Una FitzGerald, National University of Ireland - Galway, Ireland.

Comments to authors: This review summarized findings relating to heterozygous carriers of mutations in galactocerebrosidase (*GALC*) and their possible relevance to multiple sclerosis (MS). A discussion of Krabbe and Gaucher disease, two lysosomal storage disorders, leads to more detailed exposition of the effects of *GALC* mutations on the repair of cuprizone-induced demyelination and factors that mediate a defective microglial response to same. The review finishes by proposing that manipulation of triggering receptor expressed in myeloid cells-2 (*TREM-2*) and other molecules associated with myelin debris clearance by microglia, could represent a novel and productive approach to boosting remyelination and restoration of function in MS. The utility of tracking *GALC* mutation incidence amongst the MS community is also suggested as an important contributor to the understanding of the molecular mechanisms underlying MS disease incidence and course within different individuals. The authors have provided an engaging and highly informative summary of the possible importance of galactocerebrosidase function in MS incidence and disease. In what is a very well-written manuscript, the authors take the reader logically from LSDs to the specifics of *GALC* variants and their suggested impact on the repair of demyelination. Sub-headings such as 'Heterozygous loss of *GALC* function causes and impaired microglial response to myelin damage' provide informative summaries of section content and entice further enthusiastic reading. This reviewer very much enjoyed reading the review and learned some interesting important information about CNS myelin repair.

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