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Maternal immune activation modifies the course of Niemann-Pick disease, type C1 in a gender specific manner

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Abstract

Niemann-Pick disease, type C1 (NPC1) is a rare neurodegenerative lysosomal storage disease with a wide spectrum of clinical manifestation. Multiple genetic factors influence the NPC1 mouse phenotype, but very little attention has been given to prenatal environmental factors that might have long-term effects on the neuroinflammatory component of NPC1 pathology. Studies in other mouse models of cerebellar ataxia have shown that developmental exposures lead to Purkinje neuron degeneration later in life, suggesting that environmental exposures during development can impact cerebellar biology. Thus, we evaluated the potential effect of maternal immune activation (MIA) on disease progression in an *Npc1* mouse model. The MIA paradigm used mimics viral infection using the toll like receptor 3 agonist polyinosinic-polycytidilic acid during gestation. Through phenotypic and pathologic tests, we measured motor and behavioral changes as well as cerebellar neuroinflammation and neurodegeneration. We observed a gender and genotype dependent effect of MIA on the cerebellum. While the effects of MIA have been previously shown to primarily affect male progeny, we observed increased sensitivity of female mutant progeny to prenatal exposure to treatment with polyinosinic-polycytidilic acid. Specifically, prenatal MIA resulted in female NPC1 mutant progeny with greater motor deficits and a corresponding decrease in cerebellar Purkinje neurons. Our data suggest that prenatal environmental exposures may be one factor contributing to the phenotypic variability observed in individuals with NPC1.

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Author contributions

A.C. and M.F. performed the pathological evaluation, C.D.D., A.G., T.G. performed the animal phenotypic evaluations. A.C., C.D.D., F.D.P., M.F. and W.J.P. designed the experiment and wrote the manuscript.

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Keywords

Niemann-Pick disease, type C1; Lysosomal Storage Disease; Maternal Immune Activation; Neurodegeneration; Mouse model; Purkinje neurons

1. Introduction.

Niemann-Pick disease, type C (NPC) is a rare, autosomal recessive disease caused by a mutation in either *NPC1* or *NPC2* [1]. The proteins encoded by these genes are involved in the transport of cholesterol out of the endolysosomal system and pathogenic mutations lead to endolysosomal storage of sphingolipids and unesterified cholesterol [1]. NPC disease occurs at a frequency of approximately 1 in every 100,000 live births with mutations in *NPC1* accounting for 95% of cases [2]. However, this may be an underestimate since mild, late-onset NPC patients may have an atypical presentation [1, 3].

The NPC1 phenotype is heterogeneous with respect to disease manifestations and age of neurological onset. Residual NPC1 function probably explains a significant fraction of this variability; however, identifying genetic modifiers of the NPC1 phenotype is of major interest. Genetic background appears to have a significant impact on disease progression and survival in *Npc1* mutant mouse models [4, 5]. Environmental factors also appear to affect disease progression and survival in NPC1 mouse models. Liu *et al.* [4], showed that *Npc1* null mice (*Npc1^{nih}*, [6]) on the same BALB/c background but from different colonies, displayed mean survival of 89±1.4 and 80±0.8 days in University of Texas Southwestern Medical School animal facility and Jackson laboratories, respectively. Other groups have reported mean survival of *Npc1^{nih/nih}* mice on a BALB/c background ranging from ~70 to 90 days [4, 7, 8]. One potential explanation for the variable survival is genetic drift between colonies of BALB/c *Npc1^{nih}* animals; however, this has not been demonstrated. We made a similar observation in the *Npc1^{nih/nih}* mice on a C57B1/6 genetic background. These mice were reported to die at approximately 32 days of age due to hepatosplenomegaly [5]; however, *Npc1^{nih/nih}* mice on a C57B1/6 background in our facility survived almost twice as long with a mean age of death of 61±12 days (Figure S1A). On the other hand, mean age of death for *Npc1^{nih/nih}* mice on a BALB/c background in our facility was 71±7 days (Figure S1A). Consistent with our previous observation of a significant sex difference in survival on a BALB/c background [9], mean age of death was 55±16 and 65±7 days ($p<0.01$) for C57B1/6 male (N=4) and female (N=7) *Npc1^{nih/nih}* mice, respectively. Additionally, in contrast to the visceral phenotype reported by Parra *et al.* [5] in C57B1/6 *Npc1^{nih/nih}* mice, we observed a predominantly neurological phenotype with loss of cerebellar Purkinje neurons similar to what is observed in *Npc1^{nih/nih}* BALB/c animals (Figure S1B).

It is difficult to explain the substantial survival and phenotypic differences reported by Parra *et al.* and observed in our colony (Figure S1A) for homozygous *Npc1^{nih/nih}* mice on a C57B1/6 background solely by invoking genetic drift. Multiple factors may impact the lifespan of mice with a phenotypic manifestation as severe as NPC1. Among these factors are the housing conditions (i.e. presence or rodent pathogen in the facility, the diet, food accessibility, supportive care) or the diseases endpoint chosen by the investigator. We thus

hypothesized that NPC1 disease pathology might be influenced by environmental factors. Among the plethora of potential environmental modifiers, we decided to investigate the effect of maternal immune activation (MIA) on disease progression induced by a toll-like receptor (TLR) agonist. Several reports suggest that an acute immune response during pregnancy can affect brain development, with notable effects on the cerebellum [10–12]. In the present work, we used exposure to the TLR3 agonist polyinosinic-polycytidilic acid (PolyI:C) at embryonic day 9.5 (E9.5) to induce MIA. PolyI:C mimics a viral, influenza-like, infection and is the most studied experimental MIA inducer. In this MIA model, phenotypic and pathologic manifestation are triggered by IL-6 as part of the immune response [12]. Of note, IL-6 has been reported to modify NPC1 disease progression in NPC1 mutant mice. Suzuki *et al.* reported increased survival of *Npc1^{nih/nih}* mice on a mixed BALB/c and C57B1/6 background when the IL-6 gene is disrupted [13].

Cerebellar manifestations of MIA induced by PolyI:C include increased expression of inflammatory biomarkers [14, 15] and decreased Purkinje neuron density [11]. Neuroinflammation and loss of cerebellar Purkinje neurons are integral aspects of NPC1 pathology, thus we hypothesized that PolyI:C induced MIA would alter disease progression in *Npc1^{nih/nih}* mice.

2. Materials and Methods

2.1. Reagents

Unless specified all reagents were purchased from Sigma-Aldrich Millipore, St Louis, MO, USA.

2.2. Animal studies

All animal work was done according to the NIH-approved animal care and use protocols of the NHGRI (Protocol: G-94–7). Heterozygous *Npc1^{+/nih}* mice (BALB/cNctr-*Npc1^{mlN/J}* strain) [6] were bred to obtain control (*Npc1^{+/+}*) and mutant (*Npc1^{nih/nih}*) littermates. Mice were weighed weekly. A humane endpoint was reached for survival studies when at least 2 of the following 3 criteria were met: 30% decrease from maximum weight, repeated falling to side and unable to right when moving forward, and/or palpebral closure with dull eyes. Motor coordination was evaluated using the balance beam test as previously described [16] using 24 mm and 18mm wide round wooden beams. Compulsive behavior was assessed using the marble burying assay as previously described [17]. Ledge test, gait, kyphosis, and hind limb clasping were analyzed as detailed in [18].

2.3. IL-6 Enzyme linked Immunosorbent assay.

10- to 11-week-old pregnant females (E9.5) received an intra peritoneal injection of either PolyI:C (5mg/kg) or saline (n=5 per condition), 3 hours following injection, serum was collected and frozen at –80°C until analysis. Samples were diluted by 10 in reaction buffer and IL-6 concentration was quantified according to manufacturer instruction (KMC0061, ThermoFisher Scientific, Waltham, MA, USA).

2.4. Immunofluorescence staining and analysis

Mice were sacrificed at 7 weeks of age by an intraperitoneally delivered overdose of Xylazine (AnaSed, Akorn Animal Health, Lake Forest, IL, USA) and transcardially perfused with 20mL of phosphate buffer pH 7.4 followed by 20mL of 4% paraformaldehyde in phosphate buffer for immunohistochemical analysis. The brains were post-fixed for 24 hours then cryoprotected in 30% sucrose until the tissues sank. Brains were then cryostat-sectioned parasagittally (20µm) and floating sections collected in phosphate buffered saline supplemented with 0.025% Triton-X100 (PBST_x). The sections were incubated overnight at 4°C with either mouse anti-calbindin 28K (1:500, CB-955), rabbit anti-IBA1 (1:400, NCNP24, Wako, Richmond, VA, USA), Chicken anti-GFAP (1:400, NBP1-05198, Novus, Centennial, CO, USA), or rat anti-CD68 (1:1000, FA-11, Bio-Rad, Hercules, CA, USA) all in PBST_x supplement with 10% goat serum. All secondary antibodies were goat anti - mouse, -rat, -rabbit or -chicken conjugated with Alexa-488, -594 or -647 (1:1000, ThermoFisher Scientific, Waltham, MA, USA). Sections were mounted, dried, rinsed with water and coverslipped with Mowiol 4-88 mounting medium and allowed to harden overnight at room temperature in the dark. Images of the cerebellum were taken using a Zeiss Axio Observer Z1 microscope fitted with an automated scanning stage, Colibri II LED illumination and Zeiss ZEN2 software using a high-resolution AxioCam MRm camera and a 20× objective. Each fluorophore channel was pseudo-colored in ZEN2, exported as CZI, and analyzed using the FIJI distribution of ImageJ (Fiji Version 1.0) [19]. Purkinje neuron density and gliosis were analyzed as previously described [20].

2.5. Statistical analysis

Results are expressed as mean ± standard deviation, presented as box and whiskers or Kaplan-Meier survival curves. Survival curves were analyzed using the Log rank Mantel-Cox test. Direct comparison between groups were performed by Mann-Whitney test, unless specified. For all analyses, statistical significance was set at $p < 0.05$. All tests were run on GraphPad Prism 5 software (GraphPad Software, San Diego, CA, USA, www.graphpad.com).

3. Results

3.1. Phenotypic effects of Maternal Immune Activation

Administration of PolyI:C to pregnant 10- to 11-week-old *Npc1* heterozygous mice at E9.5 led to a significant 9-fold increase in maternal IL-6 serum concentration relative to saline injected mice (708 ± 420 pg.mL⁻¹ (n=5) vs. 78 ± 7 pg.mL⁻¹ (n=3); $p < 0.05$) (Figure 1A). Neither litter size (7.4 ± 2.5 vs. 6.9 ± 2.6 , n=12 litters / group, $p = 0.9$) nor the genotypic ratio differed between the control and PolyI:C treated groups. A phenotypic hallmark of MIA is the induction of compulsive behavior in male offspring [21]. Compulsive behavior can be ascertained in mice using a marble burying assay [21, 22]. *Npc1*^{+/+} male mice with a prenatal exposure to PolyI:C demonstrated increased marble burying (Figure 1B). This result, combined with the increase in IL-6, provides functional validation of our maternal PolyI:C MIA model. Mean lifespan for PolyI:C exposed *Npc1*^{inh/nih} offspring was 70 ± 7 days and did not differ significantly ($p = 0.6$) from mean lifespan of the vehicle (saline) exposed *Npc1*^{inh/nih} offspring (75 ± 8 days). No differences in lifespan were observed for

either male or female offspring (Figure 1C). In addition to survival, we investigated whether prenatal PolyI:C exposure affected the NPC1 disease phenotype. Although not significant until 8 weeks of age, the average weight of the *Npc1^{nih/nih}* animals in the male PolyI:C group was consistently reduced compared to those in the saline group at all time points. Decreased weight was observed in the PolyI:C group females after 7 weeks of age (Figure 1D). In order to assess neurological signs, we used a composite score previously applied to describe disease severity in mouse models of cerebellar ataxia [18]. This composite score is the sum of four subdomains which evaluates the mice for kyphosis, gait, hindlimb clasping and balance/coordination (ledge test) at 7 weeks of age (Figure S2). Looking at the composite score and individual subdomains, no significant differences were noted between saline and PolyI:C *Npc1^{nih/nih}* mice for kyphosis or hindlimb clasping (Figure S2). Significant impairment was noted for *Npc1^{nih/nih}* mice compared to *Npc1^{+/+}* mice for the composite score and both the gait and ledge test subdomains. However, no significant gender or maternal treatment differences were observed (Figure S2). Motor function and coordination were also assessed using a beam balance test [16]. Mice were tested on both an 18 mm (Figure 1E) and a 24 mm (Figure 1F) beam. As expected, both male and female *Npc1^{nih/nih}* mice had significantly ($p < 0.01$) more foot slips than *Npc1^{+/+}* mice when tested using either the 18 mm beam (males: 21 ± 13 vs. 0; females: 17 ± 12 vs. 0) or 24 mm beam (males: 14 ± 11 vs. 0 and females: 6 ± 5 vs. 0), but female *Npc1^{nih/nih}* mice had significantly fewer foot slips on the 24 mm beam than male *Npc1^{nih/nih}* mice ($p = 0.02$). MIA exposure had no impact on balance beam performance for male *Npc1^{nih/nih}* mice; however, MIA exposure lead to a significant increase in the number of foot slips on the 24 mm beam for female *Npc1^{nih/nih}* mice (6 ± 5 vs. 12 ± 10 ; $p = 0.04$).

3.2. Histopathological effects of Maternal Immune Activation

The functional effects of MIA were relatively minor and gender specific, thus we tested whether the increased foot slips observed in female MIA mutant mice was supported by histopathological changes. Decreased cerebellar Purkinje neuron density, increased astrogliosis and increased microgliosis are characteristic pathological findings in NPC1 disease. As gliosis accompanies Purkinje neuron loss in NPC1 and is reported in various models of MIA [14, 15], we measured the density of reactive astrocytes (GFAP⁺, Figures 2A–D and S3) and of reactive microglia (CD68⁺, Figures 2E–F and S3) in cerebellar tissue from *Npc1^{+/+}* and *Npc1^{nih/nih}* mice prenatally exposed to either saline or PolyI:C. As expected, the loss of NPC1 function induces a significant increase in both astrogliosis and microgliosis with a more pronounced effect in males (Figures 2, S3). *Npc1^{nih/nih}* males display significantly larger GFAP⁺ area and number of CD68⁺ cells in the anterior lobules I–V than females when comparing saline treated cohorts. These gender differences were lost in the PolyI:C treated cohorts. CD68 staining was undetectable *Npc1^{+/+}* mice. In the female mice no significant difference in astrogliosis nor microgliosis between treatment groups was measured (Figures 2C, 2D and 2F). In the male MIA groups, a significant decreased density of CD68⁺ cells in the anterior lobules was measured (Figures 2E).

Calbindin D immunostaining confirmed the expected anterior to posterior loss of Purkinje neurons in cerebellar tissue from both male and female *Npc1^{nih/nih}* mice (Figures 2H, 2J, S3). Purkinje neuron densities in lobules I–V were slightly increased in *Npc1^{nih/nih}* female

mice compared to *Npc1^{nih/nih}* male mice (1.8 vs. 1.1, 1.6 vs. 1.1 and 1.8 vs. 1.3 average Purkinje neurons per 100 μ m in lobules I/II, III and IV/V respectively; $p < 0.001$, $p < 0.05$ and $p < 0.05$ two-way ANOVA and Bonferroni post-tests). This observation is consistent with the 18% increase in survival of female *Npc1^{nih/nih}* mice compared to male *Npc1^{nih/nih}* mice [9]. It has previously been reported that the male offspring of PolyI:C treated mice have a generalized decrease or specific lobule VII decrease [11] in Purkinje neuron density. We observed a significant decrease in cerebellar Purkinje neurons density in MIA *Npc1^{+/+}* male mice for lobules I through VI and no difference in females (Figures 2G and 2I, S3). Unlike previous reports [11], we did not observe ectopic Purkinje neurons in the PolyI:C groups. These data are consistent with previous reports showing gender specific pathological changes in male offspring with prenatal PolyI:C exposure. Evaluation of Purkinje neuron density in saline and PolyI:C exposed *Npc1^{nih/nih}* offspring showed a significant reduction in lobules I through III in *Npc1^{nih/nih}* female mice and no difference in males (Figures 2H and J, S3). We did not observe any effect of MIA on Purkinje neuron density in *Npc1^{nih/nih}* male mice (Figure 2H); but measured a small decrease in Purkinje neuron density in the anterior lobules I/II and III of female NPC1 mutant mice (1.1 vs. 1.8 and 1.2 vs. 1.6, $p = 0.02$ and $p = 0.07$, respectively, Mann-Whitney test). The PolyI:C exposure associated decrease in Purkinje neuron density in female *Npc1^{nih/nih}* mice is consistent with our observation of decreased motor coordination in this group compared to saline treated. Interestingly, this decrease in Purkinje neuron density appeared to be independent of both astrogliosis and microgliosis in the *Npc1^{+/+}* animals.

4. Discussion

Multiple factors appear to influence the disease phenotype in *Npc1^{nih/nih}* mice with significant variation occurring between different colonies. Genetic background clearly plays a role [23, 24], but environmental exposures that could modulate neuroinflammation and Purkinje neuron density may also influence disease progression. To evaluate the potential effect of environmental exposure, we modeled prenatal maternal viral infection using PolyI:C. PolyI:C induced MIA has been extensively studied as a model for prenatal viral infection and has been proposed to have postnatal effects on autistic spectrum disorder [21, 22, 25]. We hypothesized that PolyI:C induced MIA could impact disease progression in *Npc1^{nih/nih}* mice by modulating the neuroinflammatory aspect of this lysosomal storage disorder [26]. Previous work by Vogel Ciernia *et al.* [27] demonstrated that maternal immune activation can induce lasting epigenetic changes in microglia; however, the phenotypic changes that we observed cannot be due to increased expression of *Npc1* since the *Npc1^{nih}* allele is a null allele. In addition, it is well established that the phenotypic and pathologic manifestations in the PolyI:C model are induced by IL-6, a known modifier gene in *Npc1^{nih}* mouse model [13].

To our knowledge, the present work is the first evaluation of MIA's effect on the phenotypic course of a lysosomal storage disease, many of which include a neuroinflammatory component. These data provide new insights into factors that might contribute to the heterogeneous NPC1 phenotype. This study used inbred BALB/c mice carrying the same knock-out mutation maintained in a controlled environment, a scenario designed to minimize the effect of modifier genes and additional environmental factors. Under this

paradigm, we demonstrated that gender and MIA are involved in the manifestation of NPC1 disease symptoms and pathology in the *Npc1^{nihi}* mouse model. Our results suggest that prenatal exposure to an acute immune response worsens the motor function and Purkinje loss in female NPC1 mice without significantly affecting astrogliosis nor microgliosis. This observation further strengthens our [28] and others [29] observation that microglia in the *Npc1^{nihi}* mouse model may have ambivalent, protective and deleterious, functions. Our model mimics a viral infection; however, it would be interesting to evaluate whether MIA induced by other TLR agonists such as lipopolysaccharides (TLR4) or flagellin (TLR5) which mimic bacterial infections [30] also had a postnatal effect on the NPC1 phenotype.

A significant difference in Purkinje neuron density was measured in the anterior lobules (I through IV) of *Npc1^{+/+}* male MIA treated group compared to saline (Figure 2G). Consistent with prior work [31], the decrease in Purkinje neurons was not observed in control female mice (Figure 2J). Gender influence effects are not unusual in MIA. Since no motor deficits are observed in the MIA exposed control mice, these data are consistent with the concept of a threshold of Purkinje neuron loss that has to be reached before observing a motor phenotype.

In female *Npc1* mutant mice there is delayed loss of Purkinje neurons in the anterior lobules compared to male *Npc1* mutant mice (Figures 2H and 2J). This observation is consistent with increased survival of *Npc1^{nihi/nihi}* female mice compared to *Npc1^{nihi/nihi}* male mice [9]. In male *Npc1* mutant mice MIA does not appear to influence Purkinje cell number (Figure 2I). This may be due to the fact that Purkinje neuron loss is already profound. However, in female *Npc1* mutant mice, MIA increases Purkinje neuron loss in lobules I/II and III (Figure 2J). Thus, MIA appears to abrogate the female gender advantage seen in *Npc1* mutant mice. The small, but significant, loss of Purkinje neurons caused by MIA in female *Npc1* mutant mice is reflected in the impaired motor functioning on the 24 mm wide balance beam (Figure 1F). We did not observe any motor function differences utilizing an 18 mm wide balance beam (Figure 1E). Only observing a difference on the 24 mm wide balance beam, the least stringent of the two, suggests that subtle differences are lost with more demanding test parameters. The factors contributing to increased survival in female *Npc1* mutant mice are not known; however, MIA appears to abrogate at least some of these factors leading to increased motor deficit and Purkinje neuron loss.

In a previous report evaluating the role played by the receptor interacting protein kinase 3 in *Npc1^{nihi}* mouse we observe that decreasing cerebellar gliosis, as measured with GFAP and CD68 staining, did not affect disease progression [20]. The lack of correlation between neuroinflammation level assessed by these markers and disease progression suggests that the contribution of gliosis to disease progression in NPC1 might be limited. Alternatively, these data may suggest that these widely accepted makers of neuroinflammation do not adequately discriminate between neuroprotective and neurotoxic subpopulations of astrocytes and microglia potentially present in the cerebellum of NPC1 mice. A subpopulation of disease associated microglia expressing the surface marker CD11c (*Itgax*) are present in NPC1 mice [28]. Although they remain to be studied in NPC1 cerebellum, subpopulations of astrocytes may have differential contributions to neuroinflammation [32].

Lastly, the present work supports previous reports of a gender difference in disease severity in the BALB/c *Npc1^{null}* (*Npc1^{nih/nih}*) mouse models. The repetition of this observation highlights the need to report the gender of mice used when evaluating drug treatments for NPC1 disease. MIA using TLR3 agonist is only one among the many environmental factors potentially driving the phenotypic variability of NPC1 in mouse and human. In addition to TLR agonists, environmental pollutants, such as bisphenol and phthalates which are increasingly being recognized as hazardous, could also be tested for their potential influence on progression of NPC1 and other neurodegenerative diseases.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

- [1]. Vanier MT, Niemann-Pick disease type C Orphanet J Rare Dis 5 (2010) 16. [PubMed: 20525256]
- [2]. Dvorakova L, Sikora J, Hrebicek M, Hulkova H, Bouckova M, Stolnaja L, Elleder M, Subclinical course of adult visceral Niemann-Pick type C1 disease. A rare or underdiagnosed disorder? J Inherit Metab Dis 29 (2006) 591.
- [3]. Bauer P, Balding DJ, Klunemann HH, Linden DE, Ory DS, Pineda M, Priller J, Sedel F, Muller A, Chadha-Boreham H, Welford RW, Strasser DS, Patterson MC, Genetic screening for Niemann-Pick disease type C in adults with neurological and psychiatric symptoms: findings from the ZOOM study Hum Mol Genet 22 (2013) 4349–4356. [PubMed: 23773996]
- [4]. Liu B, Li H, Repa JJ, Turley SD, Dietschy JM, Genetic variations and treatments that affect the lifespan of the NPC1 mouse Journal of lipid research 49 (2008) 663–669. [PubMed: 18077828]
- [5]. Parra J, Klein AD, Castro J, Morales MG, Mosqueira M, Valencia I, Cortes V, Rigotti A, Zanolungo S, *Npc1* deficiency in the C57BL/6J genetic background enhances Niemann-Pick disease type C spleen pathology Biochem Biophys Res Commun 413 (2011) 400–406. [PubMed: 21910975]
- [6]. Loftus SK, Morris JA, Carstea ED, Gu JZ, Cummings C, Brown A, Ellison J, Ohno K, Rosenfeld MA, Tagle DA, Pentchev PG, Pavan WJ, Murine model of Niemann-Pick C disease: mutation in a cholesterol homeostasis gene Science 277 (1997) 232–235. [PubMed: 9211850]
- [7]. Zhang M, Strnatka D, Donohue C, Hallows JL, Vincent I, Erickson RP, Astrocyte-only *Npc1* reduces neuronal cholesterol and triples life span of *Npc1*^{-/-} mice J Neurosci Res 86 (2008) 2848–2856. [PubMed: 18500759]
- [8]. Zhang JS, Erickson RP, A modifier of Niemann-Pick C 1 maps to mouse chromosome 19 Mamm Genome 11 (2000) 69–71. [PubMed: 10602996]
- [9]. Bianconi SE, Hammond DI, Farhat NY, Dang Do A, Jenkins K, Cougnoux A, Martin K, Porter FD, Evaluation of age of death in Niemann-Pick disease, type C: Utility of disease support group websites to understand natural history Mol Genet Metab (2019).
- [10]. Aavani T, Rana SA, Hawkes R, Pittman QJ, Maternal immune activation produces cerebellar hyperplasia and alterations in motor and social behaviors in male and female mice Cerebellum 14 (2015) 491–505. [PubMed: 25863812]
- [11]. Shi L, Smith SE, Malkova N, Tse D, Su Y, Patterson PH, Activation of the maternal immune system alters cerebellar development in the offspring Brain Behav Immun 23 (2009) 116–123. [PubMed: 18755264]

- [12]. Parker-Athill EC, Tan J, Maternal Immune Activation and Autism Spectrum Disorder: Interleukin-6 Signaling as a Key Mechanistic Pathway *Neurosignals* 18 (2010) 113–128. [PubMed: 20924155]
- [13]. Suzuki M, Sugimoto Y, Ohsaki Y, Ueno M, Kato S, Kitamura Y, Hosokawa H, Davies JP, Ioannou YA, Vanier MT, Endosomal accumulation of Toll-like receptor 4 causes constitutive secretion of cytokines and activation of signal transducers and activators of transcription in Niemann–Pick disease type C (NPC) fibroblasts: a potential basis for glial cell activation in the NPC brain *The Journal of neuroscience* 27 (2007) 1879–1891. [PubMed: 17314284]
- [14]. Pendyala G, Chou S, Jung Y, Coiro P, Spartz E, Padmashri R, Li M, Dunaevsky A, Maternal Immune Activation Causes Behavioral Impairments and Altered Cerebellar Cytokine and Synaptic Protein Expression *Neuropsychopharmacology* 42 (2017) 1435–1446. [PubMed: 28102228]
- [15]. Mattei D, Ivanov A, Ferrai C, Jordan P, Guneykaya D, Buonfiglioli A, Schaafsma W, Przanowski P, Deuther-Conrad W, Brust P, Maternal immune activation results in complex microglial transcriptome signature in the adult offspring that is reversed by minocycline treatment *Translational psychiatry* 7 (2017) e1120. [PubMed: 28485733]
- [16]. Luong TN, Carlisle HJ, Southwell A, Patterson PH, Assessment of Motor Balance and Coordination in Mice using the Balance Beam *J Vis Exp* (2011).
- [17]. Deacon RMJ, Digging and marble burying in mice: simple methods for in vivo identification of biological impacts *Nat Protoc* 1 (2006) 122–124. [PubMed: 17406223]
- [18]. Guyenet SJ, Furrer SA, Damian VM, Baughan TD, La Spada AR, Garden GA, A simple composite phenotype scoring system for evaluating mouse models of cerebellar ataxia *J Vis Exp* (2010).
- [19]. Schindelin J, Arganda-Carreras I, Frise E, Kaynig V, Longair M, Pietzsch T, Preibisch S, Rueden C, Saalfeld S, Schmid B, Tinevez JY, White DJ, Hartenstein V, Eliceiri K, Tomancak P, Cardona A, Fiji: an open-source platform for biological-image analysis *Nat Methods* 9 (2012) 676–682. [PubMed: 22743772]
- [20]. Cougnoux A, Clifford S, Salman A, Ng SL, Bertin J, Porter FD, Necroptosis inhibition as a therapy for Niemann–Pick disease, type C1: Inhibition of RIP kinases and combination therapy with 2-hydroxypropyl-beta-cyclodextrin *Mol Genet Metab* (2018).
- [21]. Xuan IC, Hampson DR, Gender-dependent effects of maternal immune activation on the behavior of mouse offspring *Plos One* 9 (2014) e104433. [PubMed: 25111339]
- [22]. Smith SEP, Li J, Garbett K, Mirnics K, Patterson PH, Maternal immune activation alters fetal brain development through interleukin-6 *J Neurosci* 27 (2007) 10695–10702. [PubMed: 17913903]
- [23]. Maue RA, Burgess RW, Wang B, Wooley CM, Seburn KL, Vanier MT, Rogers MA, Chang CC, Chang TY, Harris BT, Graber DJ, Penatti CA, Porter DM, Swergold BS, Henderson LP, Totenhagen JW, Trouard TP, Borbon IA, Erickson RP, A novel mouse model of Niemann–Pick type C disease carrying a D1005G-Npc1 mutation comparable to commonly observed human mutations *Hum Mol Genet* 21 (2012) 730–750. [PubMed: 22048958]
- [24]. Praggastis M, Tortelli B, Zhang J, Fujiwara H, Sidhu R, Chacko A, Chen Z, Chung C, Lieberman AP, Sikora J, Davidson C, Walkley SU, Pipalia NH, Maxfield FR, Schaffer JE, Ory DS, A murine Niemann–Pick C1 I1061T knock-in model recapitulates the pathological features of the most prevalent human disease allele *J Neurosci* 35 (2015) 8091–8106. [PubMed: 26019327]
- [25]. Atladottir HO, Thorsen P, Ostergaard L, Schendel DE, Lemcke S, Abdallah M, Parner ET, Maternal Infection Requiring Hospitalization During Pregnancy and Autism Spectrum Disorders *J Autism Dev Disord* 40 (2010) 1423–1430. [PubMed: 20414802]
- [26]. Fiorenza MT, Moro E, Erickson RP, The pathogenesis of lysosomal storage disorders: beyond the engorgement of lysosomes to abnormal development and neuroinflammation *Hum Mol Genet* 27 (2018) R119–R129. [PubMed: 29718288]
- [27]. Ciernia AV, Careaga M, LaSalle JM, Ashwood P, Microglia from offspring of dams with allergic asthma exhibit epigenomic alterations in genes dysregulated in autism *Glia* 66 (2018) 505–521. [PubMed: 29134693]

- [28]. Cougnoux A, Drummond RA, Collar AL, Iben JR, Salman A, Westgarth H, Wassif CA, Cawley NX, Farhat NY, Ozato K, Lionakis MS, Porter FD, Microglia activation in Niemann-Pick disease, type C1 is amendable to therapeutic intervention *Hum Mol Genet* 27 (2018) 2076–2089. [PubMed: 29617956]
- [29]. Gabande-Rodriguez E, Perez-Canamas A, Soto-Huelin B, Mitroi DN, Sanchez-Redondo S, Martinez-Saez E, Venero C, Peinado H, Ledesma MD, Lipid-induced lysosomal damage after demyelination corrupts microglia protective function in lysosomal storage disorders *EMBO J* (2018).
- [30]. Kawai T, Akira S, The role of pattern-recognition receptors in innate immunity: update on Toll-like receptors *Nat Immunol* 11 (2010) 373–384. [PubMed: 20404851]
- [31]. Haida O, Al Sagheer T, Balbous A, Francheteau M, Matas E, Soria F, Fernagut PO, Jaber M, Sex-dependent behavioral deficits and neuropathology in a maternal immune activation model of autism *Transl Psychiatry* 9 (2019) 124. [PubMed: 30923308]
- [32]. Liddelow SA, Guttenplan KA, Clarke LE, Bennett FC, Bohlen CJ, Schirmer L, Bennett ML, Munch AE, Chung WS, Peterson TC, Wilton DK, Frouin A, Napier BA, Panicker N, Kumar M, Buckwalter MS, Rowitch DH, Dawson VL, Dawson TM, Stevens B, Barres BA, Neurotoxic reactive astrocytes are induced by activated microglia *Nature* 541 (2017) 481–487. [PubMed: 28099414]

Figure 1: Phenotypic consequences of maternal immune activation in NPC1 mice.

(A) IL-6 concentrations in the serum of pregnant *Npc1*^{+/nih} female mice treated with either saline or PolyI:C. N=5, two samples were below detection level in the saline treated control group. *p<0.05. (B) Assessment of marble burying. Each group had between 15 and 18 animals, **p<0.01. -/- indicates homozygosity for the *Npc1*^{nih} mutant allele. (C) Kaplan-Meier survival curve of *Npc1*^{+/+} and *Npc1*^{nih/nih} mice treated with either saline or PolyI:C. Male saline (N=6); Male polyI:C (N=7); Female saline (N=6); Female PolyI:C (N=8). No groups were significantly different using a Log rank Mantel-Cox test. (D) Weight in grams over the course of the disease in *Npc1*^{nih/nih} mice. N 6 per group. Males and females are in black and gray respectively, Saline and PolyI:C are indicated by the plain and dash lines respectively. Foot slips were quantified on an 18 mm (E) and 24 mm (F) beam. PolyI:C groups have the descending diagonal stripes. For B, E-F all mice were 7 weeks old, N 12/group. ** p<0.01 Mann-Whitney test.

Figure 2: Cerebellar pathology at 7 weeks.

(A-D) GFAP staining percent area per lobule. (E-F) density per mm² of lobule of CD68⁺ cells. (G-J) Purkinje neuron density per 100µm. Dashed lines indicate PolyI:C groups, plain lines indicate saline groups. Results are displayed as median±range N 3, each N is an average of 3–4 cerebellum sections analyzed per animal.