HHS Public Access

Author manuscript

*Exp Eye Res*. Author manuscript; available in PMC 2024 April 01.

Published in final edited form as:

*Exp Eye Res*. 2023 April ; 229: 109433. doi:10.1016/j.exer.2023.109433.

# Histological Characterization of Retinal Degeneration in Mucopolysaccharidosis Type IIIC

**Jessica Ludwig**[1], **Onkar B. Sawant**[1,*], **Jill Wood**[2], **Srikanth Singamsetty**[2], **Xuefang Pan**[3], **Vera L. Bonilha**[4], **Sujata Rao**[4], **Alexey V. Pshezhetsky**[3,5]

[1]Center for Vision and Eye Banking Research, Eversight, Cleveland, OH 44103, USA.

[2]Phoenix Nest INC, Brooklyn, New York, USA.

[3]Sainte-Justine University Hospital Research Center, University of Montreal, Montreal, Quebec, Canada.

[4]Department of Ophthalmic Research, Cole Eye Institute, Cleveland Clinic, Cleveland, OH, USA.

[5]Department of Anatomy and Cell Biology, McGill University, Montreal, Quebec, Canada.

## Abstract

Heparan-α-glucosaminide N-acetyltransferase (HGSNAT) participates in lysosomal degradation of heparan sulfate. Mutations in the gene encoding this enzyme cause mucopolysaccharidosis IIIC (MPS IIIC) or Sanfilippo syndrome type C. MPS IIIC patients exhibit progressive neurodegeneration, leading to dementia and death in early adulthood. Currently there is no approved treatment for MPS IIIC. Incidences of non-syndromic retinitis pigmentosa and early signs of night blindness are reported in some MPS IIIC patients, however the majority of ocular phenotypes are not well characterized. The goal of this study was to investigate retinal degeneration phenotype in the *Hgsnat* knockout mouse model of MPS IIIC and a cadaveric human MPS IIIC eye. Cone and rod photoreceptors in the eyes of homozygous 6-month-old *Hgsnat* knockout mice and their wild-type counterparts were analyzed using cone arrestin, S-opsin, M-opsin and rhodopsin antibodies. Histological observation was performed on the eye from a 35-year-old MPS IIIC donor. We observed a nearly 50% reduction in the rod photoreceptors density in the *Hgsnat* knockout mice compared to the littermate wild-type controls. Cone photoreceptor density was unaltered at this age. Severe retinal degeneration was also observed in the MPS IIIC donor eye. To our knowledge, this is the first report characterizing ocular phenotypes arising from deleterious variants in the *Hgsnat* gene associated with MPS IIIC clinical phenotype. Our findings indicate retinal manifestations may be present even before behavioral manifestations. Thus, we speculate that ophthalmological evaluations could be used as diagnostic indicators of early disease, progression, and end-point evaluation for future MPS IIIC therapies.

[*]Corresponding author Onkar B. Sawant, Ph.D. osawant@eversightvision.org.

## Short communication

Glycosaminoglycans (GAGs or mucopolysaccharides) are long-chain sugar molecules attached to proteoglycans found throughout the body including the connective tissue, liver, spleen, skin, cartilage, brain and eye. Their catabolism requires participation of multiple enzymes. Genetic defects in these enzymes result in accumulation of partially degraded GAGs (mainly heparan sulfate, dermatan sulfate or keratan sulfate) in lysosomes. Eventually, this build up disrupts cellular function and leads to the development of Mucopolysaccharidoses (MPS), a subfamily of lysosomal storage disorders. There are 12 subtypes of MPS, each with a distinct defective gene and corresponding impacted enzyme. MPS IIIC, or Sanfilippo Syndrome type C, is caused by a deficiency in the Heparan-α-glucosaminide N-acetyltransferase (*HGSNAT*) gene. The HGSNAT enzyme transfers an acetyl group from cytoplasmic acetyl-CoA to the terminal N-glucosamine of heparan sulphate within the lysosomes (Klein, Kresse et al. 1978). This *HGSNAT* deficiency causes the accumulation of heparan sulphate (Sun 2018). MPS IIIC is unique among the nearly 50 known LSDs because it is caused by deficiency of a transferase and not a hydrolase. There is no approved treatment for MPS IIIC. New trials are underway, but like the other members of MPS III, MPS IIIC currently is not treatable by enzyme replacement therapy or stem cell therapy, the two most common treatments for other forms of MPS (Welling, Marchal et al. 2015) (Jones, Breen et al. 2016).

Clinical phenotype for the four members of MPS III (A, B, C, and D) appears in early childhood. Neurologic deterioration causes developmental delays and severe sleep and behavioral disturbances may be the first diagnostic symptoms. The disease rapidly progresses and intellectual development typically plateaus in early childhood, followed by decline. All subtypes of MPS report visual impairment, either through corneal clouding or retinopathy (Sun 2018). Interestingly, *HGSNAT* variants causing only partial deficiency of the enzyme have been identified as a genetic cause for retinitis pigmentosa (Comander, Weigel-DiFranco et al. 2017, Van Cauwenbergh, Van Schil et al. 2017, Schiff, Daich Varela et al. 2020). In MPS IIIC patients with less severe disease, retinal degeneration becomes apparent with age (Nijmeijer, van den Born et al. 2019).

Mouse models were first developed for the more prevalent MPS III subtypes, A and B (Li, Yu et al. 1999, Bhattacharyya, Gliddon et al. 2001). Severe retinal degeneration has been reported in both (Crawley, Gliddon et al. 2006, Heldermon, Hennig et al. 2007, Tse, Lotfi et al. 2015, Intartaglia, Giamundo et al. 2020). In the MPS IIIA model, photoreceptor dysfunction is apparent before any impact on the CNS when the mice are 3 months old. As they age, the outer nuclear layer (ONL) and rod density both decrease (Intartaglia, Giamundo et al. 2020). In the MPS IIIB model, the dark-adapted retinal response is dampened by the age of 5 weeks, and continues to decline. This is reflective of lost rod function that becomes significant at 15 weeks of age, when the ONL is beginning to decrease in thickness. By 34 weeks the number of photoreceptors is reduced to about 50% of the wildtype (WT) level (Tse, Lotfi et al. 2015). *Hgsnat* is expressed in all types of mouse cells that have lysosomes; the first model of MPS IIIC created by *Hgsnat* germline knockout (KO) was reported in 2015 (Martins, Hulkova et al. 2015). Several mouse models of MPS IIIC have been reported. They show somewhat heterogeneous phenotype but all exhibit

undetectable or near undetectable levels of *HGSNAT* activity, and anatomical and behavioral defects resembling those seen in human patients (Martins, Hulkova et al. 2015, Marco, Pujol et al. 2016, Pan, Taherzadeh et al. 2022).

No examination has thus far been made of visual impairment/pathology in any MPS IIIC model. This first report for Hgsnat KO analyzes retinal degeneration will exclusively focus on the 6 month time point. The goal of this study was to evaluate the retinal phenotype of *Hgsnat* KO mice (*Hgsnat-Geo* strain) (Martins, Hulkova et al. 2015) before behavioral manifestations arise, but after the eye has fully developed. We also sought to determine how the mouse defects compare to those in a human cadaveric eye from a MPS IIIC donor. All animal experiments complied with the Guide for the Care and Use of Laboratory Animals and were approved by the Animal Care and Use Committee of the Ste-Justine Hospital Research Center. The animals were cared for in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Mice of both sexes were sacrificed at six months of age and whole eyes were collected and fixed with 4% paraformaldehyde in PBS. Immunohistochemistry was performed to identify the expression of rhodopsin (Abcam #AB9887), S-Opsin (Santa Cruz Biotechnology #SC-14363), M-Opsin (Millipore #AB5405) and cone arrestin (Millipore #AB15282), as described previously (Sawant, Horton et al. 2017, Sawant, Jidigam et al. 2020). Rod photoreceptor loss was estimated based on the thickness of the ONL following an established procedure (Sawant, Horton et al. 2015). A cadaveric eye was obtained from a 35-year-old MPS IIIC eye donor. The donation process and evaluations were performed in compliance with the Declaration of Helsinki and Eye Bank Association of America (EBAA) and Food and Drug Administration (FDA) regulations. Legal consent for research was obtained prior to procurement from the donor families. Whole eye globes were fixed in a mixture of 4% paraformaldehyde and 0.5% glutaraldehyde in PBS. A small area of the retina/RPE/choroid tissue from the periphery of the MPS IIIC donor and an age/sex-matched control donor were cut and further processed as previously described (Bonilha, Bell et al. 2020). Toluidine blue stained sections were photographed with a Leica DMi8 microscope.

The *Hgsnat* KO model used here, shows first pathological changes in the CNS as early as at two months of age (Martins, Hulkova et al. 2015). Behavioral changes, including hyperactivity and reduced fear, begin to appear at 6 months and become significant by 8 months of age. Also at 8 months neuronal changes become apparent while memory and learning capabilities begin to diminish. As mice continue to age the hyperactivity and reduced fear give way to loss of balance and hesitancy to walk. Finally, as the animals pass one year of age urine retention, tremors, and gait impairment typically establish the need to euthanize (Martins, Hulkova et al. 2015). For this study, *Hgsnat* KO mice were examined at 6 months of age to determine if any ocular phenotypes developed before significant behavioral, or measurable neuronal loss arose. Such results would indicate that MPS IIIC patients may experience visual symptoms relatively early in their disease progression.

Our results show that *Hgsnat* KO mice exhibit severe rod degeneration at 6 months of age. The outer nuclear layer (ONL) of the retina was significantly thinner in *Hgsnat* KO (Figure 1 A and C) than their WT control littermates (Figure 1 B and D). Additionally, the ONL

showed sparse nuclear staining with DAPI in the *Hgsnat* KO retinas (Figure 1 C) compared to the litter mate WT control retinas (Figure 1 D). Closer analysis revealed that the number of rows of photoreceptor nuclei in the ONL is reduced at least by 50%, indicating severe rod photoreceptor degeneration (Figure 1 E and F). The row of rod cells stained for rhodopsin was also both thinner and less dense when compared to that in WT mice indicating reduced outer segment (OS) thickness (Figure 1 C' and D').

The cone cells appeared to be largely unaffected at this age in the *Hgsnat* KO mice. We observed no alteration in cone arrestin positive cone density in KO (Figure 1 B") and WT retinas (Figure 1 A"). Retinal flat mounts presented similar density and distribution of S and M opsin (Figure 1 I–J). Similarly, cross-section staining for nuclei, S-opsin and M-opsin did not show any differences between KO (Figure 1 H) and WT retinas (Figure 1 G). Further studies may determine if cones are impacted later in the disease progression. Taken together, these results indicate that *Hgsnat* KO mice at 6 months of age exhibit severe rod degeneration but cone density is unaffected compared to the litter mate WT controls.

To determine the relevance of retinal pathology observed in the MPS IIIC mouse model to human disease, eye tissue from an adult MPS IIIC donor was analyzed. A sex, age, and race matched healthy donor was used as a control. The eye cup of the MPS IIIC patient was noticeably smaller in size (Figure 2 A). Histological examination showed that like in the mouse model, the MPS IIIC peripheral retina photoreceptor layer (ONL) was considerably thinner and less densely populated with cells (Figure 2 C) than the healthy control (Figure 2 B). Hence, confirming that MPS IIIC donor also exhibited severe rod degeneration.

This result demonstrates for the first time that *Hgsnat* KO mice experience retinal degeneration in a manner that might be similar to human patients providing validity for the data obtained in the mouse model, and establishing these mice as an invaluable model to study retinal pathology in MPS IIIC.

In conjunction, we find that MPS IIIC *Hgsnat-Geo* mice exhibit severe retinal degeneration at the age when pathological manifestations in cerebral neurons are still relatively mild. Human MPS IIIC cadaveric donor tissue also demonstrates rod degeneration. Thus, we speculate that MPS IIIC patients likely experience retinal symptoms which are overshadowed by other manifestations and that ocular disease may manifest before other symptoms arise. As diagnostic delays are common in MPS III, ocular phenotype may be the first truly distinctive symptom, and better disease awareness among ophthalmologists could play a critical role in early detection and diagnosis of patients. Ophthalmologic evaluation may also be useful as an indicator of MPS IIIC progression and for end-point evaluation of efficacy for future therapies.

Further studies are needed to thoroughly characterize retinal degeneration in this MPS IIIC model. Specifically, following these significant findings at 6 months of age, future research will be conducted to determine the earliest detectable phenotype and how disease manifestation in the eye then progresses. Similarly, further studies are also needed to comprehensively understand vision impairment for MPS IIIC patients, and how disease progression impacts the retina, including cone cells. This knowledge could inform treatment

options. Additionally, future gene therapies targeting this gene for MPS IIIC patients may potentially serve a second purpose for retinitis pigmentosa patients' disease caused by partial disruptions to HGSNAT.

## Acknowledgements/Funding

The authors thank the family of the MPS IIIC donor and the MPS National Society for the precious gift of tissue provided for this work. Research activities at Eversight are supported by funding from LC Industries (Durham, NC), Eye Bank Association of America (EBAA), Connecticut Lions Eye Research Foundation, Connecticut Eye Bank and Visual Research Foundation, Blue Cross Blue Shield of Michigan Foundation, The Louise H. and David S. Ingalls Foundation (Cleveland, OH), Lowell Johnson Foundation (Pittsburgh, PA), and William G. and Helen C. Hoffman Foundation (Las Vegas, NV). AVP was partially supported by operating grant PJT-156345 from the Canadian Institutes of Health Research, Elisa Linton Sanfilippo Research Laboratory endowed fund. Supported by grants from the U.S. National Institutes of Health/National Eye Institute EY027077-01 (SR), RPB1503 (SR), National Eye Institute P30-EY025585 Core Grant and Research to Prevent Blindness Challenge Grant.

**Declarations of interests:**

AVP received research grants and honoraria from Phoenix Nest Inc.

## References

Bhattacharyya R, Gliddon B, Beccari T, Hopwood JJ and Stanley P.(2001). "A novel missense mutation in lysosomal sulfamidase is the basis of MPS III A in a spontaneous mouse mutant." Glycobiology 11(1): 99–103. [PubMed: 11181566]

Bonilha VL, Bell BA, Hu J, Milliner C, Pauer GJ, Hagstrom SA, Radu RA and Hollyfield JG (2020). "Geographic Atrophy: Confocal Scanning Laser Ophthalmoscopy, Histology, and Inflammation in the Region of Expanding Lesions." Invest Ophthalmol Vis Sci 61(8): 15.

Comander J, Weigel-DiFranco C, Maher M, Place E, Wan A, Harper S, Sandberg MA, Navarro-Gomez D.and Pierce EA (2017). "The Genetic Basis of Pericentral Retinitis Pigmentosa-A Form of Mild Retinitis Pigmentosa." Genes (Basel) 8(10).

Crawley AC., Gliddon BL, Auclair D, Brodie SL, Hirte C, King BM, Fuller M, Hemsley KM and Hopwood JJ (2006). "Characterization of a C57BL/6 congenic mouse strain of mucopolysaccharidosis type IIIA." Brain Res 1104(1):117.

Heldermon CD, Hennig AK, Ohlemiller KK, Ogilvie JM, Herzog ED, Breidenbach A, Vogler C, Wozniak DF and Sands MS (2007). "Development of sensory, motor and behavioral deficits in the murine model of Sanfilippo syndrome type B." PLoS One 2(8): e772. [PubMed: 17712420]

Intartaglia D, Giamundo G, Marrocco E, Maffia V, Salierno FG, Nusco E, Fraldi A, Conte I.and Sorrentino NC (2020). "Retinal Degeneration in MPS-IIIA Mouse Model." Front Cell Dev Biol 8: 132. [PubMed: 32195255]

Jones SA, Breen C, Heap F, Rust S, de Ruijter J, Tump E, Marchal JP, Pan L, Qiu Y, Chung JK, Nair N, Haslett PAJ, Barbier AJ and Wijburg FA (2016). "A phase 1/2 study of intrathecal heparan-N-sulfatase in patients with mucopolysaccharidosis IIIA." Mol Genet Metab 118(3): 198–205. [PubMed: 27211612]

Klein U, Kresse H.and von Figura K.(1978). "Sanfilippo syndrome type C: deficiency of acetyl-CoA:alpha-glucosaminide N-acetyltransferase in skin fibroblasts." Proc Natl Acad Sci U S A 75(10): 5185–5189. [PubMed: 33384]

Li HH, Yu WH, Rozengurt N, Zhao HZ, Lyons KM, Anagnostaras S, Fanselow MS, Suzuki K, Vanier MT and Neufeld EF (1999). "Mouse model of Sanfilippo syndrome type B produced by targeted disruption of the gene encoding alpha-N-acetylglucosaminidase." Proc Natl Acad Sci U S A 96(25): 14505–14510. [PubMed: 10588735]

Marco S, Pujol A, Roca C, Motas S, Ribera A, Garcia M, Molas M, Villacampa P, Melia CS, Sanchez V, Sanchez X, Bertolin J, Ruberte J, Haurigot V.and Bosch F.(2016). "Progressive neurologic and somatic disease in a novel mouse model of human mucopolysaccharidosis type IIIC." Dis Model Mech 9(9): 999–1013. [PubMed: 27491071]

Martins C, Hulkova H, Dridi L, Dormoy-Raclet V, Grigoryeva L, Choi Y, Langford-Smith A, Wilkinson FL, Ohmi K, DiCristo G, Hamel E, Ausseil J, Cheillan D, Moreau A, Svobodova E, Hajkova Z, Tesarova M, Hansikova H, Bigger BW, Hrebicek M.and Pshezhetsky AV (2015). "Neuroinflammation, mitochondrial defects and neurodegeneration in mucopolysaccharidosis III type C mouse model." Brain 138(Pt 2): 336–355. [PubMed: 25567323]

Nijmeijer SCM, van den Born LI, Kievit AJA, Stepien KM, Langendonk J, Marchal JP, Roosing S, Wijburg FA and Wagenmakers M.(2019). "The attenuated end of the phenotypic spectrum in MPS III: from late-onset stable cognitive impairment to a non-neuronopathic phenotype." Orphanet J Rare Dis 14(1): 249. [PubMed: 31718697]

Pan X, Taherzadeh M, Bose P, Heon-Roberts R, Nguyen ALA, Xu T, Para C, Yamanaka Y, Priestman DA, Platt FM, Khan S, Fnu N, Tomatsu S, Morales CR and Pshezhetsky AV (2022). "Glucosamine amends CNS pathology in mucopolysaccharidosis IIIC mouse expressing misfolded HGSNAT." J Exp Med 219(8).

Sawant O, Horton AM, Shukla M, Rayborn ME, Peachey NS, Hollyfield JG and Rao S.(2015). "Light-Regulated Thyroid Hormone Signaling Is Required for Rod Photoreceptor Development in the Mouse Retina." Invest Ophthalmol Vis Sci 56(13): 8248–8257. [PubMed: 26720479]

Sawant OB, Horton AM, Zucaro OF, Chan R, Bonilha VL, Samuels IS and Rao S.(2017). "The Circadian Clock Gene Bmal1 Controls Thyroid Hormone-Mediated Spectral Identity and Cone Photoreceptor Function." Cell Rep 21(3):692–706. [PubMed: 29045837]

Sawant OB, Jidigam VK, Wilcots K, Fuller RD, Samuels I.and Rao S.(2020). "Thyroid Activating Enzyme, Deiodinase II Is Required for Photoreceptor Function in the Mouse Model of Retinopathy of Prematurity." Invest Ophthalmol Vis Sci 61(13): 36.

Schiff ER, Daich Varela M, Robson AG, Pierpoint K, Ba-Abbad R, Nutan S, Zein WM, Ullah E, Huryn LA, Tuupanen S, Mahroo OA, Michaelides M, Burke D, Harvey K, Arno G, Hufnagel RB and Webster AR (2020). "A genetic and clinical study of individuals with nonsyndromic retinopathy consequent upon sequence variants in HGSNAT, the gene associated with Sanfilippo C mucopolysaccharidosis." Am J Med Genet C Semin Med Genet 184(3): 631–643. [PubMed: 32770643]

Sun A.(2018). "Lysosomal storage disease overview." Ann Transl Med 6(24): 476. [PubMed: 30740407]

Tse DY, Lotfi P, Simons DL, Sardiello M.and Wu SM (2015). "Electrophysiological and Histological Characterization of Rod-Cone Retinal Degeneration and Microglia Activation in a Mouse Model of Mucopolysaccharidosis Type IIIB." Scientific Reports 5(1): 17143. [PubMed: 26607664]

Van Cauwenbergh CK., Van Schil, Cannoodt R, Bauwens M, Van Laethem T, De Jaegere S, Steyaert W, Sante T, Menten B, Leroy BP, Coppieters F and De Baere E (2017). "arrEYE: a customized platform for high-resolution copy number analysis of coding and noncoding regions of known and candidate retinal dystrophy genes and retinal noncoding RNAs." Genet Med 19(4): 457–466. [PubMed: 27608171]

Welling L, Marchal JP, van Hasselt P, van der Ploeg AT, Wijburg FA and Boelens JJ (2015). "Early Umbilical Cord Blood-Derived Stem Cell Transplantation Does Not Prevent Neurological Deterioration in Mucopolysaccharidosis Type III." JIMD Rep 18: 63–68. [PubMed: 25256447]

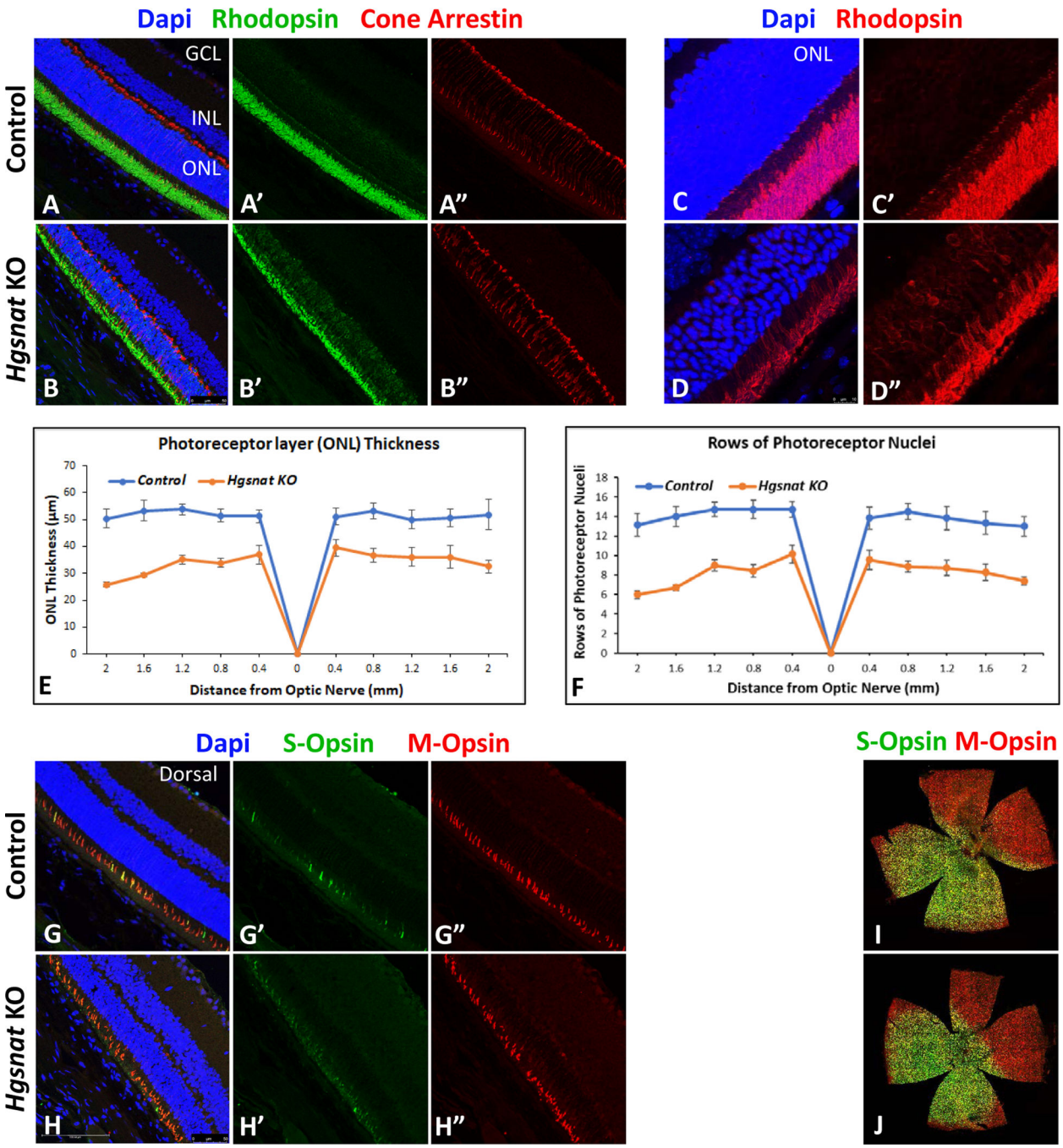


**Figure 1. *Hgsnat* KO mice exhibit rod photoreceptor degeneration phenotype at 6 months of age.**

At 6 months of age, *Hgsnat* KO mice exhibited approximately 50% reduction in the rod photoreceptor density (A', B') while cone photoreceptors (A", B") are mainly unaffected. High magnification images demonstrated reduced levels of rod pigment rhodopsin in *Hgsnat* KO retinas (D, D') compared to the control retinas (C, C'). (E, F) Graphs indicating significant reduction in the photoreceptor layer (ONL) thickness and number of photoreceptor nuclei in the *Hgsnat* KO retinas. At 6 months of age, *Hgsnat* KO mice exhibited fairly normal cone photoreceptor (G) density compared to the control (H) animals.

Cross-sections of the mouse eyes were labeled with two types of cone photopigments, S-opsin (short wavelength) and M-opsin (medium wavelength). (I-J) In the mouse retina, dorsal (superior) portion is devoid of S-opsin and enriched with M-opsin. On contrary, ventral (inferior) portion of the retina is enriched with S-opsin and relatively sparse in M-opsin expression. Overall gradient and density of the cone opsins were unaffected in the *Hgsnat* KO animals compared to the control animals. Error bars are ± SEM. Sample size = 7 eyes/group.

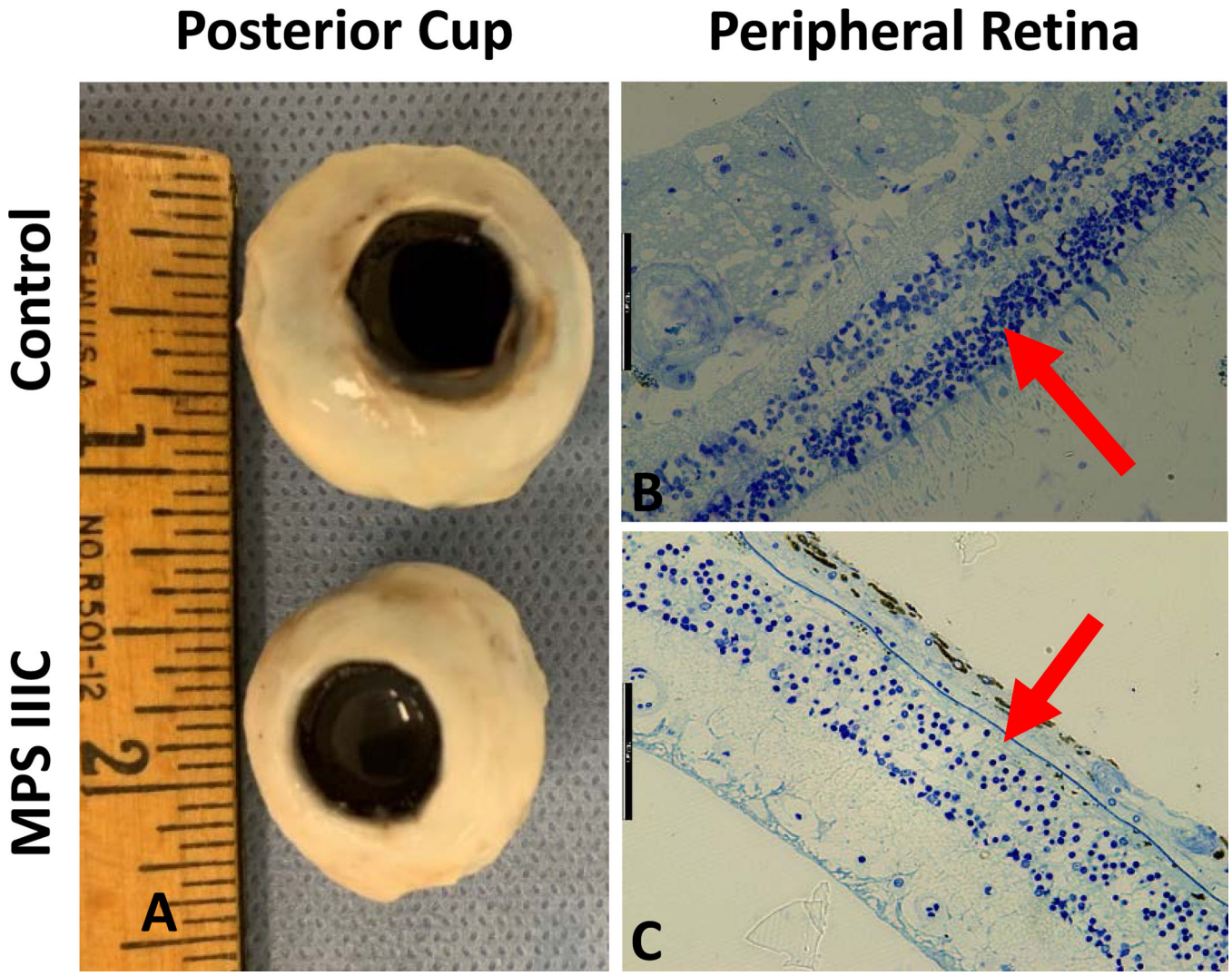


**Figure 2. Disease manifestations in MPS IIIC donor eye.**

Gross anatomical images of posterior eye cups from race, sex, and age-matched control and MPS IIIC donors demonstrating size difference (**A**). Peripheral retinal thickness of photoreceptor layer (red arrows) was significantly reduced in the MPS IIIC eye (**C**) compared to the control (**B**). Scale bar = 100μm