C57BL/6 (B6) congenital anomalies and strain associated phenotypes - are there grey areas?

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Keywords: Hydrocephalus, Microphthalmia, Portosystemic Shunts.

C57BL/6 (B6) mice are the most widely used mouse strain in research. They are great breeders, live long, have a low susceptibility to neoplasia, they are immunocompetent and seemingly resistant to some infections and are the first inbred mouse strain to have their genome sequenced.[1]

B6 mice are predisposed to a variety of congenital anomalies and have known strain associated phenotypes, which include several conditions including hydrocephalus, microphthalmia and portosystemic shunts, among many others. This presentation will focus on hydrocephalus and microphthalmia as well as a review of the recent literature on portosystemic shunts in B6J mice.

1. Hydrocephalus

Hydrocephalus in B6 mice can be observed in young mice and in most cases are recognised by the age of weaning, as hydrocephalus develops before the calvarium sutures fuse. Often breeding institutions will cull the parents of affected mice. In some colonies, prevalence may be up to 10%[2] , although Jackson Laboratories reports hereditary hydrocephalus around 1-4%.[1]

Hydrocephalus is caused by a complex interaction of many factors and is known to be a complex polygenic trait with multifactorial pathogenesis.[3] Excessive amounts of cerebrospinal fluid (CSF) accumulates in the brain ventricles, leading to dilatation and subsequent compression and damage of brain parenchyma.[3] Clinical signs include an enlarged dome-shaped head, seizures, reduced mental ability and death.[3] Normal CSF flow travels from the production site at the choroid plexus from the lateral ventricles to the third ventricle and then to fourth ventricle through the aqueduct of Sylvius. Thereafter from the fourth ventricle to subarachnoid space via the foramina and then drained via nasal lymphatics or absorption via the arachnoid villi. Movement is helped along by pulsations of the choroid plexus and by the beating of motile cilia, which are on the apical surface of ependymal cells.[3]

Hydrocephalus may be initiated by one of four factors[3]

- Aqueduct blockage (called non-communicating hydrocephalus), which is the most common
- Impaired CSF flow
- Reduction in CSF absorption (communicating hydrocephalus)

- Excess CSF production.

In mice, hydrocephalus is considered to probably be caused due to the dysfunction of motile cilia in many lines, which is therefore known as motile ciliopathy. Cilia are composed of microtubules and sit on the apical surface of most cells as either motile cilia (respiratory system, reproductive tract, brain and embryonic node in the embryo) or non-motile sensory cilia (present on most cells).[4] If there is inadequate ependymal flow (movement of CSF) due to defective motile cilia movement, the aqueduct may not develop normally nor stay patent, leading to stenosis and thereafter hydrocephalus.[4]

Hydrocephalus can be caused by various reasons, however if other ciliopathy associated conditions are present a motile ciliopathy is highly likely. Other concurrent conditions may include rhinosinusitis, spermatozoa that are defective or situs inversus.[4]

What about the grey area?

- It is thought that B6 mice probably carry mutated alleles that predispose them to motile ciliopathies and hydrocephalus. Further research is needed.

2. Microphthalmia

Microphthalmia, a small, underdeveloped eye(s), can be seen in young mice. There seems to be a predilection for the right eye (5.8x), for unknown reasons, to be more commonly affected than the left, and microphthalmia is seen more commonly in females (6.2x). [2,5,6] One source affirms that incidence may be as high as 12%[7] while others report 5-10%.[8,9]

Mesodermal dysgenesis syndrome, stemming from problems in the optic cup and lens vesicle formation, seem to be responsible for microphthalmia induction.[4] The developmental period between day 8 of gestation (optic vesicle development) and foetal fissure closure at day 12 seem to be the most important period involved in abnormal development, with subsequent microphthalmia.[10] If tear drainage is also affected, in severely microphthalmic mouse eyes, then secondary bacterial dacryocystitis or ocular infection is possible,[4]even with retrobulbar abscess formation[9]

Gross examination can be used to diagnose microphthalmia. Corneal opacities and cataracts can be examined by slit lamp biomicroscopy.[6]

Histopathology shows variability in presentation, from severe anophthalmia to microphthalmia. Foetal alcohol exposure or maternal vitamin A deficiency in humans[8], during critical developmental points in embryogenesis (optic cup and lens vesicle development), seem to worsen presentation and incidence. B6 mice have also served as a model for Foetal Alcohol Syndrome[6], with a 4x elevation in microphthalmia/anophthalmia. Additional pathologies associated with microphthalmia include central corneal opacity, iridocorneal and corneal-lenticular adhesions, ciliary

body and iris malformations, lens degeneration (cataracts), lens cortical material extrusion, vitreous body failure of development and folding of the retina.[7,9]

What about the grey areas?

- It is unknown why the right eye is more commonly affected, as well as why females present with microphthalmia more readily.
- B6 mice are commonly used for eye studies and spontaneous lesions should not be overinterpreted.

3. Portal Vein Hypoplasia

Portal vein hypoplasia (PVH) is the histologic indicator of intra- or extrahepatic portosystemic shunting (PSS). In C57Bl/6 and C57Bl/6 background mice, males are affected more frequently compared to females. Congenital PSS is believed to be from incomplete involution/closure of fetal vessels during embryonic development or after birth for the ductus venosus (intrahepatic). In a study of C57Bl/6J mice, the shunts resembled incomplete ductus venosus closure (intrahepatic shunts)[11] which in mice should be complete within 48 hours of life.[12]Some knockout mice may have increased frequency of PSS including aryl hydrocarbon receptor (Ahr), nuclear factor erythroid 2-related factor 2 (Nrf2) and fucosyltransferase 2 (Fut2) knockouts.[13]

Portal vein hypoplasia appears to be a polygenetic trait with variable expression. When comparing affected and unaffected mice, affected mice appear to be a bit smaller in size with smaller livers than their counterparts, but significant overlap exits between them.[14]Neurological signs or other clinical signs were not noted in the reported cases, although an abnormal neurochemical profile was reported.[11]The pathophysiology involves blood bypassing the liver, without filtering by the liver and removal of noxious substances and then entering systemic circulation.[11] If early assessment is desired, bile acid testing has been successful in identifying affected mice as young as 15 days old.[12]

A 2024 study reported a PSS incidence of 9.2%[15]while other authors concur, reporting approximately 1 out of 10 C57BL/6J mice are affected.[12]

Histologic appearance of PVH[14]

- Portal vein absence or small / slit-like vein
- Arteriolar reduplication (multiple hepatic arteriolar profiles) with frequent tunica media thickening
- Dilated periportal lymphatics
- Irregularly spaced small portal triads
- Mildly dilated hepatic sinusoids

- +/- periportal fibrosis, biliary ductular reaction, lipogranulomas
- Possible random, multifocal perivascular lymphocytes (rarely neutrophils) with focal loss or degeneration of hepatocytes
- Not all triads have to be affected, and it is important to look at multiple sections before ruling PVH out.

Guidelines for diagnosis

Testing for portal vein hypoplasia in mice is possible by performing blood total bile acids. Serum total bile acids >40uM serves as a useful marker for PVH.[12] This allows determination of non-affected compared to possibly affected mice.

If screening for total bile acids ante-mortally before starting a study is not possible, it is recommended to add additional mice to studies (e.g. 10%+) and then plan to remove any mice from the study after histopathology has determined which mice have portal vein hypoplasia. Otherwise, consider colorimetric assays like the phenol red assay.[12,15]

Shunting may not be visible grossly. Published studies have used specialized imaging techniques to visualize the shunts, which may be another advanced useful method.[11]

Additionally, urine-based detection has also been researched[12] and urinary iron-chelation assay, pH strip testing and the phenol red assay were investigated.

Research implications and confounders

- Smaller livers with rapid onset of cholestatic liver injury and hepatocellular carcinoma[12] due to an intolerance to purified food.
- Altered behavioural parameters[15] due to the abnormal neurochemical profile[11,12]
- Altered blood cell counts and blood biochemical parameters[15]
- Altered neurochemistry[15] with higher glutamine levels[16]
- Altered drug metabolism[15]
- Altered xenobiotic metabolism [15]
- Higher lipid content in liver[16] and lower fasting glucose levels[16]

What about the grey area?

Ongoing research is needed in PVH in mice and this condition may have gone unrecognized in studies over time, including the systemic effects of shunting physiologically and introduced experimental variation.

References

1. The Jackson Laboratory. C57BL/6J. 2025. *https://www.jax.org/strain/000664*. Retrieved June 4 2025.
2. Brayton C. MICE NON infectious Spontaneous disease and Background lesions. *22 Brayton 2 Mice NON infectious ECVP*. 2022. *https://www.ecvpath.org/sites/www.ecvdi.org/files/medias/documents/ECVP/22%20BRAYTON%20MICE%202%20NONinfectious%20POLA%20ECVP%200717.pdf*.
3. Vogel P, Read RW, Hansen GM et al. Congenital hydrocephalus in genetically engineered mice. *Vet Pathol* 2012;49:166–181.
4. Sundberg JP., Vogel Peter, Ward JMichael. *Pathology of genetically engineered and other mutant mice*. John Wiley & Sons, Inc., 2022.
5. Barthold SW, Griffey SM, Percy DH. *Pathology of Laboratory Rodents and Rabbits*. 4th ed. Wiley Blackwell, Ames, 2016.
6. The Jackson Laboratory. Microphthalmia and ocular infections in inbred C57 black mice. 1995. *https://www.jax.org/news-and-insights/1995/october/microphthalmia-and-ocular-infections-in-inbred-c57-black-mice*. Retrieved June 4 2025.
7. Smith R, Roderick T, Sundberg J. Microphthalmia and associated abnormalities in inbred black mice. *Lab Anim Sci* 1994;44:551–60.
8. Harding P, Cunha DL, Moosajee M. Animal and cellular models of microphthalmia. *Therapeutic Advances in Rare Disease* SAGE Publications Ltd, 2021.
9. Zeiss CJ. Translational models of ocular disease. *Vet Ophthalmol* 2013:15–33.
10. Ladd Beaumont S. Ocular disorders of pet mice and rats. *The Veterinary Clinics Exotic Animal Practice* 2002;311–324.
11. Cudalbu C, McLin VA, Lei H et al. The C57BL/6J Mouse Exhibits Sporadic Congenital Portosystemic Shunts. *PLoS One* 2013;8.
12. Yeoh BS, Golonka RM, Saha P et al. Urine-based Detection of Congenital Portosystemic Shunt in C57BL/6 Mice. *Function* Oxford University Press, 2023;4.
13. Meng L, Goto M, Tanaka H et al. Decreased Portal Circulation Augments Fibrosis and Ductular Reaction in Nonalcoholic Fatty Liver Disease in Mice. *American Journal of Pathology* Elsevier Inc., 2021;191:1580–1591.

14. JPC. Wednesday Slide Conference 2022-2023 Conference 15. *ASKJPC* 2022. *https://www.askjpc.org/wsco/wsc_showcase2.php?id=d0JZTTlwUktocHZtbmQzR2xaSS8vQT09*. Retrieved June 4 2025.

15. Lehtimäki KK, Rytkönen J, Pussinen R et al. Physiological and behavioural implications of the portosystemic shunt in C57Bl/6J mice. *J Physiol* 2024;602:5353–5373. *https://doi.org/10.1113/JP287237#support-information-section*.

16. Soares AF, Lei H. Non-invasive diagnosis and metabolic consequences of congenital portosystemic shunts in C57BL/6 J mice. *NMR Biomed* John Wiley and Sons Ltd, 2018;31.