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New pigmentation genes linked to iris transillumination defects in BXD glaucoma murine strains using systems genetics and mathematical modeling.

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Abstract

Pigmentation abnormalities are clinically significant; as they can lead to albinism, iris transillumination defects (TIDs), and potentially pigment dispersion glaucoma. We investigated the contributions of *Tyrp1* and *Gpnmb* to the TID in five age cohorts of BXD mice. Using systems genetics, we demonstrate for the first time the simultaneous contribution of multiple genes to the TID phenotype. The ability to separate the mice into five distinct age cohorts is one of the major highlights of the study that allowed us to study pigmentation genes as a function of age. Our model accounted for 71–88% of the explained variance of the TID phenotype across the age bins.

Keywords: iris transillumination defect, systems biology, genetic modulation, BXD, recombinant inbred strains, pigment dispersion glaucoma

The iris and iridial melanin are important influencers of vision. The mouse iris, due to its dense pigmentation, is unique and presents a powerful opportunity for studying pigment cell biology. Pigmentation abnormalities are clinically significant; as they can lead to albinism, iris transillumination defects (TIDs), and potentially pigment dispersion glaucoma. Despite its well-documented biological significance, there is a clear knowledge gap surrounding the basic molecular and cellular processes influencing pigmentation of the iris. It is a complex genetic trait controlled by an intricate network of genes whose coordination and regulation are poorly understood.

Over a decade ago, the John laboratory determined that mutations in two interacting genes—*Tyrp1* and *Gpnmb*—cause severe iris atrophy and pigmentary dispersion, respectively, in D2 mice (1-3). Other groups have documented that the expression of this phenotype is variable (4). In our recent paper published in Pigment Cell and Melanoma Research (http://onlinelibrary.wiley.com/doi/10.1111/pcmr.12106/abstract) (5), we hypothesized that other pigmentation genes modulate the primary causal genes (*Tyrp 1* and *Gpnmb*) to generate this variability. Our study utilized systems biology tools to assess the contributions of these new genetic modulators with an emphasis on known pigmentation genes. We used a large repository of phenotypic and genetic data along with the bioinformatics tools available on GeneNetwork.org (http://www.genenetwork.org/webqtl/main.py), for generating a mathematical model that accounted for 71–83% of the genetic factors modulating the TID. The parental strains from which the BXD recombinant inbred mice were generated—B6 and D2—vary in their degree of TID. B6 mice have no ocular aberrations, while D2 harbor mutations in *Tyrp1* and *Gpnmb* (1-3) both of which are required for manifestations of

severe and progressive TID. Because the BXD progeny segregate these and all other genes, the TID also segregates, thus providing a powerful genetic reference panel.

Interestingly, we found that *Tyrp1* contributed to the phenotype at all ages, yet the TID mapped to the gene *Gpnmb* only in the oldest cohort. Composite interval mapping revealed several secondary loci viz. *Oca2*, *Myo5a*, *Prkcz*, and *Zbtb20* that modulate the phenotype in the age groups up to 10–13 months. We accounted for 71–88% of the explained variance of the TID phenotype across the age bins leaving only approximately 10% of the genetic contribution toward the TID unaccounted for by these six genes. The contributions of *Tyrp1* and *Gpnmb* were highly significant in all age cohorts. Moreover, in young mice, all the six gene candidates had substantial interactions in our model. These results demonstrate that along with *Tyrp1* and *Gpnmb*; *Oca2*, *Myo5a*, *Prkcz*, and *Zbtb20* modulate the TID in an age-dependent manner.

The ability to separate the mice into five distinct age cohorts is one of the major highlights of the study that allowed us to study pigmentation genes as a function of age. Identifying the genetic modifier(s) regulating the disease during its progression would lead to new drug targets and therapeutic options.

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