Anterior segment dysgenesis and the developmental glaucomas are complex traits

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Glaucoma refers to a heterogeneous group of disorders that involve retinal ganglion cell death, optic nerve damage, and loss of visual field. Glaucoma is a leading cause of vision loss worldwide, affecting an estimated 67 million people. Elevated intraocular pressure is a major risk factor for glaucoma. Individuals with malformations of structures of the anterior segment of the eye frequently develop elevated intraocular pressure and glaucoma. In this review, we focus on the developmental glaucomas, the subset of glaucomas associated with anterior segment dysgenesis. To minimize overlap with other reviews in this issue and elsewhere, we highlight the complex, multifactorial nature of these diseases and recent advances using mice.

FEATURES OF GLAUCOMA
Glaucoma is a complex disease process involving loss of retinal ganglion cells, specific visual field defects, and degeneration of the optic nerve head (1). This degeneration produces a characteristic excavation or cupping of the optic nerve head. Glaucoma accounts for a significant proportion of blindness worldwide (2,3) and is the second leading cause of blindness in the United States (4). Elevated intraocular pressure (IOP) is a major risk factor for glaucoma, and experimental elevation of IOP causes glaucoma in animal models. Many forms of glaucoma exist and IOP is not the only important risk factor. Some individuals who have high IOP for extended periods do not develop optic nerve and retinal damage, while others develop nerve damage despite normal IOP values (1). The multiple factors contributing to IOP elevation and determining the level of susceptibility to neural damage in glaucoma are not well defined.

It is clear that many forms of glaucoma have a genetic component (5). Genes have been identified for primary open-angle glaucoma (6), normal tension glaucoma (7), and developmental glaucoma (see below). In this review, we focus on developmental glaucomas that are associated with malformations of the anterior segment of the eye.

ANTERIOR SEGMENT DYSGENESIS (ASD) IS A SPECTRUM OF DISORDERS
In various conditions involving anterior segment dysgenesis, multiple tissues are affected. These tissues include the iris, cornea, and lens. The Schlemm’s canal and trabecular meshwork drainage structures located at the anterior segment angle where the iris and cornea meet may also be affected. These abnormalities may result from a primary defect in the migration and/or differentiation of the mesenchymal cells that contribute to development of the cornea, iris, and drainage structures (8-10). Owing to important roles of the lens in anterior segment formation, these abnormalities also may result from primary defects in the lens (11).

In severe ASD, readily apparent dysgenesis (including iris hypoplasia, irregular and misplaced pupils, hazy corneas, and attachments of the iris to the cornea) can impede vision and have important cosmetic and accompanying psychological consequences. Traditionally, physicians have classified ASD into different subtypes based on the specific clinical phenotype. These subtypes include aniridia, Axenfeld’s anomaly, Rieger’s anomaly, iridogoniodysgenesis, Peters’ anomaly, and posterior embryotoxon. However, the presence or absence of specific phenotypes such as iridocorneal attachments and misplaced pupils varies considerably even between patients within a family. It is now evident that each of these disorders is part of a spectrum of expressivity of disease phenotypes (12-14).

Primary congenital glaucoma (PCG) is not traditionally grouped with other forms of ASD that associate with glaucoma because patients do not present with obvious malformations of readily visible anterior chamber structures such as the iris and pupil. In this review, we group PCG in the spectrum of ASD phenotypes because it involves abnormal development of the Schlemm’s canal and trabecular meshwork drainage structures, mutations in ASD genes sometimes cause PCG, and PCG genes can cause obvious ASD (see below).

MULTIPLE FACTORS CONTRIBUTE TO HUMAN ASD
Although ASD phenotypes often exhibit autosomal dominant inheritance, variable expressivity and incomplete penetrance
point to a complex etiology. Factors suggested to contribute to phenotypic variability include mutations in different genes, allelic heterogeneity, and genetic modifiers that differ between patients.

A number of genes cause ASD

Different genes are known to cause ASD in different patients. To date nine genes that affect ocular development are associated with ASD or glaucoma in humans (PAX6, PITX2, PITX3, FOXC1, FOXE3, EYA1, CYP1B1, LMX1B and MAF, see Table 1). There is evidence that even more genes are involved. Some families show linkage to other loci, and mutations in known ASD genes are not present in many patients indicating that other unidentified genes contribute to human ASD (15–17). As occurs in other diseases caused by multiple genes, some ASD genes may cause disease in only a small proportion of patients. The heterogeneity of genes causing ASD is only one factor contributing to phenotypic heterogeneity.

Specific mutant genes result in different phenotypes

Specific genes do not uniquely associate with specific phenotypes within the ASD spectrum. Patients with mutations in PAX6, PITX2, and FOXC1 have a range of ASD phenotypes (18–29). For example, patients with FOXC1 mutations have phenotypes including Axenfeld–Rieger anomaly, iridogoniodysgenesis, and PCG. Even mutant CYP1B1, which typically associates with PCG (16), can associate with Peters’ anomaly (30), with glaucoma of later onset than typical PCG (31,32), and is suggested to be a factor altering the presentation of adult-onset glaucoma (33). The fact that mutations in the same gene result in different phenotypes highlights the complexity of anterior segment development and its disturbances that result in ASD and glaucoma.

Possible factors underlying phenotypic heterogeneity

Why mutations in the same gene result in different phenotypes is not proven. There is evidence that the nature of the mutation affects the phenotype. Expression and analysis of different PITX2 mutations in COS-7 cells suggests that mutant PITX2 proteins that retain partial function result in milder ASD phenotypes than nonfunctional PITX2 (34). Interestingly, mutations that increase transactivation by PITX2 seem to cause severe dysgenesis (35) and a duplication involving FOXC1 (and possibly other contributing genes) associates with severe glaucoma in one family (36). Individuals with the same mutation can have different phenotypes, however, indicating that factors other than the specific mutation are also important (37).

Another possibility is that other genes interact with the known mutant gene to modify the phenotype, and that different patients have different alleles of these modifier genes. The best evidence for multigenic interactions and modifier genes is for PCG. Although sometimes considered to be a simple recessive condition, cases of both non-penetrance and variable expressivity underscore that PCG is multifactorial. Complete penetrance in families from a highly consanguineous population (38,39) and frequent reduced penetrance (sometimes 40 to 50%) in families from more outbred populations suggests that at least some of the multifactorial influence is genetic (31,32,38,40). A dominant modifier locus was proposed to influence the presence or absence of PCG in individuals homozygous for CYP1B1 mutations based on data from Saudi Arabian families but has not been identified (32).

INSIGHTS ABOUT THE MULTIFACTORIAL NATURE OF ASD FROM MICE

The many genetic differences between individuals and the effects of varying lifestyle and environment confound genotype to phenotype associations when studying multifactorial phenotypes in humans. Together with the typically small number of affected individuals in specific families, this makes it difficult to study and also to draw firm conclusions about the underlying complexity of multifactorial human phenotypes. An effective way to study these complex traits is with inbred mouse strains, where it is possible to dramatically reduce and highly control the number of genetic and environmental variables. The generation and study of mouse models with mutations in ASD genes is helping to understand the complex etiology of these diseases. These studies identify multigenic interactions, stochastic developmental events and likely environmental interactions with genotype as factors determining the final ASD phenotype. Additionally, they show that null mutations in some genes tend to associate with different severities of ASD independently of effects of other genetic differences.

Genetic modifiers contribute to phenotypic heterogeneity

Haploinsufficiency of mouse Foxc1 results in multiple, clinically obvious abnormalities, including eccentric irregularly shaped pupils and displaced Schwalbe’s line (41,42). The penetrance and nature of these abnormalities depends upon strain background. Importantly, there are consistent phenotypic differences between mice with the same Foxc1 mutation that are housed in the same highly controlled environmental conditions but that have different genetic backgrounds (42). For example, the pupil is irregular and misplaced in the majority of Foxc1+/− mice of the C57BL/6J (B6) background but in no Foxc1+/− mice of the 129S6/SvEvTac background (Fig. 1). Similarly, the iris is abnormal in Bmp4+/− mice of some but not other backgrounds (43), and sometimes Cyp1b1−/− mice (44) of a mixed background including CAST/Ei genes, but not other backgrounds, have irregular pupils (Fig. 1 and unpublished data). These observations clearly demonstrate that the effects of multiple genes interact to determine the phenotypic outcome, since strain specific modifier genes consistently alter the phenotype resulting from a particular mutation. Identification and characterization of these modifier genes will help to understand anterior segment development and will provide new insight about ASD.

Stochastic and/or environmental factors contribute to overall heterogeneity

A another important observation from Foxc1+/− and Bmp4+/− mice is that the phenotype resulting from the same mutation is
Table 1. Genes and candidate genes for ASD

<table>
<thead>
<tr>
<th>Mouse gene (Human)</th>
<th>Type</th>
<th>Mouse ocular phenotype Heterozygous</th>
<th>Human disease</th>
<th>Human location Chr cM OMIM number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atf4</td>
<td>Transcription factor</td>
<td>Normal (72) Microphthalmia/aphakia (72)</td>
<td>22q13</td>
<td>15 43 604064</td>
</tr>
<tr>
<td>Bmp4</td>
<td>Growth factor</td>
<td>ASD (43,73) Embryonic lethal (74)</td>
<td>14q22</td>
<td>14 15 112262</td>
</tr>
<tr>
<td>Bmp7</td>
<td>Growth factor</td>
<td>Normal (75,76) Anophthalmia/microphthalmia (75,76)</td>
<td>20q13</td>
<td>2 102 112267</td>
</tr>
<tr>
<td>Chx10</td>
<td>Transcription factor</td>
<td>Ocular retardation (77)</td>
<td>14q24</td>
<td>12 38 142993</td>
</tr>
<tr>
<td>Cyp1b1</td>
<td>Enzyme</td>
<td>Normala Iridode medial angle dysgenesisa</td>
<td>2p22</td>
<td>17 601771</td>
</tr>
<tr>
<td>Eya1</td>
<td>Nuclear protein</td>
<td>Open eyelids (80)</td>
<td>8q13</td>
<td>1 10 601653</td>
</tr>
<tr>
<td>Fox1</td>
<td>Transcription factor</td>
<td>ASD (41,42) Congenital glaucoma (79)</td>
<td>14q22</td>
<td>13 20 601090</td>
</tr>
<tr>
<td>Fox2</td>
<td>Transcription factor</td>
<td>Normal (87) Microphthalmia and ASD (66,87)</td>
<td>16q24</td>
<td>8 66 602402</td>
</tr>
<tr>
<td>Fox3</td>
<td>Transcription factor</td>
<td>Microphthalmia and ASD (dysegnetic lens) (85)</td>
<td>1p32</td>
<td>4 50 601094</td>
</tr>
<tr>
<td>Hesx1</td>
<td>Transcription factor</td>
<td>Anophthalmia/microphthalmia (86)</td>
<td>3p21</td>
<td>14 601802</td>
</tr>
<tr>
<td>Lmx1b</td>
<td>Transcription factor</td>
<td>Microphthalmia and ASD (66,87)</td>
<td>9q34</td>
<td>2 21 602575</td>
</tr>
<tr>
<td>Maf</td>
<td>Transcription factor</td>
<td>Microphthalmia (63)</td>
<td>16q23</td>
<td>8 61 177075</td>
</tr>
<tr>
<td>Mitf</td>
<td>Transcription factor</td>
<td>Hypopigmentationd (90)</td>
<td>1p14</td>
<td>6 40 156845</td>
</tr>
<tr>
<td>Pax6</td>
<td>Transcription factor</td>
<td>Microphthalmia (57)</td>
<td>1p13</td>
<td>2 58 106210</td>
</tr>
<tr>
<td>Pitx2</td>
<td>Transcription factor</td>
<td>ASD (94,95) Axenfeld-Rieger and ASD (24)</td>
<td>4q25</td>
<td>3 58 601542</td>
</tr>
<tr>
<td>Pitx3</td>
<td>Transcription factor</td>
<td>Apikia (96)</td>
<td>10q25</td>
<td>19 47 602669</td>
</tr>
<tr>
<td>RXRαe</td>
<td>Nuclear receptor</td>
<td>ASD, coloboma, rotated globe, other (97)</td>
<td>9q34</td>
<td>2 17 180245</td>
</tr>
<tr>
<td>Tcfap2a (TFAP2A)</td>
<td>Transcription factor</td>
<td>Normal (98,99) Anophthalmia/microphthalmia (65,98,99)</td>
<td>6p24</td>
<td>13 25 107580</td>
</tr>
</tbody>
</table>

A partial list of genes is presented, focusing on genes for which mutation of the endogenous gene has known ocular effects. A number of other genes that affect the behavior of neural crest cells, for example (101–104), or that contribute to roles of the developing lens in anterior segment formation may also prove important for ASD (11,105). Various transgenic mouse models also have ASD (for example, 106–108), as do mice with chromosomal deletions (109). In a number of cases, there are severe defects in homozygous mutants and it is possible that heterozygotes will have milder ASD phenotypes of more relevance to human ASD and glaucoma. In most cases, heterozygotes were reported to be grossly normal but detailed analysis of the angle and drainage structures was not performed and the effects of altering genetic background not always assessed. Abbreviations are as follows: OMIM Online Mendelian Inheritance in Man (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM); Atf4 activating transcription factor 4; Bmp4 and 7 bone morphogenetic protein 4 and 7; Chr chromosome; Ceh-10 homeodomain containing homolog; cM centiMorgan; Cyp1b1 cytochrome P450 1b1; Eya1 eyes absent 1 homolog; FoxC1, C2, and E3 forkhead box C1, C2, and E3; Hes1 homeobox gene expressed in ES cells; Lmx1b LIM homeobox transcription factor beta; Maf avian musculoaponeurotic fibrosarcoma (v-maf); Mitf microphthalmia-associated transcription factor; Pax6 paired box gene 6; Pitx2 and 3 paired-like homeodomain transcription factor 2 and 3; RXRα retinoid X receptor, alpha; Tcfap2a transcription factor AP-2, alpha. Where the human gene name differs from the mouse, it is included in parentheses.

aIndicates the mi allele of Mitf.
bFOXC2 was associated with some cases of human lymphedema-distichiasis syndrome. Owing to the potential effects of genetic background on phenotype, this does not rule it out as a human ASD candidate.

cIndicates the mi allele of Mitf.
dThere are different subtypes of retinoid receptors that belong to the retinoic acid receptor class (RARz, RARβ, and RARγ) or the retinoid X receptor class (RXRα, RXRβ, and RXRγ). Additionally, there are multiple isoforms of these receptor subtypes and different receptors often bind to target genes as heterodimers (111,112). This complexity and functional redundancy protects from drastic developmental effects of a mutation in a single retinoic acid receptor gene. Importantly: roles of a number of these genes in ocular development are revealed in double mutants (112–115). Although double null mutants are severely affected, it seems likely that more subtle mutations may contribute to ASD and the eyes of single mutant adult mice need thorough ocular evaluation. Additionally, the genotype for a specific receptor subtype may modify phenotypes resulting from mutations in different ASD genes (including other retinoic acid receptor genes). Deficiency of vitamin A often leads to ocular malformation and so dietary vitamin A levels may also interact with these factors to modify the phenotype.
variable between mice of the same inbred strain that are essentially genetically identical (42,43). For example, the anterior chamber of Bmp4+/− mice of the B6 background varies from no obvious dysgenesis to severely abnormal with irregular misplaced pupils and scleralization of the cornea (Fig. 2). Furthermore, not all mice with clinically obvious iris malformations are affected bilaterally (42,43). Since the eyes of mice of the same inbred strain and of individual mice are genetically identical, the differences in phenotype must be attributable to non-genetic factors. Stochastic developmental events and/or the local environment during development may influence the outcome for each eye.

Wild-type B6 mice are weakly susceptible to very severe anterior segment dysgenesis, microphthalmia and anophthal-mia. The etiology of these abnormalities is complex, with incidence varying between mouse colonies (<1 to 12% of mice) (45). Although not the case for Foxc1 and Bmp4, there is a strong bias for these abnormalities to occur in the right eye (45). The reason for this bias is not understood but may reflect environmental differences between the developing eyes. Importantly, these lesions are more common in females (45) and their incidence is greatly increased by environmental factors such as ethanol administration at specific stages of gestation (46). Other genetically distinct strains are not as susceptible to ethanol-related abnormalities, indicating that interactions between environment and a susceptible genotype are important (46).
Stochastic events lead to heterogeneity within individual eyes.

Phenotypic variability also occurs within an individual eye. Both Foxc1 and Bmp4 heterozygous mutant mice have dysgenesis of the ocular drainage structures in the anterior chamber angle (42,43) (Fig. 2). The dysgenesis is focal rather than affecting 360 degrees of the angle. Within an eye there are isolated regions of normal and abnormal trabecular meshwork and Schlemm's canal, and the severity of dysgenesis varies between abnormal regions. Stochastic developmental events must explain the intermittent nature of drainage structure anomalies. With current knowledge, all possible explanations are speculative. Among the possibilities are stochastic developmental events related to the timing and level of expression of important developmental control genes. It is possible that decreased steady-state levels of FOXC1 or BMP4 decrease the probability that an important downstream regulator(s) of development is correctly activated at the appropriate time, and in the correct location. Phenotypic variability may result from correct activation of this regulator in some groups of cells and incorrect or no activation in groups of cells at separate locations.

**MULTIPLE FACTORS CONTRIBUTE TO IOP ELEVATION**

Many patients with ASD develop elevated IOP, a major risk factor for glaucoma (47). The severity of clinically visible dysgenesis does not correlate with IOP, however, and the etiology of glaucoma development is not understood.

To facilitate mouse studies of IOP elevation, we determined the anatomy and developmental sequence of the anterior chamber angle drainage structures (48). With this information, we evaluated the angle structures in Foxc1, Foxc2 (42), Bmp4 (43), and Cyp1b1 (unpublished data) mutant mice and identified malformations in all cases. These abnormalities include small or absent Schlemm's canal and aberrantly developed trabecular meshwork. As discussed above, these defects are focal and affected by multiple factors. Together, our results are showing that angle dysgenesis presents as a complex, quantitative trait, which can cause elevated IOP after reaching a certain severity. For example, Bmp4 mutant mice with 80% or more of the circumferential drainage structures affected had high IOP. Mice with less extensive abnormalities had normal IOP. That drainage structure malformations within an eye can be focal may partially explain why some patients develop early-onset IOP elevation while others do not.

Another important result is that genetic factors that modify clinically obvious dysgenesis of the iris do not modify drainage structure malformations (42). Foxc1+/− mice of some genetic backgrounds are highly susceptible to clinical abnormalities of the iris whereas Foxc1+/− mice of other backgrounds do not develop these iris abnormalities. The extent or severity of the drainage structure malformations in Foxc1+/− mice does not alter between backgrounds that are susceptible or resistant to iris abnormalities (42). That factors altering clinically obvious dysgenesis are separable from those affecting the drainage structures helps to explain why the severity of clinically detected dysgenesis does not correlate with IOP elevation in many patients.

Many ASD patients do not develop elevated IOP in infancy. Their IOP becomes elevated in late childhood or adulthood (12). Thus, the extent of developmental drainage structure malformations cannot explain all aspects of IOP elevation. It is possible that there are metabolic abnormalities in the cells of the drainage structures. These abnormalities may extend to the tissue that appears to have developed normally. The extracellular matrix of the drainage structures is important in determining resistance to drainage (49), and mutation-induced cellular abnormalities may result in altered extracellular matrix synthesis or metabolism that results in IOP elevation over time. Alternatively, metabolic abnormalities or simply the reduced amount of functional drainage tissue may predispose affected human eyes to IOP-elevating effects of environmental factors or aging. The development and study of mouse models of ASD with progressive IOP elevation will likely be valuable in understanding the etiology of IOP elevation in these patients.

Factors affecting the ciliary body likely contribute to the complex interactions affecting IOP. In addition to producing the aqueous humor, the ciliary body secretes antioxidant enzymes into the aqueous that may protect the TM from damage that could elevate IOP. The ciliary body may also act as a neuroendocrine tissue secreting various molecules that have a paracrine effect on the drainage tissues (50). Obvious ciliary body abnormalities were detected in some mice with ASD (42,43) and more widespread cellular abnormalities may be present. Thus, the combination of both ciliary body and drainage angle phenotypes may contribute to variability in the age of onset or presence of IOP elevation. The study of mouse mutants affecting anterior segment development will help to address this possibility.

**RELEVANCE OF MULTIFACTORIAL COMPLEXITY WHEN ASSESSING CANDIDATE GENES**

Due to the complexity of ASD, several points are important when considering candidate genes. First, it is evident that ASD phenotypes are very sensitive to the level of gene activity and gene dosage (21,35,36,42,43,51,52). To assess fully the role of a specific gene in disease it is therefore important to assess regulatory regions and to check for duplications or deletions that can be missed using some approaches (21,36). Second, since mutations in specific genes may contribute to disease in a small proportion of patients and the relative importance of specific genes will differ between different patient populations, it will be important to evaluate the gene in significant numbers of patients and in different populations (53). Third, if the gene in question causes ASD in an animal model but is not found by itself to cause human ASD it cannot be discounted as a risk factor for human disease. Some genes will alter the phenotype resulting from a mutation in another gene. Assessing patients with mutations in known ASD genes for mutations in other genes may reveal important interactions. Fourth, the complexity of ocular development necessitates flexibility when defining human candidates based on mouse models. Owing to the complexity of ocular development, mouse phenotypes may not be exactly the same and may have features not typically present in human patients. For example, on the B6 background Bmp4+/− mice have optic nerve and other posterior segment...
DEFINING ASD AND GLAUCOMA GENES AND PATHWAYS

Experiments in mice and other species provide information about developmental roles and pathways affected by ASD genes, and complement human studies (54–56). Mice are useful to identify new genetic causes of ASD by testing candidate genes. Abnormalities in mouse models have supported the search for mutations in specific human candidate genes and led to the identification of human mutations (11,22,57–59). Candidate gene approaches based on known roles in development and expression patterns have identified two new ASD genes, Foxc2 and Bmp4, that cause dominant ASD phenotypes in mice and are candidates to assess in human patients (42,43). The identification of ASD genes can implicate other genes as potential candidates. For example, developmental and signaling roles of BMP4 have been studied intensely, and so a number of genes related to BMP function (including multiple BMP antagonists, receptors, and SMADs) are potential candidates for involvement in ASD (60,61).

Table 1 presents a list of genes that are known to cause ASD or are candidates that need to be assessed. Included are a number of cases with severe defects in homozygous mutants. As for Pax6 and Foxc1, even though homozygous mutants have very severe developmental abnormalities or do not survive, heterozygotes may have ASD and IOP phenotypes that are relevant to human ASD. Further analysis of these mutants, focusing on heterozygotes, will be important. Additionally, the effects of a number of these mutations on the ocular drainage structures and IOP are not yet reported.

Animal models and culture systems are also being used to assess the effects of specific mutations on the expression of other developmentally important genes in an attempt to characterize developmental pathways (42,62–66). Importantly, many of the currently known ASD genes are transcription factors. The search for genes both regulating and regulated by these transcription factors is important. Recently, microarray technology has been employed to aid in the identification of genes downstream of Pax6 (67).

Modifier gene identification and the generation of new mutants using mutagens will also help to define ASD and glaucoma pathways (68,69). CYP1B1 is an enzyme whose role in ocular development is not understood. However, modulated by a developmental signaling pathway. Cyp1b1<sup>-/-</sup> mice provide an opportunity to search for strain-specific modifier genes and to perform a sensitized mutagenesis screen to characterize pathways affecting PCG. Interestingly, CYP1B1 oxidizes all-trans-retinol to all-trans-retinol, the rate-limiting step for retinoic acid biosynthesis, and retinoic acid receptor mutations cause ASD (70,71). Testing the effects of existing mutations on ocular development and IOP in Cyp1b1 mutant mice may also be of value.

CONCLUSION

ASD and glaucoma are multifactorial phenotypes. The mouse provides an experimental, mammalian platform to complement human studies in advancing understanding of the complex etiology of these conditions.

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