Genetic causes of optic nerve hypoplasia

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Abstract:

Optic nerve hypoplasia (ONH) is the most common congenital optic nerve anomaly and a leading cause of blindness in the USA. Although most cases of ONH occur as isolated cases within their respective families, the advancement in molecular diagnostic technology has made us realise that a substantial fraction of cases has identifiable genetic causes, typically de novo mutations. An increasing number of genes has been reported, mutations of which can cause ONH. Many of the genes involved serve as transcription factors, participating in an intricate multistep process critical to eye development and neurogenesis in the neural retina. This review will discuss the respective genes and mutations, human phenotypes, and animal models that have been created to gain a deeper understanding of the disorders. The identification of the underlying gene and mutation provides an important step in diagnosis, medical care and counselling for the affected individuals and their families. We envision that future research will lead to further disease gene identification, but will also teach us about gene–gene and gene–environment interactions relevant to optic nerve development. How much of the functional impairment of the various forms of ONH is a reflection of altered morphogenesis versus neuronal homeostasis will determine the prospect of therapeutic intervention, with the ultimate goal of improving the quality of life of the individuals affected with ONH.

Introduction:

The optic nerve, which is composed of retinal ganglion cell axons and supporting glial cells, transmits visual information from the retina to the brain. Optic nerve hypoplasia (ONH) is a non-progressive congenital abnormality characterized by underdevelopment of the optic nerve. Typically, the anomalous optic nerve head appears pale and small, with a pale or pigmented peripapillary halo or double-ringed sign that is visible with ophthalmoscopy. ONH may occur bilaterally (in both eyes) or unilaterally (in one eye), although it is more commonly bilaterally (80%). Individuals with ONH manifest different levels of visual impairment, which spans a wide range. Some eyes with ONH have no light perception, while others have acceptable levels of functional vision. Severe bilateral ONH can be diagnosed within the first few months of life. Affected patients typically manifest early-onset sensory nystagmus (involuntary, rapid eye movements), followed by strabismus (inability to

align the eyes simultaneously) often related to the visual impairment due to defective communication between eye and brain. Individuals with unilateral ONH are often diagnosed at an older age, compared to those with bilateral ONH, due to preserved vision in the one normal eye.

The first recorded case of ONH was documented in 1884 in a small child by Magnus[1]. Magnus noticed a small, pale optic nerve in a boy with nystagmus and a high degree of amblyopia. However, some researchers considered Briere's report to be the first case in 1877[2]. He described a 7-year-old girl who was born blind, with no observed or documented optic disc, but normal choroid and central retinal vessels. The first schematic illustration of optic disc appearance of ONH was done by Schwarz in 1915[3]. To date, OHN is the third leading cause of blindness and is the most common congenital optic nerve anomaly in the United States[4,5]. The reported prevalences of ONH ranges from 2-17.3 per 100,000[6-11], with a considerable rise in prevalence starting in the 1980s. The cause of this increase remains disputed; however, it may be due to heightened physician awareness and improved observation and thus diagnosis of the disorder. Given that most ONH cases are isolated within their respective families, research into the causal factors of ONH has focused on environmental effects. Many environmental risk factors for ONH have been reported, including maternal alcohol abuse during pregnancy[12,13], young maternal age, primiparity[14-16], maternal use of recreational drugs[10,17], anticonvulsants[18], or antidepressant[19,20]. However, familial cases of ONH also exist[21,22], suggesting genetic etiologies. With the recent advancement in molecular diagnostic technology, mutations in an increasing number of genes have been reported to cause ONH.

Although rare, the description of ONH may sometimes be confused with optic atrophy[23,24], especially when serial examinations have not been done, and the examiner cannot appreciate whether there has been a progression or degeneration over time. Optic atrophy is a condition in which the optic nerve tissues had developed normally and either are damaged and/or degenerate secondarily.

Although ONH can occur as an isolated finding, it is seen much more frequently as part of a syndrome. ONH is usually accompanied by other anterior segmental defects of the eye (OMIM 607108)[25], structural abnormalities of the brain, hypopituitarism (OMIM 184429)[26] , developmental delay/intellectual disability[15,27], and autism spectrum disorders[28-30]. This review will focus on the genetic mutations that are currently known to cause ONH. Herein, we will define ONH broadly as any optic disc that shows decreased neuronal area and small optic nerves, congenitally. The reviews of the more narrowly defined septo-optic dysplasia, also known as DeMorsier syndrome, can be found elsewhere[23,31,32].

To date, variants in genes involved in transcription regulation, chromatin remodeling, α-dystroglycan glycosylation, cytoskeleton and scaffolding protein, RNA splicing, and the MAP kinase signaling pathway, have been associated with ONH. In the first part of the review, we will introduce the basic functions of the genes

involved, the clinical features of the affected individuals, and the animal models used to study the respective disorders. In the second part of the review, we will delineate the common developmental pathway shared by some of the key transcription factors in eye development, and we will discuss briefly the genes associated with optic nerve atrophy.

Transcription factors

HESX1

HESX1 homeobox 1 (*HESX1*) encodes a homeobox protein, which plays an important role in normal forebrain development[33] and the early determination and differentiation of the pituitary gland[34] in murine models. Dattani et al. reported the first genetic mutation in *HESX1* (homozygous p.R160C) in a familial case (siblings from a consanguineous family) of Septo-Optic Dysplasia (SOD)[35]. SOD (MIM 182230) is defined by any combination of i) ONH, ii) pituitary hypoplasia, and iii) midline neuro-radiological abnormalities, such as defects in the corpus callosum and septum pellucidum. Although the diagnosis of ONH in the study of Dattani et al. remained controversial[31,36], subsequent novel heterozygous *HESX1* mutations were identified in patients with ONH diagnosed with combined pituitary hormone deficiency[37] or SOD[38]. In addition, *HESX1* constitutive knockout mice (*Hesx1*$^{-/-}$) show substantial perinatal and postnatal lethality and display variable anterior CNS defects and pituitary dysplasia. Anophthalmia, microphthalmia, defective olfactory development, and abnormalities in the corpus callosum, hippocampal commissures, and septum pellucidum were described in *Hesx1*$^{-/-}$ mice, while the milder phenotype was found at very low frequency in *Hesx1*$^{+/-}$ mice (1%)[35].

PAX6

Paired box gene 6 (*PAX6*) encodes a transcription factor that is involved in eye morphogenesis during embryonic development[39]. It was first implicated in human aniridia[40,41] but was later found to be involved in other eye anomalies, including microphthalmia, cataracts, foveal hypoplasia, and ONH (OMIM 607108). Although ONH is not common in individuals with *PAX6* mutations, Azuma et al. reported heterozygous *PAX6* mutations (p.Q205X, p.S292I, and p.M381V) in three individuals with ONH, one of whom had developmental delay and intellectual disability. In the same study, they also identified heterozygous *PAX6* mutations (p.Q378R and p.T391A) in two patients with optic nerve agenesis[25]. Melanie et al. summarized ophthalmologic evaluations in 43 individuals with variable anomalies of the iris, carrying heterozygous mutations in *PAX6*. They reported that nystagmus and foveal hypoplasia were the most common clinical findings, present in 41 and 37 individuals, respectively. In comparison, ONH was found in 10 individuals in this cohort, most of whom carried frameshift mutations[42]. Given that PAX6 is also a master regulator of neurogenesis[43], abnormalities other than ocular defects have also been reported, including developmental delay[25], cognitive impairment, autism, epilepsy, and structural brain abnormalities of white matter, mostly the corpus callosum[44-47].

To date, more than forty Pax6 mutant mouse models are available (Mouse Genome Informatics (http://www.informatics.jax.org/)), most of which have phenotypes affecting the eyes/vision, the nervous system, craniofacial development, and/or mortality. One of the first characterized Pax6 mouse models is the *Sey/Sey* (Small eye) mouse. *Sey/Sey* pups are born without both eyes and nasal cavities, and they have a high prevalence of perinatal mortality due to breathing problems, associated with the absence of the nose[48]. Later studies showed the homozygous knockout animals also had forebrain defects [49]. Heterozygous *Sey/+* mice have milder phenotypes. These mice display microphthalmia, frequently with cataracts, which manifest within a few weeks of age, retinal abnormalities, and partial or complete absence of the iris[50]. The morphogenesis of the optic nerve is also affected in *Sey/+* mice. The cross sectional area and the myelinated fiber counts of optic nerves are decreased significantly in heterozygous mice, although the effect is more severe in male mice when compared to female heterozygous mice; the mechanism is unclear[51].

Similar to the mouse model, a *Pax6* mutant rat model, *rSey2* (rat Small eye), shows that homozygotes (*rSey/rSey*) do not develop lens and nasal placodes and are perinatal lethal, while heterozygotes (*rSey/+*) have small eyes[52]. Later in life, heterozygotes are shown to have impaired prepulse inhibition, altered social interaction, and low performance in fear-conditioned memory tests. Thus the authors suggested that the heterozygotes likely have some phenotypic components of autism[53].

The pleiotropic role of PAX6 during development can be appreciated through the detailed investigation of several *Pax6* mutant mouse models. Pax6 consists of three functional domains: the paired domain (PD), the homeodomain (HD), and the transactivating Proline-Serine-Threonine domain (PST). Pax6 mutations in different functional domains yield different phenotypes. For example, Haubst et al. compared the $Pax6^{4Neu}$ mutants (HD domain mutation) and $Pax6^{Aey18}$ mutants (PD domain mutation), and found out that while the former had only subtle effects on forebrain development, the latter had severe impairment of neurogenesis, cell proliferation, and patterning in the developing forebrain[54]. The molecular consequences of $Pax6^{Leca2}$ and $Pax6^{Leca4}$ mutants, which carry point mutations in two different subdomains within the PD domain, also differed greatly. While $Pax6^{Leca2}$ mutants increased the number of mitoses in the developing cerebral cortex, $Pax6^{Leca4}$ mutants showed the opposite. Moreover, neurogenesis was only affected in $Pax6^{Leca4}$, but not $Pax6^{Leca2}$ mutants[55]. Acknowledging that the total number of unique human *PAX6* variants reported to date is more than 400 (Leiden Open Variation Database (http://lsdb.hgu.mrc.ac.uk/home.php?select_db=PAX6)), and recognizing the diversity of clinical phenotypes in human patients, one needs to consider carefully the specific mutation to provide accurate diagnosis, counseling, and management.

SOX2

SRY (Sex determining region Y)-box 2 (*SOX2*) encodes a transcription factor that is essential for embryonic development of multiple organs, including the brain and the eyes[56]. Heterozygous *SOX2* mutations are the most common genetic cause for bilateral anophthalmia and severe microphthalmia. They account for a total of 10-20% of all patients with microphthalmia/anophthalmia[57-59]. Among 235 individuals diagnosed with congenital hypothalamic-pituitary disorders, Kelberman et al. reported two patients (patient 7; p.G130A and patient 8; p.A191T) with bilateral ONH and nystagmus[26]. The authors discussed the possible neutral nature of these two *SOX2* variants, given that both were inherited from unaffected fathers, but it should be noted that incomplete penetrance for *SOX2* mutations has been reported elsewhere[60]. In the same study, another six patients with microphthalmia/anophthalmia were found to carry *de novo SOX2* mutations, and four out of five, for whom MRI data was available, showed either hypoplastic optic nerves or complete agenesis of the optic nerves. Other phenotypes shared by these patients included hippocampus and structural anomalies of the corpus callosum. Bakrania et al. reported a female patient (case 2; p.N63fs101X) with ONH in one eye, and cataract, atypical coloboma, and esotropia in the other eye. Other extraocular abnormalities included a thin corpus callosum. Ragge et al. reported two patients (case 6; L314fs436X and case 7; G23fs85X) with similar phenotypes: bilateral anophthalmia, attenuated optic nerves without visible chiasm, global developmental delay, and malformation of the hippocampus[61]. Jayakody et al. reported a patient (patient 1; p.L97P), diagnosed with bilateral microphthalmia, left sclerocornea, and aphakia. MRI data showed right microphthalmos with a prosthesis *in situ*, a colobomatous left globe, and small optic nerves and chiasm[62].

Homozygous knockout of *Sox2* causes embryonal lethality in mice, with the products of conception dying shortly after implantation. Heterozygous knockout mice appear normal, except for a noticeable reduction in male fertility[63]. Although heterozygous knockout mice do not seem to recapitulate the human eye phenotypes, *Sox2* hypomorphic/null compound heterozygous adult mice (expressing 40% of *Sox2* compared to wild type) show hypoplasia of optic nerves and chiasmata, with eye phenotypes ranging from mild bilateral microphthalmia to severe anophthalmia[56], much like heterozygous patients. The authors argued that the striking similarity between heterozygous patients and compound heterozygous mouse models could be explained by species differences; alternatively, it has been suggested that humans with SOX2-related eye phenotypes may indeed be compound heterozygotes, carrying a null allele and a hypomorphic allele that brings the total functional level of *SOX2* down to a range that results in phenotypic expression[56].

NR2F1

Nuclear receptor subfamily 2 group F member 1 (*NR2F1*) encodes a conserved orphan nuclear receptor, which plays a critical role in cortical patterning[64,65], axon guidance[66], neurogenesis[66], and eye and optic nerve development[67,68]. We and others identified heterozygous *NR2F1* mutations in individuals manifesting global developmental delay/intellectual disability and ONH/optic atrophy (Bosch-

Boonstra-Schaaf Optic Atrophy syndrome, BBSOAS, OMIM 615722)[69-71]. The phenotypic spectrum of BBSOAS included hypotonia, seizures, autism, oromotor dysfunction, thinning of the corpus callosum, and hearing defects[72]. To date, pathogenic *NR2F1* variants have been reported as missense, translation initiation variants, frameshifting indels, and whole gene deletions. The missense mutations were enriched in the two functional domains of NR2F1: the DNA-binding domain (DBD) and the ligand-binding domain (LBD). Notably, patients with missense mutations in the DBD generally had more severe phenotypes compared to those carrying a heterozygous whole gene deletion. Given that NR2F1 binds to DNA in the form of dimers[73], a dominant negative effect may be the cause for this phenomenon. A genotype-phenotype correlation was proposed: patients with severe clinical phenotypes carry NR2F1 variants that tend to have almost completely blunted transcriptional activity, as assessed by *in vitro* luciferase assay[69].

Homozygous knockout *Nr2f1*$^{-/-}$ mice die within the first two days after birth due to starvation and dehydration. Defects in the formation of the glossopharyngeal nerve in *Nr2f1*$^{-/-}$ pups have been proposed as the cause[74], and this observation may relate to the oromotor dysfunction seen in BBSOAS patients. Abnormal development of the corpus callosum and the hippocampal commissure also is seen in embryonic *Nr2f1*$^{-/-}$ brains[75]. Eye-specific double knockout of *Nr2f1* and *Nr2f2*, a paralog of *Nr2f1*, leads to ocular colobomata, microphthalmia, and abnormal optic cups. The expression of several regulatory genes critical for early optic vesicle development, such as *Pax6* and *Otx2*, are altered in double knockout mice, resulting in abnormal differentiation of the progenitor cells at the optic vesicle[68]. Although all the BBSOAS patients identified so far carry heterozygous *NR2F1* variants, a heterozygous knockout (*Nr2f1*$^{+/-}$*)* mouse model has not been reported.

OTX2

Orthodenticle homeobox 2 (*OTX2*) encodes a homeodomain-containing transcription factor that plays an important role in forebrain and eye development[76,77]. Heterozygous mutations in *OTX2* are responsible for 2-8% of patients with microphthalmia/anophthalmia[78,79], which makes them the second most common genetic cause of microphthalmia/anophthalmia (after *SOX2*). Using a candidate-gene approach, Ragge et al. first reported eight patients with *OTX2* variants from 333 patients with ocular malformation spectrum defects. All eight patients identified in the study had microphthalmia/anophthalmia. The optic nerves and chiasm were absent or reduced in four out of the six patients who had MRI, CT, or ultrasound data available for review[80]. Later studies showed that 35% of the patients with *OTX2* mutations had ONH, while other eye defects such as coloboma and retinal dystrophies were less common[79]. In addition to ocular abnormalities, the phenotypic spectrum of *OTX2* mutations included structural and functional abnormalities of the pituitary, global developmental delay, autism, attention deficit disorder, feeding difficulties, seizures, microcephaly, and other structural brain anomalies, affecting the corpus callosum and hippocampus[79-81]. Incomplete penetrance and variable expressivity have been reported for *OTX2* mutations. In the original study, three out of eight affected individuals inherited their mutations from

a phenotypically normal parent, including frameshift or nonsense mutations. Variable expressivity has been documented extensively, including individuals sharing the same variant, even among siblings[80]. Homozygous knockout mice (*Otx2*$^{-/-}$) are embryonically lethal and display abnormal development of the forebrain, midbrain, and rostral hindbrain[76]. Heterozygous mice show variable phenotypes, including anencephaly, holoprosencephaly, microphthalmia/anophthalmia, micrognathia/agnathia, and short nose[82].

VAX1

Ventral anterior homeobox 1 (*VAX1*) encodes a homeobox transcription factor, which plays an important role in the forebrain and visual system[83,84]. Slavotinek et al. identified a homozygous missense *VAX1* variant (p.R152S) in a screen of 70 individuals with microphthalmia/anophthalmia. The affected male patient was born to phenotypically normal, consanguineous parents, who were confirmed as heterozygous carriers of the variant. In addition to microphthalmia, other clinical features included ONH, global developmental delay, hippocampal malformations, agenesis of the pineal gland and corpus callosum, and cleft lip/palate[85]. Homozygous knockout *Vax1*$^{-/-}$ mice are perinatally lethal, and the observed phenotypes bear remarkable similarity to the human patient: optic nerve dysgenesis, coloboma, and abnormalities in brain structures (corpus callosum, hippocampus, and anterior commissure)[83,84]. It should be noted that heterozygous knockout *Vax1*$^{+/-}$ mice appear normal, except for subfertility[86].

ATOH7

Atonal homolog BHLH transcription factor 7 (*ATOH7*) encodes a basic helix-loop-helix transcription factor, which is critical for retinal ganglion cell and optic nerve formation[87]. Khan et al. reported two homozygous *ATOH7* variants (one missense and the other as frameshift variant) in two consanguineous families with isolated microphthalmia. Two affected siblings from the family with frameshift mutations also displayed ONH, corneal opacity, and retinal detachment. Co-segregation of this mutation with the clinical phenotype was confirmed in the family[88]. The finding was supported by two genome-wide association studies, which identified SNP (rs3858145, p=3.4 x 10^{-10}) and SNP (rs1900004, p=2.67 x 10^{-33}) within 20 kb and 10 kb of *ATOH7*, respectively[89,90]. However, pathogenic *ATOH7* variants were not discovered in an investigation of 34 patients with ONH[91] and 76 other patients with microphthalmia/anophthalmia/coloboma[92]. Homozygous knockout *Atoh7*$^{-/-}$ mice are viable, fertile, and appear normal externally. Although they tend to have normal sized eyes, there is a 80-95% reduction in the number of retinal ganglion cells, with very thin or absent optic nerves[87,93].

Chromatin remodeling

KANSL1 (17q21.31 microdeletion)

KAT8 regulatory NSL complex subunit 1 (*KANSL1*) encodes a nuclear protein that is a key component of a histone acetyltransferase (HAT) complex[94,95]. *KANSL1* is one of

the five known protein-coding genes in the 17q21.31 microdeletion syndrome locus (MIM 610443). It was later found that haploinsufficiency of *KANSL1* was sufficient to cause the phenotypes of 17q21.31 microdeletion syndrome, also known as Koolen-de Vries syndrome (KdVS)[96]. KdVS is a multisystem disorder, characterized by developmental delay/intellectual disability, hypotonia, and characteristic facial dysmorphism. Additional features include overly social and friendly behavior, epilepsy, congenital heart defects, urogenital malformations, musculoskeletal anomalies, and ectodermal anomalies. Zollino et al. reported a deletion patient (patient 28) with hypoplastic ocular globe, unilateral ONH, strabismus, hearing impairment, and craniosynostosis. Koolen et al. reported another deletion patient (case 32) with ONH, hypermetropia, strabismus, esotropia, and corpus callosum abnormality[97]. The third deletion patient reported manifested cataracts, optic atrophy, hearing impairment, and a small hippocampus[98]. Currently no mouse model for *Kansl1* deletion exists. Knockout of *wah* (*KANSL1* ortholog in *Drosophila*) is embryonal lethal in flies. Tissue-specific knockdown of *wah* in muscles and mushroom bodies (the learning and memory centers in *Drosophila* brains) leads to neuromuscular-junction defects and decreased learning ability[96,99]. The function of *wah* in eye development is unknown currently.

α-dystroglycan glycosylation

B3GALNT2

Beta-1,3-N-Acetylgalactosaminyltransferase 2 (*B3GALNT2*) encodes a glycosyltransferase that helps to synthesize α-dystroglycan, which is an integral component of the dystrophin glycoprotein complex. Defects in glycosylation reduce the binding ability of α-dystroglycan to extracellular matrix ligands, causing a dystroglycanopathy. Stevens et al. reported seven individuals with congenital muscular dystrophy-dystroglycanopathy, and a phenotype defined as "brain and eye anomalies type A11" (MDDGA11, MIM 615181). These individuals carried either homozygous or compound heterozygous mutations in *B3GALNT2*. Two of the affected individuals had ONH. Currently, there is no murine model for *B3galnt2* loss-of-function studies; however, knockdown of *b3galnt2* in zebrafish showed retinal degeneration, impaired motility, and brain abnormalities[100]

ISPD

Isoprenoid synthase domain-containing protein (*ISPD*) encodes a protein that is required for proper α-dystroglycan modification. Similar to *B3GALNT2*, *ISPD* mutations were identified in patients diagnosed with congenital muscular dystrophy- dystroglycanopathy with brain and eye anomalies, in this case type A7 (MDDGA7, MIM 614643). Willer et al. reported seven individuals, including a pair of siblings, carrying homozygous or compound heterozygous mutations in *ISPD*. Two of the seven patients had ONH. One of the affected siblings was diagnosed with bilateral ONH, and the other had bilateral microphthalmia (status of optic nerves not mentioned in the study)[101]. Roscioli et al. identified two individuals with MDDGA7, one with a homozygous and the other with compound heterozygous

mutations in *ISPD.* However, instead of being described with ONH, they were diagnosed with optic atrophy[102]. In addition to eye abnormalities, the four individuals also shared brain malformations, including hydrocephalus, cerebellar hypoplasia, and cobblestone lissencephaly. In a forward genetic screen for axon guidance defects, it was found that a homozygous loss-of-function mutation in a mouse model *Ispd*$^{L79*/L79*}$ (leucine to premature stop codon) leads to neonatal lethality, abnormal axon guidance in the hindbrain, and abnormal fasciculation of the funiculus of the spinal cord. However, eye-related phenotypes were not reported in that mouse study[103].

Cytoskeleton and Scaffolding protein

TUBA8

Tubulin alpha 8 (*TUBA8*) encodes a member of the α-tubulin protein family. α-and β–tubulins heterodimerize and then assemble to form microtubules. Abdollahi et al. reported homozygous indel in an intron of *TUBA8* in three affected members of a consanguineous Pakistani family. The same homozygous deletion was found in an affected child from a second Pakistani family. The 14 bp deletion lies 11 bp upstream of the exon 2 splice junction. The deletion was predicted to interfere with correct splicing, which was verified by the reverse transcription polymerase chain reaction of lymphoblastoid cell line RNA from the patient. All four affected children have ONH with polymicrogyria. Other shared clinical phenotypes include seizures, thin or absent corpus callosum, and brainstem abnormalities[104]. Surprisingly, follow-up studies showed that the expression of *Tuba8* was relatively low in developing mouse and human brains, even though the clinical phenotypes mainly present as brain malformations[105]. Currently, no mouse model exists for *Tuba8* mutations.

CASK

Calcium/calmodulin-dependent serine protein kinase (*CASK*) encodes a member of the membrane-associated guanylate kinase (MAGUK) protein family, which consists of scaffolding proteins associated with intercellular junctions[106]. Heterozygous or hemizygous mutations in the X-linked *CASK* gene in patients lead to microcephaly with pontine and cerebellar hypoplasia (MICPCH, MIM 300749)[107]. The phenotypic spectrum of *CASK* mutations includes autistic traits, developmental delay/intellectual disability, axial hypotonia and/or peripheral hypertonia, movement and behavioral disorders, and seizures. Although optic nerve hypoplasia/optic atrophy is not fully expressed in MICPCH patients, it has been reported in multiple studies[108-111]. Other ophthalmological abnormalities include hyperopia, strabismus, astigmatism, and nystagmus. Constitutive knockout mice (*Cask*$^{-/y}$) and (*Cask*$^{-/-}$) die within the first day after birth[106], but deletion of a single *Cask* allele (*Cask*$^{+/-}$) in female mice has been found to recapitulate many human phenotypes, including microcephaly, hypotonia, ataxia, and ONH without microphthalmia or malformation of the brain midline[112]. The study suggested that Cask regulates oxidative metabolism in the brain, and the rate of glucose oxidation

was reduced by 20% in the brain of *Cask*$^{+/-}$ mice compared with wild type littermates[112].

RNA splicing

PUF60

Poly(U) binding splicing factor 60 (*PUF60*) encodes a ribonucleoprotein-binding protein, which is involved in pre-mRNA splicing and transcriptional regulation. Dauber et al. identified the first patient with a *de novo* heterozygous variant, p.H169Y in *PUF60*[113]. El Chehadeh et al. reported another five patients with *de novo* heterozygous variants in *PUF60*[114]. All six patients identified to date manifest the same facial gestalt as seen in individuals with 8q24.3 microdeletion syndrome, also known as Verheij syndrome (MIM 615583). Other shared clinical features include bilateral ONH (2/6), developmental delay (6/6), cardiac defects (5/6), short stature (5/6), joint laxity and/or dislocation (5/6), vertebral anomalies (3/6), and feeding difficulties (3/6). Currently, there is no mouse model for *Puf60* mutations.

MAPK signaling pathway

BRAF

B-Raf proto-oncogene (*BRAF*) encodes a serine/threonine-protein kinase that plays a role in regulating the MAP kinase signaling pathway. Mutations in *BRAF* are one of the causes of cardio-facio-cutaneous (CFC) syndrome (MIM 115150). Armour et al. compiled clinical data on 38 individuals with CFC, which was characterized clinically by heart defects, distinctive facial features, and skin abnormalities. The analysis showed that 32/38 individuals carried autosomal dominant variants in *BRAF*. The remaining 6 individuals carried either *MEK1* or *MEK2* variants. Among the patients with *BRAF* mutations, optic nerve hypoplasia/dysplasia was diagnosed in 9 out of the 20 individuals who had provided ophthalmological assessments for review. Meanwhile, 2 out of 6 individuals carrying *MEK1* or *MEK2* variants had such diagnoses[115]. Note that both MEK1 and MEK2, like BRAF, are also integral parts of the MAP kinase pathway. As a result, mutations in any of these genes lead to similar phenotypes in CFC. Although the signs of CFC overlap substantially with Noonan syndrome, it was estimated that only 2% (3/139) of Noonan syndrome patients had ONH[116]. Given that most *BRAF* mutations found in CFC patients were predicted to result in hyperactivation of BRAF, multiple corresponding mouse models have been generated. *Braf*$^{+/V600E}$ and *Braf*$^{+/L597V}$ mouse models, both carrying an allele expressing constitutively active Braf protein, recapitulate certain patient phenotypes, including small body size, facial dysmorphism, cardiomegaly, and eye abnormalities (cataracts)[117,118].

Others

ALDH1A3

Aldehyde dehydrogenase 1 family member A3 (*ALDH1A3*) encodes an aldehyde dehydrogenase enzyme that uses retinal (retinaldehyde) as a substrate to synthesize retinoic acid, which plays a critical role in eye development[119]. Fares-Taie et al. identified two homozygous missense mutations (p.R89C and p.A493P) and one homozygous splice-site mutation in four individuals from three consanguineous families. These mutations segregated with microphthalmia/anophthalmia along with occasional orbital cystic, neurological, and cardiac anomalies in the family. Available MRI data from two individuals showed hypoplastic optic nerves[120]. Yahyavi et al. identified a homozygous nonsense mutation, p.L389*, which they predicted to cause nonsense-mediated decay, in a patient with microphthalmia/anophthalmia and hypoplasia of the optic nerve and optic chiasm[121]. Homozygous knockout *Aldh1a3*$^{-/-}$ mice are lethal perinatally and show defects in the invagination of the ventral optic cup, closure of the choroid fissures, and abnormal axonal projections of retinal cells into the brain[119,122,123]. Interestingly, Aldh1a3 is also critical for the development of nasal structures, and null mice die of respiratory distress at birth, resulting from choanal atresia (blockage of nasal passage). However, all the human patients reported to date do not display these nasal or choanal abnormalities, suggesting residual protein function of the pathogenic alleles, or species-dependent differences in mice vs. humans.

DDHD2

DDHD domain-containing protein 2 (*DDHD2*) encodes a phospholipase enzyme and is a major brain triglyceride hydrolase. Schuurs-Hoeijmakers et al. reported five individuals diagnosed with complex hereditary spastic paraplegia (SPG54, MIM 615033) carrying biallelic mutations in *DDHD2*. Three out of five individuals had ONH. Other shared features included developmental delay/intellectual disability, hypotonia, strabismus, and thin corpus callosum[124]. *Ddhd2* constitutive knockout mice (*Ddhd2*$^{-/-}$) exhibit defects in movement and cognitive function, and show selective elevation in triacylglycerols through the CNS. However, the visual ability in these mice is not different substantially from wildtype controls in the optomotor test[125].

Discussion

Molecular network of transcription factors in eye development

Eye morphogenesis in vertebrates is an intricate multi-step process, including the formation of the eye pit, optic vesicle, optic cup, lens, and neurogenesis in the neural retina **(Figure 1)**. Many key transcription factors participate in these sequential events to ensure the proper formation and maturation of the eye[126,127]. While most knowledge described here is derived from murine studies, a high level of conservation exists for the sequence and function of these key transcription factors

in eye development between human and murine models, so the described processes carry significance in humans as well. In mice, the evagination of optic pits leads to the formation of the early optic vesicle at E9.5. Then, the invagination of optic vesicle at E10.5 results in the formation of a dual-layered optic cup, which consists of three main domains: the neural retina (NR), retinal pigmented epithelium (RPE), and optic stalk (OS). The OS later becomes the optic nerve when the axons of the retinal ganglion cells fill the cavity of the stalk and complete the closure of the choroid fissure **(Figure 1)**.

The boundary between optic stalk and neural retina is established at the optic disc, which serves an entrance for the blood vessels to the eye and as an exit for the axons of retinal ganglion cells from the eye[128]. Pax6, an NR marker, and Pax2, a ventral OS marker, antagonize each other to establish the sharp boundary **(Figure 1)**[129]. Reciprocal expansion of Pax2 and Pax6 gene expressions is seen in the corresponding loss-of-function mutant mice. Disruption of this boundary causes defects in the optic nerve, optic stalk, retina, lens, and optic fissure closure[130,131]. Vax1 and Vax2 are also involved in the boundary formation, by inhibiting Pax6 expression, given that the expression of Pax6 and Pax2 is rapidly acquired and lost from the optic stalk in Vax1 and Vax2 double knockouts at E10[132].

Another example of boundary formation defects is reported in *Nr2f1* and *Nr2f2* double knock mice. Increased expression of Pax6 in NR and decreased expression of Pax2 in ventral NR, accompanied by reduced expression of Pax2/Vax1 in the OS, shifts the NR-vOS boundary proximally, eventually leading to abnormal differentiation of the optic stalk[68]. These data suggested that Nr2f1 and Nr2f2 are the key regulators that balance the expression of Pax6 and Pax2 during the early development of the optic cup. However, the eye phenotypes in the individuals with heterozygous *PAX6* mutations are generally more severe than those with heterozygous *NR2F1* mutations. This could be explained by the functional redundancy between NR2F1 and NR2F2, given that eye-specific double knockout of *Nr2f1* and *Nr2f2* in mice leads to major eye abnormalities but does not do so in eye-specific single knockout of *Nr2f1*[68].

Two transcription factors synergistically regulating lens development are Pax6 and Sox2, again indicating the intricate interaction between transcription factors during eye development[133,134]. Pax6 and Sox2 form a co-DNA-binding complex, which activates cooperatively the lens-specific enhancer elements DC5 in order to differentiate embryonic ectoderm into lens ectoderm. In contrast to the synergistic relationship, the same two transcription factors antagonize each other in the developing optic cup, to specify the multipotent optic cup progenitors toward a neurogenic fate (Sox2) or a non-neurogenic fate (Pax6)[135]. This suggests that the interplay between transcription factors may be highly cell type-specific and developmental stage-specific during eye development.

Adding to the complexity of eye development is the fact that the aforementioned transcription factors are also involved in the morphogenesis of the forebrain and other brain regions, such as the midline brain, hypothalamus, and pituitary gland. This involvement may explain why ONH is more commonly a part of a syndrome involving other structural brain anomalies, rather than an isolated finding. Similarly, most mutant mouse models manifest more extensive systemic

defects, in addition to isolated ONH. To date, multiple genes are implicated in ONH; however, most of them are associated with other eye anomalies and/or brain structural defects. Nevertheless, the possibility that one single gene mutation leads to isolated ONH should not be discounted.

Mitochondrial optic neuropathy

In contrast to ONH, the genetic causes of optic atrophy are mostly mitochondria-related. Leber hereditary optic neuropathy (LHON) and dominant optic atrophy (DOA) are the two most common inherited optic atrophy disorders[136]. Mutations in primary mitochondrial DNA (mtDNA) affect the respiratory chain complexes in LHON[137]. Mutations in *OPA1*, which encodes an inner mitochondrial membrane protein important for oxidative phosphorylation and mtDNA maintenance, account for three-quarters of all DOA patients[138,139]. Other genes implicated in optic atrophy include *FXN*[140], *TIMM8A*[141], *SPG7*[142], *MFN2*[143], *DNM1L*[144], *OPA3*[145], *SDHA*[146], *CISD2*[147], and *SLC25A46*[148,149]. All these genes encode mitochondrial proteins and presumably are involved in energy metabolism, although genes not directly related to mitochondria exist, such as *KIF1A*[150], *KLC2*[151], and *ATP1A3*[152]. Tissues with high-energy demand, such as those found in the nervous system and skeletal muscles are more vulnerable to mitochondrial dysfunction. This could explain why patients with these mutations typically manifest optic atrophy and/or myopathy at its onset. Nevertheless, the association of ONH with mitochondrial cytopathies has been reported and will require further investigation[153].

Conclusion

With ONH being the second most common cause of congenital visual impairment[154], the investigation of the causes and risk factors of ONH is of great importance. Although previous studies had mostly attributed ONH to the prenatal environment, the identification of an increasing number of genetic causes of ONH suggests multiple etiological mechanisms, including gene-environment interactions, as well as monogenic causes with high penetrance. The consideration of gene-environment interactions and potential genetic modifiers is based, in part, on the presence of variable expressivity of related phenotypes among individuals carrying the same genetic variants.

Eye morphogenesis in mammalian species is an intricate process. Many key transcription factors relevant for eye development also play roles in the morphogenesis of forebrain. This interplay causes more complex clinical phenotypes, many of which involve cognitive deficits and behavioral alterations. With the advancement and clinical availability of genome-wide sequencing technology, we anticipate that more genes will be implicated in the etiology of ONH. In some cases, the modes of inheritance may go well beyond Mendelian genetics, e.g., digenic or oligogenic inheritance. These steps will lead to an increased molecular diagnostic rate for individuals with ONH *per se*. Additional research will investigate whether the functional consequences associated with the respective disorders are all due to structural deficits from altered neurodevelopment, or whether some function(s) could be regained, with therapeutic intervention, even started later in life.

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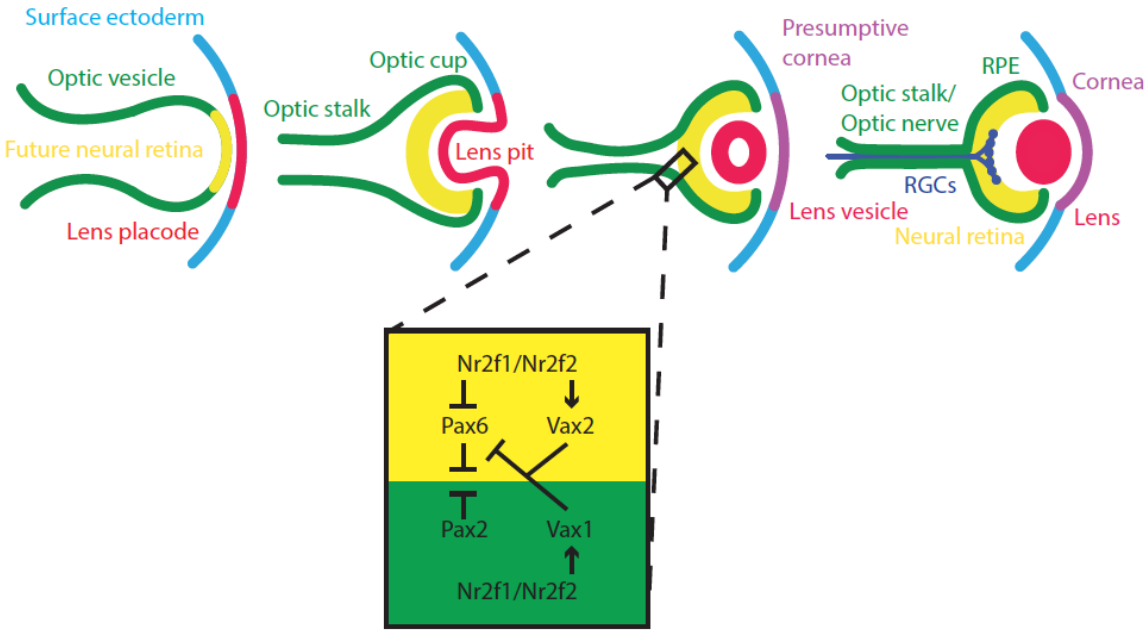

Figure 1. Eye development and key transcription factors. In early eye development, the evagination of optic pits leads to the formation of the early optic vesicle. The surface ectoderm and the underlying neuroepithelium invaginate to form the lens vesicle and bilayered optic cup, respectively. The outer layer of the optic cup gives rise to the retinal pigment epithelium, while the inner layer gives rise to the neural retina. The optic stalk later becomes the optic nerve when the axons of the retinal ganglion cells fill the cavity of the stalk and complete the closure of the choroid fissure. Many key transcription factors, mutations of which cause ONH, participate in the proper development of the optic stalk/optic nerve. RPE, retinal pigment epithelium; RGC, retinal ganglion cell.