Genetic Advances in Microphthalmia

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Abstract

Congenital ocular anomalies such as anophthalmia and microphthalmia (AM) are severe craniofacial malformations in human. The etiologies of these ocular globe anomalies are diverse but the genetic origin appears to be a predominant cause. Until recently, genetic diagnosis capability was rather limited in AM patients and only a few genes were available for routine genetic testing. While some issues remain poorly understood, knowledge regarding the molecular basis of AM dramatically improved over the last years with the development of new molecular screening technologies. Thus, the genetic cause is now identifiable in more than 50% of patients with a severe bilateral eye phenotype and in around 30% of all AM patients taken together. Such advances in the knowledge of these genetic bases are important as they improve the quality of care, in terms of diagnosis, prognosis, and genetic counseling delivered to the patients and their families.

Keywords
- microphthalmia
- anophthalmia
- eye development
- genetic advances

Introduction

Congenital ocular anomalies are common craniofacial malformations in human as they are most often compatible with life and procreation. Their nature varies depending on the ocular structure affected by the developmental defect. Abnormal development processes can affect the formation of the different segments of the eye, resulting in anterior and/or posterior segment dysgenesis, as well as the entire ocular globe, leading to anophthalmia and microphthalmia (AM).

The AM malformations are the most severe ocular anomalies and they are relatively frequent with an estimated prevalence of 1 per 7,000, and 1 per 30,000 births, respectively.[1–3] AM describe the absence or reduced size of the ocular globe, respectively. These anomalies can be isolated or associated with other malformations affecting the face and the cranium, as well as other systems. Extraocular malformations are associated in 33 to 95% AM patients.[4] The etiologies of these ocular globe anomalies are diverse but the genetic origin appears to be a predominant cause.[5]

Until recently, genetic diagnosis capability was rather limited in AM patients and only a few genes were available for routine genetic testing. Recent advances in genetics, especially in DNA sequencing, henceforth allow identification and testing of many new genes that contribute to human eye congenital diseases, especially in AM patients. Thus, the genetic cause is now identifiable in 50 to 80% of the patients with a severe bilateral eye phenotype and in around 30% of all the AM patients taken together.[6,7] Such advances in the knowledge of these genetic bases are important as they improve the quality of care, in terms of diagnosis, prognosis, and genetic counseling delivered to the patients and their families.

Early Eye Development

The AM phenotypic spectrum is the consequence of a defect in the ocular globe formation that happens between the 4th and the 6th week of gestation in human.

The morphogenesis of the eye is a well-conserved process among vertebrates,[8] which starts with the specification of the eye field, as a single domain of cells in the most anterior part

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of the neuroectoderm neural plate. Then, cells evaginate laterally, splitting the eye field into right and left optic vesicles. After this, each optic vesicle interacts with the overlying surface ectoderm that will give rise to the lens and part of the cornea. The optic vesicle extension leads to the invagination of their distal surface into proximal and distal territories, forming the optic stalk and optic cup, respectively. This invagination causes the formation of a furrow from the ventral side of the optic cup into the optic stalk, called the choroid fissure. This transient opening enables the entrance of the hyaloid artery to irrigate the developing eye and the exit of the retinal ganglion cell axons to reach their target tissues in the brain. The nasal and temporal edges of the choroid fissure subsequently fuse to close the globe of the eye.[8] With the closure of the embryonic fissure, the basic structure of the eye is then established.

Thus, the formation of a properly shaped eye is the consequence of coordinated induction processes and differentiation pathways, and, any disruption in one of these events has the potential to generate an AM phenotype.

Clinical Diagnosis

AM can affect one or both eyes, and be associated with extraocular features. Anophthalmia describes the complete absence of ocular tissue in the orbit (true anophthalmia) or the absence of ocular tissue on clinical examination (clinical anophthalmia). Ocular adnexa (eyelids, conjunctiva, and lacrimal apparatus) are present. Microphthalmia consists of reduced size of the ocular globe and is defined as a globe with a total axial length that is at least two standard deviations below the mean for the patient's age ($<$ 19 mm in a 1-year-old child; $<$ 21 mm in an adult).[9,10] Microphthalmia is classified as simple microphthalmia or complex microphthalmia when associated with anterior and/or posterior segment dysgenesis, and graded depending on the degree of axial length reduction.

Imaging studies such as ultrasonography, computed tomography scan or magnetic resonance imaging of the eyes (but also of the orbits and the brain) help to characterize the eye malformation and determine the ocular diagnosis and management (for instance, the indication of prosthetic intervention and surgery).

A global evaluation is also required, as AM phenotype frequently occurs with other anomalies, possibly as a part of a well-defined syndrome.[4,11] A total of 269 different syndromes with AM are retrieved by the London Dysmorphology Database.

Causes

Prenatal exposure to viral infections such as rubella, or toxic substances such as alcohol or retinoic acid, are known causes of the AM phenotype. Nonetheless, family history and/or parental eye phenotype may orientate toward a genetic condition. It is likely that genetic defects are the major contributors of AM.[5]

The genetic causes of AM can be divided into chromosome abnormalities and gene disorders. Conventional cytogenetics with karyotyping may detect chromosomal aneuploidies (especially trisomy 13 and 18) in 5 to 10% of patients. In such cases, AM is often associated with multiple malformations. Molecular karyotyping (comparative genomic hybridization array), allows identification of unbalanced chromosomal rearrangements in an additional 3 to 10% of patients depending on the presence of extraocular features.[12,13]

In addition, AM phenotype can be the consequence of mutations that affect the function of genes known to play a crucial role in eye development. Since the last decade, many genes have been implicated in isolated and syndromic forms of AM phenotype and all patterns of inheritance have been reported (dominant, recessive, and X-linked transmission manner). SRY (sex determining region Y)-box 2 (*SOX2*) is the gene most frequently involved in AM phenotype, accounting for approximately 10 to 15% of patients,[6,14] followed by orthodenticle homeobox 2 (*OTX2*) and retina and anterior neural fold homeobox (*RAX*), which are implicated in 3% of AM patients.[6] Numerous other genes are involved in only a very small percentage of AM patients.

Some genes, mostly encoding transcription factors (such as *SOX2*, *RAX*, *OTX2*, forkhead box E3 [*FOXE3*], and visual system homeobox 2 [*VSX2*]), are known to be responsible for mainly nonsyndromic AM phenotype. Several other genes predominantly feature microphthalmia and anophthalmia in a syndromic form when mutated. The first example is the stimulation by retinoic acid 6 (*STRA6*) gene for which mutated patients present with anophthalmia or microphthalmia and, in most cases, various degrees of pulmonary, diaphragmatic and cardiac defects, thus defining PDAC (pulmonary agenesis/dysgenesis/ hypoplasia, microphthalmia/anophthalmia, and a diaphragmatic defect) spectrum or Matthew–Wood syndrome.[15,16] Other examples can be quoted such as the SPARC-related modular calcium binding 1 (*SMOC1*) gene leading to ophthalmoacromelic syndrome, also called Waardenburg anophthalmia; the BCL6 corepressor (*BCOR*) gene responsible for oculofaciocardiodental syndrome; the holocytochrome c synthase (*HCCS*) gene associated with MIDAS syndrome (microphthalmia, dermal aplasia, and sclerocornea) in which patients display linear skin defects; and finally, the porcupine homolog (Drosophila) (*PORCN*) gene causing Goltz–Gorlin syndrome featuring focal dermal hypoplasia.[4]

However, the clinical presentation (ocular and extraocular features) associated with mutations in most of these ocular development genes can be extremely variable between patients and within families. Moreover, the limit between nonsyndromic and syndromic AM genes is not clear and rather artificial, as part of the involved genes can lead to isolated or syndromic AM phenotypes. This is becoming more pronounced with the genetic progress that has been made (see the section on Genetic Advances for discussion).

Genetic Counseling

The existence of a range of modes of inheritance, germline mosaicism, and incomplete penetrance pose altogether particular challenges for genetic counseling. The empiric risk to siblings without a clear etiology or family history has been estimated ranging between 10 and 15%.[3] Identifying the underlying genetic cause of the AM phenotype allows to

precise this recurrence risk to siblings (which varies from < 1–50%, depending on the inheritance manner) and to propose prenatal molecular diagnostic in at-risk families.

Genetic Advances

Recent advances have been made in the knowledge of the AM genetic bases. Indeed, such progress is necessary, as it improves the quality of care in terms of diagnosis, prognosis, and genetic counseling of the AM patients and second, the genetics of the eye has benefited from the broad progress that has been made during the last few years in the field of genetics.

The first level of genetic advances has consisted of a better analysis of the genes already known to be involved in AM phenotype, mainly by combining the Sanger sequencing of the coding regions of these genes with the detection of exonic rearrangements,[6,7,17,18] thus improving the mutation detection rate. As an example, it shows that deletion involving the major AM genes (*SOX2* and *OTX2*) represent more than a quarter of the mutations identified in these genes.[6] These deletions are not recognized by classical direct sequencing and need specific semiquantitative methods to be revealed.

Nevertheless, the main progress made in AM genetics lies in the development of new DNA sequencing technologies that allow identification and testing of many genes that contribute to human eye congenital diseases. Next-generation sequencing (NGS) is a high-throughput sequencing technique, enabling the parallel and massive sequencing of genes. The approaches mainly used in patients are targeted exome sequencing (to test a panel of genes, and for instance, a panel of genes already known to be involved in eye diseases and/or ocular development) and whole exome sequencing (which aim to screen all coding genes).[19]

The spread of these kinds of approaches in many laboratories has contributed to the spectacular increase, in a short period, of new genes involved in AM phenotype, whose number is still continuously growing. For instance, the aldehyde dehydrogenase 1 family, member A3 (*ALDH1A3*) gene,[20] the retinoic acid receptor, β (*RARB*) gene[21] and very recently, the mab-21-like 2 (*Caenorhabditis elegans*) (*MAB21L2*) gene[22] are newly discovered causal genes in AM phenotype. These genes can be analyzed in patients who have had no molecular diagnosis until now. Many other genes, with more or less clear pathogenicity, have also been quite recently involved in AM patients, either by classic genetic approaches such as bone morphogenetic protein 4 (*BMP4*),[23] *BMP7*,[24] growth differentiation factor 6 (*GDF6*),[25] *GDF3*,[26] and ventral anterior homeobox 1 (*VAX1*)[27] or, in a short period of time, by NGS approaches such as atonal homolog 7 (Drosophila) (*ATOH7*),[28] chromosome 12 open reading frame 57 (*C12orf57*),[29] odz, odd Oz/ten-m homolog 3 (Drosophila) (*ODZ3*),[30] high mobility group box 3 (*HMGB3*),[31] and yes-associated protein 1 (*YAP1*).[32]

However, complementary data will be needed to add further support for the pathogenicity and the transmission manner of the mutations in these latter genes, before making a molecular diagnosis on which genetic counseling can be delivered to the patients. This will be principally allowed by the analysis of more AM pedigrees and specific disease modeling. Notably, new sophisticated reverse genetic approaches, such as TALENs (transcription activator-like effector nucleases)[33] and especially the CRISPR/CRISPR-associated (Cas) system,[34] now allow to study data from these human studies and to model congenital ocular pathologies in an animal, by assessing the role of orthologs of genes mutated in human.[35,36]

Another aspect of these genetic advances is that they have generated an enormous volume of genetic data for the AM phenotype, but also for many hereditary eye conditions. The AM phenotype is part of the wide eye anomalies spectrum and the genetics of the eye is particularly complex and heterogeneous at the clinical and molecular level. For many eye congenital diseases, several genes can contribute to the same ocular phenotype and on the other hand, a mutated gene can be responsible for different eye phenotypes.[4,6] This heterogeneity is now becoming more complex through the emergence of NGS data that provide molecular information about genes and mutations underlying different eye phenotypes.[19] Indeed, NGS projects allow discovery of new genes involved in a specific disease, but can also highlight a new implication of a known gene in a distinct or overlapping eye phenotype. This can be illustrated by the example of cytochrome P450, family 1, subfamily B, polypeptide 1 (*CYP1B1*), initially thought to be a primary congenital glaucoma gene[37] that may acts as a modifying gene for the AM phenotype.[19] The application of NGS approaches in AM patients has also served to extend the clinical implication of known AM genes; the best example is the *STRA6* gene, initially thought to be solely associated with PDAC syndrome,[16] which can lead, although more rarely, to isolated forms of AM phenotype.[38]

Therefore, the genetic borders between clinical forms of inherited eye diseases tend toward fading with the progress made in eye genetics, making the diagnosis and the genetic counseling more complex.

Unresolved Questions

Despite the involvement of approximately 20 genes in AM, mutations in each of these genes explain the symptoms in only a very small percentage of AM patients (apart for *SOX2*, which is involved in 10 to 15% of AM patients). Thus, less than half of the AM patients have an underlying cause identified, and it seems likely that only a small proportion of AM causative genes have been identified so far. While this represents a vast genetic heterogeneity, this is not entirely surprising in the developmental process of such a complex organ.

Another issue that is not well understood is the important phenotypic variability between families harboring a mutation in the same gene, within patients in the same family, and even within individuals when ocular involvement is unilateral or clearly asymmetric. Some factors such as modifier genes, environmental factors, and stochastic effects are thought to explain this variability, even if mechanisms involved are not yet clearly understood.

Finally, one major issue that should be raised is how to manage and treat these patients. Follow-up of a cohort of AM patients is needed to determine the frequency and nature of associated malformations, as the visual and neurological

outcome. A recent improvement on the pathophysiology of AM could lead to the development of various therapeutic approaches. Identification of several genes involved in the retinoic acid signaling pathway such as *STRA6*, *ALDH1A3*, and *RARB* may open therapeutic perspectives for AM conditions, perspectives that can be extended for degenerative ophthalmic diseases.[39] Feasibility and efficiency of postnatal manipulation of dosage of genes involved in AM phenotype using topical application have been demonstrated for *Pax6* in the mouse[40] and may thus also become a possibility to improve the visual prognosis.

Conclusion

In conclusion, knowledge regarding the molecular basis of AM dramatically improved over the past years with the development of new molecular screening technologies. Some issues remain poorly understood such as phenotypic variability. This variability reflects the complexity of the eye developmental process, driven by interacting networks, and modulated by stochastic events and environmental influences. Deciphering factors determining the clinical variability represent a further essential step to achieve to improve management and genetic counseling. The progress in the molecular basis of eye genetics also provides the impetus for advances in many other areas such as eye pathophysiology, animal and cellular modeling, and therapeutic research.

References

1 Morrison D, FitzPatrick D, Hanson I, et al. National study of microphthalmia, anophthalmia, and coloboma (MAC) in Scotland: investigation of genetic aetiology. J Med Genet 2002;39(1):16–22
2 Shah SP, Taylor AE, Sowden JC, et al; Surveillance of Eye Anomalies Special Interest Group. Anophthalmos, microphthalmos, and Coloboma in the United kingdom: clinical features, results of investigations, and early management. Ophthalmology 2012;119(2):362–368
3 Verma AS, Fitzpatrick DR. Anophthalmia and microphthalmia. Orphanet J Rare Dis 2007;2:47
4 Slavotinek AM. Eye development genes and known syndromes. Mol Genet Metab 2011;104(4):448–456
5 Bermejo E, Martínez-Frías ML. Congenital eye malformations: clinical-epidemiological analysis of 1,124,654 consecutive births in Spain. Am J Med Genet 1998;75(5):497–504
6 Chassaing N, Causse A, Vigouroux A, et al. Molecular findings and clinical data in a cohort of 150 patients with anophthalmia/microphthalmia. Clin Genet 2014;86(4):326–334
7 Williamson KA, FitzPatrick DR. The genetic architecture of microphthalmia, anophthalmia and coloboma. Eur J Med Genet 2014;57(8):369–380
8 Graw J. Eye development. Curr Top Dev Biol 2010;90:343–386
9 Weiss AH, Kousseff BG, Ross EA, Longbottom J. Simple microphthalmos. Arch Ophthalmol 1989;107(11):1625–1630
10 Weiss AH, Kousseff BG, Ross EA, Longbottom J. Complex microphthalmos. Arch Ophthalmol 1989;107(11):1619–1624
11 Forrester MB, Merz RD. Descriptive epidemiology of anophthalmia and microphthalmia, Hawaii, 1986-2001. Birth Defects Res A Clin Mol Teratol 2006;76(3):187–192
12 Delahaye A, Bitoun P, Drunat S, et al. Genomic imbalances detected by array-CGH in patients with syndromal ocular developmental anomalies. Eur J Hum Genet 2012;20(5):527–533
13 Raca G, Jackson CA, Kucinskas L, et al. Array comparative genomic hybridization analysis in patients with anophthalmia, microphthalmia, and coloboma. Genet Med 2011;13(5):437–442
14 Fantes J, Ragge NK, Lynch SA, et al. Mutations in SOX2 cause anophthalmia. Nat Genet 2003;33(4):461–463
15 Chitayat D, Sroka H, Keating S, et al. The PDAC syndrome (pulmonary hypoplasia/agenesis, diaphragmatic hernia/eventration, anophthalmia/microphthalmia, and cardiac defect) (Spear syndrome, Matthew-Wood syndrome): report of eight cases including a living child and further evidence for autosomal recessive inheritance. Am J Med Genet A 2007;143A(12):1268–1281
16 Pasutto F, Sticht H, Hammersen G, et al. Mutations in STRA6 cause a broad spectrum of malformations including anophthalmia, congenital heart defects, diaphragmatic hernia, alveolar capillary dysplasia, lung hypoplasia, and mental retardation. Am J Hum Genet 2007;80(3):550–560
17 Gerth-Kahlert C, Williamson K, Ansari M, et al. Clinical and mutation analysis of 51 probands with anophthalmia and/or severe microphthalmia from a single center. Mol Genet Genomic Med 2013;1(1):15–31
18 Schilter KF, Reis LM, Schneider A, et al. Whole-genome copy number variation analysis in anophthalmia and microphthalmia. Clin Genet 2013;84(5):473–481
19 Prokudin I, Simons C, Grigg JR, et al. Exome sequencing in developmental eye disease leads to identification of causal variants in GJA8, CRYGC, PAX6 and CYP1B1. Eur J Hum Genet 2014;22(7):907–915
20 Fares-Taie L, Gerber S, Chassaing N, et al. ALDH1A3 mutations cause recessive anophthalmia and microphthalmia. Am J Hum Genet 2013;92(2):265–270
21 Srour M, Chitayat D, Caron V, et al. Recessive and dominant mutations in retinoic acid receptor beta in cases with microphthalmia and diaphragmatic hernia. Am J Hum Genet 2013;93(4):765–772
22 Rainger J, Pehlivan D, Johansson S, et al; UK10K; Baylor-Hopkins Center for Mendelian Genomics. Monoallelic and biallelic mutations in MAB21L2 cause a spectrum of major eye malformations. Am J Hum Genet 2014;94(6):915–923
23 Bakrania P, Efthymiou M, Klein JC, et al. Mutations in BMP4 cause eye, brain, and digit developmental anomalies: overlap between the BMP4 and hedgehog signaling pathways. Am J Hum Genet 2008;82(2):304–319
24 Wyatt AW, Osborne RJ, Stewart H, Ragge NK. Bone morphogenetic protein 7 (BMP7) mutations are associated with variable ocular, brain, ear, palate, and skeletal anomalies. Hum Mutat 2010;31(7):781–787
25 Asai-Coakwell M, French CR, Berry KM, et al. GDF6, a novel locus for a spectrum of ocular developmental anomalies. Am J Hum Genet 2007;80(2):306–315
26 Ye M, Berry-Wynne KM, Asai-Coakwell M, et al. Mutation of the bone morphogenetic protein GDF3 causes ocular and skeletal anomalies. Hum Mol Genet 2010;19(2):287–298
27 Slavotinek AM, Chao R, Vacik T, et al. VAX1 mutation associated with microphthalmia, corpus callosum agenesis, and orofacial clefting: the first description of a VAX1 phenotype in humans. Hum Mutat 2012;33(2):364–368
28 Khan K, Logan CV, McKibbin M, et al. Next generation sequencing identifies mutations in Atonal homolog 7 (ATOH7) in families with global eye developmental defects. Hum Mol Genet 2012;21(4):776–783
29 Zahrani F, Aldahmesh MA, Alshammari MJ, Al-Hazzaa SA, Alkuraya FS. Mutations in c12orf57 cause a syndromic form of colobomatous microphthalmia. Am J Hum Genet 2013;92(3):387–391
30 Aldahmesh MA, Mohammed JY, Al-Hazzaa S, Alkuraya FS. Homozygous null mutation in ODZ3 causes microphthalmia in humans. Genet Med 2012;14(11):900–904

31 Scott AF, Mohr DW, Kasch LM, et al. Identification of an HMGB3 frameshift mutation in a family with an X-linked colobomatous microphthalmia syndrome using whole-genome and X-exome sequencing. JAMA Ophthalmol 2014;132(10):1215–1220
32 Williamson KA, Rainger J, Floyd JA, et al; UK10K Consortium. Heterozygous loss-of-function mutations in YAP1 cause both isolated and syndromic optic fissure closure defects. Am J Hum Genet 2014;94(2):295–302
33 Joung JK, Sander JD. TALENs: a widely applicable technology for targeted genome editing. Nat Rev Mol Cell Biol 2013;14(1):49–55
34 Hwang WY, Fu Y, Reyon D, et al. Efficient genome editing in zebrafish using a CRISPR-Cas system. Nat Biotechnol 2013;31(3):227–229
35 Gestri G, Link BA, Neuhauss SC. The visual system of zebrafish and its use to model human ocular diseases. Dev Neurobiol 2012; 72(3):302–327
36 Lieschke GJ, Currie PD. Animal models of human disease: zebrafish swim into view. Nat Rev Genet 2007;8(5):353–367
37 Stoilov I, Akarsu AN, Sarfarazi M. Identification of three different truncating mutations in cytochrome P4501B1 (CYP1B1) as the principal cause of primary congenital glaucoma (Buphthalmos) in families linked to the GLC3A locus on chromosome 2p21. Hum Mol Genet 1997;6(4):641–647
38 Casey J, Kawaguchi R, Morrissey M, et al. First implication of STRA6 mutations in isolated anophthalmia, microphthalmia, and coloboma: a new dimension to the STRA6 phenotype. Hum Mutat 2011;32(12):1417–1426
39 Koriyama Y, Takagi Y, Chiba K, et al. Requirement of retinoic acid receptor β for genipin derivative-induced optic nerve regeneration in adult rat retina. PLoS ONE 2013;8(8): e71252
40 Gregory-Evans CY, Wang X, Wasan KM, Zhao J, Metcalfe AL, Gregory-Evans K. Postnatal manipulation of Pax6 dosage reverses congenital tissue malformation defects. J Clin Invest 2014;124(1): 111–116