Through the Looking Glass – eye anomalies in the age of molecular science

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This Special Issue, devoted to the Genetics of Ocular Developmental Disorders, appears 27 years after the first description of *PAX6* involvement in human aniridia (Jordan 1992). This initial discovery paved the way to an understanding of the importance of this 'master' control gene in human eye development. A wealth of work on *PAX6* led to publications demonstrating that homologous genes were involved in ocular development in other species (Quiring 1994). Eight years later, Alward (2000) proposed that we reconsider anterior segment dysgenesis as a single variable entity, namely Axenfeld-Rieger syndrome, caused by mutations in two genes, *PITX2* and *FOXC1*. This was a visionary approach, providing a glimpse of the genetic heterogeneity that we recognize in eye developmental disorders today and strengthening the need for collaboration between clinicians and scientists.

With the exception of compound heterozygous variants in *PAX6* (Glaser 1994), there was no molecular etiology for humans born with eye anomalies such as anophthalmia, microphthalmia and coloboma, until 2003, when *SOX2* emerged as a critical gene for whole eye development (Fantes 2003; Ragge 2005). Variants in this gene turned out to be responsible for up to 15% of cases of anophthalmia or severe microphthalmia, in many cases associated with a pervasive neurodevelopmental disorder and other anomalies. Identification of this key gene started to awaken further interest in the genetics of structural eye anomalies.

Since that time, studies conducted in humans have led to the identification of a wealth of genes and emerging pathways in eye development. Quite apart from such an accumulation of knowledge of novel genes, an overwhelming genetic heterogeneity was recognized making the journey into the labyrinth of diagnosis really challenging. Coinciding with the increasing number of genes involved in ocular developmental disorders, new sequencing techniques allowed easier and faster screening for variants. This progress has been limited to a certain extent by the complexity of ascertaining the pathogenic effects of gene sequence variants. Overall, the newer technology provided increased capacity to analyse samples, making it possible to adopt less stringent clinical criteria. This in turn revealed a new clinical complexity, with the existence of complex and overlapping phenotypes.

It is well known that a variant protein can, in cascade fashion, impede numerous functions of the network to which it belongs. Several papers in this Special Issue detail the major role of key gene regulatory networks. This increases our understanding of the various or overlapping phenotypes triggered by mutations in different genes acting in the same network or located

at nodes on several interconnecting pathways (Cavodeassi 2018; Nédélec 2019). Whatever the progress made, even building *in vitro* organoids, our knowledge on the basic mechanisms that shape the eye and render it functional is still nascent. Multiple questions have to be addressed to understand regulatory mechanisms and their effectors, such as the role of non-coding RNAs, a topic presented in this issue (Karali 2018). We remain exceptionally curious about the mechanisms underlying variable penetrance and expressivity, particularly those modulating the expression of major genes that occasionally cause rather mild or unilateral malformations. For instance, an understanding of the ways the developmental pathways can buffer the effect of deleterious variants may lead us towards therapeutic approaches.

Suffice it to say, it is clear that the diagnosis of such complex eye disorders cannot simply rely only on molecular data and that a close collaboration between clinicians and scientists is more critical than ever before.

The ophthalmologist can accurately diagnose the patient with an absence of the iris, peripheral keratopathy, foveal hypoplasia and nystagmus as having aniridia and will know to test for variants in *PAX6* or for deletions in the WAGR 11p- region. Difficulties arise in the clinical identification of *PAX6*-related ocular anomalies in patients with significant residual iris tissue, mild reduction of vision and no nystagmus. Vital clues as to the diagnosis of 'aniridia' in such patients remain to be identified to avoid such patients being commonly mistaken as having anterior segment dysgenesis (ASD) of the Axenfeld-Rieger spectrum (ARS). In such cases, assessing the presence of congenital cataract, more characteristic of aniridia, could help to clarify the situation and indicate targeted analysis of candidate genes.

Despite the development of high throughput sequencing, it remains important to decide when to devote time and resources to the analysis of *PAX6* non-coding regions in the search for genetic diagnosis (Hall 2018) Such functional analyses may require a long biological process, sometimes using cellular or animal models, to assess the pathogenic effect of a variant. This underscores the importance of different animal models not only as a basis for our knowledge of pathogenesis, but also to ascertain variant consequences (Bovolenta 2018; Cavodeassi 2019; Gaspar 2018; Graw 2019).

When considering early onset cataract, molecular analysis of a at least a large gene panel is the most effective way to reveal the diagnostic cause within a set of conditions with impressive genetic heterogeneity. Nonetheless, the isolated nature of the initial ocular lesion cannot be determined without the expert systemic evaluation of a pediatric or genetics specialist (Reis 2018)

On the other hand, grouping relevant phenotypes under the terminology of ASD syndromes can also be confusing. While ASD is an all-encompassing term that describes anomalies of the iris, anterior chamber angle and peripheral Descemet membrane, it can also include other conditions, including Peters' anomaly, anterior polar cataract and some forms of cornea plana. One of the common complications of all disorders that interfere with anterior chamber angle development is glaucoma, with patients having at least 50% lifetime risk of developing elevated intraocular pressure at any time between birth and adulthood, necessitating vigilant surveillance. This complication is also common in patients with *PAX6*-related aniridia and following surgery for early onset cataract. Patients with ARS have other systemic neural crest-

related malformations that indicate more generalized sequelae of underlying variants, as opposed to predominant ocular manifestations. Rare patients with *FOXC1* variants have an aniridia phenotype, while the occasional patient with a *PAX6* variant has an anterior segment phenotype that can be described as Peters' anomaly (Hanson 1994) or even severe microphthalmia (Deml 2015). These authors believe that as long as one is aware of the uncommon presentations of *FOXC1* and *PAX6* mutations, the terminology of ARS and of aniridia respectively are quite acceptable and widely understood by the ophthalmic and genetic communities. It is, however, imperative to identify the underlying genetic variants and to anticipate associated systemic and ocular anomalies and complications to provide effective management (Ma 2018).

Perhaps the most clinically heterogeneous and sometimes inaccurate diagnosis is that of Peters' anomaly. Here corneal opacification is accompanied by central defects in Descemet membrane and variable degrees of lenticulo-irido-corneal adhesion. Such patients very commonly have systemic anomalies (Peters' Plus) (Traboulsi 1992) and posterior segment ocular defects that compound the visual axis impediment and the high prevalence of glaucoma. Sclerocornea and Peters' anomaly are often and understandably confused, although ultrasound biomicroscopy, where available, distinguishes the two. Accurate phenotyping will improve the ability to identify a causative mutation, thus helping to guide follow-up and genetic counselling.

Patients with small or undeveloped eyes also present challenges in clinical diagnosis. They are often referred to as having the anophthalmia-microphthalmia-coloboma spectrum (Plaisancié 2019; Slavotinek 2018). The term clinical anophthalmia is used to describe the situation where the eyes are not visible clinically, but may nonetheless be present in a rudimentary form subconjunctivally on ultrasound examination, and may even contain functioning tissue sufficient for light perception. True anophthalmia would describe a situation where the optic vesicles fail to form altogether, and is often associated with absence of optic nerve and/or other optic pathway and CNS defects (Slavotinek 2018). Many individuals whose axial diameter is reduced also have typical colobomas of the iris, choroid and sometimes the optic nerve head in the inferior-nasal area of closure of the fetal fissure. Occasionally such fetal fissure anomalies manifest as orbital cysts, sometimes connecting with the globe. Occasional patients will have colobomas in the presence of a normal sized globe, while others have small eyes, but no colobomas, and hypermetropia calling for a clinical diagnosis of nanophthalmos. Whilst individuals with nanophthalmos usually only exhibit ocular findings, up to half of individuals with colobomatous microphthalmia have systemic anomalies relating to variants in a wide variety of underlying genes, or larger complex chromosomal abnormalities (Chambers 2018). Patients with anophthalmia or colobomatous microphthalmia need extensive systemic work-up and molecular testing to identify the responsible gene variant or chromosomal anomalies (Al Somiry 2019; Cavodeassi 2018; Ceroni 2018; Nédélec 2019; Plaisancié 2019; Slavotinek 2018).

At a time when molecular mechanisms of embryologic development of the eye and other organ systems are beginning to be understood, and the genetic mutations underlying anomalies are more easily tested for, one has to consider the next logical step of using this knowledge towards treatment of these conditions. The eye continues to develop after birth, and a knowledge of how to capitalize on this attribute and an understanding of networks and

pathways of developmental eye genes are fundamental to any therapeutic approach. Emboldened by the initial success of gene therapy relating to retinal degenerative diseases, perhaps related approaches could be adopted to harness the existing pathways for development postnatally. The exciting paradigm that the eye with aniridia could be responsive to treatment postnatally provides that initial milestone and paves the way for emerging future therapies (Gregory-Evans 2019).

Another consequence of this increasing knowledge is that of housekeeping classification and nomenclature, in that one has to revisit terms and criteria used to describe congenital anomalies. Furthermore, the importance of clinical diagnostic precision cannot be overemphasized to ensure the correct path is taken to the identification and management of the individual patient. In a recent meeting in the European Reference Network on Eye Diseases (ERN-EYE), a consensus was to go back toward the simplest way possible of using the HPO terms, and when possible the 'old' syndrome delineations with the limitation that is briefly noted above. The report stated that to build an "intelligent" database that would be able to integrate comprehensive molecular data with extensive phenotypic description was currently out of reach (Sergouniotis 2019).

Based upon the advances made in our basic knowledge of ocular development, the clinician now more than ever has the unique advantage of making accurate observations supported by ocular imaging, systemic investigations, and interpreting molecular tests. They can use these to guide the clinical management of the individual patient with eye anomalies, whether isolated or in the context of a systemic syndrome. When this is provided in the context of a multidisciplinary collaborative approach, the best service can be provided to the affected individual and their family. We thank the contributors to the Special Issue for providing such up to date knowledge that will enable us to do this.

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