PERSPECTIVE

Hope and major strides for genetic diseases of the eye

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There have been dramatic advances in the elucidation of the genetic etiology of inherited eye diseases and their underlying pathophysiology in the last two to three decades. This was made possible by the exponential development of powerful molecular biology instrumentation and techniques, the completion of the human genome project, an increasing interest in the study of these diseases worldwide, and a push by the lay public to find cures for these rare but devastating conditions. The genes for a wide range of eye diseases have been identified and have led to a rethinking and a reclassification of disorders that is based not only on classical clinical signs, but also on underlying genetic etiology. Examples of these include the corneal dystrophies, rare forms of strabismus now designated as the cranial dysinnervation disorders, ocular malformations that result from mutations in transcription factors, cataracts that result from mutations in crystallins and other structural lens components, and finally retinal dystrophies that result from defects in phototransduction or visual cycle defects. This article is a perspective on recent advances in the field of ophthalmic genetics.

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What allowed gene discovery in most of these conditions were the concerted efforts to collect families and refine phenotypic delineation over decades by clinicians and clinician-scientists interested in genetics. This permitted genetic mapping and, through classic candidate gene or other approaches, the identification of the responsible gene(s).

The practising ophthalmologist is appreciating the impact of genetic factors on common disorders such as age-related macular degeneration and the potential for gene therapy after its success in RPE65-related Leber congenital amaurosis (LCA) (Bainbridge *et al*. 2008). Medical journals are devoting increasing page space to articles on genetic aspects of disease, and several ophthalmic journals have established regular rubrics on the subject and have appointed section editors for this field.

We are at a point of time in which it is possible not only to make a correct clinical diagnosis of an inherited ocular disorder, but also to substantiate this diagnosis with molecular evidence, identifying the individuals responsible mutation and assigning specific molecular subtypes of genetically heterogeneous disorders. Laboratories around the world are offering genetic testing for increasing numbers of ocular malformations and retinal dystrophies. This is done either through direct sequencing of the whole gene or of exons most likely to carry mutations (Stone 2007), or through the use of microarrays that detect mutations that have been reported in the literature (Zernant *et al*. 2005; Henderson *et al*. 2007). Costs of clinical molecular testing, the speed at which it is conducted and results returned to the clinical services are de-

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creasing. In USA, insurance companies are accepting to bear the cost of such tests in majority of cases.

Definitive diagnosis of the retinal dystrophies, for example, is accomplished through genetic testing and the identification of the causal mutations (Koenekoop *et al*. 2007). The yield of finding causative mutations in patients with retinal dystrophies varies between disorders and is dependent on the familiarity of the ophthalmologist with characteristic clinical findings of the individual dystrophy, and the availability of clinical testing for the particular disorder and gene. For example, the yield of genetic testing for retinitis pigmentosa in the adult patient with autosomal dominant disease is around 25% if rhodopsin mutations are tested for; there is around a 70% chance of finding the responsible mutation in patients with LCA, and this yield is even higher if the individual phenotype is suggestive of a particular gene such as *CRB1* in patients with preserved paraarteriolar RPE, thickened and disorganized retina. The experience of the ophthalmologist who is ordering the test based on the clinical phenotype cannot be overemphasized. It is possible that in the future there will be microarrays that would test for a number of genes that cause autosomal recessive or autosomal dominant retinitis pigmentosa, for example. I recommend the utilization of testing facilities that provide full sequencing of individual genes, whenever possible, as well as professional interpretation of sequence variations. This approach has the highest chance of finding all mutations, but is lengthy and leads to delays in returning a report, and to extra costs. Microarrays (or chips) allow rapid testing, are relatively cheap, robust and based on sequence variations presumably or definitely causing disease in reported patients. Their disadvantage lies in the fact that they will not detect unreported mutations. Almost 60%–70% of new LCA patients will have at least one causal mutation identified by the latest versions of the microarrays (Koenekoop *et al*. 2007). All genetic testing has to differentiate benign gene sequence variants or benign polymorphisms from causal mutations. Co-segregation of the variant with the disease trait in the family, new *in silico* prediction programmes, and rapid laboratory functional testing of variants assist the testing laboratories in their interpretation of sequence variations in individual patients and families.

OCT and emerging retinal imaging techniques will take increasing importance in the evaluation of patients with retinal dystrophies before therapies are instituted. The high resolution that is possible with the new generation scanners allows the evaluation of retinal architecture, the preservation of retinal layers and measurement of retinal thickness. When this information is added to psychophysical data, it provides a more complete baseline overview of a patient's retinal (potential) function as they are evaluated for medical or gene therapies.

I will restrict my next comments to the retinal dystrophies and to recent advances in the treatment of RPE65-related LCA, since gene therapy for strabismus, congenital malformations, cataracts and corneal dystrophies are either impossible or unlikely to be developed in the near future. Many of these disorders, however, are amenable to surgical and other interventions that improve visual function, and diagnostic genetic testing plays an important role in the assignment of specific genotypes to the individual patient and family.

As a group, retinal dystrophies constitute a significant cause of childhood and adult blindness. They are the leading cause of childhood blindness in developed and developing countries. Since the discovery that mutations in the rhodopsin gene cause some cases of autosomal dominant retinitis pigmentosa (Dryja *et al*. 1990), more than 180 genes that are associated with retinal dystrophies have been mapped and cloned (www.retnet.org). Many of these genes code for proteins that are involved in the normal development and functioning of photoreceptors, RPE and other retinal neurons. A recent development in the field of retinal dystrophies and associated disorders is the discovery that mutations of genes coding for proteins involved in the structure and function of the cilia of photoreceptors and other ciliated cells in the body lead to retinal dysfunction. These inherited disorders have been termed 'ciliopathies' and include Joubert syndrome, the Usher syndromes, Bardet–Biedl syndromes, as well as some forms of LCA and retinitis pigmentosa (Adams *et al*. 2007). The cilium connects the inner and outer segments of photoreceptors and is intimately involved in intra-cellular signalling, in the Wnt and Sonic hedgehog signalling pathways, and cilia are thus involved in tissue patterning and development (Adams *et al*. 2007).

While the diagnostic aspects of genetic eye diseases and retinal dystrophies in particular have made major strides, therapy remains limited but very promising. Animal studies have demonstrated that many retinal dystrophies can be significantly improved with gene replacement therapy (Cideciyan *et al*. 2009), pharmacological intervention (Van Hooser 2000; Maeda *et al*. 2009), or nanoparticle delivery (Cai *et al*. 2008). None of this would have been possible without gene identification, technological advances and an improved understanding of underlying pathophysiology. Most exciting is the successful and so far safe treatment of RPE65-related LCA by viral-mediated normal gene transfer using subretinal injections (Bainbridge *et al*. 2008; Maguire *et al*. 2008; Cideciyan *et al*. 2009). Several European and USA research teams are currently administering this therapy within very strict research protocols. While initial trials involved only adults, the promising results have encouraged the enrollment of children in the hope of preventing progressive and irreversible vision loss. This therapeutic model will undoubtedly be used in other retinal disorders.

With the current detailed delineation of the clinical manifestations of genetic eye diseases, and with the availability of precise gene testing, sophisticated retinal imaging and electrophysiologic testing modalities, accurate diagnosis of individual retinal dystrophies is possible in many but not all

patients. This knowledge allows a better prediction of the clinical course and the planning of possible and emerging therapies. One should provide the most precise diagnosis and determine the stage and extent of the disease using available diagnostic modalities. There is good reason to be hopeful that therapy will be available soon, at least for some of these devastating diseases. Comprehensive eye examinations, low vision evaluation with the prescription of corrective lenses and other low vision devices as needed, allow more optimal patient function. A healthy diet, UV light protection and modest vitamin supplementation should be encouraged, as all these modalities have a potential beneficial role in retinal health.

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