Chromosomal Rearrangements and Novel Genes in Disorders of Eye Development, Cataract and Glaucoma

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Disorders of eye development such as microphthalmia and anophthalmia (small and absent eyes respectively), anterior segment dysgenesis where there may be pupillary and iris anomalies, and associated cataract and glaucoma, often lead to visual impairment or blindness. Currently treatment options are limited, as much is unknown about the molecular pathways that control normal eye development and induce the aberrant processes that lead to ocular defects. Mutation detection rates in most of the known genes are generally low, emphasizing the genetic heterogeneity of developmental ocular defects. Identification of the disease genes in these conditions improves the clinical information available for affected individuals and families, and provides new insights into the underlying biological processes for facilitation of better treatment options. Investigation of chromosomal rearrangements associated with an ocular phenotype has been especially powerful for disease gene identification. Molecular characterization of such rearrangements, which pinpoints the region by physically disrupting the causative gene or its regulatory sequences, allows for rapid elucidation of underlying genetic factors that contribute to the phenotype. Genes including *PAX6*, *PITX2*, *FOXC1*, *MAF*, *TMEM114*, *SOX2*, *OTX2* and *BMP4* have been identified in this way to be associated with developmental eye disorders. More recently, new methods in chromosomal analysis such as comparative genomic hybridization (CGH) microarray, have also enhanced our ability in disease gene identification.

Keywords: chromosomal rearrangements, disease genes, cataract, glaucoma, microphthalmia

Congenital ocular anomalies include debilitating eye conditions such as anophthalmos (no eye), microphthalmos (small eye), coloboma (optic fissure defect), congenital cataract (clouding of the lens) and anterior segment dysgenesis (iris and corneal abnormality) which confers a high risk of blindness due to glaucoma. These disorders can occur separately in patients if an underlying disease gene affects only one part of the eye (Figure 1A, 1B, Figure 2), or if the abnormality occurs very early on so that there is no eye (anophthalmia; Figure 1C). Patients may also have combinations of eye anomalies with concomitant features such as coloboma and cataract in the one eye (Figure 1D). For combinations of eye anomalies to occur some underlying disease genes are expressed in many parts of the developing eye. For instance, *PAX6* is expressed in the early developing optic vesicle and lens placode (4th to 5th week of development in the human) (Figure 2A, 2B), and continues to be expressed in the developing retina, iris and lens (Figure 2C, 2D), so that mutations in *PAX6* may lead to disease affecting both the anterior and posterior segments of the eye (Glaser et al., 1994; Grindley et al., 1995). In contrast, other genes are principally expressed in one part of the eye and the development of this component may influence the development of other eye structures (Beebe & Coats, 2000). As an example, *MAF* is expressed in the developing lens (Figure 2B; Kawauchi et al., 1999), and the presence of iris colobomata in some *MAF* mutation patients indicates that development of the lens may influence development of the iris (Hansen et al., 2007; Jamieson et al., 2002). These possible combinations of ocular anomalies emphasize the complexity of the genetic and developmental pathways in eye formation, perhaps accounting in part for the

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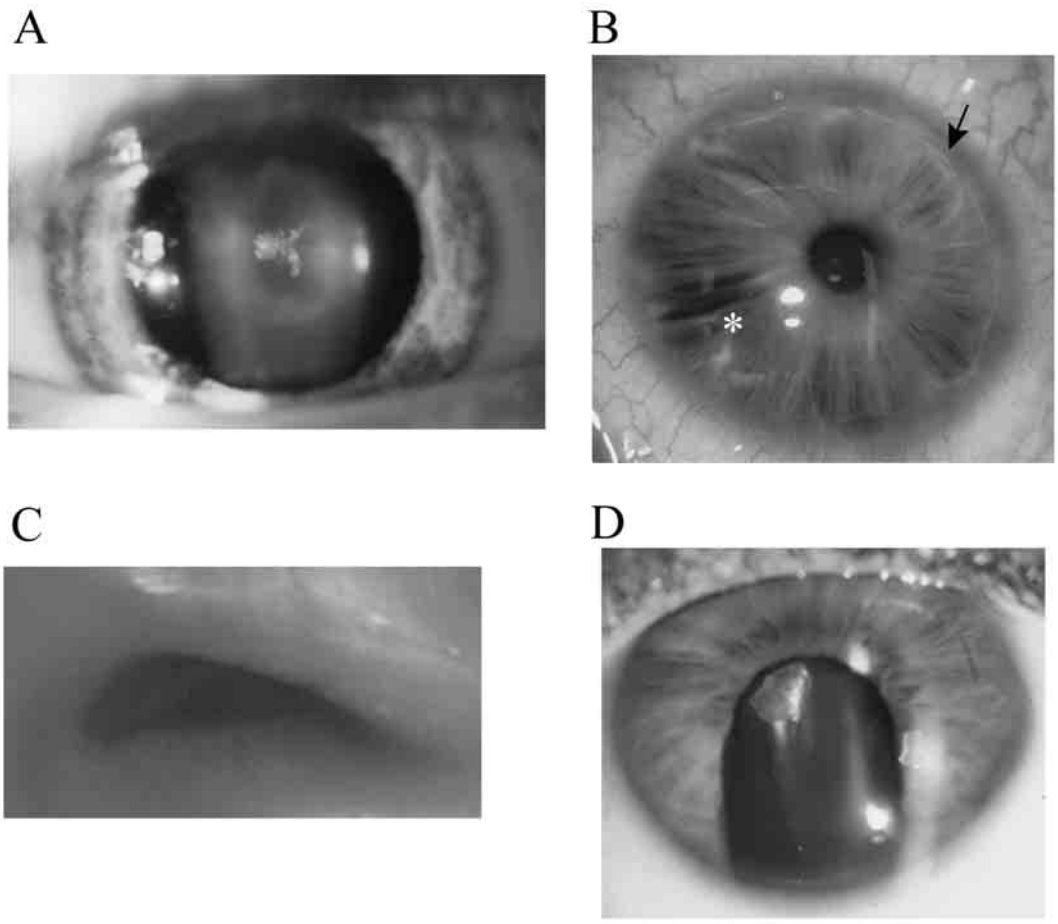

Figure 1

Developmental ocular anomalies may affect one or several parts of the eye. A. Hereditary congenital cataract causing a sutural and lamellar cataract in the lens. Disruption of *TMEM114* in a chromosomal translocation family is associated with lamellar cataracts (Jamieson et al., 2007). B. Rieger anomaly of the eye with prominent posterior embryotoxon (arrow) and iris hypoplasia (*). Mutations in *FOXC1* may lead to isolated eye anterior segment dysgenesis (Nishimura et al., 2001). C. Eye anophthalmos. *SOX2* mutations can be found in patients with anophthalmia (Fantes et al., 2003). D. Coloboma, cataract and microphthalmia present in the one eye. Cataract and iris coloboma have been identified in two *MAF* mutation patients (Hansen et al., 2007; Jamieson et al., 2002).

marked genetic heterogeneity in these conditions. While a number of disease genes have been identified, the underlying genetic causes in the majority of patients are unknown.

Chromosomal Rearrangements and Disease Gene Identification

Patients with chromosomal translocations or other rearrangements provide a rapid pointer in novel eye disease gene identification. This approach can be particularly effective for investigating severe ocular disorders such as anophthalmia (Fantes et al., 2003), where it may be difficult to identify families large enough for other approaches such as linkage analysis. It can also prove successful in less severe ocular disorders where there is a familial translocation (Jamieson et al., 2007). Chromosomal rearrangements such as translocations, inversions or microdeletions may result in a loss- or gain-of-function phenotype either by physically disrupting the coding region of a gene, or by dissociating it from its regulatory elements and/or placing it under the control of other regulatory elements (Kleinjan & van Heyningen, 2005). Molecular characterization of the chromosomal rearrangement therefore provides an opportunity to isolate gene/s or regulatory element/s disrupted by the rearrangement which may contribute to the disease phenotype in the patient. In this review, we discuss the ocular developmental disorders where underlying disease genes have been identified in this way (Table 1), as well as comparative genomic hybridisation (CGH) microarray analysis which may provide an additional avenue for novel ocular disease gene identification.

Eye Disorder Genes Identified Through Chromosomal Rearrangements

Anterior Segment Anomaly and Glaucoma Genes

PAX6

Heterozygous mutations in *PAX6* underlie the panocular condition aniridia where patients have absence

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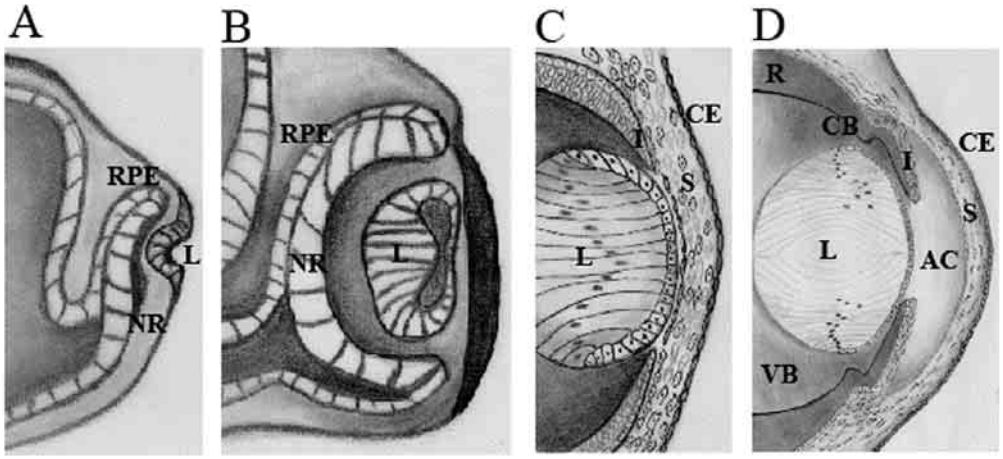

Figure 2

Spatial relationship of developing structures in eye development and eye components. In the developing mammalian eye the optic vesicle is of neuroectodermal origin and it arises from the forebrain at embryonic day (E) 8.5 in the mouse (approximately beginning of 4th week of development in the human) (Moore & Persaud, 1993; Smith et al., 2002). A. The optic vesicle approaches the surface ectoderm, which starts to thicken forming the lens (L) placode which at E10.5 (early 5th week of development in the human) invaginates into the optic cup. The optic vesicle folds so that the anterior layer forms the future neuroretina (NR) and the posterior layer forms the retinal pigment epithelium (RPE). B. The lens pit then separates from the surface ectoderm and becomes the lens (L) vesicle at E11 in the mouse (end of 5th week of development in the human) and the cornea begins to form and retinal structures undergo differentiation. The layers of the optic cup forming the optic fissure close inferiorly at E12.5 in the mouse (during the 6th week of development in the human). C. At E14 to E15.5 in the mouse (6th to 7th week in the human), the mesenchymal cells closest to the lens condense to form the corneal endothelium. Corneal epithelium (CE) originates from the overlying surface ectoderm, while differentiating mesenchyme that forms the corneal stroma (S) is located between the epithelium and endothelium. The primary lens fibres elongate and fill the lumen of the lens (L) vesicle. The tip of the optic cup extends and the formation of the future iris (I) begins. D. By E16.5 in the mouse the anterior chamber (AC) has formed (7th week of development in the human). The anterior edge of the optic cup extends forward in between the lens and cornea to provide the base for the iris (I) and ciliary body (CB). Further mesenchymal migration onto the anterior surface of the iris and posterior CB forms the iris and ciliary body stroma. Once the primary lens fibres have elongated to fill the lens (L) lumen, the secondary lens fibres start to elongate from the lens equator. Secondary fibres from each side of the lens meet at the anterior and posterior ends and form the Y-shaped lens sutures. At this stage the components of the eye are clearly present with an anterior segment that contains the cornea (CE, corneal epithelium and S, corneal stroma), which is the clear front part of the eye, as well as the iris (I) or colored part of the eye, the anterior chamber (AC) and associated drainage structures and the lens (L). The posterior segment contains the retina (R), which is composed of several cell layers including the light-sensitive photoreceptor cells, as well as the optic nerve, and the vitreous body (VB) within the cavity of the optic cup behind the lens.

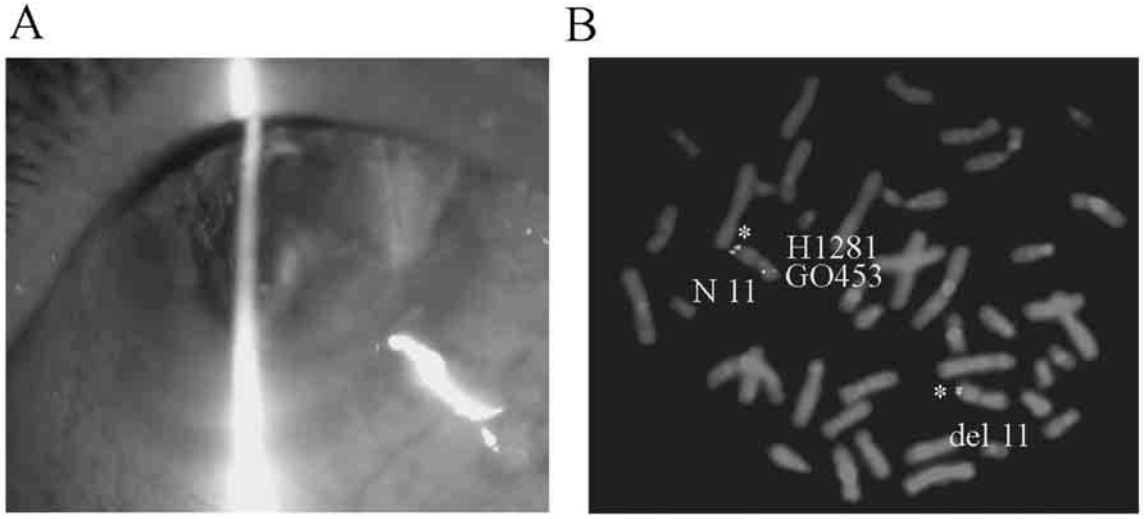

Figure 3

Familial cryptic 11p13 deletion in aniridia. A. A man with aniridia had a normal karyotype and on eye examination there was almost complete absence of the iris, as well as corneal vascularisation. B. FISH experiments were conducted using several probes for the 11p13 loci *PAX6* and *WT1*. The first of these experiments detected loss of signal for the probes G0453 and H1281, on one copy of chromosome 11 ("del 11"). These two probes lie approximately 10kb and 70kb (respectively) 3' to *PAX6*, in a critical control region for *PAX6* (Crolla & van Heyningen, 2002). The other chromosome 11 copy ("N 11") was normal for these two probes. An 11q subtelomere probe also showed normal signals on both chromosomes 11 (*). Other FISH experiments (data not shown) found normal results for *PAX6* exons 1 to 4, and the *WT1* locus on both chromosomes 11. This same cryptic deletion was also found in this man's son and daughter, both also having aniridia.

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Table 1

Eye Anomaly Genes Identified Through Chromosomal Rearrangements and Estimates of Mutation Detection Frequency Where Possible

Eye anomaly phenotype groups and gene symbols and names	Locus	Phenotype, frequency of mutation detection
Anterior segment anomaly & glaucoma		
PAX6, (paired box gene 6)	11p13	Aniridia, up to 90% Peters anomaly, occasional mutation reports (Crolla & van Heyningen, 2002; Hanson et al., 1994; Robinson et al., 2008)
PITX2, (paired-like homeodomain transcription factor 2)	4q25	Rieger syndrome, 37–42% Isolated eye anterior segment dysgeneses eg Rieger, Axenfeld anomalies, 1% (Amendt et al., 2000; Perveen et al., 2000)
FOXC1, (Forkhead box C1)	6p25.3	Isolated eye anterior segment dysgeneses eg Rieger, Axenfeld anomalies, 13% (Nishimura et al., 2001)
Congenital and hereditary cataracts		
MAF, (V-MAF avian musculoaponeurotic fibrosarcoma oncogene homologue)	16q23	Cataracts — pulverulent, cerulean or lamellar, with small corneae, +/– iris colobomata (Hansen et al., 2007; Jamieson et al., 2003; Vanita et al., 2006)
TMEM114, (transmembrane protein 114)	16p13.3	Cataracts — lamellar (Jamieson et al., 2007)
Anophthalmia and severe microphthalmia		
SOX2, (SRY-BOX 2)	3q26.3-q27	Anophthalmia, 10%, frequent associated learning difficulties, pituitary, oesophageal & other anomalies (Bakrania et al., 2007; Ragge et al., 2005a)
OTX2, (orthodenticle, Drosophila, homologue of, 2)	14q22-23	Anophthalmia, 2%, associated learning difficulties (Ragge et al., 2005b)
BMP4, (bone morphogenetic protein 4)	14q22-23	Anophthalmia, 2%, associated learning difficulties and hand anomalies (Bakrania et al., 2008)

or hypoplasia of the iris associated with cataracts, glaucoma, corneal vascularisation and macular hypoplasia (Glaser et al., 1992; Hanson et al., 1993). Wilms tumor and aniridia in patients with the WAGR (Wilms tumor, Aniridia, Genitourinary abnormalities, mental Retardation) syndrome as well as those with isolated aniridia, in association with 11p13 deletions facilitated the long range physical mapping and cloning of *PAX6* (Ton et al., 1991; van Heyningen et al., 1985). *PAX6* codes for a highly conserved transcription factor containing two DNA binding domains, a paired domain and a paired-type homeodomain.

Numerous pathological mutations in PAX6 have been described producing predominantly aniridia, but other phenotypes are also described such as Peters anomaly (anterior segment abnormality with central corneal clouding) and autosomal dominant keratitis (Glaser et al., 1992; Hanson et al., 1994; Mirzayans et al., 1995). Approximately 37% of patients with sporadic aniridia have deletions or rearrangements of the *PAX6* region at 11p13 with approximately half of these being visible cytogenetically and the remaining half detectable by FISH for cryptic deletions (Crolla & van Heyningen, 2002; Gronskov et al., 2001; Robinson et al., 2008). Since *WT1* is only approximately 700kb from *PAX6*, *PAX6* deletion patients may have a *WT1* deletion and so require surveillance for the development of Wilms tumor. Patients with sporadic aniridia may also have intragenic mutations and these are predominantly mutations which may cause loss of function of the protein such as nonsense, frameshift and splice-site mutations (Tzoulaki et al., 2005). Familial aniridia is more usually caused by intragenic mutations present in approximately 70 to 90% of cases (Crolla & van Heyningen, 2002; Gronskov et al., 2001; Robinson et al., 2008); (Table 1). However, chromosomal abnormalities may also be responsible, and we have identified a family where partial deletion of the 3' region of the PAX6 gene is present in an affected father and two affected children (Figure 3A, 3B). Intragenic missense mutations may be found in patients with less classical features of aniridia including ectropion uveae (Figure 4); (Willcock et al., 2006).

PITX2

PITX2 at 4q25 codes for a transcription factor belonging to the paired-bicoid homeobox family and mutations in this gene cause Rieger syndrome, which is characterized by eye anterior segment dysgenesis plus dental, umbilical and other anomalies. The association of Rieger syndrome in patients with 4q deletions and translocations led to localisation and cloning of *PITX2* (Semina et al., 1996; Vaux et al., 1992). Eye anterior segment dysgenesis includes such features as adhesions between the iris and cornea, as

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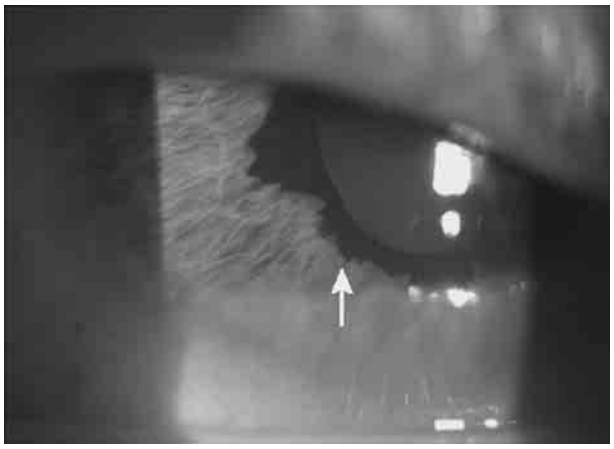

Figure 4

Ectropion uveae in variant aniridia with a PAX6 X423L mutation. Ectropion uveae is present where there is extension of the posterior pigment epithelium of the iris onto the anterior iris surface causing darkening around the pupil (arrow). This patient and his similarly affected infant son also had hypoplastic anomalous optic discs and macular hypoplasia. This combination of anterior and posterior segment ocular findings suggested a diagnosis of variant aniridia and an X423L mutation was identified in PAX6 (Willcock et al., 2006).

well as possible iris hypoplasia and raised intraocular pressure and glaucoma. When these eye findings occur in isolation, without associated systemic features such as dental and umbilical anomalies, they are referred to as Rieger or Axenfeld anomaly (Amendt et al., 2000).

Heterozygous intragenic mutations including frameshift, nonsense, splice-site and missense mutations in *PITX2* are identified in approximately 37-42% of patients with Rieger syndrome. In contrast, the mutation detection rate in patients with isolated eye anterior segment dysgenesis (e.g., Rieger anomaly and other isolated anterior chamber anomalies) is low, in the vicinity of 1% (Amendt et al., 2000; Perveen et al., 2000) (Table 1). Reported mutation detection series may not routinely include karyotypic or microdeletion detection techniques to determine chromosomal abnormalities in these patients. We have recently seen a 20 year old intellectually normal man with Rieger syndrome whose karyotype showed a *de novo* 4q inversion. The inversion breakpoints were difficult to precisely define on karyotype analysis and subsequent CGH microarray analysis revealed a deletion which included *PITX2* (Figure 5A, 5B). This emphasizes the need for karyotypic investigation in patients with Rieger syndrome and the additional benefit of CGH microarray in detection and clarification of the precise genetic anomaly.

FOXC1

Two chromosomal translocations involving chromosome 6p25 were mapped to reveal *FOXC1* as a candidate disease gene in patients with anterior segment dysgenesis (Nishimura et al., 1998). FOXC1 belongs to the forkhead family of transcription factors distinguished by a highly conserved DNA-binding domain, called the forkhead domain.

In a series of 70 patients with non-syndromic eye anterior segment dysgenesis, seven (10%) patients were found to have heterozygous intragenic mutations in *FOXC1*, including frameshift, nonsense and missense mutations, while two (2.9%) had 6p25 duplications, one of which was detectable cytogenetically (Nishimura et al., 2001; Table 1). *FOXC1* mutations have also been detected in some patients with Rieger syndrome, although the detection rate is difficult to determine due to small numbers of patients examined and lack of Rieger syndrome definition in some series (Cella et al., 2006; Honkanen et al., 2003; Mears et al., 1998). Additional syndromic features including distinct facial features, cardiac anomalies and deafness are recognized with more extensive deletions of the 6p25 region (Gould et al., 2004; Maclean et al., 2005).

Congenital and Hereditary Cataract Genes

MAF

Chromosomal breakpoint mapping in a family with a t(5;16)(p15.3;q23.2) translocation in balanced and unbalanced forms led to the identification of *MAF*, at 16q23, as a disease gene in cataract and anterior segment development (Jamieson et al., 2002; Jamieson et al., 2003). *MAF* belongs to the basic region leucine zipper (bZIP) transcription factor superfamily and is expressed in the lens (Blank & Andrews, 1997).

Heterozygous intragenic mutations in *MAF* have been found in patients with pulverulent cataracts in association with reduced corneal diameters and occasionally iris colobomata (Hansen et al., 2007; Jamieson et al., 2003; Vanita et al., 2006; Table 1). Mutations identified so far are missense mutations principally affecting conserved amino acids in the DNA-binding domain. A missense mutation in the transactivation domain in the mouse also leads to a cataract phenotype (Perveen et al., 2007).

TMEM114

A balanced t(16;22)(p13.3;q22.1) translocation in a family with congenital lamellar cataracts was mapped to reveal a cluster of lens-derived expressed sequence tags (ESTs) in a previously unsequenced region of the human genome at 16p13.3 (Jamieson et al., 2007). Breakpoint mapping, bioinformatic analysis and sequencing revealed that the 16p breakpoint physically disrupted the 5' promoter region of the novel *TMEM114* (*Transmembrane protein 114*) gene. *TMEM114* is a newly identified member of the PMP-22/EMP/MP20 transmembrane protein family, members of which have previously been implicated in cataract formation (Pras et al., 2002; Steele et al., 1997; Table 1). Expression studies confirmed *Tmem114* presence in the developing eye and postnatal lens anterior epithelium of the mouse. Sequencing of the coding exons of *TMEM114* identified heterozygous missense variants in conserved amino acids in two unrelated cataract patients, which were absent in 200 control chromosomes but present

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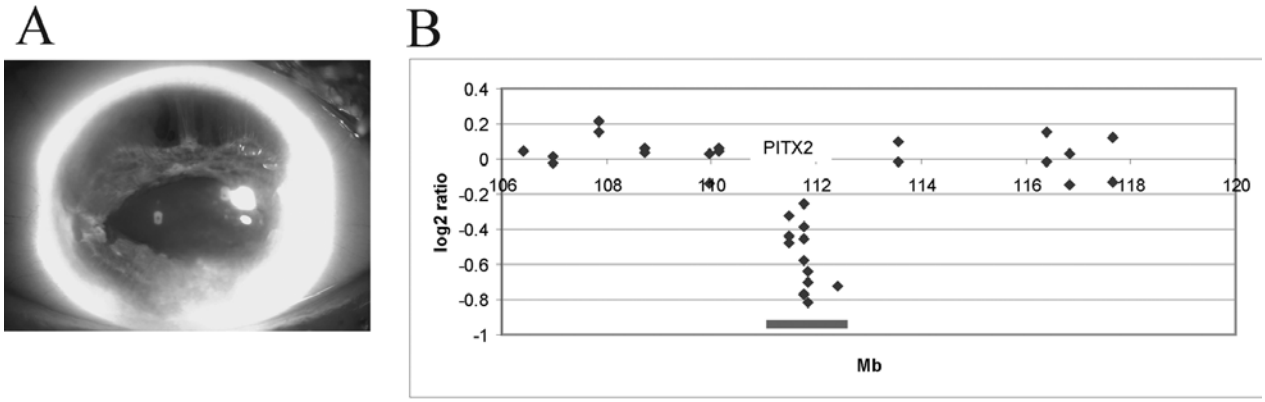

Figure 5

Rieger syndrome in a patient with a de novo 4q inversion and PITX2 microdeletion. A. This patient had iris absence and hypoplasia, but no macular or optic nerve abnormalities. He also had absent teeth and redundant periumbilical skin, and a clinical diagnosis of Rieger syndrome was made. A karyotype revealed a 4q inversion, which was not found in his parents. The breakpoints and a possible deletion region seen on karyotyping were difficult to define and were reported as 46,XY,der(4)?del(4)(q?31.?2q?31.?3)inv(4)(q21.3.q3?3). B. CGH microarray on this patient using a Bluegnome cytochip (Cambridge, UK) 1 Mb array, is shown plotted from 106-120Mb on chromosome 4. Each data point represents an individual BAC clone with between 1 & 5 replicates. The deleted region, indicated by the solid line at the bottom of the figure, extends from approximately 111.5–112.4 Mb and includes the PITX2 gene at 111.8 Mb. In view of this array finding, the karyotype description of the 4q inversion was amended in retrospect, to 46,XY,inv(4)(q25q33).

in nonaffected first degree relatives, indicating they may be rare normal variants or associated with congenital cataracts with reduced penetrance (Jamieson et al., 2007).

Anophthalmia and Microphthalmia Genes

SOX2

Reports of deletions and translocations of chromosome 3q27 associated with anophthalmia and microphthalmia, followed by FISH investigation in an infant with anophthalmia and a t(3;11)(q27;p11.2) translocation, led to the identification of *SOX2* as a disease gene in bilateral anophthalmia (Driggers et al., 1999; Fantes et al., 2003; Male et al., 2002). *SOX2* is a member of the sex determining region Y-box (SOX) transcription factor gene family and its ocular expression is evident at critical stages of eye development starting from the early optic vesicle and the lens field of the surface ectoderm. It continues to be expressed in the proliferating cells of the neuroretina and developing lens (Kamachi et al., 1998).

Heterozygous mutations in *SOX2* are identified in approximately 10% of patients with bilateral anophthalmia or severe microphthalmia (Table 1). These mutations include frameshift and nonsense mutations expected to lead to a truncated mutant protein, as well as whole gene deletions identified by MLPA (multiplex ligation-dependent probe amplificiation) and FISH (Bakrania et al., 2007; Fantes et al., 2003; Ragge et al., 2005a). Associated ocular features in a microphthalmic eye in these patients include sclerocornea and cataracts. Non-ocular features are frequently present and may include learning difficulties, as well as neurological, pituitary, oesophageal and male genital anomalies (Bakrania et al., 2007; Fantes et al., 2003; Williamson et al., 2006).

OTX2

Analysis of patients with interstitial deletions of 14q22-23 and a t(3;14)(q28;q23.2) patient led to the identification of *OTX2* as a candidate disease gene in anophthalmia (Bennett et al., 1991; Elliott et al., 1993; Lemyre et al., 1998; Nolen et al., 2006). *OTX2* is a bicoid-type homeodomain transcription factor important in retinal differentiation and fore and midbrain development (Bovolenta et al., 1997; Simeone et al., 1993). Heterozygous loss of function mutations in *OTX2* are a rare cause of the anophthlamia or severe microphthalmia phenotype occurring in approximately 2% of patients (Table 1). Associated learning difficulties may be present (Ragge et al., 2005b).

BMP4

Analysis of the severe phenotype in 14q22-23 deletion patients and the t(3;14)(q28;q23.2) patient also indicated the likelihood of *BMP4* as a candidate disease gene in anophthalmia (Nolen et al., 2006). BMP4 is a member of the transforming growth factor-β1 superfamily of secretory signalling molecules. It is expressed in the early forming optic vesicle and is necessary for lens induction (Furuta & Hogan, 1998) and anterior and posterior segment development (Chang et al., 2001). *BMP4* mutation is a rare cause of the anophthalmia phenotype with a heterozygous intragenic frameshift mutation identified in one out of 215 ocular developmental anomaly probands, and in this familial case there was also learning difficulty and polydactyly. A patient with bilateral microphthlamia in this cohort had a missense variant with associated learning difficulty and hand anomalies, although the unaffected father also had this variant (Bakrania et al., 2008). Variable penetrance and gonadal mosaicism in unaffected parents of children with anophthalmia

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makes recurrence risk prediction difficult even when an underlying mutation is identified in *SOX2*, *OTX2* or *BMP4* (Bakrania et al., 2008; Faivre et al., 2006; Ragge et al., 2005b).

Translocation Patients and CGH Microarray for Future Novel Gene Detection in Ocular Anomalies

Investigation of chromosomal rearrangements and deletions detected by standard karyotype analysis, has been successful in novel gene identification in a number of ocular disorders. The efficiency of the analysis of chromosomal aberrations has been enhanced by improved FISH detection methods, availability of BAC probes for labeling for breakpoint mapping, and the application of bioinformatic analyses of the breakpoint and deletion regions for prioritization of candidate genes enabled by resources such as the UCSC genome browser (http://genome.ucsc.edu/; Kent et al., 2002). This emphasizes the extremely valuable nature of chromosomal aberrations that are identified in association with an ocular phenotype.

CGH microarray technology is particularly effective for identifying novel candidate genes in subtle deleted or duplicated regions. Standard karyotypic analyses, while valuable in detecting balanced chromosomal translocations and inversions, are limited in their resolution of duplications or deletions to about 5 to 10 Mb. CGH microarrays provide the means for disease gene identification at a greater resolution in whole genome analysis (Bakrania et al., 2008; Nolen et al., 2006; Vissers et al., 2004). In CGH microarray, genomic DNA from the patient and a control case are labeled with two different fluorescent dyes and then co-hybridized to the array slide, which is imprinted with a DNA probe set, arrayed as many thousands of discrete spots. Genomic imbalances are quantified by analysing the ratio of the two fluorescent signals for each spot (Jarmuz et al., 2006). The resolution of the array is determined by the density (across all the chromosomes) of the genomic clone set printed on the array. Studies using CGH microarray to assess patients with mental retardation and/or other malformations with a normal karyotype +/- normal sub-telomere FISH, have revealed that 10-14% of the patients have a chromosomal abnormality responsible for the phenotype (de Vries et al., 2005; Menten et al., 2006; Shaw-Smith et al., 2004; Thienpont et al., 2007; Vissers et al., 2003). Most of these studies used a resolution of approximately 1Mb across the genome. Our application of this technology in a pilot study of patients with normal karyotypes with ocular anomalies plus mental retardation, dysmorphic features or other congenital anomalies has identified microdeletions in three out of 26 (11.5%) Australian patients. Two patients had microdeletions in known disease regions and one has revealed a novel region for investigation in anterior segment dysgenesis and glaucoma. These findings, in addition to two other novel balanced chromosomal translocation patients with anterior segment dysgenesis and glaucoma recently identified, indicates that chromosomal aberration patients must not be ignored and can continue to provide valuable starting points for novel disease gene identification. Combination of this approach, with linkage analysis where it is possible with large patient families, and analyses of animal models for these disorders, will optimize the likelihood of identifying novel disease genes and understanding of the functional impact of mutations in these genes in eye disease.

Acknowledgments

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