REVIEW

Insights into keratoconus from a genetic perspective

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Keratoconus is a progressive and non-inflammatory thinning of the cornea, which may result in severe visual impairment due to irregular curvature and scarring. It can occur in isolation but is often seen in association with other systemic or ocular disorders. There is a well-recognised genetic component to keratoconus, as evidenced by family and twin studies; however, the aetiology of the disease is complex with both genetic and environmental factors playing a role. Over the last decade significant progress has been made in identifying genetic risk factors for keratoconus. Multiple approaches have been taken including candidate gene studies and genome-wide studies. *VSX1* remains as the best characterised keratoconus gene but only accounts for rare cases. Other candidate genes with a role to play include *SOD1*, other corneal dystrophy genes such as *ZEB1* and *TGFBI* and collagen genes. Family-based studies have recently led to the identification of the *MIR184* gene for keratoconus with cataract and to the *DOCK9* gene in a family with isolated keratoconus. Numerous other linkages have been reported and new sequencing technologies are set to rapidly expand the number of identified keratoconus genes in these regions. Similarly, recent genome-wide association studies in case-controlled cohorts have identified common variations in and around *HGF*, *RAB3GAP1* and *LOX* as candidate risk factors for keratoconus. These gene identifications are beginning to reveal the molecular aetiology of keratoconus but despite this recent progress, there remain numerous genetic risk factors to be identified for this relatively common yet complex disease.

Keratoconus most likely represents a multigenic disease with a complex mode of inheritance and environmental factors contributing to the disease manifestation. The relative contribution of each is subject to debate and likely varies between individuals. It appears that at one end of the spectrum, disease in some individuals is probably due entirely to environmental influences, as seen following trauma,[1] yet at the other end may be solely under the control of genetic mechanisms, as evidenced by strong autosomal dominant inheritance in certain families.[2,3] The majority of cases will most likely be the result of a genetic predisposition with precipitating environmental stressors or modifiers. This review article summarises the historical and current level of knowledge of the genetic risk factors for keratoconus.

PHENOTYPIC SPECTRUM

Early descriptions of keratoconus were focused on advanced disease, that is, severe corneal disease causing marked visual impairment or other clinical symptoms. The advent of more sophisticated corneal imaging modalities, such as computerised corneal topography and *in vivo* confocal microscopy has led to a greater understanding of the vast variation in expressivity of corneal diseases. This allows objective measurement of earlier and/or milder changes in the disease process. In particular, corneal topography of 'unaffected' family members in keratoconus demonstrated abnormalities of corneal shape that were not within the 'normal' range, yet did not meet the existing clinical criteria for keratoconus.[4] Clinicians now refer to this entity as 'forme fruste' keratoconus, with a re-definition of the refractive and topographic criteria. Similarly, imaging with the *in vivo* confocal microscope now allows micro-structural analysis, demonstrating alterations in very early or mild corneal disease, rather than only at the end-stage.[5] Large variations in intra- and inter-familial 'disease' are antici-pated when careful, expert phenotyping is undertaken as recently demonstrated in a New Zealand cohort and in twins.[6,7]

HEREDITY OF KERATOCONUS

The role of heredity in keratoconus is well documented,[4,8–10] with concordance in twins and a positive family history reported in six to 23 per cent of patients with keratoconus.[11,12] Using more sophisticated corneal imaging modalities, such as computerised corneal topography, prevalence in first-degree relatives is 15 to 67 times higher than the general population.[10]

The mode of inheritance is reported as sporadic, autosomal recessive[10] and autosomal dominant.[13] Other studies[14] have established linkage by using a model of inheritance that lies somewhere between these two.

Twin studies have emerged as a powerful tool to determine the effect of heredity on disease manifestation. Comparing cohorts

of monozygotic (MZ) twins, with a cohort of dizygotic (DZ) twins, an assessment can be made of the relative contributions of the genotype and environment to the phenotype. A recent small study demonstrated higher concordance of keratoconus in monozygotic than in dizygotic twins, with a greater similarity of phenotype in the monozygotic twins, consistent with a strong genetic component to this disorder.[7]

EPIDEMIOLOGY

The incidence of keratoconus is estimated to be between 29 and 229 per 100,000 depending on the population examined[7] and the rates among different ethnic groups are documented to vary.[11,15–18] A study of incidence and severity of keratoconus at the Leicester Royal Infirmary in the United Kingdom identified a four-fold greater incidence of keratoconus among Asians (predominantly Indian) living in the catchment area than among Caucasians.[18] The severity of the disease, defined as age of onset and time from presentation to graft was also found to be greater among the Asian group. Although race was assigned on the basis of the patient's name rather than collected directly from the patient, the comparisons between races in this study are strong, as all patients were resident in the same area and a single definition of disease was used. It is widely believed that keratoconus is more prevalent and aggressive in New Zealand, especially in the Maori and Polynesian populations, although exact figures are not available.[19] Keratoconus is the leading indication for corneal transplantation in both adults and children in New Zealand and Australia.[20–22] Of the affected Maori and Pacific peoples, 31 per cent have a positive family history.[6] Many multi-generational families are identified that show many members affected in many generations. The strong familial aggregation of keratoconus observed within this population is likely to be due to a major gene effect.

ASSOCIATION WITH OTHER DISORDERS

The occurrence of keratoconus in association with a wide range of other diseases may also provide clues as to the underlying genetic mechanisms. Connective tissue disorders are over-represented among patients with keratoconus, suggesting an underlying

structural abnormality and include Ehlers Danlos syndrome[23] osteogenesis imperfecta, mitral valve prolapse[12] and craniosynostoses, such as Crouzon.[24] Keratoconus is frequently reported as a manifestation of Marfan syndrome,[25] although the cornea in Marfan syndrome is clearly characterised as being flatter in curvature and generally thinner.[26–28] This may suggest that given the right environmental stressor, the inherent corneal structure reaches a tipping point to become keratoconic.

Keratoconus frequently occurs in isolation, but is often associated with a wide range of ocular disorders, including cataract,[29] atopy,[30] Leber congenital amaurosis,[31] other corneal dystrophies and retinal dystrophies.[32] Other associations include X-linked hyphidrotic ectodermal dysplasia, with changes in the *EDA* gene[33] and Williams–Beuren syndrome.[34] The observation of keratoconus occurring in association with an unusual syndrome or disorder, does not in itself mean the underlying genetic cause may be common to both concurrent diseases but it may suggest that a manifestation of the syndrome is an environmental agonist for keratoconus. As keratoconus is not uncommon, it feasibly may occur independently of the coexistent disorder.

Many genetically determined corneal dystrophies can also occur in association with keratoconus including posterior polymorphous corneal dystrophy (PPCD),[35] lattice dystrophy,[36] granular dystrophy[37] and Fuchs' endothelial dystrophy.[38] The association of keratoconus with so many corneal dystrophies and ocular abnormalities implies either a similarity in the underlying genetic defect or a tightly linked network of interacting proteins, with a final common developmental pathway. Keratoconus also occurs with a range of chromosomal abnormalities, most commonly in Down syndrome due to trisomy 21[39] and also in Turner syndrome,[40] chromosome 13 ring anomaly[41] and translocation 7;11.[42]

CANDIDATE GENE ANALYSIS

Depending on knowledge of the underlying biology of the trait, it may be possible to predict genes involved in particular diseases. In keratoconus, potential candidates are those associated with other corneal dystrophies, connective tissue disorders or located on chromosomes where chromosomal aneuploidy or breakpoints are associated with the disease, for example, chromosome 21

| Gene	Locus	Reference
VSX1	20p11.2	43
ZEB1	10p11.22	44
SOD1	21q22.11	45
TGFBI	5q31.1	46
COL4A3/COL4A4	2q36.3	47
FLG	1q21	48

Table 1. Candidate genes with mutations identified in patients with keratoconus

in Down syndrome. Bioinformatic databases are used to determine if putative genes make biological sense and are expressed in the relevant tissue (cornea). Genes are usually prioritised according to their likelihood as disease-causing genes in keratoconus on the basis of their expression patterns and any known functions or mutations, which are relevant to the eye. If the ocular expression of any of the most promising genes is not clear, expression studies using reverse transcription-polymerase chain reaction (RT-PCR) and/or Western blotting of corneal tissue may be performed to assist in evaluating the significance of any variants found in genes that are expressed in the cornea.

Mutations in a number of candidate genes have been determined in a small percentage of patients with keratoconus. These[43–48] are listed in Table 1 and their genomic location indicated in Figure 1.

VISUAL SYSTEM HOMEOBOX 1 GENE

The visual system homeobox 1 gene (*VSX1*) is a member of the 'paired-like' homeodomain transcription factors. This family plays a role in craniofacial and ocular development. Human *VSX1* has been mapped to 20p11.2. It was initially reported to consist of five exons and be approximately 6.2 kb in size.[49] An additional two exons were later characterised,[50,51] which encode alternative isoforms of the *VSX1* transcript. The expression of *VSX1* has been detected in embryonic craniofacial tissues, adult retina and adult corneas.[49,52] Mutations in *VSX1* were reportedly associated with craniofacial abnormalities, empty sella tunica and abnormal retinal cells[53] but more frequently and controversially to a number of corneal dystrophies and ectasias, specifically keratoconus and PPCD.
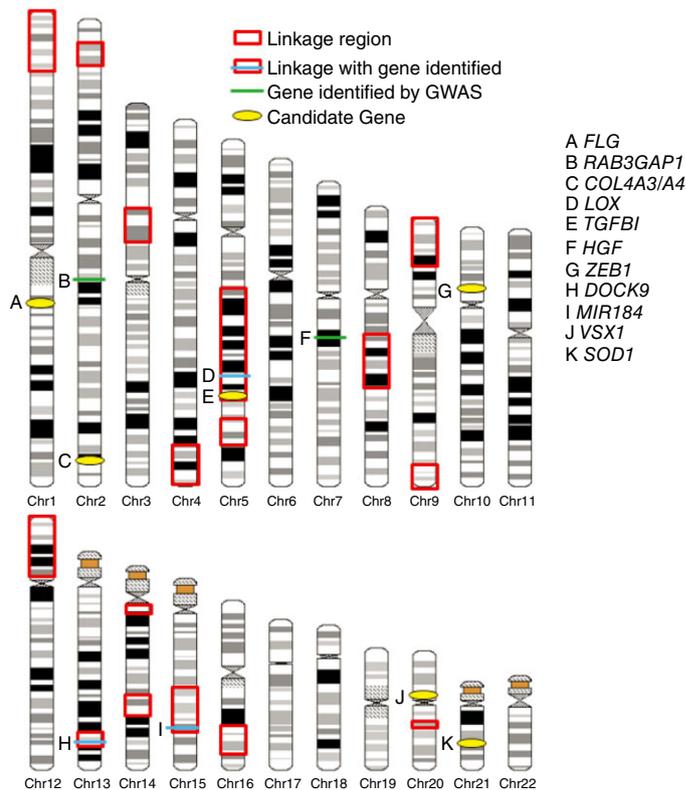
Figure 1. Loci identified for keratoconus through genome-wide and candidate gene studies. Linkage regions are boxed in red. The names of identified genes are given according to the key.

The relationship between keratoconus and *VSX1* was first reported in the study by Héon and colleagues.[43] Several mutations linked to keratoconus have since been identified.[50,54–57] The role of *VSX1* in the pathogenesis of keratoconus has been controversial, as a number of other studies have failed to identify an association between *VSX1* variants/polymorphisms and keratoconus.[58–62] These contradictory results may be attributed partly to the low frequency of changes, ethnic variation, and the mounting evidence that keratoconus is likely to be a multifactorial and polygenic disease.[62]

VSX1 expression in keratocytes has been characterised both *in vitro* and *in vivo* using RT-PCR, immunohistochemistry and *in situ* hybridisation. Although not observed in resting or quiescent human keratocytes, in wounded corneas, or when cultured in serum to mimic wounded conditions, the keratocytes express *VSX1* and this is also associated with fibroblastic transformation.[52] These observations add strength

to the hypothesis that *VSX1* is involved in the wound healing response and thus may contribute to the underlying pathology in corneal disease.

The largest published series on this subject looked at an Italian cohort of 302 individuals with keratoconus and found changes in *VSX1* in 3.2 per cent of the affected population.[63] A study of Iranian families with keratoconus also showed His244Arg segregating with disease in a two generation pedigree. Four affected individuals were heterozygous, whereas five unaffected were not, and the mutation was not present in extensively phenotyped controls.[64] Analysis of the pedigrees has demonstrated a 58 per cent reduced penetrance in general amongst the Iranian families with keratoconus, which could explain the finding of Tang and colleagues,[61] who reported the presence of this same variant in unaffected family members. Other recent studies also highlight segregation of other *VSX1* changes.[63,65]

ASSOCIATION WITH PPCD AND THE *ZEB1* GENE

The association of PPCD with keratoconus is also well documented with many cases of these two conditions occurring in the same cornea.[8,66–71] PPCD and keratoconus share a potential common mode of involvement of the posterior surface of the cornea, specifically Descçemet's membrane and/or an underlying commonality in the pathophysiology of corneal dystrophies. A recent paper characterised the cornea in patients with PPCD showing the topographic parameters are significantly steeper but with no clinical or topographical evidence of keratoconus.[72] This group of patients was not genetically characterised but another study demonstrated that in six patients with *ZEB1* (zinc finger E-box binding homeobox 1) gene mutations, three had steep corneas but no evidence of keratoconus.[73] Another group reported changes in the *ZEB1* gene in patients with keratoconus[44] indicating there may be genetic overlap between these corneal diseases.

OXIDATIVE STRESS GENES

Another explanatory hypothesis is that oxidative stress plays a role in the aetiology of keratoconus. A superoxide dismutase isoenzyme *SOD1* was considered a candidate because of its location on chromosome 21, given the association of trisomy 21 with keratoconus, and a differential level of expression in healthy corneas compared with keratoconic corneas. An intronic sequence deletion was found to segregate with disease in two families with keratoconus[45] but it remains unclear whether this is a truly pathogenic association, as it has only been detected in a further two patients of a total of 430 (0.9 per cent).[60,63,64]

To explore the putative role of oxidative stress, another group undertook mitochondrial complex 1 gene analysis (*ND1–6*, encoded by the mitochondrial genome) in a group of 20 patients with keratoconus negative for *VSX1* mutations.[74] Complex 1 variations may result in an increase in reactive oxygen species (ROS). An extraordinarily large number of changes (n = 84) were found in the ND group of genes, with the majority occurring in *ND5* and included two novel frameshift mutations.[74] Some of the changes detected are reported as also occurring in other mitochondrial related disorders. With such diverse variation and the

complexities associated with mitochondrial disease, the true impact of these changes will require larger studies to verify the interesting findings.

EXTRACELLULAR MATRIX GENES

The tissue inhibitors of metalloproteinases (TIMPs) are naturally occurring inhibitors of the matrix metalloproteinases and the balance between the two regulates extracellular matrix remodelling. As *TIMP3* showed differential expression in the keratoconic cornea, it has also been screened for mutations with none detected.[63]

A recent study looked at the role of the transforming growth factor beta-induced (*TGFBI*) gene which is an extracellular matrix gene responsible for many dominant corneal dystrophies.[75] The gene was investigated in a Chinese cohort of 30 patients with keratoconus and a novel nonsense mutation, G535X, was observed in one individual.[46] The relevance of this gene to keratoconus more broadly is yet to be determined.

COLLAGENS

Another theory of disease pathogenesis is an underlying alteration in the corneal collagen structure, function and/or development during embryology. Thus, a number of collagen genes have been investigated, as well as genes thought to be involved in the collagen pathways. Sequencing *COL4A3* and *COL4A4* failed to detect any pathological variants in 107 patients with keratoconus but significant allele frequency differences at the D326Y variant in *COL4A3* and M1237V and F1644F in *COL4A4* were distinctive of patients with keratoconus.[47]

Another study analysed *COL4A1* and *COL4A2* in 15 Ecuadorian families with keratoconus and although a number of missense variants were detected in both genes, none segregated with disease in the families, suggesting some other genetic cause for their disease.[76] *COL8A1* and *COL8A2* were also investigated in 50 patients but yet again no pathogenic sequence variants were detected.[77]

APOPTOSIS RELATED PATHWAYS

The occurrence of atopic/allergic eye disease in association with keratoconus is well documented in up to 50 per cent of individuals.[48] One postulated mechanism is the mechanical stimulation from eye rubbing causes epithelial damage resulting in keratocyte apoptosis, via the Fas-ligand or Interleukin 1 pathway,[62] although it cannot be excluded that the genetic cause for the two conditions is more tightly linked. Filaggrin (*FLG*) mutations are a strong genetic risk factor for atopic dermatitis, with the protein expressed in the corneal epithelium, rendering this gene a good candidate. Two prevalent loss-of-function *FLG* alleles (R501X and 2282del4) were screened for in a keratoconic population, and five of 89 demonstrated at least one *FLG* mutation.[48] This was lower than expected with the mutation frequency in a population with atopic dermatitis around 12 to 15 per cent.

GENOME-WIDE STUDIES

Genome-wide studies aim to take an unbiased approach towards gene mapping and the identification of candidate genes for disease. They have the advantage of not relying on prior knowledge of the function of a gene, as illustrated above with candidate gene studies but have the disadvantage of a more stringent level of statistical significance to prevent the reporting of false positive findings due to chance and multiple hypothesis testing. Both linkage and association studies[2,3,13,14,78–87] have been used at the genome-wide level in keratoconus (Table 2, Figure 1). Increasing evidence suggests copy number variation (CNV) is a significant genetic mechanism in Mendelian disorders. Such variants can be detected on a genome-wide level using array CGH; however, in a recent study of 20 keratoconic patients from Saudi Arabia, no changes were detected that would account for keratoconus.[88]

LINKAGE STUDIES IN EXTENDED FAMILIES

Linkage analysis has been used extensively to map susceptibility loci for keratoconus. The approach of using large extended pedigrees has proven the most successful with two potential genes identified though this approach.

MIR184 IN A NORTHERN IRISH FAMILY

In 2003, Hughes and colleagues reported a single extended pedigree from Northern Ireland with keratoconus and cataract co-segregating in an autosomal dominant inheritance pattern. Using microsatellite markers, the authors demonstrated linkage of the phenotype to chromosome 15q22–q24,[29] which was further refined by subsequent fine mapping to a critical region of 5.5 Mb.[89] Recently introduced high throughput ('next generation') DNA sequencing technology was then used to sequence the entire region. This resulted in the identification of three novel variants (in genes *DNAJA4*, *IREB2* and *MIR184*) that segregated with the phenotype in this family.[78] The r.57 c > u mutation in the microRNA gene *MIR184*, was considered to be the most likely cause, as the *IREB2* mutation was in the 3'UTR and *DNAJA4* was not known to play a role in the eye. *MIR184* is abundantly expressed in the cornea and lens epithelia and the mutation was located in the seed region and thus, highly likely to affect the function of the microRNA. The study took over a decade to identify the causative variant and was eventually achieved only through the use of cutting edge technology and relying on the well-annotated human genome reference sequence. A subsequent report has identified the same mutation in a family with EDICT syndrome (corneal endothelial dystrophy, iris hypoplasia, congenital cataract and corneal stromal thinning),[90] expanding and further defining the phenotype associated with this mutation. The primary known role of microRNA in the cell is the regulation of gene expression, through inhibition of translation by binding to complementary sequences in the 3'UTR of target mRNA. Only a few direct disease associations have been reported in microRNA genes; however, as the catalogue of known microRNA grows and these genes are more routinely assayed in gene discovery projects, their relative contribution to disease is likely to grow. This finding represented the first successful outcome from a linkage/positional cloning study for keratoconus, although it is not yet known if *MIR184* is relevant in isolated keratoconus.

DOCK9 in an Ecuadorian family

In 2009, Gajecka and colleagues[3] reported a study of 18 Ecuadorian families with autosomal dominant keratoconus. They identified linkage in one of these families to a 5.59 Mb region of chromosome 13q32 using a whole-genome single-nucleotide polymorphism (SNP) array with 250,000 SNPs. Eight candidate genes of the 25

Locus	Method	Cohort type	Marker type	Ethnicity	Comments	Ref
1p36	Linkage	Extended pedigree	SNPs, 10 K	Australian		81
2p24	Linkage	Small families	Microsatellites, 10 cM	Caucasian and Arab		82
3p14–q13	Linkage	Extended pedigree	Microsatellites, 10 cM	Italian		2
5q14–q21	Linkage	Extended pedigree	Microsatellites, 10 cM	Caucasian from USA		80
8q13–q21	Linkage	Extended pedigree	SNPs, 10 K	Australian		81
9q34	Linkage	Small families	Microsatellites, 10 cM	Caucasian and Hispanic		83
13q32	Linkage	Extended pedigree	SNPs, 250 K	Ecuadorian	*DOCK9* c.2262A > C	3,79
14q24	Linkage	Multiple families	SNPs, 10 K	Mixed		85
15q22–q24	Linkage	Extended pedigree	Microsatellites, 10 cM	Northern Ireland	*MIR184* r.57C > U	29,78
16q22–q23	Linkage	Small families	Microsatellites, 10 cM	Finland		13
20q12	IBD	Genetic isolate	Microsatellites, 10 cM	Tasmanian of UK descent		14
4q, 5q, 12p, 14q	Linkage	Small families	Microsatellites, 10 cM	Caucasian and Hispanic	suggestive	83
5q21, 5q32–33, 14q11	Linkage	Small families	Microsatellites, 10 cM	Italian	suggestive	84
HGF gene	GWAS	Case-controlled	SNPs, 1 M	Australian and USA	suggestive	86
RAB3GAP1 gene	GWAS	Case-controlled	SNPs, 610 K	USA	suggestive	87

GWAS: genome-wide association studies, IBD: identity-by-descent, SNP: single-nucleotide polymorphism

Table 2. Loci identified through genome-wide analysis

located in the linkage region were subsequently sequenced in the family.[79] They identified a novel variant, c.2262A (Gln745His) in the *DOCK9* (dedicator of cytokinesis 9) gene that segregated in the family and was absent from ethnically matched controls. This mutation is predicted to be 'possibly damaging' to protein function by the PolyPhen algorithm[91] and is possibly the cause of keratoconus in this family. *DOCK9* specifically activates the G-protein, CDC42. It is expressed in both keratoconic and normal corneas by RT-PCR. The reported mutation is located in the DHR1 domain, which binds phospholipids and is likely involved in the recruitment of the protein to the cell membrane.[79] It is not clear how mutations in this gene might cause keratoconus specifically and there have been no further reports of mutations in this gene in patients with keratoconus.

Other loci identified in extended families

Four other chromosomal loci have been identified using extended pedigrees. In 2004, Brancati and colleagues[2] reported a three-generation Italian family with 11 affected members showing linkage to a 53 Mb region of chromosome 3, flanking the centromere. Tang and colleagues[80] described a four-generation pedigree from the United States of America with multiple affected founders and demonstrated linkage in one branch of the pedigree to chromosome 5q14–q21. A further two loci were reported by Burdon and colleagues[81] in a single three generation Australian pedigree showing potential digenic inheritance, with affected individuals displaying segregation of haplotypes on chromosomes 1p36 and 8q13–q21. Although all these studies screened likely candidate genes within the linkage regions, causative mutations are yet to be identified. Modern sequencing technology opens the way for gene identification in these families in the near future.

IDENTITY-BY-DESCENT APPROACH TO GENE MAPPING

A novel approach to keratoconus gene mapping was taken by Fullerton and colleagues,[14] who reported a locus on chromosome 20q12. This study was based on measuring identity-by-descent in apparently unrelated individuals from a single clinic, in a small regional city in the island state of Tasmania, Australia. This approach assumed that patients with keratoconus in the area were distantly related due to a founder effect. This study identified a region on 20q12 that was shared between seven affected individuals and also showed association in a larger cohort, with the allele frequency differing significantly from the normal Tasmanian population.

LINKAGE STUDIES IN SMALL FAMILIES

Several linkage studies for keratoconus have been conducted using cohorts of multiple small families. These studies have identified multiple risk loci through the use of both parametric and non-parametric linkage analysis.

In 2002, Tyynismaa and colleagues[13] reported a linkage study in 20 families from northern Finland. Linkage was detected on chromosome 16q22–q23 over a region of 6.0 cM. Hutchings and colleagues[82] conducted a genome-wide linkage study initially using a small cohort of seven families of Caucasian and Arab origin. Putative linkage to chromosome 2p24 was identified but did not meet the required Logarithm of the odds (LOD) score of 3.3 for statistical significance. To improve the power, they investigated this region in a further 21 families from the same ethnic groups. Multipoint parametric linkage analysis gave a LOD score of 5.13, when 52 per cent of the families were included in the analysis (maximum

hierarchical LOD or max HLOD). No specific mutations have been reported in either cohort.

A larger study of 67 families of both European (Caucasian) and Hispanic origin containing 110 sib-pairs was reported in 2006 by Li and colleagues.[83] The most significant association identified was on chromosome 9q34 in the Caucasian families alone but was strengthened by the inclusion of the Hispanic cohort. Other suggestive loci included regions on 4q, 5q, 12p and 14q. The 5q locus is in close proximity to the previously reported linkage in the large family by Tang and colleagues,[80] although the peaks did not overlap. Further support for linkage to chromosome 5 was gained from the study by Bisceglia and colleagues,[84] which reported a study of 25 families from Southern Italy. They showed replication of linkage at 5q21 overlapping with the significant finding of Tang and colleagues.[80] In addition, they reported suggestive linkage at 5q32–q33 and 14q11, in both cases overlapping with the suggestive linkage reported by Li and colleagues.[83] Although neither of the latter peaks reached formal statistical significance in either study, the similar findings in two independent studies provides further confidence that these regions do harbour keratoconus susceptibility loci. As with most other loci reported, the causative genes are yet to be identified.

The most recent reported linkage study for keratoconus was published in 2010 by Liskova and colleagues.[85] The study consisted of six moderately sized families from mixed ethnic backgrounds recruited at Moorfields Eye Hospital in London. The study identified significant linkage to chromosome 14q24. The authors investigated one candidate gene from the region, *VSX2*, which is known to cause other ocular phenotypes and is related to *VSX1*, which has been previously implicated in keratoconus. No coding mutations were identified in this gene.

LIMITATIONS OF FINDINGS BY LINKAGE ANALYSIS

It is of note, that although some of the loci identified by linkage analysis of keratoconus in collections of small families have been identified in multiple studies (5q21, 5q32 and 14q11), few if any causative mutations have been reported from these familial cohorts. This may be due to a lack of resources to screen the large numbers of genes under each peak but could also indicate the limitations of using multiple families to map complex disease traits by linkage, particularly in small numbers. The heterogeneity of the disease can mask linkage signals in such cohorts, particularly where individual families within the study do not have sufficient power to detect significant linkage in their own right. In contrast, the studies of extended pedigrees have led to the identification of mutations in *MIR184* and *DOCK9*; however, further work on these genes in other cohorts is required to determine the mutation frequency of these genes in keratoconus in general and determine the overall contribution of these genes to the disease.

ASSOCIATION STUDIES

Genome-wide association studies (GWAS) in case-controlled cohorts have been a valuable addition to the techniques available to interrogate the genetics of complex disease. These studies aim to identify single-nucleotide polymorphisms, at which the allele frequency differs significantly between cases and controls. The finding of such a single-nucleotide polymorphism then implies that a causative variant is located in linkage disequilibrium with that SNP (that is, usually inherited with the SNP due to physical proximity and disinclination towards recombination). Two such studies have been reported for keratoconus, although neither has identified loci reaching strict statistical significance of $p < 5 \times 10^{-8}$ to account for multiple testing. The first report by Burdon and colleagues[86] used cohorts from Australia, Northern Ireland and the USA in parallel and identified association between keratoconus and SNPs in the promoter region of the hepatocyte growth factor (*HGF*) gene. The finding was consistent across three cohorts, with the fourth replication cohort trending in the same direction. On meta-analysis of all four cohorts, a p-value of 9.9×10^{-7} was obtained, falling slightly short of genome-wide significance. The study also showed a relationship between genotype at the associated SNP (rs3735520) and serum hepatocyte growth factor levels in normal individuals. In addition, this gene has been associated with refractive error and specifically with myopia in multiple studies,[16,92,93] making it an attractive candidate for keratoconus.

The second GWAS study for keratoconus was reported by Li and colleagues[87] and described the findings from the USA cohorts that also contributed to the *HGF*-association results. This study of 222 Caucasian cases compared to 3,324 controls identified multiple putative loci that were then followed in a further 304 cases and 518 controls, as well as a panel of 307 patients from 70 families. After typing of 4,905 SNPs of high priority in the follow-up panels, SNP rs4954218 located near *RAB3GAP1* was the most consistently and significantly associated. This gene has been reported previously in association with corneal malformation and thus, is an excellent candidate for a keratoconus susceptibility locus.

COMBINING GWAS AND LINKAGE STUDIES

The amount of data generated through a GWAS is a valuable resource, which can be mined well beyond the top few significant associations. The USA GWAS of Li and colleagues[87] has recently shed light on a gene that may have led to the linkage signal observed in a large family at chromosome 5q by the same group.[83] All the genes under the previously described linkage peaks were reviewed for their likely role in keratoconus, based on their expression pattern and described roles in the literature and biological databases. One gene in particular, *LOX*, located under the 5q peak stood out as a particularly relevant candidate. *LOX* is involved in cross-linking collagen and elastin fibres in the corneal stroma. Artificial collagen fibre cross-linking using riboflavin and UV light is a procedure currently being explored for the treatment of keratoconus with some success.[94] The GWAS data was examined for evidence of association in the *LOX* gene and SNPs with nominally significant p-values were identified.[95] These SNPs and others nearby were then genotyped in a confirmation cohort and also in the family-based cohort. Evidence of association at similar significance was also seen in these cohorts, suggesting that the *LOX* locus is associated with keratoconus and may have contributed to the linkage signal seen in this region. Other genes and rare variants under the linkage peak have not yet been assessed in the family cohort and may also be contributing to the signal.

SUMMARY

Although it is clear that keratoconus has a major genetic component, identification of

the specific causative genes has been slow. Studies of individual large pedigrees have provided the clearest results but these findings are yet to be expanded to keratoconus in the general population. Further findings in such families are expected in the near future through the use of the advanced technology, high throughput sequencing, to screen large numbers of genes in parallel. Candidate gene studies have also identified some associations but again, they tend to be rare variants with limited applicability to the broader population. Genome-wide association studies also hold promise but require larger cohorts than those analysed to gain sufficient power to detect the small effects expected at individual loci. An alternative approach is to take information from genome-wide association studies of relevant quantitative traits to help select candidate genes for analysis. In particular, assessment of genes known to be involved in the determination of normal central corneal thickness or corneal curvature could be relevant in keratoconus. Much progress has been made recently in the understanding of the genetics of central corneal thickness[96-98] in particular and this opens the way for detailed analysis of such genes in keratoconus cohorts. In the era of genomics and international co-operation between research groups, the stage is set for a rapid expansion of knowledge in the area of genetics keratoconus.

GRANTS AND FINANCIAL ASSISTANCE

REFERENCES

1. Bareja U, Vajpayee RB. Posterior keratoconus due to iron nail injury-a case report. *Indian J Ophthalmol* 1991; 39: 30.
2. Brancati F, Valente EM, Sarkozy A, Feher J, Castori M, Del Duca P, Mingarelli R et al. A locus for autosomal dominant keratoconus maps to human chromosome 3p14–q13. *J Med Genet* 2004; 41: 188–192.
3. Gajecka M, Radhakrishna U, Winters D, Nath SK, Rydzanicz M, Ratnamala U, Ewing K et al. Localization of a gene for keratoconus to a 5.6-Mb interval on 13q32. *Invest Ophthalmol Vis Sci* 2009; 50: 1531–1539.
4. Rabinowitz YS, Garbus J, McDonnell PJ. Computer-assisted corneal topography in family members of patients with keratoconus. *Arch Ophthalmol* 1990; 108: 365–371.
5. Grupcheva CN, Malik TY, Craig JP, Sherwin T, McGhee CN. Microstructural assessment of rare corneal dystrophies using real time in vivo confocal microscopy. *Clin Experiment Ophthalmol* 2001; 29: 281–285.

6. Jordan CA, Zamri A, Wheeldon C, Patel DV, Johnson R, McGhee CN. Computerized corneal tomography and associated features in a large New Zealand keratoconic population. *J Cataract Refract Surg* 2011; 37: 1493–1501.
7. Tuft SJ, Hassan H, George S, Frazer DG, Willoughby CE, Liskova P. Keratoconus in 18 pairs of twins. *Acta Ophthalmol* 2012; 90: e482–486.
8. Bechara SJ, Waring GO, 3rd, Insler MS. Keratoconus in two pairs of identical twins. *Cornea* 1996; 15: 90–93.
9. Redmond KB. The role of heredity in keratoconus. *Trans Ophthalmol Soc Aust* 1968; 27: 52–54.
10. Wang Y, Rabinowitz YS, Rotter JI, Yang H. Genetic epidemiological study of keratoconus: evidence for major gene determination. *Am J Med Genet* 2000; 93: 403–409.
11. Kennedy RH, Bourne WM, Dyer JA. A 48-year clinical and epidemiologic study of keratoconus. *Am J Ophthalmol* 1986; 101: 267–273.
12. Rabinowitz YS. Keratoconus. *Surv Ophthalmol* 1998; 42: 297–319.
13. Tyynismaa H, Sistonen P, Tuupanen S, Tervo T, Dammert A, Latvala T, Alitalo T. A locus for autosomal dominant keratoconus: linkage to 16q22.3–q23.1 in Finnish families. *Invest Ophthalmol Vis Sci* 2002; 43: 3160–3164.
14. Fullerton J, Paprocki P, Foote S, Mackey DA, Williamson R, Forrest S. Identity-by-descent approach to gene localisation in eight individuals affected by keratoconus from north-west Tasmania, Australia. *Hum Genet* 2002; 110: 462–470.
15. Ihalainen A. Clinical and epidemiological features of keratoconus genetic and external factors in the pathogenesis of the disease. *Acta Ophthalmol* 1986; Suppl 178: 1–64.
16. Kok YO, Tan GF, Loon SC. Review: keratoconus in Asia. *Cornea* 2012; 31: 581–593.
17. Nielsen K, Hjortdal J, Aagaard Nohr E, Ehlers N. Incidence and prevalence of keratoconus in Denmark. *Acta ophthalmol Scand* 2007; 85: 890–892.
18. Pearson AR, Soneji B, Sarvananthan N, Sandford-Smith JH. Does ethnic origin influence the incidence or severity of keratoconus? *Eye (Lond)* 2000; 14: 625–628.
19. Owens H, Gamble GD, Bjornholdt MC, Boyce NK, Keung L. Topographic indications of emerging keratoconus in teenage New Zealanders. *Cornea* 2007; 26: 312–318.
20. Edwards M, McGhee CN, Dean S. The genetics of keratoconus. *Clin Experiment Ophthalmol* 2001; 29: 345–351.
21. Patel HY, Ormonde S, Brookes NH, Moffatt LS, McGhee CN. The indications and outcome of paediatric corneal transplantation in New Zealand: 1991–2003. *Br J Ophthalmol* 2005; 89: 404–408.
22. Williams KA, Lowe MT, Keane MC, Jones VJ, Loh RS, Coster DJ. The Australian Corneal Graft Registry 2012 Report. Adelaide: Flinders Universtiy, 2012.
23. Cameron JA. Corneal abnormalities in Ehlers-Danlos syndrome type VI. *Cornea* 1993; 12: 54–9.
24. Perlman JM, Zaidman GW. Bilateral keratoconus in Crouzon's syndrome. *Cornea* 1994; 13: 80–81.
25. Bass HN, Sparkes RS, Crandall BF, Marcy SM. Congenital contractural arachnodactyly, keratoconus, and probable Marfan syndrome in the same pedigree. *J Pediatr* 1981; 98: 591–593.
26. Heur M, Costin B, Crowe S, Grimm RA, Moran R, Svensson LG, Traboulsi EI. The value of keratometry and central corneal thickness measurements

in the clinical diagnosis of Marfan syndrome. *Am J Ophthalmol* 2008; 145: 997–1001.
27. Maumenee IH. The eye in the Marfan syndrome. *Trans Am Ophthalmol Soc* 1981; 79: 684–733.
28. Sultan G, Baudouin C, Auzerie O, De Saint Jean M, Goldschild M, Pisella PJ. Cornea in Marfan disease: Orbscan and in vivo confocal microscopy analysis. *Invest Ophthalmol Vis Sci* 2002; 43: 1757–1764.
29. Hughes AE, Dash DP, Jackson AJ, Frazer DG, Silvestri G. Familial keratoconus with cataract: linkage to the long arm of chromosome 15 and exclusion of candidate genes. *Invest Ophthalmol Vis Sci* 2003; 44: 5063–5066.
30. Harrison RJ, Klouda PT, Easty DL, Manku M, Charles J, Stewart CM. Association between keratoconus and atopy. *Br J Ophthalmol* 1989; 73: 816–822.
31. Godel V, Blumenthal M, Iaina A. Congenital Leber amaurosis, keratoconus, and mental retardation in familial juvenile nephronophtisis. *J Pediatr Ophthalmol Strabismus* 1978; 15: 89–91.
32. Wilhelmus KR. Keratoconus and progressive cone dystrophy. *Ophthalmologica* 1995; 209: 278–279.
33. Piccione M, Serra G, Sanfilippo C, Andreucci E, Sani I, Corsello G. A new mutation in EDA gene in X-linked hypohidrotic ectodermal dysplasia associated with keratoconus. *Minerva Pediatr* 2012; 64: 59–64.
34. Pinsard L, Touboul D, Vu Y, Lacombe D, Leger F, Colin J. Keratoconus associated with Williams-Beuren syndrome: first case reports. *Ophthalmic Genet* 2010; 31: 252–256.
35. Gasset AR, Zimmerman TJ. Posterior polymorphous dystrophy associated with keratoconus. *Am J Ophthalmol* 1974; 78: 535–537.
36. Sassani JW, Smith SG, Rabinowitz YS. Keratoconus and bilateral lattice-granular corneal dystrophies. *Cornea* 1992; 11: 343–350.
37. Wollensak G, Green WR, Temprano J. Keratoconus associated with corneal granular dystrophy in a patient of Italian origin. *Cornea* 2002; 21: 121–122.
38. Lipman RM, Rubenstein JB, Torczynski E. Keratoconus and Fuchs' corneal endothelial dystrophy in a patient and her family. *Arch Ophthalmol* 1990; 108: 993–994.
39. Shapiro MB, France TD. The ocular features of Down's syndrome. *Am J Ophthalmol* 1985; 99: 659–663.
40. Macsai M, Maguen E, Nucci P. Keratoconus and Turner's syndrome. *Cornea* 1997; 16: 534–536.
41. Heaven CJ, Lalloo F, McHale E. Keratoconus associated with chromosome 13 ring abnormality. *Br J Ophthalmol* 2000; 84: 1079.
42. Morrison DA, Rosser EM, Claoue C. Keratoconus associated with a chromosome 7,11 translocation. *Eye (Lond)* 2001; 15: 556–557.
43. Heon E, Greenberg A, Kopp KK, Rootman D, Vincent AL, Billingsley G, Priston M et al. VSX1: a gene for posterior polymorphous dystrophy and keratoconus. *Hum Mol Genet* 2002; 11: 1029–1036.
44. Muszynska D, Lechner J, Dash D, Heon E, Hughes A, Willoughby C. Identification and characterisation of a novel missense homeodomain mutation in ZEB1 resulting in keratoconus. *Invest Ophthalmol Vis Sci* 2011; 52: 1077; abstract #149.
45. Udar N, Atilano SR, Brown DJ, Holguin B, Small K, Nesburn AB, Kenney MC. SOD1: a candidate gene for keratoconus. *Invest Ophthalmol Vis Sci* 2006; 47: 3345–3351.
46. Guan T, Liu C, Ma Z, Ding S. The point mutation and polymorphism in keratoconus candidate gene

TGFBI in Chinese population. *Gene* 2012; 503: 137–139.

47. Stabuc-Silih M, Ravnik-Glavac M, Glavac D, Hawlina M, Strazisar M. Polymorphisms in COL4A3 and COL4A4 genes associated with keratoconus. *Mol Vis* 2009; 15: 2848–2860.

48. Droitcourt C, Touboul D, Ged C, Ezzedine K, Cario-Andre M, de Verneuil H, Colin J et al. A prospective study of filaggrin null mutations in keratoconus patients with or without atopic disorders. *Dermatology* 2012; 222: 336–341.

49. Semina EV, Mintz-Hittner HA, Murray JC. Isolation and characterization of a novel human paired-like homeodomain-containing transcription factor gene, VSX1, expressed in ocular tissues. *Genomics* 2000; 63: 289–293.

50. Dash DP, George S, O'Prey D, Burns D, Nabili S, Donnelly U, Hughes AE et al. Mutational screening of VSX1 in keratoconus patients from the European population. *Eye (Lond)* 2010; 24: 1085–1092.

51. Hosseini SM, Herd S, Vincent AL, Heon E. Genetic analysis of chromosome 20-related posterior polymorphous corneal dystrophy: genetic heterogeneity and exclusion of three candidate genes. *Mol Vis* 2008; 14: 71–80.

52. Barbaro V, Di Iorio E, Ferrari S, Bisceglia L, Ruzza A, De Luca M, Pellegrini G. Expression of VSX1 in human corneal keratocytes during differentiation into myofibroblasts in response to wound healing. *Invest Ophthalmol Vis Sci* 2006; 47: 5243–5250.

53. Mintz-Hittner HA, Semina EV, Frishman LJ, Prager TC, Murray JC. VSX1 (RINX) mutation with craniofacial anomalies, empty sella, corneal endothelial changes, and abnormal retinal and auditory bipolar cells. *Ophthalmology* 2004; 111: 828–836.

54. Bisceglia L, Ciaschetti M, De Bonis P, Campo PA, Pizzicoli C, Scala C, Grifa M et al. VSX1 mutational analysis in a series of Italian patients affected by keratoconus: detection of a novel mutation. *Invest Ophthalmol Vis Sci* 2005; 46: 39–45.

55. Eran P, Almogit A, David Z, Wolf HR, Hana G, Yaniv B, Elon P et al. The D144E substitution in the VSX1 gene: a non-pathogenic variant or a disease causing mutation? *Ophthalmic Genet* 2008; 29: 53–59.

56. Mok JW, Baek SJ, Joo CK. VSX1 gene variants are associated with keratoconus in unrelated Korean patients. *J Hum Genet* 2008; 53: 842–849.

57. Paliwal P, Singh A, Tandon R, Titiyal JS, Sharma A. A novel VSX1 mutation identified in an individual with keratoconus in India. *Mol Vis* 2009; 15: 2475–2479.

58. Aldave AJ, Yellore VS, Salem AK, Yoo GL, Rayner SA, Yang H, Tang GY et al. No VSX1 gene mutations associated with keratoconus. *Invest Ophthalmol Vis Sci* 2006; 47: 2820–2822.

59. Liskova P, Ebenezer ND, Hysi PG, Gwilliam R, El-Ashry MF, Moodaley LC, Hau S et al. Molecular analysis of the VSX1 gene in familial keratoconus. *Mol Vis* 2007; 13: 1887–1891.

60. Stabuc-Silih M, Strazisar M, Hawlina M, Glavac D. Absence of pathogenic mutations in VSX1 and SOD1 genes in patients with keratoconus. *Cornea* 2010; 29: 172–176.

61. Tang YG, Picornell Y, Su X, Li X, Yang H, Rabinowitz YS. Three VSX1 gene mutations, L159M, R166W, and H244R, are not associated with keratoconus. *Cornea* 2008; 27: 189–192.

62. McGhee CN. 2008 Sir Norman McAlister Gregg Lecture: 150 years of practical observations on the conical cornea–what have we learned? *Clin Experiment Ophthalmol* 2009; 37: 160–176.

63. De Bonis P, Laborante A, Pizzicoli C, Stallone R, Barbano R, Longo C, Mazzilli E et al. Mutational screening of VSX1, SPARC, SOD1, LOX, and TIMP3 in keratoconus. *Mol Vis* 2011; 17: 2482–2894.

64. Saee-Rad S, Hashemi H, Miraftab M, Noori-Daloii MR, Chaleshtori MH, Raoofian R, Jafari F et al. Mutation analysis of VSX1 and SOD1 in Iranian patients with keratoconus. *Mol Vis* 2011; 17: 3128–3136.

65. Paliwal P, Tandon R, Dube D, Kaur P, Sharma A. Familial segregation of a VSX1 mutation adds a new dimension to its role in the causation of keratoconus. *Mol Vis* 2011; 17: 481–485.

66. Blair SD, Seabrooks D, Shields WJ, Pillai S, Cavanagh HD. Bilateral progressive essential iris atrophy and keratoconus with coincident features of posterior polymorphous dystrophy: a case report and proposed pathogenesis. *Cornea* 1992; 11: 255–261.

67. Cremona FA, Ghosheh FR, Rapuano CJ, Eagle RC Jr., Hammersmith KM, Laibson PR, Ayres BD et al. Keratoconus associated with other corneal dystrophies. *Cornea* 2009; 28: 127–135.

68. Lam HY, Wiggs JL, Jurkunas UV. Unusual presentation of presumed posterior polymorphous dystrophy associated with iris heterochromia, band keratopathy and keratoconus. *Cornea* 2010; 29: 1180–1185.

69. Mazzotta C, Baiocchi S, Caporossi O, Buccoliero D, Casprini F, Caporossi A, Balestrazzi A. Confocal microscopy identification of keratoconus associated with posterior polymorphous corneal dystrophy. *J Cataract Refract Surg* 2008; 34: 318–321.

70. Weissman BA, Ehrlich M, Levenson JE, Pettit TH. Four cases of keratoconus and posterior polymorphous corneal dystrophy. *Optom Vis Sci* 1989; 66: 243–246.

71. Bechara SJ, Grossniklaus HE, Waring GO, 3rd, Wells JA, 3rd. Keratoconus associated with posterior polymorphous dystrophy. *Am J Ophthalmol* 1991; 112: 729–731.

72. Raber IM, Fintelmann R, Chhabra S, Ribeiro MP, Eagle RC Jr, Orlin SE. Posterior polymorphous dystrophy associated with nonkeratoconic steep corneal curvatures. *Cornea* 2011; 30: 1120–1124.

73. Liskova P, Filipec M, Merjava S, Jirsova K, Tuft SJ. Variable ocular phenotypes of posterior polymorphous corneal dystrophy caused by mutations in the ZEB1 gene. *Ophthalmic Genet* 2010; 31: 230–234.

74. Pathak D, Nayak B, Singh M, Sharma N, Tandon R, Sinha R, Titiyal JS et al. Mitochondrial complex 1 gene analysis in keratoconus. *Mol Vis* 2011; 17: 1514–1525.

75. Munier FL, Frueh BE, Othenin-Girard P, Uffer S, Cousin P, Wang MX, Heon E et al. BIGH3 mutation spectrum in corneal dystrophies. *Invest Ophthalmol Vis Sci* 2002; 43: 949–954.

76. Karolak JA, Kulinska K, Nowak DM, Pitarque JA, Molinari A, Rydzanicz M, Bejjani BA et al. Sequence variants in COL4A1 and COL4A2 genes in Ecuadorian families with keratoconus. *Mol Vis* 2011; 17: 827–843.

77. Aldave AJ, Bourla N, Yellore VS, Rayner SA, Khan MA, Salem AK, Sonmez B. Keratoconus is not associated with mutations in COL8A1 and COL8A2. *Cornea* 2007; 26: 963–965.

78. Hughes AE, Bradley DT, Campbell M, Lechner J, Dash DP, Simpson DA, Willoughby CE. Mutation altering the miR-184 seed region causes familial keratoconus with cataract. *Am J Hum Genet* 2011; 89: 628–633.

79. Czugala M, Karolak JA, Nowak DM, Polakowski P, Pitarque J, Molinari A, Rydzanicz M et al. Novel mutation and three other sequence variants segregating with phenotype at keratoconus 13q32 susceptibility locus. *Eur J Hum Genet* 2012; 20: 389–397.

80. Tang YG, Rabinowitz YS, Taylor KD, Li X, Hu M, Picornell Y, Yang H. Genomewide linkage scan in a multigeneration Caucasian pedigree identifies a novel locus for keratoconus on chromosome 5q14.3–q21.1. *Genet Med* 2005; 7: 397–405.

81. Burdon KP, Coster DJ, Charlesworth JC, Mills RA, Laurie KJ, Giunta C, Hewitt AW et al. Apparent autosomal dominant keratoconus in a large Australian pedigree accounted for by digenic inheritance of two novel loci. *Hum Genet* 2008; 124: 379–386.

82. Hutchings H, Ginisty H, Le Gallo M, Levy D, Stoesser F, Rouland JF, Arne JL et al. Identification of a new locus for isolated familial keratoconus at 2p24. *J Med Genet* 2005; 42: 88–94.

83. Li X, Rabinowitz YS, Tang YG, Picornell Y, Taylor KD, Hu M, Yang H. Two-stage genome-wide linkage scan in keratoconus sib pair families. *Invest Ophthalmol Vis Sci* 2006; 47: 3791–3795.

84. Bisceglia L, De Bonis P, Pizzicoli C, Fischetti L, Laborante A, Di Perna M, Giuliani F et al. Linkage analysis in keratoconus: replication of locus 5q21.2 and identification of other suggestive Loci. *Invest Ophthalmol Vis Sci* 2009; 50: 1081–1086.

85. Liskova P, Hysi PG, Waseem N, Ebenezer ND, Bhattacharya SS, Tuft SJ. Evidence for keratoconus susceptibility locus on chromosome 14: a genome-wide linkage screen using single-nucleotide polymorphism markers. *Arch Ophthalmol* 2010; 128: 1191–1195.

86. Burdon KP, Macgregor S, Bykhovskaya Y, Javadiyan S, Li X, Laurie KJ, Muszynska D et al. Association of polymorphisms in the hepatocyte growth factor gene promoter with keratoconus. *Invest Ophthalmol Vis Sci* 2011; 52: 8514–8519.

87. Li X, Bykhovskaya Y, Haritunians T, Siscovick D, Aldave A, Szczotka-Flynn L, Iyengar SK et al. A genome-wide association study identifies a potential novel gene locus for keratoconus, one of the commonest causes for corneal transplantation in developed countries. *Hum Mol Genet* 2012; 21: 421–429.

88. Abu-Amero KK, Hellani AM, Al Mansouri SM, Kalantan H, Al-Muammar AM. High-resolution analysis of DNA copy number alterations in patients with isolated sporadic keratoconus. *Mol Vis* 2011; 17: 822–826.

89. Dash DP, Silvestri G, Hughes AE. Fine mapping of the keratoconus with cataract locus on chromosome 15q and candidate gene analysis. *Mol Vis* 2006; 12: 499–505.

90. Iliff BW, Riazuddin SA, Gottsch JD. A single-base substitution in the seed region of miR-184 causes EDICT syndrome. *Invest Ophthalmol Vis Sci* 2012; 53: 348–353.

91. Adzhubei IA, Schmidt S, Peshkin L, Ramensky VE, Gerasimova A, Bork P, Kondrashov AS et al. A method and server for predicting damaging missense mutations. *Nat Methods* 2010; 7: 248–249.

92. Veerappan S, Pertile KK, Islam AF, Schache M, Chen CY, Mitchell P, Dirani M et al. Role of the hepatocyte growth factor gene in refractive error. *Ophthalmology* 2010; 117: 239–245.

93. Yanovitch T, Li YJ, Metlapally R, Abbott D, Viet KN, Young TL. Hepatocyte growth factor and myopia: genetic association analyses in a Caucasian population. *Mol Vis* 2009; 15: 1028–1035.

94. Suri K, Hammersmith KM, Nagra PK. Corneal collagen cross-linking: ectasia and beyond. *Curr Opin Ophthalmol* 2012; 23: 280–287.

95. Bykhovskaya Y, Li X, Epifantseva I, Haritunians T, Siscovick D, Aldave A, Szczotka-Flynn L et al. Variation in the lysyl oxidase (LOX) gene is associated with keratoconus in family-based and case-control studies. *Invest Ophthalmol Vis Sci* 2012; 53: 4152–4157.

96. Lu Y, Dimasi DP, Hysi PG, Hewitt AW, Burdon KP, Toh T, Ruddle JB et al. Common genetic variants near the Brittle Cornea syndrome locus ZNF469 influence the blinding disease risk factor central corneal thickness. *PLoS Genet* 2010; 6: e1000947.

97. Vitart V, Bencic G, Hayward C, Skunca Herman J, Huffman J, Campbell S, Bucan K et al. New loci associated with central cornea thickness include COL5A1, AKAP13 and AVGR8. *Hum Mol Genet* 2010; 19: 4304–4311.

98. Vithana EN, Aung T, Khor CC, Cornes BK, Tay WT, Sim X, Lavanya R et al. Collagen-related genes influence the glaucoma risk factor, central corneal thickness. *Hum Mo Genet* 2011; 20: 649–658.