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Relevance of Molecular Diagnosis of Corneal Dystrophies

Preeti Paliwal and Arundhati Sharma

Laboratory of Cyto-Molecular Genetics, Department of Anatomy, All India Institute of Medical Sciences, New Delhi, India

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ABSTRACT Corneal dystrophies are defined as a group of inherited corneal disorders characterized by opacification of the cornea. Initial classification of corneal dystrophies was based on the layer of cornea involved but with the advent of better technology, a larger picture has evolved that includes both phenotypic and genotypic variants. With the evolving knowledge, a revised classification has been proposed by the International Committee for Classification of Corneal Dystrophies (IC3D). This classification has taken into account the clinical, histologic and genetic basis of the disease, integrating them into one. Our understanding of corneal dystrophies has reached new heights with mutations identified in at least 14 genes. Even though there has been a vast addition of information to the database, we still come across new variants which may seem enigmatic phenotypically. With the additional new information refining the molecular basis, there is a need to explore further the underlying molecular pathology so as to enable the possibility of better treatment modalities to the affected patients and their families. In view of the recent advances, we hereby review the dystrophies with an aim to provide updated information on the clinical and molecular aspects of corneal dystrophies which will aid in their differential diagnosis and management.

INTRODUCTION

Genetics is rapidly entering the realm of clinical medicine. Genetic elucidation of various disorders has provided deep insight into biological pathways and encoded protein function. These advancements have led us into an era of new definitions, advanced diagnostics and translational research. It is because of these new developments that a revised classification of corneal dystrophies has been proposed by International Committee for Classification of Corneal Dystrophies (IC3D) (Weiss et al. 2008). Corneal dystrophies are defined as a of group corneal disorders that are primary bilateral, inherited, alterations of cornea affecting its transparency and refraction (American Academy of Ophthalmology 2007–2008). With clinical investigations, accurate diagnosis of corneal dystrophies is possible in approximately 50% of cases and histopathological correlation can be done in about 60% of them. A better understanding of the disease process has been possible using candidate gene and related approaches to identify genes and causative mutations specific for each type of corneal dystrophy and their phenotypic variants. This also aids in identification of overlapping and atypical cases. It is now possible to confirm the diagnosis of a corneal dystrophy in cases associated with an atypical phenotype such as unilateral dystrophies (Aldave et al. 2008) , dystrophies that involve more than a single layer of the cornea (Aldave et al. 2005) and dystrophies that are associated with extra ocular involvement (Battisti et al. 1998). With the current understanding there is a need to incorporate molecular genetic analysis into everyday clinical practice to enhance the clinician's ability to differentiate between dystrophic and non-dystrophic corneal disorders. We, hereby, review the dystrophies with an aim to provide updated information on the genes and the underlying mutations that are involved in the causation of various corneal dystrophies which aids in their differential diagnosis and provides better treatment and management options.

**Corresponding author*
Dr. Arundhati Sharma
Associate Professor,
Laboratory of Cyto-Molecular Genetics,
Room No. 1015, Department of Anatomy,
All India Institute of Medical Sciences
New Delhi 110 029, India
E-mail: arundhati_sharma@yahoo.com,
arundhatisharma1@gmail.com

1. TGFBI (TRANSFORMING GROWTH FACTOR BETA INDUCED) GENE

TGFBI gene encodes an Arg-Gly-Asp (RGD)-containing protein that binds to type I, II and IV collagens (Runager et al. 2008). The RGD motif modulates the cell adhesion and is found in many extracellular matrix proteins. It also serves as a ligand recognition sequence for several integrins (Munier et al. 1997). This protein plays a role in cell-collagen interactions and acts to inhibit cell adhesion. Since its discovery by Munier et al. in 1997, several mutations in

TGFBI gene have been identified to cause a group of corneal dystrophies that involves Epithelial Basement Membrane Dystrophy (EBMD), Bowmans layer dystrophies (Reis Bucklers corneal dystrophy/ GCDIII and Thiel Bhenke corneal dystrophy) and stromal dystrophies (granular and lattice corneal dystrophy). The discovery of the genetic basis of many corneal dystrophies has also shown that mutations in the same gene can cause different phenotypes. The association of gene mutations with specific phenotypes has given the clinician the opportunity to use molecular genetic analysis to diagnose dystrophic corneal disorders.

1) Epithelial Basement Membrane Dystrophy, *also known as* ***Map Dot Fingerprint Dystrophy (OMIM 121820):*** The disorder is characterized by a map pattern appearing as diffuse gray geographic patches that contain oval clear zones. Majority of the cases are sporadic, but autosomal dominant inheritance has been reported in several studies. Boutboul et al. in 2006 identified a heterozygous 1526T-G transversion in the TGFBI gene that caused a leu509-to-arg (L509R) amino acid substitution. Dominant inheritance has been documented in a family from North Ireland and in a Swiss patient harbouring R666S mutation in TGFBI gene.

2) Dystrophies of Bowman's Layer and Stroma: The changes induced in Bowman's layer are secondary to the events occurring in the epithelium or the subjacent stroma, thus there are no primary dystrophies of Bowman's layer. Many variants of these dystrophies have been recognized as separate clinico-pathologic entities based on the nature of deposits, but because of similarities to their original dystrophy, the designation has been retained for the variants but with addition of a roman numeral.

Honeycomb Dystrophy or Thiel Behnke Dystrophy (CDB2) (OMIM 602082) is an autosomal dominant dystrophy affecting Bowman's layer in early life in a characteristic bilateral, honeycomb arrangement of sub epithelial corneal opacities (Thiel and Behnke 1967). Histopathologically, the thickness of epithelium is markedly irregular, with occasional vacuolization of basal epithelial cells and focal iron deposition (Kanai et al. 1973; Haddad et al. 1976). Electron microscopic analysis with characteristic curly fibrils can distinguish the two dystrophies. Confirmation of Thiel Behnke can be done by molecular analysis of underlying Arg 555 Gln (R555Q) mutation in TGFBI (Munier et al. 1997). A study using linkage mapping has shown association to another locus on chromosome 10q23-q24 (Yee et al. 1997) in Thiel-Behnke corneal dystrophy.

3) Granular Dystrophy: This is bilaterally sym-metrical dystrophy characterized by small, discrete, sharply demarcated, grayish white opacities. The ubiquitous phenotypes are transmitted as non-pleiotropic autosomal dominant traits with complete penetrance.

Granular dystrophy is categorized into the following subtypes:

3 a) Groenouw Granular Dystrophy Type I (OMIM 121900): was originally described by Groenouw as *noduli cornae* is transmitted as autosomal dominant trait. Eosinophilic, hyaline deposits are seen in the stroma and beneath the epithelium; these deposits stain intense red with Masson's trichrome. Missense mutation Arg 555 Trp in TGFBI is seen to occur in Groenouw Granular dystrophy type I (Munier et al.1997). The severity of the disorder increases in the presence of a homozygous mutation. Other mutations associated with GCDI are listed in table 1.

3b) Granular Dystrophy Type II or Avellino Corneal Dystrophy (OMIM 607541): **ACD** shares features with both Lattice and Granular corneal dystrophy as both granular and amyloid deposits are seen. The dystrophy presents in the first and second decade of life with flat, sub epithelial, or crumb shaped stromal lesions. Lattice lines appear in later life. The number and type of lesions may vary widely within a sibship. Missense mutation at Arg124 His (R124H) in TGFBI is responsible for GCD II (Munier et al.1997).

3c) Granular Corneal Dystrophy Type III or Reis-Buckler's Dystrophy RBCD (OMIM 608470): Clinical features involve focal gray white sub epithelial opacities that occupy the central cornea and spread paracentrally, assuming a variety of forms. There is more diffuse presence of band shaped granular dystrophy that stain intensely red with masson's trichrome. The dystrophy is characterized by presence of Arg 124 Leu mutation (Munier et al.1997). Several other mutations like trinucleotide deletion in exon 12 are also reported to be associated (Rozzo et al. 1998).

4) Lattice Corneal Dystrophy (LCD): Fine irregular lines and dots, mainly in the anterior

Fig. 1. Composite image showing representative images of granular corneal dystrophy (I); macular corneal dystrophy (II); congenital hereditary endothelial dystrophy (III)

Table 1: Mutations identified till date in TGFBI gene in granular and lattice corneal dystrophies.

S.No.	*Nucleotide change*	*Amino acid change*	*Reference*
1	c.337G>A	Val113Ile	Zenteno et al. 2006
2	c.367G>C	Asp123His	Ha et al. 2003
3	c.370C>A	Arg124Ser	Munier et al. 2002
4	c.370C>T	Arg124Cys	Munier et al. 1997
5	c.371G>T	Arg124Leu	Munier et al. 1997
6	c.371G>A	Arg124His	Munier et al. 1997; Paliwal et al. 2009[$]
7	c.371G>A+ c.1631A>G	Arg124His+N544Ser	Yamada et al. 2009
8	c.371G>T & c.373_378 delACGGAG	Arg124Leu DeltaThr 125_DeltaGlu126del	Dighiero et al. 2000
9	c.1501C>A	Pro501Thr	Ha et al. 2000; Fujiki et al 2001; Tsujikawa et al. 2002
10	c.1514T>A	Val505Asp	Tian et al. 2005
11	c.1548C>G	Ser516Arg	Paliwal et al. 2010[$]
12	c.1553T>C	Leu518Pro	Fujiki et al 2001
13	c.1565T>A	Ile522N	Zhang et al. 2009
14	c.1580T>G	Leu527Arg	Fujiki et al 2001
15	c.1612A>C	Thr538Pro	Yu et al. 2006; Paliwal et al. 2010[$]
16	c.1613C>G	Thr538Arg	Munier et al. 2002
17	c.1616T>A	Val539Asp	Chakravarthi et al. 2005*
18	c.1618_1620delTTT	Phe540del	Munier et al. 2002
19	c.1619T >C	Phe540Ser	Stix et al. 2005
20	c.1631A>G	Asn544Ser	Mashima et al. 2000
21	c.1636G>A	Ala546Thr	Dighiero et al. 2000
22	c.1637C>A	Ala546Asp	Klintworth et al. 2004
23	c.1640T>C	Phe547Ser	Takacs et al. 2007
24	c.1652C>A	Pro551Gln	Klintworth et al. 2004
25	c.1663C>T	Arg555Trp	Munier et al. 1997
26	c.1664G.>A	Arg555Gln	Munier et al. 1997
27	c.1675T>G	Leu559Val	Paliwal et al. 2010[$]
28	c.1706T>G	Leu569Arg	Warren et al. 2003
29	c.1714_1716delCAC	His572del	Aldave et al 2006
30	c.1715A>G	His572Arg	Atchaneeyasakul et al 2007
31	c.1781G>T	Gly594Val	Chakravarthi et al. 2005*
32	c.1903T>A	Met619Lys	Aldave et al 2008
33	c.1864A>C	Asn622His	Stewart et al. 1999
34	c.1866T>A	Asn622Lys	Munier et al. 2002
35	c.1866T>G	Asn622Lys	Munier et al. 2002
36	c.1868G>A	Gly623Asp	Munier et al. 2002
37	c.1870A>G	Val624Met	Afshari et al. 2008
38	c.1870_1875delGTGGTC	Val624_Val625del	Chakravarthi et al. 2005*
39	c.1874T>A	Val625Asp	Tian et al. 2007
40	c.1877A>G	His626Arg	Munier et al. 2002
41	c.1877A>C	His626Pro	Munier et al. 2002
42	c.1879delG	Val627SerfsX44	Munier et al. 2002
43	c.1886_1894dup	Thr629_Asn630insAsnValPro	Schmitt-Bernard et al. 2000
44	c.1892T>A	Val631Asp	Munier et al. 2002

The asterix * mark the mutations identified in south and [$] marks the ones identified in North Indian patients

cornea involving anterior stroma and Bowman's layer are characteristic of this dystrophy. In some cases it appears as diffuse axial and anterior stromal haze and with progression, the lesions can appear as small nodules, dots or thread like spicules. On histologic examination, the epithelial layer seems to be irregular, with areas of thickening and thinning. The basal epithelial cells degenerate and the basement membrane is thickened and discontinuous without normal hemidesmosmes. Bowman's layer may be absent, thick or thin in different areas. Deposits of eosinophilic material are seen between the epithelium, Bowman's layer and deep in the stroma. These deposits stain with Congo red. Lattice corneal dystrophy is categorized into the following subtypes:

4a) Lattice Dystrophy Type (LCDI) (OMIM 122200): results from a missense mutation R124C (Munier et al.1997) with features of typical LCD and is accompanied by amyloid deposits, suggesting that the R124-mutated keratoepithelin forms amyloidogenic intermediates that precipitate in the cornea.

4b) LCDIIIA (OMIM 608471): is a late onset form of LCD, characterized by recurrent corneal erosions and cause of visual failure between fourth and sixth decade. Lattice like lines are thick and ropy. Amyloid deposits are found in anterior mid stroma, including areas between Bowman's layer and stroma. C to A transversion at nucleotide 501 of TGFBI gene converting codon 501 from CCA (Pro) to ACA (Thr) is reported (Ha et al. 2000; Tsujikawa et al. 2002) suggested that the P501T mutation in heterozygous form is responsible for most cases of lattice corneal dystrophy in Japan which has been confirmed by other studies since then.

4c) LCD IIIB (OMIM 204870): was reported by (Stewert et al. 1999) with a mutation in codon 622 and 626 of TGFBI gene. Onset is usually unilateral and early as compared to LCD III and LCDIIIA.

4d) LCDIV: is associated with L527R mutation (Fujiki et al. 2001) in heterozygous form with high phenotypic variation in affected individuals. A number of other mutations are known based on which they are classified as separate entities as described in Table 1.

2. GELSOLIN GENE

The gene is located on chromosome 9q34 and mutation Asp187Asn (Afshari et al. 2001) and Asn187Tyr (Stewart et al. 2000) are known to cause amyloid deposition in a phenotype resembling lattice corneal dystrophy. Gelsolin is known to participate in the cleaving of actin filaments. Actin being a part of the cellular skeleton regulates cell movement and other functions. In addition, gelsolin is also secreted into the blood, and it is this secreted form that is found in the amyloid fibrils in the cornea of the affected (Stewart et al. 2000). LCD II accompanies the Finnish type of familial systemic amyloidosis of the Meretoja syndrome. Patients exhibit a progressive cranial and peripheral neuropathy with dry skin, blepharochalasis, protruding lips, and a mask like facies. (Stewart et al. 2000).

3. TACSTD2 GENE (TUMOR ASSOCIATED CALCIUM SIGNAL TRANSDUCER 2)

The gene is located at 1p32 and codes for membrane protein that transduces calcium signal and acts as a cell surface receptor. Several mutations in this gene have been identified in association with gelatinous drop like corneal dystrophy (GDLD), the most common of which is Q118X mutation (Tian et al. 2004). The disease is characterized by sub-epithelial lesions that may be seen as a group of small multiple nodules, in mulberry configuration. Associated mutations are described in Table 2.

4. KRT3 AND KRT12 GENES

Mutations have been identified in the two genes which encode cornea specific keratins K3 and K12 and causes Meesmann's epithelial dystrophy and Stocker Holt dystrophy. The KRT3 (12q13) and KRT12 (17q12) proteins are keratin intermediate filaments, expressed in the anterior corneal epithelium to form heterodimers required for structural integrity of the cell. Mutations are seen to occur in the conserved helix initiation motif in exon 1 of KRT12 and helix termination motifs of exon 7 in KRT3. Two mutations are reported to occur in KRT3 gene, Glu498Val (Szaflik et al. 2008), Arg503Pro (Chen et al. 2005) and Glu509Lys (Irvine et al. 1997).12 mutations have been reported in KRT12 gene (Table 3). Except for one, all causative mutations are missense substitution which has a domain negative effect causing an aberrant as-

Table 2: Mutations identified till date in gelatinous drop like corneal dystrophy in TACSTD2 gene

S.No	*Mutation*	*Amino acid change*	*References*
1	c.2T>G	Met1Arg	Ren et al. 2002
2	c.198C>A	Cys66X	Alavi et al. 2007
3	c.250A>T	Lys84X	Murakami et al. 2004
4	c.322T>C	Cys108Arg	Murakami et al. 2004
5	c.341T>G	Phe114Cys	Alavi et al. 2007
6	c.352C>T	Gln118X	Tsujikawa et al. 1999
7	c.352C>G	Gln118Glu	Ren et al. 2002
8	c.356G>A	Cys119Tyr	Paliwal et al. 2010[$]
9	c.355T>A	Cys119Ser	Ren et al. 2002
10	c.493_494insCCACCGCC	Gly165AlafsX15	Ren et al. 2002
11	c.509C>A	Ser170X	Tsujikawa et al. 1999
12	c.519dupC	Ala174ArgfsX43	Tasa et al. 2001
13	c.526_576del51	del.176_192	Jing et al. 2009
14	c.551A>G p	Tyr184Cys	Tian et al. 2005
15	c.557T>C	Leu186Pro	Alavi et al. 2007
16	c.564delC	Lys189SerfsX82	Ren et al. 2002
17	c.581T>A	Val194Glu	Ren et al. 2002
18	c.619C>T	Gln207X	Tsujikawa et al. 1999
19	c. 632delA	Gln211ArgfsX60	Tsujikawa et al. 1999
20	c.653delA	Asp218ValfsX53	Markoff et al. 2006
21	c.679G>A	Glu227Lys	Alavi et al. 2007
22	c.772_783delATCTATTACCTGinsT	lle258X 772 to 783del	Ha et al. 2003
23	c.811delA	Lys271SerfsX26	Ren et al. 2002

The [$]marks the mutation identified in North Indian patients.

sembly of intermediate filaments thereby leading to disruption of keratinocyte filament architecture and cell lysis. Meesmann's epithelial dystrophy (OMIM 122100) is a juvenile, autosomal dominant disorder with incomplete penetrance characterized by minute, spherical micro cysts, disposed with varying symmetry. Stocker Holt dystrophy resembles Meesmans dystrophy in the phenotype and was initially reported by stocker and Holt as grayish white, closely packed punctuate opacities present bilaterally. R19I mutation in Arg 19 Ile gene is reported to be associated with this disorder.

5. CHST6 (CARBOHYDRATE 6-SULFOTRANSFERASE) GENE

Macular Corneal Dystrophy MCD (OMIM 217800): is associated with mutations in CHST6 gene mapped to chromosome 16 (16q22) by Akama et al. in 2001. Macular corneal dystrophy is characterized by bilateral corneal opacification. The corneal stroma is often thinner than the normal. This is a recessive disorder of corneal sulfate (KS) metabolism that results in deposition of unsulfated keratan sulfate in the extra cellular corneal stroma leading to bilat-

Table 3: Mutations identified till date in KRT12 gene in epithelial basement membrane dystrophy

S.No.	*Mutation*	*Amino acid change*	*Référence*
1	c.386T>C	Met129Thr	Corden et al. 2000
2	c.389A>C	Gln130Pro	Corden et al. (BJO) 2000
3	c.394C>G	Leu132Val	Aldave 2005
4	c.399T>G	Asn133Lys	Irvine et al. 2002
5	c.403A>G	Arg135Gly	Nishida et al. 1997
6	c.404G>T	Arg135Ile	Nishida et al. 1997
7	c.404G>C	Arg135Thr	Irvine et al. 1997
8	c.405A>C	Arg135Ser	Yoon et al. 2004
9	c.409G>C	Ala137Pro	Takahashi et al. 1997
10	c.419T>G	Leu140Arg	Nishida et al. 1997
11	c.427G>C	Val143Leu	Irvine et al. 1997
12	c.1171_1197dup	Ile391_Leu399dup	Yoon et al. 2004
13	c.1276A>G p	Ile 426Val	Coleman et al 1999
14	c.1277T>G	Ile 426Ser	Nichini et al. 2005
15	c.1285T>G	Tyr429Asp	Nishida et al. 1997
16	c.1286A>G	Tyr429Cys	Chen et al. 2005

eral, progressive corneal opacification. Histopathologically the deposits stains blue with colloidal iron. The product of CHST6 gene, corneal N- acetyglucosamine in KS. The amino acid substitution resulting from nucleotide changes in MCD patients cause loss of enzymatic activity and the resulting unsulfated KS leads to loss of transparency in the corneas of the affected. Patients have corneal deposits typical of MCD. Subtypes are distinguished based on the presence of antigenic keratan sulfate. MCDI the commonest form has no detectable antigenic keratin sulfate in the serum or cornea while in MCDII normal amount of antigenic keratan sulfate is seen in serum and accumulated in the cornea. Type IA serum lacks a detectable amount of antigenic keratin sulfate but keratocyte shows abnormal accumulation. Unlike TGFBI related dystrophies, MCD does not show any mutation hot spots or genotype phenotype correlation though more than 100 mutations are known to be associated with it (Table 4).

6. COL8A2 GENE

Mutations in COL8A2 gene are reported to be associated with Fuch's Endothelial Dystrophy. Ultra-structural studies in early 1970s identified it as primary endothelial dystrophy. Its clinical and pathological hallmark is focal thickening of Descemets membrane. Genome wide search for an early onset or autosomal recessive form of Fuch's Endothelial Dystrophy identified a locus at chromosome 1p34.3-p32 which includes the COL8A2 gene coding for the alpha-2 chain of type VIII collagen, a short-chain collagen that is a component of endothelial basement membranes. Analysis of its coding sequence has revealed till date two underlying mutations Leu 450 Trp and Gln455Lys in early onset cases (Gottsch et al. 2005), suggestive of the role of type VIII collagen in influencing the terminal differentiation of the neural crest-derived corneal endothelial cell. Several genome-wide linkage studies performed recently in families with the classic late-onset form of FECD (Sundin et al. 2006) have demonstrated evidence of link-age to different loci at chromosome 13 (13pTel- 13q12.13) and a 6.9- megabase region on chromosome 18 (18q21.2-18q21.32), although no pathogenic mutations have been identified in the positional candidate genes screened to date. Thus, it appears that locus heterogeneity may exist for FECD, in which mutations in several genes on different chromosomes may produce a common disease phenotype.

7. TCF8 GENE

Polymorphous Corneal Dystrophy (PPCD) (OMIM 122000): It is a rare endothelial dystrophy characterized clinically by bilateral vesicular or linear lesions at the level of Descemet's membrane and pathologically by epithelium like cells replacing it. Although mutations in the visual system homeobox gene 1 (VSX1) (MIM No. 605020) (Heon et al. 2007), COL8A2 (John et al. 2005) and the gene for transcription factor 8 (*TCF8*) have been reported to play a role in the pathogenesis of posterior polymorphous corneal dystrophy (PPCD) (MIM No. 122000), convincing evidence exists to support the role of only *TCF8* mutations in this autosomal dominant corneal endothelial dystrophy. The TCF8 gene at locus11p20 encodes a zinc finger protein (Nil-2-a). Nil-2-a inhibits T-lymphocyte-specific interleukin 2 (IL2) gene expression by binding to a negative regulatory domain 100 nucleotides 5' of the IL2 transcription start site. Krafchak and colleagues in 2005 reported frameshift and nonsense mutations in *TCF8* in 5 of 11 families with Posterior Polymorphous Corneal Dystrophy (PPCD) (OMIM 122000). Each mutation was demonstrated to segregate with the affected phenotype in each family examined with the exception of 2 unaffected individuals from 1 large family. This exception was attributed by the authors to incomplete penetrance**.** Autosomal dominant mode of inheritance is known for PPCD. Several other mutations have since been described that are listed in (Table 5).

8. SLC4A11 GENE

Mutations in SLC4A11 gene are associated with autosomal recessive Congenital Hereditary Endothelial Dystrophy (CHED). CHED is a clinically characterized by bilateral diffuse corneal edema with both the eyes usually symmetrically affected without any other developmental abnormality in the anterior segment. Profound thickening of Descemet's membrane and degenerated corneal endothelial cells are seen with

Table 4: Mutations identified till date in CHST6 gene in MCD

S.No.	*Nucleotide Change*	*Amino acid change*	*Reference*
1	c.1A>T	Met1?	Liskova et al. 2007
2	c.6G>A	Trp2X	Sultana et al. 2005*
3	c.7C>A	Leu3Met	Sultana et al. 2005*
4	c.15_16delCG	Arg5fs	Warren et al. 2003*
5	c.15_16insATGCTGTGCG	Val6fs	Sultana et al. 2005*
6	c.16_40del	Val6fs	Liu et al. 2006
7	c.44T>C	Leu15Pro	Niel et al. 2003
8	c.51delG	Gln18fs	Klintworth et al. 2006
9	c.52C>T	Gln18X	Sultana et al. 2005*
10	c.65T>G	Leu22Arg	Warren et al. 2003*
11	c.91C>T	Pro31Ser	El-Ashry et al. 2002
12	c.92C>T	Pro31Leu	Birgani et al. 2009
13	c.94_100del	Ser32fs	Sultana et al. 2005*
14	c.124C>T	His42Tyr	Warren et al. 2003*
15	c.137T>C	Leu46Pro	Klintworth et al. 2006
16	c.143C>A	Ser48X	Birgani et al. 2009
17	c.148C>A	Arg50Cys	Akama et al. 2000
18	c.148C>T	Arg50Cys	Warren et al. 2003
19	c.149G>T	Arg50Leu	Warren et al. 2003
20	c.152C>T	Ser51Leu	Aldave et al. 2004
21	c.155G>A	Gly52Asp	Sultana et al. 2005*
22	c.158C>T	Ser53Leu	Warren et al. 2003
23	c.161C>T	Ser54Phe	Sultana et al. 2005*
24	166_167delGTinsAG	p.Val56Arg	Sultana et al. 2005*
25	c.165C>A	Phe55Leu	Birgani et al. 2009
26	c.172C>T	Gln58X	Niel et al. 2003
27	c.176T>C	Leu59Pro	Ha et al. 2003
28	c.180delC	Phe60fs	Sultana et al. 2005*
29	c.182A>C	Asn61Thr	Niel et al. 2003
30	c.189C>G	His63Gln	Klintworth et al. 2006
31	c.196G>T	Val66Phe	Gulias-Canizo et al. 2006
32	c.196G>C	Val66Leu	Ha et al. 2003
33	c.197T>A	Val66Asp	Gruenauer-Kloevekorn et al. 2008
34	c.198delC	Phe67fs	Warren et al. 2003*
35	c.199T>A	Phe67Ile	Gruenauer-Kloevekorn et al. 2008
36	c.199T>G	Phe67Val	Gruenauer-Kloevekorn et al. 2008
37	c.202T>C	Tyr68His	Niel et al. 2003
38	c.209T>A	Met70Leu	Niel et al. 2003
39	c.213G>T	Glu71Asp	Gruenauer-Kloevekorn et al. 2008
40	c.214C>T	Pro72Ser	El-Ashry et al. 2002
41	c.217G>A	Ala73Thr	Sultana et al. 2005*
42	c.217G>C	Ala73Pro	Klintworth et al. 2006
43	c.226G>A	Val76Met	Ha et al. 2003
44	c.231G>A	Trp77X	Klintworth et al. 2006
45	c.231G>C	Trp77Cys	Gruenauer-Kloevekorn et al. 2008
46	c.244C>T	Gln82X	Niel et al. 2003
47	c.271_273delGCTinsA	Ala91fs	Niel et al. 2003
48	c.274G>C	Val92Leu	Klintworth et al. 2006
49	c.277C>A	Arg93Ser	Aldave et al. 2004
50	c.278G>A	Arg93His	Warren et al. 2003*
51	c.290G>C	Arg97Pro	Warren et al. 2003*
52	c.293C>G	Ser98Trp	Sultana et al. 2005*
53	c.304T>G	Cys102Gly	Niel et al. 2003
54	c.305G>A	Cys102Tyr	Warren et al. 2003
55	c.310A>G	Met104Val	Aldave et al. 2004
56	c.320T>C	Phe107Ser	Sultana et al. 2005*
57	c.329A>G	Tyr110Cys	Aldave et al. 2004
58	c.340C>T	Arg114Cys	Klintworth et al. 2006
59	c.363C>G	Phe121Leu	Sultana et al. 2005*
60	c.363_364insC	Gln122fs	Niel et al. 2003
61	c.365A>C	Gln122Pro	Aldave et al. 2004
62	c.369G>A	Trp123X	Sultana et al. 2005*
63	c.375_376insGGCCGTGC	Ser126fs	Ha et al. 2003
64	c.379C>T	Arg127Cys	Warren et al. 2003
65	c.383C>T	Ala128Val	Liu et al. 2006
66	c.391T>C	Ser131Pro	Sultana et al. 2005*

Table 4: Contd.....

S.No.	*Nucleotide Change*	*Amino acid change*	*Reference*
67	c.392C>T	Ser131Leu	Klintworth et al. 2006
68	c.395C>T	Pro132Leu	Birgani et al. 2009
69	c.406A>G	Ser136Gly	Birgani et al. 2009
70	c.414_415insTT	Pro139fs	Akama et al. 2000
71	c.418C>T	Arg140X	El-Ashry et al. 2005
72	c.446G>A	Cys149Y	Birgani et al. 2009
73	c.455T>C	Leu152Pro	Niel et al. 2003
74	c.459C>A	Cys153X	Sultana et al. 2005*
75	c.494G>C	Cys165Ser	Sultana et al. 2005*
76	c.495C>G	Cys165Trp	Sultana et al. 2005*
77	c.497G>C	Arg166Pro	Niel et al. 2003
78	c.500C>T	Ser167Phe	Sultana et al. 2005*
79		Leu173Phe+ Leu200Arg	Gruenauer-Kloevekorn C et al. 2008
80	c.521A>G	Lys174Arg	Akama et al. 2000
81	c.529C>T	Arg177Cys	Klintworth et al. 2006
82	c.530G>A	Arg177His	Iida-Hasegawa et al. 2003
83	c.533T>G	Phe178Cys	Sultana et al. 2005*
84	c.545delA	Gln182fs	Sultana et al. 2005*
85	c.573_574insC	Ala192fs	Klintworth et al. 2006
86	c.578T>C	Leu193Pro	Sultana et al. 2005*
87	c.581_586delACCTACinsGGT	Asn194_Arg196delinsArgCys	Warren et al. 2003*
88	c.585_587insACG	Arg196_Ile197insArg	Sultana et al. 2005*
89	c.593T>A	Val198Glu	Abbruzzese et al. 2004
90	c.599T>G	Leu200Arg	Niel et al. 2003
91	c.604C>A	Arg202Ser	Niel et al. 2003
92	c.607G>A	Asp203Asn	Klintworth et al. 2006
93	c.609C>A	Asp203Glu	Akama et al. 2000
94	c.611C>A	Pro204Gln	Sultana et al. 2005*
95	c.611C>G	Pro204Arg	Sultana et al. 2005*
96	c.612_614delGCGinsAT	Arg205fs	Warren et al. 2003*
97	c.614G>A	Arg205Gln	Warren et al. 2003*
98	c.614G>T	Arg205Leu	Iida-Hasegawa et al. 2003
99	c.616G>A	Ala206Thr	Warren et al. 2003*
100	c.617C>T	Ala206Val	El-Ashry et al. 2005
101	c.629C>T	Ser210Phe	Sultana et al. 2005*
102	c.631C>T	Arg211Trp	Akama et al. 2000
103	c.632G>A	Arg211Gln	Ha et al. 2003
104	c.649G>A	Ala217Thr	Iida-Hasegawa et al. 2003
105	c.656_657insCTG	Ala219_Arg220insTrp	Sultana et al. 2005*
106	c.661G>T	Asp221Tyr	Sultana et al. 2005*
107	c.663C>G	Asp221Glu	Sultana et al. 2005*
108	c.668G>A	Gly223Asp	Liu et al. 2006
109	c.682A>G;683C>A	Thr228Asp	Klintworth et al. 2006
110	c.696G>A	Trp232X	Ha et al. 2003
111	c.738C>G	Cys246Trp	Klintworth et al. 2006
112	c.740delG	Arg247fs	Klintworth et al. 2006
113	c.744C>G	Ser248Arg	Klintworth et al. 2006
114	c.746A>C	His249Pro	Warren et al. 2003
115	c.746A>G	His249Arg	Birgani et al. 2009
116	c.803A>G	Tyr268Cys	Ha et al. 2003
117	c.814C>A	Arg272Ser	Sultana et al. 2005*
118	c.815G>A	Arg272His	Klintworth et al. 2006
119	c.820G>A	Glu274Lys	Warren et al. 2003*
120	c.827T>C	Leu276Pro	Sultana et al. 2005*
121	c.891_892insC	Pro297fs	Birgani et al. 2009
122	c.892C>G+ c.1734_1735delTG	Glu298X	Gruenauer-Kloevekorn C et al. 2008
123	c.925G>T	Gly309X	Sultana et al. 2005*
124	c.985G>C	Val329Leu	Liu et al. 2006
125	c.991C>T	Gln331X	Aldave et al. 2004
126	c.993G>T	Gln331His	Liu et al. 2006
127	c.1000C>T	Arg334Cys	Sultana et al. 2005*
128	C.1001G>A	Arg334His	Gulias-Canizo et al. 2006
129	c.1002_1012delinsTTG	His335fs	Sultana et al. 2005*
130	c.1039G>T	Glu347X	Sultana et al. 2005*

Table 4: Contd.....

S.No.	*Nucleotide Change*	*Amino acid change*	*Reference*
131	c.1046G>A	Cys349Tyr	Klintworth et al. 2006
132	c.1047C>G	Cys349Trp	Klintworth et al. 2006
133	c.1052_1059dupCTGCGCTG	Gln354ValfsX30S	Aldave et al. 2004
134	c.1056_1078del	p.Ala352AlafsX5;Leu353fs	Warren et al. 2003*
135	c.1060_1061insGTGCGCTG	Gln354fs	Klintworth et al. 2006
136	c.1072T>G	Tyr358Asp	El-Ashry et al. 2005
137	c.1072T>C	Tyr358His	Dang et al. 2009

Asterix * mark the mutations identified in south Indian patients

Table 5: Mutations identified till date in TCF8 gene in PPCD

S.No.	*Nucleotide change*	*Amino acid change*	*Reference*
1	c.2T>G	Met1Arg	Aldave et al. 2007
2	c.34C>T	Gln12X	Aldave et al. 2007
3	c.640C>T	Gln214X	Aldave et al. 2007
4	c.929dupA	Cys311ValfsX25	Aldave et al. 2007
5	c.973C>T	Arg325X	Aldave et al. 2007
6	c.1124delT	Phe375SerfsX31	Liskova et al. 2007
7	c.1332_1335delCAAT	Ile444MetfsX48	Krafchak et al. 2005
8	c.1348C>T	Gln450X	Krafchak et al. 2005
9	c.1482dupA	Glu495ArgfsX10	Aldave et al. 2007
10	c.1568delA	Val526X	Aldave et al. 2007
11	c.1576dupG	Val526GlyfsX3	Krafchak et al. 2005
12	c.2157C>G	Tyr719X	Liskova et al. 2007
13	c.2182G>T	Glu728X	Krafchak et al. 2005
14	c.2324dupA	Glu776GlyfsX44	Liskova et al. 2007
15	c.2916_2917delTG	Gly973ValfsX14	Krafchak et al. 2005
16	c.2988_2989delAG	Glu997AlafsX7	Aldave et al. 2007

and the phenotype may vary in different individuals. Vision may be severely impaired, and many patients require corneal transplantation. Both autosomal dominant (AD) and autosomal recessive (AR) forms of the disorder have been described. The gene encodes transporter involved in borate homeostasis. In the absence of borate, it functions as a Na(+) and OH(-); H(+) channel. In the presence of borate functions as an electrogenic Na(+) coupled borate cotransporter. The gene responsible for AD CHED (OMIM 121700) (HGMW-approved symbol CHED1) has been mapped to the pericentromeric region of chromosome 20 (Toma et al. 1995). With genome-wide search using homozygosity mapping and DNA pooling, a region of homozygosity in all affected individuals was identified, narrowing the disease gene locus to an 8-cM region flanked by markers D20S113 and D20S882. This AR CHED (OMIM 217700) (HGMW-approved symbol CHED2) disease gene locus is physically and genetically distinct from the AD CHED locus. The CHED2 locus is situated as little as 4.0 cM from the telomere, thus CHED2 locus is located in the cytogenetic band 20p13. Since the initial reports of the mutations in SLC4A11 gene (Vithana et al. 2006) several studies have been conducted to identify the underlying mutations (Table 6).

9. UBIAD1 GENE

Mutations in UBIAD1 gene located on chromosome 1p36 were recently found to be associated with **Central Crystalline Dystrophy (OMIM 121800)** (Orr et al. 2007). SCCD is associated with a number of non-ocular manifestations, the most important of which is hypercholesterolemia found in approximately 40% of affected patients. More than 10 underlying mutations in UBAID1 have reported been since its initial discovery resulting in SCCD.

10. PIPK3 GENE

Fleck Corneal Dystrophy (OMIM 121850): is characterized by the presence of multiple, non- progressive minute opacities in corneal stroma with varying phenotype present in the same family (Assi et al. 1999). The dis-order is inherited in autosomal dominant form with mutation in PIPK3 gene (Li et al. 2005) that is known to be involved in the phosphorylation in lipid metabolism pathway.

Table 6: Mutations identified till date in CHEDII cases.

S.No.	*Nucleotide change*	*Amino acid change*	*Reference*
1	c.140delA	Tyr47SerfsX69	Sultana et al. 2007*
2	c.246_247delTTinsA	Phe84LeufsX32	Jiao et al. 2007
3	c.306delC	Gly103ValfsX13	Sultana et al. 2007*
4	c.334C>T	Arg112X	Sultana et al. 2007*
5	c.353_356delAGAA	Lys118ThrfsX12	Vithana et al. 2006
6	c.473_480delGCTTCGCC	Arg158ProfsX4	Sultana et al. 2007*
7	c.520delGCTTCGCC	Arg158fs	Aldahmesh et al. 2009
8	c.618_619delAG	Val208AlafsX38	Sultana et al. 2007*
9	c.625C>T	Arg209Trp	Sultana et al. 2007*
10	c.637T>C	Ser213Pro	Desir et al. 2007
11	c.638C>T	Ser213Leu	Sultana et al. 2007*
12	c.695G>A	Ser232Asn	Aldave et al. 2007
13	c.697C>T	p.Arg233Cys	Sultana et al. 2007*
14	c.859_862delGAGAinsCCT	Glu287ProfsX21	Kumar et al. 2007*
15	c.878_889del	Glu293_Glu296del	Sultana et al. 2007*
16	c.985A>T	Arg329X	Aldave et al. 2007
17	IVS-7 c.996 + 26C_+44Cdel19	-	Sultana et al. 2007*
18	c.1044+25del19nt	-	Aldahmesh et al. 2009
19	IVS-8 c.1091-1G.C	-	Sultana et al. 2007*
20	c.1202C>A	Thr401Lys	Sultana et al. 2007*
21	c.1228G<177C	Gly394Arg	Aldahmesh et al. 2009
22	c.1253G>A	Gly418Asp	Sultana et al. 2007*
23	c.1317_1322del6ins8	Leu440ValfsX6	Sultana et al. 2007*
24	c.1378_1381delTACGinsA	Tyr460_Ala461delinsThr	Desir et al. 2007
25	c.1391G>A	Gly464Asp	Vithana et al. 2006
26	c.1418T>G	Leu473Arg	Sultana et al. 2007*
27	c.1463G>A	Arg488Lys	Desir et al. 2007
28	12 c.1466C>T	Ser489Leu	Sultana et al. 2007
29	c.1704_1705delCT	Ser569ArgfsX177	Jiao et al. 2007
30	c.1751C>A	His568HisfsX177	Sultana et al. 2007*
31	c.1813C>T	p.Arg605X	Jiao et al. 2007
32	c.1894G>T	Glu632X	Sultana et al. 2007*
33	c.2017_2019delTTC	Phe672del or Phe673del	Kumar et al. 2007*
	c.2014_2016delTTC or		
35	IVS 15 c.2067 -6_-16 delinsGGCCGGCCGG	Inactivation of spliceacceptor site IVS15 –6_-16 delins	Vithana et al. 2006
36	c.2114+1G>A	Inclusion of intron 15	Aldahmesh et al. 2009 -
37	c.2233_2240 dupTATGACAC	Ile748MetfsX5	Desir et al. 2007
38	c.2263C>T	Arg755Trp	Sultana et al. 2007*
39	c.2264G>A	Arg755Gln	Jiao et al. 2007
40	c.2236C>T+c.2547G>C	Arg757X+Thr833Thr	Aldahmesh et al. 2009
41	c.2318C>T	Pro773Leu	Sultana et al. 2007*
42	c.2389_2391delGAT	Asp797del	Sultana et al. 2007*
43	c.2407C>T	Gln803X	Sultana et al. 2007*
44	c.2411G>A	Arg804His	Jiao et al. 2007
45	c.2420delTinsGG	Leu807ArgfsX71	Jiao et al. 2007
46	c.2423_2454del	Leu808ArgfsX110	Desir et al. 2007
47	c.2470G>A	Val824Met	Sultana et al. 2007*
48	c.2498C>T	Thr833Met	Jiao et al. 2007
49	c.2528T>C	Leu843Pro	Desir et al. 2007
50	c.2566A>G	Met856Val	Desir et al. 2007
51	c.2606G>A	Arg869His	Jiao et al. 2007
52	c.2605C>T	Arg869Cys	Sultana et al. 2007*
53	c.2623C>T	Arg875X	Sultana et al. 2007*

Asterix* mark the mutations identified in south Indian patients

OTHER DYSTROPHIES WITH UNKNOWN GENES

1) Congenital Hereditary Stromal Dystrophy: It is an autosomal dominant non progressive disorder that was observed in one large family with descendants in France and Germany in 1939. The dystrophy is characterized with tightly packed lamellae with small diameter of collagen fibre. Decorin gene was found to be involved with mutation leading to abnormal binding of dermatan sulfate to collagen resulting in the opaque cornea.

2) X- Linked Endothelial Corneal Dystro-

phy: Corneal opacification was seen only in male patients in this dystrophy in which the transmission of trait from father to carrier daughters was seen. On linkage analysis a region on long arm of chromosome was seen (Xq25) (Bredrup et al. 2005).

RELEVANCE OF MOLECULAR TESTING

With the present level of knowledge and understanding of the molecular processes involved in the causation of different dystrophies, it is recognized that distinct clinico-pathologic phenotypes result from specific mutations in a particular gene or different mutations in the same gene (genetic heterogeneity), leading to a better understanding of their pathogenesis. Genetic analysis can be introduced on a routine basis along with other ophthalmologic investigations as there has been a profound decrease in the cost of mutation screening in our country.

AIIMS is a centre providing these services and catering to the patient population from North India. On similar lines, several studies have been conducted on corneal dystrophies in South India, mainly at three centers - L V Prasad eye Institute (Hyderabad), Arvind Eye Hospital (Chennai) and Shankar Nethralaya (Chennai). There is a difference in distribution of the dystrophies and the mutations among the North (Paliwal et al. 2009; Paliwal et al. 2010) and South Indian population, with autosomal recessive CHED and MCD being more common in South India as compared to autosomal dominant TGFBI related dystrophies being more prevalent in the North. Several novel as well as known mutations have been identified in the South Indian population for CHED (Table 6) and MCD (Table 4). Additionally, Warren et al. (2003) have also reported the mutations in CHST6 gene in MCD (Table 4) and Chakravarthi et al. (2005) in TGFBI.

Molecular analysis can be used to confirm a suspected diagnosis in cases with an atypical phenotype, for genotype- phenotype correlations, precise classification of the dystrophy based on which appropriate treatment can be planned i.e. lamellar or penetrating keratoplasty, identifying the carrier status and predicting the underlying risk of disease in the family. Incorporation of such analyses will facilitate accurate diagnosis and counseling and provide better treatment and management options to the affected.

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