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Autosomal Dominant Corneal Dystrophy with TGFBI Mutations: Lessons Learned from a Chinese Pedigree

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Abstract

Corneal dystrophies are rare autosomal dominant genetic diseases with diverse anatomic classification. The complexity of this disease reflects both the heterogeneity of causative gene mutations and the diversity of clinical presentations. Here, we studied and report a four-generation Chinese pedigree with corneal dystrophy, in which the clinical symptoms resemble Meesmann corneal dystrophy without the characteristic gene mutations of either KRT3 or KRT12. Targeted exon sequencing with PCR methods identified an Arg124Cys mutation in the TGFBI gene that concordant with phenotype in all affected individuals. This TGFBI mutation has been associated with multiple subtypes of corneal dystrophy. We reviewed the global reported TGFBI mutations in different ethnic groups and geographically mapped TGFBI mutations previously reported in China. The distribution of TGFBI allelic mutations may assist the clinical diagnosis of the heterogeneous, autosomal dominant corneal dystrophies.

Keywords: Corneal dystrophy; TGFBI; Genetic heterogeneity; Phenotypic heterogeneity

Introduction

The relationship between phenotype and genotype is a central element for accurate clinical diagnosis of disease and assessment of its potential for treatment. This is especially true with the extensive genotypic heterogeneity that underlies phenotypic expression of the corneal dystrophies. We illustrate our experience with a large family that came to our clinical attention with a dominant corneal dystrophy that was initially assessed as Meesmann corneal dystrophy [1].

Corneal dystrophies are rare and usually autosomal dominant genetic disorders with bilateral, symmetrical, and non-inflammatory progressive corneal opacities that lead to varying degrees of visual impairment [2]. Although they are rare, the corneal dystrophies are phenotypically complex with the involvement of one or more of the five distinctive corneal layers and they have an extensive eponymous classification. Structural location has categorized the phenotypic heterogeneity of corneal dystrophy into anterior or superficial dystrophy (including the epithelium and Bowman membrane), middle or stromal dystrophy and posterior dystrophy (including the Decemet membrane and endothelium). Different gene mutations have been identified worldwide in families of different ancestries with corneal dystrophy and these different gene mutations have added a layer of genetic complexity to the existing phenotypic complexity. Multiple and independent allelic mutations have been identified so far in at least 12 different genes that map to 9 different chromosomes (Table 1). These genes are beginning to assist clinical assessment because they are associated with different categories and subtypes of corneal dystrophy as well as with their more historical eponymous classifications [2,3].

Eponymous names, structural locations of anatomical change, and biological functions of the associated genes create layers of complexity for rare genetic disorders, such as the corneal dystrophies, and this complexity widens the gap between the presentation of clinical phenotype in individual patients and an accurate diagnosis. Phenotypic complexity arises not just from the heterogeneity of different gene loci but also from an increasing number of different allelic forms of the known genes that influence the corneal dystrophies. Nevertheless, prevalence and expected incidence of new cases of genetically different corneal dystrophies are unknown because registries of affected cases have not been established and global rarity of the corneal dystrophies complicates their diagnosis if it is based solely on a clinical phenotypic impression. Most practitioners, except in specialty clinics, will only see new cases in genetically related individuals and, rarely, will they see a wide range of completely new and novel cases of different corneal dystrophies. However, rapid and accurate diagnosis is critical for appropriate patient management.

Genetic heterogeneity in the same type of corneal dystrophy may result from different allelic mutations in various regions of the same gene and defects in the same genomic region may lead to different types of corneal dystrophy, possibly through epigenetic events. One example is the mutations in the KRT3/KRT12 genes and alterations in the keratin protein heterodimers in Meesmann's corneal dystrophy as discussed in our recent paper [1]. An extreme example might be the apparent absence of KRT3 and KRT12 mutations in several patients who were reported to have the clinical phenotype of Meesmann corneal dystrophy [4].

Another example of genetic and phenotypic heterogeneity is found with corneal dystrophies that are associated with the TGFBI (transforming growth factor, betainduced)- locus. A wide spectrum of TGFBI mutations and their clinical consequences have been investigated in corneal dystrophy patients in different ethnic populations [5].

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Gene locus	Gene name	Chromosome location
KRT3	keratin 3	12q13.13
KRT12	keratin 12	17q21.2
TGFBI	transforming growth factor, beta induced (keratoepithelin)	5q31
TACSTD2	tumor-associated calcium signal transducer 2	1p32
CHST6	carbohydrate (N-acetylglycosamine 6-O) sulfotransferase 6	16q22
GSN	gelsolin	9q33
UBIAD1	UbiA prenyltransferase domain containing 1	1p36.22
DCN	decorin	12q21.33
PIKFYVE	phosphoinositide kinase, FYVE finger containing	2q34
COL8A2	collagen, type VIII, alpha 2	1p34.2 -p32.3
SLC4A11	solute carrier family 4, sodium borate transporter, member 11	20p13
ZEB1	zinc finger E-box binding homeobox 1	10p11.22

Table 1: Genes and chromosome locations associated with corneal dystrophy (names from HUGO Gene Nomenclature Committee).

The same mutation in TGFBI (e.g., Arg124His) may contribute to different subtypes of corneal dystrophy phenotypes, on the other hand, two distinct mutations of TGFBI (Arg124Ser and Arg555Trp) have been found to be responsible for the same subtype of corneal dystrophy, known as granular corneal dystrophy type 1 (GCD1) [6].

Here we report an in-depth analysis of corneal dystrophy in four-generation Chinese kindred, which had been clinically assessed with Meesmann-type corneal dystrophy, but for which there was no exon mutation in either the KRT3 or KRT12 gene [1]. If the clinical phenotype were, in fact, a variant of Meesmann corneal dystrophy, absence of a protein-coding mutation suggested that novel regulatory elements might be responsible for the clinical phenotype. However, before embarking on a search for potential regulatory elements in the genome, we screened the family for other corneal dystrophy related genes, beginning with the more polymorphic TGFBI. We found an Arg124Cysmutation in TGFBI that is the result of a C>T transition in all tested affected patients and, since this rare dominant-negative disorder is genetically heterogeneous in the Chinese population, we also mapped the geographic spectrum of known TGFBI mutations. This geographic spectrum was helpful to focus our attention on TGFBI and, therefore, we suggest that, in the absence of registries for these rare, phenotypically and genotypically heterogeneous disorders, the geographic distribution of allelic forms of the different corneal dystrophies might serve as a useful guide for future clinical genetic investigations of new cases by giving these rare traits a geographic context.

Methods

The Institutional Review Board of the Central Hospital, affiliated with Zhengzhou University, China, approved this study. All subjects or their parents provided written informed consent.

Patients

We studied a four-generation Chinese family including six living individuals with corneal dystrophy. The proband is a 20 year-old boy (IV-4) who was initially brought to hospital in Zhengzhou for evaluation of blurred vision, irritation, tearing and often photophobia. Visual acuity was normal (1.2 in both eyes). Multiple small cysts were seen with slit-lamp examination. His father (III-4) has a similar eye problem, which drew the family to clinical genetic attention. Subsequently, the extended four-generation family was assessed. Peripheral blood samples were obtained from patients II-2, II-3, III-4, III-6, III-12 and IV-4 and unaffected individuals III-1, -5, -7, -11, IV-2, -3, -5, -6 (including two non-genetic relatives III-5 and III-7).

exon 1	caggaggcctaagggaccta ctccatgctgcaaggttttt	607
exon 2	tcaattgcccatgtcaaaga gccctgaaaaatgtctccaa	607
exon 3	ccagttggttggctgtaggt gaggagcagctcaggaaatg	514
exon 4	ccccagaggccatccctcct ccgggcagacggaggtcatc	358
exon 5	ggcatgatgaatgggagtct gagaagcaggcacaaagagg	579
exon 6	tctccttgggccctctatt tcaggggaacctgctctatg	416
exon 7	aggaagaggaaaggcaggtt agcaacaggacaggatgacc	532
exon 8	agaaggcgaggaggatctg gtcacaacccacacatttgc	527
exon 9	tgactgttcccctgatgaca ttttggttgagctgagtgga	434
exon 10	ttggcagcttcacttggttt ttccttccttgtcagcaacc	409
exon 11	tcccagccttaataacccatc cttttccccatcccaagtct	433
exon 12	tccagtggcctggactctac gatgtgccaactgtttgctg	337
exon 13	tgctttgtgtcctctgacca catcctgggggtgagatatg	402
exon 14	ggcgacaagattgaaactcc cccaattcactctgcaatca	405
exon 15	tgtgcattcacctttcttgg agtgggagtggggagaagtt	406
exon 16	gtccacctgaaggcacactt ccaagtcaccctgctgttct	393
exon 17	cacctgctatgtgcaggaga ggctggattgcttgattcat	532

Table 2: Primers for PCR Amplification Direct Sequence of TGFBI gene.

DNA collection, isolation, amplification and sequencing

Genomic DNA was isolated from peripheral blood leukocytes by standard procedures using a blood DNA kit (Qiagen). The coding regions of the TGFBI genes were screened for mutations by direct sequencing using DNA amplified with primers (Table 2). Polymerase chain reaction (PCR) was performed at 94 °C for 5 min followed by 35 cycles at 94 °C for 30 s, 57 °C for 30 s, 72 °C for 30 s, and 72 °C for 5min. Primer sequences and annealing temperatures for each primer set are given in (Table 2). PCR products were examined on 1% agarose gels and then sequenced using an ABI PRISM 377 DNA sequencer (Applied Biosystems, Foster City, CA) and BigDye Termiantion cycle sequencing kit v. 3.1 (Applied Biosystems).

Results

The pedigree (Figure 1) shows eight affected individuals, all males, ranging in age from 20 (IV-4) to 80 (II-2) years; affected individuals I-1 and III-3 were identified from family description. Detailed clinical assessments were previously reported [1]. We note that vision was very poor in both eyes for individuals II-2, II-3, III-4, -6, and -12. Slit-lamp examination of all affected living individuals showed multiple fine vesicles (microcysts) within the corneal epithelia in both eyes. In addition, two individuals (III-1 and IV-2) were clinically diagnosed with pathological myopia. The diagnosis was autosomal dominant, corneal dystrophy, Meesmann subtype, based purely on clinical impression from physical examination.

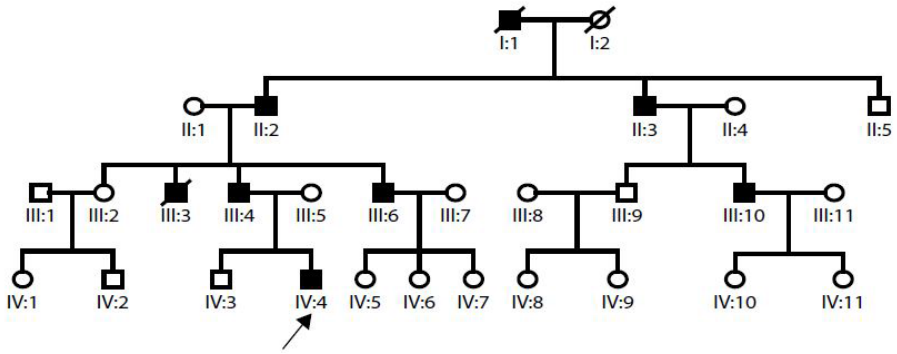

Figure 1: Pedigree of a Chinese family with corneal dystrophy, showing autosomal dominant inheritance. Affected individuals have a solid symbol and proband (indicated by an arrow) is IV-4.

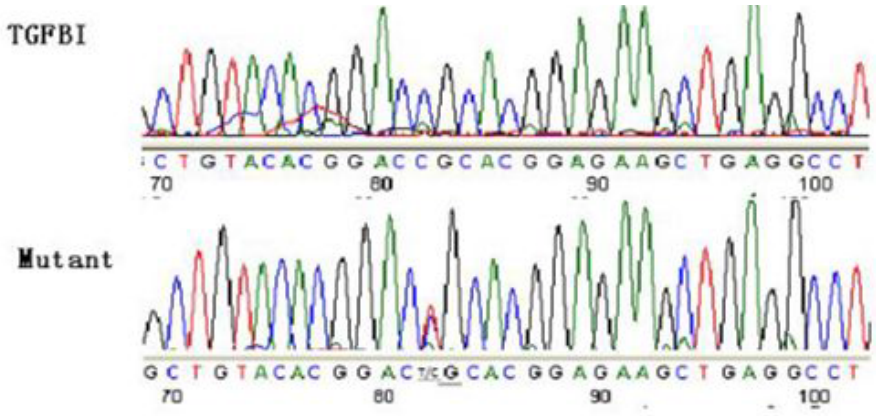

Figure 2: Sequencing analysis of PCR products from exon 4 of TGFBI in affected patients. The arrow indicates a nucleotide change in codon 124, which resulted in a change from wild type (CGC) to mutant (TGC) C>T, Arg124Cys.

Genomic DNA from peripheral blood leucocytes of affected living individuals (II-2, -3, III- 4, -6, -12, IV-4), unaffected individuals (III-1, -11, IV-2, -3, -5, -6) and two nongenetic relatives (III-5 and III-7), was assessed for exon mutations in the TGFBI genes. The mutation of Arg 124 Cys at exon 4 of TGFBI was found. A heterozygous c 418 C>T transition mutation in exon 4 of TGFBI (Figure 2) was identified in all affected and tested individuals but not in unaffected family members. This mutation leads to a substitution at codon 124 (Arg124Cys, R124C) and is consistent with the literature on different subtypes of corneal dystrophy.

Discussion

The corneal dystrophy in this large Chinese pedigree was initially diagnosed as Meesmann epithelial corneal dystrophy, but no common KRT3 or KRT12 gene mutation was found with whole exome sequencing [1]. Either the clinical diagnosis was incorrect or some other gene is responsible for the autosomal dominant corneal dystrophy phenotype in this pedigree. Therefore, we started a search for mutations in other common genes that are associated with corneal dystrophy. We selected the TGFBI gene as a candidate for targeted whole exon sequencing. The TGFBI (chr5:135,364,584-135,399,507) comprises 17 exons and encodes a 68 kDa extracellular matrix protein (TGFBI kerato-epithelin), which is expressed in the cornea and other tissues. TGFBI is an important disease gene related to different type of corneal dystrophy (Table 3) because disease-related mutations are very polymorphic. More than 50 mutations have been found in exon 4, 11, 12, 13 or 14 of TGFBI; however, two mutations in the TGFBI protein at Arg 124 and Arg 555 appear to be hotspots and account for >50% of all known TGBFI mutations. In our study, a nucleotide 418 C>T mutation in exon 4 of TGFBI (R124C) was identified and cosegregated with phenotype in all affected individuals and it was absent in unaffected individuals. This mutation leads to an amino acid substitution at codon 124 (R124C) (Figure 3). This pedigree is another example of classical Arg124Cyc TGFBI mutation reported in lattice corneal dystrophy according to International Committee Classification of Corneal Dystrophy (IC3D) [3].

In a broader sense, we also considered population-type questions, such as whether the same mutations of TGFBI gene share the same ancestry (co-ancestry), in other words they are transmitted by lineage, or they are independent spontaneous mutations. In order to address this question, haplotype analysis would have to be done with the families that shared the same mutations, but that would be beyond the scope of our study. In the meantime, we ask a more simple question of what is the geographic distributions of reported TGFBI mutations (including the present study) in the Chinese population. Interestingly, the profile of reported TGFBI mutations varies geographically and these mutations are associated with multiple subtypes of corneal dystrophy. It appears that patients from the south part of China tend to have TGFBI mutation at the 3'-end of the gene, while patients from the north part of China frequently have mutations at the 5'-end (exon 4) of TGFBI gene.

Patient origin	TGFBI mutations in corneal dystrophy			Subtype	Reference
	Gene/Exon	Nucleotide	Codon		
China	4	418 G>A	A124H	RBCD	[9] [10]
	12	1664G>C	A555G		
Taiwan	4	418G>T	R124C	LCD1	[11]
	4	418G>A	R124H	GCD2	
	12	1646G>C	A546D	vLCD	
	12	1664G>A	R555Q	TBCD	
	12	1664G>T	R555W	GCD1	
Korea	12	1580T>G	L527R	LCD 4	[12] [13]
	12	1620T>C	F540F		
	12	1664G>A	R555Q	TBCD	
	4	371G>A	R124H	GCD2	
	4	370C>T	R124C	LCD1	
	12	1580T>G	L527R	LCD2	
	12	1625C>G	P542R	LCD2	
Poland	4	417C>T	R124C	LCD1	[14] [15]
	4	418G>A	R124H	GCD2	
	12	1711G>A	R555Q	TBCD	
	12	1710C>T	R555W	GCD1	
	14	1924A>G	H626R	vLCD	
	13	1694T>C	L565P	LCD	
Japan			P501T	LCD 3	[16]
Mexico	4	337G>A	V113I	aGCD	[17]
	11	1504A>G	M502V	unknown	
	12	1637C>A	A546D	PCA	
	12	1649T>C	L550P	GCD2	
	12	1663C>T	R555W	GCD1	
	14	1877A>G	H626R	LCD	
America	14	1877A>G	H626R	LCD	[18]
New Zealand	12	1664C>T	R555W	GCD1	[19]
	14	1877A>C	H626P	CDB	
Middle East	4	418G>A	R124H	ACD	[20]
Italy	14	1892G>T	V631N	LCD	[21]

Table 3: Review of geographic origins of TGFBI mutations in corneal dystrophy.

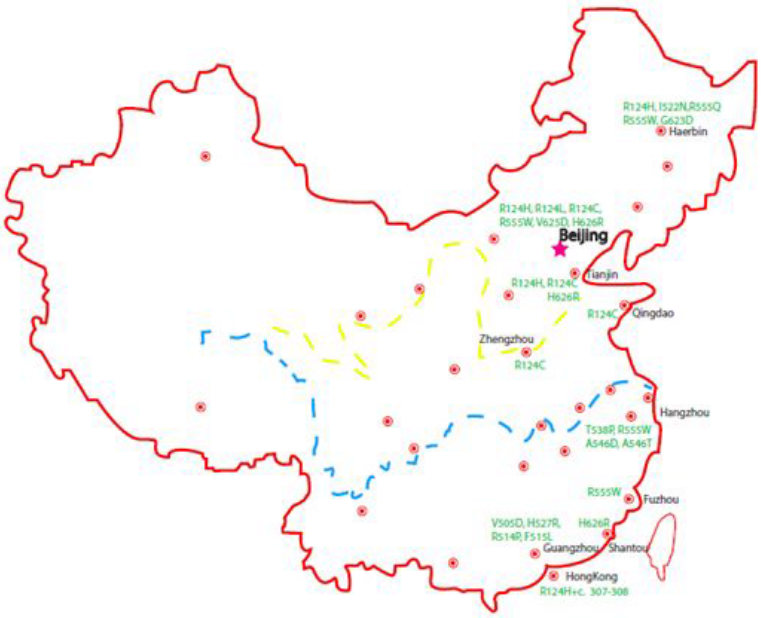

Figure 3: Geographic distribution of TGFBI mutations in Chinese population. The spectrum of TGFBI mutations, as reported in literature [6,9,10, 22,23], is depicted on the geographical map. Yellow River, yellow dashed line; Yangchi River, blue dashed line.

How mutations in TGBFI mutations cause corneal dystrophy is still unknown. Several studies indicate various TGFBI mutations change the structural conformation of the TGFBI protein and disrupt protein-protein interaction, particularly the function of cell adhesion [7,8]. For example, the R555W mutant of the fourth fasciclin 1 (FAS1-4) domain of the protein (TGFBIp/keratoepithelin/βig-h3), associated with granular corneal dystrophy type 1, is significantly less susceptible to proteolysis by thermolysin and trypsin than the normal or wildtype domain of the protein. Therefore, a functional hypothesis is that the R555W mutation may disrupt the normal degradation and turnover of the corneal TGFBI protein, leading to its accumulation and increased propensity to aggregate through electrostatic interactions [8]. In our pedigree, the initial diagnosis was based on phenotypic similarity to Meesmann epithelial corneal dystrophy, but it was without a KRT3 or KRT12 mutation [1]. Our discovery of the same TGFBI gene mutation (A124C) in all affected patients caused us to re-evaluate the clinical diagnosis that suggested GCD or LCD types of corneal dystrophy.

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