Genetics

Association of *TCF4* Gene Polymorphisms with Fuchs' Corneal Dystrophy in the Chinese

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Purpose. To test the association between *TCF4*, a gene recently found to confer susceptibility to Fuchs' corneal dystrophy (FCD) in Caucasian populations, and Chinese patients with FCD.

Methods. Fifty-seven Chinese subjects with clinically diagnosed FCD and 121 normal control subjects were recruited. Genomic DNA was extracted and the 18 single nucleotide polymorphisms (SNPs) within *TCF4* were genotyped (Sequenom MassArray primer extension system; Sequenom, Inc., San Diego, CA). Statistical association between individual SNPs and FCD was evaluated using 1 *df* additive genetic models, and verified with 2 *df* unguided genotype tests of association. $P <$ 0.002 was considered statistically significant after accounting for the 18 SNPs.

Results. The affected individuals ranged in age from 48 to 87 years, with an average age of 67 years. There was no statistical difference in the demographic information between the FCD and the control group (mean age of 65.1 years; range, 39–85, $P = 0.12$). Two SNPs within *TCF4* (rs17089887 and rs17089925) were significant experiment-wide ($P = 7.34 \times 10^{-5}$ and $P = 0.00045$ respectively) with an increase in disease risk of >2.3-fold per copy of the risk allele compared with individuals who were wild type. However, the most significantly associated SNP from the original report (rs613872) was not found to be present in Chinese FCD subjects.

Conclusions. Polymorphisms within *TCF4*, a gene which has been implicated in FCD susceptibility among Europeans, was also found to be strongly associated with FCD in Chinese. (*Invest Ophthalmol Vis Sci.* 2011;52:5573–5578) DOI:10.1167/iovs.11-7568

F uchs' corneal dystrophy (FCD) (MIM 136800) is a bilateral, often asymmetric, progressive disorder affecting the corneal endothelium[1] that afflicts approximately 4% of the population over the age of 40 years.[2] FCD is characterized by the development of guttae, excrescences of the Descemet membrane that typically appear in the fourth or the fifth decade of life and increase in number over time. From the time of onset, there is typically slow progression, caused by impaired endothelial dysfunction, eventually leading to stromal edema and impaired vision.[3]

Few studies have examined the prevalence of FCD on a large scale. Cross-sectional studies however suggest a relatively higher prevalence of the disease in European countries relative to other parts of the world including Asia. A population based study conducted in Reykjavik, Iceland revealed 11% of females and 7% of males with guttae.[4] Corneal guttae and FCD are rare in the Japanese population.[5] An examination of 107 patients with cataract in Japan revealed four (3.8%) with 'primary cornea guttata,' described as early signs of FCD.[6] However, FCD appears to be more common in Chinese compared with Japanese. A study that compared the prevalence of cornea guttata between Japan and Singapore found a significantly increased prevalence of disease in Singapore (5.5% vs. 8.5%). This was associated with a lower mean endothelial cell count among the affected individuals in Singapore compare with the Japanese.[7] A Singaporean study also found 7.1% of the penetrating keratoplasty (PK) transplants to be secondary to FCD, further indicating that FCD is not as uncommon as previously thought among Chinese people.[8]

FCD is likely to be complex in etiology, with genetic as well as environmental factors playing a role in its causation. Although it has often been described in the literature as an autosomal dominant inherited condition,[9–11] the precise mode of inheritance for most typical cases remain unknown. FCD may be classified into an early-onset variant (familial FCD) or a late-onset (classic) variant, the latter may be further subclassified into late-onset familial FCD or the more common late-onset sporadic FCD.[12] The much rarer early onset variant has been found to be caused by mutations in the *COL8A2* gene.[13,14] This gene encodes the α2 subtype of collagen VIII which is a major component of Descemet's membrane, and has recently been shown to strongly associate with central corneal thickness.[15] Linkage studies have identified several chromosomal loci associated with common late-onset familial FCD, including chromosomes 5q33.1–q35.2, 9p22.1–9p24.1, 13ptel–13q12.13, and 18q21.2–q21.32.[16–19] In addition, rare missense mutations in genes *SLC4A11* and *TCF8,* associated with congenital hereditary endothelial dystrophy and posterior polymorphous corneal dystrophy, respectively, have also been identified in late onset FCD cases.[19–21]

Very recently, a genome-wide association study conducted in 130 European FCD cases and 260 matched control subjects found that common alleles at *TCF4* contribute substantively to the risk of FCD.[22] Here, we conducted an independent validation study reassessing the role of *TCF4* in 57 unrelated patients with late-onset FCD of Chinese ethnicity in Singapore and show that at least 2 variants in *TCF4* are independently associated with FCD at an experiment-wide significance level. The

single nucleotide polymorphism (SNP) rs613872, most highly associated with FCD in Caucasians, was found to be nonpolymorphic in the Chinese.

METHODS

Ascertainment of Cases and Control Subjects

The study protocol had the approval of the institutional review board of Singapore National Eye Centre and was in compliance with the tenets of the Declaration of Helsinki. All patients underwent complete ophthalmic examination including funduscopy and slit lamp examination and confocal specular microscopy. The diagnosis of FCD was based on the presence of greater than 5 mm of confluent central corneal endothelial guttae in each eye (Krachmer grade 4 or higher)[2] or histopathologically confirmed FCD after performing corneal transplantation. A detailed history was recorded for all subjects, including any family history and duration of onset of symptoms. After the nature and possible consequences of study enrollment were explained to each patient, written informed consent to participate in the research was obtained from each study participant. Chinese subjects with no corneal guttae (Krachmer grade 0) on slit lamp examination and confocal specular microscopy were recruited as control subjects. We excluded individuals in whom the corneal endothelium could not be evaluated.

Genotyping

Genomic DNA was extracted from leukocytes of the peripheral blood (Nucleon Blood Extraction Kit, Amersham, Biosciences, Buckinghamshire, UK). We genotyped 18 single nucleotide polymorphisms (SNPs) across *TCF4*, selected based on Chinese Hapmap data (Sequenom MassArray iPLEX primer extension system; www.sequenom.com; Sequenom, Inc., San Diego, CA). In SNP selection, we also considered the *TCF4* gene regions from which the original association signals were observed in the European population,[22] and chose SNPs appropriate for a Chinese population (Table 1).T1

Statistical Analysis

Logistic regression (1 *df*) trend and unguided (2 *df*) genotype tests were used to model statistical associations of all the SNPs with FCD,

adjusted for age and sex. Conditional analysis using logistic regression was also done to examine the independent effect of an SNP conditional on another SNP. Hardy-Weinberg equilibrium of the genotypic frequencies among cases and separately among the controls was also examined. Haplotype association tests for the joint effect of all the haplotypes and haplotype-specific effects were performed. All the afore-mentioned analyses were performed using the package PLINK.[23] Haploview[24] was used to compute the linkage disequilibrium (LD) statistics and the LD plot. Single SNP *P* value <0.002 was considered statistically significant after correcting for 18 independent tests.

To understand the discriminatory power of the SNPs within the gene *TCF4*, we used logistic regression as the model for classification. Area under the receiver operating characteristic curve (AUC), sensitivity, and specificity were used to identify the best subset of SNPs for classification. The R[25] package Epi[26] was used to perform the classification analysis based on logistic regression.

RESULTS

Demographics of Subjects

Fifty-seven unrelated patients with a diagnosis of late onset FCD and 121 normal control subjects were enrolled into the study. The affected probands ranged in age from 48 to 87 years, with an average age of 67 years. There was no statistical difference in the demographic information between the FCD and the control group (mean age of 65.1 years; range, 39–85; *P* = 0.12). There were 45 females and 12 males among the FCD cases. There were 70 females and 51 males within the control group. All the patients and control subjects were Chinese. Twelve of the FCD patients had a family history of FCD and the remaining 45 cases were 'late-onset' sporadic FCD.

Single Nucleotide Polymorphism (SNP) Association Analysis

Characteristics of the entire set of SNPs genotyped in this study are shown in Table 1. The allelic and genotype frequencies of the most significant SNPs within *TCF4* investigated in Chinese patients with FCD are shown in Table 2. The genotype fre-

TABLE 1. Characteristics of Genotyped SNPs within *TCF4*

SNP ID	Position†	Position	Allele	Freq* Cases	Freq* Controls	OR‡	P‡
rs7235583	53747212	Upstream	A/C	0.07018	0.08678	0.808	0.6131
rs1945737	53744545	Upstream	C/T	0.4035	0.3967	0.982	0.9369
rs17806802	53718138	Upstream	A/G§	NA	NA	NA	NA
rs2286812	53717464	Upstream	C/T	0.1404	0.125	1.136	0.6964
rs1477440	53696010	Upstream	A/G	0.5263	0.4298	1.423	0.1468
rs17089925	53400834	Upstream	C/T	0.6228	0.4174	2.381	0.0004
rs2123392	53214865	Intron 3	C/T	0.2719	0.4000	0.469	0.0047
rs613872	53210302	Intron 3	G/T§	NA	NA	NA	NA
rs17089887	53208256	Intron 3	C/T	0.6491	0.4504	2.567	0.0003
rs1319637	53207325	Intron 3	A/G	0.0351	0.0785	0.420	0.1439
rs1452787	53207207	Intron 3	A/G	0.2719	0.3926	0.479	0.006
rs2123389	53201972	Intron 3	A/G	0.0351	0.0703	0.575	0.336
rs7233312	53143573	Intron 3	C/T	0.3333	0.4008	0.692	0.127
rs17089826	53126993	Intron 5	C/T	0.0088	0.00413	4.763	0.2836
rs2919450	53084545	Intron 5	A/C	0.6228	0.4959	0.630	0.03985
rs17594358	53050489	Intron 6	A/G§	NA	NA	NA	NA
rs1348047	53050058	Intron 6	G/T	0.6140	0.4339	1.993	0.0046
rs17594301	53048885	Intron 6	C/T§	NA	NA	NA	NA

Most significant SNPs within *TCF4* are highlighted in bold. NA, not applicable; OR, odds ratio.
* Frequencies corresponding to minor allele in controls are shown.
† SNP position is based on Genome Build: 37.1.
‡ *P* values and OR after adjusting for age and sex using logistic regression.
§ SNPs that were nonpolymorphic in Chinese.

TABLE 2. Most Significant SNPs within *TCF4* Investigated in Chinese Patients with Fuchs' Corneal Dystrophy

SNP ID		Allele Distribution (%)		Trend Test (P)	Per Allele Odds Ratio (95% CI)	Genotype Distribution (%)			Unguided 2 df Genotype P
		Cases	Controls				Cases	Controls	
rs1348047	G	70 (0.614)	105 (0.434)	0.0046	1.99 (1.24–3.21)	GG*	23 (0.403)	24 (0.198)	0.01408
	T	44 (0.386)	137 (0.566)			GT	24 (0.421)	57 (0.471)	
						TT	10 (0.175)	40 (0.331)	
rs1452787	G	31 (0.272)	95 (0.393)	0.006	0.48 (0.28–0.81)	GG†	8 (0.140)	20 (0.165)	0.0042
	A	83 (0.728)	147 (0.607)			GA	15 (0.263)	55 (0.454)	
						AA	34 (0.596)	46 (0.380)	
rs17089887	C	74 (0.649)	109 (0.450)	0.0003	2.57 (1.54–4.29)	CC*	28 (0.491)	22 (0.182)	7.34E-05
	T	40 (0.351)	133 (0.550)			TC	18 (0.316)	65 (0.537)	
						TT	11 (0.193)	34 (0.281)	
rs17089925	T	71 (0.623)	101 (0.417)	0.0004	2.38 (1.47–3.85)	TT*	26 (0.456)	22 (0.182)	0.00045
	C	43 (0.377)	141 (0.583)			TC	19 (0.333)	57 (0.471)	
						CC	12 (0.210)	42 (0.347)	
rs2123392	C	31 (0.272)	96 (0.400)	0.0047	0.47 (0.28–0.79)	CC†	8 (0.140)	20 (0.167)	0.00316
	T	83 (0.728)	144 (0.600)			CT	15 (0.263)	56 (0.467)	
						TT	34 (0.596)	44 (0.367)	

Cases, $n = 57$; control subjects, $n = 121$. Primary analysis was conducted using the trend test and accompanying per allele odds ratio.

$P < 0.002$ was considered statistically significant after accounting for the 18 SNPs. The unguided 2 df genotype tests was performed to verify that no significant departure occurred from the trend assumption. All P values and odds ratios shown are adjusted for age and sex. CI, confidence interval.

* Homozygous for risk allele.

† Homozygous for protective allele.

quencies of the controls and cases are in keeping with Hardy-Weinberg equilibrium. Single-locus analysis conducted on the 18 SNPs within *TCF4* revealed four of them to be nonpolymorphic in Chinese. This included the most significantly associated SNP from the original report (rs613872).[22] After accounting for the testing of 18 SNPs, only association signals at rs17089887 and rs17089925 remained significant experiment-wide (genotype $P = 7.34 \times 10^{-5}$ and $P = 0.00045$, respectively; Table 2). Each copy of the C allele of rs17089887 and the T allele of rs17089925 conferred a 2.57- (95% CI, 1.54–4.29) and 2.38-fold (95% CI, 1.47–3.85) increased risk of FCD, respectively.

Linkage Disequilibrium and Haplotype Association Analysis

The LD plot (Fig. 1) shows three blocks within *TCF4* based on the 14 typed SNPs; block 1 (rs1348047 and rs2919450), block 2 (rs2123389 to rs17089925), and block3 (rs2286812 to rs7235583). The association signals arose from blocks 1 and 2 only. Baratz et al.[22] also showed extensive LD between individual SNPs across the entire *TCF4* gene for Caucasian samples, using imputed genotypes for 720 SNPs across the chromosome 18 locus. According to the position and identity of SNPs within LD blocks, blocks 1 and 2 here may correspond with blocks 7 to 8 and 17 of the Caucasian study, respectively. However, as the set of SNPs used for LD plots in these two studies is different, any comparison of LD patterns between Caucasians and Chinese need to be interpreted with caution. To examine the joint association of SNPs within these blocks, haplotype association tests were performed. The P values for global tests of haplotype associations for block 1, 2, and 3, respectively, are 0.0041, 0.0030, and 0.67. These associations are of similar magnitude to the association signals from blocks 7 to 8 and
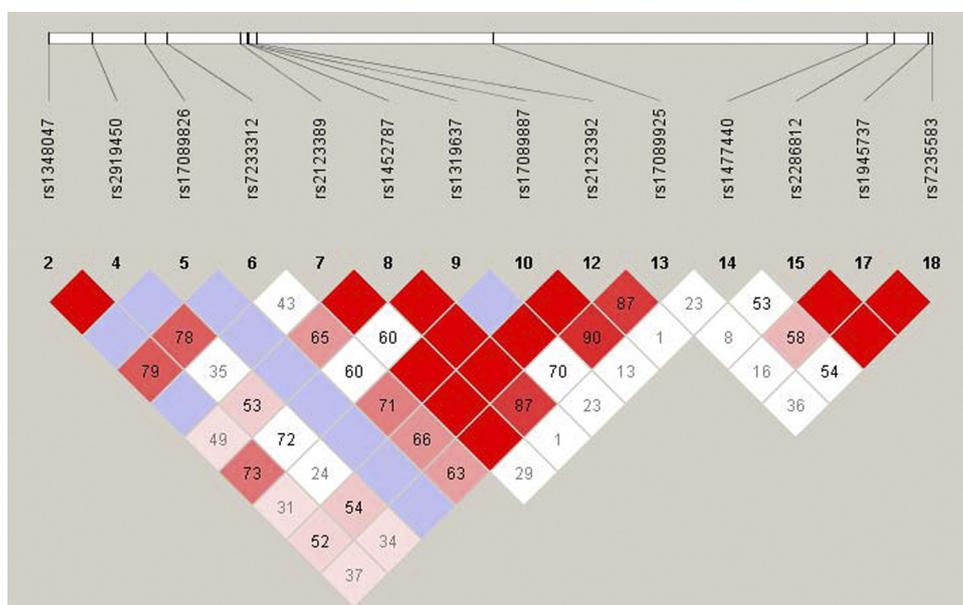

FIGURE 1. Plot of pairwise linkage disequilibrium (D prime) across 14 *TCF4* SNPs in the Chinese control subjects.

TABLE 3. Association between *TCF4* Variants and Fuchs' Corneal Dytrophy

| | Conditional on | | | | | | | |
| | rs1348047 | | rs17089887 | | rs17089925 | | rs2123392 | |
SNP	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)
rs1348047	NA	NA	0.008	1.93 (1.19–3.13)	0.012	1.82 (1.14–2.91)	0.0163	1.78 (1.11–2.86)
rs17089887	0.008	1.93 (1.19–3.13)	NA	NA	0.391	1.47 (0.61–3.52)	0.0136	2.70 (1.23–5.88)
rs17089925	0.012	1.82 (1.14–2.91)	0.391	1.47 (0.61–3.52)	NA	NA	0.014	2.14 (1.17–3.95)
rs2123392	0.0163	1.78 (1.11–2.86)	0.0136	0.37 (0.17–0.81)	0.014	2.14 (1.17–3.95)	NA	NA

block 17 reported in the Caucasian study.[22] Of note, the upstream haplotype block associations described in Baratz et al.[22] (in blocks 31 and 34), were not statistically significant ($P > 0.05$) in our study. We are mindful that the haplotype-based association tests did not show evidence of association exceeding that observed by single SNP analysis, suggesting that single SNP analysis is sufficiently informative to account for the association signals observed in our study. In addition, no evidence for increased risk for any specific haplotype was observed based on haplotype specific association tests within these blocks (results not shown).

Conditional Analysis

Among the four associated SNPs, pairwise conditional logistic regression analysis was performed to identify the marginal independence of the association signals (Table 3). The SNP rs1348047 and rs2123392 are associated independently of the SNPs rs17089887 and rs17089925. The rs17089887 and

rs17089925 are highly correlated ($r^2 = 0.736$) and hence these two SNPs are not independently associated with the FCD.

Classification Analysis

The associated SNPs rs1348047 from block 1 and the SNPs rs17089887, rs2123392, and rs17089925 from block 2 were examined for their ability to classify FCD subjects from control samples. All possible subsets using these four SNPs were examined for the classification performance using sensitivity, specificity, and the AUC metrics. The best result was obtained for the combination of SNPs rs1348047 and SNPs rs17089887 which are from the two independent blocks within *TCF4*. The receiver operating characteristic (ROC) curve and the corresponding AUC values for the model with and without the covariate sex are given in Figure 2. The risk model distinguished between case and control subjects with 71% accuracy when sex was included in the model. Due to limited sample size, the analysis was restricted to the complete
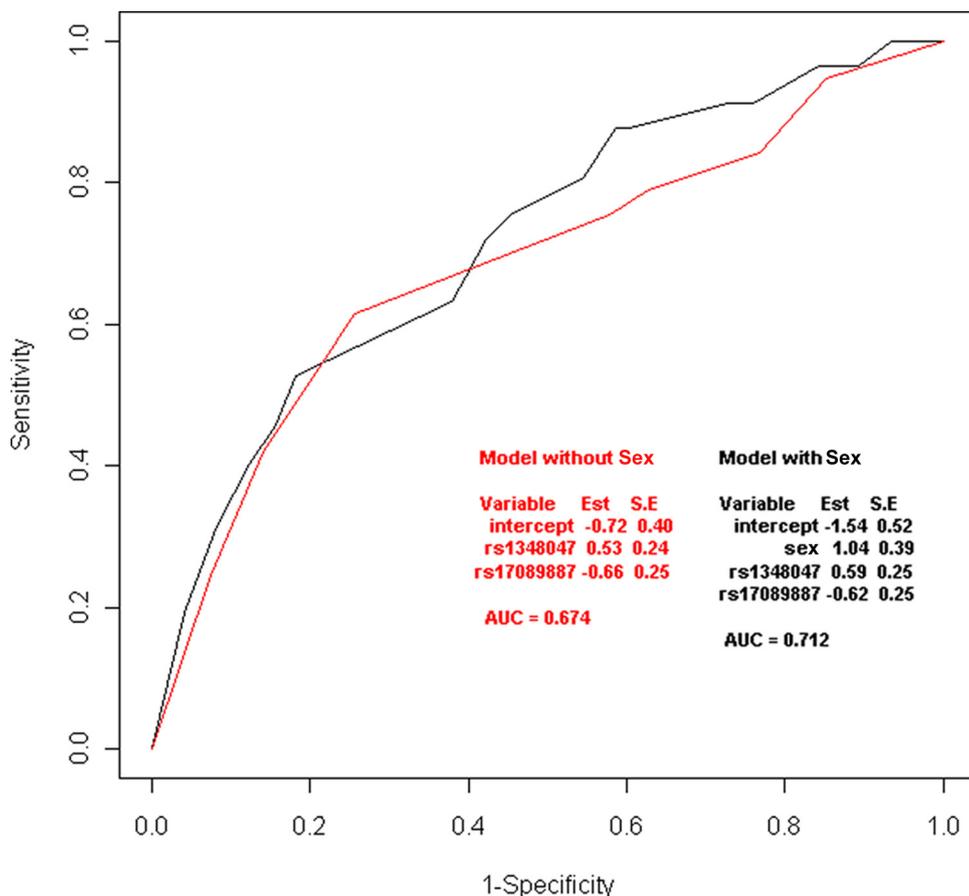

Model without Sex

Variable	Est	S.E
intercept	-0.72	0.40
rs1348047	0.53	0.24
rs17089887	-0.66	0.25

AUC = 0.674

Model with Sex

Variable	Est	S.E
intercept	-1.54	0.52
sex	1.04	0.39
rs1348047	0.59	0.25
rs17089887	-0.62	0.25

AUC = 0.712

FIGURE 2. Plot of ROC curves for classification models based on two independent *TCF4* SNPs. The ROC curves with and without the covariate sex are shown in *black* and *red* respectively. Beta coefficients of the two logistic regression models used for classification are shown.

samples and no test sets were used to evaluate classification accuracy.

DISCUSSION

The *TCF4* gene located on chromosome 18q21.1 is a large gene that spans across 366,861 bases of genomic DNA. It consists of 20 exons and encodes a 667-amino acid protein. We observed strong evidence of association between two *TCF4* SNPs and susceptibility to FCD in Singaporean Chinese. The index SNP reported in Europeans (rs613872) was found to not be present in individuals of Chinese descent, and instead, a SNP located just 2000 base pairs away (rs17089887) and another correlated SNP (rs17089925) showed strong association in Chinese. This is in keeping with the phenomenon that SNPs tagging a given functional causal variant may differ from one population to another, often attributed to differences in patterns of linkage disequilibrium. However in this case the tagging SNP rs613872 was simply not polymorphic in the Chinese and the association signal, in all likelihood arising from the same functional locus as in the Europeans, was detected via SNPs adjacent to rs613872. Similar to the original report, our study also indicated at least two regions of the *TCF4* locus, to be associated independently with FCD. A larger sample cohort may be required to give adequate power to improve the association with some of the independent SNPs that showed marginal association with FCD (Table 2). The odds ratios observed here in Chinese (>2.3 per copy of the risk allele) are consistent with that observed in Europeans (>4 per copy of the risk allele).[22] These findings clearly highlight the importance of *TCF4* as a genetic determinant of FCD susceptibility with global significance.

The magnitude of association observed both here and in the European studies are noteworthy and of substantial effect sizes (per allele OR >2-fold increase), reminiscent of that conferred by Complement Factor H polymorphisms and age-related macular degeneration.[27–29] In comparison, the vast majority of robustly replicated loci for other complex diseases only confer per allele ORs of <1.50.[30,31] These observations suggest *TCF4* to be a major susceptibility gene for FCD. The protein encoded by *TCF4*, referred to as E2-2, is a member of the ubiquitously expressed class 1 basic helix-loop-helix (bHLH) transcription factors that are involved in cellular growth and differentiation.[32] *TCF4* is expressed predominantly in pre-B-cells but expression has also been shown in adult heart, brain, placenta, skeletal muscle, and lung. *TCF4* expression has also been shown in the developing corneal endothelium of the mouse.[22] In man, haploinsufficiency of *TCF4* is a cause of Pitt-Hopkins syndrome (PHTS; MIM 610,954).[33] PHTS is a rare, autosomal dominant, syndromic encephalopathy characterized by severe psychomotor delay, epilepsy, daily bouts of diurnal hyperventilation starting in infancy, mild postnatal growth retardation, postnatal microcephaly, and distinctive facial features. It is unusual to have the same gene being associated with two such divergent phenotypes as PHTS and late onset FCD. However, several plausible hypotheses have been put forth for the molecular mechanisms underlying FCD associated with *TCF4* risk variants.[22] It has been suggested that the risk variants of *TCF4* may alter the expression of *TCF8*. *TCF4* is known to up regulate the expression of *TCF8*, missense variations of which are associated with late-onset FCD cases. The *TCF8* gene encodes a transcription factor that plays a critical role in embryonic development. Of particular significance are previous reports of *TCF8* gene playing a role in the regulation of type I collagen expression

and in the repression of the epithelial phenotype, which is critical in the maintenance of an endothelial phenotype.[34,35]

Our results, observed here in individuals of Chinese descent, strongly support data reported from previous European studies which show strong association between *TCF4* variants and FCD. Further study should now focus on the functional disease mutations responsible for the strong association signal to dissect the molecular mechanisms underlying FCD pathogenesis.

References

1. Adamis AP, Filatov V, Tripathi BJ, Tripathi RC. Fuchs' endothelial dystrophy of the cornea. *Surv Ophthalmol.* 1993;38:149–168.
2. Krachmer JH, Purcell JJ Jr, Young CW, Bucher KD. Corneal endothelial dystrophy. A Study of 64 families. *Arch Ophthalmol.* 1978; 96:2036–2039.
3. Waring GO, Bourne WM, Edelhauser HF, Kenyon KR. The corneal endothelium. Normal and pathologic structure and function. *Ophthlamology.* 1982;89:531–590.
4. Zoega GM, Fujisawa A, Sasaki H, et al. Prevalence and risk factors for cornea guttata in the Reykjavik Eye Study. *Ophthalmology.* 2006;113:565–569.
5. Hogan MJ, Wood I, Fine M. Fuch's endothelial dystrophy of the cornea: 29th Sanford Gifford Memorial Lecture. *Am J Ophthlamol.* 1974;78:363–383.
6. Nagaki Y, Hayasaka S, Kitagawa K, Yamamoto S. Primary cornea guttata in Japanese patients with cataract: specular microscopic observations. *Jpn J Ophthalmol.* 1996;40:520–525.
7. Kitagawa K, Kojima M, Sasaki H, et al. Prevalence of primary cornea guttata and morphology of corneal endothelium in aging Japanese and Singaporean subjects. *Ophthalmic Res.* 2002;34: 135–138.
8. Tan DT, Janardhanan P, Zhou H, et al. Penetrating keratoplasty in Asian eyes: the Singapore Corneal Transplant Study. *Ophthalmology.* 2008;115:975–982.
9. Cross HE, Maumenee AE, Cantolino SJ. Inheritance of Fuchs' endothelial dystrophy. *Arch Ophthalmol.* 1971;85:268–272.
10. Rosenblum P, Stark WJ, Maumenee IH, Hirst LW, Maumenee AE. Hereditary Fuchs' dystrophy. *Am J Ophthalmol.* 1980;90:455–462.
11. Magovern M, Beauchamp GR, Mc Tigue JW, et al. Inheritance of Fuchs' combined dystrophy. *Ophthalmology.* 1979;86:1897–1923.
12. Aldave AJ, Rayner SA, Salem AK, et al. No pathogenic mutations identified in the COL8A1 and COL8A2 genes in familial Fuchs corneal dystrophy. *Invest Ophthalmol Vis Sci.* 2006;47:3787–3790.
13. Gottsch JD, Sundin OH, Liu SH, et al. Inheritance of a novel COL8A2 mutation defines a distinct early-onset subtype of fuchs corneal dystrophy. *Invest Ophthalmol Vis Sci.* 2005;46:1934–1939.
14. Biswas S, Munier FL, Yardley J, et al. Missense mutations in COL8A2, the gene encoding the alpha2 chain of type VIII collagen, cause two forms of corneal endothelial dystrophy. *Hum Mol Genet.* 2001;10:2415–2423.
15. Vithana EN, Aung T, Khor CC, et al. Collagen-related genes influence the glaucoma risk factor, central corneal thickness. *Hum Mol Genet.* 2011;20:649–658.
16. Sundin OH, Jun AS, Broman KW, et al. Linkage of late-onset Fuchs corneal dystrophy to a novel locus at 13pTel-13q12.13. *Invest Ophthalmol Vis Sci.* 2006;47:140–145.
17. Sundin OH, Broman KW, Chang HH, et al. A common locus for late-onset Fuchs corneal dystrophy maps to 18q21.2-q21.32. *Invest Ophthalmol Vis Sci.* 2006;47:3919–3926.
18. Riazuddin SA, Eghrari AO, Al Saif A, et al. Linkage of a mild late-onset phenotype of Fuchs corneal dystrophy to a novel locus at 5q33.1-q35.2. *Invest Ophthalmol Vis Sci.* 2009;50: 5667–5671.
19. Riazuddin SA, Zaghloul NA, Al-Saif A, et al. Missense mutations in TCF8 cause late-onset Fuchs corneal dystrophy and interact with FCD4 on chromosome 9p. *Am J Hum Genet.* 2010;86:45–53.

20. Vithana EN, Morgan PE, Ramprasad V, et al. SLC4A11 mutations in Fuchs endothelial corneal dystrophy. *Hum Mol Genet.* 2008;17: 656–666.

21. Riazuddin SA, Vithana EN, Seet LF, et al. Missense mutations in the sodium borate cotransporter SLC4A11 cause late-onset Fuchs corneal dystrophy. *Hum Mutat.* 2010 Nov;31:1261–1268.

22. Baratz KH, Tosakulwong N, Ryu E, et al. E2–2 protein and Fuchs's corneal dystrophy. *N Engl J Med.* 2010;363:1016–1024.

23. Purcell S, Neale B, Todd-Brown K, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet.* 2007;81:559–575.

24. Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics.* 2005;21:263–265.

25. R Development Core Team. *R: A Language and Environment for Statistical Computing.* Vienna, Austria: R Foundation for Statistical Computing; 2008.

26. Carstensen B, Plummer M, Laara E, et al. Epi: a package for statistical analysis in epidemiology. R package version 1.0.8. Available at: http://www.pubhealth.ku.dk/~bxc/Epi/. Accessed January 31, 2011.

27. Klein RJ, Zeiss C, Chew EY, et al. Complement factor H polymorphism in age-related macular degeneration. *Science.* 2005;308:385–389.

28. Haines JL, Hauser MA, Schmidt S, et al. Complement factor H variant increases the risk of age-related macular degeneration. *Science.* 2005;308:419–421.

29. Edwards AO, Ritter R, Abel KJ, et al. Complement factor H polymorphism and age-related macular degeneration. *Science.* 2005; 308:421–424.

30. Thomas G, Jacobs KB, Kraft P, et al. A multistage genome-wide association study in breast cancer identifies two new risk alleles at 1p11.2 and 14q24.1 (RAD51L1). *Nat Genet.* 2009;41:579–584.

31. Purdue MP, Johansson M, Zelenika D, et al. Genome-wide association study of renal cell carcinoma identifies two susceptibility loci on 2p21 and 11q13.3. *Nat Genet.* 2011;43:60–65.

32. Murre C, Bain G, van Dijk MA, et al. Structure and function of helix-loop-helix proteins. *Biochim Biophys Acta.* 1994;1218:129–135.

33. Amiel J, Rio M, de Pontual L, et al. Mutations in TCF4, encoding a class I basic helix-loop-helix transcription factor, are responsible for Pitt-Hopkins syndrome, a severe epileptic encephalopathy associated with autonomic dysfunction. *Am J Hum Genet.* 2007;80: 988–993.

34. Terraz C, Toman D, Delauche M, Ronco P, Rossert J. Delta Ef1 binds to a far upstream sequence of the mouse pro-alpha 1(I) collagen gene and represses its expression in osteoblasts. *J Biol Chem.* 2001;276:37011–37019.

35. Frisch SM. E1a induces the expression of epithelial characteristics. *J Cell Biol.* 1994;127:1085–1096.