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Evaluating the association of VSX1 mutation with keratoconus and the granular corneal dystrophy in an Iranian family

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评价 VSX1 突变与圆锥角膜合并角膜颗粒状营养不良在伊朗家族中的关联

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摘要

目的:为了评估视觉系统同源框 1(VSX1)基因的突变和圆锥角膜(KCN)以及颗粒状角膜营养不良(GCD)之间的关联。

方法:对一个同时患有 KCN 和 GCD 的四代伊朗人家系进行了直接测序,鉴别出一个包含四代人同时患有 GCD 的伊朗 KCN 家系。从全血样品中提取基因组 DNA。然后,为了研究 KCN 和 GCD 之间可能的连锁关系,通过 PCR 在每个样品中扩增 VSX1 基因的整个编码区和内含子-外显子边界。随后,对 PCR 产物进行直接测序,并在患者和对照组中进行突变分析。

结果:VSX1 基因突变分析未发现 KCN 和 GCD 疾病与 VSX1 基因相关的证据。我们的数据排除了 VSX1 作为该特定家系中 KCN / GCD 致病基因的可能性。

结论:尽管患有 GCD 的 KCN 患者与 VSX1 基因变异无关联,但是仍需要对其它可能与 KCN 合并 GCD 发病机制相关的基因进行研究。

关键词:视觉系统同源框;圆锥角膜;颗粒状角膜营养不良症;伊朗家系; 聚合酶链反应;突变

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Abstract

• AIM: To evaluate association between mutations in the visual system homeobox 1 (VSX1) gene and keratoconus (KCN) complicated with granular corneal dystrophy (GCD), direct sequencing was performed in an Iranian family affected by KCN and GCD in four generations.

• METHODS: An Iranian pedigree with keratoconus spanning four generations along with GCD was identified. Whole blood sample was used for genomic DNA extraction. The molecular analysis by using polymerase chain reaction (PCR) of the entire coding region and intron-exon boundaries of VSX1 gene was preformed to investigate the possible linkage between KCN and GCD. Subsequently, direct sequencing was used for PCR products and mutation analysis was conducted in the patients and controls.

• RESULTS: Mutation analysis in VSX1 gene did not detect evidence for association between KCN and GCD diseases and VSX1 gene. Our data excluded VSX1 as the disease-causing gene for KCN/GCD in this specific pedigree.

• CONCLUSION: Despite of no association between KCN patients with GCD and VSX1 gene variations, other probable genes involved in pathogenesis of the KCN and GCD diseases need to be investigated in the patients.

• KEYWORDS: granular corneal dystrophy; Iranian family; keratoconus; mutation; polymerase chain reaction; visual system homeobox

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INTRODUCTION

Keratoconus (KCN) is a bilateral, non-inflammatory, slowly progressive, corneal ectasia characterized disease with corneal thinning and myopia and irregular astigmatism disease that

leads to vision impairment. The incidence of this disorder is estimated about 1 in 500 - 2000 individuals in the general population and its prevalence is about 54.5 per 100000[1]. The prevalence and incidence rates of KCN in Asians are higher than Caucasians[2-3]. Based on the results of various studies, the prevalence of KCN in first-degree relatives is significantly higher than the general population[2-3]. Six to eight percentage of patients with KCN show a positive family history, and also it has been shown the concordance of this disorder is high among monozygotic twins[4-5].

The pathogenetic process related to KCN is still unknown; however, most of scientists do not have any doubt about the role of genetic background in this disorder. It has been shown that KCN is a heterogenic inherited disease[6] and an autosomal dominant inheritance pattern has been described for eight different types of it[7]. To date, many different chromosomal loci as well as genes such as visual system homeobox 1 (VSX1) and superoxide dismutase (SOD1) have been implicated in KCN pathogenesis[8-9]. The VSX1 gene is pathogenic in type Ⅰ of the disease [10].

Granular corneal dystrophy (GCD), an IC3D category 1 dystrophy, is a bilateral, noninflammatory condition that results in deposition of discrete, irregularly shaped opacities in the cornea by adulthood. It specifically affects the middle portion of the cornea (stroma) and can eventually cause decreased vision and eye discomfort. The inheritance patterns of GCD include autosomal dominant, autosomal recessive and x-linked[11].

It has been suggested that there may be a genetic linkage between keratoconus and granular dystrophy based on the evidences that show the presence of these disorders in two generations[12]. Molecular studies in two families affected by keratoconus demonstrated two distinct heterozygous mutations in the VSX1 gene[10]. Moreover, the major problem in linkage analysis of these disorders is the lack of multiple familial cases. In this study, we conducted mutation analysis of the entire coding region and intron-exon boundaries of VSX1 gene in an Iranian family with combined keratoconus and granular corneal dystrophy. To confirm the association of the identified VSX1 mutation with KCN and GCD, we performed a genetic analysis in affected family members. To our knowledge, this is the first report of an association study between VSX1 gene mutation and KCN and GCD in Iranian patients.

SUBJECTS AND METHODS

Ophthalmological Examination This study was approved by the local Institutional Review Board (IRB) and informed consent was obtained from all affected individuals. An Iranian pedigree was recognized with keratoconus spanning four generations along with GCD. Initially the patients were under genetic counseling and their family pedigree was drawn (Figure 1). Ophthalmological and slit lamp examination were used for all family members. Corneal topography and central or paracentral corneal thinning or conical protrusion of the cornea with or without stromal scar tissue, or both was used for KCN diagnosis. Corneal imaging with Pentacam (Oculus, Wetzlar, Germany) showed a typical characteristic pattern of KCN. Although corneal topography showed abnormalities such as inferior-superior asymmetry, skewing of the steepest radial axes above and below the horizontal meridian, differences between right and left central corneal power, and central steepening but the slit lamp examination did not show any signs of KCN in some patients[13]. In these patients, forme fruste diagnosis or latent keratoconus was established. Furthermore, white, dot-like opacities were noted in the central cornea of affected cases along with clear intervening areas but the limbus was spared, which is the typical features of granular corneal dystrophy (GCD).

Molecular Genetic Studies As shown in the pedigree, in the four generations there are 16 patients (8 females and 8 males) affected by KCN and GCD. Test tubes containing 0.5mL EDTA were used for peripheral blood (5mL) collection. A QIAamp DNA mini kit (Qiagen, Hilden, Germany) was used for DNA extraction. PCR with specific primers (Table 1) was used for amplification of all coding exons and intron/exon boundaries areas of the gene. The final volume of mixture for each reaction was 25-μL which contained 0.2μM each primer, 0.5U DNA polymerase (RedTaq, Sigma-Aldrich, Germany), 2.5μL 10× PCR buffer with 2.5mM $MgCl_2$, and approximately 100ng genomic DNA. The temperature-time series of 30 cycles of PCR were 94℃-30s, 60-61℃-30s, and 72℃-60s for denaturation, annealing, and extension processes. Five minutes extension temperature was preformed after 30 cycles. BigDye terminator (Applied Biosystems, USA) and the ABI PRISM 377 genetic sequencer were used for DNA sequencing of all PCR products.

After sequencing, the nucleotide sequences were extracted and compared with Homo sapiens VSX1 gene (NC_000020.11).

RESULTS

Figure 1 shows a simplified pedigree of studied family. As shown in this diagram, 8 males and also 8 females in 4 generations are suffering from KCN and GCD simultaneously. According to this pedigree, the inheritance of simultaneous presence of KCN and GCN seems to be autosomal dominant. After comparing sequences with the RefSeq record (NC_000020.11), result showed no pathogenic mutation in the VSX1 gene.

Even though there was no definite pathogenic mutation in the VSX1 in this family, there are a number of gene variants that had been detected by gene sequencing of the VSX1 gene (Table 2).

DISCUSSION

A clinical and molecular investigation of Iranian family members suffered from simultaneous KCN and GCD has been reported in this article. This is the first report that investigated the VSX1

Table 1 Primer Sequence used in this study

Exon	Primer Sequence	Tm (℃)	Product size (bp)
1	F: 5'-AGGTGACTGAGGGGACTGC-3' R: 5'-TGGAGATGGTTGTTGAAGACC-3'	61	950
2-3	F: 5'-CCCAAGAGGTTCATAACTTCAATC-3' R: 5'-AAAAGGCATGAGGGTCATAGG-3'	60	961
4	F: 5'-GATCATGCTCGGGAGAGAAG-3' R: 5'-TCTGTGAAAAATGAGGCAACC-3'	60	489
5	F: 5'-CCCCAGAGATAGGCACTGAC-3' R: 5'-TCCCTGCTCAAGCTACAAAAG-3'	60	688

TM: Melting point temperature; bp: Base pair.

Table 2 VSX1 mutations reported in other studies

Reference sequence	SNP ID	c. DNA change	Aminoacide change	Reference
NM-014588	rs12480307	c.546A>G	p. A182A	Dehkordi *et al*[14]
NM-199452	rs6138482	c.650G>A	p. R217H	
NT-011387.8	-	-	p. H244R	
-	-	c.715T>A	p. S251T	Shetty *et al*[15]
-	-	c.803T>A	p. L268H	
NM-014588	rs12480307	c.546A>G	p. A182A	Verma *et al*[16]
NM-014588	rs6138482	c.627+23G>A	-	
NM-014588	rs56157240	c.627+84T>A		
NM-014588	IVS3-24C	c.504-24C>T		
NM-014588.5	rs74315436	c.50T>C	p. L17P	Semina *et al*[17]
NM-014588.5	rs74315433	c.479G>A	p. G160D	
NM-014588.5	rs369865672	c.173C>T	p. P58L	Jeoung *et al*[18]
NM_014588.5	rs74315433	c.479G>T	p. G160V	
NM-014588.5	rs74315434	c.475T>A	p. L159M	Czugala *et al*[19]
NM-014588.5	rs74315432	c.496C>T	p. R166 W	Saee-Rad *et al*[9]

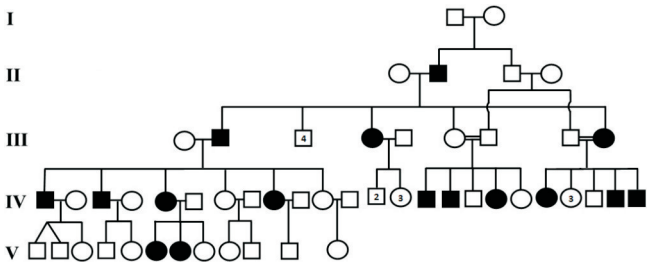

Figure 1 Family tree. Shading: combined granular dystrophy and keratoconus; A circle or a square with a number on the inside: Multiple individuals of each sex; Double lines: Consanguineous mating.

mutation in a family during 4 generations. The phenotype mainly was characterized by slowly progressive visual impairment and ocular symptoms, such as photophobia and tearing. Family members showed widely various onset age, clinical presentation, and disease progression. In this family, 16 patients were evaluated by both slit lamp examination and video keratography examination.

The gene frequency of autosomal dominant keratoconus is not known[20]; on the other hand, the incidence of sporadic cases is about 1 per 2000 in the general population which is more than families with clear Mendelian inheritance[1]. Although the majority of patients with KCN have a sporadic form of the disease, but there are some evidences that shows the involvement of genetic factors in incidence of KCN. Based on previous prospective studies, it has been revealed that the prevalence of undiagnosed KCN is high in relatives of KCN patients. KCN will be assumed as a familial disease instead of sporadic one when it has been diagnosed in 11% - 14% of apparently unaffected relatives due to complete slit - lamp examination, refraction, and corneal topography[21-22].

It has been shown that KCN has autosomal dominant inheritance[20-23] with reduced penetrance in 90% of pedigrees with familial KCN[24-25]. It is notable that autosomal recessive mode of inheritance has been described in families with consanguineous marriage[26-27]. In the family evaluated in this study, all patients have an affected parent, except in generation III family No. 4 who are disease-free but three of their sons/daughters are affected. Therefore, the family pedigree in this study shows a clear autosomal dominant inheritance pattern with reduced penetrance.

Multiple gene involvement theory in the development and progression of KCN has been supported by pervious genetics studies on this disease. As an example, no mutations have been identified for the KCN loci such as 5q32-q33, 5q21.2,

14q11.2, and 15q2.32[28] which were candidate regions to studying the mutations for this disorder. On the other hand, other reports show mutations in various genes such as VSX1 (KCN1, MIM605020, locus 20p11.2) and SOD1 (MIM 147450, locus 21q22.11) have an enrollment in the etiology of KCN. However, the role of these changes has not confirmed by other subsequent studies[14,29]. Various genes such as COL6A1, COL8A1, MMP9, and MMP2, which were candidate genes for KCN, have been examined and excluded as causative genes[25].

Due to expression of VSX1 in the retina, it was thought this gene plays a role in retinal bipolar interneurons development[30-31]. Based on pervious researches, the role of VSX1 gene was analyzed in corneal dystrophy[32]. Posterior polymorphous corneal dystrophy (PPCD) is a type of this disease which inherited as an autosomal dominant with extremely variable expression[33]. Although the implication of VSX1 gene has been proved, its involvement is questionable in PPCD yet[33]. Heon *et al*[10] (2002) introduced two mutations in VSX1 (p. Leu159Met and p. Gly160Asp) which are involved in PPCD and KCN, but linkage analysis and sequencing of VSX1 gene in two Czech families exclude the role of this gene in PPCD[34].

According to the possible role of VSX1 in PPCD and KCN, we tried to find a particular function for VSX1 mutation in association of KCN and GCD. Not only we did not investigate a specific mutation in VSX1 gene in this family, but also no pathogenic mutations have been detected in the coding sequences and exon-intron boundaries of VSX1 related to GCD and KCN. In conclusion, this study excluded VSX1 as the disease-causing gene for KCN/GCD in this specific pedigree. Considering other possible genes involved in pathogenesis of the KCN and GCD disease is recommended in this family.

REFERENCES

1 Gajecka M, Radhakrishna U, Winters D, Nath SK, Rydzanicz M, Ratnamala U, Ewing K, Molinari A, Pitarque JA, Lee K, Leal SM, Bejjani BA. Localization of a gene for keratoconus to a 5.6-Mb interval on 13q32. *Invest Ophthalmol Vis Sci* 2009;50(4):1531-1539

2 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

3 Vazirani J, Basu S. Keratoconus: current perspectives. *Clin Ophthalmol* 2013;7:2019-2030

4 Parker J, Ko WW, Pavlopoulos G, Wolfe PJ, Rabinowitz YS, Feldman ST. Videokeratography of keratoconus in monozygotic twins. *J Refract Surg* 1996;12(1):180-183

5 Rabinowitz YS. Keratoconus. *Surv Ophthalmol* 1998;42(4):297-319

6 Gordon-Shaag A, Millodot M, Shneor E. The Epidemiology and Etiology of Keratoconus. *International Journal of Keratoconus and Ectatic Corneal Diseases* 2012;1(1):7-15

7 Nielsen K, Hjortdal J, Pihlmann M, Corydon TJ. Update on the keratoconus genetics. *Acta Ophthalmol* 2013;91(2):106-113

8 Moschos MMI, Kokolakis N, Gazouli M, Chatziralli IP, Droutsas D, Anagnou NP, Ladas ID. Polymorphism Analysis of VSX1 and SOD1 Genes in Greek Patients with Keratoconus. *Ophthalmic Genet* 2015;36(3):213-217

9 Saee-Rad S, Hashemi H, Miraftab M, Noori-Daloii MR, Chaleshtori MH, Raoofian R, Jafari F, Greene W, Fakhraie G, Rezvan F, Heidari M. Mutation analysis of VSX1 and SOD1 in Iranian patients with keratoconus. *Mol Vis* 2011;17:3128-3136

10 Héon E, Greenberg A, Kopp KK, Rootman D, Vincent AL, Billingsley G, Priston M, Dorval KM, Chow RL, McInnes RR, Heathcote G, Westall C, Sutphin JE, Semina E, Bremner R, Stone EM. VSX1: a gene for posterior polymorphous dystrophy and keratoconus. *Hum Mol Genet* 2002;11(9):1029-1036

11 Vincent AL. Corneal dystrophies and genetics in the International Committee for Classification of Corneal Dystrophies era: a review. *Clin Experiment Ophthalmol* 2014;42(1):4-12

12 Jadidi K, Mosavi SA, Morovvati S. Familial case of keratoconus with corneal granular dystrophy in a family of Iranian origin. *J Clin Exp Ophthalmol* 2014;5:369

13 Rabinowitz YS. Videokeratographic indices to aid in screening for keratoconus. *J Refract Surg* 1995;11(5):371-379

14 Dehkordi FA, Rashki A, Bagheri N, Chaleshtori MH, Memarzadeh E, Salehi A, Ghatreh H, Zandi F, Yazdanpanahi N, Tabatabaiefar MA, Chaleshtori MH. Study of VSX1 mutations in patients with keratoconus in southwest Iran using PCR-single-strand conformation polymorphism/heteroduplex analysis and sequencing method. *Acta Cytol* 2013;57(6):646-651

15 Shetty R, Nuijts RM, Nanaiah SG, Anandula VR, Ghosh A, Jayadev C, Pahuja N, Kumaramanickavel G, Nallathambi J. Two novel missense substitutions in the VSX1 gene: clinical and genetic analysis of families with Keratoconus from India. *BMC Med Genet* 2015;16:33

16 Verma A, Das M, Srinivasan M, Prajna NV, Sundaresan P. Investigation of VSX1 sequence variants in South Indian patients with sporadic cases of keratoconus. *BMC Res Notes* 2013;6:103

17 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(2):289-293

18 Jeoung JW, Kim MK, Park SS, Kim SY, Ko HS, Wee WR, Lee JH. VSX1 gene and keratoconus: genetic analysis in Korean patients. *Cornea* 2012;31(7):746-750

19 Czugala M, Karolak JA, Nowak DM, Polakowski P, Pitarque J, Molinari A, Rydzanicz M, Bejjani BA, Yue BY, Szaflik JP, Gajecka M. Novel mutation and three other sequence variants segregating with phenotype at keratoconus 13q32 susceptibility locus. *Eur J Hum Genet* 2012;20(4):389-397

20 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(10):3160-3164

21 Karimian F, Aramesh S, Rabei HM, Javadi MA, Rafati N. Topographic evaluation of relatives of patients with keratoconus. *Cornea* 2008;27(8):874-878

22 Kaya V, Utine CA, Altunsoy M, Oral D, Yilmaz OF. Evaluation of corneal topography with Orbscan II in first-degree relatives of patients with keratoconus. *Cornea* 2008;27(5):531-534

23 Brancati F, Valente EM, Sarkozy A, Fehèr J, Castori M, Del Duca P, Mingarelli R, Pizzuti A, Dallapiccola B. A locus for autosomal

dominant keratoconus maps to human chromosome 3p14-q13. *J Med Genet* 2004;41(3):188-192

24 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(12):5063-5066

25 Nowak DM, Gajecka M. The genetics of keratoconus. *Middle East Afr J Ophthalmol* 2011;18(1):2-6

26 Gordon-Shaag A, Millodot M, Essa M, Garth J, Ghara M, Shneor E. Is consanguinity a risk factor for keratoconus? *Optom Vis Sci* 2013;90(5):448-454

27 Kirby D, Jackson AP, Karbani G, Crow YJ. Mental retardation, keratoconus, febrile seizures and sinoatrial block: a previously undescribed autosomal recessive disorder. *Clin Genet* 2005;67(5):448-449

28 Bisceglia L, De Bonis P, Pizzicoli C, Fischetti L, Laborante A, Di Perna M, Giuliani F, Delle Noci N, Buzzonetti L, Zelante L. Linkage analysis in keratoconus: replication of locus 5q21.2 and identification of other suggestive Loci. *Invest Ophthalmol Vis Sci* 2009;50(3):1081-1086

29 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(8):3345-3351

30 Hayashi T, Huang J, Deeb SS. Expression of rinx/vsx1 during postnatal eye development in cone-bipolar, differentiating ganglion, and lens fiber cells. *Jpn J Ophthalmol* 2005;49(2):93-105

31 Ohtoshi A, Wang SW, Maeda H, Saszik SM, Frishman LJ, Klein WH, Behringer RR. Regulation of retinal cone bipolar cell differentiation and photopic vision by the CVC homeobox gene Vsx1. *Curr Biol* 2004;14(6):530-536

32 Aldave AJ, Han J, Frausto R F. Genetics of the corneal endothelial dystrophies: an evidence-based review. *Clin Genet* 2013;84(2):109-119

33 Klintworth GK. Corneal dystrophies. *Orphanet J Rare Dis* 2009;4:7

34 Gwilliam R, Liskova P, Filipec M, Kmoch S, Jirsova K, Huckle EJ, Stables CL, Bhattacharya SS, Hardcastle AJ, Deloukas P, Ebenezer ND. Posterior polymorphous corneal dystrophy in Czech families maps to chromosome 20 and excludes the VSX1 gene. *Invest Ophthalmol Vis Sci* 2005;46(12):4480-4484