A study on the association between – 31T/C and – 511C/T polymorphisms in interleukin-1β gene in Egyptian patients with keratoconus

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Aim

To study the association of interleukin (IL) 1B promoter polymorphisms rs1143627 (–31T>C) and rs16944 (–511C>T) and the risk of keratoconus (KC) in the Egyptian population, establishing a biomarker for managing of KC cases and to give convenient genetic counseling.

Materials and methods

The association between – 31T/C and – 511C/T polymorphisms in the *IL1B* promoter and Egyptian KC patients were investigated. This study included 159 unrelated patients with affected cases and 159 healthy individuals for rs16944 (–511C>T) polymorphism, 144 blood samples that belong to unrelated affected cases, and 141 healthy individuals for rs1143627 (–31T>C) polymorphism. All cases had been examined in the Research Institute of Ophthalmology and written informed consents were obtained from all participating individuals. PCR–restriction fragment length polymorphism analyses were used for screening of rs1143627 (–31T>C) and rs16944 (–511 C>T) polymorphisms.

Results

The – 511 (rs16944) and –31 (rs1143627) polymorphisms in the promoter region of *IL1B* have been analyzed. The C/C genotype frequency of rs1143627 (–31T>C; $P = 0.001$) had a statistically significant association with increasing risk of KC. The C/C genotype frequency of rs16944 (–511C>T; $P = 0.066$) was not statistically associated with increasing risk of KC. However, the TT genotype is more frequent in patients than controls (22.6 vs7.5%).

Conclusion

This is the first report and research of *IL1B* polymorphism screening in Egyptian KC patients. This result associates –31 (rs1143627) polymorphism with increasing the risk of KC. So, this polymorphism may play a role in developing KC among Egyptian families.

Keywords:
genotyping, interleukin 1B, keratoconus, polymorphism

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Introduction

Keratoconus (KC) is a corneal ectatic disease that results in bilateral and asymmetrical corneal distortion (Abu-Amero *et al.*, 2014) characterized by altered refractive powers and reduced vision. Symptoms appear by the age of early 20's or late teens. Clinical signs of KC are extremely variable depending on the stage of progression of the disease. KC is also characterized by stromal thinning, conical protrusion, Fleischer's ring, Vogt's striae, increased visibility of nerve fibers, and rupture in Bowman's layer (Krachmer *et al.*, 1984; Bron, 1988). Histologically, the keratoconic corneal stroma may become less than one-quarter its normal thickness (Kenney and Brown, 2003).

Recent studies have shown that the KC is associated with eye rubbing in atopic patients (Krachmer *et al.*, 1984; Bawazeer *et al.*, 2000), contact lens wearing (Macsai *et al.*, 1990), increased proteinase activity, decreased levels of proteinase inhibitors, increased oxidative damage, and keratocyte apoptosis. Prevalence rates for KC range from 0.0002 to 2.34% (Gordon-Shaag *et al.*, 2012). Prevalence and incidence rates of KC are higher in Asians than the Caucasian population (Pearson *et al.*, 2000; Georgiou *et al.*, 2004). In the Middle East Population studies (including the Arabs and non-Arabs) it has been suggested that the incidence of KC is between 20/100 000 and 24.9/100 000 (Georgiou *et al.*, 2004; Ziaei *et al.*, 2012). On the basis of a previous study done in Egypt, it was found that the prevalence of KC was 1.12%. Of all the affected cases, 5.5% had unilateral, and the other cases (94.5%) had bilateral KC (Elbedewy *et al.*,2019). Another study found a higher incidence of KC for Asians (25/100 000/year) as compared with White

people (3.3/100 000/year). The higher incidence of KC in the Asian population is because this community has a tradition of consanguineous marriages (Althomali *et al.*, 2018). A previous study found that the percentage of parental first-cousin marriage was 35.4% among patients with KC and 18.3% among controls (Krachmer *et al.*, 1984).

KC is a multifactorial disorder, controlled by both genetic and environmental factors, that is, it is a complex disease; consanguinity has been found to be a risk factor for KC which proves that it is a genetic disease. To date, 17 distinct genomic loci have been mapped for KC showing that there is a high degree of genetic heterogeneity in this disease (Burdon and Vincent, 2013). Recent studies have shown that there is a lot of candidate genes associated with KC like the VSX1 gene that have been proven to be significantly associated with KC in the Han Chinese population. ZEB1 mutations in the European population were found to be associated with KC (Lechner *et al.*, 2013a, 2013b). D326Y variant in *COL4A3* and M1237V and F1644F variants in COL4A4 show significant allelic effects. Filaggrin (OMIM 135940) mutations are a strong genetic risk factor for atopic dermatitis (Bawazeer *et al.*, 2000).

In addition to these genes other genes like superoxide dismutase 1 (lipoxygenase-transforming growth factor, β1), secreted protein acidic and rich in cysteine, human leukocyte antigen, mitochondrial complex I genes, and collagen type IV, alpha 3 and collagen type IV, and alpha four are considered to be candidate genes for KC. Meta-analysis study mentioned that single-nucleotide polymorphism (SNP) rs4954218, located at the 2q21.3 genomic region, is associated with KC. RAB3GAP1 is considered to be a candidate gene for KC (Li *et al.*, 2011). The results of two genome-wide association studies found that hepatocyte growth factor (HGF) is considered to be a candidate gene for KC. Two SNPs located upstream of the HGF gene on chromosome 7 showed repeated association with KC (Burdon *et al.*, 2011).

Interleukin (IL)-1 is a proinflammatory cytokine that is responsible for the production of cytokines and chemokines, which play an important role in inflammatory processes. IL-1 control many different cellular activities, including cell proliferation, differentiation, and apoptosis (Mikami *et al.*, 2013). The IL-1 family consists of two proinflammatory cytokines (IL1A and *IL1B*) and the IL-1 receptor antagonist (IL-1Ra). These proteins are encoded by IL1A, *IL1B*, and IL1RN, respectively; they are present in chromosome 2q14. Recent studies have shown an association of polymorphisms in IL1A, *IL1B*, and IL1RN with KC in a Korean population and this study also proved that *IL1B* promoter polymorphisms rs1143627 (–31T>C) and rs16944 (–511C>T) are significantly associated with an increased risk of KC (Kim *et al.*, 2008). These two polymorphisms were found to be associated with KC in Korean and Japanese populations.

In this study, our aim was to show if *IL1B* promoter polymorphisms rs1143627 (–31T>C) and rs16944 (–511C>T) are significantly associated with an increased risk of KC in the Egyptian population.

Materials and methods

This study included 159 blood samples that belong to unrelated affected cases and 159 healthy individuals for rs16944 (–511C>T) polymorphism, 144 blood samples that belong to unrelated affected cases, and 141 healthy individuals for rs1143627 (–31T>C) polymorphisms. All cases had been examined in The Research Institute of Ophthalmology with written informed consent obtained from all participants.

The patients were diagnosed with KC based on the following criteria or symptoms of KC including:

(1) Medical history included: age, sex, progression, contact lens use, and eye rubbing behavior. There is often a history of frequent changes in eye glasses which do not adequately correct vision. Another common progression is from soft contact lenses, to Toric or astigmatism correcting contact lens, to rigid gas permeable contact lens
    The majority of cases of KC are bilateral and are often asymmetric. The less affected eye may show a high amount of astigmatism. Onset is typically in early adolescence and progresses into the mid-20s and mid-30s. There is variable progression for each individual
(2) Diagnosis can be made by slit-lamp examination and observation of central or inferior corneal thinning. The Pentacam is a rotating Scheimpflug camera system for anterior segment analysis. It measures topography and elevation of the anterior and posterior corneal surface and corneal thickness. Ultrasound pachymetry can also be used to measure the thinnest zone on the cornea
(3) All patients were subjected to full history taking including pedigree construction paying stress on parental consanguinity and other affected family members; investigations were done accordingly to reach precise diagnoses of cases. The parents of each patient were informed about the aims, methods, and possible results of DNA analysis and gave their informed consent to the study.

Genomic DNA was extracted from peripheral blood leukocytes of all patients and their parents using the salting out procedure (Štabuc-Šilih *et al.*, 2009). Genotyping screening was carried out using PCR and restriction fragment length polymorphism analysis (RFLP) for two SNPs –511 (rs16944) and –31(rs1143627) in the *IL1B* promoter region. Polymerase chain reaction was done using 3.5 μg genomic DNA, (2.5 μl) 10x buffer, (2.5 μl) 0.25 mmol/l dNTPs, (2.5 μl) 2.5 pmol of each primer Metabion (Planegg, Germany), and (0.5 μl) of Taq polymerase (Recombient; Thermo, Waltham, MA, USA), and (9 μl) of sterile H_2O (Steinkasserer *et al.*, 1992; Cantagrel *et al.*, 1999; Timms *et al.*, 2004). PCR conditions started with denaturation at 96°C for 1 min, followed by 35 cycles of 94°C for 50 s, 60.1°C for 1 min, 72°C for 40 s, and final elongation at 72°C for 10 min for rs1143627 (–31T>C) and denaturation at 94°C for 1 min followed by 35 cycles, 94°C for 5 min, 55.3°C for 30 s, and 72°C for 30 s, and a final extension at 72°C of 5 min for rs16944 (–511C>T). Successful amplification was confirmed by the appearance of PCR products at the expected band, which is 420-bp for rs1143627 (–31T>C) and 304 bp for rs16944 (–511C>T). The digestion of PCR products was carried out in a 15 μl total volume containing 5 μl of PCR product, 0.5 μl (5 units) of the enzyme, AluI (fast digest) for rs1143627 (–31T>C) and AvaI (fast digest) for rs16944 (–511C>T), 1.5 μl of 10x buffer (10 mmol/l Tris-HCl, 100 mmol/l KCl, 1 mmol/l DTT, 1 mmol/l EDTA, 0.2 mg/ml BSA, and 50% glycerol, 7 μl sterile H_2O, and incubated at 37°C for 15 min. For rs1143627 (–31T>C), the allele T has three cut sites resulting in four fragments of the following sizes: 247, 97, 57, and 20 bp. The C allele has two cut sites, resulting in fragments of sizes 344, 57, and 20 bp, while the T/C show 344, 247, 97, 57, and 20 bp. For rs16944 (–511C>T), allele C results in 190 bp and 114 bp and for T allele results in 304 bp and for T/C allele it results in 304, 190, and 114 bp.

The Hardy–Weinberg equilibrium was calculated using the GenePop web version 4.0 program. Fisher's exact test and χ^2-test were used to detect and determine the statistically significant differences in genotype between patients and control group. Odds ratio of risk and confidence intervals (CI) were used to determine the strength of the association (Kim *et al.*, 2008). Haploview that uses the expectation maximization algorithm (Barrett *et al.*, 2004) was used to calculate associations and haplotype frequencies.

Results

Two SNPs (rs1143627 and rs16944) among patients and control were analyzed for *IL1B*. Genotypic genotypic frequencies in KC patients and control are mentioned in Tables 1 and 2. Table 3 mentioned the allele frequencies for the two SNPs of *IL1B* and their genomic location. The distribution of rs1143627 (–31C>T) T/T, T/C, and C/C genotype frequencies in patients were 35.4, 43.8, and 20.8% while in control were 40.4, 51.1, and 8.5%, respectively. The C/C genotype frequency for rs1143627 (–31C>T) in patients is higher than that in control and as a result the C/C genotype suggests to be a risk factor for KC and also proof that there is significant association between rs1143627 (–31C>T) and KC.

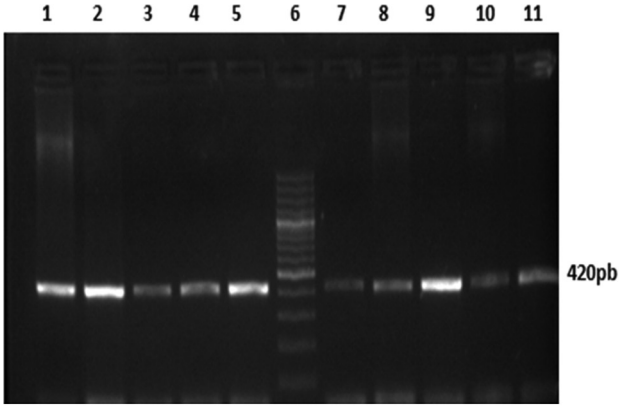
C allele frequency for rs1143627 (–31C>T) in patients was 85.41% while in control it was 68.08% (P = 0.22, odds ratio = 1.44, 95% CI = 0.81–2.558).

For rs16944 (–511T>C) T/T, T/C, and C/C genotype frequencies in patients were 22.6, 64.2, and 13.2%, respectively, while in control they were 7.5, 69.8, and 22.6%. The frequency of the C/C for rs16944 (–511T>C) in control is higher than that in patients which is proof that there is no significant association. The C allele frequency at rs16944 in patients was 45.28% while in control it was 57.84% (P = 0.46, odds ratio = 1.13, 95% CI = 0.82–1.58).

Fig. 1 showed a 3.5% agarose gel illustrating the PCR product of SNP – 31 (rs1143627), Lanes 1, 2, 3, 4, 5, 7, 8, 9, 10, 11: PCR product 420-bp, Lane 6: Mis 100 bp DNA ladder.

Fig. 2 showed a 3.5% agarose gel illustrating the PCR RFLP analysis of SNP –31 (rs1143627) digested with AluI (Fast digest). Lanes 1, 2, 8: patients with homozygous genotyping T/T 247 pb, 97 pb, 57 bp, 20 pb; Lane 3, 4, 6: patients with heterozygous genotyping T/C 344 pb, 247 bp, 97 bp, 20 pb; Lane 7: patients with homozygous wild-type C/C 344 bp, 57 pb, 20 pb; Lane 5: 100bp DNA ladder.

Figure 1



PCR of SNP rs1143627 (–31T>C).

Table 1 Genotype frequency of rs1143627 (–31C>T) polymorphism in keratoconus patients

IL1B (SNPs)	Genotypes	Keratoconus frequency (*n*=144) [*n* (%)]	Control frequency (*n*=141) [*n* (%)]	*P*
–31 T>C (rs1143627)	C/C	30 (20.8)	12 (8.5)	0.001
	T/C	63 (43.8)	72 (51.1)	
	T/T	51 (35.4)	57 (40.4)	

IL, interleukin; SNP, single-nucleotide polymorphism.

Table 2 Genotype frequency of rs16944 (–511C>T) polymorphism in keratoconus patients

IL1B (SNPs)	Genotypes	Keratoconus frequency (*n*=159) [*n* (%)]	Control frequency (*n*=159) [*n* (%)]	*P*
–511C>T (rs16944)	C/C	21 (13.2)	36 (22.6)	0.066
	T/C	102 (64.2)	111 (69.8)	
	T/T	36 (22.6)	12 (7.5)	

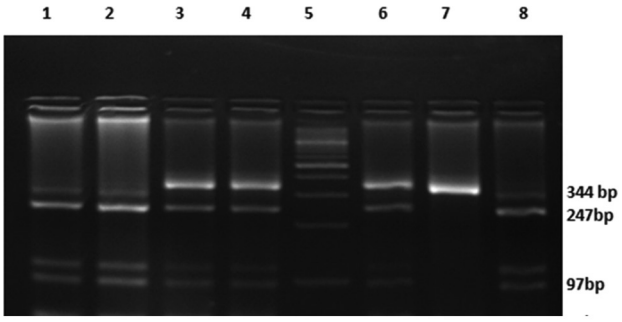
IL, interleukin; SNP, single-nucleotide polymorphism.

Table 3 Allele frequencies of rs16944 (–511C>T) and rs1143627 (–31C>T) polymorphisms in keratoconus patients

SNPs	Chr	Gene	Gene location	Alleles (1>2)	Risk allele	Risk allele frequency, % cases	Risk allele frequency, % controls	*P*	OR	OR (95%CI)
rs1143627 (–31 T>C)	2	*IL1B*	Promoter	T>C	C	85.41	68.08	0.22	1.44	0.81-2.558
rs16944 (–511 C>T)	2	*IL1B*	Promoter	C>T	C	45.28	57.84	0.46	1.13	0.82-1.58

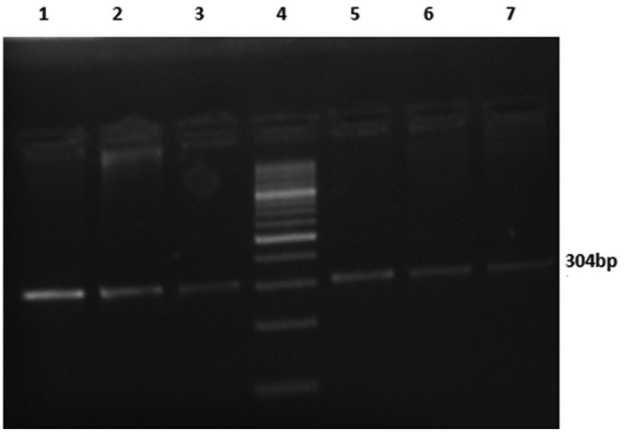
CI, confidence interval; IL, interleukin; OR, odds ratio; SNP, single-nucleotide polymorphism.

Figure 2



PCR restriction fragment length polymorphism analysis of SNP –31(rs1143627).

Figure 3



PCR of SNP rs6944 –511(C>T).

Fig. 3 showed a 3.5% agarose gel illustrating the PCR product of SNP – 511(C>T), Lanes 1, 2, 3, 4, 5, 6: PCR product 304 bp; Lane: 4M is 100 bp DNA ladder.

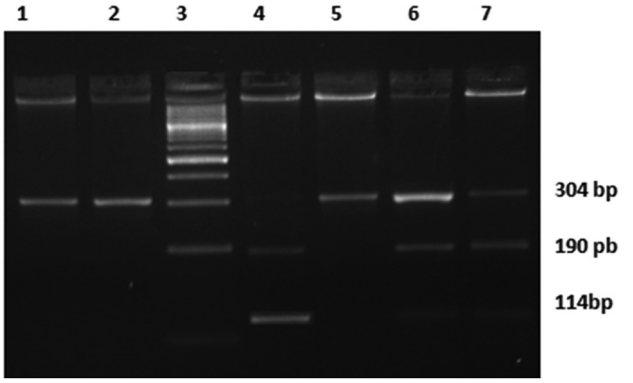
Fig. 4 showed a 3.5% agarose gel illustrating the PCR RFLP analysis of SNP rs16944 – 511 (C>T) digested with AvaI; Lanes 1, 2, 5: patients with homozygous wild-type T/T 304 bp; Lane 4: patients with homozygous genotyping C/C 190 pb, 114 pb; Lanes 6, 7: patients with heterozygous genotyping T/C 304 pb, 190 pb, 114 pb; Lane3: M is 100 bp DNA ladder.

Discussion

The target of this study was to investigate whether the polymorphisms of *IL1B* promoters (–31C>T), (–511T>C) had a role in the development of KC among Egyptian population. Two SNPs (rs1143627, rs16944) were genotyped and screened in the *IL1B* promoter region. This study reported a significant association between –31T/C polymorphism in the *IL1B* promoter region and KC in Egyptian patients, proofing and suggesting that polymorphism –31C>T in the *IL1B* promoter region contributes to the risk of KC in the Egyptian population. On the other hand, the study reported that there is no significant association between –511T>C in the *IL1B* promoter and KC in the Egyptian population.

The rs1143627 SNPs located at the – 31 position in the *IL1B* promoter region. As a result, promoter sequences considered to be potential sources of polymorphisms controlling gene expression suggest that rs1143627 may play an important and vital role in *IL1B* gene expression; that is why it may be considered to be a risk factor for KC. This result is supported by several previous studies showing that polymorphisms located in the *IL1B* promoter region, especially the T allele of rs1143627 in the TATA box, can enhance the expression of *IL1B* (Hall *et al.*, 2004; Landvik *et al.*, 2009). In the human

Figure 4



PCR restriction fragment length polymorphism analysis of SNP rs16944 –511(C>T).

corneal epithelial, stromal fibroblast, and endothelial cells *IL1B* protein has been detected and discovered (Weng *et al.*, 1997), and expression of this protein has reportedly been enhanced in KC corneas than that in normal ones (Zhou *et al.*, 1996). In KC, keratocyte apoptosis results in corneal thinning and is considered to have a role in this process (Wilson and Kim, 1998; Kaldawy *et al.*, 2002), suggesting that the enhanced *IL1B* expression caused by the promoter polymorphism rs1143627 result in overexpression of the *IL1B* protein that results in the increased corneal apoptotic activity in KC patients (Kim *et al.*, 2008). In this study, *IL1B* polymorphism (–31C>T) is considered to be a risk factor for KC among Egyptians. A previous study reported that the IL1 protein level in the epithelium and endothelium was higher in KC corneas than that in normal ones (Zabaleta *et al.*, 2006). IL1 plays a vital role in the regulation of expression of collagenases, metalloproteinases, and other enzymes (Strissel *et al.*, 1997; West-Mays *et al.*, 1997). The two SNPs (rs1143627, rs16944) located in the *IL1B* promoter region have been repeatedly associated with multiple clinical conditions in several studies (Momiyama *et al.*, 2001; Camargo *et al.*, 2004) as cardiovascular disease (Iacoviello *et al.*, 2005) and gastric cancer (Chang *et al.*, 2005). A study has shown that the IL1 genotype has a great effect on protein expression in the stratum corneum (DeJongh *et al.*, 2008). The – 31*C allele was accompanied by an increased level of *IL1B* and decreased level of IL1A (Hall *et al.*, 2004). In a previous study, it has been reported that the T and C variants at the *IL1B* –31 position control gene expression and binding of proteins (Kim *et al.*, 2008).

The C allele at –31 and T allele at –511 are associated significantly with increasing LPS (lipopolysaccharide) by two- or three-folds result in inducing *IL1B* protein secretion (Chen *et al.*, 2006). There was a complete linkage disequilibrium between – 511C>T and –31T>C in *IL1B* based on a cis interaction (Hulkkonen *et al.*, 2000; Chen *et al.*, 2006). Previous studies shed the lights on the presence of proinflammatory cytokines such as IL-6, IL-1β, interferon-γ, and tumor necrosis factor-α in the tear film of KC patients (Ionescu *et al.*, 2018).

Conclusion

In conclusion, this recent study has suggested that the *IL1B* promoter polymorphisms 1143627 was associated with KC in the Egyptian population and the C/C genotype was considered to be a risk factor for KC. This finding is in line with a previous study on Chinese Han populations (Wang *et al.*, 2016), while the *IL1B* promoter polymorphisms 16944 was found to be not associated with KC in the Egyptian population. This result was consistent with a previous study done in the Turkish population, suggesting that the rs16944 polymorphism do not play a role in the development of KC (Palamar *et al.*, 2014). On the other hand, these results were in contrast with other studies done in Korean (Kim *et al.*, 2008) and Japanese populations (Mikami *et al.*, 2013).

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Conflicts of interest

There are no conflicts of interest.

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