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Genetic association of the *COL1A1* gene promoter –1997 G/T (rs1107946) and Sp1 +1245 G/T (rs1800012) polymorphisms and keloid scars in a Jeddah population

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Background/aim: Scars develop at the end of the natural wound-healing process and are characterized by excessive collagen deposition, particularly types I and III collagen. This study aimed to investigate the genetic association of *COL1A1* –1997 G/T (rs1107946) and *COL1A1* Sp1 +1245 G/T (rs1800012) polymorphisms with the incidence of scars.

Materials and methods: A case-control association study was conducted with 84 volunteers from Jeddah, Saudi Arabia (47 patients and 37 controls). The allele frequency distribution and nucleotide genotypes of –1997 G/T, +1245 G/T were ascertained by polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) analysis.

Results: Our results indicated that the distribution of *COL1A1* (rs1107946) genotypes was significantly different between patients and controls (P = 0.00). The incidence of *COL1A1* (rs1107946) genotype GG was significantly associated with a risk of scars. The distribution of the (rs1107946) genotype was drastically higher in women with scars (P= 0.00). One haplotype block in *COL1A1* was documented by the pair-wise linkage disequilibrium between the single nucleotide polymorphisms (SNPs). The frequency of the GG haplotype constructed by the two SNPs was robustly high and associated with risk of scars.

Conclusion: Our results strengthen the evidence for the association between polymorphisms of –1997 G/T, +1245 G/T of the *COL1A1* gene in the genetic etiology of keloid scars.

Key words: Keloid, collagen, *COL1A1*, restriction fragment length polymorphism, polymerase chain reaction, single nucleotide polymorphism

1. Introduction

Keloid scars are benign fibroproliferative cutaneous lesions resulting from the overproduction of all components of the healing process, including fibroblasts, collagen, elastin, and proteoglycans; they can occur following burn injuries, lacerations, abrasions, surgery, piercings, vaccinations, and even minor skin trauma. They are unique in humans, and especially in genetically susceptible individuals from nonwhite populations (1–3). It has been estimated that about 15%–20% of Blacks, Hispanics, and Orientals suffer from keloids and there appears to be a genetic predisposition to keloid formation (4). Keloids can develop at every age, but they have a higher incidence between 10 and 30 years (1,3). A slight female predominance is also noted, but this could be related to the higher rate of earlobe piercing in females (3,5). The incidence rates of hypertrophic scarring vary from 40% to 70% following surgery to up to 91% following burn injuries (4). The alterations responsible for keloid formation are still unknown, but it is thought that injury to the reticular layer of the dermis, which consists mainly of collagen and fibroblasts, can contribute to the formation of keloids. The end result of this is the failure of wound healing and accumulation of excess matrix and collagen, called scarring (2,6,7).

Collagen consists of triple helix proteins and represents approximately 70% of the dry weight of the human skin (8–10). The prominent collagens are types I and III. Type I collagen constitutes 80%–90% and type III constitutes 10%–15% of the total collagen present in the skin (9). In humans the gene coding for the α 1 chain of type I collagen is located on the long arm of chromosome 17 (17q21.3-q22), has a size of 18 kb, and consists of 51 exons (12,13). The transcription of *COL1A1* of type I collagen is regulated by the promoter and the first intron (12). The absolute amount of collagen increases in keloids, indicating increased collagen synthesis or decreased cross linking; mature scars have a higher content of cross linked collagen than keloids (2,13).

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There is a common single nucleotide polymorphism (SNP) in the specificity protein 1 (Sp1) binding site within the promoter region of intron 1 of *COL1A1* (1). Most studies of *COL1A1* have focused on the polymorphism that affects the Sp1 binding site in the first intron at position +1245 (rs1800012) (G/T). More recently, two polymorphisms have been identified in the proximal promoter of *COL1A1* at positions –1997 (rs1107946) (G/T) and –1663 (rs2412298) (indel T). These polymorphisms have been associated with bone mineral density (BMD) in several studies, and in some populations have been reported to interact with the Sp1 polymorphism to regulate BMD (14–16).

The current study was designed to evaluate the association of –1997 G/T (rs1107946) and *COL1A1* Sp1 +1245 G/T (rs1800012) of the *COL1A1* gene polymorphisms with the incidence of scars in a Jeddah population in Saudi Arabia. Genomic DNA extraction from the collected samples was performed, followed by polymerase chain reaction (PCR) amplification with specific primers, and then restriction fragment length polymorphism (RFLP) analysis, sequencing, and alignment of the obtained sequence. Statistical analysis and data interpretation were carried out in the patients with scars and in the normal group.

2. Materials and methods

2.1. Study of the population

A total of 84 participants were recruited, 47 patients with scars (25 men and 24 women) and 37 healthy controls (19 men and 18 women), with ages ranging from 7 to 60 years old. The patients with scars consisted of 32 individuals with keloid scars and 15 with hypertrophic scars. The scar samples were collected from the plastic surgery clinic of King Fahad Hospital, the Military Forces Hospital, and the tissue culture unit in the King Fahad Medical Research Center in Jeddah. These samples were evaluated by a clinician based on the following clinical criteria: hypertrophic scars tend to be red and raised scars, limited to the borders of the original lesion, and often presenting contractures; keloid scars are raised, sometimes painful and pruritic, and are not limited to the borders of the original lesion. Seven samples from circumcision surgeries at the Maternity Hospital Surgical Clinic in Jeddah and 30 blood samples from students at King Abdulaziz University were obtained as normal samples. This study was approved by the Ethical Committee Unit of Biomedical Ethics, affiliated with the Directorate of Health Affairs, Jeddah, and informed consent was obtained from each participant.

2.2. Genotyping study

Blood samples were collected into tubes containing the anticoagulant ethylenediamine tetraacetic acid, tissue samples were collected in normal saline, and cell cultures were collected in 1.5-mL microcentrifuge tubes containing 200 µL of potassium phosphates PBS. The samples were then stored at 4 °C until used.

Genomic DNA was extracted from whole samples using the QIAamp DNA Mini Kit (QIAGEN) according to the manufacturer's instructions. The concentration of genomic DNA was determined using a Jenway 6800 UV/Visible spectrophotometer (JENWAY).

Primer sets were designed to amplify fragments encompassing the SNPs. The primers used for genotyping of polymorphisms in –1997 G/T of *COL1A1 (*rs1107946) and Sp1 +1245 G/T of *COL3A1* (rs1800012) are listed in Table 1. Amplification was carried out using a programmable thermal cycler PCR instrument (Eppendorf Master Cycler Personal, Eppendorf AG). A final volume of 50 µL was prepared as follows: 2 µL (0.2 µg/µL) of genomic DNA, 25 µL of Hot Start Green Master Mix (Promega), 22 µL of nuclease-free water, and 2.5 pmol of each primer. Thermal cycling conditions for –1997 G/T (rs1107946) and +1245 G/T (rs1800012) are given in Table 1. The –1997 G/T (rs1107946) and Sp1 +1245 G/T (rs1800012) SNPs in the *COL1A1* gene were analyzed by PCR amplification, followed by RFLP analysis. The genotypes of these SNPs were determined by DNA digestion using *BsaI* (New England BioLabs, Inc.) for –1997 G/T (rs1107946) and *PflMI* (New England BioLabs, Inc.) for +1245 G/T (rs1800012). Results were visualized on 3% (w/v) agarose gel electrophoresis. Sequencing was performed following the instructions of the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) and using the Sanger dideoxy sequencing method.

2.3. Haplotype structure and statistical analysis

The data analysis was performed using SPSS for Windows, version 16 (SPSS Inc.). Descriptive data are given as means ± standard deviation (SD). Associations between groups and genotypes were assessed using the nonparametric Mann–Whitney test. A 2-by-3 chi-square test was applied to test the association between genotypes and clinical groups, and a 2-by-2 test was used to test the association between alleles in clinical groups. Contingency analysis was applied to calculate the odds ratio (OR) and risk ratio (RR) at a 95% confidence interval (CI) to estimate the relative risk and strength of association for the various genotypes or their combinations. Statistical significance was defined as $P < 0.05$. The Hardy–Weinberg equilibrium was tested by a goodness-of-fit $\chi 2$ test to compare the observed genotype frequencies with the expected genotype frequencies among the scar patients and the normal group, with the chi-square test featuring one degree of freedom.

Table 1. Restriction enzymes employed to distinguish between alleles and primers used to amplify genomic DNA around the polymorphic sites in *COL1A1* –1997 and +1245.

Site of polymorphism	Primer sets (5'→3')	Annealing temperature (°C)	Allele size (bp)	Ref.
BsaI –1997 G/T (rs1107946) Genomic DNA	TCACTAACCCTCATACTACCAAGC CCCGACCATGTGGCAGCA	61	G: 151+124 T: 275	(16)
PflMI +1245 G/T (rs1800012) Genomic DNA	CCAATCAGCCGCTCCCATTC CATCGGGAGGGCAGGCTC	60	G: 99+57 T: 156	(1)

3. Results

3.1. Characteristics of the population and genotyping

The average age and SD of the participants was 27.06 ± 13.17 years old and 19.62 ± 18.59 years old for the scar patients and the normal group, respectively. The results showed that there was a significant difference between the patients with scars and the normal group in terms of age (P = 0.03) (data not shown). For the –1997 G/T *COL1A1* rs1107946 polymorphism genotyping, the T allele produced 270 bp, the G allele formed fragments of 151 bp and 124 bp, the G allele of +1245 G/T *COL1A1* rs1800012 created fragments of 99 and 57 bp, and the T allele produced 156 bp (Figures 1 and 2). Direct DNA sequence analysis of the amplified PCR products revealed T/G and G/G substitutions at –1997 (rs1107946) of the *COL1A1* gene (Figures 3A and 3B).

The prevalence of each genotype of the *COL1A1* –1997 G/T (rs1107946) was 37.8% for GG, 62.2% for GT, and 0.0% for TT for healthy controls, and 66% for GG, 29.8% for GT, and 4.2% for TT for the patients with scars. For the *COL1A1* +1245 G/T (rs1800012), the prevalence was 67.6% for GG, 32.4% for GT, and 0.0% for TT for the controls, and 49.9 for GG, 51.5% for GT, and 0.0% for TT for the patients with scars (Table 2). These genotype distributions were in the Hardy–Weinberg equilibrium. It is remarkable that all obtained results of the +1245 G/T polymorphism significantly illustrated the absence of allele TT in all subjects (Table 2).

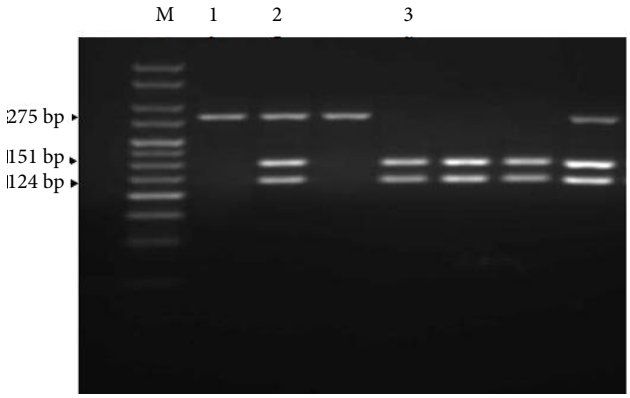

Figure 1. A photograph of 3% (w/v) agarose gel showing the digested PCR products for –1997 G/T *COL1A1* polymorphism genotyping. Lane M: DNA marker; lane 1: homozygous TT, the genotype that produces one 275-bp band; lane 2: heterozygous GT, the genotype that produces three bands, a 275-bp band, a 151-bp band, and a 124-bp band; lane 3: homozygous GG, the normal genotype that produces two bands, a 151-bp band and a 124-bp band.

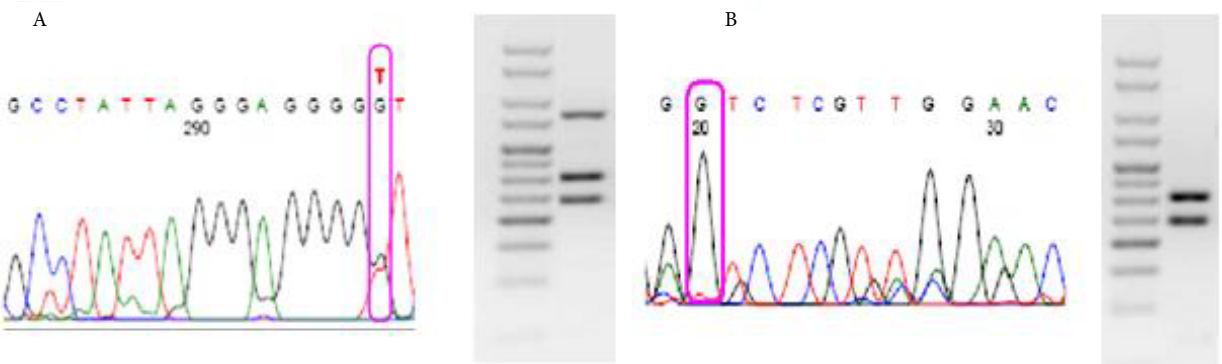

Figure 2. A photograph of a 3% (w/v) agarose gel showing the digested PCR products for +1245 G/T *COL1A1* polymorphism genotyping. Lane M: DNA marker; lanes 1 and 2: heterozygous GT, the genotype that produces three bands, a 156-bp band, a 99-bp band, and a 57-bp band; lane 3: homozygous GG, the normal genotype that produces two bands, a 99-bp band and a 57-bp band.

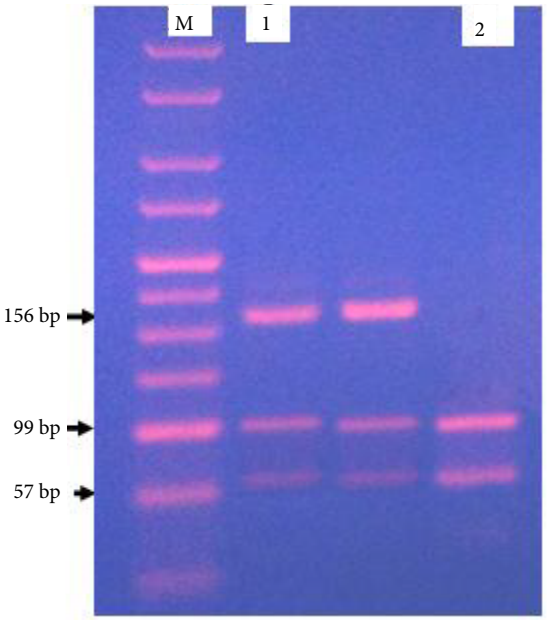

Figure 3. (A) Sequence analysis of –1663 to –1997 in the *COL1A1* gene (a normal case). The box indicates T/G nucleotide substitution. (B) Sequence analysis of –1663 to –1997 in the *COL1A1* gene (a scar case). The box indicates two copies of G allele.

There was a highly significant difference in genotype distribution and allele frequencies of the –1997 G/T (rs1107946) polymorphism between the patients with scars and the normal group using the 2-by-3 general test of association (P = 0.00), which means that most of the patients with scars tended to have the GG genotype and most of the normal subjects tended to have the GT genotype. The OR and the RR were 0.28 (95% CI: 0.11–0.69). In contrast, no significant association was noted between the genotype and the subject group (patients with scars or normal subjects) (P = 0.08) for the +1245 G/T SNP. The OR and RR were 2.17 (95 % CI: 0.89–5.32) (Table 2). The genotype distributions of polymorphism met the Hardy–Weinberg expectations in both groups (the patients with scars and the normal group) for the –1997 G/T SNP (P = 0.006) and for the +1245 G/T SNP (P = 0.01) (Table 2).

The frequencies of G and T alleles for the scar group were 80.9% and 19.1%, respectively, while for the normal group they were 68.9% and 31.1%, respectively. Thus, the results demonstrated that there was a significant difference in allele frequencies between the scar group and the normal group for the –1997 G/T SNP (P = 0.05) (Table 2). In the case of the +1245 G/T SNP, the frequencies of

Table 2. The association between the presence of *COL1A1* –1997 G/T (rs1107946) or +1245 G/T (rs1800012) polymorphisms and the incidence of scars.

SNP	Group	*COL1A1* genotype distribution			Allele frequency	HWE	P-value[a]	P-value[b]	P-value[c]
		GG	GT	TT					
–1997 G/T (n = 84)	Normal (n = 37)	(n = 14) 37.8%	(n = 23) 62.2%	(n = 0)	G: 68.9% (n = 51)	0.006	0.02**	0.00**	0.05*
					T: 31.1% (n = 23)				
	Scar (n = 47)	(n = 31) 66%	(n = 14) 29.8%	(n = 2) 4.2%	G: 80.9% (n = 76)	0.79			
					T: 19.1% (n = 18)				
+1245 G/T (n = 84)	Normal (n = 37)	(n = 25) 67.6%	(n = 12) 32.4%	(n = 0)	G: 83.8% (n = 62)	0.23	0.08	0.08	0.14
					T: 16.2% (n = 12)				
	Scar (n = 47)	(n = 23) 48.9%	(n = 24) 51.1%	(n = 0)	G: 74.5% (n = 70)	0.01			
					T: 25.5% (n = 24)				

n: number of samples, HWE: Hardy–Weinberg equation.
P-value[a]: expressed as frequencies, and were compared by the Mann–Whitney test.
P-value[b]: correspond to genotype distribution using a 2-by-3 chi-square test.
P-value[c]: correspond to allele frequency using a 2-by-2 chi-square test.
*Significance (P = 0.05); **Highly significant results (P < 0.05).

G and T alleles for the scar group were 74.5% and 25.5%, respectively, while for the normal group they were 83.8% and 16.2%, respectively; no significant difference in allele frequencies between the scar group and the normal group was observed (P = 0.07) (Table 2). The association between the presence of –1997 G/T (rs1107946) or +1245 G/T (rs1800012) polymorphisms and the incidence of scars is shown in Table 2. A highly significant difference in variation among genotypes (P = 0.02) between the scar group and the normal group was found in the –1997 G/T SNP, while no significant difference (P = 0.08) was found in the +1245 G/T SNP.

In addition, the association between the presence of either –1997 G/T (rs1107946) or +1245 G/T (rs1800012) polymorphisms and the incidence of scars in male patients were studied (Table 3). The genotype distributions of polymorphism met the Hardy–Weinberg expectations in both groups (males with scars and normal males) (P = 0.23 in the –1997 G/T SNP, 0.36 in the +1245 G/T SNP). No significant difference in variation among the genotypes (P = 0.51 in –1997 G/T) and (P = 0.22 in +1245 G/T) was observed between males with scars and normal males (Table 3). The distribution of genotypes among the scar group in the –1997 G/T SNP was 66.7% homozygous GG (n = 16), 25% heterozygous GT (n = 6), and 8.3% homozygous TT (n = 2), while among the normal group it was 52.6% homozygous GG (n = 10), 47.4% heterozygous GT (n = 9), and the homozygous recessive TT was undetected in this group as shown in Table 3. For the +1245 G/T SNP, the distribution of genotypes among the scar group was 50% homozygous GG (n = 12) and 50% heterozygous GT (n = 12), while among the normal group it was 68.4% homozygous GG (n = 13) and 31.6% heterozygous GT (n = 6). The homozygous recessive TT was undetected in both groups as shown in Table 3. Therefore, there was no significant difference in genotype distribution and allele frequencies of the –1997 G/T (rs1107946) polymorphism between the scar and the normal male groups (P = 0.17 in –1997 G/T) and (P = 0.22 in +1245 G/T). Moreover, the results demonstrated that there was no significant difference in allele frequencies between male patients with scars and normal male subjects (P = 0.75 for –1997 G/T) and (P = 0.29 for +1245 G/T).

The association between the presence of –1997 G/T (rs1107946) or +1245 G/T (rs1800012) polymorphisms and the incidence of scars in female patients is shown in Table 4. The genotype distributions for polymorphism met the Hardy–Weinberg expectations in both groups (females

Table 3. An association between the presence of *COL1A1* –1997 G/T (rs1107946) or +1245 G/T (rs1800012) polymorphisms and the incidence of scars in males.

SNP	Group	*COL1A1* genotype distribution			Allele frequency	HWE	P-value[a]	P-value[b]	P-value[c]
		GG	GT	TT					
–1997 G/T (n = 43)	Normal (n = 19)	(n = 10) 52.6%	(n = 9) 47.4%	(n = 0)	G: 76.3% (n = 29)	0.17	0.51	0.17	0.75
					T: 23,7% (n = 9)				
	Scar (n = 24)	(n = 16) 66.7%	(n = 6) 25%	(n = 2) 8.3%	G: 79.2% (n = 38)	0.23			
					T: 20.8% (n = 10)				
+1245 G/T (n = 43)	Normal (n = 19)	(n = 13) 68.4%	(n = 6) 31.6%	(n = 0)	G: 84.2% (n = 32)	0.36	0.22	0.22	0.29
					T: 15.8% (n = 6)				
	Scar (n = 24)	(n = 12) 50%	(n = 12) 50%	(n = 0)	G: 75% (n = 36)	0.02			
					T: 25% (n = 12)				

n: number of samples.
HWE: Hardy–Weinberg expectations.
P-value[a]: expressed as frequencies, and were compared by Mann–Whitney test.
P-value[b]: correspond to genotype distribution using a 2-by-3 chi-square test.
P-value[c]: correspond to allele frequency using a 2-by-2 chi-square test.

Table 4. An association between the presence of *COL1A1* –1997 G/T (rs1107946) or +1245 G/T (rs1800012) polymorphisms and the incidence of scars in females.

SNP	Group	*COL1A1* genotype distribution			Allele frequency	HWE	P-value[a]	P-value[b]	P-value[c]
		GG	GT	TT					
–1997 G/T (n = 41)	Normal (n = 18)	(n = 4) 22.2%	(n = 14) 77.8%	(n = 0)	G: 61.1% (n = 22)	0.008	0.00**	0.00**	0.02**
					T: 38.9% (n = 14)				
	Scar (n = 23)	(n = 15) 65.2%	(n = 8) 34.8%	(n = 0)	G: 82.6% (n = 38)	0.09			
					T: 17.4% (n = 8)				
+1245 G/T (n = 41)	Normal (n = 18)	(n = 12) 66.7%	(n = 6) 33.3%	(n = 0)	G: 83.4% (n = 30)	0.39	0.23	0.22	0.30
					T: 16.6% (n = 6)				
	Scar (n = 23)	(n = 11) 47.8%	(n = 12) 52.2%	(n = 0)	G: 73.9% (n = 34)	0.03			
					T: 26.1% (n = 12)				

n: number of samples.
HWE: Hardy–Weinberg expectations.
P-value[a]: expressed as frequencies, and compared by the Mann–Whitney test.
P-value[b]: correspond to genotype distribution using a 2-by-3 chi-square test.
P-value[c]: correspond to allele frequency using a 2-by-2 chi-square test.
**Highly significant results (P < 0.05).

with scars and normal females) (P = 0.008 in the –1997 G/T SNP) and (P = 0.03 in the +1245 G/T SNP).There was a highly significant difference in variation among genotypes in the –1997 G/T SNP (P = 0.00) between female patients with scars and normal females; however, no significant difference in variation among genotypes in +1245 G/T (P = 0.23) was found between these groups. Furthermore, the distribution of genotypes in the scar group was 65.2% homozygous GG (n = 15) and 34.8% heterozygous GT (n = 8), while in the normal group it was 22.2% homozygous GG (n = 4) and 77.8% heterozygous GT (n = 14). The homozygous recessive TT was undetected in both groups. In the +1245 G/T SNP, the distribution of genotypes in the scar group was 47.8 % homozygous GG (n = 11) and 52.2 % heterozygous GT (n = 12), while in the normal group it was 66.7% homozygous GG (n = 12) and 33.3 % heterozygous GT (n = 6). The homozygous recessive TT was undetected in both groups. When the results in the two female groups were compared, a highly significant association between groups and genotypes was observed (P = 0.00) in –1997 G/T; there was no significant association between groups and genotypes in +1245 G/T (P = 0.22). Furthermore, a highly significant difference in allele frequencies between the scar and the normal group in females was detected (P = 0.02) in the –1997 G/T SNP, while there were no significant differences in allele frequencies between the scar and the normal group (P= 0.30) in the +1245 G/T SNP (Table 4).

3.2. Haplotype analysis

When we combined the two polymorphisms together, we found five –1997 G/T, +1245 G/T genotype alleles (GGGG, GTGG, GGGT, GTGT, and TTGG) in our population. The other genotypes (GGTT, GTTT, TTGT, and TTTT) were undetected. Genotype combination frequencies for all subjects are presented in Table 5. It is obvious that there was a strong association in terms of linkage disequilibrium (LD) between the –1997 G/T (rs1107946) and +1245 G/T (rs1800012) polymorphisms (P = 0.001). From the obtained results, we found that the haplotype GG (–1997 G, +1245 G) was the most common (with a frequency of 63.10%). The frequency of the rare haplotype TT was 8.93%. Haplotype analysis yielded a highly significant association with scars (P = 0.005, Table 6).

Table 5. Genotype frequencies for both polymorphisms in all 84 subjects.

Genotype	Derived alleles	N	Frequency (%)	X^2	Alleles	N
GGGG	GG/GG	24	28.57	10.8194	GG	106
GGGT	GG/GT	21	25		GT	26
GTGG	GT/GG	22	26.19		TG	21
GTGT	GT/GT	15	17.86		TT	15
TTGG	TT/GG	2	2.38			
Total		84	100%			168

Data are presented as the number of samples and the frequency.
X^2 indicated a significant value in genotype data at P = 0.001.

Table 6. Linkage disequilibrium (LD) and inferred haplotype frequencies for *COL1A1* –1997 G/T and +1245 G/T in all subjects.

SNP1		SNP2	D'	r^2	X^2	Haplotype	
						Haplotype	Frequency (%)
–1997 G/T (rs1107946)	+1245 G/T (rs1800012)	–1	0.088	7.39		GG GT TG TT Total	63.10 12.50 15.48 8.93 100

D' = the deviation of the observed frequency of a haplotype from the expected.
X^2 = 7.39 indicated that there were significantly higher frequencies of the *COL1A1* allele haplotypes in the subjects (P = 0.005).

4. Discussion

Until now, the mechanism of keloid formation remains unknown, underscoring the vital need to understand and control these disfiguring maladaptive processes, characterized by excessive collagen accumulation. Many previous studies of alleles in relation to osteoporosis-related phenotypes focused on the intron 1 Sp1 binding site polymorphism (16). A promoter polymorphism, –1997 G/T, was also associated with BMD (17). To date, no published studies have investigated the association between the –1997 G/T, +1245 G/T *COL1A1* polymorphisms and keloid disease.

The present study was undertaken to clarify the genetic factors associated with susceptibility to keloid, elucidate the molecular mechanisms for keloid formation, and investigate the relation between –1997 G/T, Sp1 +1245 G/T in the regulatory region of the *COL1A1* gene polymorphisms and the prevalence of scars in Jeddah. The obtained results demonstrate that there was a significant difference across genotypes in the scar group, as compared with the normal group, in the –1997 G/T polymorphism, while no association was found between the +1245 G/T polymorphism and the incidence of scars. The –1997 G/T and +1245 G/T polymorphisms were associated with BMD in several studies. Our results were in agreement with bone studies (18) in which a significant association between perimenopausal bone mass and –1997 G/T was shown, but no association between the +1245 G/T polymorphism and peri- or postmenopausal bone mass could be demonstrated.

On the other hand, our findings differed from results reported in Denmark in which +1245 G/T was found to have a negative influence on BMD, whereas –1997 G/T was not significantly associated with BMD (14). Furthermore, the present results were in disagreement with those reported in the Rotterdam study (17), which indicated that the *COL1A1* Sp1 polymorphism influenced BMD and the risk of fracture in postmenopausal Caucasian women. In contrast, no independent effect of the –1997 G/T promoter polymorphism on BMD or fractures was detected. A recent study showed that the *COL1A1* gene was associated with reduced anterior cruciate ligament (ACL) injury in professional soccer players and found no association between the *COL1A1* –1997 G/T polymorphism and the

incidence of ACL ruptures; the *COL1A1* gene was found to be in high degree of LD with the +1245 G/T loci (19).

A higher frequency distribution of the *COL1A1* –1997 GG genotype has been shown to be associated with scar formation. The GG genotype was significantly higher in the scar group than in the normal one (P = 0.00). The combined analysis of two studies (16,20) suggested that the GG genotype association with BMD was reduced in TT homozygotes compared with the other genotype groups.

In the Sp1 +1245 G/T SNP, our results were in accordance with those reported by González-Bofill et al. (18). On the other hand, Enoch and Leaper (13) conducted a study in Denmark on the association of promoter and intron 1 polymorphisms of *COL1A1* gene haplotypes with increased risk of osteoporosis and found that the T-allele of the +1245 G>T polymorphism was associated with a decrease in BMD (P = 0.02). They suggested that alleles at the Sp1 site (+1245 G/T) interact with alleles at the –1997 G/T site to regulate BMD (16).

When the subjects were divided by sex, the obtained results were in agreement with the Rotterdam study that found no association between the Sp1 or the –1997 G/T polymorphisms with BMD or fractures in men (17). The SNP rs1107946 was found to be only significant in females but not in males.

In a study of 308 postmenopausal Caucasian women from the United States, the –1997 and +1245 G/T polymorphisms were found to be associated with BMD, such that higher values were found in carriers of the G allele at both polymorphic sites (21). These observations were in agreement with the present observations in –1997 G/T, but not in +1245 G/T. Another study of 1100 Japanese postmenopausal women reported a significant association between –1997 G/T alleles and BMD in GG homozygotes (22). Furthermore, a study in India showed that an allele affecting BMD was exhibited by the major allele (G) of –1997 G/T and the minor allele (T) of +1245 G/T (23), whereas a population of Chinese women showed no association between –1997 G/T alleles and BMD (24). Other studies (8,25) reported higher BMD values in relation to the –1997 T allele in Japanese postmenopausal women. In contrast, studies in Spanish and American postmenopausal women reported that the –1997 T allele was associated with reduced BMD (16,17,21). The differences between these studies could be due to the fact that the polymorphisms studied here are in LD with other functional polymorphisms at the *COL1A1* locus that regulate BMD and that the patterns of LD differ in these different populations (16).

In the present study, two locus haplotypes were tested for an association with the scars. It was assumed that the predisposition for scars might be a polygenic trait; therefore, the haplotypes in two candidate polymorphisms would provide more information than those in any single polymorphism on the complex relationship between DNA sequence variations and traits. In fact, studies have shown that haplotype analysis is more powerful when the genotypes are in LD with the causative locus (19).

The present results showed also that the GG haplotype (with an estimated frequency among subjects of 65.96%) was significantly higher in the scar group compared with the normal group, suggesting that participants with two copies of this haplotype have an increased risk for scars. Ficek et al. (19) reported that higher frequency of the GT haplotype was associated with reduced risk of ACL injury. Yazdanpanah et al. (17) indicated a significantly high association of the GT haplotype with an increased risk of fragility fracture in women. Another study suggested that the GT haplotype of the *COL1A1* gene was associated with a higher risk of postmenopausal osteoporosis in women from Northwest India (24). These results were in agreement with a study on *COL1A1* haplotypes and hip fractures, which found a significant association between the GT haplotype and hip fractures in older people, and especially in women (26).

Over all, regulatory polymorphisms at the DNA level can strongly affect variations in gene expression. Any SNP in the regulatory DNA binding site can modify the affinity with the regulatory protein, leading to different levels of gene expression. Promoter polymorphisms are suspected to affect gene transcription activity, and thereby gene functions. That, together with the assumption that the –1997 G/T and +1245 G/T polymorphisms have never been tested with respect to scars, led us to consider these polymorphisms separately, as candidates to influence the risk of sustaining scars. Our results suggested that the –1997 G/T polymorphism was strongly associated with the incidence of scars. The –1997 G/T polymorphism belongs to the proximal promoter elements that modulate the efficiency of the basal level of transcription.

In conclusion, there was an association between the –1997 G/T *COL1A1* gene polymorphism and scars, especially in female subjects. No association between scars and +1245 G/T was found. Although functional analyses of *COL1A1* 5' flank suggested that the GG haplotype was responsible for enhancing the transcriptional activity of the *COL1A1* gene, we recommend conducting further investigations, including the evaluation of the type I/type III collagen ratio, to explain the relationship between *COL1A1* expression and susceptibility to scars.

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