

## Genetic basis of keloid formation in wounds after cardiac surgery

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### Abstract

Keloid disease is the abnormal formation of scar tissue in genetically predisposed people. Among many genes which may be related to the development of keloids, transforming growth factor  $\beta 1$  (*TGF- $\beta 1$* ) is one of the most mentioned. It encodes cytokinin, which is responsible for the production of extracellular matrix and takes part in healing. Any abnormalities which arise during synthesis of the protein as a result of polymorphism or gene mutation may be the cause of healing disorders (scarring of the body); thus it is responsible for the development of keloids. The objective of this study is to determine the single nucleotide polymorphism of the gene *TGF- $\beta 1$* , at the position -509(rs1800469)509, to compare the obtained results in the form of three different genotypes within the analysed group (keloids) and within the control group (healthy scars), and to analyse the correlation between obtained genotypes and the occurrence of keloid disease.

Seventy-three patients after cardiac surgery with scars on their sternums were examined (22 women and 51 men) in the age group from 38 to 84 years. Two groups of patients were distinguished: 37 with keloids and 36 with healthy scars. DNA taken from patients was analysed and polymorphism C(-509)T of the gene *TGF- $\beta 1$*  was determined.

On the basis of the study it was found that the allele T in the position -509 of the gene *TGF- $\beta 1$*  is associated with a lower risk of keloid formation regardless of age and gender.

**Key words:** keloid scarring, keloid disease, gene polymorphism, gene mutation.

### Streszczenie

Choroba keloidowa polega na nieprawidłowym tworzeniu się tkanki bliznowej u osób genetycznie predysponowanych. Wśród wielu genów, które mogą mieć związek z rozwojem keloidu, jednym z najczęściej wymienianych jest gen kodujący transformujący czynnik wzrostu  $\beta 1$  (*TGF- $\beta 1$* ). Koduje on cytokinę odpowiedzialną za tworzenie macierzy pozakomórkowej i uczestniczy w procesie gojenia. Wszelkie nieprawidłowości pojawiające się w czasie syntezy białek w wyniku polimorfizmów lub mutacji genów mogą być przyczyną zaburzeń gojenia i powstawania blizn na ciele, powodując rozwój keloidów. Celem tej pracy jest zbadanie jednonukleotydowego polimorfizmu genu *TGF- $\beta 1$*  w pozycji -509(rs1800469)509 i porównanie uzyskanych wyników w formie trzech różnych genotypów w ramach grupy badanej (keloidy) i grupy kontrolnej (zdrowe blizny) oraz analiza korelacji pomiędzy uzyskanymi genotypami i występowaniem keloidów.

Badaniem objęto 73 pacjentów po operacjach chirurgicznych z bliznami na mostku: 22 kobiety i 51 mężczyzn w wieku od 38 do 84 lat. Wyróżniono dwie grupy: 37 pacjentów z keloidami oraz 36 pacjentów ze zdrowymi bliznami. Przebadano próbki DNA pacjentów i ujawniono polimorfizm C(-509)T genu *TGF- $\beta 1$* . Na podstawie badania wykazano, że allel T w pozycji (-509) genu *TGF- $\beta 1$*  związany jest z niższym ryzykiem powstawania keloidów niezależnie od wieku i płci.

**Słowa kluczowe:** blizny keloidowe, choroba keloidowa, polimorfizmy genów, mutacje genowe.

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## Introduction

Keloid (keloid disease) is a mild form of skin neoplasm which appears as a result of abnormal wound healing. It arises from the excessive accumulation of extracellular matrix in the forming scar and it often extends beyond the area of the primary skin lesion [1-5]. The cause of the disease has not been explained yet. It concerns predisposed persons in whom, as a result of injury or surgery, symptomatic, extensive, unsightly and disfiguring scars may develop. The scars cause disfigurement or in extreme cases organ dysfunction. Family history of keloids and more frequent occurrence of the disease in some races, ethnic groups and in twins indicate that there is a genetic basis [1, 6, 17]. Many reports of keloid disease linked with genetic defects and related connective tissue such as Ehlers-Danlos syndrome, Goeminne syndrome, Rubinstein-Taybi syndrome or Dupuytren's contracture [2, 7-10, 17] can be found in the scientific literature. In the genetic defects with mutations, one or several genes may be responsible for the formation of keloids. *TGF-β1* (transforming growth factor β1), a gene located on chromosome 19 which encodes the cytokine TGF-β1, is one of the most frequently mentioned among many genes that may have a key role in the formation of keloids. Transforming growth factor β1 as one of the three isoforms of the protein (TGF-β1, -β2 and -β3) [11-13, 17] has a key role in the wound healing processes [14, 15] and therefore all the irregularities of the cytokine result-

ing from gene polymorphism or mutation of *TGF-β1* may be a direct cause of keloid disease development.

## Aim of the study

The aim of the study was to investigate the polymorphism C(-509)T in the promoter region of the *TGF-β1* gene (rs1800469) among patients with a healthy scar and those with a keloid and to analyse the relationship between genotypes and the occurrence of keloid disease.

## Material and methods

One hundred and eleven people were invited to participate in the study. The people were treated between 2009 and 2010 when surgical closure was carried out. Within the group of patient enrolled in the study 50 people with keloids (Fig. 1) were distinguished and 50 people with healthy scars (Fig. 2) formed a control group.

The eligibility of patients in each group was determined using the Vancouver Scar Scale assessing colour, elasticity and convexity of scars based on the subjective experience of a disease, i.e. pain, burning, itching (Tables I and II).

The minimum total number of points that can be achieved during the test is 0 points. The maximum is 16 points. Patients enrolled in the control group (healthy scar) obtained a score ≤ 3 points, whereas patients with a score ≥ 9 points were enrolled in the study group (keloids).



Fig. 1. Keloid



Fig. 2. Healthy scar

Tab. I. Vancouver Scar Scale

Characteristic of the scar	Type	Points
Vascularity	Normal	0
	Pink	1
	Red	2
	Purple	3
Pliability	Normal	0
	Supple	1
	Yielding	2
	Firm	3
	Ropes	4
	Contracture	5
Pigmentation	Normal	0
	Hypo	1
	Hyper	2
Height	Flat	0
	< 2 mm	1
	2-5 mm	2
	> 5 mm	3

Max. 13 points.

**Tab. II.** Discomfort related to the scar

Type of discomfort	Lack of discomfort	Pain	Burning	Itching
	0 points	1 points	1 points	1 points

Max. 3 points

Each of the patients participating in the study had a photograph of the scar taken with a millimetre scale and the code number assigned to the patient. Biological material in the form of a double swab from mucosa was collected from all participants using a PrepFiler Forensic DNA Extraction Kit (Applied Biosystems) for DNA research.

Polymorphism C(-509)T TGF-β1 (rs1800469) was identified by PCR-RFLP (polymerase chain reaction – restriction fragment length polymorphism) using specific primer pairs. The sense primer with the following sequence was used for amplification.

5'-CAGACTCTAGAGACTGTCAG-3'

And the antisense primer:

5'-GTCACCAGAAAGAGGAC-3'.

In the preliminary experiments the optimal PCR conditions were established. All amplifications were performed in thermocycler Mastercycler gradient (Eppendorf), in a volume 20 µl of reaction mixture containing:

- 40 ng genomic DNA,
- buffer PCR [10 mM Tris-HCl, 50 mM KCl, 0.08% Nonidet P40] (MBI Fermentas),
- dNTP [200 mM] (MBI Fermentas),
- MgCl<sub>2</sub> [1.5 mM] (MBI Fermentas),
- 4 pmol sense and antisense primer (synt. TIB MOLBIOL, Poznań),
- 0.5 U polymerase *Taq* (MBI Fermentas).

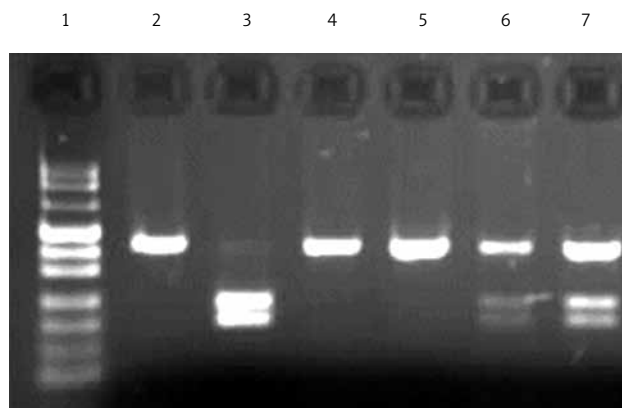
The following temperature-time profile was used in PCR:

- Phase I: initial denaturation 940°C – 5 min,
- Phase II (38 cycles):
  - denaturation: 940°C – 20 seconds,
  - hybridization of primers: 600°C – 40 seconds,
  - elongation: 720°C – 40 seconds.
- Phase III: final elongation 720°C – 8 minutes.

The products of amplification (length = 419 bp) were subjected to restriction analysis with the enzyme *Bsu36I* (37°C/16 h). Within the amplified gene fragment *TGF-β1*, allele (-509)C has one restriction area for the enzyme *Bsu36I*, and as a result of digestion of the amplicon two restriction fragments of the 228 bp and 191 bp are created. Allele (-509)T does not have such a restriction site and the digestion of the amplicon forms a restriction fragment of 419 bp.

The obtained restriction forms were separated by electrophoresis on a 2% agarose gel with staining ethidium bromide. The separation was conducted in 1 × TBE buffer (0.089 M Tris, 0.089 M boric acid, 2 mM EDTA) at a temperature of 200°C, at a voltage of 80 V. The lengths of the restriction fragments were determined pursuant to the DNA marker pUC Mix Marker 8 (MBI Fermentas).

The final step was gel documentation using G:BOX Bio-Imaging System (*SYNGENE*) (Fig. 3).



Electrophoretic separation of the restriction fragments. Paths: 1 – lengths of DNA marker – pUC Mix Marker 8 (MBI Fermentas), 2, 4, 5 – homozygotes TT, 3 – homozygous CC, 6, 7 – heterozygotes CT

**Fig. 3.** Identification of polymorphism C(-509)T of *TGF-β1* gene by PCR-RFLP

**Statistical analysis**

The statistical significance of differences between qualitative variables was analysed with the exact two-sided Fisher test, and between measurable variables with the Kruskal-Wallis test or Mann-Whitney *U* test. The value of the odds ratio (OR) and its 95% confidence interval (95% CI) were used to assess the strength of the association between genotype and keloid formation. The logistic regression model was used in multivariate analysis. *P* < 0.05 was taken into consideration as the threshold of statistical significance. The calculations were performed in Statistica 10 software.

**Results**

Of the 100 people who were examined, DNA was isolated in 73 patients (22 women and 51 men) aged between 38 to 84. The average age was 64.75 and the standard deviation 9.37. The age was not significantly different (*p* = 0.46, Mann-Whitney *U* test) between 36 patients with keloids (63.9 ± 10.0 years) and 37 patients with healthy scars (65.6 ± 8.7 years).

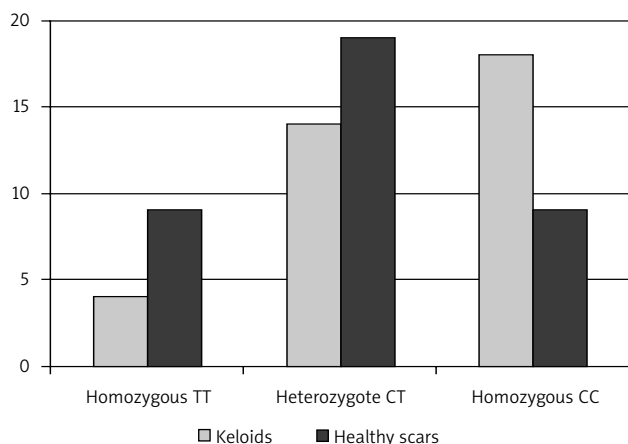
The percentage distribution of the genders in each group is presented in Table III. There were no significant differences between groups (*p* = 1.0, exact Fisher test).

**Tab. III.** Distribution of genders of the analysed groups of patients

	Women	Men	Together
Keloids	11 29.73%	26 70.27%	37
Healthy scars	11 30.56%	25 69.44%	36
Total	22	51	73

**Tab. IV.** Distribution of frequency of genotypes and alleles of polymorphic *TGF-β1* T(-506)C in the analysed groups of patients

Group	Homozygous CC	Heterozygote CT	Homozygous TT	Allele C	Allele T
Healthy scars	9 24.32%	19 51.35%	9 24.32%	37 50.00%	37 50.00%
Keloids	18 50%	14 38.89%	4 11.11%	50 69.44%	22 30.56%



**Fig. 4.** Distribution of the number of *TGF-β1* polymorphisms T(506)C in the analysed groups of patients

The act of isolating DNA failed in 27 patients. The incorrect way of taking the samples, e.g. too small amount of DNA or improper storage condition of the swabs, could be the cause of the failure.

The results of the analysis of the polymorphism C(-509)T *TGF-β1* are presented in Table IV. The distribution of genotypes was consistent with the Hardy-Weinberg law ( $p = 0.70$  and  $p = 1.0$ ) in both groups, in the test as well as in the control group (Fig. 4).

There was no relationship between the genotype and the age of the patients ( $p = 0.48$ , Kruskal-Wallis test). In contrast, a significant correlation between genotype and the occurrence of keloids was observed: the presence of allele T (i.e. CT or TT genotype) was related to significantly lower risk of keloid formation (OR = 0.32). The dependence of the various alleles and genotypes relative to each other is presented in Table V.

**Discussion**

Scar formation is a physiological process which enables healing of wounds. In some cases excessive overgrowth of scar tissue occurs while the wounds are healing. It extends beyond the area of the wound and causes severe pain and itching and a keloid develops.

The frequency of the occurrence of keloids is estimated from 5% to 16%, particularly in people with dark skin colour. There is no single effective method of treating keloids [3, 16], and all those previously used such as surgical removal, laser therapy, cryotherapy, pressotherapy, injecting, corticosteroids, radiation or the use of silicone materials only allow one to minimize the size of the scar and related ailments. If it cannot be effectively treated, can it be prevented?

There are literature reports of a relationship between keloid disease and changes in DNA where the mutations and polymorphisms of various genes may influence the de-

**Tab. V.** The relationship between genotype *TGF-β1* T(506)C and the presence of keloid

Compared genotypes <i>TGF-β1</i> T(506)C	Odds ratio for presence of keloid OR	Confidence interval OR 95% CI	P
TT + CT vs. CC	0.3214	0.1188-0.8697	0.030
TT vs. CT + CC	0.3889	0.1079-1.4020	0.22
TT vs. CC	0.2222	0.0535-0.9229	0.046
T vs. C	0.4400	0.2235-0.8664	0.019
CT vs. CC	0.3684	0.1281-1.0600	0.073
TT vs. CT	0.6032	0.1540-2.3623	0.52

It was found in the multivariate analysis (Table VI) that the presence of the allele T (i.e. CT or TT genotype) was related to a significantly lower risk of keloid formation (OR = 0.32), regardless of age and gender.

**Tab. VI.** Multivariate analysis in the logistic regression model of the relationship between age, gender and carrier of allele *TGF-β1* (-509)T (genotypes CT or TT) with the presence of keloid

Independent variables	OR	95% CI	p
Age (years)	0.9773	0.9276-1.0296	0.38
Gender (male)	0.9225	0.3186-2.6712	0.88
Carrier of allele <i>TGF-β1</i> (-509)T	0.3153	0.1136-0.8746	0.024

velopment of keloid disease [1, 2, 6-10, 17]. There are also studies which find no relationship between gene polymorphism of *TGF-β1* and keloid disease [11].

Regarding the different reported studies, we attempted to find out whether by analysing the specific gene *TGF-β1* and obtaining the specific genotype of a person we can determine the risk of keloid formation.

On the basis of the results demonstrated above, the relationship between occurrence of nucleotide T (-509) *TGF-β1* and decreased risk of developing keloid scars was proven. This would help to identify patients who in the case of any interference (trauma or surgery) are prone to additional complications, i.e. the development of keloids. Having such knowledge before the scheduled surgery, a method of operating with minimum invasiveness (e.g. laparoscopy) could be used.

The results encourage further genetic research aimed at searching for new mutations and polymorphisms of the specific genes responsible for the development of keloids. It would allow us to predict the development of keloid disease and its course and, as a result, to treat it successfully and to prevent it in the future.

### Conclusions

1. On the basis of this analysis, it was found that the presence of allele I at position -509 of the gene *TGF-β1* is associated with a lower risk of keloid formation.
2. The age of the patients participating in the study did not differ significantly between the groups and did not influence the risk of keloids.
3. No evidence of a relationship between the gender of patients and risk of developing keloid disease was found.

### Disclosure

The authors report no conflict of interest.

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