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Expression of the *GZMB* Gene Polymorphism, SNP rs8192917, in 990 Han Chinese Patients with Postoperative Keloids

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Background: A keloid is a pathological scar hyperplasia that is affected by genetic and environmental factors. Although the involvement of cytotoxic granzyme B in keloids has been recognized, there is almost no research on granzyme B (*GZMB)* gene polymorphisms and keloids. This study aimed to explore the relationship between genetic polymorphisms of *GZMB* and postsurgical keloid susceptibility in the Han Chinese population.

Material/Methods: A total of 3078 participants, including 990 patients with postsurgical keloids and 2088 controls without postsurgical keloids, were enrolled. We selected 15 common DNA variants in the *GZMB* gene for analysis. Associations were analyzed in both single marker-based and haplotype-based methods. The Genotype-Tissue Expression database was used to examine the biological significance of the targeted single nucleotide polymorphisms (SNPs).

Results: SNP rs8192917 was found to be associated with the susceptibility of keloids (*t* statistic=4.82, $P=1.47\times10^{-6}$). An increased risk of keloids was significantly associated with the minor allele (C allele) of rs8192917 (odds ratio=1.33; 95% CI=1.18-1.49], $P=1.47\times10^{-6}$). In addition, a significant association was reported for genotypes of rs8192917 and clinical severity of keloids ($\chi^2=10.61$, $P=0.03$).

Conclusions: The results suggested there are significant associations between common genetic variants in *GZMB* and the susceptibility of postsurgical keloids in the Chinese Han population. These genetic polymorphisms were also related with the severity of postsurgical keloid symptoms in participants with keloids. The current study can contribute to future etiological and clinical research of keloids.

Keywords: Case-Control Studies • Disease Susceptibility • Keloid • Polymorphism, Single Nucleotide

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Background

Keloid and hypertrophic scars represent an aberrant response to the wound healing process [1]. These scars are caused by cutaneous injuries, such as trauma, burns, surgeries, or uncertain origins, which is different from the origins of other scar types. Keloids are clinically treated as a benign skin tumor [2], and their incidence in the Asian population can be as high as 4% to 16% [3]. Keloid tissue is usually accompanied by chronic inflammation, and it is prone to infection and ulceration, which can lead to scar carcinoma [4,5]. Furthermore, for keloids, the prognosis of squamous cell carcinoma is poor and mortality is high [6]. Keloids can occur in locations including the ears, chin, chest, back, and vulva and are accompanied by unbearable itching and tingling and a serious negative impact on the quality of life and mental state of patients [7,8]. Due to the unclear pathogenesis of keloids, they have always posed a difficult problem in the field of plastic surgery.

There are currently many hypotheses about the formation of keloids, such as the immunology, tension, and endocrine hypotheses. Early studies have found that compared with in normal tissues, the concentrations of T lymphocytes, B lymphocytes, macrophages, and immune complexes in the peripheral circulation of keloids are significantly increased, and it is speculated that the proliferation of keloids may be caused by immune stimulation [9,10]. Genetic and molecular epidemiologic studies have suggested that the polymorphisms of many genes that affect the proliferation and apoptosis of keloid fibroblasts or extracellular deposition and degradation are associated with keloid risk, including TGF-β, Bcl-2, Smad7, IL-6, IL-4, IL-10, and Fas [11,12]. TGF-β is recognized as one of the most representative cytokines closely related to wound healing [13]. Given that hyperproliferation of fibroblasts is a major feature of keloids and that inflammation is associated with poor prognosis, the Fas/FasL pathway, which plays an important role in cell apoptosis, is thought to be associated with keloids. Some single nucleotide polymorphism (SNP) sites in exons 7 to 9 of the *Fas* gene are very likely to be related to functional changes in the Fas protein, leading to the formation of local keloids [14,15].

The cytotoxic granzyme B (GzmB)/perforin pathway shows a similar function to the Fas/FasL pathway in apoptosis and inflammation, leading to poor healing of damaged tissues. There are 5 granzymes in humans, of which granzyme B is a serine protease encoded by the *GZMB* gene (located at 14q12) that is mainly secreted by natural killer cells, $CD8^+$ T lymphocytes (CTLs), and tumor cells. It can induce the expression of inflammatory factors and promote tissue fibrosis by releasing TGF-β, activating interleukin, and degrading decorin [16]. Therefore, granzyme B is thought to be involved in inflammatory skin diseases [17]. Research has observed decreased decorin levels in keloid scar tissue compared with in normal skin, while reduced decorin is associated with collagen disorders in animal models, and *GzmB* knockout could inhibit this phenomenon to some extent [18,19]. Consistently, another report demonstrated that using *GzmB* inhibitors can also speed up wound healing [20]. However, *GzmB* appeared to have no physiological function in healthy GzmB-knockout mice or in young, healthy human skin [21,22]. Although 2 gene association mapping studies have been published on keloids [23,24], there is almost no research focused on *GZMB* gene polymorphisms. As basic patient characteristics, such as age, sex, and recurrence history, can play an important role in keloid treatment, there is an urgent need for more efficacious options.

To date, no study in Han Chinese individuals examining the association of the *GZMB* gene with postsurgical keloids is available. Thus, our study aimed to explore the correlation between the *GZMB* gene and postsurgical keloid genetic susceptibility in the Chinese Han population based on a candidate gene association mapping study design to provide more insights into the etiology of keloids.

Material and Methods

Ethics Statement

Written informed consent was obtained from all participants. This study was approved by the Ethics Committee of the First Affiliated Hospital of Xi'an Jiaotong University per reference 20171016YG dated October 18, 2017, and the study procedures were performed following the tenets of the Declaration of Helsinki (version 2002).

Study Participants

A total of 3078 study participants, including 990 patients with postsurgical keloids and 2088 control participants without postsurgical keloids, were consecutively recruited from the Plastic and Aesthetic Surgery Outpatient Department of the First Affiliated Hospital of Xi'an Jiaotong University between Jan 2018 and July 2021. All participants were unrelated Han Chinese individuals (at least 3 generations were of Han descent and had no history of migration). All participants were examined carefully by at least 2 dermatologists. According to the shape of the scars, proliferation outside the original wound boundary, and invasion of the surrounding normal skin, keloids were diagnosed. The diagnosis of the hypertrophic scar was confirmed when the height of the scar was more than 2 mm above the normal surrounding skin [25]. Patients with hypertrophic scars and some keloid syndromes were excluded from the study. The severity of keloids was assessed according to color, scar height, pliability, pain, and itching of keloid

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Table 1. Characteristic and demographic information of the study participants.

Variables	Cases (N=990)	Controls (N=2,088)	Statistics	*P*
Age, mean±sd	40.3±15.0	40.3±17.2	t=-0.11	0.91
Gender (%)				
Male	584 (59)	1,234 (59)		
Female	406 (41)	854 (41)	χ^2=0.0003	0.99
Family History (%)				
Yes	121 (12)	246 (12)		
No	869 (88)	1,842 (88)	χ^2=0.0857	0.77
Clinical Severity (%)				
Mild	273 (28)	–		
Moderate	503 (51)	–		
Severe	214 (21)	–	–	–

scars [26]. All healthy controls without keloids were from the same clinics, and all participants were free of obesity and autoimmune and systemic diseases in the study. Peripheral blood samples were collected from the study participants and preserved for further genotyping experiments. Demographic information, including age, sex, and family history of the study participants, was collected from questionnaires and medical records and is presented in **Table 1**.

SNP Choice and Experiment for Genotyping

GZMB is a short gene of only approximately 3.2 kb that includes untranslated regions. We extracted all common DNA variants (MAF ≥0.05) from the gene region based on data from the 1000 Genomes Project. We selected a total of 15 SNPs for genotyping (**Supplementary Table 1**). Genomic DNA was extracted using a commercial DNA kit (Axygen Scientific Inc, Union City, CA, USA). The candidate SNPs were genotyped based on the Sequenom MassARRAY platform, and the raw data were processed by a Typer Analyzer. Labels of samples (cases or controls) were blinded to technicians performing the genotyping experiments. Five percent of the study samples were replicated for genotyping experiments, and a 100% concordance rate was achieved.

Statistical Methods

Power analysis was conducted using the GAS genetic power calculator (https://csg.sph.umich.edu/abecasis/gas_power_calculator/), and the result is summarized in **Supplementary Figure 1**. Characteristic and demographic variables were observed between participants with keloids and healthy controls. Genotyping quality was estimated by Hardy-Weinberg equilibrium tests conducted in the control group. Logit models were applied to each genetic marker for examining the association signals between genotypes and susceptibility to keloids. In the logistic model, the genotypes of each SNP were coded in additive mode. For 0, 1, or 2 copies of the minor allele, the SNP was coded as 0, 1, or 2. Plink [27] was utilized for fitting logistic models. Multiple testing was addressed by Bonferroni corrections through which the threshold *P* value was determined by 0.05 divided by the number of tests. Linkage disequilibrium (LD) patterns were analyzed using Haploview [28]. The standard method was applied to constructing LD blocks based on our genotyped SNPs [29]. Haplotypic frequencies in participants with keloids and controls were estimated and compared to identify significant haplotypes related to risk of keloids. In addition to the susceptibility to keloids, the relationship between genotypes of targeted SNPs and the clinical features of keloids were also investigated. The statistical computing program R was used for descriptive and general statistical analyses.

Methods for In Silico Analyses

In silico analyses were performed to investigate the biological significance of the top hit association signals identified in the association analyses. Several bioinformatics tools and databases were utilized. SIFT [30] and polyphen2 [31] were used to examine the biological significance of nonsynonymous variants on protein structures. The Genotype-Tissue Expression database was used to depict the potential expression quantitative trait loci (eQTL) pattern for candidate genes in various kinds of human tissues [32].

Results

Demographic Characteristics of the Participants

With the formation of dermal hyalinized collagens visible under a microscope, keloids are seen to expand beyond the

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Table 2. Results of the single marker-based association analyses.

CHR	SNP	BP	A1	OR [95%CI]	*T*-statistics	*P*
14	rs2236337	24631041	C	0.89 [0.79-1.00]	-2.00	0.05
14	rs2236338	24631076	G	1.13 [1.27-2.03]	2.04	0.04
14	rs74345106	24631185	T	0.87 [0.59-1.27]	-0.73	0.46
14	rs6573910	24631676	T	0.89 [0.79-1.00]	-1.98	0.05
14	rs6573911	24631727	T	1.13 [1.01-1.26]	2.09	0.04
14	rs71405867	24632191	G	1.03 [0.90-1.19]	0.43	0.66
14	rs1126639	24632342	A	0.89 [0.79-1.00]	-1.99	0.05
14	rs11539752	24632383	C	0.88 [0.78-1.00]	-2.01	0.04
14	rs10909625	24632423	C	1.13 [1.01-1.27]	2.08	0.04
14	rs10873219	24632500	T	1.03 [0.90-1.18]	0.41	0.69
14	rs59268439	24632691	T	0.94 [0.80-1.11]	-0.70	0.48
14	**rs9671454**	**24632850**	**C**	**0.90 [0.69-1.19]**	**-0.74**	**0.46**
14	**rs8192917**	**24632954**	**C**	**1.33 [1.18-1.49]**	**4.82**	**1.47×10^{-6}**
14	rs2273843	24634203	C	1.04 [0.90-1.20]	0.49	0.63
14	rs2273844	24634208	A	1.03 [0.92-1.16]	0.55	0.58

CHR – chromosome; A1 – tested allele; AFF – number of patients with keloids in different genotype groups; UNAFF – uumber of controls in different genotype groups. OR [95% CI] – odds ratio with 95% confidence interval. Significant results are highlighted in bold.

boundaries of the initial lesions. Hypertrophic scars, on the other hand, tend to form within the limits of wounds and present histologically as dermal nodules (**Supplementary Figure 2**). A total of 3078 participants, including 990 patients with keloids and 2088 healthy controls, were enrolled in this study. No significant differences were identified for age (t=-0.11, P=0.91), sex (χ^2=0.0003, P=0.99), or family history (χ^2=0.0857, P=0.77) between patients with keloids and healthy controls (**Table 1**). In the 990 patients with keloids, 273 patients (28%) had mild symptoms, 503 (51%) had moderate symptoms, and 214 (21%) had severe symptoms.

Genetic Associations Between Susceptibility to Keloids and GZMB Genetic Polymorphisms

All of the 15 candidate SNPs were included in the Hardy-Weinberg equilibrium tests in the control group, and the results are summarized in **Supplementary Table 1**. SNP rs8192917 was found to be significantly related with the susceptibility of keloids (**Table 2**). An increased risk of keloids was associated with the C allele (minor allele) of rs8192917 (odds ratio [OR]=1.33; 95%CI=1.18-1.49, P=1.47×10^{-6}). In addition to rs8192917, a couple of other SNPs achieved nominal significance despite their strong correlations with rs819291 (**Figure 1**). A large LD block comprising 9 SNPs was constructed (**Figure 1**). Significant associations were obtained from multiple haplotypes within these LD blocks (**Table 3**, χ^2=329.60, P=2.81×10^{-67}).

Genetic Associations for Clinical Severity of Keloids

A significant association was observed for genotypes of rs8192917 and clinical severity of keloids (χ^2=10.61, P=0.03). The proportion of patients with TT genotypes (homozygote of major alleles) that had mild and severe symptoms was 26% and 10%, respectively. On the other hand, the proportion of patients with CC genotypes (homozygote of minor alleles) that had mild and severe symptoms was 24% and 17%, respectively (**Table 4**). The proportion of patients with mild symptoms was lower for patients with TT genotypes than for patients with CC genotypes, while the proportion of patients with severe symptoms was higher for patients with TT genotypes than for patients with CC genotypes. The copy number of the C allele was associated with more severe symptoms in patients with keloids.

Functional Consequences of SNP rs8192917

SNP rs8192917 is located in the exonic region of the *GZMB* gene. This SNP is a nonsynonymous variant, and its minor allele alters amino acids from Arg to Gln. Further bioinformatics tools were used to investigate whether this change in amino acids would in turn have significant functional consequences on proteins encoded by *GZMB*. The SIFT score was 0.189, and its prediction was "tolerated". However, in Polyphen2, this SNP was predicted to be "possibly damaged". Additionally, potential

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Figure 1. Linkage disequilibrium structure of the 15 genotyped single nucleotide polymorphisms. Values of linkage disequilibrium are indicated in each cell. Linkage disequilibrium blocks are indicated in bold.

Table 3. Results of haplotype-based association analyses.

Locus	SNPs	Haplotype	F_A	F_U	χ^2	DF	*P*
GZMB	rs2236338- rs6573910- rs6573911- rs71405867- rs1126639- rs11539752- rs10909625- rs10873219	OMNIBUS	NA	NA	329.60	7	2.81×10^{-67}
		ACCAGGTG	0.573	0.672	55.00	1	1.20×10^{-13}
		GTTGACCT	0.115	0.101	2.81	1	0.0936
		GTTGACCG	0.048	0.058	2.12	1	0.1456
		GTTAACCT	0.072	0.080	1.07	1	0.3017
		ACCAGGCG	0.037	0.002	117.50	1	2.18×10^{-27}
		ACTAGGTG	0.081	0.038	48.52	1	3.26×10^{-12}
		GTTAACCG	0.032	0.048	7.36	1	0.0067
		GCCAGGTG	0.042	0.002	137.30	1	1.01×10^{-31}

F_A – haplotype frequency in patients with postsurgical keloids; F_U – haplotype frequency in controls; DF – degrees of freedom.

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Table 4. Association between genotypes of rs8192917 and clinical severity of postsurgical keloids.

Clinical severity (%)	Genotypes of SNP rs8192917			χ^2	*P*
	TT (N=378)	CT (N=398)	CC (N=150)		
Mild	100 (26)	137 (34)	36 (24)		
Moderate	242 (64)	198 (50)	88 (59)		
Severe	36 (10)	63 (16)	26 (17)	10.61	0.03

eQTL effects of this SNP on *GZMB* were also observed using the Genotype-Tissue Expression database. In tissue of the tibial nerve, a significant eQTL signal was observed (**Supplementary Figure 3**). The CC genotype of rs8192917 was significantly associated with a lower gene expression level of *GZMB*. Nevertheless, this eQTL signal was only identified in tissues of the tibial nerve, not in the other 45 kinds of human tissues, including the targeted tissue of keloids (**Supplementary Table 2**).

Discussion

Although multiple lines of evidence from studies of animal models have indicated that the *GZMB* gene might be involved in the pathogenesis mechanisms of keloids [21,22], few studies have been conducted to investigate the distribution of genetic polymorphisms of the *GZMB* gene in patients with keloids and controls. In our study, a significant association signal was identified between rs8192917 of *GZMB* and the susceptibility to keloids in the Chinese Han population. To the best of our knowledge, the present report is the first to validate this relationship in human populations. Several GWA studies have linked the *GZMB* gene to vitiligo [33,34], and a significant signal was also obtained from SNP rs8192917. Interestingly, even the effect directions reported in these previous GWA studies on vitiligo were the same as those in the present study on keloids. This observation suggests a potential common genetic basis between vitiligo and keloids, although very few studies on this topic have been conducted.

A previous study indicated that granzyme B (protein encoded by *GZMB*) inhibits the wound healing process by cleaving extracellular matrix proteins during chronic inflammation [19]. Furthermore, a recent study showed that serpina3n, which attenuates granzyme B-mediated decorin cleavage, can also accelerate the wound healing process in type II diabetic mice [20]. In this sense, if an allele of a SNP in the *GZMB* gene is related with increased susceptibility of keloids, the same allele should also be related to a lower expression of *GZMB* in relevant human tissues. Our findings agreed with this hypothesis. The minor allele (C allele) of rs8192917 was associated with increased susceptibility of keloids, and the CC genotype of rs8192917 was associated with a lower gene expression level of *GZMB*. Additionally, we identified a significant association signal between the copy number of C alleles and the clinical severity of keloids in patients. This dosage-dependent pattern could also be considered evidence in support of the pathogenesis mechanism proposed here.

The functional consequence of SNP rs8192917 was reported inconsistently in SIFT and Polyphen2. The SIFT score was 0.189, and its prediction was "tolerated". However, in Polyphen2, this SNP was predicted to be "possibly damaged". This is not very surprising, and a recent report focusing on *BRCA1/2* has shown that due to low accuracies, current bioinformatics based tools might not be able to provide satisfying prediction results for variants with unknown significance [35]. Therefore, it is still too early to determine the functional consequence of this SNP solely based on in silico tools. Although this SNP was located at the exonic region and altered the amino acid in its encoded protein, it may only be a proxy for some ungenotyped susceptible DNA variants in the current report. Nevertheless, since *GZMB* is a relatively short gene and we genotyped all the common DNA variants, not just tag SNPs, we could deduce that those rare or low-frequency variants may be the ones that contribute to the risk and clinical severity of keloids. Furthermore, in the haplotype-based association analyses, a significant association signal was identified for haplotypes located within an LD block comprised of 9 SNPs. Although SNP rs8192917 was not included in this LD block, it was in strong LD with almost all 9 of its SNPs. Therefore, it was quite likely that this association signal obtained from haplotype analyses was dependent on rs8192917.

With the development of biotechnology, such as sequencing, genetic association analyses and multi-omics integrative analyses can help reveal the pathogenesis of complex diseases [36-39] and provide promising therapeutic candidates for the development of new drugs [40,41]. Therefore, sequencing-based integrative analysis might be a promising direction for determining the genetic architectures of *GZMB* on the susceptibility of keloids in the future.

It is worth mentioning the limitations of the present study. Although all common DNA variants located within the gene region of *GZMB*, including untranslated regions, were extracted, some key functional regions might still have been missed. The

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10 kb to 15 kb regions upstream and downstream of a gene are considered to play important roles in gene expression regulation. However, in the present study, these regions were not scanned. In addition, necessary cautions are still needed in analyzing the eQTL signal observed from the publicly available database, because this signal was only identified in the tibial nerve, which is not a target tissue for keloids. Finally, this study had a small sample size, which might have affected the results.

Conclusions

The results of this study suggest there are significant associations between the common genetic variants in the *GZMB* gene and the susceptibility of postsurgical keloids in the Han Chinese population. These genetic polymorphisms were also related with the severity of symptoms in participants with postsurgical keloids. The results of this study can contribute to future etiological and clinical research of keloids.

Acknowledgements

We would like to thank all the study participants for their cooperation.

Declaration of Figures' Authenticity

All figures submitted have been created by the authors, who confirm that the images are original with no duplication and have not been previously published in whole or in part.

Supplementary Materials

Supplementary Table 1. Basic information of the 15 genotyped SNPs.

CHR	Position	SNP	A1	A2	Locus	Av Het	Av HetSE	FUNC	MAF	HWE
14	24631041	rs2236337	C	T	GZMB	0.369	0.220	Untranslated-3	0.35	0.44
14	24631076	rs2236338	G	A	GZMB	0.371	0.219	Missense	0.30	0.60
14	24631185	rs74345106	T	G	GZMB	0.003	0.040	Missense	0.02	0.62
14	24631676	rs6573910	T	C	GZMB	0.421	0.182	Intron	0.29	0.75
14	24631727	rs6573911	T	C	GZMB	0.421	0.183	Intron	0.33	1.00
14	24632191	rs71405867	G	A	GZMB	0.223	0.249	Intron	0.16	0.58
14	24632342	rs1126639	A	G	GZMB	0.353	0.228	Coding-synon	0.29	0.60
14	24632383	rs11539752	C	G	GZMB	0.369	0.220	Missense	0.29	0.96
14	24632423	rs10909625	C	T	GZMB	0.362	0.224	Codings-ynon	0.29	0.87
14	24632500	rs10873219	T	G	GZMB	0.319	0.240	Intron	0.18	0.56
14	24632691	rs59268439	T	C	GZMB	0.113	0.209	Intron	0.12	0.92
14	24632850	rs9671454	C	G	GZMB	0.332	0.236	Intron	0.04	0.18
14	24632954	rs8192917	C	T	GZMB	0.377	0.215	Missense	0.29	0.55
14	24634203	rs2273843	C	T	GZMB	0.259	0.250	Untranslated-5	0.16	0.63
14	24634208	rs2273844	A	G	GZMB	0.428	0.175	Untranslated-5	0.29	1.00

CHR – chromosome; A1 – minor allele; A2 – major allele; avHet – average heterogeneity; avHetSE – standard error of average heterogeneity; FUNC – function; MAF – minor allele frequency; HWE – P values for Hardy-Weinberg equilibrium tests conducted in controls.

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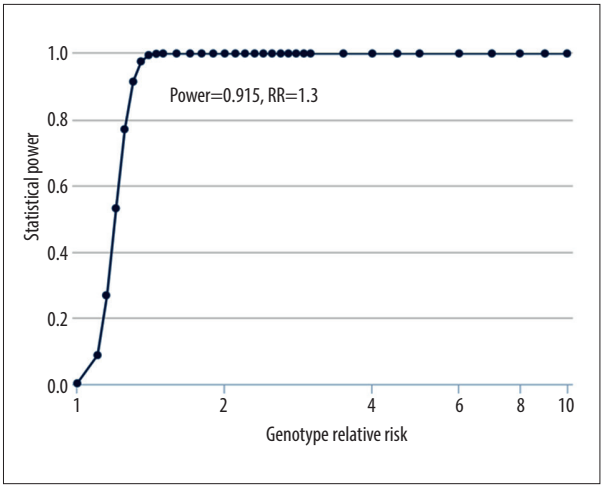
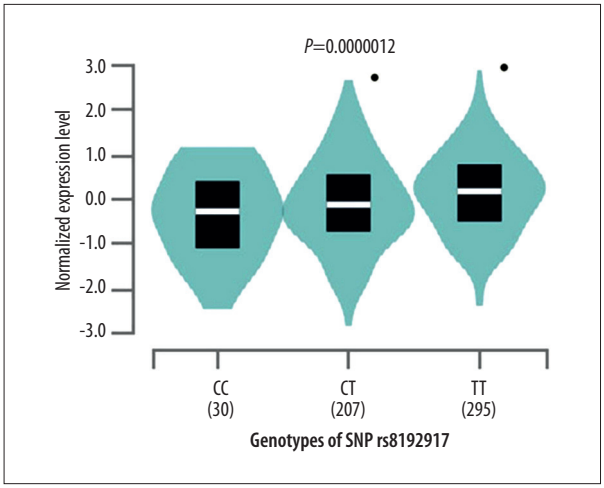


Supplementary Figure 1. Results of the power analysis for the present study.



Supplemental Figure 3. Violin plot for gene expression levels of GZMB in human tissue of tibial nerve grouped by genotypes of SNP rs8192917.

Supplementary Figure 2. The clinical characteristics of keloid scars and hypertrophic scars. (**A**) A 21-year-old man with postsurgical keloid scars. (**B**) A 24-year-old man with arm hypertrophic scars.

Supplementary Table 2. eQTL signals obtained for SNP rs8192917 on GZMB in 46 types of human tissues.

Gene	SNP	P	NES	T-statistic	Tissue
GZMB	rs8192917	1.20×10^{-5}	0.240	4.400	Nerve – Tibial
GZMB	rs8192917	0.002	0.360	3.100	Brain – Cerebellar Hemisphere
GZMB	rs8192917	0.006	0.130	2.800	Adipose – Visceral (Omentum)
GZMB	rs8192917	0.008	0.054	2.600	Whole Blood
GZMB	rs8192917	0.024	0.140	2.300	Artery – Aorta
GZMB	rs8192917	0.032	0.160	2.200	Liver
GZMB	rs8192917	0.035	0.140	2.100	Pancreas
GZMB	rs8192917	0.040	-0.110	-2.100	Skin – Sun Exposed (Lower leg)
GZMB	rs8192917	0.048	0.092	2.000	Muscle – Skeletal
GZMB	rs8192917	0.064	0.240	1.900	Brain – Substantia nigra

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Supplementary Table 2 continued. eQTL signals obtained for SNP rs8192917 on GZMB in 46 types of human tissues.

Gene	SNP	P	NES	T-statistic	Tissue
GZMB	rs8192917	0.070	0.076	1.800	Adipose – Subcutaneous
GZMB	rs8192917	0.074	0.079	1.800	Esophagus – Mucosa
GZMB	rs8192917	0.090	0.140	1.700	Spleen
GZMB	rs8192917	0.095	0.065	1.700	Lung
GZMB	rs8192917	0.120	0.170	1.600	Minor Salivary Gland
GZMB	rs8192917	0.130	0.074	1.500	Stomach
GZMB	rs8192917	0.190	0.095	1.300	Artery – Coronary
GZMB	rs8192917	0.190	0.160	1.300	Cells – EBV-transformed lymphocytes
GZMB	rs8192917	0.200	0.100	1.300	Pituitary
GZMB	rs8192917	0.210	0.073	1.200	Esophagus – Muscularis
GZMB	rs8192917	0.260	0.059	1.100	Breast – Mammary Tissue
GZMB	rs8192917	0.280	0.056	1.100	Heart – Left Ventricle
GZMB	rs8192917	0.300	0.110	1.100	Brain – Hypothalamus
GZMB	rs8192917	0.340	-0.077	-0.950	Testis
GZMB	rs8192917	0.350	0.100	0.950	Brain – Putamen (basal ganglia)
GZMB	rs8192917	0.370	0.054	0.890	Skin – Not Sun Exposed (Suprapubic)
GZMB	rs8192917	0.390	0.065	0.850	Adrenal Gland
GZMB	rs8192917	0.420	0.066	0.800	Small Intestine – Terminal Ileum
GZMB	rs8192917	0.460	0.062	0.740	Prostate
GZMB	rs8192917	0.490	0.067	0.700	Brain – Caudate (basal ganglia)
GZMB	rs8192917	0.500	-0.074	-0.670	Brain – Cortex
GZMB	rs8192917	0.580	-0.064	-0.560	Vagina
GZMB	rs8192917	0.590	-0.060	-0.550	Brain – Frontal Cortex (BA9)
GZMB	rs8192917	0.620	0.023	0.500	Thyroid
GZMB	rs8192917	0.640	-0.051	-0.470	Ovary
GZMB	rs8192917	0.660	-0.059	-0.450	Uterus
GZMB	rs8192917	0.790	0.028	0.270	Brain – Nucleus accumbens (basal ganglia)
GZMB	rs8192917	0.800	-0.012	-0.260	Colon – Transverse
GZMB	rs8192917	0.800	0.013	0.250	Heart – Atrial Appendage
GZMB	rs8192917	0.820	0.034	0.230	Brain – Spinal cord (cervical c-1)
GZMB	rs8192917	0.840	0.014	0.200	Colon – Sigmoid
GZMB	rs8192917	0.910	0.012	0.110	Brain – Anterior cingulate cortex (BA24)
GZMB	rs8192917	0.930	-0.012	-0.090	Brain – Amygdala
GZMB	rs8192917	0.940	-0.003	-0.073	Artery – Tibial
GZMB	rs8192917	0.950	0.008	0.069	Brain – Cerebellum
GZMB	rs8192917	0.980	0.003	0.030	Brain – Hippocampus

NES – normalized effect size. Threshold of *P* value was 0.05/46≈0.001.

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