Association Study Confirmed Susceptibility Loci with Keloid in the Chinese Han Population

Fei Zhu1,2,3,4, Baoyu Wu1,2,3,9, Ping Li1,2,9, Jianbo Wang1,2,3, Huayang Tang1,2,3, Ye Liu4, Xianbo Zuo2,3, Hui Cheng1,2,3, Yantao Ding1,2,3, Wen Wang1,2,3, Yujuan Zhai1,2,3, Fangfang Qian1,2,3, Wenju Wang1,2,3, Xiangfeng Yuan1,2,3, Jing Wang1,2,3, Weiwei Ha1,2,3, Junsheng Hou1,2,3, Fusheng Zhou2,3, Yin Wang4, Jiping Gao1,2,3, Yujun Sheng1,2,3, Liangdan Sun1,2,3, Jianjun Liu2,3, Xuejun Zhang1,2,3

1 Institute of Dermatology and Department of Dermatology, NO.1 Hospital, Anhui Medical University. 2,944 controls were recruited consecutively from the outpatients at the Department of Dermatology, NO.1 Hospital, Anhui Medical University. 3,944 controls were recruited consecutively from the outpatients at the Department of Dermatology, NO.1 Hospital, Anhui Medical University. All subjects were of self-reported Chinese Han ancestry (Table 1). The clinical diagnosis of all cases was confirmed by at least two experienced dermatologists.

Materials and Methods

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* E-mail: ayzxj@vip.sina.com (SY); liuj3@qis.a-star.edu.sg (JL)

† These authors contributed equally to this work.

Abstract

Keloid is benign, proliferative dermal collagen growth with unknown etiology. Recently, a genome-wide association study (GWAS) in Japanese population has identified 3 susceptibility loci (rs873549 at 1q41, rs940187 and rs1511412 at 3q22.3, rs8032158 at 15p21.3) for keloid. In order to examine whether these susceptibility loci are associated with keloid in the Chinese Han population, we recruited 714 patients with keloid and 2,944 controls. We found three SNPs in two regions showed significant association with keloid in the Chinese Han population: 1q41 (rs873549, P = 3.03 × 10−33, OR = 2.05, 95% CI: 1.82–2.31 and rs1442440, P = 9.85 × 10−18, OR = 0.56, 95% CI: 0.49–0.64, respectively) and 15q21.3 (rs2271289 located in NEDD4, P = 1.36 × 10−33, OR = 2.02) and two protective haplotypes of GA and AA (GA, P = 1.94 × 10−19, OR = 0.53, AA, P = 0.00043, OR = 0.78, respectively) from the two SNPs (rs873549 and rs1442440). Our study confirmed two previously reported loci 1q41 and 15q21.3 for keloid in the Chinese Han population, which suggested the common genetic factor predisposing to the development of keloid shared by the Chinese Han and Japanese populations.

Introduction

Keloid is a benign, proliferative dermal collagen growth that represents a pathologic wound-healing response to skin injury. It is characterized by an excessive accumulation of extracellular matrix and especially by overabundant collagen formation, which has escaped the boundaries of the original wound to invade the surrounding normal skin and causes aesthetically displeasing and functionally disabling, even leading to the patients to suffer from both physical and psychological distress [1,2,3,4,5]. Keloid is unique to human and affects some proportion of people in all ethnic populations [1]. Prevalence of keloid varies among different populations, it affects a higher proportion of people of African-Americans and Asians, especially in dark-skinned individuals [6,7,8]. There are limited data on Chinese patients with keloid. Several lines of evidence show the importance of genetic factors in keloid [3,7,9]. Keloid is more common in ethnicities with darker pigmented skins; the familial heritability and prevalence in twins and especially by overabundant collagen formation, which has escaped the boundaries of the original wound to invade the surrounding normal skin and causes aesthetically displeasing and functionally disabling, even leading to the patients to suffer from both physical and psychological distress [1,2,3,4,5]. Keloid is unique to human and affects some proportion of people in all ethnic populations [1]. Prevalence of keloid varies among different populations, it affects a higher proportion of people of African-Americans and Asians, especially in dark-skinned individuals [6,7,8]. There are limited data on Chinese patients with keloid. Several lines of evidence show the importance of genetic factors in keloid [3,7,9]. Keloid is more common in ethnicities with darker pigmented skins; the familial heritability and prevalence in twins also support the concept of the genetic predisposition to keloid. Previous linkage study and candidate gene study have identified genetic factors predisposing to keloid [6,10,11,12], however the results of keloid genetic studies have not been very satisfactory. Recently, GWAS have been proven to be a powerful tool to identity susceptibility genes for common diseases [13]. Nakashima et al [14] performed a GWAS of keloid and identified 3 disease susceptibility loci for keloid in Japanese population. Despite the convincing evidence of its association with keloid in Japanese population, it is not yet known whether these loci play a role in the development of keloid in other populations such as Chinese Han population. The importance of replication in different population should not be overlooked [15].

In this study, we aim to investigate association pattern of these 12 previously reported SNPs for keloid in the Chinese Han population.

Materials and Methods

Subjects

A total of 714 patients with keloid and 2,944 controls were recruited consecutively from the outpatients at the Department of Dermatology, NO.1 Hospital, Anhui Medical University. All subjects were of self-reported Chinese Han ancestry (Table 1). The clinical diagnosis of all cases was confirmed by at least two experienced dermatologists.

These authors contributed equally to this work.
SNP Selection and Genotyping
We selected 12 SNPs with at least marginal association evidence (P < 0.05) based on previous keloid GWAS and other Keloid candidate gene studies and genotyped them in 714 keloid patients. Specifically, 5 SNPs within 3 loci (rs8032158 at NEDD4, rs873549 and rs1442440 at 1q41, rs940187 and rs1511412 at 3q22.3, P = 5.0 × 10^-3) and 3 SNPs within 3 loci (rs2271289 at NEDD4, rs2983632 at 20p11.21, rs12629284 at 3q23, 5.0 × 10^-8 < P < 0.05) based on the Japanese keloid GWAS [14], as well as other four SNPs (rs1866744 at SMAD6, rs11071932, rs9806504 and rs2118610 at SMAD5) based on Afro-Caribbean studies [16]. MAF for 12 SNPs distribution in CHB and JPT (HapMap data) were showed in Table 2. SNPs were genotyped using the Sequenom MassArray system (Sequenom IPLEX assay) at State Key Laboratory Incubation Base of Dermatology, Ministry of National Science and Technology, Hefei, Anhui, China. Approximately 15 ng of genomic DNA was used to genotype each sample. Locus-specific PCR and detection primers were designed using the MassARRAY Assay Design 3.0 software (Sequenom). The DNA samples were amplified by multiple PCR reactions, and the PCR products were then used for locus-specific single-base extension reaction. The resulting products were desalted and transferred to a 384-element SpectroCHIP array. Allele detection was performed using MALDI-TOF MS. The mass spectrometers were analyzed by the MassARRAY Typer software (Sequenom, San Diego, USA).

Statistical Analyses
The distributions of MAF for all SNPs in cases and the controls were assessed by Chi-square test and additive model were used for association. Deviation from Hardy–Weinberg equilibrium (HWE) in controls were calculated and all attained P values > 0.05. Disease associations were analyzed by allelic test, as well as logistic regression and OR and 95% CI were calculated. Independence test of SNPs in the same locus was performed by logistic regression, as well as haplotype-based association test. All statistical analyzis were performed by PLINK 1.07 software [17], unless otherwise specified. Linkage disequilibrium patterns and values were obtained by Haplovie v4.2 [18]. Ten SNPs that passed quality control (P_{HWE} > 0.05 in the control and call rate > 90%) were included for further analysis.

Results
We found significant association evidence at 1q41 (rs873549, P = 3.03 × 10^-33, OR = 2.05, 95% CI: 1.82–2.31 and rs1442440, P = 9.85 × 10^-16, OR = 0.56, 95% CI: 0.49–0.64, respectively) and 15p21.3 (rs2271289, P = 1.02 × 10^-11, OR = 0.66, 95% CI: 0.58–0.74). The other SNPs did not reach the threshold significant association for keloid (P_{heterozygous} > 0.05) in this study. The statistical results of 10 SNPs were summarized in Table 3.

At 1q41 locus, logistic regression analysis indicated two association signals (rs873549, P_{conditional} = 1.82 × 10^-16, OR = 1.81, rs1442440, P_{conditional} = 0.025, OR = 0.78). Haplotype analysis with rs873549 and rs1442440 showed that significant association evidence for one risk haplotype of AG (P = 1.36 × 10^-31, OR = 2.02) and two protective haplotypes of GA and AA (GA, P = 1.94 × 10^-19, OR = 0.53, AA, P = 0.00043, OR = 0.78, respectively, Table 4).

Discussion
We carried out an association study and confirmed the association of three previously reported SNPs within two susceptibility loci 1q41 (rs873549 and rs1442440) and 15p21.3 (rs2271289) for keloid in the Chinese Han population.

At the locus 1q41, the contributions of rs873549 and rs1442440 were confirmed to show stronger association with keloid in the Chinese population (P = 3.03 × 10^-33, OR = 2.05, P = 9.85 × 10^-16, OR = 0.56, respectively) than in populations of Japanese ancestry.
consistent association evidence for keloid ([14,20]). Previously studies demonstrate that phosphatase and tensin homolog (PTEN) [21], insulin-like growth factor I receptor (IGF-IR) [22] and SMAD4 [23] are substrates of NEDD4. The E3 ubiquitin ligase plays a pivotal role in the TGF-β signaling pathway [24,25]. NEDD4 was previously suggested to negatively regulate TGF-β signaling with ubiquitin-mediated degradation of SMAD4 [14]. The TGF-β family is upregulated in keloid tissue and stimulates the proliferation of ESTs in skin by semiquantitative RT-PCR and not identified any initiation codon recognition sequence or any open reading frames.

Hence we should further investigate potential implications of this region for keloid and need functional analysis of these transcripts to clarify their roles on the development of keloid.

At 15p21.3, the SNP rs8032158 within NEDD4 was significant associated within keloid in populations of Japanese ancestry [14]. In this study, we found rs2271289 located in the intron region of NEDD4 associated with keloid in the Chinese Han population (P = 1.02 × 10^{-11}, OR = 0.66), rs8032158 had moderately LD with rs2271289 based on HapMap3 (CHB, D' = 0.96, r^2 = 0.41, and JPT, D' = 1.0, r^2 = 0.34). The results suggested NEDD4 that might be a common genetic factor for the development of keloid within multiple populations in terms of Chinese Han and Japanese, although the most significant SNPs were different among them.

Biologically, NEDD4 is an E3 ubiquitin ligase composed of a C2 domain, three or four WW domains and an ubiquitin ligase Hect domain [19]. NEDD4 is highly expressed in the skin, skeletal muscle, the liver, the bladder, placenta and cancer cell lines [14,20]. Previously studies demonstrate that phosphatase and tensin homolog (PTEN) [21], insulin-like growth factor I receptor (IGF-IR) [22] and SMAD4 [23] are substrates of NEDD4, which have been reported to be associated with keloid. Some studies were indicated NEDD4 may be involved in cellular proliferation or differentiation through various signaling pathways including P53, MAPK or TGF-β [11]. The E3 ubiquitin ligase plays a pivotal role in the TGF-β signaling pathway [24,25]. NEDD4 was previously suggested to negatively regulate TGF-β signaling with ubiquitin-mediated degradation of SMAD4 [14].

### Table 3. Summary of association results 10 SNPs within 7 loci replicated in the Chinese Han population with keloid.

| SNP          | Chr | Gene | Allele(minor/major) | MAF  | P-value | OR   | 95% Cl |
|--------------|-----|------|---------------------|------|---------|------|--------|
| rs873549     | 1q41| G/A  | 0.53                | 0.35 | 3.03 × 10^{-33} | 2.05 | 1.82–2.31 |
| rs1442440    | 1q41| G/A  | 0.25                | 0.37 | 9.85 × 10^{-18} | 0.56 | 0.49–0.64 |
| rs940187     | 3q22| A/G  | 0.04                | 0.03 | 0.01291 | 1.48 | 1.08–2.017 |
| rs1511412    | 3q22| A/G  | 0.014               | 0.007| 0.01596 | 1.90 | 1.12–3.24 |
| rs12629284   | 3q22| T/C  | 0.49                | 0.50 | 0.5417  | 0.96 | 0.86–1.08  |
| rs2271289    | 15q23| T/C | 0.35                | 0.45 | 1.02 × 10^{-11} | 0.66 | 0.58–0.74 |
| rs1866744    | 15q23| T/C | 0.46                | 0.44 | 0.2423  | 1.07 | 0.95–1.21 |
| rs11071932   | 15q23| A/G  | 0.002               | 0.001| 0.194   | 2.50 | 0.597–10.48 |
| rs2118610    | 15q23| A/G  | 0.114               | 0.106| 0.5168  | 1.06 | 0.88–1.28 |
| rs2983632    | 20p11.21| A/G | 0.4076              | 0.4007| 0.6348  | 1.03 | 0.91–1.16 |

MAF, minor allele frequency; OR, odds ratio. 95% CI, 95% confidence intervals.

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### Table 4. Haplotype association analysis between rs873549 and rs1442440 in patients and controls.

| SNP         | Haplotype | Cases frequency | Controls frequency | OR      | P value |
|-------------|-----------|----------------|--------------------|---------|---------|
| rs873549 G/A | GG        | 0.019          | 0.013              | 1.57    | 0.1126  |
|             | AG        | 0.506          | 0.337              | 2.02    | 1.36 × 10^{-31} |
| rs1442440 G/A | GA        | 0.229          | 0.356              | 0.53    | 1.94 × 10^{-19} |
|             | AA        | 0.247          | 0.294              | 0.78    | 0.00043 |

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fibroblasts. TGF-β is also known to promote type I collagen synthesis and inhibit the transcription of collagenase [26]. These facts suggest that the genetic variation(s) in NEDD4 might affect fibroblast proliferation and keloid formation. However, further study is warranted to explore its exact role in the development of keloid.

At 3p22.3, the SNPs rs940187 and rs1511412 within FOXL2 were significant associated with keloid in Japanese population ($P = 1.80 \times 10^{-13}$, OR = 1.98, and $P = 2.31 \times 10^{-13}$, OR = 1.87, respectively) [14]. In this study we did not observed significant association for keloid in Chinese Han population ($P = 0.013$, OR = 1.48, and $P = 0.016$, OR = 1.87, respectively) ($P_{\text{Bonferroni}} > 0.05$). OR indicates that these two SNPs probably are associated with keloid in the Chinese Han population. It’s probable that the frequency of these two variants in Chinese Han population was relatively low and the sample size was not very large in this study, therefore the statistical power of association tests was limited. Of course, it also might be due to the existence of susceptibility heterogeneity for keloid between Chinese Han and Japanese populations.

In summary, we not only confirmed two susceptibility loci for keloid (1q41 and NEDD4 at 15q21.3) in the Chinese Han population but also indicated common genetic factors shared by both Chinese Han and Japanese populations.

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Author Contributions

Conceived and designed the experiments: F. Zhu BYW PL SY JYL. Performed the experiments: JBW YL YTD Wen Wang YJZ. Analyzed the data: XBZ LDS HC HVT. Contributed reagents/materials/analysis tools: F. Zhou XJZ. Wrote the paper: BYW.

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