Original Article
SNP rs1511412 in FOXL2 gene as a risk factor for keloid by meta analysis

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Abstract: Objective: Determine whether SNP rs1511412 is associated with keloid. Design and methods: One large-scale GWAS identified association between SNP rs1511412 in the FOXL2 gene and keloid disease in the Japanese population. However, researchers didn’t observe significant association for keloid in Chinese Han population (P(Bonferroni)>0.05). It’s probable that the frequency of this variant in Chinese Han population was relatively low and the sample size was not very large in this study (power =45.5). We performed an independent case control association study in the Chinese Han population and a follow-up large scale meta-analysis for SNP rs1511412. Results: Our study included 309 keloid patients and 1080 controls of the Chinese Han population. A significant association was found between SNP and keloid (P=0.02, OR=2.23). Meta-analysis included 1847 keloid patients and 7229 controls combined from five Asian populations. The association between SNP rs1511412 and keloid became highly significant (P<1×10^-8 OR=1.89). Conclusion: We conclude that SNP rs1511412 in FOXL2 is indeed a genetic risk factor for keloid across different ethnic populations.

Keywords: Meta analysis, keloid, SNP

Introduction

Keloid is a dermal fibroproliferative growth caused by pathologic wound healing following skin injury. Keloid is defined as a scar growing continuously and invasively beyond the confines of the original wound and is characterized by excessive fibroblast proliferation and deposition of extracellular matrix and collagen fibers. The fact that the incidence of keloid is higher in darker-skinned individuals suggests that genetic factors play an important role.

With linkage analyses, researchers have revealed that a keloid susceptibility locus is present on chromosomes 7p11, 2q23, and 18q21.1 in African-American, Japanese [1], and Chinese ethnic lineages, respectively [2]. But the responsible genes have not been identified [1]. After that, Nakashima et al. [3] performed a multistage genome wide association study in 824 Japanese individuals with keloid and 3,205 Japanese controls and identified significant associations of keloid with 4 SNPs (single nucleotide polymorphisms) in 3 new chromosomal regions, especially rs1511412 in FOXL2 within loci 3q22.3. However, cause by the P value couldn’t pass the threshold of bonferroni correction, Fei Zhu et al. [4] failed to identify the similar effect in separate Chinese population, which may due to the small sample size.

In this study, we carried out a case control study to assess the association between SNP rs1511412 in the FOXL2 gene on chromosome 3q22.3 and keloid in another independent Chinese population, and used meta-analysis method combining data together to make sample size exponential growth to get enough power to clarify inconsistent results of this association study [5]. Here, we performed such a study as well as a follow-up meta-analysis with five combined Asian populations to provide a more precise estimate of this association.
### Table 1. Association result of current study and the characteristics of the populations used for meta-analysis

<table>
<thead>
<tr>
<th>Studies</th>
<th>population</th>
<th>number (case/control)</th>
<th>Age, year (case/control)</th>
<th>Male % (case/control)</th>
<th>Minor allele frequency (case/control)</th>
<th>OR (95%CI)</th>
<th>P</th>
<th>p-hwe</th>
<th>power</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zhu et al. (2013)</td>
<td>China</td>
<td>3658 (714/2944)</td>
<td>30.72±12.99/30.38±9.73</td>
<td>44.7/48.8</td>
<td>0.015/0.008</td>
<td>1.962 (1.152-3.339)</td>
<td>1.14E-02</td>
<td>0.682</td>
<td>45.5</td>
</tr>
<tr>
<td>Current study</td>
<td>China</td>
<td>1389 (309/1080)</td>
<td>30.57±12.56/61.09±13.99</td>
<td>46.6/56.1</td>
<td>0.01471/0.006648</td>
<td>2.23 (1.093-4.552)</td>
<td>2.37E-02</td>
<td>0.678</td>
<td>31.8</td>
</tr>
<tr>
<td>Mitsuko et al. (2010)-first stage</td>
<td>Japan</td>
<td>1122 (188/934)</td>
<td>26.7±6.2/52.2±14.6</td>
<td>34/73.7</td>
<td>0.14/0.08</td>
<td>1.92 (1.36-2.72)</td>
<td>2.20E-04</td>
<td>0.386</td>
<td>74.8</td>
</tr>
<tr>
<td>Mitsuko et al. (2010)-second stage</td>
<td>Japan</td>
<td>1780 (329/1451)</td>
<td>53.69±18.1/52.36±11.8</td>
<td>42.9/46.6</td>
<td>0.15/0.08</td>
<td>1.85 (1.43-2.40)</td>
<td>2.32E-06</td>
<td>0.307</td>
<td>88.3</td>
</tr>
<tr>
<td>Mitsuko et al. (2010)-replication</td>
<td>Japan</td>
<td>1127 (307/820)</td>
<td>46.9±20.9/59.55±15.6</td>
<td>36.8/55.7</td>
<td>0.14/0.08</td>
<td>1.86 (1.39-2.48)</td>
<td>2.48E-05</td>
<td>0.963</td>
<td>82.6</td>
</tr>
</tbody>
</table>


Table 2. Association power detect of Chinese and Japanese group

<table>
<thead>
<tr>
<th>Group</th>
<th>Minor allele frequency (case/control)</th>
<th>P-hwe</th>
<th>OR (95% CI)</th>
<th>Power</th>
</tr>
</thead>
<tbody>
<tr>
<td>Japan</td>
<td>0.1416/0.0812</td>
<td>0.626</td>
<td>1.870 (1.580-2.210)</td>
<td>99.8</td>
</tr>
<tr>
<td>China</td>
<td>0.0149/0.007</td>
<td>0.478</td>
<td>2.104 (1.3846-3.1978)</td>
<td>66.5</td>
</tr>
</tbody>
</table>

Materials and methods

Keloid and controls

Based on the criterion that a scar escaped the boundaries of the original wound to invade the surrounding normal skin, all the patients diagnosed as keloids were recruited from the department of Dermatology, Anhui Provincial Hospital, China. Besides, patients with hypertrophic scars or some syndromes (e.g., Rubinstein-Taybi syndrome) were excluded from our study. Finally, in this study, we collected 309 keloid patients and 1080 controls of the Chinese Han population to investigate the association signal between SNP rs1511412 in the FOXL2 gene and keloid disease.

Genomic DNA extracting and SNP genotyping

After informed consent, genomic DNA was isolated from peripheral blood of the patients using a Qiagen kit (Hilden, Germany). In addition, genomic DNA of 1080 unrelated healthy individuals was extracted as a control.

We genotyped SNP rs1511412 using the Sequenom iPLEX platform (Sequenom, Inc., San Diego, CA, USA).

Statistical analyses

Hardy Weinberg equilibrium tests of the genotyping data were performed using PLINK (version 1.07). The power of the study population was estimated using a free Power and Sample (PS) size calculation program (PS version 3.1.2).

For allelic association between a SNP and keloid, the P value and corresponding odds ratio (OR) with a 95% confidence interval were computed by Chi-square tests using Pearson’s 2X2 contingency tables as implemented in PLINK version 1.07.

For meta-analysis, we searched publically PubMed database (the US National Library of Medicine) using key words of “rs1511412”, “keloid”, “association”, or “GWAS”. Three reports were found for analyses of association between the SNP rs1511412 and keloid. Only those published studies with full-text articles in English and enough sample size were included in this meta-analysis, one study was excluded (204 Japanese patients and HapMap Japanese controls, power =9) [6]. We also extracted some basic information, such as ethnicity, publication years, gender ratios and other relevant information, if any, for both cases and controls in each study.

Meat-analysis was performed using the Comprehensive Meta-Analysis V2 software program. All the allelic data or genotypic data, ORs and 95% confidence intervals from different studies were inputted into the software program for running statistical analyses. The heterogeneity between different studies was tested using Q tests and I-square (I2) and P values were computed.

Results

Results of case-control study

We used a case-control population to determine whether SNP rs1511412 is associated with keloid in the Chinese population. This population consists of 309 keloid patients and 1080 controls (Table 1). The call rate of genotyping was 99.2% for the SNP rs1511412. Genotypes in controls were in accordance with Hardy-Weinberg equilibrium (P=0.678). Significant difference was observed in distribution of genotypes between cases and controls (x²= 5.119, P=0.0237).

Result of power calculation

We detected the power of each study population, and In the Japanese populations, the power is bigger than 70 percent, but in Chinese populations, the power all small than 50 percent (Table 1). Besides, we also detected the power of Chinese and Japanese group, and the result shows that the Japanese get power of 99.8 percent while the Chinese get 66.5 percent (Table 2).
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Table 3. Meta-analysis in combined Asian populations and Japanese/Chinese group

<table>
<thead>
<tr>
<th>Subgroups</th>
<th>Study characters</th>
<th>Z test for pooled effect size</th>
<th>Q test for heterogeneity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N (study)</td>
<td>N (case/control)</td>
<td>Z_value</td>
</tr>
<tr>
<td>Asian</td>
<td>5</td>
<td>1847/7229</td>
<td>7.981</td>
</tr>
<tr>
<td>Japanese group</td>
<td>3</td>
<td>824/3205</td>
<td>7.275</td>
</tr>
<tr>
<td>Chinese group</td>
<td>2</td>
<td>1023/4024</td>
<td>3.308</td>
</tr>
</tbody>
</table>

Meta-analysis

Two previous independent studies had analyzed the association between rs1511412 and keloid in Asian population. One is the large scan GWAS association study with Japanese population shows positive result, the other is a replication study with Chinese population get a negative result cause by couldn’t pass the threshold of bonferroni correction P value. To conclusively assess the association between rs1511412 and keloid, we performed a meta-analysis in the Asian populations. The characteristics of the populations used for meta-analysis are shown in Table 1.

We performed a heterogeneity analysis with a Q test, and found no heterogeneity among the Asian populations or each subgroup populations (Table 3).

The meta-analysis in all five combined Asian populations (1847 cases and 7229 controls) revealed a highly significant association between SNP rs1511412 and keloid (P<1×10⁻⁸, OR=1.894) (Table 3; Figure 1). Similar analysis also identified significant association result in Japanese and Chinese group (Table 3). These results provide strong genetic evidence that rs1511412 confers a highly significant risk of keloid in the Asian population.

Discussion

In our case-control study, a significant association between the SNP rs1511412 and keloid risk was revealed in Chinese population. Additionally, the following meta-analysis integrating our current study and 2 previous studies in five Asian populations with a total of 1847 cases and 7229 controls demonstrated the association between rs1511412 and keloid.

The rs1511412 is located on chromosome 3q22.3 involved gene FOXL2, which according to the Gene Expression Omnibus (GEO) is highly expressed in the pituitary gland, ovary and extraocular muscle and encodes a fork-head transcription factor that can binding DNA and stimulating the expression of gonadotropin-releasing hormone (GnRH) [7] and regulates cholesterol metabolism and steroidogenesis.
A nationwide population-based study describes that since women outnumbered men with a 1.33 ratio, and women with uterine leiomyoma have a 2.25-fold greater risk of keloids compared with women without leiomyoma [9]. In addition, keloids appear more often in puberty, enlarge during pregnancy and tend to decrease in size of keloid after menopause [10]. Besides, it has been reported that mutation of FOXL2 may cause blepharophimosis-ptosis-epicanthus inversus syndrome or premature ovarian failure [11]. It also has been reported that tamoxifen and nonsteroidal anti-estrogens inhibit keloid fibroblast proliferation and reduce collagen production through down regulation of TGF-β [12, 13]. Therefore, it was assumed that genetic variations in FOXL2 might contribute to keloid susceptibility through effects on the levels of GnRH and/or steroid hormones [3], and the gonadal hormones and pregnancy estrogens might influence keloid formation.

In conclusion, the current case-control study clarify the significant association between rs1511412 and keloid, and in spite of the power of Chinese group calculated in this study is not big enough (<80), the follow-up meta-analysis confirmed that rs17465637 is a risk factor for keloid. However, it is needed to recollect more Chinese sample and implement fine-mapping of 3q22.3 region or function analysis to identify causal variant.

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Disclosure of conflict of interest

None.

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