Replication study of four keloid-associated polymorphisms in patients of European descent – a single centre study

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SUMMARY Keloid is defined as a benign dermal fibro-proliferative growth that extends outside the original wound and invades adjacent dermal tissue. Its pathogenesis is complex and much evidence suggests the influence of genetic factors, including the rs873549, rs1511412, rs940187 and rs8032158 polymorphisms associated with keloid risk in Japanese patients. The aim of our study was to investigate possible associations between rs873549, rs1511412, rs940187 and rs8032158 variants and the risk of keloid in Polish patients of European descent. The genetic polymorphisms were identified by sequencing genomic DNA extracted from peripheral blood leukocytes from 86 keloid patients and from newborn cord blood leukocytes from 100 newborns as a control group. No significant differences (p > 0.05) in the distributions of rs873549, rs1511412, rs940187 and rs8032158 alleles were found between keloid patients and newborn controls (26.7% vs 25.5%, 9.9% vs 7.0%, 19.8% vs 12.5%, and 41.9% vs 33.5%, respectively). Logistic regression with adjustment for gender revealed that only the CC homozygous genotype of rs8032158 polymorphism was significantly more frequent in keloid patients as compared with controls (19.8% vs 11.0%, respectively). Our results suggest that in contrast to Asian populations only the rs8032158 polymorphism at locus 15q21.3 is associated with the susceptibility to keloid scarring in patients of European descent.

Keywords association study, genetic polymorphism, Caucasians, keloid

1. Introduction

Keloid is defined as benign proliferative scars that grow beyond the confines of original insults to the skin, invading into adjacent normal tissue. This differentiates keloid from hypertrophic scars, which are raised scars that remain within the boundaries of the inciting cutaneous insult (1). The pathogenesis of keloid is complex, and much evidence suggests the influence of genetic factors. The main sources of evidence indicating genetic predisposition to keloid formation are as follows: familial inheritance, ethnic differences in keloid prevalence, and the identification of chromosomal loci or SNPs (Single Nucleotide Polymorphisms) for keloid via linkage analysis or association studies, respectively (1,2).

In 2010 Nakashima et al. through a multistage genome-wide association study identified four SNPs, which significantly predisposed to keloid in the Japanese population. The authors reported that the most significant association with keloid was with rs873549 on chromosome 1q41, with rs1511412 and rs940187 in two separate linkage disequilibrium (LD) blocks on chromosome 3q22.3-23, and with rs8032158 on chromosome 15q21.3 (3). So far the associations of SNPs on chromosomes: 1q41, 3q22.3-23 and 15q21.3 with keloid have been confirmed in Asian patients (4-8) and also that the latter locus was also associated with predisposition to keloid in Afro-Americans (9). Therefore, this raises the question whether the four SNPs also predispose to keloid formation in patients of European descent. To further address this issue we decided to investigate the possible associations of rs873549, rs1511412, rs940187 and rs8032158 polymorphisms with the risk of keloid in Polish patients.

2. Materials and Methods

2.1. Keloid group and controls

The study group consisted of 86 consecutive patients
with keloid (17 males and 69 females, aged from 18 to 70 years old), who were treated with surgical excision at a cosmetic surgery clinic (Aesthetic Med, Szczecin, Poland). Forty eight patients had been diagnosed with keloid scarring as a postoperative complication from previous surgery; multiple keloid scars were present in 26 patients; and 10 subjects reported the presence of keloid in first degree relatives. The control group consisted of 100 healthy, full-term newborns (52 males and 48 females) randomly chosen from the Newborn DNA Repository at the Pomeranian Medical University in Szczecin. All patients in the study and control groups were Poles of European descent. The study was conducted in accordance with the Declaration of Helsinki (2013) and was approved by bioethics committee at the Pomeranian Medical University in Szczecin. Patients' informed consent for cases and parental informed consent for newborn controls were obtained.

2.2. Genotyping

The rs873549, rs15111412, rs940187 and rs8032158 polymorphisms were identified by sequencing amplicons from genomic DNA (sequences of primers for polymerase chain reaction are available upon request) extracted from peripheral blood leukocytes from keloid patients and from newborn cord blood leukocytes from newborns, as described previously (10).

2.3. Statistical analyses

Possible divergence of genotype frequencies from Hardy-Weinberg equilibrium and differences in alleles frequencies between groups were assessed using χ² tests. Genotype frequencies between groups were compared by logistic regression with adjustment for gender (note that the controls/cases were not matched for gender) in additive, dominant or recessive modes of inheritance for the risk allele. Calculations were performed using a data analysis software system (Dell Statistica, version 13, Dell Inc. 2016, software.dell.com). A two-tailed \( p < 0.05 \) was considered statistically significant.

Table 1. Association analysis of four SNPs (rs873549, rs15111412, rs940187 and rs8032158) with keloid in Caucasians

| SNP (Position) | Allele (1/2) | Cases 1/2 | Controls 1/2 | \( p \) | Cases 11 | 12 | 22 | Controls 11 | 12 | 22 | \( p^c \) | OR (95% CI) |
|---------------|-------------|-----------|-------------|-------|---------|----|-----|-----------|----|-----|-------|-----------|
| rs873549 (1: 222,271,767) | TC | 126/46 | 149/51 | 0.785 | 52 | 22 | 12 | 56 | 37 | 7 | 0.453 | 1.48 (0.53-4.13) |
| rs15111412 (3: 138,713,704) | GA | 155/17 | 186/14 | 0.316 | 69 | 17 | 0 | 86 | 14 | 0 | 0.236 | 1.65 (0.72-3.80) |
| rs940187 (3: 138,841,593) | CT | 138/34 | 175/25 | 0.056 | 55 | 28 | 3 | 78 | 19 | 3 | 0.896 | 1.12 (0.20-6.35) |
| rs8032158 (15: 56,194,877) | TC | 100/72 | 133/67 | 0.096 | 31 | 38 | 17 | 44 | 45 | 11 | 0.046 | 2.47 (1.01-6.08) |

\( ^a \)SNP position was indexed to the NCBI build 37 (GRCh37.p13). \(^b \)Allele 1 and allele 2 were defined as the non-susceptible allele or the risk allele, respectively. \(^c \)\( p \) values for logistic regression with adjustment for gender in recessive mode of inheritance for the risk allele. \(^d \)OR (Odds Ratios) and CI (Confidence Intervals) were calculated using the non-susceptible allele as a reference.

3. Results and Discussion

The rs873549, rs15111412, rs940187 and rs8032158 genotype distributions in the control group conformed to expected Hardy-Weinberg distributions (\( p = 0.793, p = 0.452, p = 0.189 \) and \( p = 0.920 \), respectively). No significant differences in the distributions of rs873549, rs15111412, rs940187 and rs8032158 alleles were found between keloid patients and newborn controls (Table 1). Logistic regression with adjustment for gender revealed no significant associations between rs873549, rs15111412, rs940187 or rs8032158 and predisposition to keloid in additive or dominant modes of inheritance for the risk allele (data not shown). There were also no significant associations between rs873549, rs15111412 or rs940187 polymorphisms and keloid risk in recessive mode of inheritance for the risk allele. Only the CC homozygous genotype of rs8032158 polymorphism was significantly more frequent in keloid patients as compared with controls (Table 1). Our results confirm the association of rs8032158 polymorphism at locus 15q21.3 with keloid, as well as showing its occurrence in another ethnic group. However, we are fully aware that a major limitation of our study is relatively low statistical power due to small sample size, and further studies might well show associations for the other polymorphisms. Our calculation for rs8032158 in the recessive mode of inheritance (performed with the Genetic Power Calculator; http://zzz.bwh.harvard.edu/gpc/) revealed that a minimum of 238 keloid patients would be necessary to achieve 80% statistical power under the assumption of a 5% type I error rate (\( \alpha \)), and therefore the discovery of an association was fortuitous but substantial.

The rs8032158 SNP maps to intron 4 of the NEDD4 (Neural Precursor Cell Expressed, Developmentally Down-Regulated 4) gene encoding a ubiquitin ligase (3). In addition, rs8032158 is in linkage disequilibrium with rs2271289 in intron 5 of NEDD4, which was revealed to be associated with keloid in two independent studies of Chinese Han (4, 8). On the other hand, the DNA sequencing of the NEDD4 region in 94 keloid patients has excluded linkage of rs8032158 with hidden causative mutations (3). Using an in silico approach (https://
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