Original Paper

Association of ADAM33 Gene Polymorphisms with Keloid Scars in a Northeastern Chinese Population

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Key Words

Keloid scar • ADAM33 • Single nucleotide polymorphism • Association study

Abstract

Objective: To study the association between ADAM33 and keloid scars in the northeastern Chinese population. **Methods:** A total of 283 keloid scar patients and a control group of 290 healthy volunteers were recruited for this study. Six polymorphic loci (V4, T+1, T2, T1, S2 and Q-1) of ADAM33 were selected for genotyping. Genotypes were determined by using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. **Results:** We observed the frequency of the rs612709 A allele exhibited a significantly decreased frequency in cases than in controls (22 vs. 39.6%, $P < 0.0001$). We also found that the frequencies of H2 (GGAAGA) haplotypes was significantly higher in the case group than in the control group ($P = 0.041$). In contrast, the haplotype H8 (GGGAGG) was more common in the control group than in the case group ($P = 0.022$). **Conclusions:** Our data suggest that the ADAM33 polymorphisms may be associated with keloid scars in the northeastern Chinese population.

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Introduction

The formation of keloid scars (KS) after skin trauma is a significant clinical problem. They can actively persist for many years. Keloid scars and hypertrophic scars have a similar clinical appearance of excessive scarring. They are characterized by overproduction

of the extracellular matrix, mainly type I collagen, fibronectin and proteoglycans, but keloids, unlike hypertrophic scars, show a tendency to grow beyond the sites of original injury without spontaneous regression [1]. After early stage of granulation, with predominance of macrophages and increasing number of fibroblasts synthesizing new extracellular matrix, tissue remodelling occurs through granulation tissue contraction. Keloid scar is a familial condition [2], occurring more commonly in ethnic groups with darker skin [3]. The highest incidence of keloids is found in the black population, where it has been estimated to be around 4–6% and up to 16% in random samples of black Africans [4]. The mechanism of keloid scar formation is largely unknown [5, 6]. As increased familial aggregation, a higher prevalence in certain races, parallelism in identical twins, and alterations in gene expression favor the contribution of genetic risk factors to the development of keloid scar [7], researchers proposed that the mode of inheritance in keloid scar is autosomal recessive; [8] however, no single gene has yet been identified. Some genetic markers have been reported to have significant importance in diagnosis, prognosis, and development of keloid scar.

A disintegrin and metalloprotease 33 (ADAM33) gene, is a member of ADAM family. ADAM33 protein is a zinc-dependent endopeptidase, with pro-domain, catalytic, disintegrin-like, cysteine rich and epidermal growth factor-like domain [9]. It is abundantly expressed in smooth muscle and fibroblasts [10]. ADAM proteins are involved in cell adhesion, cell fusion, cell signaling, and proteolysis [9, 11]. The latter can be illustrated by the capacity to shed cytokines, growth factors, or their receptors from the cell surface and the remodeling of extracellular matrix components. Garlisi demonstrated that ADAM33 is an active proteinase that is able to cleave α 2-macroglobulin [12] and synthetic peptides [13]. The enzymatic activity of ADAM33 can be inhibited by tissue inhibitor of metalloproteinase-3 and -4 (TIMP-3 and -4, respectively) as well as several small molecules [13]. This suggests that ADAM33 is involved in pulmonary defenses and tissue remodeling. Van Eerdewagh found the SNPs in ADAM33 gene was associated with asthma and bronchial hyper-responsiveness, it is the first time that ADAM33 gene was identified as a susceptibility gene for bronchial hyper-responsiveness by positional cloning techniques [11]. Furthermore, ADAM33 has been correlated with many cutaneous inflammatory and immune-mediated disorders [14–16]. We used a case-control study to systematically investigate the association between ADAM33 polymorphisms and keloid scar. We determined the prevalence of single nucleotide polymorphisms (SNPs) and haplotypes of the ADAM33 gene using 573 samples (283 keloid scar patients and 290 healthy controls) from the Han population of northeastern China. Our study provides significant data to support the involvement of ADAM33 in the pathogenesis of keloid scar.

Materials and Methods

The case-control study consisted of 283 northeastern Chinese individuals with keloid, and 290 healthy Chinese individuals as controls. 283 keloid patients whose diagnosis was confirmed by dermatologists in the First Affiliated Hospital of Harbin Medical University. Rigorous clinical criteria were used to differentiate KD from HTS, to limit the potentially confounding effects of misdiagnosis. We defined a keloid scar as a dermal lesion that had spread beyond the margins of the original wound, continued to grow over time, not regressed spontaneously commonly following excision and been present for a minimum of 1 year.

All the patients were interviewed, and a full medical history was obtained using a proforma. Every patient's scar was examined and photographed. 290 healthy individuals with no personal or family history of abnormal scar, were selected from Medical Examination Center of the First Affiliated Hospital of Harbin Medical University as controls. The detail characters were shown in Table 1. All individuals both in case and control groups were informed consent. The study was carried out with the approval of the clinical research ethics committee of Harbin Medical University. Genomic DNA was extracted from 200 μ l of peripheral blood using the Qiamp DNA Blood Mini Kit (Qiagen, Germany) and following the manufacturer's protocols.

Table 1. Description of the studied populations

	case	Control
Number of patients	283	290
age	43.6(7.1)	52.6(12.8)
male	101	124

Table 2. Description of the investigated ADAM33 SNPs

Chromosome	Position	SNP ID	Reference	SNP Name	Allels	Primer Sequences
3589161			2787094	V4	C/G	F: 5'-ACACACAGAATGGGGGAGAG-3' R: 5'-CCAGAAGCAAAGGTCACACA-3'
3590127			2280089	T+1	A/G	F: 5'-CTGAGCCCAGAAACCTGATT-3' R: 5'-AGAAGGGAAGGGCTCATGC-3'
3590205			2280090	T2	A/G	F: 5'-TTCTCAGGGTCTGGGAGAAA-3' R: 5'-GCCAACCTCCTGGACTCTTA-3'
3590234			2280091	T1	A/G	F: 5'-ACTCAAGGTGACTGGGTGCT-3' R: 5'-GAGGGCATGAGGCTCACTTG-3'
3591742			528557	S2	C/G	F: 5'-AGAGCTCTGAGGAGGGGAAC-3' R: 5'-TGTGCAGGCTGAAAGTATGC-3'
3592207			612709	Q-1	A/G	F: 5'-GGATTCAAACGGCAAGGAG-3' R: 5'-GTTTACCTAGATGGCCAGGA-3'

Table 3. The frequencies of genotypes in ADAM33

SNP	Genotype	Frequency (%)		P-Value	Odds ratio and Confidence intervals (95% CI)
		Case (283)	Control (290)		
rs2787094 (V4)	CC	69 (0.2438)	78 (0.2689)	0.491	0.876(0.602-1.276)
	CG	94 (0.3321)	97 (0.3345)	0.953	0.990(0.699-1.401)
	GG	120 (0.4240)	115(0.3966)	0.504	1.120(0.803-1.563)
rs2280089 (T+1)	AA	30 (0.1060)	25(0.0862)	0.422	1.257(0.719-2.196)
	AG	64(0.2261)	51(0.1759)	0.133	1.370(0.908-2.066)
	GG	189(0.6678)	214(0.7379)	0.067	0.714(0.498-1.024)
rs2280090 (T2)	GG	189(0.6678)	182 (0.6276)	0.314	1.193(0.846-1.682)
	AG	86(0.3039)	99 (0.3414)	0.338	0.842(0.593-1.196)
	AA	8(0.0283)	9(0.0310)	0.845	0.908(0.345-2.388)
rs2280091 (T1)	AA	215(0.7597)	236(0.8138)	0.114	0.723(0.484-1.082)
	AG	63(0.2226)	50(0.1724)	0.131	1.375(0.909-2.079)
	GG	5(0.0177)	4(0.0138)	0.709	1.286(0.342-4.839)
rs528557 (S2)	CC	28(0.0989)	17(0.0586)	0.073	1.763(0.943-3.299)
	CG	79(0.2792)	88(0.3034)	0.523	0.889(0.620-1.275)
	GG	176(0.6219)	185(0.6379)	0.691	0.934(0.665-1.311)
rs612709 (Q-1)	AA	12(0.0424)	53(0.1828)	<0.0001	0.198(0.103-0.379)
	AG	101(0.3569)	124(0.4276)	0.083	0.743(0.531-1.040)
	GG	170(0.6007)	113(0.3897)	<0.0001	2.356(1.686-3.295)

SNP Genotyping

Genotyping was performed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis. The polymorphic region was amplified by PCR using a T-Gradient Thermoblock PCR System (Iometra, Germany) in a 25 µl reaction solution containing 0.3µg genomic DNA, 10× PCR buffer, 0.3 mM MgCl₂, 0.2mM dNTPs, 2 U TaqDNA polymerase (Takara, Japan), and 0.1 µmol of each primer (Invitrogen, USA). Six SNPs in ADAM33 (V4[rs2787094], T+1[rs2280089], T2[rs2280090], T1[rs2280091], S2[rs528557] and Q-1[rs612709]) were investigated in this case-control study. SNP names and primer sequences are listed in Table 2. According to the manufacturer's instructions, PCR products were digested overnight with restriction enzymes (NEB, UK) and analyzed by agarose gel electrophoresis.

Table 4. The frequencies of alleles of ADAM33

SNP	Allele	Minor allele frequency (%) case(283)	control(290)	P-Value	Odds ratio and Confidence intervals (95% CI)
rs2787094 (V4)	C:G	0.4099	0.4362	0.368	0.898(0.710-1.135)
rs2280089 (T+1)	A:G	0.2191	0.1741	0.056	1.330(0.993-1.783)
rs2280090 (T2)	A:G	0.1802	0.2017	0.355	0.870(0.648-1.168)
rs2280091 (T1)	G:A	0.1290	0.1000	0.123	1.333(0.924-1.922)
rs528557 (S2)	C:G	0.2385	0.2103	0.253	1.176(0.891-1.553)
rs612709 (Q-1)	A:G	0.2208	0.3966	<0.0001	0.431(0.333-0.559)

Table 5. The distribution of haplotypes of ADAM33

haplotype	rs2787094	rs2280089	rs2280090	rs2280091	rs528557	Rs612709	Frequency (%)	Frequency (%)	P-Value	Odds ratio and Confidence intervals (95% CI)
	V4	T+1	T2	T1	S2	Q-1	case(283)	control(290)		
H1	G	G	G	A	G	A	0.174	0.159	0.334	0.808(0.524-1.246)
H2	G	G	A	A	G	A	0.168	0.201	0.041	1.583(1.617-2.463)
H3	C	G	G	G	C	A	0.122	0.113	0.512	0.845(0.512-1.396)
H4	C	G	G	G	G	A	0.063	0.057	0.540	0.809(0.410-1.595)
H5	G	G	G	A	C	A	0.057	0.053	0.642	0.846(0.418-1.713)
H6	G	G	G	G	G	A	0.031	0.035	0.595	1.291(0.502-3.320)
H7	G	G	G	G	G	G	0.023	0.028	0.376	1.658(0.536-5.131)
H8	C	G	G	A	G	G	0.021	0.007	0.022	0.199(0.043-0.918)
H9	G	A	A	G	C	A	0.020	0.021	0.730	1.235(0.373-4.092)
H10	C	G	G	A	G	A	0.018	0.018	0.969	1.025(0.294-3.580)
H11	G	G	G	A	C	G	0.011	0.007	0.429	0.509(0.092-2.801)

Statistical analysis

Each SNP was tested for deviation from Hardy-Weinberg equilibrium (HWE) using chi-square with expected frequencies derived from allele frequencies. Frequencies of each allele and genotypes were calculated directly and chi-square tests were carried out using SPSS software for windows (Version 13.0; SPSS Inc, Chicago, IL), significance was defined as $p < 0.05$. Haplotype block was constructed in Haploview 4.1 software (<http://www.broad.mit.edu/mpg/haploview>). The relative association with rare alleles was estimated as an odds ratio (OR) with a 95% confidence interval (CI).

Results

Frequency distributions in alleles and genotypes of ADAM33 polymorphisms

Deviations from Hardy-Weinberg equilibrium were not seen in case and healthy groups for any of six SNPs. The genotype and allele frequencies of all six ADAM33 SNPs in the two groups are shown in Table 3 and Table 4. The genotype frequencies of Q-1[rs612709] AA, CG were higher in keloid scar groups than those in controls ($P < 0.0001$, OR = 0.198, 95% CI = 0.103–0.379 and $p < 0.0001$, OR = 2.356, 95% CI = 1.686–3.295, respectively) (shown in Table 3). As shown in Table 4, the frequency of the rs612709 A allele exhibited a significantly

decreased frequency in cases than in controls (22 vs.39.6%, $P<0.0001$). In contrast, we did not find statistically significant differences in the allele frequencies in V4[rs2787094], T+1[rs2280089], T2[rs2280090], T1[rs2280091], S2[rs528557] between cases and controls and these polymorphisms were not associated with keloid scar.

ADAM33 haplotype analysis

The haplotypes of cases and controls were constructed using Haploview 4.1 software. The haplotypes were selected for further analysis which frequencies were not less than 1.0%. There were 11 haplotypes in all samples. Haplotype analysis showed that the frequencies of H2 (GGAAGA) haplotypes was significantly higher in the case group than in the control group ($P=0.041$). In contrast, the haplotype H8 (GGGAGG) was more common in the control group than in the case group ($P=0.022$). The details were seen in Table 5.

Discussion

The present study is the first demonstration of an association between ADAM33 polymorphisms and keloid scar in an East Asian population. Using a case-control design, we investigated the relationship between human peripheral blood ADAM33 polymorphisms and keloid scar in northeast China. Our results showed that the Q-1 SNPs in blood was significantly associated with keloid scar. As multiple SNPs may act in combination to increase the risk of keloid scar, haplotypes were constructed, and their frequencies were compared between the case and control groups. The haplotype data suggests that the GGAAGA haplotypes may be risk factor for keloid scar. In contrast, the GGGAGG haplotype may be a protective factor for the disease.

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 [17]. Although the familial nature of the disease and its reported presence in identical twins has long indicated a genetic component to keloid scar pathology, the exact aetiopathogenesis of keloid scar is unknown [18, 19]. Genetic studies have yet to identify a gene or genes involved in keloid scar. Using a case-control association study approach, it is possible to attempt to test candidate gene loci that may be involved in keloid scar pathogenesis.

There are more than 30 members of the ADAM (adisintegrin and metalloprotease) family of proteins with orthologues in several species. ADAM33 gene, mapped on chromosome 20p13, is a member of ADAM family. ADAMs are membrane-anchored metalloproteases that play key roles in cell-to-cell interactions and shedding of pro-protein ectodomains [20, 21]. ADAM33 protein is a zinc-dependent endopeptidase, characterized by a pro-domain, metallo-protease, and disintegrin domains, a cysteine-rich region sometimes containing an EGF repeat, a transmembrane domain, and a cytoplasmic carboxyl-terminus. [9]. ADAM33 gene was identified to be associated with asthma and airway hyperresponsiveness (AHR) by positional cloning [11]. Furthermore, ADAM33 has been correlated with many immune mediated disorders [14-16]. And that, many researchers assessed the expression of ADAM33 protein in human lung tissue, primary bronchial smooth muscle cells, and a fibroblast cell line (MRC-5). ADAM33 belongs to a family of type I transmembrane metalloproteinases. These integral membrane glycoproteins play important physiological roles in fertilization, myogenesis, and neurogenesis due to their participation in cell-cell interactions and proteolytic release of cell surface membrane proteins such as cytokines, growth factors, and receptors [22, 23]. Q-1 is located in the intron immediately before exon 16, which contains an epidermal growth factor (EGF) domain [24]. ADAM33 is closely related to matrix metalloproteases, but may bind EGF directly. A disturbance in the EGF domain will likely affect regulation of ADAM33. Through alternative splicing, exon 16 can be spliced out, giving rise to the β -variant of ADAM33. This variant was found in 30% of ADAM33 mRNA transcripts in pulmonary fibroblasts [23]. Because the EGF domain is incomplete, it has been suggested that the β -variant prevents maturation of ADAM33 and may exert a dominant negative effect

on its protease activity [25]. The intronic Q-1 SNP may therefore influence the splicing of the β -variant and disturb the maturation of ADAM33. Subsequent effects on protease activity may result in a defect in tissue repair after inflammation-induced damage. This may be involved in the mechanism of keloid scar formation.

Several limitations in this study need to be addressed. This study was a single-center cohort investigation on a relatively small scale, and thus, replication studies with large independent cohorts are warranted. Secondly, we did not detect the ADAM33 expression in keloid scar tissue. Based on the results in blood sample, next we will perform the experiment in human tissue. The detection of ADAM33 protein will help to clarify the effect of the genetic variants of ADAM33 gene on keloid scar formation.

Conclusion

In summary, our results suggest that the ADAM33 gene polymorphism was associated with keloid scars in the northeastern Chinese Han population.

Disclosure Statement

The authors declare no competing interests.

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