Longer TA repeat but not V89L polymorphisms in the SRD5A2 gene may confer acne risk in the Chinese population

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Abstract

Introduction: Several studies have reported that the V89L and TA repeat polymorphisms [(TA)n] of the SRD5A2 gene were associated with SRD5A2 activity. The activity of dihydrotestosterone, which is converted from testosterone by SRD5A2, is responsible for sebum secretion and the formation of acne. We hypothesized that abnormalities in SRD5A2 action could contribute to the formation of acne.

Aim: To study whether the structural change of the SRD5A2 gene may affect the risk of acne in patients with normal serum testosterone levels.

Material and methods: Genotyping of rs523349 and (TA)n of SRD5A2 was performed in 49 Chinese acne patients with significant improvements with SRD5A2 inhibitor-finasteride but normal serum testosterone levels, and in 50 healthy Chinese age-matched controls without acne.

Results: There was no significant difference between the two groups in the frequencies of V and L alleles and VV, VL, and LL genotypes of V89L (χ^2 test, $p > 0.5$). (TA)n polymorphic repeat sites are 5 alleles (TA0, TA3, TA6, TA9, TA12) in our population. The differences in S and L allele frequencies between the two groups were statistically significant ($p < 0.005$). People with a longer ($n \geq 6$) allele of the (TA)n repeat polymorphism had a higher risk of having acne than those with a shorter ($n < 6$) allele (OR = 3.52, 95% CI: 1.73–7.16).

Conclusions: This study suggests that SRD5A2 polymorphisms might be associated with acne risk. This is the first report focusing on the Chinese population according to our knowledge. Further large sample studies may be required to confirm the association and to assess any interactions with environmental factors.

Key words: acne, SRD5A2 gene, V89L polymorphism, (TA)n repeat polymorphism.

Introduction

Acne is a common chronic inflammatory disease of sebaceous glands, which are exocrine glands in the skin that secrete sebum (an oily substance) to lubricate the skin and hair. Its pathogenesis appears to be multifactorial, including hypersecretion of sebaceous glands, keratinization of the sebaceous gland ducts, and microbial infections and inflammatory mediators in pilosebaceous units [1, 2]. Androgens, as a prerequisite for sebaceous gland development and secretion of sebum, play an important role in the pathogenesis of acne. Steroid 5α-reductase is crucial to androgen action, which converts testosterone to the more potent androgen dihydrotestosterone (DHT). The activity of 5α-reductase is higher in sebaceous glands of the scalp and facial skin than in sebaceous glands of non-acne-prone skin in other locations [3]. Many studies have shown that most acne patients have normal circulating androgen levels, which suggests that androgen acts on sebaceous glands independently of circulating androgen [4]. Local increased DHT synthesis caused by increased 5α-reductase activity in the sebaceous glands may be associated with this phenomenon.

There are two subtypes of 5α-reductase, steroid 5 α-reductase type 1 (SRD5A1) and steroid 5 α-reductase type 2

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(SRD5A2), encoded by two distinct genes. SRD5A2 is located within the inner walls of open and closed comedones, and in endothelial cells from sections of inflammatory lesions from acne patients [5]. Acne patients obtain improvement with the treatment of finasteride-SRD5A2 inhibitor by reducing 5α-reductase activity and thus inhibiting the conversion of testosterone into DHT [6]. Therefore, we hypothesized that excessive local androgen production due to increased SRD5A2 activity in acne-prone skin plays a key role in the development of acne. Familial aggregation suggests the possibility of genetic susceptibility in such patients to develop acne [7, 8]. Therefore, we hypothesized that genetic variation in the gene coding for SRD5A2 may be associated with acne pathogenesis.

The SRD5A2 gene is located on chromosome 2 at locus 2p23, containing 5 exons and 4 introns, encoding 254 amino acids, with a long 3′ untranslated region. Several studies have reported that V89L (rs523349) polymorphism of the SRD5A2 gene was associated with the enzyme activity. Replacement of the amino acid valine with leucine downregulated the conversion rates of testosterone to DHT in the prostate, which reduced the risk of prostate cancer [9–11]. The (TA)n marker is located in the 3′ untranslated region (3′ UTR) of the SRD5A2 gene, and its functional consequence is thought to result in instability of mRNA transcripts with UA-rich 3′ UTRs (transcribed from TA-rich regions of DNA), which may lead to dysregulated levels of reductase [12, 13]. A study of 30 prostate cancer patients showed that almost 57% of the samples examined showed evidence of somatic mutations at the 3′ UTR of the SRD5A2 locus [14].

Aim

In this study, genotyping of rs523349 and (TA)n of SRD5A2 was conducted in 49 acne patients with normal serum testosterone levels, who had significant improvements with SRD5A2 inhibitor-finasteride, and in 50 healthy age-matched Chinese controls without acne. Our focus is to investigate whether there is any association between V89L, TA dinucleotide repeat polymorphisms of the human SRD5A2 gene and the risk of developing acne in patients with a normal testosterone level, which has never been reported.

Material and methods

Subjects

A total of 99 individuals younger than 40 years old were recruited to participate in the study in Shanghai, China. They were from the Han ethnic group: 49 were patients presenting with acne and 50 were healthy control individuals with no acne history. The selection criteria for the acne patients for the study were: (1) the duration of symptoms was more than 6 months; (2) normal serum total testosterone (TT) and free testosterone (FT); (3) normal serum level of prolactin (PRL); (4) no evidence of polycystic ovarian syndrome, which is ruled out by regular menstrual cycles, normal ultrasound examination, and serum luteinizing hormone/follicle-stimulating hormone (LH/FSH) ratio < 1; (5) absence of chronic renal disease, diabetes mellitus, and hepatic disease; (6) after 3 months of treatment (5 mg/day) with finasteride (a synthetic 5α-reductase inhibitor from ProscarR; Merck, Sharp, Dohme Ltd, Hertfordshire, UK) in both men and women, skin lesions were improved significantly in both men and women. Female patients were informed that finasteride could increase the risk of birth defects in a male fetus and consequently pregnancy was contraindicated during the treatment and reliable contraception measures must be used such as the rhythm method and use of condoms, while any use of contraceptives with anti-androgen effects would be excluded. The exclusion criteria were the following: (1) occupational acne or drug-induced acne-like rash; (2) pregnant or lactating women or 6 months of pregnancy requirements. Urine pregnancy tests were performed to exclude pregnant women; (3) subjects who had used any other drugs for treatment of acne in the last month, such as antibiotics, other anti-androgen drugs, glucocorticoids, and isotretinoin soft capsules.

All the patients and controls were enrolled with informed written consent. The study was approved by the Ethics Committee of Changhai Hospital and the procedures followed were in accordance with the Helsinki Declaration of 1975 as revised in 2000. All the patients signed informed consent for their participation in our study after reviewing the protocol of this experiment. Female patients were informed that finasteride could increase the risk of birth defects in a male fetus and consequently pregnancy was contraindicated during the treatment and the following 6 months, so reliable contraception must be used. We recorded the following information of the subjects: name, gender, age, height, weight, duration of disease, acne severity assessed by the Global Acne Grading System, acne genetic history, serum testosterone levels.

Hormonal evaluation

Blood samples were collected before the treatment with finasteride to evaluate estradiol (E), TT, FT, PRL, LH, FSH, and insulin. All blood samples from female patients were obtained on the fifth day of the menstrual cycle.

Efficacy evaluation of finasteride in the treatment of acne

All patients were evaluated by Doshi's Global Acne Grading System (GAGS) [15]. We calculated the total score before and after the treatment, and evaluated the therapeutic effects according to changes in scores. The

formula is efficacy index = (pre-treatment score – post-treatment score) pre-treatment score × 100%. An efficacy index more than 60% means significant effects.

DNA extraction and genotype analysis

DNA was extracted from whole blood samples using QIAamp DNA blood Kits (Qiagen, Valencia, CA). Polymerase chain reaction (PCR) was performed to determine V89L substitution. Primers were designed using the Primer-3 software as follows: a forward primer (5′-CAGCCGCTTGTCAACTCTCT-3′), a reverse primers (5′-ACGGTACTTCTGGGCCTCTT-3′), and amplification length was 133 bp. Amplifications were performed in a T-Gradient thermocycler (Biometra, Germany) as follows: (i) initial denaturation of 96°C for 5 min; 5 cycles of 96°C for 20 s, 68°C for 1 min; (ii) 10 cycles of 96°C for 20 s, 64°C for 50 s, 72°C for 45 s; (iii) 15 cycles of 96°C for 20 s, 61°C for 50 s, 72°C for 45 s; (iv) an final extension at 72°C for 5 min. Polymerase chain reaction reactions contained genomic DNA 0.5 µl, the primer (upstream and downstream primer; 20 µM) 0.5 µl, Premix Ex Taq (Code: D335A, TaKaRa Japan) 10 µl, and sterile distilled water 9 µl. Agarose gel electrophoresis was performed to confirm the purity of each reaction product. The PCR product was then sequenced by Bio Corporation.

Assay SRD5A2 gene (TA)n dinucleotide repeat polymorphism was determined with the real-time SYBR Green PCR-HRM (polymerase chain reaction – high-resolution melting) method. Primers were designed using Primer 3Plus according to the SRD5A2 sequence. The forward primer was 5′-GCTGATGAAAACTGTCAAGCTGCT-GA-3′, the reverse primer was 5′-GCCAGCTGGCAGAAC-GCCAGGAGAC-3′, and amplification length was 116 bp. Amplification was performed in a Rotor-Gene 6000 (Corbett, Australia) as follows: initial denaturation of 95°C for 10 min; 40 cycles of 95°C for 10 s and then 60°C for 30 s; melt 72°C→95°C, 1°C each step; high-resolution melting 75°C→84°C 0.1°C each step. Polymerase chain reaction reactions contained genomic DNA 0.5 µl, the primers (upstream and downstream primer, 20 µM), 0.5 µl, Realtime PCR Master Mix (Realtime PCR Master Mix, SYBR Green, ABI, USA) 10 µl, sterile distilled water 9 µl. Those samples with different DNA melting curves were sequenced to identify their genotypes. Then the genotypes of the rest samples were identified by analysis of DNA melting curves during the polymerase chain reaction.

Statistical analysis

We calculated the allelic percentages for SRD5A2 polymorphisms and used the χ^2 test to calculate the significance of the differences between cases and controls. The χ^2 values were calculated with SPSS 17.0 software. Two sided *p*-values of less than 0.05 were considered significant. Different alleles at V89L and the (TA)n polymorphic sites were not investigated for linkage disequilibrium in the cases and the controls, because the sample size was too small.

Results

There were no significant differences between acne cases and controls in age, gender or BMI (Table 1).

The V89L site was highly polymorphic. However, there was no significant difference between acne cases and controls in the frequency distribution of various alleles at this site (Table 2). For genotype analyses, Val/Val genotype was considered to be a high activity genotype, while Val/Leu and Leu/Leu were considered to be low activity genotypes. We did not find any differences between the acne patient group and the control group in the distribution of high and low activity genotypes (Table 2).

At the (TA)n repeat site, five alleles of the (TA)n repeat – (TA)0, (TA)3, (TA)6, (TA)9 and (TA)12 – were detected in this study, labeled as alleles A, B, C, D and E. Eleven genotypes were present in the participants. Table 3 shows the distribution of the genotypes. The TA repeat number in the acne patient group was from 3 to 12 (mean: 7.19, median: 6). In the control group the number of TA repeats was from 0 to 12 (mean: 5.94, median: 6). By using the Cochran and Cox approximate *t* test, a statistically significant difference of TA repeat numbers was found between the acne patient group and the control group (the Cochran and Cox

Table 1. Age, gender and BMI distribution among acne patients and healthy controls

Group	Age [years]		Gender			BMI [kg/m^2]	
	Mean ± SD	*P*-value	Females	Males	*P*-value	Mean ± SD	*P*-value
Cases (*n* = 49)	21.55 ±3.88	> 0.10	38	11	> 0.50	20.50 ±1.82	> 0.5
Controls (*n* = 50)	22.18 ±3.37		36	14		21.06 ±1.65	

Table 2. SRD5A2 rs523349 allele and genotype frequencies among acne patients and healthy controls

Group	Allele frequencies			Genotype frequencies			
	V (%)	L (%)	*P*-value	VV (%)	VL (%)	LL (%)	*P*-value
Cases (*n* = 49)	39.80	60.20	> 0.5	20.40	38.78	40.82	> 0.5
Controls (*n* = 50)	43.00	57.00		18.00	50.00	32.00	

Table 3. Distribution of the different genotypes between control and case groups

Genotypes	Controls	Cases
AA	0	4
AB	0	2
BB	6	9
BC	0	7
BD	2	0
CC	17	7
CD	8	5
CE	0	1
DD	9	9
DE	3	4
EE	4	2

approximate *t* test, $p < 0.05$). With the TA repeat median number of 6 in the control group as the split point, we defined short TA repeat numbers $n < 6$ as allele S and long TA repeat numbers $n \geq 6$ as allele L. We found a significant difference between the acne patient group and the control group in the frequency distribution of various alleles and genotypes (Table 4). Individuals carrying the L allele of (TA) n had a higher risk of acne than those carrying the S allele (OR = 3.52, 95% confidence interval: 1.73–7.16).

Discussion

This study represents a preliminary investigation of the association between the variants of the androgen metabolic enzyme SRD5A2 gene and the risk of developing acne in the Chinese population. Subjects for the study were limited to patients with normal serum testosterone levels who had significant improvement after treatment with the SRD5A2 inhibitor finasteride. This suggests that the SRD5A2 enzyme activity may be an important mechanism in the pathogenesis of acne in these patients. Therefore, we were more likely to discover potential association(s) between polymorphism of the SRD5A2 gene and the risk of developing acne in the these patients, which would make our study practical in a reduced sample size.

SRD5A2 plays an important role in the conversion of testosterone to the more potent DHT, but it cannot function without NADPH as its cofactor [16]. Russell *et al.* [17] studied 22 nucleotide missense mutations of SRD5A2 gene and demonstrated that change of certain amino acids might affect activity of SRDSA2 through decreasing binding affinity of the enzyme for its substrate or NADPH, or by affecting the optimum pH (which is pH 5.0–5.5). Given the important role of SRD5A2 in androgen metabolism, many investigators have hypothesized that increased 5α-reductase activity might contribute to hyperandrogenism, and many studies have investigated the association of SRD5A2 gene variants and androgen-related disorders. There have been reports on the V89L polymorphism and SRD5A2 activity and prostate cancer (an androgen-dependent neoplasm) risk, which showed that individuals carrying the V allele of the SRD5A2 gene had higher levels of plasma 5α-androstan-3β,17β-diol (a DHT metabolite) than those carrying the L allele and that V89L substitution was associated with risk of prostate cancer [10, 18]. An *in vitro* transfection experiment confirmed that the activity of 5α-reductase with leucine at codon 89 was 47% lower than that with valine [19]. But the relationship between the V89L polymorphism and 5α-reductase enzyme activity and prostate cancer risk remained controversial. Wang *et al.* conducted a recent meta-analysis of 25 reports (including 8615 prostate cancer cases and 9089 controls) on V89L polymorphism and prostate cancer risk, and revealed that males with VV or VL genotype were at increased risk for prostate cancer compared with those with LL genotype in the European population. But such a phenomenon was not found in populations from Asia and Africa [20].

Our study showed no significant difference between the acne group and the control group in either the frequency of V and L alleles of V89L or the frequency of VV, VL and LL genotypes of V89L, which implied that V89L might be a polymorphism with no effect on the binding affinity of the enzyme for the substrate or NADPH or the optimum pH. However, there was a relatively small sample size in our study, so a study with a much larger sample size would be required to determine the significance of the V89L polymorphism for risk of acne.

SRD5A2 is primarily expressed in genital skin and the prostate. The polymorphisms of SRD5A2 have been studied in prostate diseases mostly. Reichardt *et al.* [21] reported 10 genotypes of fragments of (TA)n from 87 bp to 131 bp in prostate cancer patients of different races, including 37 low-risk Asian-Americans, 94 high-risk African-Americans, and 68 intermediate-risk non-Hispanic Whites. The longest fragments, from 121 bp to 131 bp, were detected only in African-Americans, who were the

Table 4. (TA)n allele and genotype distribution frequencies of SRD5A2 among acne patients and healthy controls

Group	Allele frequencies			Genotype frequencies			
	S (%)	L (%)	*P*-value	SS (%)	SL (%)	LL (%)	*P*-value
Cases (n = 49)	14.29	85.71	< 0.005	12.24	4.08	83.67	< 0.025
Controls (n = 50)	37.00	63.00		30.00	14.00	56.00	

high-risk population. Rajender *et al.* [22] analyzed the (TA)n repeat of the SRD5A2 gene in 87 histologically confirmed prostate cancer patients, 40 benign prostatic hyperplasia cases and 96 control samples from southern parts of India, and revealed that the (TA)9 allele might confer a certain prostate cancer risk in south Indian men. Thus, it has been proposed that longer TA repeat lengths may result in an elevation of enzyme activity, leading to an increased prostatic level of DHT. Our study showed five alleles of TA dinucleotide repeat polymorphism – (TA)0, (TA)3, (TA)6, (TA)9, and (TA)12 – by PCR-HRM. The acne patients had longer TA repeats than controls. Our previous study demonstrated that young male acne patients with normal testosterone levels had higher DHT levels than controls, and with DHT levels significantly decreased after the treatment of finasteride, the disease was significantly improved [23]. That previous study has some relevance to this study. (TA)n polymorphism plays a role in the pathogenesis of acne in patients with elevated DHT levels.

This is the first genetic association analysis of the 5α-reductase gene as a candidate gene for acne pathogenesis. This study showed that the V89L polymorphism in the SRD5A2 gene may have little effect on acne in the Chinese Han population, but the (TA)n repeat polymorphism in SRD5A2 may be associated with acne pathogenesis and longer (TA)n repeats may increase the risk of developing acne in the Chinese Han population. The (TA)n repeat polymorphism in SRD5A2 may be one of the genetic mechanisms involved in the pathogenesis of acne. Thus our study provides some mechanistic insight into the potential importance of SRD5A2 in acne pathogenesis. Simultaneous detection of TA repeat polymorphism and 5α-reductase activity may provide a more accurate evaluation of the biological significance of this polymorphism. A study with a much larger sample size may be needed to determine such interactions in both normal people and acne patients.

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Conflict of interest

The authors declare no conflict of interest.

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