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ORIGINAL RESEARCH

The Associations of Androgen-Related Genes CYP21A2 and CYP19A1 with Severe Acne Vulgaris in Patients from Southwest China

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Objective: Androgens acting through the androgen receptor play a crucial role in the pathogenesis of acne. This study aimed to identify whether two key genes (*CYP21A2* and *CYP19A1*) involved in the synthesis and metabolism of androgens were associated with Pillsbury III-IV severe acne vulgaris.

Methods: We carried out a standard questionnaire survey about acne and enlisted 600 Pillsbury III-IV severe acne vulgaris patients and 652 healthy controls of Han Chinese descent from Yunnan, China in the study. Twenty-two single nucleotide polymorphisms (SNPs) were genotyped by SNaPshot assay and analyzed for association with severe acne.

Results: There was no significant difference in gender between the two groups ($P = 0.085$), and the age of the acne case group was significantly lower than that of the control group ($P < 0.001$). Our results revealed that only two SNPs, rs6474 (p.Arg102Lys) ($P = 0.001$) and rs6465 ($P = 0.025$) of the *CYP21A2* gene were significantly associated with severe acne among the Han Chinese. When subjects were divided into males and females, significant associations were observed only in male patients with severe acne vulgaris for four variants: *CYP21A2* rs6474 (p.Arg102Lys) ($P = 0.002$); *CYP21A2* rs6465 ($P = 0.012$); *CYP19A1* rs8023263 ($P = 0.037$); and *CYP19A1* rs2470152 ($P = 0.007$). Haplotype analyses showed that the distribution of *CYP21A2* haplotypes was significantly associated with male patients, while no association of *CYP19A1* haplotypes was observed. The structure of the human *CYP21A2* consists of two substrate binding sites and one substrate access channel.

Conclusion: This study shed a light on a potentially important effect of *CYP21A2* and *CYP19A1* genes in severe acne vulgaris in the Han Chinese, especially for male patients. Future studies using independently verified datasets from a broader geographical spectrum will be valuable in identifying the causal and functional variants responsible for severe acne vulgaris within the *CYP19A1* and *CYP21A2* genes.

Keywords: androgen receptor, severe acne, synthesis, metabolism, genetic risk

Introduction

Acne is estimated to affect 9.4% of the global population, making it the eighth most prevalent disease worldwide.[1] Acne can be a painful and disfiguring disease, which leaves some individuals with permanent physical and psychological scars.[2,3] Likewise among those suffering from severe acne, suicidal ideation is markedly more common,[4] highlighting the severe psychological toll that this disease can take. Thus, severe acne can be considered a public health problem. Several factors have been implicated in the development of acne, including androgen, sebum over-production, abnormal follicular infundibular function, proliferation of Cutibacterium

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acnes, and inflammation, as well as lifestyle and heredity.[3,5] In particular, Cutibacterium acnes play an important role in promoting the inflammatory responses by enhancing the secretion of cytokines, such as tumor necrosis factor (TNF)-α, interleukin (IL)-8 and IL-12 from the phagocytes and keratinocytes.[6] The heritability of the disease suggests a probable genetic mechanism.

Androgens, which enhance sebum production and follicular keratosis, play an essential role in the development of acne.[7] However, the exact genetic mechanisms underlying how androgens affect acne development remain unclear. Certain androgen-related genes were found to be the risk factors of acne, especially for severe acne, although support for these associations has not been unanimous. Previous studies have focused on genes, such as *CYP1A*1,[8] *CYP17*,[5] and androgen receptors (*AR*s).[9] Consequently, in this study, we opted to focus on examining whether *CYP21A2* and *CYP19A1* are associated with acne vulgaris.

CYP21A2 is localized on the chromosome 6p21.3 in the region of the major histocompatibility complex of class III. An earlier study of *CYP21A2* polymorphism among random acne patients found that alterations of the *CYP21A2* gene were more common in patients with acne than in the controls, but there is a poor correlation between these changes and increased steroids and acne.[10] To date, there are no other associational studies that explore the relationship between *CYP21A2* and acne.

CYP19A1 is located on the long arm of chromosome 15 at position 15q21.1, and it encodes aromatase, a key steroidogenic enzyme that catalyzes the final step of estrogen biosynthesis through the aromatization of testosterone and androstenedione.[11] Polymorphisms of the *CYP19A1* gene encoding aromatase have been reported to be correlated with plasma testosterone levels, and some studies proposed that the polymorphisms of the *CYP19A1* gene had a positive association with some androgen-related diseases, such as hyperandrogenism, PCOS,[12,13] prostate cancer,[14] female pattern hair loss,[15] and some estrogen dependent diseases such as breast cancer,[16,17] endometrial cancer,[18] and endometriosis,[19,20] and changes in the timing of the menarche.[21,22]

Taking into account these reports on the potential effects of both *CYP21A2* and *CYP19A1*, we hypothesized that *CYP21A2* and *CYP19A1*, being two key genes involved in the synthesis and metabolism of androgens, may be related to the occurrence of acne, in particular of severe acne vulgaris, and we performed a systematic genetic analysis, with a relatively large sample size of Han Chinese, based on a case-control study.

Materials and Methods

Subject Selection and Information Gathering Protocols

A total of 1252 unrelated Han Chinese individuals, 600 patients and 652 controls, were recruited from Yunnan, in the southwest of China, for this study. All the patients were examined in succession in the outpatient unit of dermatology at the first affiliated hospital of Kunming Medical University, by dermatologists using the Pillsbury Classification Scale.[23] Patients who present with Pillsbury III–IV according to the criteria were recruited. Severe acne lesions were characterized predominantly as inflammatory papules, pustules, nodules, scars and cysts.[24] In this study, we enrolled those patients who presented with inflammatory nodules, scars and cysts, or accompanied with large pus-filled cysts, substantial swelling and exfoliation around the infections, in addition to comedones, papules and pustules. We then collected 652 gender-matched healthy people as controls. The subjects were then divided into a male and a female group. Written informed consent was obtained from all subjects, and this study was approved by the ethics board of Kunming Medical University. This study was conducted in accordance with the Declaration of Helsinki.

Content of Questionnaire

The subjects recruited for the case group were given a standardized questionnaire concerning their personal information (age, gender, ethnicity, occupation, place of birth and family residence, weight, and height), socioeconomic situation (religion, dietary habits, smoking and alcohol, skin type, genetic factors, drug history, and past medical history), acne status (age at onset, duration, location and type of skin lesions, season at onset, and aggravated season), risk factors (menstrual cycle, sun exposure, emotional impact, and agrypnia), familial hereditary history, and hobbies. The data were collected from 539 (89.8%) cases using these questionnaires.

Standard for Exclusion

The exclusion criteria were as follows: those with 1) endocrine diseases, such as polycystic ovary syndrome, diabetes, hyperthyroidism, CAH or thyropenia; 2) other genetic diseases; 3) androgen-related diseases; 4) serious digestive diseases; 5) infectious diseases; 6) occupational

Table 1 The Basic Characteristics of All SNPs for the *CYP21A2* and *CYP19A1* Genes

Number	SNP ID	Position (bp)[a]	Allele	MAF[b]	Location/Annotation
CYP21A2					
1	rs6464	32114316	A/C	0.158	Exon1/tag SNP
2	rs6467	32114837	T/G	0.300	Intron2/tag SNP
3	rs6474	32114865	G/A	0.222	Exon3/tag SNP
4	rs6465	32115740	C/T	0.162	Intron6/tag SNP
CYP19A1					
1	rs4646	49290136	C/A	0.280	3-UTR
2	rs10046	49290278	T/C	0.439	3-UTR
3	rs700519	49295260	C/T	0.146	Exon7
4	rs8023263	49304889	G/T	0.415	Intron4/tag SNP
5	rs2899473	49306365	C/T	0.146	Intron4/tag SNP
6	rs12594287	49311199	G/A	0.232	Intron3/tag SNP
7	rs2414096	49317071	G/A	0.425	Intron2
8	rs727479	49321839	T/G	0.237	Intron2/tag SNP
9	rs767199	49327679	G/A	0.488	Intron1/tag SNP
10	rs11636667	49329485	C/T	0.488	Intron1/tag SNP
11	rs749292	49346023	G/A	0.476	Intron1/tag SNP
12	rs730154	49378496	A/G	0.305	Exon 1.4/tag SNP
13	rs28757111	49380750	T/C	0.159	Intron1/tag SNP
14	rs2470152	49382264	T/C	0.500	Intron1/tag SNP
15	rs41399553	49383121	C/T	0.134	Intron1/tag SNP
16	rs1902584	49398946	A/T	0.122	Intron1/tag SNP
17	rs1004984	49400821	C/T	0.295	Intron1/tag SNP
18	rs28757078[c]	49412515	C/T	0.207	Intron1/tag SNP

Notes: [a]Postion are based on NCBI web site, [b]The second allele is the minor allele. [c]The SNP which is unsuccessfully genotyped.

acne or pharmacological acne tetter; and those who had 7) had acne for less than six months; 8) taken tretinoin or other hormones within two months prior to possible enrollment.[3]

SNP Selection and Genotyping

Genomic DNA was extracted from the whole blood of all patients and controls using the AxyPrep™ Blood Genomic DNA Miniprep Kit (Axygen, USA), following the procedure detailed in the kit. The DNA samples were stored at –20°C. The information of 18 *CYP19A1* and 4 *CYP21A2* single nucleotide polymorphisms (SNPs) was acquired from public databases NCBI dbSNP, (http://www.ncbi.nlm.nih.gov/projects/SNP/); and HapMap, (http://hapmap.ncbi.nlm.nih.gov/, Phase 3, CHB), under a rationale of minor allele frequency (MAF) >10%. Among the 22 SNPs, 18 (*CYP19A1*:14 SNPs; *CYP21A2*:4 SNPs) were marked as tag SNPs in the HapMap dataset for CHB. Another 4 SNPs of the *CYP19A1* gene, rs4646 and rs10046 (in the 3′-UTR), rs700519 (Arg264Cys), and rs2414096, have been extensively studied in hyperandrogenism diseases. The basic characteristics of the selected SNPs for the *CYP21A2* and *CYP19A1* genes are presented in Table 1.

All the SNPs were genotyped using SNaPshot assay, for multiplex polymerase chain reactions (PCRs). PCR primers and extension primers for all 21 successfully genotyped SNPs are presented in Table 2 (rs28757078 of *CYP19A1* was abandoned because of insufficient power to detect its effects). GeneMarker (Holland and Parson, 2011) was used to read the genotyping results. For quality control, a 4% masked random sample of cases and controls was tested repetitively by direct sequencing, and all the results were 100% concordant.

Functional Implications of the SNP rs6474 (p.Arg102Lys) from Structure Modeling

In order to infer the functional implications of the SNP rs6474 (p.Arg102Lys) the structure of human *CYP21A2*

Table 2 Primers for All Genotyped SNPs of the *CYP21A2* and *CYP19A1* Genes

Num.	SNP ID	Primer (5′-3′)	
CYP21A2			
1	rs6464	Forward Reverse Extension	CTGCTGTGGAACTGGTGGAAG TGTAGATGGGCCCGAATTTCTG TTTTTTTTTTTTTTTTTTTTAGTCAGGCCAAGCAGATAGAT
2	rs6467	Forward Reverse Extension	CTCAGCTGCCTTCATCAGTTC GTGAGCTTCTTGTGGGCTTTC TTTTTTTTTTTTTTTTTCCAGCTTGTCTGCAGGAGGAG
3	rs6474	Forward Reverse Extension	CTCAGCTGCCTTCATCAGTTC GTGAGCTTCTTGTGGGCTTTC TTTTTAAGGACAGGTCCGGGTAGTTC
4	rs6465	Forward Reverse Extension	TTTGCATACCCCAGTTATGGGC ATGTAGTCCATCATGTCCCTC TTTTCCTGCAGAGGGTGAAAGGAGC
CYP19A1			
1	rs4646	Forward Reverse Extension	GCTGGAAATGATCTTTACCCC TTCACCGACTATTTCTCCCTC TTTTTTTTTTTTTTTGTGTGAACAGGAGCAGATGAC
2	rs10046	Forward Reverse Extension	GCTGGAAATGATCTTTACCCC TTCACCGACTATTTCTCCCTC TTTTTTTTTTTGATGAGAAATGCTCCAGAGT
3	rs700519	Forward Reverse Extension	CAGCAAGGATTTGAAAGATGCC TAGTTCAGGTCAGTACCTCTG TTTTTTTTTTTTTTTTCTCTTCTGTGGAAATCCTGC
4	rs8023263	Forward Reverse Extension	CCTAATACACCTGAGCCAAATG TTCCCCTATCCACAAAAGGTG TTTTTTTTTTTTTTTTTTTTTTGAAATAATGCTATAAGATCC
5	rs2899473	Forward Reverse Extension	CTGGATAAGGAAGCTTGCAAC CCATATCTGTCATCTAGCCTC TTTTTTGAGGAAATAAAGTTCCAAC
6	rs12594287	Forward Reverse Extension	CTCGGTTAAATTCAAGTGGGC GGAAATAAAGTCTTCAGCTGGG TTTTTTTTTTTGACATGCAGTAGCATTGCCAG
7	rs2414096	Forward Reverse Extension	GGAGAATGTCCAATCCAAGAAC TTCAAAGACCCATTGCCTGAC TTTTTTTTTTTTTTTTTTGCTTAAGAGCCTTTTCTTAAA
8	rs727479	Forward Reverse Extension	CTGGAACATCTTCTTCACTGC CACTATCACCACATTCCCAAG TTTTTTTTTTTTTTTTTTTTTTTTCAAGACAAAGAGGGGGCATGG
9	rs767199	Forward Reverse Extension	CCAAGCTCTAGTGTCTTCAAG TGGAGAGATGGTTTGTTTGGC TTTTTGTGCTGCAGTCCATTCCCCAC

(*Continued*)

Table 2 (Continued).

Num.	SNP ID	Primer (5′-3′)	
10	rs11636667	Forward Reverse Extension	TCATGACACTTGAGGTTCCAG CACACCATGTGTATCTAGCTG TTTTTTTTTTTTTTTTTGAGCAAGACAATAGGAACCAA
11	rs749292	Forward Reverse Extension	TATGGAAGGAGGACTGAGTGG GGCCTGATAGAAATTGTGCAG TTTTTTTTTTCCTTCTTCAAACCTCGGAGTC
12	rs730154	Forward Reverse Extension	TTGCCGGTTCCAGCAAAACTTC CCTGAGCTCATTGCTAATGTG TTTTTCCAGCAAAACTTCATGGAGC
13	rs28757111	Forward Reverse Extension	CTTGGAAAGGAAGCTTTGTGC TACTGGACTTGGCTATGTTGC TTTTTTTTTTTTTTTTTAGAACAAAGAATCTCAGGGTA
14	rs2470152	Forward Reverse Extension	CAATTTCAAGGGTTGTGGGAC AATCTCTGCCTGTGGAAAGTC TTTTTTTTTTTTTTTTTTTCTTCTTTGATGTCCAGCCCAC
15	rs41399553	Forward Reverse Extension	TTGAGGCATCTGCCTTCTTAG CTACTTATCTGCCCCTTAGAG TTTTTTTTTTTCCTTGTATTTGCTCAGACA
16	rs1902584	Forward Reverse Extension	TCCTGTTAGATACAGATGCAC GGTGATGGGTTATGAGGATTAG TTTTTTTTTTTTTTTTTTTTTTTTCACATACAATTCTTATGAACA
17	rs1004984	Forward Reverse Extension	AAATTGGATTGTGGCAGAGGG AATCATCACTGATGGACCCTG TTTTTTTTTTTTTTCCCCCATGACTGCCTACTGTT

was modeled by using the bovine *CYP21A2* structure as a template. The bovine *CYP21A2* crystal structure (PDB: 3QZ1) complexed with the substrate 17-OHP was obtained from the Protein Data Bank. Discovery Studio 3.1 (Accerlrys, San Diego, CA) was used to perform homology modeling.

Statistical Analysis

All statistical analyses were performed using SPSS v.17.0 (IBM Corp., Armonk, NY, USA). The Hardy–Weinberg equilibrium (HWE) test was carried out for each SNP in the control group using Chi-square tests. Genotype frequency differences in each SNP between Pillsbury III–IV severe acne vulgaris patients and the corresponding control subjects were estimated by the unconditional logistic regression model, adjusted for age. The pairwise linkage disequilibrium (LD) between the *CYP19A1* gene and the *CYP21A2* gene in the control group was performed using Haploview software version 4.2.[25] Haplotype block structures were defined as previously described,[26] and haplotype frequency was estimated using PHASE 2.0. The global difference in haplotype frequencies between the cases and controls was estimated using Chi-square tests. Haplotype frequencies of the two candidate genes were further subject to Bonferroni correction to account for multiple comparisons. The conservative significance threshold for a single test was assessed at a type I error rate of 0.05/N, where N was the number of tested markers for each haplotype.

Results

Clinical Features

Overall, both groups were similar with respect to gender, while the mean age of the control group was higher than that of the acne case group, so as to mitigate the possibility that some of the younger controls might develop acne later on. The clinical characteristics of patients with severe acne

vulgaris are summarized in Table 3. In general, age of acne onset, skin type, and severity of acne symptoms were significantly different between males and females; the frequency of cysts or nodules, hypertrophic scarring and atrophic scarring is higher in male patients. In other words, the clinical symptoms of acne were more severe among male patients. Risk factors among the males were males included smoking, alcohol consumption, and a diet heavy in lard and oil. Females were more affected by anxiety and depression and suffered from poor quality of sleep. Additionally, 56.8% of acne in female acne patients was reported to be related to their menstrual cycles. A large portion of both male patients (75.3%) and female patients (62.7%) had a family history of acne dating back within one generation.

Basic Characteristics

A total of 569 patients (94.8%, 22.62 ± 6.30 years old; 348 males and 221 females) and 631 controls (96.8%, 26.76 ± 8.05 years old; 355 males and 276 females) were successfully genotyped and included for further analysis in the present study. Age and gender characteristics of both the case and control groups are shown in Table 4.

CYP21A2 rs6474 (p.Arg102Lys) and rs6465 are Associated with Pillsbury III-IV Severe Acne Vulgaris

The linkage disequilibrium map of the tested SNPs among the control populations is shown in Figure 1. The genotypes of selected polymorphisms of *CYP19A1* and *CYP21A2* followed the Hardy–Weinberg equilibrium, with significant values ($P<0.01$) except for rs6465 in the control group ($P = 0.0006$), which was accordingly excluded for further analysis. Two *CYP21A2* SNPs and two *CYP19A1* SNPs showed significant associations with severe acne vulgaris (Table 5). The other 17 SNPs showed no significant association in male- or female-severe acne (Table 6). Genotype AA of rs6474 (p.Arg102Lys) of the *CYP21A2* gene had a significantly higher frequency in severe acne vulgaris patients (OR = 5.431, 95% CI: 2.060–14.318, $P = 0.001$). By contrast, the genotype TT of rs6465 (intron 6) of the *CYP21A2* gene had a significantly lower frequency in severe acne vulgaris patients (OR = 0.417, 95% CI: 0.194–0.896, $P = 0.025$). However, there was no apparent association with severe acne vulgaris in the four reported SNPs of *CYP19A1* gene, rs4646 and rs10046 (in the 3′-UTR), rs700519 (Arg264Cys), rs2414096, which have been extensively studied in hyperandrogenism diseases (Table 6).

Associations of *CYP21A2* and *CYP19A1* with Severe Acne Vulgaris Among Males

Our previous studies suggested that the existence of shorter *AR* gene CAG repeat polymorphism and the *CYP17* –34C/T homozygote in males results in a significantly increased risk of developing severe acne. As such, we grouped the subjects into males and females with severe acne vulgaris and paired them with their corresponding controls. There were significant differences between male patients and controls for the genotype AA of rs6474 (p.Arg102Lys) and the genotype TT of rs6465 of *CYP21A2*, as well as the genotype GT of rs8023263 and the genotype CT of rs2470152 of the *CYP19A1* gene (rs6474, OR = 11.7 $P = 0.002$; rs6465, OR = 0.272, $P = 0.012$; rs8023263, OR = 0.658, $P = 0.037$; rs2470152, OR = 1.675, $P = 0.007$). Similarly, the allele A of rs6474 (p.Arg102Lys) and the allele T of rs6465 in the *CYP21A2* gene showed significant differences in the incidence of severe acne vulgaris between male subjects and controls (rs6467 A allele, OR = 1.542, $P = 0.006$; rs6465 T allele, OR = 0.717, $P = 0.039$). The allele frequencies of both SNPs rs8023263 and rs2470152 of *CYP19A1* were similar between the males with severe acne vulgaris and the male controls (Table 5).

Lack of Association of *CYP21A2* and *CYP19A1* with Severe Acne Vulgaris in Females

There was no evidence of association of the 21 SNPs with risk for female severe acne vulgaris ($P > 0.05$) for both *CYP21A2* and *CYP19A1* at either the genotype or the allele level. The data of the above mentioned 4 SNPs are shown in Table 5, and the data of the other 17 SNPs are shown in Table 6.

Haplotype Analysis of *CYP21A2* and *CYP19A1*

The linkage disequilibrium plot of the two candidate genes is presented in Figure 1. We reconstructed haplotypes of the 3 SNPs of the *CYP21A2* gene. For the *CYP19A1* gene, a total of 17 variants were considered and divided into three haplotype blocks, with the aggregate data from the control group. Also presented in Table 7 are the association results of risk of severe acne with common haplotypes in each haplotype block. The analyses include all subjects, as well as analyses conducted in the male or female population. We pooled together those haplotypes with a frequency of <3% in the case or control groups and compared distribution

Table 3 The Clinical Characteristics of Severe Acne in Male and Female Patients

Characteristics	Number of Patients*		χ^2	*P*
	Male (325)	**Female (214)**		
Age (years)	21.3±5.45	24.5±6.79		
Age of onset (years) 10–15 16–20 21–25 26–30 >30	 151 (46.6%) 137 (42.3%) 27 (8.3%) 3 (0.9%) 6 (1.9%)	 87 (40.8%) 91 (42.7%) 15 (7.0%) 9 (4.2%) 11 (5.2%)	11.957	**0.018**
Skin types Oil type Dry type Mixed type	 296 (91.1%) 13 (4.0%) 16 (4.9%)	 173 (80.8%) 10 (4.7%) 31 (14.5%)	15.223	**<0.001**
Type of skin lesions				
Comedones (n) 0 1–50 51–100 >100	 13 (4.0%) 238 (73.2%) 52 (16.0%) 22 (6.8%)	 6 (2.8%) 147 (68.7%) 44 (20.6%) 17 (7.9%)	2.649	0.449
Papule (n) 0 1–50 59–100 >100	 3 (0.9%) 254 (78.2%) 49 (15.1%) 19 (5.8%)	 6 (2.8%) 151 (70.9%) 38 (17.8%) 18 (8.5%)	5.537	0.136
Cyst or nodules (n) 0 1–10 >10	 46 (14.2%) 195 (60.0%) 84 (25.8%)	 79 (36.9%) 105 (49.1%) 30 (14.0%)	40.134	**<0.001**
Hypertrophic scar (n%) 0% 1–25% 25–50% >50%	 127 (39.2%) 171 (52.8%) 20 (6.2%) 6 (1.9%)	 148 (69.2%) 58 (27.1%) 5 (2.3%) 3 (1.4%)	46.83	**<0.001**
Atrophic scar (n%) 0% 1–25% 25–50% >50%	 63 (19.4%) 216 (66.7%) 29 (9.0%) 16 (4.9%)	 85 (39.7%) 111 (51.9%) 14 (6.5%) 4 (1.9%)	28.103	**<0.001**
Season at onset Spring Sunmmer Fall Winter Reversal of season	 14 (4.4%) 286 (89.1%) 5 (1.6%) 4 (1.2%) 12 (3.7%)	 14 (6.5%) 185 (185) 3 (1.4%) 0 (0.0%) 12 (5.6%)	4.956	0.292

(*Continued*)

Table 3 (Continued).

Characteristics	Number of Patients*		χ^2	*P*
	Male (325)	**Female (214)**		
Aggravate season				
Spring	101 (31.4%)	75 (35.0%)	3.01	0.390
Sunmmer	200 (62.1%)	125 (58.4%)		
Fall	3 (0.9%)	0 (0.0%)		
Winter	18 (5.6%)	14 (6.5%)		
Dietary habits				
Smoking				
Never	217 (71.4%)	191 (90.5%)	29.213	**<0.001**
1–10 cigarette/d	57 (18.8%)	16 (7.6%)		
10–20 cigarette/d	23 (7.6%)	4 (1.9%)		
>20 cigarette/d	7 (2.3%)	0 (0.0)		
Alcohol				
Never	257 (82.4%)	192 (93.2%)	13.876	**0.001**
Once-twice/week	42 (13.5%)	13 (6.3%)		
More than third week	13 (4.2%)	1 (0.5%)		
Eggs				
Less than once/week	73 (22.9%)	56 (26.3%)	0.959	0.619
Once-thrid/week	122 (38.2%)	81 (38.0%)		
More than third week	124 (38.9%)	76 (35.7%)		
Vegetables				
Less than once/week	20 (6.2%)	8 (3.7%)	1.61	0.447
Once-thrid/week	34 (10.6%)	23 (10.7%)		
More than third week	267 (83.2%)	183 (85.5%)		
Friuts				
Less than once/week	47 (14.6%)	11 (5.1%)	25.517	**<0.001**
Once-thrid/week	102 (31.8%)	44 (20.6%)		
More than third week	172 (53.6%)	159 (74.3%)		
Sweet food				
Less than once/week	150 (46.7%)	89 (41.6%)	1.87	0.393
Once-thrid/week	100 (31.2%)	68 (31.8%)		
More than third week	71 (22.1%)	57 (26.6%)		
Lard oil				
Less than once/week	85 (26.5%)	77 (36.0%)	14.86	**0.001**
Once-thrid/week	63 (19.6%)	58 (27.1%)		
More than third week	173 (53.9%)	79 (36.9%)		
Spicy food				
Less than once/week	101 (31.3%)	68 (31.8%)	0.024	0.988
Once-thrid/week	103 (31.9%)	67 (31.3%)		
More than third week	119 (36.8%)	79 (36.9%)		
Pork				
Less than once/week	25 (7.8%)	19 (8.9%)	4.002	0.135
Once-thrid/week	49 (15.2%)	46 (21.5%)		
More than third week	248 (77.0%)	149 (69.6%)		

(*Continued*)

Table 3 (Continued).

Characteristics	Number of Patients*		χ^2	*P*
	Male (325)	Female (214)		
Beef Less than once/week Once-thrid/week More than third week	 153 (47.7%) 112 (34.9%) 56 (17.4%)	 104 (48.6%) 77 (36.0%) 33 (15.4%)	0.383	0.826
Family history Yes No	 209 (71.3%)	 87 103 (54.2%)		
Aggravate factors				
Sun exposure Yes No	 93 (30.4%) 213 (69.6%)	 70 (33.0%) 142 (67.0%)	0.401	0.527
Menstrual cycle Yes No	 0 (0.0%) 325 (100.0%)	 121 (56.8%) 92 (43.2%)	238.196	**<0.001**
Nervous Yes No	 132 (40.7%) 192 (59.3%)	 127 (59.3%) 87 (40.7%)	17.869	**<0.001**
Depressed Yes No	 70 (21.6%) 254 (78.4%)	 83 (38.8%) 131 (61.2%)	18.691	**<0.001**
Agrypnia Yes No	 93 (28.7%) 231 (71.3%)	 93 (43.5%) 121 (56.5%)	12.403	**<0.001**

Notes: *There are some missing data. *P* values <0.05 were marked in bold.

Table 4 Age and Gender Characteristics of Successfully Genotyped Cases and Controls

	Gender (n)		Total	*P* +	Age (Mean±SD) of Years	t*	*P**
	Male	Female					
Control	355	276	631	0.085	26.76 ± 8.05	−5.37	< 0.001
Case	348	221	569		22.62 ± 6.30		

Notes: +χ^2 test. *Student's *t*-test.

frequencies between the two groups (Table 7). We performed an overall haplotype test to analyze the global difference in haplotype frequencies between the case and control groups, which showed a significant difference (case vs control, $P = 0.032$; male case vs male control, $P = 0.011$). In particular, haplotype AGG was significantly associated with a lower risk of severe acne vulgaris in male patients (OR = 0.697, $P = 0.009$). Inversely, haplotype AGA was significantly associated with a higher risk of severe acne vulgaris in male patients (OR = 1.822, $P = 0.002$), and haplotype AGA also affected risk in the whole patient group, but the *P* value was only marginally significant (OR = 1.350, $P = 0.044$), and this positive association disappeared after Bonferroni correction. For *CYP19A1*, we could not find any significant heterogeneity using either the overall

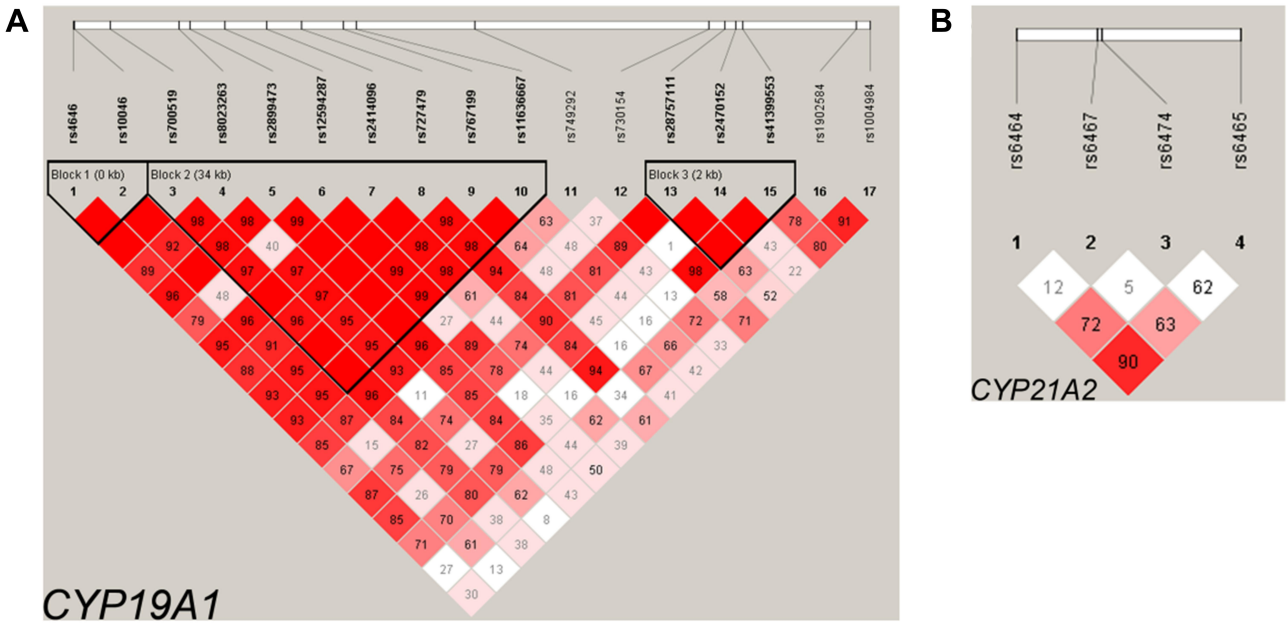

Figure 1 Linkage disequilibrium (LD) structures of the CYP19A1 gene (**A**) and the CYP21A2 gene (**B**) in controls from Yunnan Province in China. The results are based on the data obtained in this study. Red squares represent high LD as measured by D', which gradually desaturate to white squares of low LD. Individual squares show the 100 × D' value for each SNP pair.

haplotype test or single haplotype test for all subjects or after stratification by gender ($P > 0.05$).

Functional Implications of the SNP rs6474 (p. Arg102Lys) from Structure

Bovine and human sequences share 79% sequence identity. The overall structure of the human *CYP21A2* exhibited the typical P450 fold consisting of α-helical and β-sheet domains. The structure consists of two substrate binding sites (S1 and S2) and one substrate access channel (Figure 2A). Residues K98, L99, V100, S101, R102, N103, Y104, R223 and D234 lie within 5 Å of the substrate-binding site (S1) (Figure 2B).

Discussion

Both genetic factors and androgens play an important role in the predisposition to acne vulgaris,[3,5] which is one of the clinical features of hyperandrogenemia. Furthermore, acne occurs earlier and is more severe in those with a family history of acne.[4] To date, however, there are few reports on the association between polymorphisms or mutations of genes and acne.

Most studies have been population-based case and control studies, based on a small number sample, of certain steroid hormone-related genes, *ARs*,[9] human cytochrome P450 *1A1* genes (*CYP1A1*),[8] steroid 21-hydroxylase (*CYP21A2*),[10] steroid 17-hydroxylase (*CYP17*),[5] and innate immunity genes, such as toll-like receptors type 2 (*TLR2*),[3] toll-like receptors type 4 (*TLR4*),[27] tumor necrosis factor-alpha (*TNF-α*),[28] tumor necrosis factor receptor type 2 (*TNFR2*) 2, and interleukin-10 (*IL-10*).[28] Androgens/*AR*s signaling pathway is essential for the formation of acne. Testosterone can convert to 5α-dihydrotestosterone (DHT) by the 5α-reductase. Three kinds of 5α-reductase have been identified with different patterns of expression. Sebocytes and keratinocytes are the cells mainly expressing the type I 5α-reductase. Type II 5α-reductase is mainly observed in seminal vesicles, prostate, and epididymis. The third type of 5α-reductase is mostly found in prostate cancer. Infections lead to generation of acne in the hair follicle, which induces activation and migration of neutrophils and macrophages to the follicles. These cells are activated by *AR*-mediated signals and secret pro-inflammatory factors including IL-6, IL-12 and TNF-α, thereby aggravating the inflammatory responses and infection.[29,30] In this study, we successfully genotyped 21 gene variants of the *CYP19A1* and *CYP21A2* genes. We found that two tag SNPs (rs6474 and rs6465) in the *CYP21A2* gene were significantly associated with Pillsbury III–IV severe acne vulgaris, particularly among male patients with severe acne vulgaris. Genotype AA of rs6474 (p.Arg102Lys) of the *CYP21A2* gene showed a high risk for severe acne vulgaris and male severe acne vulgaris, while genotype TT of rs6465 conferred a weak protective effect against severe acne vulgaris and male severe acne

Table 5 A Comparison of the Genotype and Allele Frequency of Positive SNPs in the *CYP21A2* and *CYP19A1* genes Between Severe Acne Patients and Controls

SNP	Genotype/ Allele	All Subjects				Male Subjects				Female Subjects			
		Case (%) N=569	Control (%) N=631	OR (95% CI)	*P*-value*	Case (%) N=348	Control (%) N=355	OR (95% CI)	*P*-value*	Case (%) N=221	Control (%) N=276	OR (95% CI)	*P*-value*
CYP21A2													
rs6474	GG	415 (72.9)	472 (74.8)	1.000 (reference)	–	248 (71.3)	272 (76.6)	1.000 (reference)	–	167 (75.6)	200 (72.5)	1.000 (reference)	–
	AG	131 (23.0)	153 (24.2)	0.983 (0.744–1.299)	0.904	85 (24.4)	81 (22.8)	1.186 (0.829–1.696)	0.350	46 (20.8)	72 (26.1)	0.718 (0.455–1.132)	0.154
	AA	23 (4.0)	6 (1.0)	5.431 (2.060–14.32)	**0.001**[#]	15 (4.3)	2 (0.6)	11.66 (2.470–55.04)	**0.002**[#]	8 (3.6)	4 (1.4)	2.175 (0.555–8.531)	0.265
	G allele	961 (84.4)	1097 (86.9)	1.000 (reference)	–	581 (83.5)	625 (88.0)	1.000 (reference)	–	380 (86.0)	472 (85.5)	1.000 (reference)	–
	A allele	177 (15.6)	165 (13.1)	1.260 (0.992–1.601)	0.058	115 (16.5)	85 (12.0)	1.542 (1.131–2.103)	**0.006**[#]	62 (14.0)	80 (14.5)	0.894 (0.607–1.318)	0.573
rs6465	CC	441 (77.5)	472 (74.8)	1.000 (reference)		273 (78.4)	264 (74.4)	1.000 (reference)	–	168 (76.0)	208 (75.4)	1.000 (reference)	–
	CT	118 (20.7)	135 (21.4)	0.953 (0.713–1.274)	0.744	70 (20.1)	73 (20.6)	0.927 (0.637–1.351)	0.694	48 (21.7)	62 (22.5)	0.991 (0.626–1.571)	0.971
	TT	10 (1.8)	24 (3.8)	0.417 (0.194–0.896)	**0.025**	5 (1.4)	18 (5.1)	0.272 (0.099-0.751)	**0.012**[#]	5 (2.3)	6 (2.2)	0.886 (0.250–3.133)	0.850
	C allele	1000 (87.9)	1079 (85.5)	1.000 (reference)		616 (88.5)	601 (84.6)	1.000 (reference)		384 (86.9)	478 (86.6)	1.000 (reference)	–
	T allele	138 (12.1)	183 (14.5)	0.809 (0.633–1.035)	0.092	80 (11.5)	109 (15.4)	0.717 (0.523–0.983)	**0.039**	58 (13.1)	74 (13.4)	0.973 (0.655–1.445)	0.892
CYP19A1													
rs8023263	GG	130 (22.8)	125 (19.8)	1.000 (reference)	–	85 (24.4)	64 (18.0)	1.000 (reference)	–	45 (20.4)	61 (22.1)	1.000 (reference)	–
	GT	273 (48.0)	319 (50.6)	0.788 (0.580–1.070)	0.126	163 (46.8)	188 (53.0)	0.658 (0.444–0.975)	**0.037**	110 (49.8)	131 (47.5)	0.996 (0.604–1.644)	0.989
	TT	166 (29.2)	187 (29.6)	0.904 (0.646–1.265)	0.556	100 (28.7)	103 (29.0)	0.791 (0.512–1.221)	0.289	66 (29.9)	84 (30.4)	1.065 (0.618–1.833)	0.821
	G allele	533 (46.8)	569 (45.1)	1.000 (reference)	–	333 (47.8)	316 (44.5)	1.000 (reference)	–	200 (45.2)	253 (45.8)	1.000 (reference)	–
	T allele	605 (53.2)	693 (54.9)	0.965 (0.816–1.140)	0.675	363 (52.2)	394 (55.5)	0.911 (0.735–1.128)	0.391	242 (54.8)	299 (54.2)	1.036 (0.790–1.358)	0.800
rs2470152	TT	126 (22.1)	158 (25.0)	1.000 (reference)	–	73 (21.0)	101 (28.5)	1.000 (reference)	–	53 (24.0)	57 (20.7)	1.000 (reference)	–
	CT	289 (50.8)	291 (46.1)	1.300 (0.967–1.748)	0.083	184 (52.9)	152 (42.8)	1.675 (1.149–2.442)	**0.007**	105 (47.5)	139 (50.4)	0.878 (0.538–1.430)	0.600
	CC	154 (27.1)	182 (28.8)	1.131 (0.813–1.574)	0.463	91 (26.1)	102 (28.7)	1.231 (0.808–1.873)	0.333	63 (28.5)	80 (29.0)	1.011 (0.588–1.736)	0.970
	T allele	541 (47.5)	607 (48.1)	1.000 (reference)	–	330 (47.4)	354 (49.9)	1.000 (reference)	–	211 (47.7)	253 (45.8)	1.000 (reference)	–
	C allele	597 (52.5)	655 (51.9)	1.057 (0.894–1.249)	0.516	366 (52.6)	356 (50.1)	1.101 (0.890–1.363)	0.377	231 (52.3)	299 (54.2)	1.014 (0.773–1.330)	0.920

Notes: *All data were calculated by using the unconditional logistic regression, with an adjustment for age. The major alleles of all the SNPs were chosen as references. [#]Considering multiple testing correction, a more stringent cut-off *P* value was set as 0.0125 (0.05/4, Bonferroni correction) for the data set. SNP rs6474 of *CYP21A2* remain significant with severe acne after the stringent Bonferroni correction. For male severe acne, rs6474 of *CYP21A2* and rs2470152 of *CYP19A1* remain significant, while rs6465 of *CYP21A2* show a marginal significant difference after the stringent Bonferroni correction. *P* values <0.05 were marked in bold.

Table 6 A Comparison of the Genotype and Allele Frequency of 2 SNPs of *CYP21A2* and 15 SNPs of *CYP19A1* Between Severe Acne Patients and Controls

SNP	Genotype/ Allele	All Subjects				Male Subjects				Female Subjects			
		Case (%) N=569	Control (%) N=631	OR (95% CI)	P*	Case (%) N=348	Control (%) N=355	OR (95% CI)	P*	Case (%) N=221	Control (%) N=276	OR (95% CI)	P*
CYP21A2													
rs6464	AA	344 (60.5)	387 (61.3)	1.000 (reference)	–	204 (58.6)	218 (61.4)	1.000 (reference)	–	140 (63.3)	169 (61.2)	1.000 (reference)	–
	AC	200 (35.1)	215 (34.1)	1.013 (0.789–1.302)	0.917	128 (36.8)	126 (35.5)	1.041 (0.758–1.430)	0.805	72 (32.6)	89 (32.2)	1.032 (0.683–1.557)	0.882
	CC	25 (4.4)	29 (4.6)	0.924 (0.519–1.643)	0.787	16 (4.6)	11 (3.1)	1.272 (0.572–2.827)	0.556	9 (4.1)	18 (6.5)	0.680 (0.280–1.655)	0.396
	A allele	888 (78.0)	989 (78.4)	1.000 (reference)	–	536 (77.0)	562 (79.2)	1.000 (reference)	–	352 (79.6)	427 (77.4)	1.000 (reference)	–
	C allele	250 (22.0)	273 (21.6)	0.991 (0.810–1.213)	0.932	160 (23.0)	148 (20.8)	1.068 (0.825–1.381)	0.619	90 (20.4)	125 (22.6)	0.928 (0.669–1.288)	0.655
rs6467	TT	217 (38.1)	240 (38.0)	1.000 (reference)	–	137 (39.4)	139 (39.2)	1.000 (reference)	–	80 (36.2)	101 (36.6)	1.000 (reference)	–
	GT	251 (44.1)	285 (45.2)	0.992 (0.765–1.287)	0.954	145 (41.7)	149 (42.0)	0.991 (0.709–1.385)	0.956	106 (48.0)	136 (49.3)	0.964 (0.635–1.465)	0.865
	GG	101 (17.8)	106 (16.8)	1.006 (0.715–1.415)	0.972	66 (19.0)	67 (18.9)	0.976 (0.641–1.487)	0.910	35 (15.8)	39 (14.1)	1.095 (0.609–1.968)	0.763
	T allele	685 (60.2)	765 (60.6)	1.000 (reference)	–	419 (60.2)	427 (60.1)	1.000 (reference)	–	266 (60.2)	338 (61.2)	1.000 (reference)	–
	G allele	453 (39.8)	497 (39.4)	1.001 (0.844–1.187)	0.989	277 (39.8)	283 (39.9)	0.987 (0.794–1.227)	0.905	176 (39.8)	214 (38.8)	1.027 (0.779–1.354)	0.852
CYP19A1													
rs4646	CC	295 (51.8)	324 (51.3)	1.000 (reference)	–	168 (48.3)	180 (50.7)	1.000 (reference)	–	127 (57.5)	144 (52.2)	1.000 (reference)	–
	AC	240 (42.2)	262 (41.5)	0.929 (0.727–1.188)	0.558	157 (45.1)	150 (42.3)	1.088 (0.796–1.488)	0.597	83 (37.6)	112 (40.6)	0.722 (0.482–1.080)	0.113
	AA	34 (6.0)	45 (7.1)	0.849 (0.518–1.391)	0.515	23 (6.6)	25 (7.0)	0.989 (0.534–1.832)	0.973	11 (5.0)	20 (7.2)	0.711 (0.305–1.657)	0.430
	C allele	830 (72.9)	910 (72.1)	1.000 (reference)	–	493 (70.8)	510 (71.8)	1.000 (reference)	–	337 (76.2)	400 (72.5)	1.000 (reference)	–
	A allele	308 (27.1)	352 (27.9)	0.929 (0.771–1.120)	0.442	203 (29.2)	200 (28.2)	1.037 (0.819–1.312)	0.765	105 (23.8)	152 (27.5)	0.784 (0.574–1.071)	0.127
rs10046	TT	170 (29.9)	182 (28.8)	1.000 (reference)	–	105 (30.2)	100 (28.2)	1.000 (reference)	–	65 (29.4)	82 (29.7)	1.000 (reference)	–
	CT	279 (49.0)	326 (51.7)	0.821 (0.624–1.081)	0.160	161 (46.3)	189 (53.2)	0.761 (0.534–1.083)	0.129	118 (53.4)	137 (49.6)	0.888 (0.571–1.381)	0.597
	CC	120 (21.1)	123 (19.5)	0.987 (0.701–1.388)	0.939	82 (23.7)	66 (18.6)	1.112 (0.720–1.716)	0.632	38 (17.2)	57 (20.7)	0.839 (0.476–1.478)	0.544
	T allele	619 (54.4)	690 (54.7)	1.000 (reference)	–	371 (53.3)	389 (54.8)	1.000 (reference)	–	248 (56.1)	301 (54.5)	1.000 (reference)	–
	C allele	519 (45.6)	572 (45.3)	0.976 (0.826–1.154)	0.780	325 (46.7)	321 (45.2)	1.027 (0.829–1.272)	0.807	194 (43.9)	251 (45.5)	0.917 (0.698–1.203)	0.530
rs700519	CC	410 (72.1)	453 (71.8)	1.000 (reference)	–	258 (74.1)	254 (71.5)	1.000 (reference)	–	152 (68.8)	199 (72.1)	1.000 (reference)	–
	CT	144 (25.3)	161 (25.5)	0.974 (0.742–1.278)	0.850	79 (22.7)	93 (26.2)	0.802 (0.564–1.142)	0.222	65 (29.4)	68 (24.6)	1.302 (0.846–2.006)	0.231
	TT	15 (2.6)	17 (2.7)	1.012 (0.486–2.104)	0.975	11 (3.2)	8 (2.3)	1.328 (0.516–3.416)	0.556	4 (1.8)	9 (3.3)	0.699 (0.201–2.432)	0.573
	C allele	964 (84.7)	1067 (84)	1.000 (reference)	–	595 (85.5)	601 (84.6)	1.000 (reference)	–	369 (83.5)	466 (84.4)	1.000 (reference)	–
	T allele	174 (15.3)	195 (15.5)	0.984 (0.782–1.240)	0.894	101 (14.5)	109 (15.4)	0.907 (0.673–1.223)	0.523	73 (16.5)	86 (15.6)	1.129 (0.783–1.628)	0.515
rs2899473	CC	387 (68.0)	439 (69.6)	1.000 (reference)	–	244 (70.1)	248 (69.9)	1.000 (reference)	–	143 (64.7)	191 (69.2)	1.000 (reference)	–
	CT	165 (29.0)	172 (27.3)	1.059 (0.813–1.378)	0.672	91 (26.1)	98 (27.6)	0.891 (0.633–1.255)	0.509	74 (33.5)	74 (26.8)	1.372 (0.901–2.088)	0.141
	TT	17 (3.0)	20 (3.2)	1.073 (0.539–2.135)	0.841	13 (3.7)	9 (2.5)	1.559 (0.639–3.804)	0.329	4 (1.8)	11 (4.0)	0.612 (0.182–2.053)	0.427
	C allele	939 (82.5)	1050 (83)	1.000 (reference)	–	579 (83.2)	594 (83.7)	1.000 (reference)	–	360 (81.4)	456 (82.6)	1.000 (reference)	–
	T allele	199 (17.5)	212 (16.8)	1.051 (0.843–1.311)	0.659	117 (16.8)	116 (16.3)	1.007 (0.756–1.341)	0.962	82 (18.6)	96 (17.4)	1.137 (0.800–1.615)	0.475

rs12594287	GG	320 (56.2)	363 (57.5)	1.000 (reference)	–	207 (59.5)	206 (58.0)	1.000 (reference)	–	113 (51.1)	157 (56.9)	1.000 (reference)	–
	AG	212 (37.3)	216 (34.2)	1.092 (0.849–1.404)	0.492	118 (33.9)	120 (33.8)	0.956 (0.691–1.324)	0.787	94 (42.5)	96 (34.8)	1.286 (0.860–1.922)	0.220
	AA	37 (6.5)	52 (8.2)	0.875 (0.549–1.395)	0.574	23 (6.6)	29 (8.2)	0.831 (0.459–1.505)	0.542	14 (6.3)	23 (8.3)	0.970 (0.455–2.065)	0.936
	G allele	852 (74.9)	942 (74.6)	1.000 (reference)	–	532 (76.4)	532 (74.9)	1.000 (reference)	–	320 (72.4)	410 (74.3)	1.000 (reference)	–
	A allele	286 (25.1)	320 (25.4)	1.003 (0.828–1.215)	0.975	164 (23.6)	178 (25.1)	0.926 (0.722–1.188)	0.545	122 (27.6)	142 (25.7)	1.113 (0.821–1.506)	0.490
rs2414096	GG	172 (30.2)	183 (29.0)	1.000 (reference)	–	104 (29.9)	101 (28.5)	1.000 (reference)	–	68 (30.8)	82 (29.7)	1.000 (reference)	–
	AG	273 (48.0)	307 (48.7)	0.928 (0.705–1.221)	0.593	168 (48.3)	172 (48.5)	0.954 (0.670–1.359)	0.795	105 (47.5)	135 (48.9)	0.871 (0.558–1.359)	0.542
	AA	124 (21.8)	141 (22.3)	0.972 (0.698–1.354)	0.866	76 (21.8)	82 (23.1)	0.921 (0.603–1.407)	0.704	48 (21.7)	59 (21.4)	1.091 (0.636–1.873)	0.751
	G allele	617 (54.2)	673 (53.3)	1.000 (reference)	–	376 (54.0)	374 (52.7)	1.000 (reference)	–	241 (54.5)	299 (54.2)	1.000 (reference)	–
	A allele	521 (45.8)	589 (46.7)	0.981 (0.830–1.159)	0.823	320 (46.0)	336 (47.3)	0.958 (0.774–1.187)	0.697	201 (45.5)	253 (45.8)	1.030 (0.785–1.350)	0.832
rs727479	TT	300 (52.7)	350 (55.5)	1.000 (reference)	–	173 (49.7)	198 (55.8)	1.000 (reference)	–	127 (57.5)	152 (55.1)	1.000 (reference)	–
	GT	232 (40.8)	237 (37.6)	1.040 (0.812–1.332)	0.753	154 (44.3)	135 (38.0)	1.238 (0.905–1.695)	0.182	78 (35.3)	102 (37.0)	0.786 (0.522–1.184)	0.249
	GG	37 (6.5)	44 (7.0)	1.009 (0.621–1.640)	0.970	21 (6.0)	22 (6.2)	1.071 (0.562–2.041)	0.834	16 (7.2)	22 (8.0)	0.939 (0.441–1.999)	0.870
	T allele	832 (73.1)	937 (74.2)	1.000 (reference)	–	500 (71.8)	531 (74.8)	1.000 (reference)	–	332 (75.1)	406 (73.6)	1.000 (reference)	–
	G allele	306 (26.9)	325 (25.8)	1.022 (0.846–1.234)	0.825	196 (28.2)	179 (25.2)	1.128 (0.886–1.435)	0.329	110 (24.9)	146 (26.4)	0.877 (0.643–1.197)	0.409
rs767199	GG	174 (30.6)	183 (29.0)	1.000 (reference)	–	107 (30.7)	101 (28.5)	1.000 (reference)	–	67 (30.3)	82 (29.7)	1.000 (reference)	–
	AG	269 (47.3)	313 (49.6)	0.883 (0.671–1.162)	0.374	159 (45.7)	177 (49.9)	0.855 (0.601–1.216)	0.383	109 (49.3)	136 (49.3)	0.901 (0.578–1.405)	0.647
	AA	126 (22.1)	135 (21.4)	1.040 (0.745–1.451)	0.817	81 (23.3)	77 (21.7)	1.020 (0.669–1.556)	0.927	45 (20.4)	58 (21.0)	1.127 (0.651–1.951)	0.668
	G allele	617 (54.2)	679 (53.8)	1.000 (reference)	–	373 (53.6)	379 (53.4)	1.000 (reference)	–	243 (55.0)	300 (54.3)	1.000 (reference)	–
	A allele	521 (45.7)	583 (46.2)	1.008 (0.853–1.192)	0.922	321 (46.1)	331 (46.6)	0.998 (0.806–1.236)	0.988	199 (45.0)	252 (45.7)	1.045 (0.797–1.371)	0.750
rs11636667	CC	174 (30.6)	183 (29.0)	1.000 (reference)	–	106 (30.5)	101 (28.5)	1.000 (reference)	–	68 (30.8)	82 (29.7)	1.000 (reference)	–
	CT	273 (48.0)	311 (49.3)	0.908 (0.690–1.194)	0.489	163 (46.8)	176 (49.6)	0.886 (0.622–1.260)	0.499	110 (49.8)	135 (48.9)	0.924 (0.594–1.437)	0.725
	TT	122 (21.4)	137 (21.7)	0.979 (0.701–1.367)	0.902	79 (22.7)	78 (22.0)	0.989 (0.648–1.510)	0.958	43 (19.5)	59 (21.4)	1.004 (0.580–1.737)	0.989
	C allele	621 (54.6)	677 (53.6)	1.000 (reference)	–	375 (53.9)	378 (53.2)	1.000 (reference)	–	246 (55.7)	299 (54.2)	1.000 (reference)	–
	T allele	517 (45.4)	585 (46.4)	0.983 (0.831–1.161)	0.836	321 (46.1)	332 (46.8)	0.986 (0.797–1.221)	0.900	196 (44.3)	253 (45.8)	0.994 (0.758–1.304)	0.965
rs749292	GG	177 (31.1)	180 (28.5)	1.000 (reference)	–	114 (32.8)	98 (27.6)	1.000 (reference)	–	63 (28.5)	82 (29.7)	1.000 (reference)	–
	AG	280 (49.2)	312 (49.4)	0.906 (0.689–1.191)	0.479	163 (46.8)	175 (49.3)	0.809 (0.570–1.149)	0.236	117 (52.9)	137 (49.6)	1.040 (0.666–1.623)	0.863
	AA	112 (19.7)	139 (22.0)	0.847 (0.605–1.187)	0.335	71 (20.4)	82 (23.1)	0.746 (0.488–1.140)	0.175	41 (18.6)	57 (20.7)	1.115 (0.635–1.957)	0.706
	G allele	634 (55.7)	672 (53.2)	1.000 (reference)	–	391 (56.2)	371 (52.3)	1.000 (reference)	–	243 (55.0)	301 (54.5)	1.000 (reference)	–
	A allele	504 (44.3)	590 (46.8)	0.919 (0.777–1.086)	0.322	305 (43.8)	339 (47.7)	0.855 (0.690–1.059)	0.151	199 (45.0)	251 (45.5)	1.051 (0.801–1.379)	0.718
rs730154	AA	233 (40.9)	276 (43.7)	1.000 (reference)	–	148 (42.5)	150 (42.3)	1.000 (reference)	–	85 (38.5)	126 (45.7)	1.000 (reference)	–
	AG	255 (44.8)	265 (42.0)	1.116 (0.865–1.439)	0.399	151 (43.4)	154 (43.4)	0.998 (0.721–1.381)	0.988	104 (47.1)	111 (40.2)	1.292 (0.854–1.954)	0.225
	GG	81 (14.2)	90 (14.3)	1.061 (0.740–1.523)	0.746	49 (14.1)	51 (14.4)	0.986 (0.621–1.566)	0.953	32 (14.5)	39 (14.1)	1.172 (0.654–2.100)	0.594
	A allele	721 (63.4)	817 (64.7)	1.000 (reference)	–	447 (64.2)	454 (63.9)	1.000 (reference)	–	274 (62.0)	363 (65.8)	1.000 (reference)	–
	G allele	417 (36.6)	445 (35.3)	1.054 (0.886–1.253)	0.553	249 (35.8)	256 (36.1)	0.994 (0.796–1.241)	0.957	168 (38.0)	189 (34.2)	1.137 (0.859–1.506)	0.369

(*Continued*)

Table 6 (Continued).

SNP	Genotype/ Allele	All Subjects				Male Subjects				Female Subjects			
		Case (%) N=569	Control (%) N=631	OR (95% CI)	*P**	Case (%) N=348	Control (%) N=355	OR (95% CI)	*P**	Case (%) N=221	Control (%) N=276	OR (95% CI)	*P**
rs28757111	TT	371 (65.2)	429 (68.0)	1.000 (reference)	–	229 (65.8)	241 (67.9)	1.000 (reference)	–	142 (64.3)	188 (68.1)	(reference)	–
	CT	182 (32.0)	177 (28.1)	1.134 (0.875–1.469)	0.341	107 (30.7)	103 (29.0)	1.023 (0.734–1.425)	0.895	75 (33.9)	74 (26.8)	1.376 (0.903–2.096)	0.137
	CC	16 (2.8)	25 (4.0)	0.830 (0.426–1.618)	0.584	12 (3.4)	11 (3.1)	1.231 (0.521–2.906)	0.636	4 (1.8)	14 (5.1)	0.454 (0.139–1.479)	0.190
	T allele	924 (81.2)	1035 (82.)	1.000 (reference)	–	565 (81.2)	585 (82.4)	1.000 (reference)	–	359 (81.2)	450 (81.5)	1.000 (reference)	–
	C allele	214 (18.8)	227 (18.0)	1.047 (0.845–1.298)	0.675	131 (18.8)	125 (17.6)	1.052 (0.799–1.386)	0.717	83 (18.8)	102 (18.5)	1.066 (0.753–1.508)	0.720
rs41399553	CC	388 (68.2)	435 (68.9)	1.000 (reference)	–	241 (69.3)	238 (67.0)	1.000 (reference)	–	147 (66.5)	197 (71.4)	1.000 (reference)	–
	CT	168 (29.5)	181 (28.7)	1.031 (0.795–1.338)	0.815	99 (28.4)	108 (30.4)	0.937 (0.672–1.307)	0.702	69 (31.2)	73 (26.4)	1.142 (0.749–1.741)	0.537
	TT	13 (2.3)	15 (2.4)	0.976 (0.441–2.160)	0.952	8 (2.3)	9 (2.5)	1.012 (0.368–2.876)	0.981	5 (2.3)	6 (2.2)	0.809 (0.226–2.891)	0.744
	C allele	944 (83.0)	1051 (83.)	1.000 (reference)	–	581 (83.5)	584 (82.3)	1.000 (reference)	–	363 (82.1)	467 (84.6)	1.000 (reference)	–
	T allele	194 (17.0)	211 (16.7)	1.018 (0.815–1.271)	0.873	115 (16.5)	126 (17.7)	0.958 (0.721–1.272)	0.767	79 (17.9)	85 (15.4)	1.064 (0.743–1.524)	0.736
rs1902584	AA	394 (69.2)	452 (71.6)	1.000 (reference)	–	249 (71.6)	266 (74.9)	1.000 (reference)	–	145 (65.6)	186 (67.4)	1.000 (reference)	–
	AT	164 (28.8)	163 (25.8)	1.098 (0.843–1.431)	0.488	91 (26.1)	81 (22.8)	1.125 (0.792–1.599)	0.511	73 (33.0)	82 (29.7)	1.003 (0.664–1.515)	0.989
	TT	11 (1.9)	16 (2.5)	0.928 (0.410–2.098)	0.857	8 (2.3)	8 (2.3)	1.311 (0.468–3.671)	0.607	3 (1.4)	8 (2.9)	0.463 (0111–1.932)	0.291
	A allele	952 (83.7)	1067 (84)	1.000 (reference)	–	589 (84.6)	613 (86.3)	1.000 (reference)	–	363 (82.1)	454 (82.2)	1.000 (reference)	–
	T allele	186 (16.3)	195 (15.5)	1.057 (0.842–1.327)	0.634	107 (15.4)	97 (13.7)	1.132 (0.831–1.532)	0.421	79 (17.9)	98 (17.8)	0.919 (0.646–1.306)	0.637
rs1004984	CC	246 (43.2)	294 (46.6)	1.000 (reference)	–	158 (45.4)	162 (45.6)	1.000 (reference)	–	88 (39.8)	132 (47.8)	1.000 (reference)	–
	CT	255 (44.8)	272 (43.1)	1.085 (0.845–1.393)	0.524	150 (43.1)	158 (44.5)	0.969 (0.705–1.333)	0.848	105 (47.5)	114 (41.3)	1.244 (0.827–1.871)	0.294
	TT	68 (12.0)	65 (10.3)	1.243 (0.837–1.846)	0.282	40 (11.5)	35 (9.9)	1.282 (0.763–2.155)	0.348	28 (12.7)	30 (10.9)	1.062 (0.569–1.983)	0.850
	C allele	747 (65.6)	860 (68.1)	1.000 (reference)	–	466 (67.0)	482 (67.9)	1.000 (reference)	–	281 (63.6)	378 (68.5)	1.000 (reference)	–
	T allele	391 (34.4)	402 (31.9)	1.105 (0.926–1.319)	0.269	230 (33.0)	228 (32.1)	1.071 (0.853–1.345)	0.553	161 (36.4)	174 (31.5)	1.092 (0.821–1.451)	0.545

Notes: *All data were calculated by using the unconditional logistic regression, with an adjustment for age. The major alleles of all the SNPs were chosen as references.

Table 7 The Association of the *CYP21A2* and *CYP19A1* Haplotypes with Severe Acne Patients and Controls in the Han Chinese

Haplotype	All Subjects				Male Subjects				Female Subjects			
	Case N=1138 (%)	Control N=1262 (%)	OR (95% CI)	*P*-value*	Case N=696 (%)	Control N=710 (%)	OR (95% CI)	*P*-value*	Case N=442 (%)	Control N=552 (%)	OR (95% CI)	*P*-value*
CYP21A2 gene: rs6464, rs6467, rs6474												
ATG	45.3	46.4	0.954 (0.812–1.120)	0.566	44.7	46.2	0.941 (0.763–1.161)	0.592	46.2	46.7	0.977 (0.760 – 1.25)	0.898
AGG	17.3	19.7	0.856 (0.696–1.053)	0.156	15.9	21.4	0.697 (0.531–0.913)	**0.009**#	19.5	17.4	1.147 (0.831–1.58)	0.410
CGG	12.4	11.3	1.115 (0.870–1.430)	0.410	12.6	11.5	1.108 (0.804–1.528)	0.567	12.0	10.9	1.117 (0.754–1.65)	0.616
CTG	9.5	9.6	0.989 (0.753–1.299)	0.945	10.2	8.9	1.167 (0.817–1.667)	0.415	8.4	10.5	0.778 (0.505–1.20)	0.278
AGA	10.1	7.7	1.350 (1.017–1.792)	0.044	11.2	6.5	1.822 (1.245–2.665)	**0.002**#	8.4	9.2	0.897 (0.576–1.39)	0.655
ATA	5.4	4.6	1.176 (0.813–1.700)	0.398	5.2	5.1	1.021 (0.635–1.641)	1.000	5.7	4.0	1.444 (0.803–2.59)	0.232
Others	0.1	0.8	0.110 (0.014–0.862)	**0.013**#	0.1	0.4	0.339 (0.035–3.268)	0.625	0.0	1.3	0.552 (0.522–0.58)	**0.019**
Global	10.1	7.7		**0.032**				**0.011**				0.158
CYP19A1 gene												
Block1: rs4646, rs10046												
CT	54.4	54.7	0.989 (0.842–1.161)	0.902	53.3	54.8	0.942 (0.764–1.162)	0.593	56.1	54.5	1.066 (0.828–1.371)	0.653
AC	27.1	27.9	0.959 (0.802–1.148)	0.680	29.2	28.2	1.050 (0.833–1.323)	0.680	23.8	27.5	0.820 (0.615–1.093)	0.190
CC	18.5	17.4	1.078 (0.875–1.328)	0.489	17.5	17.0	1.035 (0.785–1.364)	0.833	20.1	17.9	1.154 (0.839–1.586)	0.415
Global				0.752				0.875				0.349
Block2: rs700519 rs8023263 rs2899473 rs12594287: rs2414096 rs727479 rs767199 rs11636667												
CTCGATAT	42.8	45.2	0.905 (0.770–1.064)	0.233	43.0	45.4	0.908 (0.735–1.120)	0.390	42.3	44.7	0.906 (0.703–1.166)	0.479
CGCGGGGC	25.0	24.7	1.013 (0.841–1.219)	0.927	26.1	24.1	1.116 (0.877–1.421)	0.389	22.9	25.5	0.863 (0.644–1.157)	0.334
TGTAGTGC	14.5	14.7	0.981 (0.782–1.231)	0.908	13.5	14.8	0.899 (0.666–1.215)	0.492	16.1	14.7	1.113 (0.787–1.574)	0.595
CTCAGTGC	7.5	8.3	0.889 (0.660–1.198)	0.450	6.6	8.5	0.767 (0.514–1.143)	0.225	8.8	8.5	1.040 (0.667–1.621)	0.910
Others[a]	10.3	7.0	1.529 (1.145–2.041)	**0.034**	10.8	7.3	1.528 (1.055–2.213)	**0.026**	10	6.5	1.585 (1.001–2.509)	0.060
Global				0.059				0.104				0.289

(*Continued*)

Table 7 (Continued).

Haplotype	All Subjects				Male Subjects				Female Subjects			
	Case N=1138 (%)	Control N=1262 (%)	OR (95% CI)	*P*-value*	Case N=696 (%)	Control N=710 (%)	OR (95% CI)	*P*-value*	Case N=442 (%)	Control N=552 (%)	OR (95% CI)	*P*-value*
Block3: rs28757111 rs2470152 rs41399553												
TCC	33.5	33.9	0.981 (0.828–1.162)	0.829	33.6	32.5	1.05 (0.841–1.312)	0.691	33.3	35.7	0.898 (0.690–1.169)	0.461
TTC	30.7	31.4	0.967 (0.813–1.150)	0.724	31.0	32.1	0.951 (0.760–1.191)	0.688	30.1	30.4	0.984 (0.749–1.292)	0.945
CCC	18.7	18.0	1.050 (0.854–1.291)	0.673	18.7	17.6	1.075 (0.819–1.410)	0.628	18.8	18.5	1.020 (0.740–1.406)	0.935
TTT	16.8	16.7	1.005 (0.811–1.245)	1.000	16.2	17.7	0.898 (0.680–1.187)	0.478	17.6	15.4	1.177 (0.841–1.648)	0.345
Others[a]	0.4	0.0	0.473 (0.454–0.494)	0.050	0.4	0.0	0.494 (0.468–0.521)	0.121	0.2	0	0.444 (0.414–0.476)	0.445
Global				0.325				0.424				0.696

Notes: *P-values were calculated by using the Fisher's exact test. Person's chi-square test was used for estimating global *P*-value. *P* values <0.05 were showed in bold. #Indicated significant *P*-value after Bonferroni correction (The significant threshold for *CYP21A2* is P<0.0167 (0.05/3)). [a]Haplotypes with a frequency of <3% in the case or control groups were pooled together.

vulgaris. The minor allele A of rs6474 (p.Arg102Lys) conferred a strong predisposition to male severe acne vulgaris and minor allele T of rs6465 showed a weak protective effect on male severe acne vulgaris (Table 5).

Unfortunately, we failed to find any association between severe acne vulgaris and four well-reported SNPs [rs4646 and rs10046 (in the 3′-UTR), rs700519 (Arg264Cys), rs2414096] of other androgen-related diseases. However, we found genotypes of two tag SNPs (rs8023263 and rs2470152) in the noncoding region of the *CYP19A1* gene were associated with male patients with severe acne vulgaris. The Genotype GT of rs8023263 conferred a weak protective effect on male severe acne vulgaris patients, whereas heterozygote CT of rs2470152 showed a significant risk for male severe acne vulgaris patients. We found that the most frequent haplotype AGG of the *CYP21A2* gene tended to provide a protective effect for male patients with severe acne vulgaris, whereas haplotype AGA conferred a risk-effect towards male patients with severe acne vulgaris. This disparity suggests that the gene variant rs6474 (p.Arg102Lys) may be a causative SNP in severe acne vulgaris, especially for among males. However, for *CYP19A1*, we could not find any significant heterogeneity.

A growing number of studies of gene variants of *CYP21A2* focus on an autosomal recessive inherited disorder of steroid metabolism, known as congenital adrenal hyperplasia (CAH), which has been classified into a classical (C-CAH) and a nonclassical (NC-CAH) form.[31] Further studies using molecular screening techniques found that the mutation of *CYP21A2* was more common in unselected acne patients than in controls, further supporting the possibility that the *CYP21A2* gene may contribute to the variability of the clinical phenotype in hyperandrogenic states, including acne.[10,32]

In this study, we found that the two novel SNPs of *CYP21A2*, rs6474 (p.Arg102Lys) and rs6465, may confer a susceptibility to severe acne vulgaris, particularly for males. Rs6474 (p.Arg102Lys), located on the extron 3 of *CYP21A2*, may reduce the activity of 21-hydroxylase, which can result in adrenal androgen excess and contribute to the clinical manifestations of male severe acne vulgaris. Bovine shares 79% sequence identity with humans. The typical P450 fold, which was composed of α-helical and β-sheet domains, was showed in the structure of human *CYP21A2*. The structure consists of two substrate binding sites (S1 and S2) and one substrate access channel (Figure 2A). These local structures are critical for the function of *CYP21A2*. Mutations at these local structures may affect binding and converting of the substrate 17-OHP, thus causing multiple related diseases,

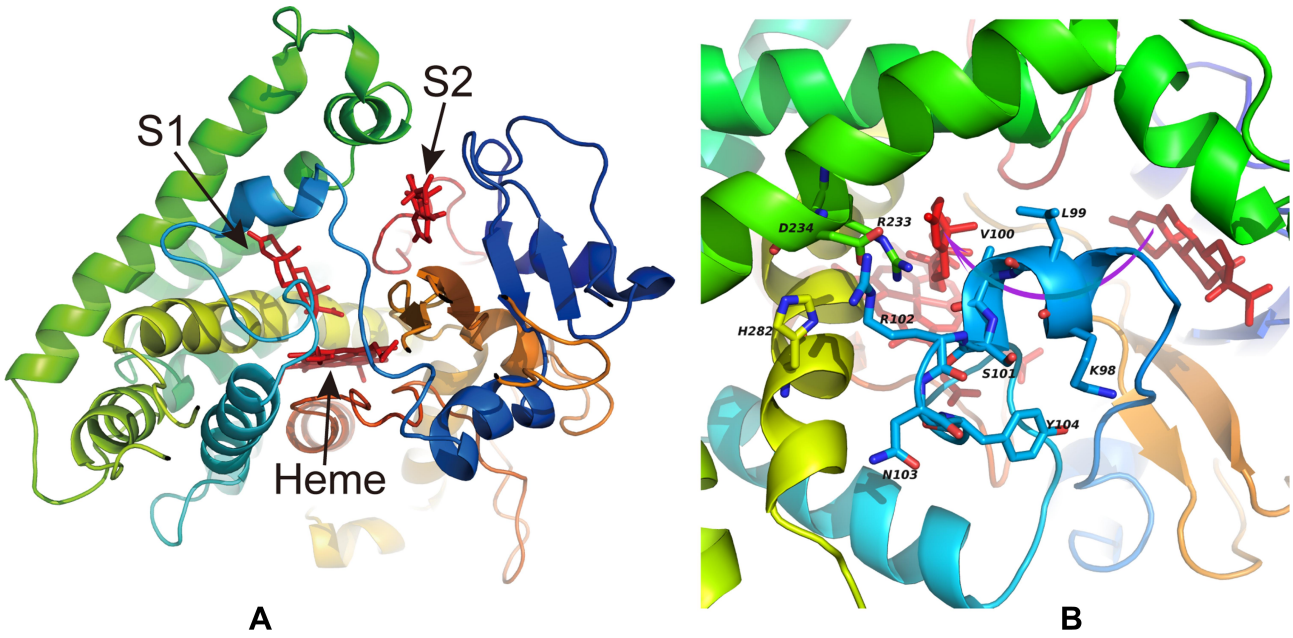

Figure 2 The structure of human CYP21A2 complexed with 17-OHP. (**A**) The overview of the binding mode of 17-OHP to CYP21A2. Secondary structural elements are colored from blue (N-terminus) to red (C-terminus). The ligand 17-OHP (S1 and S2) and heme are colored red. (**B**) A close-up view of the binding mode of 17-OHP to the binding cavity (S1) of CYP21A2. The residues involved in binding and accessing of substrate are labeled and shown as sticks. The purple arrow represents the substrate access channel.

including severe acne vulgaris. Residues K98, L99, V100, S101, R102, N103, Y104, R223, and D234 lie within 5 Å of substrate-binding site (S1) (Figure 2B). In our study, the SNP rs6474 (p.Arg102Lys) was identified in severe acne vulgaris patients. The structure analysis above suggests a strong association between this SNP and severe acne vulgaris.

However, the SNP of rs6465 in the intron region of the *CYP21A2* gene has a protective effect on male severe acne vulgaris, and variations in the introns may affect regulatory sequences in close proximity or in combination with some other functional polymorphisms, resulting in a change in the protein sequence of 21-hydroxylase, which may cause a dominant-negative effect of 21-hydroxylase activity in acne patients. The analysis of haplotypes corroborated our single-marker results by showing that the haplotypes were significant in male patients with severe acne vulgaris, thus confirming the importance of the *CYP21A2* gene in severe acne vulgaris in males. Accordingly, we speculate that risk alleles and genotypes of rs6467 and risk haplotypes of the *CYP21A2* gene might have a greater influence on 21-hydroxylase expression. Taking these findings into account, our data suggest that the *CYP21A2* gene might be actively involved in male severe acne vulgaris. Further independent replication analysis and functional assays should be carried out to further clarify the exact role of the *CYP21A2* gene in this disease.

The polymorphisms of *CYP19A1* have been evaluated in relation to androgen-related diseases (prostate cancer, PCOS) and estrogen-related diseases (breast cancer, endometrial cancer, endometriosis) with mixed results. The polymorphisms of the *CYP19A1* gene encoding aromatase have been correlated with plasma testosterone levels, so *CYP19A1* may therefore act as a genetic modifier of the hyperandrogenic phenotype of severe acne vulgaris. Despite these results and our strong suspicions of their potential role in acne, we found no significant association with severe acne vulgaris of the reported SNPs, rs4646 and rs10046 (in the 3′-UTR), rs700519 (Arg264Cys), and rs2414096, which had been implicated in hyperandrogenism diseases.

However, we identified a heterozygous genotype of two tag SNPs (the GT of rs8023263 and CT of rs2470152) of this gene as being significantly associated with severe acne among Han Chinese males. SNP rs8023263, located in an intron region, was similarly associated, and it could be linked to some other functional polymorphisms, or it might influence the level of gene expression related to aromatase activity. Published data that suggested the association of those polymorphisms in the intron region or a synonymous mutation of *CYP19A1* associated with aromatase activity is in line with our hypothesis that SNP rs8023263 might be related with aromatase activity involved in the pathophysiology of acne formation. Therefore, the genotypes GT of rs8023263 and CT of

rs2470152 may have a potential effect on the activity of aromatase and be involved in the development of severe acne vulgaris in males.

The findings of significant association between these genes and male patients with severe acne vulgaris, though interesting, do leave some questions to be resolved, foremost being why significant associations were only observed among severe acne vulgaris patients. This observation, along with our previous findings that androgen-related genes *CYP17* –34 C/T also contribute to severe acne pathogenesis, largely rests on the position that the genetic elements are more important in severe acne and not in mild acne, the latter appearing to be more related to environmental factors and individual lifestyle. Acne is also more severe in those with a positive family history,[4] suggesting that hereditary factors are potentially responsible for severe acne. The second major question to consider is what accounts for the gender-based differences we observed. In females, the polymorphisms with acne are not as clear and obvious as those in males, since the genetic, metabolic and hormonal factors differ between the two of them. Moreover, estrogen and estrogen-related genes, as well as the homeostatic balance between androgens and estrogens, may play an important role in female acne. The clinical data in the present study showed that females are more prone to be affected by the emotions of stress and depression and may also experience poor quality of sleep and irregular menstrual cycles. In light of these differences, it may potentially be that environmental factors play a more significant role in female acne and, as such, should be more fully explored so that the differences in male and female experience of the environmental conditions related to acne susceptibility can be understood. Clearly, the multifactorial and polygenic nature of acne necessitate further study to investigate the potential mechanism between different phenotypes, including severity and gender discrepancies, confounding factors, and other genetic elements.

There are some limitations to this present study. First, although we can hypothesize as to the manner in which these genes are connected with severe acne and similar diseases, our study is merely suggestive of these underlying mechanisms, and further studies that can more fully map out the actions of these genes are needed. Second, we only analyzed the association of *CYP21A2* and *CYP19A1* with acne, without long-term studies of functional assays. Moreover, while numerous studies point out that androgens do play a role in the pathogenesis of acne vulgaris, the results were somewhat discordant. The circulating levels of these hormones were often within the normal range, and we did not estimate hormone parameters between the acne patients and healthy controls. Nevertheless, the data concerning hormone parameters may be useful in future observations of the activity of 21-hydroxylase (serum 17-OHP) and aromatase (E2/T ratio). This will greatly aid in mapping out the connections of the *CYP21A2* and *CYP19A1* genes, as well as their coding enzymes, with severe acne vulgaris and, thus, better explain our results. In addition, we only analyzed four tag SNPs for the *CYP21A2* gene, which greatly limits our ability to cover the entire gene and may have yielded a less complete picture of this gene's potential associations.

In conclusion, we found two different alleles and genotypes of rs6474 and rs6465, as well as haplotypes of the *CYP21A2* gene, positively associated with Pillsbury III-IV severe acne vulgaris in males, and the genotypes GT of rs8023263 as well as CT of rs2470152 of the *CYP19A1* gene were also associated with Pillsbury III-IV severe acne vulgaris. These results suggest that genetic variations of androgen-related genes can cause alterations either in the fine regulation of these genes or in the function of the resulting proteins, which can result in imbalances in the levels of androgen and estrogen, potentially conferring a subsequent susceptibility to androgen-related diseases.

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Disclosure

The authors declare that they have no competing interests.

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