Relationship between the CAG Repeat Polymorphism in the Androgen Receptor Gene and Acne in the Han Ethnic Group

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\textbf{Introduction}

Acne vulgaris is one of the most common skin diseases. An analysis of the 1996 census data in the USA indicated that the prevalence of acne in the age group from 12 to 24 years was 85\% \cite{1}. The role of androgens in the pathogenesis of acne vulgaris is well established \cite{2}. Their effects are mediated by binding to nuclear androgen receptors (ARs) which are localized in the basal layer of the sebaceous gland and outer root \cite{3, 4}. In a previous study, Imperato-McGinley et al. \cite{5} found that androgen-insensitive subjects who lack functional ARs do not produce sebum and do not develop acne, which suggested that ARs play a key role in the etiology of acne. However, the exact genetic mechanisms how ARs affect acne development are still unclear.

The AR gene resides on chromosome X and encodes a protein of 910 amino acids \cite{6}. Androgens, when bound
to the hormone-binding domain, activate the receptor, causing nuclear translocation of the ligand-receptor complex and a series of molecular events leading to the transactivation of androgen-regulated genes [7]. The 5’-end of exon 1 of the AR gene includes a polymorphic CAG triplet repeat that codes for a polyglutamine tract. Polyglutamine tracts are often present in transcription factors, including SPI and TATA-binding protein. They are suggested to form polar zippers, which bind specific tors, including SP1 and TATA-binding protein. They are glutamine tracts are often present in transcription factors.

Moreover, the androgens also play an important role in the development of acne. In the present study, we investigated whether there is an association between acne and the AR CAG repeat polymorphism.

**Subjects and Methods**

**Subjects**

A total of 406 individuals from the Han ethnic group in China participated in the study; 206 of them were patients presenting with acne including 120 males and 86 females, and 200 including 132 males and 68 females were healthy people used as control group. The control group was matched in age with the case group. All participants in the study signed an informed consent, and human sample handling was approved by Kunming Medical College and Yunnan University.

All subjects were examined in our outpatient unit by dermatologists. Patients with acne venenata and acne comedones were excluded from the study. The clinical grade of acne was assessed based on the Consensus Conference on Acne Classification [14]. According to these criteria, mild acne is defined by the presence of comedones, without significant inflammation and a few or no papules, moderate acne by the presence of comedones, with marked inflammatory papules and pustules, and severe acne by the presence of inflammatory nodules, in addition to comedones, papules and pustules. For analysis convenience, patients were divided into two categories, namely mild + moderate and severe acne. Statistical evaluation was performed based on these two groups.

**Polymorphism Detection and Statistical Analysis**

Genomic DNA was extracted from peripheral blood of all patients and controls by the phenol-chloroform method according to Gross-Bellard et al. [15].

The AR exon 1 CAG trinucleotide repeat was PCR amplified using primer sequences which were designed by primer 5.0 software with inclusion of a 5’-FAM-labeled forward primer to generate a fluorescent product as follows: forward primer 5’-FAM AGT TAG GCC TGG GAA GGG TC-3’ and reverse primer 5’TAC GAT GGG CCT GGG GAG A-3’. PCR was carried out in a 25-µl reaction volume containing 50–100 ng of template DNA, 1.5–2.0 mM MgCl2, 200 mM dNTPs and 15 pmol of each primer. 1× standard buffer and 2 units of AmpliTaq Gold DNA polymerase (Perkin Elmer, Warrington, UK). PCR amplification was performed on a Perkin Elmer DNA Thermal Cycler. Thermocycling consisted of an initial denaturation of 5 min at 94°C, 25 cycles of 1 min of denaturation at 94°C, 45 s of annealing at 64°C and 45 s of extension at 72°C, and a final extension of 7 min at 72°C. Appropriate amounts of PCR products (0.75 µl) were mixed with 1.75 µl of a premixed buffer solution (formamide:loading buffer:standard = 5:1:1). Genescan-350 TAMRA (6-carboxy-tetramethylrhodamine red; Applied Biosystems, USA) was used as the reference standard. Electrophoresis was performed using a 6% denaturing polyacrylamide gel and an ABI Prism 377 DNA sequencer. The sizes of the CAG-containing DNA fragments were measured using Genescan Analysis 3.0 software (Applied Biosystems). The CAG repeat number of each allele was calculated by function \( n = \text{fraglength} - 228/3 \).

Ten male samples were chosen randomly for sequencing to confirm the number of CAG repeats; primer sequences without labeled fluorescence were designed referring to the former. PCR products were purified by the Wizard Purification Kit (Promega), then directly sequenced in both directions using the Bigdye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) according to the standard protocol. The sequences were aligned using the DNAstar software.

Statistical analysis was performed using the statistical package SPSS for Windows (version 11.5, SPSS Inc., Chicago, Ill., USA). The 2-independent-sample t test was used to evaluate the significance of the difference of the number of CAG repeats between the normal control and acne group. Statistical significance was defined as a 2-side p value of <0.05.

**Results**

In the Chinese Han males we investigated, the number of AR gene CAG ranged from 14 to 28 among the controls and 13 to 26 among the cases. The numbers of CAG repeats of 10 male samples were confirmed by sequencing as well as by gene scan (fig. 1). The mean CAG repeat length was 22.07 (SD = 3.026) in the control group, and 20.61 (SD = 2.423) in the patients. The differences in CAG repeat length between the two groups were statistically significant (\( p < 0.001 \)). Additionally, when we divided the male patients into two subgroups, i.e. moderate-acne subgroup (mean = 20.79, SD = 2.434) and severe-acne subgroup (mean = 20.50, SD = 2.427), we also found that the CAG repeat length of both subgroups was significantly different from that of the controls (\( p = 0.014, p < 0.001 \); table 1). The mean CAG repeat length in the female acne group was 21.09 (SD = 2.810) with a range of 13–30, while,
in the female control group, the mean CAG repeat number was 21.32 (SD = 3.302) with a range of 12–28. There was no significant difference in the mean number of CAG repeat length among the two groups in women. Furthermore, neither did we find a difference between female patient groups and the control group when the mild-, moderate- and severe-acne subgroups were considered separately (table 2).

**Discussion**

There has previously been some evidence that acne is an inherited disease [16, 17]. The detailed mode of inheritance remains unproven; however, it is most likely polygenic, and genetic factors, *Propionibacterium acnes* infection, production of inflammation and environmental influences are involved in the etiology of acne [2]. Few acne candidate genes have been proposed, such as human cytochrome P450 1A1 gene [18], steroid 21-hydroxylase gene [19], epithelial mucin gene [20] and CYP17 [21]. Given the role of androgen in the pathogenesis of acne, the gene encoding the AR has been an important candidate for the determination of acne risk. However, only one study on CAG repeats among acne patients was reported. In 1998, Sawaya and Shalita [22] found that the AR polymorphism of CAG trinucleotide repeat length exhibited a correlation with some androgenic skin disease but not with acne in Caucasians. Since significant ethnic differences have been observed within the normal range of AR polyglutamine stretch, this means the CAG repeat number of the AR may reflect the variance on genetic background in different races. So it is interesting to study the relationship between the CAG repeat polymorphism of the AR and acne.

In the present study, we evaluated whether the CAG repeat polymorphism in the AR may contribute to acne risk in the Chinese Han ethnic group by a case-control analysis. We observed a significant relationship between the CAG repeat length and male acne risk. A smaller mean number of CAG repeats in the male patients (20.61 ± 2.423) was observed, suggesting that shorter CAG re-
peptide alleles could have a dangerous effect for male acne development. However, in female individuals with severe acne, we did not find a similar association. To our knowledge, this is the first study to investigate the role of the AR CAG repeat polymorphism in the Han ethnic group for acne. Our results were not consistent with the report of Sawaya and Shalita [22] that no association was found between CAG repeat length and acne in Caucasians. One possible explanation is such a difference may reflect the genetic differentiation between Caucasians and the Han ethnic group.

Our observation of a possible correlation between shorter CAG repeats and male acne is consistent with the association between CAG repeat number and the risk of prostate cancer. The androgen testosterone and its metabolite dihydrotestosterone exert their effects on gene expression and thus affect the sebaceous gland via the AR [23]. In vitro studies show that AR transcriptional activity is influenced by the CAG repeat length, and the length of the CAG tract is inversely correlated with transcriptional activity of the AR [24]. Although the probable increase in AR intrinsic activity with each reduction in AR CAG length is relatively small, these effects are genetically determined and therefore exert effects over a person’s entire lifetime. Small changes can therefore have significant cumulative pathological effects over time [25]. Collectively, the evidence supports the hypothesis that the glutamine repeat plays a role in AR function by fine-tuning the balance between excess and deficient receptor function.

However, in female individuals we did not find a similar phenomenon. In addition, there is a difference of 0.65 (male 22.07 vs. female 21.32) in the average number of CAG repeats between male and female controls who have no acne. This result indicated that a lower number of CAG repeats, which may be associated with acne in males, did not have the same level of effect in females. Physiological interplay between androgens and estrogens in various endocrine targets is well established [26]. Each respective hormone may affect the expression of the other’s receptor, i.e. androgens of the estrogen receptor and vice versa [27, 28], so we speculated that the proportion of ARs and estrogen receptors played an important role in the pathogenesis of female acne, which needs further study. Nevertheless, the etiology of acne is multifactorial, and a few genes are related to the condition. Beyond genetic factors, the environmental factors also deserve investigation to understand female as well as male acne. Further study on interactions between such genes and environmental factors would shed light on the possible genetic mechanisms of acne.

In conclusion, the results of our study suggest that the AR gene CAG microsatellite polymorphism may be one of the candidate genetic markers responsible for male acne in the Han population, and further study of other possible genetic markers with regard to acne will provide new information on the genetic background underlying acne. As well, the complex interaction between the expressions of these genetic loci along with the influence of environmental factors may explain the phenomenon.

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References


