ORIGINAL ARTICLE

The effect of GGC and CAG repeat polymorphisms on the androgen receptor gene in response to finasteride therapy in men with androgenetic alopecia

Mohammadreza Ghassemi, Gholam Hossein Ghaffarpour, Shiva Ghods
Department of Dermatology, Rasoul Akram Hospital, Iran University of Medical Sciences, Tehran, Iran

Background: It should be assessed whether the polymorphisms on androgen receptor gene can affect therapeutic response to androgenetic alopecia (AGA) medications. We aimed to find a link between polymorphisms on the androgen receptor gene (including the number of triple sequences of cytosine, adenine, and guanine [CAG] and guanine-guanine-cytosine [GGC]) and response to treatment with finasteride in male patients. **Materials and Methods:** This case–control study was performed on 25 consecutive male patients with hereditary AGA and 25 sex-matched healthy individuals without AGA. The complete sequence of the gene was extracted from the NCBI database. To replicate the samples, real-time polymerase chain reaction technique was used for the pointed gene and the results were confirmed by the sequencing technique. **Results:** The mean number of CAG sequences in two groups with and without baldness, was 23.16 ± 0.47 and 23.04 ± 0.67. For GGC sequencing with and without baldness, mean count was 22.22 ± 1.45 and 19.92 ± 81.2, respectively, which was significantly higher in the group with baldness. There was no association between number of CAG sequence and improvement in hair loss or the level of patients' satisfaction, but lower number of GGC sequences was associated with higher rate of stopping hair loss, more new hair growth, higher level of satisfaction, and more clinical response to finasteride and clinical improvement in AGA patients. **Conclusion:** Counting of GGC sequence in the gene encoding the androgen receptor is associated with an increase in odds of baldness and a decrease in the response rate to finasteride in AGA patients.

Key words: Androgenetic alopecia, androgenetic gene receptor, finasteride therapy, genetic sequences database, men

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INTRODUCTION

Androgenic hair loss is associated with high levels of dehydrotestosterone (DHT) and also increased expression of the androgen receptor gene.[1] Testosterone and other weaker androgens, such as dehydroepiandrosterone and androstenedione, are metabolized in many skin tissues.[2] Testosterone can easily penetrate into the cell membrane, and in the cell's cytoplasm, the 5-alpha-reductase enzyme mainly type II converts it to DHT and then DHT can powerfully connect to the androgen receptor and the complex is transferred to the nucleus.[3,4] These events result in the transcription of the target gene and ultimately the translation of the genes that carry out biological activities. The level of DHT, 5α-reductase, and androgen receptors in the scalp increases with hair loss.[5] The higher levels of androgen and androgen receptors can lead to higher expression of the controlling genes in the hair follicle cycle.[6] Signals that follow in papillary dermal and hair follicles in bald people lead to the early ending of the anagen phase and early beginning of the catagen phase.[7] Catagen occurs when anagen phase holding factors such as IGF-1, insulin-like growth factor, and VEGF are reduced. The androgenic receptor (AR) gene determines the sensitivity of the cells to the androgen.[8] The gene is

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Address for correspondence: Dr. Shiva Ghods, Department of Dermatology, Rasoul Akram Hospital, Iran University of Medical Sciences, Tehran, Iran.
E-mail: s.ghods.dermatologist@gmail.com
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located on the X chromosome at Xq11-12 and the product of the gene is of the family of the nuclear receptor.[9] The amine end of the gene comprises a region of polyglutamine with coded CAG triple repeats that are required for transcriptional activation. The number of these repeats is polymorphic in the population.[10] The variation of CAG repeat in the AR gene has different clinical consequences.[11] The low number of CAG repeats in the AR gene leads to an increased risk for coronary artery disease and prostate cancer.[12] Furthermore, its low frequency may be associated with androgens associated skin diseases such as hirsutism, AGA, and acne in men and women.[13] However, another study has shown that triple-repeat polymorphisms do not have an association with the loss of androgenetic hair loss.[14] Repetition of a triple CAG and a lesser amount of GGC has been inversely related to protein levels and androgen transcriptional activity of the gene receptor in various studies.[15] Androgenic alopecia (AGA) is caused by sensitivity of hair follicles to DHT, therefore, inhibition of 5-alpha reductase enzyme is the first-line treatment for this type of hair loss.[16] In the studies, the best treatment for men with androgenic hair loss was topical finasteride and minoxidil.[17,18] Based on the confirmed role of genetics and especially androgen gene receptor in patients with AGA, it is necessary to study the polymorphisms of this gene in Iranian population and its association with the response to treatment of AGA. The influence of these gene polymorphisms on the therapeutic response to AGA medications should also be assessed. The present study attempted to find an association between polymorphisms in the AR (including the number of triple sequences of CAG and GGC) and response to treatment with finasteride in male AGA patients.

MATERIALS AND METHODS

Study population

This case–control association study was performed on 25 consecutive male patients with hereditary AGA and 25 sex-matched healthy individuals without AGA. In the former group, all had evidence and clinical manifestations of AGA that were deliberately and voluntarily recruited on the basis of expert judgment, and their hair loss was classified according to the basic and specific classification (BASP) criteria.[19] The study was approved by the Ethics Committee of Iran University of Medical Sciences (Ethical code: IR.IUMS.REC1394.26910). All the patients signed informed consent for participation in the study. The patients were all treated with oral finasteride 1 mg with a similar order and followed up for 6 months (once every 3 months) in terms of response to treatment. Those with diffuse hair loss, coin hair loss, or any kind of inflammation on the scalp and systemic diseases result in hair loss including thyroid disorders or other hormonal disorders, or those treated with systemic drugs were all excluded. The control group was selected from personnel or relatives of patients who have no manifestations of hair loss for genetic comparison. The baseline and clinical features of patients were collected in two groups via questionnaires that included demographic characteristics (age), anthropometric indices (weight and height), clinical history of AGA, and medications.

Identifying gene sequences

After getting written informed consent, peripheral venous blood samples were taken from all the patients. In this study, the AR gene was evaluated for repeating the CAG and GGC triple sequences in both case and control groups. First, the complete sequence of the gene was extracted from the NCBI database. To replicate the samples, usual real-time-polymerase chain reaction (RT-PCR) technique was used for the pointed gene, and the results were confirmed by the sequencing technique. The details of the evaluation and identifying CAG and GGC sequences were as follows: first, DNA was extracted from blood samples using a kit and according to the manufacturer's instructions. The concentration of the genomic DNA extracted was determined by spectrophotometry. The extracted DNA was maintained at −20°C until the PCR. For DNA amplification, the forward and reverse primers were designed by Gene Runner software [Table 1]. To perform PCR, first, the DNA extracted from the blood samples was removed from the freezer and melted at room temperature. Then, specific microtubes for PCR were numbered and a microtube (per test) was numbered as negative control. Negative control has all of the material required in a PCR reaction except for the template DNA. To carry out the PCR test, the materials needed for the reaction were calculated in terms of number and volume including DNA template 50 ng/μl (1 μl), primer mix 10 pmol/μl (2 μl), PCR Master Mix 2X (12.5 μl), and distilled water (25 μl). Electrophoresis was used to detect PCR products and then the gel electrophoresis was photographed [Figures 1 and 2]. Finally, the samples were sent for sequencing to assess the number of CAG and GGC triples repeats in both case and control groups.

Statistical analysis

Results were presented as mean ± standard deviation for quantitative variables and were summarized by absolute frequencies and percentages for categorical variables.

Table 1: The primers designed for polymerase chain reaction amplification

Amplicon length	Primer length	Primer sequence	Name of primer
201	20	5'-TCCGCGAAGTGATCCAGAAC-3'	CAG-F
201	20	5'-CTTGGGGAGAACCATCCTCA-3'	CAG-R
222	20	5'-TCCTGGCACACTCTCTTCAC-3'	GGC-F
222	22	5'-GCCAGGGTACCACACATCAGGT-3'	GGC-R

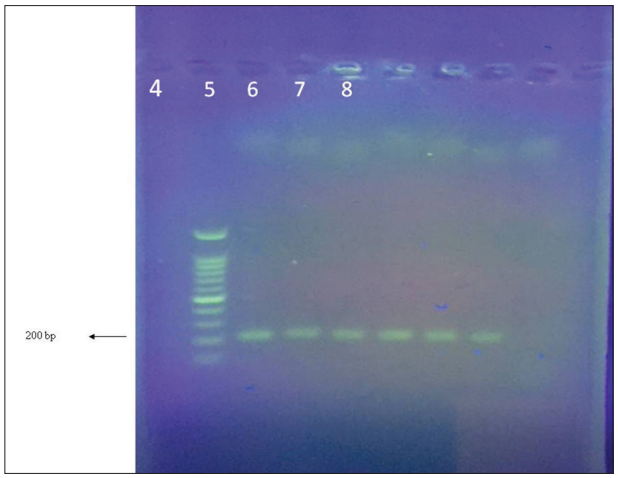

Figure 1: Polymerase chain reaction result of GGC repeat region (optimized). Column 1 = Marker 100 bp; Column 2–7 = Test samples; Column 8 = Negative control

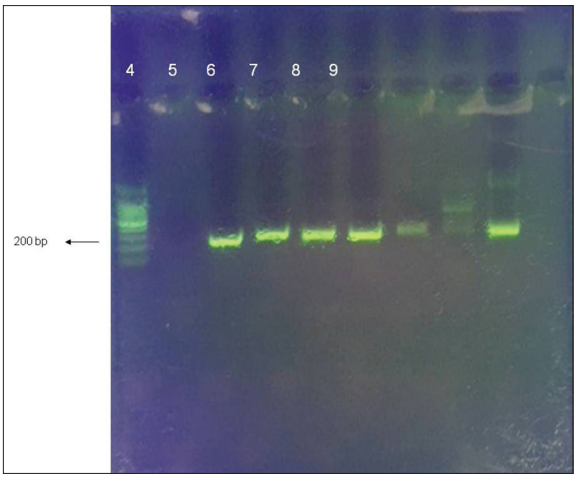

Figure 2: Polymerase chain reaction result of CAG repeat region. Column 1 = marker 100 bp; Column 2 = negative control; Columns 3–9 = test samples (column 8 has an individual band and is repeated in subsequent tests)

Normality of data was analyzed using the Kolmogorov–Smirnoff test. Categorical variables were compared using Chi-square test or Fisher's exact test when more than 20% of cells with expected count of less than 5 were observed. Quantitative variables were also compared with *t*-test or Mann U-test. For the statistical analysis, the statistical software SPSS version 16.0 for Windows (SPSS Inc., Chicago, IL, USA) was used. $P = 0.05$ or less were considered statistically significant.

RESULTS

The mean age in patients with and without AGA was 57.88 ± 7.84 years and 57.56 ± 5.98 years, respectively, with no significant difference ($P = 0.788$). In addition, the mean weight of patients in both groups with and without baldness was 81.28 ± 12.11 kg and 81.96 ± 7.99 kg, respectively, which did not differ between the two groups ($P = 0\ 805$). There was no significant difference between two groups with and without AGA in terms of history of androgen-related diseases (8% and 16%, respectively, $P = 0.384$), However, familial history of hair loss in the AGA group was significantly higher than the control group (92% and 28%, respectively, $P < 0.001$). Regarding history of drug use, the use of acetylsalicylic acid was reported in 1 case, ibuprofen in 1 case, citalopram in 1 case, zinc in 1 case, and isotretinoin in 2 cases in the group with baldness; in the control group, the use of acetylsalicylic acid was reported in 7 cases, statins in 2 cases, diclofenac in 1 case, hydroxyzine in 1 case, and metformin in 1 case with no overall difference ($P = 0.131$).

In terms of the number of CAG sequences in two groups with and without baldness, the mean number of this sequence was 23.16 ± 0.47 and 23.04 ± 0.67, which did not differ significantly between the two groups ($P = 0.470$) [Table 2]. However, in terms of GGC sequencing in both groups with and without baldness, the mean count was 22.22 ± 1.45 and 19.92 ± 81.2, which was significantly higher in the group with baldness ($P < 0.001$). Based on area under the receiver operating characteristic curve analysis, the number of GGC sequence was a good predictor for predicting AGA (area under the curve = 0.742, 95% confidence interval: 0.603–0.882, $P = 0.003$). The best cutoff value for the number of GGC sequence to predict AGA was 23 yielding a sensitivity of 92% and a specificity of 56%. In other words, in people with a GGC sequence count of more than 23, the chance for AGA will increase significantly.

In the pre- and post-finasteride treatment, the mean scores of basic and specific (BASP) classification (based on Hamilton–Norwood's Cyclic Redundancy Check [CRC]) were 3.64 ± 0.85 and 2.64 ± 1.15, respectively, indicating a significant decrease in the respective scores after treatment ($P = 0.003$). There was no significant correlation between BASP score change and number of CAG sequence (correlation coefficient = 0.189, $P = 0.366$). Similarly, there was no significant correlation between the change in BASP score and the number of GGC sequences (correlation coefficient = 0.251, $P = 0.225$).

As shown in Table 3, within 6 months after treatment with finasteride, considerable improvement was revealed with respect to stopping hair loss (complete in 68% and partial in 20%), new hair growth (64%), and satisfaction (measured by questionnaire) with the treatment (complete in 60% and partial in 8%) within 6 months after treatment with finasteride. The study showed no association between the number of CAG sequence and improvement in hair loss or the level of patients' satisfaction, but lower number of GGC sequences was associated with a higher rate of stopping or improvement in hair loss, more new hair growth, higher level of satisfaction, and more clinical improvement in AGA patients [Table 3].

Table 2: Mean number of gene sequences according to improvement after treatment

Characteristics	CAG count	GGC count
Stopping hair loss		
Improve	22.88±0.60	18.76±2.46
No improve	23.50±0.71	20.50±3.54
P	0.236	0.008
Renewing hair growth		
Positive	22.88±0.62	18.50±2.28
Negative	23.33±0.71	22.44±1.67
P	0.105	0.001
Complete satisfaction		
Positive	22.87±0.64	18.60±2.32
Negative	23.20±0.84	22.00±2.24
P	0.289	0.012
Clinical improvement		
Positive	22.88±0.62	18.50±2.28
Negative	23.33±0.71	22.44±1.67
P	0.105	0.001

CAG=Cytosine, adenine, and guanine, GGC=Guanine-guanine-cytosine

Table 3: Clinical improvement 3 and 6 months after treatment

Characteristics	3 months later (%)	6 months later (%)
Stopping hair loss		
Complete stopping	68	68
Partial stopping	24	20
Lack of stopping	8	8
Deterioration	0	4
Renewing hair growth	28	64
Satisfaction with treatment		
Complete	36	60
Partial	48	8
None	16	32
Clinical improvement		
Pubic hair growth	36	56
Terminal hair growth	0	8
No response	64	36

DISCUSSION

Early diagnosis followed by the recognition of its inheritance pattern is important in the evaluation and management of AGA. In this regard, this disease is a polygenic hereditary disorder in many cases, and thus in a small number, it also has a single-gene pattern.[20] Along with assessing the heredity of the disease, there are some gene polymorphisms or specific sequences associated with the pathogenesis of the disease. In recent years, there has been some evidence of a relationship between some gene sequences and the increased likelihood of baldness, especially in men. In some studies, however, the relationship between the number of CAG and GGC sequences on the androgens receptor coding gene and their synergistic effects and the occurrence of male pattern baldness, especially at young age, has been raised. For example, in a study by Ellis *et al*.[6] in Australia, StuI restriction site was found in all but one (98.1%) of young bald men and in 92.3% of older balding men, and the longer and more frequent recurrence of CAG and GGC was found in patients with male pattern baldness as compared to nonbald people. In another study by Wakisaka *et al*. in Japan,[21] the number of CAG + GGC duplicates contrasted with the degree of clinical symptoms of baldness, with fewer repetitive sequences, and with a greater improvement after treatment with finasteride. On the other hand, in a study which conducted on Mexican brothers, there was no relationship between the number of these sequences or their haplotypes with the incidence of baldness or the degree to which they responded to treatment with drugs such as fungicides.[22] Moreover, in some studies, the reduction in the number of two short sequences was associated with an increased risk of AGA and even male sexual dysfunction. As shown in the study by Cauci *et al*. in Italy,[23] the risk of alopecia in individuals with a GGC count of <23 was significantly higher than those with a GGC count above 23.

What we found in this study was that, first, the number and count of the short CAG sequence did not correlate with the severity of the disease or the process of recovery in alopecia, but increased number of GGC sequence was associated with a higher risk for male pattern baldness. In this regard, fewer short sequences of GGC were associated with faster disease regression, chances of new hair growth, higher patient satisfaction, and clinical improvement in hair growth. Based on the cutoff point obtained in our study, not only people with GGC counting more than 23 are more likely to develop male pattern baldness, but also fewer count of this sequence is associated with a higher probability of responding to finasteride therapy. However, it is important that the contradictory responses in different studies are due to a different genomic tendency to baldness in different population and sample size, showing that the power of studies is also different in various studies and as a result of our study, this indicator is very influential.

CONCLUSION

Finally, it can be concluded that repetitive counting of GGC sequence in the gene encoding the androgen receptor is associated with an increase in the odds of baldness, and due to our study, unlike of CAG, repetitive of GGC sequence decrease response rate to finasteride in AGA patients. In this regard, a GGC count of ≥23 increases the likelihood of AGA or reduces the response to finasteride. Therefore, in patients with high GGC sequencing, it may be necessary to increase the dose of the above medication or prolong duration of therapy or use additional treatment options.

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Conflicts of interest

There are no conflicts of interest.

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