ORIGINAL ARTICLE Egypt. J. Med. Hum. Genet. Vol. 10, No. 1, May, 2009

A Study of the androgen receptor gene polymorphism and the level of expression of the androgen receptor in androgenetic alopecia among Egyptians

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ABSTRACT

Background: Androgenetic alopecia (AGA) occurs in men and women. The nature of the genetic predisposition to androgenetic alopecia is still unresolved. The aim of the work is to study the genotype of the androgen receptor gene (StuI polymorphism) and its relationship to AGA in a case control study and to determine the level of androgen receptor expression (AR) in the balding scalp relative to the non-balding scalp area.
Subjects and Methods: This study was conducted on one hundred individuals; 60 cases with AGA (36 males and 24 females) and 40 age and sex matched control patients (20 males and 20 females). StuI restriction fragment length polymorphism(RFLP) of exon 1 was detected by PCR based assay using genomic DNA of subjects with AGA and controls. Immunohistochemical detection of the androgen receptor (AR) using antihuman AR antibody was implemented to compare its level in the balding scalp and in the non-balding area in individuals having AGA.
Results: Analysis of StuI restriction fragment length polymorphism in exon 1 of the androgen receptor (AR) gene revealed a relatively commoner incidence of the cut allele in males with AGA relative to age and sex matched controls (the association was of border line significance p=0.07. Interestingly, all persons who had maternal uncles suffering from AGA had the Stu1 cut variant of AR gene (p= 0.03 using Chi square test). Semiquantitative immunohistochemical analysis of AR in the bold scalp biopsies showed higher expression in the level of AR than the non bold bioposies within the same individual.
Conclusion: To the best of our knowledge this is the first study of AR gene polymorphism and AR expression in AGA amongst Egyptians. This study contributes in the understanding of the molecular pathogenesis of AGA which could help in finding better therapeutic alternatives for such trait in the future.

Key Words:

Androgenetic alopecia, androgen receptor, StuI polymorphism, immunohistochemical expression.

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INTRODUCTION

Androgenetic alopecia (AGA) is the most common cause of hair loss in both men and women. In men, it is often referred to as male-pattern baldness; a common form of scalp hair loss that affects most males by old age[1]. The condition can also affect females; however, this is less well characterized and it remains controversial as to whether the two conditions are the same. Certainly, the pattern of hair loss is different in women and the prevalence is lower than that in men.[2]

The onset of AGA is extremely variable and appears to be determined by the presence of sufficient circulating androgens and the degree of genetic predisposition. The condition is not a serious one from a medical perspective; however, the loss of hair is often an unwanted and stressful for the patient and therefore might have considerable psychosocial consequences.[3]

Male pattern hair loss usually follows a defined pattern, as described by the Hamilton–Norwood scale. Women; on the other hand, have lesser degrees of miniaturization of terminal hairs in affected areas and therefore, rarely any ‘balding’ when compared to men. Female pattern hair loss (FPHL) is not as easily classifiable as in men; three different clinical patterns have been described: The Ludwig pattern, the Hamilton pattern and the Christmas tree pattern[4]. Most commonly FPHL presents by the Ludwig’s pattern.[4]

Androgenetic alopecia is the result of step-wise miniaturisation of the hair follicle and alteration of the hair-cycle dynamics[5]. Pre-programmed follicles on the scalp progress through long growth (anagen) cycles and short rest (telogen) cycles. With each passage through the hair cycle, the duration of the anagen phase decreases whereas the telogen phase elongates. By time, the length of the new anagen hair becomes considerably shorter than that of its predecessor and the emerging hair does not reach the skin. In addition, the latency period between telogen hair shedding and anagen regrowth becomes longer, leading to a reduction in the number of hairs present on the scalp.[5]

Interestingly, the hair follicle may follow an alternative route during which the telogen phase, not accompanied by a coincident new early anagen, ends with teloptosis leaving the follicle empty[6]. Kenogen indicates the physiological interval of the hair cycle in which the hair follicle remains empty after the telogen hair has been extruded and before a new anagen hair emerges. Kenogen frequency and duration are greater in men and women with androgenetic alopecia (AGA).[7]

The follicular miniaturization that accompanies these hair-cycle changes is global, affecting the papilla, the matrix and ultimately the hair shaft. The dermal papilla is fundamental to the maintenance of hair growth and is probably the target for androgen-directed apoptosis or cell migration[8]. It is generally believed that there is only one androgen receptor because of the wide- ranging effects throughout the body with the absence of functional androgen receptors in testicular feminization and the results of cloning studies of the androgen receptor[9]. The concentration of androgen receptors, however, was significantly higher in dermal papilla cells derived

from the androgen- dependent follicles including beard, moustache, pubis and scrotum than in the relatively androgen- insensitive areas of 'non- balding' scalp.[10]

The androgen receptor (AR) is a typical steroid receptor and is responsible for determining the sensitivity of cells to androgens. The human AR gene localizes to the X chromosome at Xq11–12 and is encoded in 8 exons. The steroid hormone receptors regulate gene transcription by interacting with a specific DNA sequence in a ligand-dependent manner.[11]

The distribution of androgenetic alopecia has been found to follow a normal distribution, representing the full range of phenotypes, from no evidence of hair loss, to fully developed baldness. This distribution is more consistent with a polygenic trait. In addition, it is worth noting that hereditary traits determined by a single gene rarely occur at a frequency greater than 1 in 1000. Although the exact frequency of AGA is difficult to ascertain, it has been estimated to be between 40% and 60% in men, further supporting a more pervasive mode of inheritance.[12]

The sequences of several candidate genes for androgenetic alopecia were studied because of their relevance to the hypothesis that androgens are involved in this form of hair loss. Among these were the genes that encode the 5α-reductase enzymes: SRD5A1 and SRD5A2, in male-pattern baldness because 5α-reductase converts testosterone to DHT. However, analysis of these genes using case–control and familial linkage studies have shown that it is unlikely that they contribute to androgenetic alopecia[13,14]. Also, the autosomal gene encoding aromatase cytochrome P450 gene, CYP19, was compared between cases and controls but no difference was detected, suggesting that it is unlikely that the aromatase gene is involved in determining predisposition to androgenetic alopecia.[15]

Following the same trail, the observed father-to-son transmission of AGA raised the hypothesis that a gene on the Y chromosome might contribute to the condition. In addition, the Y chromosome determines sex and the concentrations of sex steroids such as testosterone and DHT[16]. However, examination of the non-recombining region of the Y chromosome has demonstrated that it is unlikely that causative mutations occur in any genes contained in this region.[15]

Owing to the fact that an increased concentration of androgen receptor is associated with the balding scalp[17], differences in the DNA sequence of the gene encoding the androgen receptor, or in the AR regulatory sequences, might lead to differences in the concentration or activity of the receptor. Such differences might increase sensitivity to DHT in balding individuals, leading to hair loss at an earlier age.

The frequency of short AR repeat lengths of both CAG and GGC has been found to be significantly greater in young bald men than in non-bald men. The ubiquity of the androgen receptor gene StuI restriction site and higher incidence of shorter triplet repeat haplotypes in bald men suggests that these markers are very close to a functional variant that is a necessary component of the polygenic determination of male pattern baldness.

Functional mutation in or near the androgen receptor gene may explain the reported high levels of expression of this gene in the balding scalp.[18]

In addition to androgen-related genes, genes that are involved in patterning, signalling and hair-follicle morphogenesis are attracting much attention from researchers. Important candidate areas include fibroblast growth factor, WNT (wingless type) proteins, β-catenin, LEF1, FOXN1 (forkhead box N1), noggin, bone morphogenic protein 2 and 4, sonic hedgehog and its cognate receptor patched, platelet-derived growth factor A, follistatin and epidermal growth factor.[19]

Without treatment, androgenetic alopecia is a progressive condition. Hairs decrease in number at a rate of ~5% per year[20]. Apart from various camouflage and surgical options, there are a number of topical and systemic drugs, individually or combined, which may be used for treating the condition[21]. These include some over the counter (OTC) products e.g. Serenoa Repens (Saw Palmetto) Permixon; a naturally occurring 5 alpha reductase inhibitor[22] and zinc in specific forms (i.e., zinc acetate, zinc sulfate) which limits the reduced cofactor NADPH of 5a-R that is necessary for the 5a reduction of testosterone to form DHT[23]. Nutrients that are promoted by health food stores to 'help hair grow' include the amino acids Arginine/L-arginine and/or cysteine / cysteine/L-Cysteine, biotin and folic acid[24]. Prescription drugs include, Spironolactone, an aldosterone antagonist that acts as a weak antiandrogen in blocking the AR, but also inhibits androgen biosynthesis. Cyproterone acetate, is another well-known antiandrogen that can be used in female AGA, hirsutism and virilizing syndromes[25]. Additionally, there is oral finasteride which has been shown to be effective in the treatment of hair loss in men, while its efficacy in women has remained controversial[26]. It is a potent and highly selective 5α-reductase type-2 inhibitor[27]. It binds irreversibly to the 5α-reductase type-2 enzyme and inhibits the conversion of testosterone to DHT. Topical formulations include Minoxidil which appears to prolong the anagen growth phase leading to a decrease in hair shedding, but it does not inhibit the biological process. Hence, once treatment is stopped, hair shedding rapidly resumes, with the loss of all minoxidil-stimulated hair growth.[28]

Failure of any of these agents to permanently reverse the hair loss process has led to the introduction of innovative modalities in this field. Namely, gene therapy[29,30], antisense chimeras[31], growth factors[32,33] and stem cell[34] technologies are among those researched for such purpose.

SUBJECTS AND METHODS

This study included 60 cases having androgenetic alopecia (AGA): 36 males and 24 females, who came to the Dermatology Department Outpatient Clinic of Alexandria University Main Hospital and forty volunteer control participants (20 males and 20 females) were also included. All cases of AGA were subjected to thorough history taking and clinical examination to determine the type and grade of alopecia and 4mm punch biopsies were taken from both the bald and the non-bald scalp areas. Informed consent was obtained from the participants following the explanation of the details of the procedures to them. Blood samples were collected from both the patients and controls. Additionally,

family history of close relatives with apparent advanced grades of hair loss was collected from the patients. Approval was obtained from the Research Review Committee of Alexandria Faculty of Medicine prior to undertaking the study.

I- Detection of StuI RFLP of exon 1 of AR gene:
Blood samples (1-2ml whole blood) were collected in EDTA tubes. The “Qiagen DNA Mini Kit” was used for the genomic DNA extraction, following the instruction of the manufacteurer. PCR amplification of a region in exon 1 of AR gene containing the polymorphism for StuI restriction endonuclease enzyme was undertaken in a 25µl reaction using 10 picomoles of the primers (F 5’-CACAGGCTACCTGGTCCTGG-3) and (R5’-CTGCCTTACACAACTCCTTGGC-3’)[35]. The PCR amplification of a 416 bp fragment of exon 1 of AR gene was detected. The reaction mixture for the amplification procedures was prepared in a final volume of 25µl using the PCR core system I PCR (Amersham Biosciences). The amplified products were digested with StuI restriction endonuclease (5units per reaction in the presence of 1x buffer B) following the manufacturer instructions (Fermentos). The products were electrophoresed through agarose 2% gels and stained with ethidium bromide.

II-Histological examination of scalp biopsies:
Four millimetre punch biopsies were taken from the occipital non- balding area (labelled as A) and three and a half millimetre punch biopsies were taken from the balding scalp area of the same patient (labelled as B) of sixty cases with AGA (36 males and 24 females). They were immediately fixed in buffered formalin saline and were processed and stained with routine H&E stain and examined microscopically for:

-The presence of intact hair follicles in biopsies taken from the non-bald area ‘A’.

-The presence of miniaturized hair follicles in biopsies taken from the scalp area of Androgenetic alopecia ‘B’.

III- Immunohistochemical detection of the androgen receptor:
The level of expression of the androgen receptor in the skin of the scalp was detected using the Avidin-Biotin indirect immunoperoxidase method[36]. Sections A (from the non-bald scalp area) were compared to sections B (from the bald area). The slides were scored independently by two examiners and an average semi-quantitative score was determined for each section. Sections were scored as Negative, 1+, 2+ or 3+ depending on the staining intensity and the proportion of positive cells. Negative (N) if < 10% of nuclei were immunoreactive, mild (1+) if 10-25% of nuclei were positive, moderate (2+) if 25-50% of nuclei were positive and strong (3+) if > 50% of nuclei were positive.

Negative control section was included on each slide, in which the primary antibody was replaced by phosphate buffered saline (PBS). A pap pen was used to avoid overflow of the Ab to the negative section. Slides of Senile prostatic hyperplasia were used as a positive control in each run. Sebaceous glands were found to be positive in all studied biopsies, thus it was considered as an internal positive control.

IV-Statistical analysis:

Androgen receptor StuI polymorphism association in the case control study was analysed using Chi square and immunohistochemical analysis of AR expression was studied using Wilcoxon signed rank test and Mann Whitney U test. Statistical significance level was considered at $p \leq 0.05$.

RESULTS

This study included 60 cases having AGA (36 males and 24 females) who sought medical advice at the Dermatology Department Outpatient Clinic of Faculty of Medicine, Alexandria University Main hospital. Forty control participants (20 males and 20 females) were also included in the study. The age of the patients, in the study Group, ranged between 16 and 58 years, with a mean age of (29.27 ± 10.58). The controls were chosen to be age and sex matched. Their ages varied from 18-50 with a mean age of (29.8 ±8.33). Grades of AGA according to the Hamilton-Norwood classification ranged from frontal alopecia (grades II & III), frontal hair loss and temporal recession (grades IV & V) and total frontal and vertex alopecia (grades VI &VII). In males, 58.3% (21/36) of patients had frontal baldness of gradeIII, 30.6% (11/36) had gradeV, and 11.1% (4/36) had total frontal and vertex baldness of gradeVII AGA. An additional classification; the Ludwig FPHL was employed for the females[37]. Ludwig (I) pattern hair loss was obvious in 16.7% (4/24) of the female patients, 75% (18/24) had L (II) FPHL. and 8.3% (2/24) of the females had L (III) FPHL.

Immunohistochemical analysis of AR expression:

Tissue samples taken from the bald area (Group B) showed higher percentage of androgen receptor (AR) expression than those taken from the non-bald region (Group A). The dermal papilla (DP) was the main part of the hair follicle showing the characteristic nuclear staining indicating the presence of AR (Figure. 1). It was observed that DP in sections taken from Group B (bald area) stained more intensely indicating a higher expression of AR (Figure. 1A) in comparison to the less-intense staining observed in non-bald areas indicating less number of AR in DP nuclei (Figure. 1B).

Semiquantitative screening of AR immunostaining scoring of scalp biopsies showed 3.3% (2/60) of cases from Group A with negative staining of the epidermis while none of Group B was negative. Whilst AR staining was scored as mild in 80 % (48/60) of cases in Group A, only 25% (15/60) of cases of Group B showed mild staining. Moderate staining was observed in 55% (33/60) of cases in Group B compared to only 15% (9/60) of cases in Group A. Additionally, 20% (12/60) of cases from Group B showed strong nuclear staining whereas only 1.7% (1/60) in Group A revealed strong staining. The difference between the intensity of nuclear staining in the two Groups was statistically highly significant $P<0.0001$ using Wilcoxon signed ranks test (Table 1).

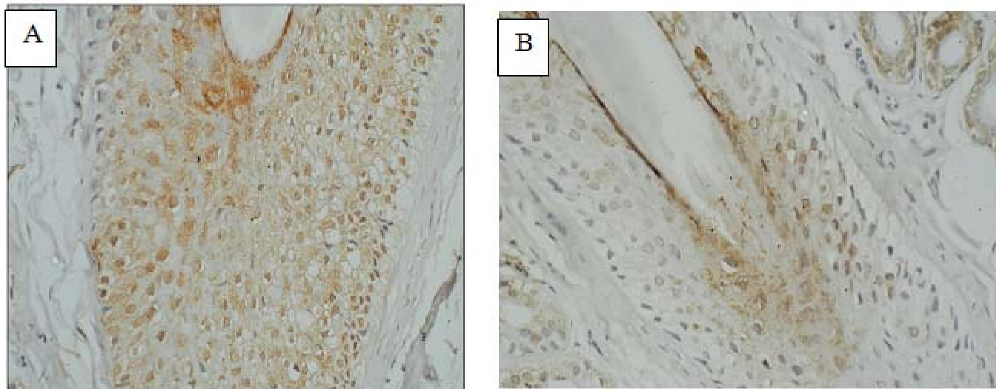

Fig. 1: Androgen Receptor (AR) Immunostaining In Scalp Biopsies. (Figure A)Bald scalp AR immunostained biopsy showing strong positive staining of nuclei of hair follicle dermal papilla(DP). (B) Non bald scalp biopsy of the same individual showing less intense staining of AR of the hair follicle DP. (Magnification is 400x).

Table 1: Comparison of intensity of nuclear staining between non bald and bald scalp tissue sections.

	Group A (Non bald) N (%)	**Group B (bald) N (%)**	**Total N (%)**
Negative	2(3.3)	-	2(1.67)
1+ (mild)	48 (80)	15(25)	63(52.5)
2+ (moderate)	9(15)	33(55)	42(35)
3+ (strong)	1(1.7)	12 (20)	13 (10.83)
Total	60(100)	60(100)	120(100)
WSRT	-6.13		
P value	< 0.001*		

*Statistically significant at P≤ 0.05.

PCR based analysis of StuI polymorphism of exon 1 of AR gene:
Sixty patients (36 males and 24 females) and forty control patients (20 males and 20 females) were included in the study. However, 9 cases and 3 controls were excluded due to poor quality of the sample which resulted in inhibition of the PCR.

PCR amplification of a region in exon 1 of AR gene was performed followed by detection of the polymorphism for StuI restriction endonuclease enzyme.

The AGGCCT sequence is cut by the StuI restriction enzyme resulting in 329/87bp (cut allele), while the AGACCT sequence is not digested by StuI, resulting in a 416bp fragment. The StuI restriction site in the AR gene, is located on the Xq12.22 chromosome. Consequently, male participants are hemizygous for the AR gene polymorphism. Females, however, presented with either a heterozygous state with two bands appearing; one at 416bp and another at 329bp or with a homocut state with only the 329bp & 87bp (Figure. 2).

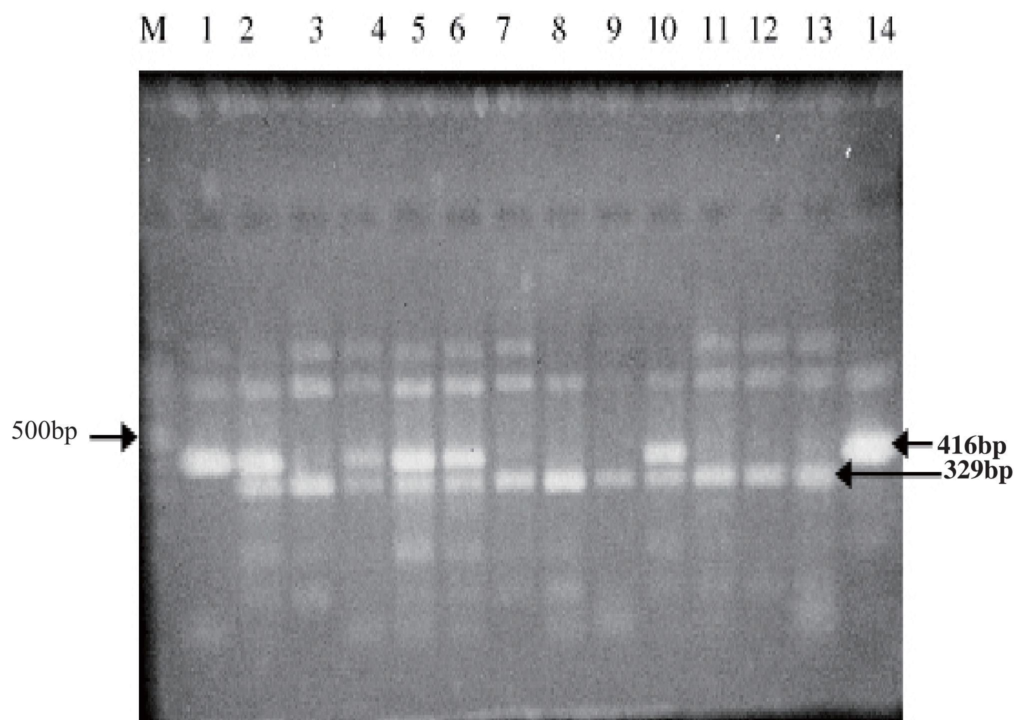

Fig. 2: Gel captured image of the StuI Restriction assay using 2% agarose gel electrophoresis. The AGGCCT sequence is cut by the StuI restriction enzyme, while the AGACCT sequence is not. Lane1: Undigested sample (band size = 416bp). Lanes 2,4,5,6,10 show two bands one at 416bp and another at 329bp indicating a heterozygous state (female cases). Lanes 3,7,8,9,11,12,13 show a transluminant band at 329bp indicating it is homo- cut by the StuI restriction enzyme Lane 14 shows a transluminant band at 416bp indicating it is homo-uncut. M: 100bp DNA marker.

Among the cases studied, 11.8% (6/51) were homo uncut indicating absence of the 'cut' allele compared to 29.7% (11/37) of the control Group. Whereas 66.7% (34/51) of the alopecic patients were homo-cut, 48.6% (18/37) of the controls. In females having FPHL, 21.6% (11/51) were heterozygous similar to the percentage of heterozygous alleles detected among the control females, (21.6%, 8/37).

The cases were sub-stratified according to the gender and the analysis was repeated. As regards the males included in the study (Table 2), 12.9% (4/31) of the alopecic males did not inherit the cut allele compared to 38.9% (7/18) of the control males. On the other hand, 87.1% (27/31) of the males having AGA had the cut allele compared to only 61.1% (11/18) of those of the control Group. This association between the AR StuI polymorphism and occurrence of AGA was found to be of borderline significance (p=0.07 using Fisher exact test).

Table 2: Comparison of genotype between cases and controls in males.

	Bald N (%)	Control N (%)	Total N (%)
Home uncut	4(12.9)	7(38.9)	11 (22.4)
Home cut	27(87.1)	11 (61.1)	38(77.6)
Total	31(100)	18(100)	49(100)
P value of fisher exact test	0.07		

Regarding the results of the genotype analysis in females (Table 3), it was observed that 10% (2/20) of the patients did not inherit the uncut allele compared to 21.1% (4/19) of the control. Thirty five percent (7/20) of the patients and 36.8% (7/19) of those not suffering of FPHL were homo-cut. Out of those who were heterozygous, 55% (11/20) had FPHL and 42.1% (8/19) had no hair loss. To conclude, the association between the AR StuI polymorphism and the occurrence of FPHL in the female cohort was not statistically significant (p= 0.57 using Chi square test).

Table 3: Comparison of genotype between cases and controls in females.

	Bald N (%)	Control N (%)	Total N (%)
Home uncut	2(10)	4(21.1)	6(15.4)
Home cut	7(35)	7(36.8)	14(35.9)
Hetero cut	11(55)	8(42.1)	19 (48.7)
Total	20(100)	19 (100)	39 (100)
Chi square	1.12		
P Value	0.57		

All clinicopathologic parameters were included in the multinomial logistic regression model which showed no relation between the age of the patient and the corresponding genotype (p value=0.661). The grade of alopecia with which a case presented, was significantly related to the genotype of the patient (p value= 0.002).

There was a significant relationship detected between the presence of family history of an affected close relative and the genotype of the individual (p value <0.0001).

Relationship between the level of expression of AR in the bald and the presence of AR polymorphism:

The level of expression of AR in the bald tissue sections of patients and the results of the PCR based analysis of StuI polymorphic site in exon 1 of the AR were undertaken blindly. Studying the relationship between the expression and the StuI AR polymorphism showed a significant relation (p=0.05 using Chi square test). Statistical analysis of our data revealed a significant level of AR detected in the bald scalp biopsies in patients having the 'cut' allele. Among

those demonstrating mild nuclear staining for the presence of the androgen receptor, 30% (3/10) did not have the "cut" allele, 30% (3/10) were homo-cut and 40% (4/10) were heterozygous. Regarding those in which moderate staining was observed, 3.4% (1/29) were uncut compared to 72.4% (21/29) homo-cut individuals and 24.2% (7/29) heterozygous ones. Those who were observed to have strong nuclear intensity, 75% (9/12) were homo-cut, 16.7% (2/12) were uncut and 8.3% (1/12) were heterozygous (Table 4).

Table 4: Relation between genotype and intensity of nuclear staining in bald scalp tissue sections.

	Intensity of reaction				Total N (%)
	Negative N (%)	1+ (mild) N (%)	2+ (moderate) N (%)	3+ strong) N (%)	
Home uncut	-	3(30%)	1(3.4%)	2(16.7%)	6(11.8)
Home cut	-	3(30%)	21(72.4%)	9(75%)	33(64.7)
Hetero cut	-	4(40%)	7(24.2%)	1(8.3%)	12 (23.5)
Total	-	10(100%)	29(100%)	12(100%)	51(100)
Chi square	9.44				
P Value	0.05*				

DISCUSSION

The common heritable loss of scalp hair known as male pattern baldness or Androgenetic alopecia affects up to 80% of males by age 80 years. According to Hamilton[38], the hair loss is heriTable, androgen dependent and occurs in a defined pattern. Ellis et al.[13] showed that the mode of inheritance remains unproven; however, it is most likely polygenic.

Studies were conducted on cultured dermal papillae derived from various parts of the human body to unravel the paradoxical responses of hair follicles to androgens[10,39,40]. This was done in the hope for finding better therapeutic modalities for androgen dependent disorders such as hirsutism and AGA[10]. Randall et al.[10] observed that cultured dermal papilla cells from androgen-dependent human hair follicles (e.g. beard) contain more androgen receptors than those from non-balding areas of the scalp. However, Hibberts et al.[40] reported that the expression of the androgen receptor is increased in balding scalp. This is in agreement with our study in which the scalp biopsy obtained from the bald area demonstrated a significantly higher level of expression of the AR in comparison to the non bald scalp biopsy.

On the other hand, Sinclair[1] has stated that since drugs such as finasteride, which inhibit the formation of the potent androgen DHT, have shown to reverse baldness in affected individuals, then tissue-specific abnormalities of the androgen receptor are not sufficient to cause baldness. This indicates that both high androgen levels and increased androgen receptor expression are required to cause baldness. Ellis et al.[18] pointed out that this could probably be attributed

to the polygenic nature of androgenetic alopecia. In fact, this could be a likely explanation to what has been found in our study. There was a borderline significant relationship detected between the grade of alopecia and the level of expression of AR in males. To the best of our knowledge, this is the first study to suggest possible correlation between the grade of AGA in males and the level of expression of the AR in the balding scalp; a point which is worth further study.

Accordingly, the tissue-specific distribution of the AR raises the possibility of functional mutations in the upstream promoter regions of its gene that causes increased transcription and translation in the affected scalp[18]. Ellis et al.[18] conducted a study on Australian males recruited from a 'Victorian Family Heart Study, in which the StuI RFLP marker was investigated to determine the link between AR gene and AGA[18]. This StuI restriction site is located in the first exon of the gene and is caused by a single third base change, Adenine (A) to Guanine (G), that does not cause an amino acid change. It was reported that a genetic mutation in or near AR could be in linkage disequilibrium with the StuI restriction site (i.e. the two alleles are found together along generations without cross-over) is a possible explanation. The relationship between AR StuI polymorphism and AGA was further documented by Levy-Nissenbaum et al.[41], in Israeli males having AGA. Hillmer et al.[42] observed association between severe form of AGA and AR Stu1 polymorphism in patients of German descent, but the relationship did not reach statistical significance. In the present study, the StuI restriction site was commonly observed among the bald, suggesting a possible association between the polymorphic site in the AR gene and the occurrence of Androgenetic alopecia, however an appreciable number of the non-bald participants also had this variant, therefore, additional genes and factors are likely to play additional role.

AR StuI polymorphism was also studied in females complaining of AGA in the present study, this restriction site has not been previously investigated in females having AGA, however no statistically significant relationship was observed. It is a fact that neither the androgen-dependent nature nor the genetic basis of female pattern hair loss (FPHL) has been clearly established[43]. Concerning the investigation of AR polymorphism in females with AGA, it is rather difficult to make concrete assumptions. AR mutations in females at the StuI restriction site were designated as being either homozygous or heterozygous. It is true that in female mammals, most genes on one X chromosome are silenced as a result of X-chromosome inactivation. However, some genes escape X-inactivation and are expressed from both the active and inactive X chromosome. Such genes are potential contributors to phenotypic variability among females heterozygous for X-linked conditions.[44] A significant relationship between the grade of alopecia and the detection of StuI polymorphic site in exon1 of the AR gene, was found in both the males and the females. In other words, individuals inheriting the cut allele had higher grades of alopecia. Our study is the first to prove such a correlation in either the males or the females. Therefore, inheritance of the StuI polymorphic allele has an additional impact on the degree of hair loss and thereby, the grade of AGA with which a case presents. This fact applies to both males in which the

Hamilton-Norwood classification of AGA is employed and to females with the Ludwig pattern of FPHL.

In an attempt to underline the fact that a strong family history predisposes to early development and rapid progression of AGA as proposed by Tosti et al.[4], family history was collected from patients included in this study. Almost all of the cases who had maternal uncles suffering from advanced stages of AGA were found to have homo-cut genotype at the AR StuI polymorphism. Also, the majority of males having only their father suffering from high grade AGA had inherited the allele. A significant correlation was found between the family history of having a relative (father or maternal uncle) with AGA and the presence of the cut allele. Therefore, it still remains to be explained why fathers and sons tend to have the same pattern of alopecia. A likely explanation would be that the remaining etiological fraction is due to other genetic variation at autosomal loci. Half of the women who had an affected mother were homo-cut and ~40% were heterozygous. From this, it becomes quite apparent that this polymorphic site of the AR ranks high among the essential factors which is inherited from the maternal side of the family and possesses a strong influence on the expression of this disease.

Our study has shown a significant association between the level of expression of AR and the presence of AR StuI polymorphism in both males and females. It was observed that patients having AR gene polymorphism at the StuI restriction site of exon 1 tend to have higher expression levels of AR in their balding scalp tissue. To the best of our knowledge, this study is the first to report such an association. Further studies on the genetic factors involved in AGA are warranted to establish genotype phenotype correlation and to pave the road to new treatment modalities. Though the development of a cream that could permanently restrict androgen receptor expression within the hair follicle is some years away, research is currently focusing in that direction. This provides another application to the finding of higher expression of AR in the balding scalp in comparison to that in the non-bald area. In the mean time, supporting the patients emotionally and ensuring that they understand the limitations of the currently available treatments, remain to be the only options in the management of AGA.[1]

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