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Androgen Receptor (AR) gene polymorphism rs6152 is associated with androgenetic alopecia

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Abstract

Androgenetic alopecia is a genetic disorder characterized by distinct forms of baldness. Main reason of alopecia is the imbalance between hair fall and regrowth. Alopecia can be either syndromic alopecia that occurs along with other clinical conditions like epilepsy, nail dystrophy or it can be is non-syndromic alopecia; having no other clinical conditions. Androgenetic alopecia (AGA) is a non-syndromic alopecia commonly known as Male Pattern Baldness. 80% of males above age of 70 and 80 years are affected by this condition. We considered rs6152, G>A polymorphism of androgen receptor (AR) gene as candidate for androgenetic alopecia in Pakistani men. A total of 60 patients with reported clinical alopecia and 30 age-matched controls were analyzed. Polymerase chain reaction and subsequent restriction fragment length polymorphism (RFLP) by StuI restriction enzyme were performed to detect rs6152 polymorphism of AR gene using. 40 patients with cosmetically reported baldness showed association rs6152 polymorphism with androgenetic alopecia, whereas 3 controls with no baldness also exhibited rs6152, G>A polymorphism. The study indicated strong association of rs6125 G>A SNP of AR gene with androgenetic alopecia.

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Introduction

Alopecia is a genetic disorder. Alopecia is basically a Greek word means “hair loss or baldness”. The term “Alopecia” is used to describe different forms of human hair loss. The reasons behind the onset of Alopecia are not known, but association of psychological stress, physical trauma, and genetic predisposition with onset of Alopecia has been strongly suggested [1]. Genomics and genetics research community tried to understand the source of common complex disorders. According to them diseases which are frequently encountered in health care are the common diseases. The monogenic subtypes of common disorders are characterized by a strong influence of germline mutations in a single gene. While the common disorders influenced by several genes and environmental factors, are most commonly referred as complex disorders [2].

The clinical form appears due to an imbalance between hair shedding and hair regeneration. Both endogenous and exogenous factors influence the condition of hair follicles that are the sources of hair growth [3]. Hair loss is a physiological process. Normally 70-100 hairs are shed everyday but the daily loss should not exceed the limit, with the exception of individual variations and seasonal increased hair loss. However, hair loss becomes problematic when daily hair loss exceeds the limit of 100 hairs and condition prevails more than a few weeks. Type of hair loss depends on its causative agent. Androgenetic alopecia normally shows autosomal recessive inheritance pattern but from a study it has been observed that families with X linked recessive trait also show autosomal dominant inheritance pattern [4].

Androgenetic alopecia (AGA) is a common reason of hair shedding affecting both men and women. Male-Pattern Baldness (MPB) is another term used to describe this condition in men. AGA mainly affects scalp hair of mostly old age men [5]. It has been suggested that degree of genetic predisposition and presence of sufficient amount of androgens circulation can be used to determine the onset of Androgenetic Alopecia. Medically androgenetic alopecia is not a serious condition, but it may be a source of stress and psychosocial consequences [6].

Hamilton–Norwood scale is a defined pattern of hair loss mostly followed to diagnose the intensity of androgenetic alopecia. It is characterized by step-wise hair follicles miniaturization due to alteration in hair cycle [7]. Initially hair cycle has long growth phase and short resting phase but with passage of time the shrinkage of growth phase and elongation of resting phase take place due to which the hair do not grow properly and due to short growth phase the anagen hairs do not reach the skin. The time between both telogen hair shedding phase and regrowth phase increases which decrease the scalp hairs [7]. Androgenetic alopecia is caused due to imbalance between hair regrowth and shedding.

Male pattern baldness is a hereditary disorder and follows the autosomal dominant trait pattern [8]. It characterized by M shaped pattern along frontal and parietal area by center vertex or top of the head hair loss due to gradual thinning of the hair. Dissatisfaction with body appearance, depression and low self-esteem are the physiological effects of Androgenetic alopecia [6].

Androgenetic alopecia affects 30% of males at the age of 30 and 80% at age of 70 [9]. Thinning of hair begins most commonly in teen age and mostly expressed fully above the age of 40 years in both males and females. It is more common in men than women [10].

AGA usually initiates from temples and proceeds towards the crown of the head with gradual thinning of scalp hair. In the extreme type of male pattern baldness only a border of hair at the both sides and back is left. It can also progress to complete baldness. According to Norwood- Hamilton scale of male pattern baldness there are seven types; in type I there is no hair loss. Type II has minor recession of frontal hair line. Type III shows further loss of frontal hair. Type IV – VI are characterized with further loss of frontal and vertex hair. In type VII only significant amount of hair are present is occipital scalp region.

Androgenetic alopecia is a common disorder in Asian men and also in women. Studies have showed approximately 73% reported instances of disorder in Asian general population [11]. In Pakistan only few research studies have been conducted on alopecia. For example, a study conducted by another group showed the clinical features and associations of alopecia areata in population of all ages [12]. Another study has also been reported by Aslam *et.al* (2004) on Localized Autosomal Recessive Hypotrichosis (LAH) which is a very rare form of alopecia [13]. This study was conducted on a Pakistani family having infected individuals of LAH and mapped the second locus of chromosome 3q27 to 7.59cM [2].

Materials and methods

Study population

All subjects under study were males from Pakistani population in Attock and Rawalpindi area (North part of Pakistan) with the appearance of hair fall and reported alopecia. The study was approved by the local ethical committee of Virtual University of Pakistan.

Polymerase Chain Reaction (PCR)

DNA extraction was performed by inorganic method from peripheral blood leukocytes [14]. The segment of AR gene (416 bp) was amplified by using the forward primer 5´-CACAGGCTACCTGGTCCTGG-3´ and reverse primer 5´-CTGCCTTACACAACTCCTTGGC-3´, previously used by Ellis et al., 2001 to study victorian population [15]. The reaction mixture contained dNTPs (0.2mM each), Taq polymerase (5 IU), PCR buffer (100mM), all from Thermofischer scientific; and 0.5mM forward and reverse primers.

Restriction Fragment Length Polymorphism (RFLP)

RFLP was performed after PCR using Stu1 (ECO1471) restriction enzyme [7]. Stu1 restriction enzyme identifies the sequence AGG^CCT and in our product, it digested the 416bp amplified product if at the position of third base Guanine (G) was present instead of Adenine (A) within the sequence (AGGCCT). Restriction of fragment resulted into two fragments with size 329bp and 87bp respectively. If at the third base position, Adenine (A) would be present (AGACCT), then the amplified product would not be digested by Stu1 enzyme. Forty patients and thirty controls were included in restriction fragment length polymorphism. However, 10 diseased and 20 controls were excluded due to poor quality of the PCR results. StuI restriction enzyme was obtained from Thermofischer scientific.

Statistical Analysis

Comparisons of allele and genotypes frequencies between diseased and controls were performed by calculating value of chi- square (x^2) using social science statistics. Statistical significance level was considered at the values below 0.05 ($p<0.05$).

Results

PCR was performed for 100 samples containing 50 normal and 50 diseased male individuals. The fragment of exon 1 (416bp) was amplified by using specific primers. Among these 100 samples 10 disease and 20 control samples showed poor results of PCR, that is why these 30 samples were not included in RFLP. PCR products were run on 2% gel along with 1Kb DNA ladder (10,000bp-250bp) to verify the product size (**Figure 1**).

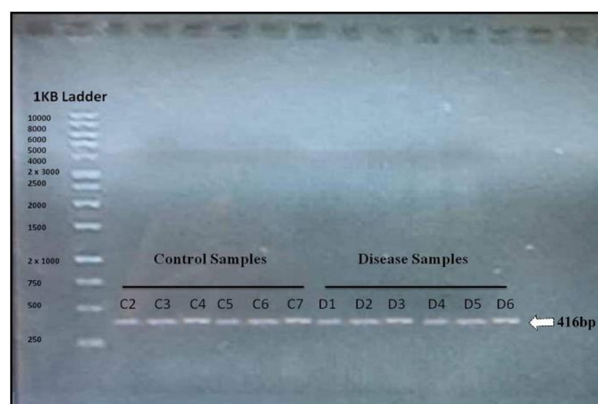

Fig. 1: Image of the polymerase chain reaction using 2% agarose gel electrophoresis. Lanes 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 indicate amplified segment of interest (band size = 416bp).

Association of rs6152 polymorphism with Androgenetic alopecia

Restriction Fragment Length Polymorphism was performed on 70 PCR products containing 40 diseased and 30 normal samples. The AR-E211 G>A (rs6152) polymorphism was detected by using Stu1 restriction endonuclease enzyme. The PCR product containing AGGCCT sequence was digested by Stu1 restriction endonuclease enzyme resulting in to two fragments of 329bp and 87bp respectively. While the AGACCT sequence remained undigested by Stu1 enzyme and the resulted fragment size was 416bp. 100% (40 samples) alopecia patients showed mutation and were digested by Stu1 restriction enzyme. Among 30 control samples 10% (3 samples) showed digestion by the enzyme, while 90% (27 samples) were not digested, which indicated the absence of mutation in those subjects. All the RFLP products were visualized on 3% gel along with 50bp ladder (BIORON) (**Figure 2**).

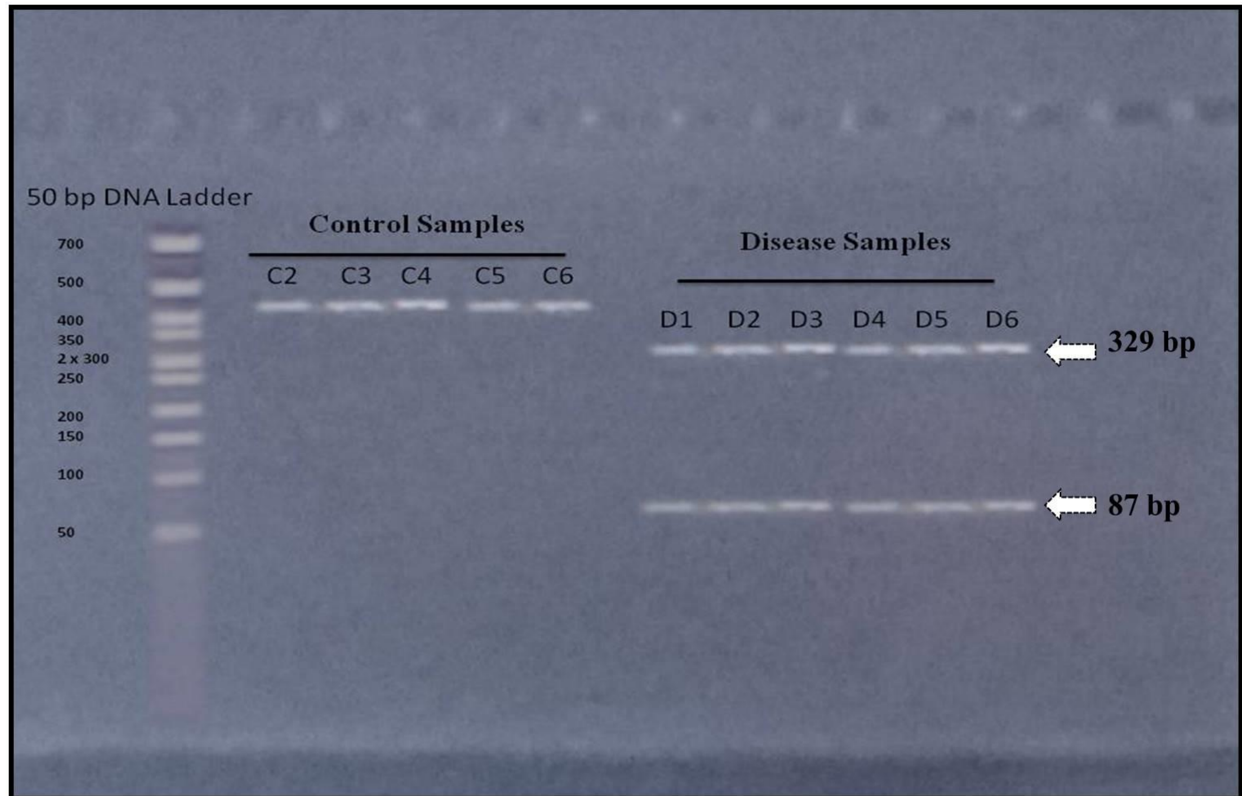

Fig. 2: Representative image of the StuI Restriction assay revealed using 3% agarose gel electrophoresis. The AGGCCT sequence is cut by the StuI restriction enzyme, while the AGACCT sequence is not. Lanes 5, 6, 7, 8, 9 show undigested samples (band size = 416bp). Lanes 10, 11, 12, 13, 14, 15 show two bands one at 329bp and another at 87bp.

As indicated in **Table 1**, *p* value obtained by applying Chi square is < 0.00001. This result is significant at $p < .05$. (expected values are displayed in square brackets and individual $\chi 2$ values are displayed in parentheses).

Table 1: Distribution of Alleles in diseased and control group

	A Allele (wild type)	G Allele (risk allele)	Marginal Row Totals
Control	54 (23.66) [38.9]	6 (36.34) [25.33]	60
Diseased	2 (32.34) [28.46]	80 (49.66) [18.53]	82
Marginal Column Total	56	86	142 (Grand Total)

Note: Chi square calculations of RFLP results (expected values are displayed in square brackets and individual $\chi 2$ values are displayed in parentheses), $p<0.05$.

Discussion

Hair follicles are the structures responsible for hair growth and formation thus all hair disorders are commonly due to hair follicles disorders. Hair growth and formation is a cyclic process with three stages Anagen, Catagen, and Telogen [2]. Androgenetic Alopecia is also known as male pattern baldness that occurs because of alteration in hair cycle which causes gradual hair follicles miniaturization [7]. High level of circulating androgens and genetic predisposition are the suggested causes of androgenetic alopecia. Androgenetic alopecia is a source of psychosocial consequences and stress otherwise it is not medically harmful [6]. Hamilton–Norwood scale is a defined pattern of hair loss mostly followed in androgenetic alopecia. Androgenetic alopecia follows autosomal dominant inheritance pattern. McElwee et.al describes many options for treating androgenetic alopecia [16]. Only two drugs Minoxidil and Finasteride are approved by

FDA. Other options include use of Anti- androgens, laser treatment, hair surgery etc.

Androgens are steroid hormones, which affect the genetic transcriptional events by binding nuclear receptors. Scalp hair follicles show sensitivity to normal circulating androgens level responsible for follicles miniaturization. Hibberts et.al (1998) reported that in baldness elevation in the expression of androgen receptor, which is a typical steroid receptor has been observed [17]. Lee W S and Lee H J (2012) reported high level of androgen dihydrotestosterone (DHT) and increased in expression level of androgen receptor [11].

SRD5A1 and SRD5A2 are the genes encodes for 5α-reductase enzymes were studied by Ellis et.al in male-pattern baldness as 5α-reductase is responsible to convert testosterone to DHT [15] and high level of DHT has been reported in alopecia. Study of CYP19 gene encodes for aromatase, which converts testosterone to oestrogens show no differences between affected and unaffected suggesting that it is doubtful that the aromatase gene is involved in determining predisposition to androgenetic alopecia [5]. Non-recombining region of the Y chromosome examination demonstrated that causative mutations do not present in this region [7]. The frequency of short repeats CAG and GGC length present on AR gene has been studied by Kassem et.al (2009) and it has been reported that higher frequency of these repeats length has been found in bald men as compared to non-bald men [7].

Cobb et.al, reported rs5919324 located upstream of AR shown highest significance association with MPB [18]. SNP rs1998076 from the 20p11 was reported in association with MPB in German population [19]. In European populations various SNPs on Xq12 have been reported as significantly associated with MPB [19].

This study focused on the association between androgen receptor and male pattern baldness as AR gene is a primary candidate of AGA. This study was conducted to check the involvement of AR-E211 G>A (rs6152) single nucleotide polymorphism present at exon 1 of AR gene in pathogeneses of androgenetic alopecia in Pakistani male population.

All the DNA sample of patients and control are used to amplify the exon 1 of AR gene using specific primers. The set of 70 PCR products containing 40 patients and 30 normal samples have been digested by using stu1 restriction enzyme to check the AR-E211 G>A polymorphism

The result of this study showed that 100% samples means all of the 40 samples of affected individuals contained AR-E211 G>A polymorphism at exon 1 of AR gene as all the 40 amplified products have been digested when incubated with stu1 restriction enzyme. As all the subjects under study were males, that are hemizygous and carry only one X chromosome as compared to females, we have to consider the fact that the AR gene is located on X chromosome, therefore, in case of patients carrying mutation, only two bands of 329 bp and 87 bp size were observed after StuI restriction and the clinical condition is more pronounced in males. In case of controls 90% (27/30 samples) showed undigested band while 10% (3/30 samples) showed mutation. It can be justified in two ways: first that mostly AGA fully expressed after 40 years and it is a very common condition present in almost 80% by the age of 80 year while these individuals are still below 30s so they may become bald in later stages of life. Secondly it is possible that along with this mutation some other polygenic causes are necessary to influence the androgen level and cause baldness and these samples, which show mutation but are still prevented from baldness may lack these other necessary polygenic factors. The second possible explanation open gates for further research to find other genetic predisposition and mutations, which can possibly affect the level of androgens and cause baldness. In 2009, Kassem et.al studied the same polymorphism in Egyptians population using same primers and restriction enzyme as used in this study [7]. In their study 66.7% alopecic patients and 48.6% control samples showed mutation and had been digested by Stu1 enzyme while 11.8% patients and 29.7% controls were remained undigested and indicating absence of AR-E211 G>A polymorphism [7]. They also performed immunohistochemistry for the androgen receptor to check its expression level in the scalp skin. In this process sections of both bald scalp area and non-bald scalp area were compared and scored independently as Negatives, 1+(mild) if <10% nuclei were immunoreactive, 2+ (moderate) if 25-50% of nuclei were positive and 3+ (strong) if >50% of nuclei were positive. Statistical analysis established that scalp biopsies of patients having rs6152 showed significantly higher expression of AR [7]. Another study performed by Ellis et.al in 2001 found the higher frequency of Stu1 restriction site in older age bald men as compared to older age controls [15]. These studies have shown the significant association of AR-E211 G>A polymorphism with AGA.

Findings of this study show strong association of AR gene and MPB. Results suggest that this mutation is

necessary for clinical formation of AGA but there is a possibility that other polygenic factors are also required to fully understand the pathogenesis of AGA.

DNA sequencing is the process of determining the precise order of nucleotides within a DNA molecule. If DNA sequencing for the mentioned polymorphism would be performed, it could confirm the restriction site sequence of Stu1 restriction enzyme and would have increased the validity of results. Although it has been reported by Hayes et.al in 2005 [20] and Ellis et.al in 2001 [15] that rs6152 single nucleotide polymorphism is associated with AGA and it is a restriction site for Stu1 enzyme, it has been performed for the first time in Pakistani population to our knowledge. In further studies, sequencing can be performed to give exact nucleotide sequence of studied region.

Conclusion

We can conclude from this study that the SNP rs6152 found in exon 1 of AR gene contributes to the clinical form of androgenetic alopecia in patients of Pakistan. Although some of the control subjects exhibit this mutation but they did not have clinical signs indicating that alopecia might be a multigenic disorder as described previously [21, 22] or some of the environmental factors might help to mask the genetic affects.

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Authors Contributions

Ayesha Maqbool conceived the study and wrote the manuscript. Sadia Kousar performed the experiments. Akhtar Ali performed statistical analysis and provided helpful suggestions. Tanveer Hussain, M. E. Babar and Yasir Ali reviewed the manuscript.

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