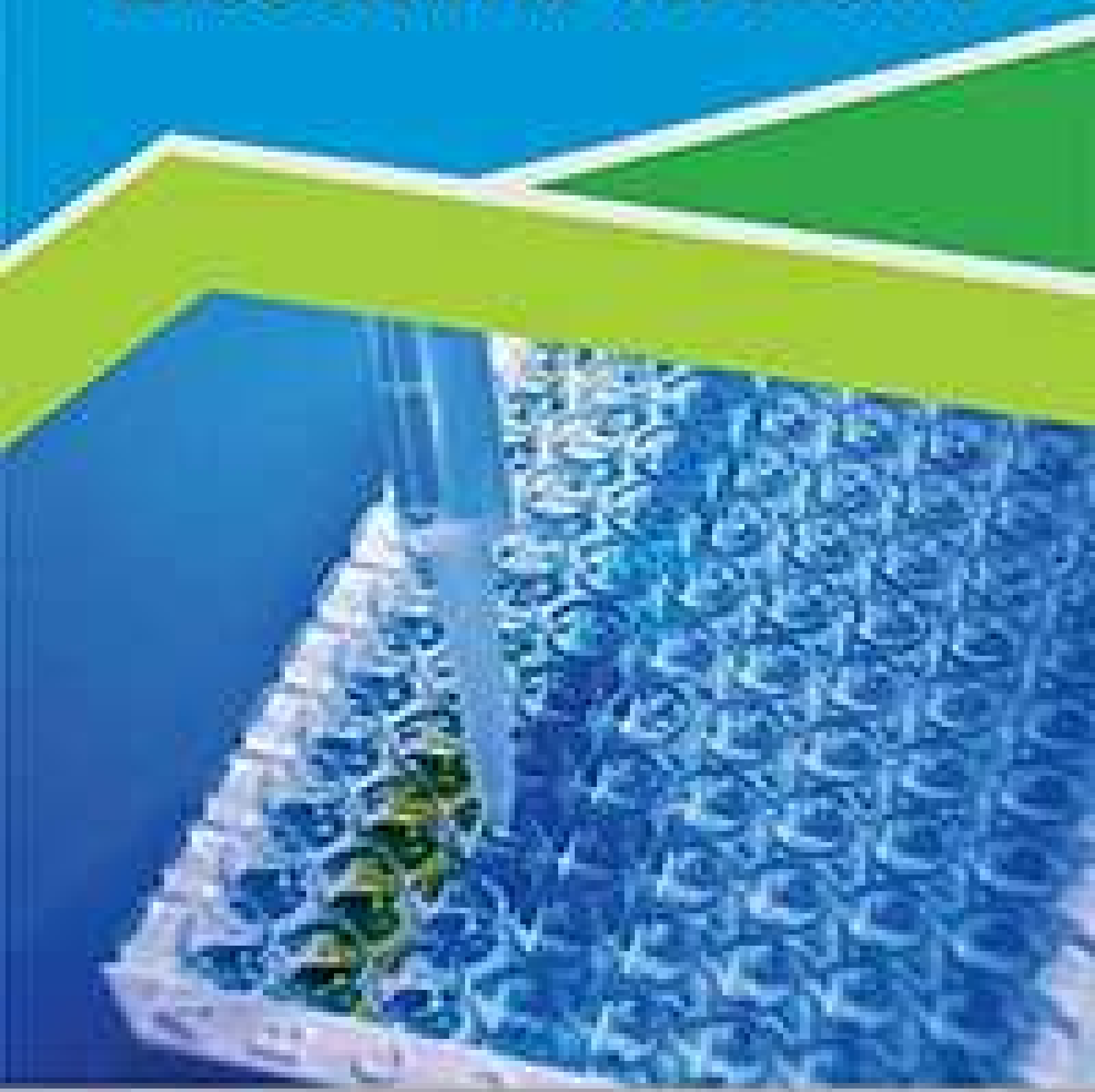


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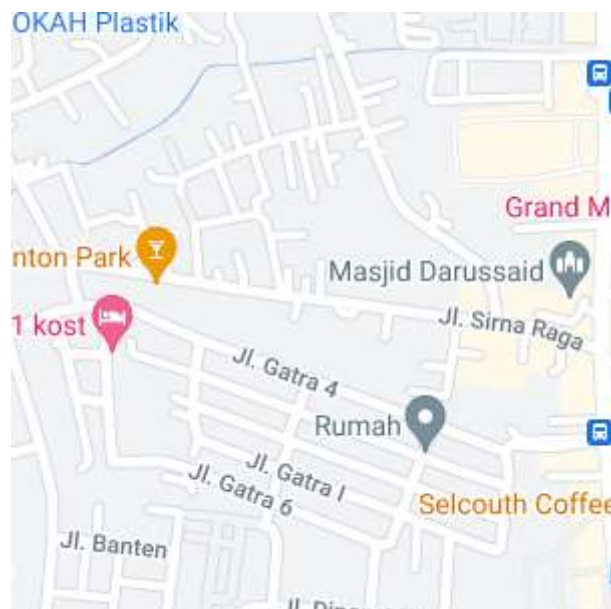


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
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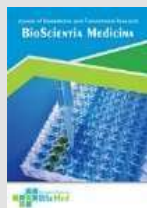
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The Role of rs6152 Allele and Non-Genetic Factors in Androgenetic Alopecia: A Pilot Study in the Indonesian Local Population

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ARTICLE INFO

Keywords:

Alopecia
Androgenetic Alopecia
Risk factors
rs6152

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All authors have reviewed and approved the final version of the manuscript.

<https://doi.org/10.37275/bsm.v8i6.1002>

ABSTRACT

Background: Androgenetic alopecia (AGA) is a common form of hair loss which inflicts progressive hair loss leading to various patterns. The cause of this disease is believed to be multifactorial, which is majorly attributed to genetic and non-genetic factors. This pilot study aimed to investigate the association of rs6152 allele, a SNP on AR gene, with AGA, as well as explore other contributing factors in the Indonesian local population. **Methods:** In this cross-sectional study, a total of 100 participants, which categorized into alopecia subjects and non-alopeia subjects, were enrolled for rs6152 SNPs detection. Anthropomorphic data such as height and weight, blood pressure and family history were obtained by measurement and questionnaire. **Results:** The study showed low frequency of individuals with rs6152 non-risk alleles (2%) and further analysis showed no significant association between rs6152 allele and AGA. However, familial history analysis revealed a strong association between family history and AGA risks. Additionally, age, gender, hypertension status and BMI were identified as significant factors associated (p-value < 0.05) with AGA. **Conclusion:** rs6152 was not a reliable genetic marker for AGA in the Indonesian local population. While familial history with AGA showed the inheritance pattern of autosomal dominant inheritance with sex limitation, non-genetic factors such as age, gender, hypertension status and BMI were strongly associated with AGA risk. This shows the complexity and multifactorial causes of AGA in the Indonesian local population.

1. Introduction

Androgenetic alopecia (AGA), also referred to as patterned baldness, is a prevalent form of hair loss affecting both genders worldwide. AGA manifests as a progressive attenuation of hair density, primarily at the crown and frontal scalp regions, resulting in a distinctive balding pattern.¹ While AGA exhibits varying prevalence rates across ethnicities, it represents a noteworthy concern within the Indonesian populace. Recent epidemiological investigations conducted at a prominent national hospital in Jakarta have underscored AGA as the

second most prevalent hair disorder after alopecia areata in this demographic.² Despite AGA's benign nature regarding physical health, its psychosocial burdens, including depression and anxiety, significantly compromised individuals' quality of life.^{3,4} Accordingly, concerted efforts aimed at enhancing awareness and implementing tailored interventions are warranted to ameliorate the impact of AGA on the Indonesian populace.⁵

Hair thinning in AGA is characterized by the miniaturization of hair follicles, which worsens progressively with age. This condition is believed to be

influenced by both genetic and hormonal factors, such as dihydrotestosterone, although the former is reported to influence more.⁶ The genetic association with androgenetic alopecia (AGA) is a complex relationship which follows polygenic inheritance which is believed to contribute to the varying degree of severity, onset, and patterns observed in affected individuals. Nevertheless, androgens and the genetics that modulate the androgen metabolism are believed to play a major role in AGA. The androgen receptor (AR) gene is one of the extensively researched genes that is believed to play a central role in AGA pathogenesis. This is due to the androgens exerting their effects through the AR by binding to the androgen response element (ARE).⁷ This has been elucidated by research utilizing mice, which demonstrates that proper delivery of dihydrotestosterone (DHT) activates the AR and leads to delayed hair growth, as well as alterations and miniaturization of hair follicles.⁸ rs6152, one of single nucleotide polymorphisms (SNPs) within the AR gene, located on X chromosomes at Xq11-12, have been associated with AGA.⁹

The influence of ethnicity on AGA was already reported. This is shown by several studies done on different major ethnicities, such as European, Asian, and Indian descent. In the Asian population, the onset of AGA usually happens later compared to European descent. While AGA in European descent was predominant by frontal baldness, the pattern of AGA in Asian descent was distinct and varied. Indian men typically experience less extensive balding patterns compared to Asians.¹⁰ Indonesia is an archipelago country with many different ethnicities. In this multiethnicity setting, the role of rs6152 as a genetic marker for AGA could elucidate the mechanisms and facilitate valuable insights into the pathogenesis of AGA.

2. Methods

In this cross-sectional study, a total of 100 volunteers were enrolled in this study which was distributed evenly into two categories named normal (control) subjects and AGA subjects. Ethical clearance

for this study was given by Universitas Tarumanagara Human Research Ethics Committee (UTHREC) with ethical clearance number PPZ20222090. Informed consent was signed by the participant after the participant received an explanation and understood the aims and methods of the study. The determination of normal subjects and AGA subjects were done by physical examination of the hair. A questionnaire was given to each participant, which included age, gender, BMI, smoking history, age of onset (for AGA subjects), family history of alopecia, and ethnicity of the participant along with the parents. Blood pressure was checked by an automated tensimeter (Omron, HEM-7121). For DNA extraction, blood was drawn from the volunteers by a physician. Blood DNA extraction was done using a Genomic DNA Midi Kit (Geneaid, Taiwan). After extraction, DNA was kept in a -20°C freezer until further use. Alleles of rs6152 were detected by Tetra arms PCR, which had already been validated.¹¹ Data analysis and statistics were processed using the R (version 4.3.3) and Rstudio (Version: 2023.12.1+402). Package ggstatsplot was used for statistical analysis and data visualization.¹² A p-value of <0.05 was considered statistically significant.

3. Results

Table 1 shows the demographic data of participants enrolled in this study. The participants, who were categorized as alopecia and non-alopecia, have significant median differences in age and body mass index between the two categories. This data also shows a significant association between gender, hypertension status, and family history of alopecia. Interestingly, a low prevalence of A alleles was found in this study, and no significant association between risk/non-risk alleles and alopecia status was found. One non-risk allele was found in a female aged 24 years old belonging to a control group with West Nusa Tenggara ethnicity, and the other was in a male aged 34 years old in the alopecia group with mixed ethnicity of Javanese and Ambonese.

Figure 1 shows the distribution of 11 ethnicities we found in this study. This ethnicity data is based on the

self-reported ancestry of the participants provided during the questionnaire. To ensure accuracy, we track back the ethnicity of participants' parents. Those with a combination of ethnic backgrounds were

grouped into mixed ethnicity, while participants who have parents with similar ethnic backgrounds were grouped according to their own ethnicity.

Table 1. Demographic data of participants.

Characteristic	Alopecia, N = 50 ¹	Non-Alopecia, N = 50 ¹	p-value ²
Allele (G/A)			>0.9
A	1 (2.0%)	1 (2.0%)	
G	49 (98%)	49 (98%)	
Age	38 (33,51)	27 (23,34)	<0.001
Gender			<0.001
Female	19 (38%)	43 (86%)	
Male	31 (62%)	7 (14%)	
Hypertension status			0.004
Hypertension	38 (76%)	24 (48%)	
Non-hypertension	12 (24%)	26 (52%)	
Body mass index	24.6 (22.9,26.3)	23.4 (20.4,25.3)	0.014
Smoking history	14 (28%)	17 (34%)	0.5

¹ n (%): Median (IQR)

² Fisher's exact test; Wilcoxon rank sum test; Pearson's Chi-squared test.

Figure 2 shows the median differences in onset age between genders, with the non-alopecia group as the control. The onset age for the alopecia group, male or female, was defined as the age when the participants first realized hair thinning. Although no statistical differences were found for each gender group

compared to the controls, this study not only shows a descriptive cut-off on the emergence of AGA in the participant's lifetime based on gender but also shows the trends that females have older onset age than males.

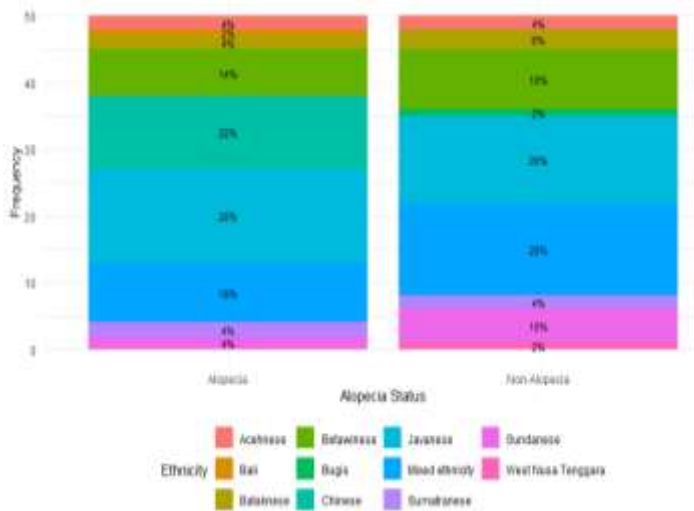


Figure 1. Ethnicity distribution based on alopecia status in this study.

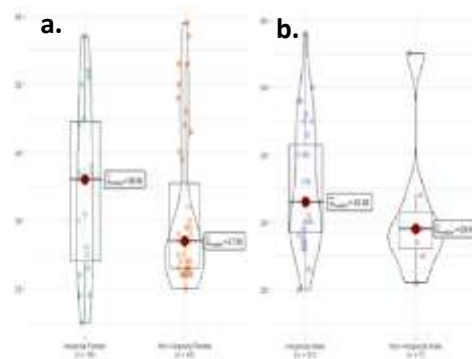


Figure 2. Alopecia onset age comparison between genders. a) Female, b) Male.

Figure 3 shows the stacked bar plot for alopecia vs non-alopecia group based on their family history of alopecia. The number inside the stacked bar shows the frequencies of the participants, which satisfies the

conditions depicted by the legends. Pearson Chi-square was used in this analysis and shows a strong significant association ($p\text{-value} < 0.001$) between family history and the risks of alopecia.

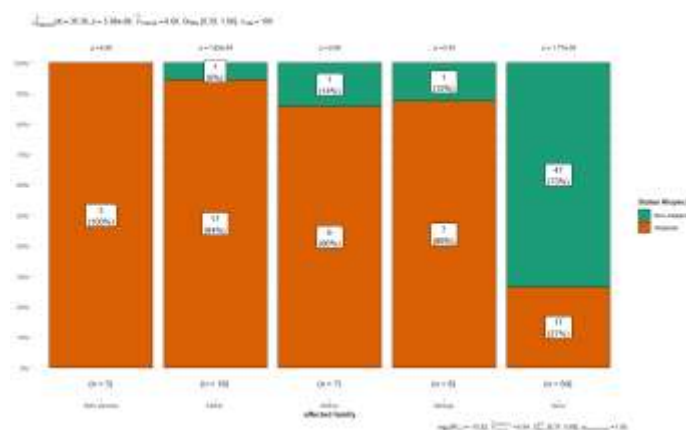


Figure 3. Distribution of affected families among alopecia status. The x-axis shows the affected family members of the participant, while the y-axis shows the frequency in percentage.

4. Discussion

This pilot study represents the first attempt in Indonesia to try to associate rs6152 with AGA in the local population. In this study, we investigated the potential role of rs6152, which has been reported in association with AGA and other androgen receptor-mediated diseases such as polycystic ovary syndrome and prostate cancer.^{9,13,14} However, contrary to previous findings, our pilot study discovered no significant association between rs6152 alleles and

alopecia status. We also found that the non-risks allele, A allele, was also found in both the alopecia and non-alopecia groups in low frequency. This study indicates that rs6152 may not be applicable as a robust predictor for AGA in the Indonesian population despite the recent popularity in commercial SNP analysis. This suggests that the genetic pattern in our population was different from other populations studied previously, which shows the importance of conducting population-specific studies before

commercializing SNPs as a disease predictor. Notably, this study may elucidate Lee and Lee's findings, which highlighted the differences in characteristics in Asian populations compared to Europeans. However, further study is necessary to explain the effect of genetics and AGA patterns between Asians and Europeans.

Aside from genetic factors examined in this study, we also explored other factors that contribute to Alopecia in the local population and found that AGA was associated with age, gender, hypertension, and BMI. This study also reports the onset age of alopecia, which differs between genders, notably 36 years for females and 33 years for males. Age and gender are two of the most determining factors in AGA development, and onset age varies based on race. The prevalence and severity of AGA increases with age and differs by gender. The prevalence of Caucasian male developing AGA was 30% in their forties and increased to 40% in their fifties,¹⁵ while their degree of severity, Norwood-Hamilton classification type III or worse, was reported to be increased in their forties.¹⁶ While a study by Takashima et al. on the Japanese population shows that the prevalence is lower than that of Europeans, As for genders, this study shows that females have lower risks in AGA development, which, in accordance with findings by Legiawati et al., amounts to only 35% of female AGA in the local population.² A study by Paik et al. on Korean women also shows a lower prevalence of female AGA, which is only 5.6% across all ages, with a notable increase, especially when reaching their sixties.¹⁷

This study also shows the association of AGA with hypertension and BMI, with the alopecia group associated with hypertension and higher BMI. AGA was also reported to be associated with hypertension and other cardiovascular diseases.¹⁸⁻²⁰ This condition is believed to be associated with aldosterone, a steroid hormone produced by the adrenal gland. This hormone, which regulates sodium and potassium levels in the body to maintain the levels of systole and diastole, was found to be higher in hypertensive cases, and its levels correlated with systole and diastole in AGA subjects. Due to this, AGA has been proposed as

a marker for hypertension and metabolic syndrome.^{19,20} Contrary to the result by El-Rahman and El-Esawy, we also found that the alopecia group has a significantly higher BMI compared to the non-alopecia group. However, Fortes et al. and Yang et al. report the association between obesity and AGA elucidate that BMI contributes more to the severity of AGA.^{21,22}

Although no association between rs6152 and AGA was found in this study, we found that family history is strongly associated with AGA risks. This study also shows the pattern of inheritance, which can be seen that all the participants whose both parents with AGA are female, 83% of the alopecia group whose mother have family history are female and 82% of the alopecia group whose father have alopecia is male (data not shown). This complex pattern of inheritance shows autosomal dominant inheritance with sex limitation which shows that there's both parents' contribution of AGA, the trait can be inherited from maternal side and paternal side. Nevertheless, this strongly supports that AGA manifestation is multifactorial and inherited.¹ We also want to notice the limitations of this study, which is the small sample size used in this study. This limitation may contribute to the clarity of the inheritance pattern of AGA. However, due to the small frequency of A alleles of rs6152 found in this study, we believed checking rs6152 for AGA was not necessary for risks in Alopecia.

5. Conclusion

This study is the first study on rs6152 SNP in their association with AGA in the Indonesian local populations, which have different ethnicities. No association of rs6152 SNP with AGA was found in this study, and A allele of rs6152 was found only in 2% of participants. Instead, AGA was strongly associated with age, gender, hypertension, status, BMI, and family history. This signifies that AGA in the Indonesian local population was multifactorial and that the AGA risk factor is inherited maternally or paternally.

6. References

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