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Posted Date: 7 August 2023

doi: 10.20944/preprints202308.0463.v1

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Investigations of 13 Genes in Non-Cicatricial Alopecia

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Abstract: *Background*: Androgenetic alopecia (AGA) and alopecia areata (AA) are most common types of non-cicatricial alopecia. Both diseases have limited effective therapeutic options and affect patients' quality of life. Pharmacogenetic tests can help predict the most appropriate treatment option by evaluating the single nucleotide polymorphisms (SNPs) corresponding to genes related to alopecia. *The objective of the study* was to evaluate and compare selected SNPs and genes in AA and AGA patients from Romania and Brazil. *Materials and methods*: We investigated associations between AA and AGA and 45 tag SNPs of 13 genes in 287 Romanian and 882 Brazilian patients. The DNA samples were extracted from saliva using qPCR technique. *Results*: GR-alpha gene, GPR44-2 gene, SULT1A1 gene and CRABP2 gene were statistically significantly different in Brazil compared to Romania. Minoxidil may be recommended in half of the cases of AGA and AA. Patients with AGA and high expression of SRD5A1 or PTGFR-2 may benefit from Dutasteride, respectively Latanoprost treatment. Most of the studied genes showed no differences between the two population. *Conclusion*: The DNA analysis of the patients with alopecia may contribute to a successful treatment.

Keywords: alopecia areata; androgenetic alopecia; genetic test; DNA analysis

1. Background

Androgenetic alopecia (AGA) and alopecia areata (AA) are among the most common types of non-cicatricial alopecia. AGA is characterized by a distinctively recedingfrontal hairline in men (Figure 1) and diffuse hair thinning with retention of the frontal hairline in women. Patient with AGA have a shorter anagen phase of the hair cycle, which will gradually diminish the hair follicle (HF) and cause the development of vellus hairs [1]. AGA's etiology and pathophysiology are not entirely understood; however, genetics [2] and androgens are incriminated [3]. AA is a complex autoimmune disease with a genetic substrate that targets the HF in the anagen phase [4]. It typically presents with round alopecic patches (Figure 2) and can affect both children and adults. AA [5] and AGA [6] evaluation and diagnostic should include a medical history, clinical examination, dermatoscopy, and hair pull test. Additional investigations for AA, such as scalp biopsy, are usually not necessary [5]. The effectiveness of most treatments for AGA and AA is variable. Consequently, utilizing DNA variants may have an impact on treatment strategies.

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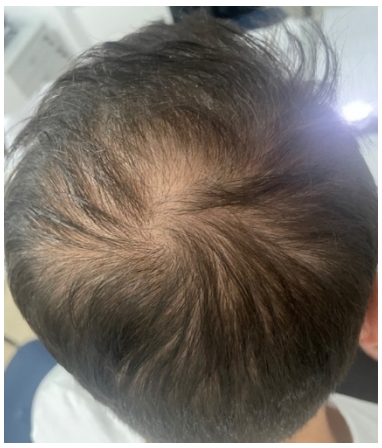

Figure 1. Androgenetic alopecia vertex area. Photographic display with patient consent.

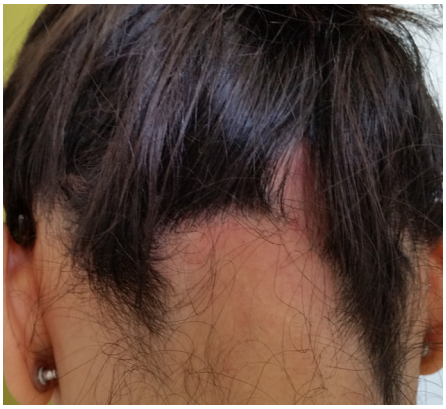

Figure 2. Alopecia areata: round alopecic patches in the occipital area (ophiasis). Photographic display with patient consent.

The current analysis evaluates and compares the associations between non-cicatricial types of alopecia (AGA and AA) and 45 tag single nucleotide polymorphisms (SNPs) and 13 genes identified from previous genome-wide AA and AGA association studies. The evaluated population comprises 287 patients from Romania and 882 from Brazil, clinically diagnosed with AA or AGA.

Treatments of AGA are limited and usually consist of 5 alpha-reductase (finasteride and dutasteride), minoxidil (2% BID or 5% QID) and antiandrogens for woman [6]. Platelet- rich plasma (PRP) and hair transplantation from non-androgenic scalp to alopecic area, are procedures that can be associated [8]. Regarding AA, topical and intralesional corticosteroids remain the first-line treatment. Other topical treatments as minoxidil, prostaglandin F2α analogues, calcineurin inhibitors, and sensitization therapy are also prescribed [5].

2. The Objective of the Study

The study aims to evaluate and compare genetic data (selected SNPs and genes) in patients from Romania and Brazil diagnosed with AA and AGA.

3. Materials and Methods

We investigated associations between AA and AGA and 45 tag single nucleotide polymorphisms (SNPs) of 13 genes in a total of 1169 patients: 287 Romanian patients (group 1) and 882 Brazilian patients (group 2). All the patients of both groups were diagnosed with AA or AGA. The diagnosis of AGA or AA was confirmed by healthcare professionals in private clinics from different cities from Romania and Brazil, based on the evolution of the disease, the clinical aspect and the trichoscopic examination. The DNA samples were extracted from saliva.

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Fagron Genomics (Spain) provided all anonymized data, and all patients signed the informed consent. The qPCR technique determined SNPs using the Taqman Technology on the QuantStudio™ 12K Flex Real-Time PCR System.

The prediction analysis evaluated single-nucleotide polymorphisms (SNPs) identified from previous genome-wide AA and AGA association studies. The evaluated SNPs and the related genes were: rs9282861(SULT1A1 gene), rs523349 (SRD5A2 gene), rs39848 (SRD5A1 gene), rs10782665 (PTGFR-3 gene), rs1328441 (PTGFR-2 gene), rs6686438 (PTGFR-1 gene), rs13283456 (PTGES2 gene), rs2229765 (IGF1R gene), rs6198 (GR-alpha gene), rs533116 (GPR44-2 gene), rs545659 (GPR44-1 gene), rs2470152 (CYP19A1 gene), rs12724719 (CRABP2 gene), rs13078881 (BTD gene), rs4343 (ACE gene). The study was approved by the Bioethics Committee of Colentina Clinical Hospital, Bucharest, Romania (approval no.17/22.12.2022). All the patients included in the study signed an informed consent for genetic research.

Statistical Analysis

Data were collected, analyzed, coded, and introduced in the Statistical Package for Social Science (IBM SPSS, NY, USA) version 23. Quantitative data were presented as a number of patients from each country and percentages of the patients for each SNP in each alopecic disease (AGA and AA), as illustrated in Table 1.

Table 1. Comparation between SNPs/Genotype in patients with androgenetic alopecia and alopecia areata from Romania and Brazil **.**

GENA - SNP/Genotype	Brasil (BR), n = 882)				Romania (RO), n = 287				Chi square test	
	N	Genotype in BR	AGA	AA	N	Genotype in RO	AGA	AA	df	P
GR-alpha- RS6198										
AA	651	73.8%	76.5%	73.6%	189	65.9%	50.0%	67.6%		
GA	208	23.6%	19.6%	23.8%	82	28.6%	46.4%	26.6%	2	0.007
GG	23	2.6%	3.9%	2.5%	16	5.6%	3.6%	5.8%		
CYP19A1- RS2470152										
AA	259	29.4%	29.4%	29.4%	89	31.0%	21.4%	32.0%		
AG	418	47.4%	37.3%	48.0%	137	47.7%	42.9%	48.3%	2	0.749
GG	205	23.2%	33.3%	22.6%	61	21.3%	35.7%	19.7%		
SRD5A1- RS39848										
CC	177	20.1%	25.5%	19.7%	52	18.1%	10.7%	18.9%		
CT	418	47.4%	43.1%	47.7%	131	45.6%	53.6%	44.8%	2	0.485
TT	287	32.5%	31.4%	32.6%	104	36.2%	35.7%	36.3%		
SRD5A2- RS523349										
CC	425	48.2%	37.3%	48.9%	138	48.1%	42.9%	48.6%		
CG	365	41.4%	51.0%	40.8%	118	41.1%	42.9%	40.9%	2	0.984
GG	92	10.4%	11.8%	10.3%	31	10.8%	14.3%	10.4%		
GPR44-1- RS545659										
AA	559	63.4%	68.6%	63.1%	184	64.1%	75.0%	62.9%		
GA	265	30.0%	27.5%	30.2%	93	32.4%	21.4%	33.6%	2	0.137
GG	58	6.6%	3.9%	6.7%	10	3.5%	3.6%	3.5%		
GPR44-2- RS533116										
AA	111	12.6%	11.8%	12.6%	48	16.7%	25.0%	15.8%		
GA	383	43.4%	41.2%	43.6%	134	46.7%	46.4%	46.7%	2	0.048
GG	388	44.0%	47.1%	43.8%	105	36.6%	28.6%	37.5%		
PTGFR-1- RS6686438										
GG	485	55.0%	60.8%	54.6%	146	50.9%	46.4%	51.4%	2	0.477
GT	326	37.0%	27.5%	37.5%	116	40.4%	42.9%	40.2%		

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GENA - SNP/Genotype	Brasil (BR), n = 882				Romania (RO), n = 287				Chi square test	
	N	Genotype in BR	AGA	AA	N	Genotype in RO	AGA	AA	df	P
TT	71	8.0%	11.8%	7.8%	25	8.7%	10.7%	8.5%		
PTGFR-2- RS1328441										
AA	238	27.0%	27.5%	27.0%	60	20.9%	21.4%	20.8%		
GA	423	48.0%	41.2%	48.4%	147	51.2%	60.7%	50.2%	2	0.118
GG	221	25.1%	31.4%	24.7%	80	27.9%	17.9%	29.0%		
PTGFR-3- RS10782665										
GG	356	40.4%	41.2%	40.3%	114	39.7%	32.1%	40.5%		
GT	381	43.2%	33.3%	43.8%	126	43.9%	50.0%	43.2%	2	0.977
TT	145	16.4%	25.5%	15.9%	47	16.4%	17.9%	16.2%		
PTGES2- RS13283456										
CC	619	70.2%	80.4%	69.6%	195	67.9%	71.4%	67.6%		
CT	230	26.1%	17.6%	26.6%	75	26.1%	21.4%	26.6%	2	0.277
TT	33	3.7%	2.0%	3.9%	17	5.9%	7.1%	5.8%		
SULT1A1- RS9282861										
CC	464	52.6%	52.9%	52.6%	152	53.0%	50.0%	53.3%		
TC	408	46.3%	43.1%	46.5%	122	42.5%	46.4%	42.1%	2	0.001
TT	10	1.1%	3.9%	1.0%	13	4.5%	3.6%	4.6%		
CRABP2- RS12724719										
AA	29	3.3%	5.9%	3.1%	2	0.7%	0.0%	0.8%		
AG	278	31.5%	43.1%	30.8%	64	22.3%	17.9%	22.8%	2	<0.001
GG	575	65.2%	51.0%	66.1%	221	77.0%	82.1%	76.4%		
BTD- RS13078881										
CC	2	0.2%	0.0%	0.2%	0	0.0%	0.0%	0.0%		
CG	60	6.8%	13.7%	6.4%	20	7.0%	7.1%	6.9%	2	0.719
GG	820	93.0%	86.3%	93.4%	267	93.0%	92.9%	93.1%		
ACE- RS4343										
AA	213	24.1%	29.4%	23.8%	54	18.8%	25.0%	18.1%		
AG	424	48.1%	33.3%	49.0%	158	55.1%	57.1%	54.8%	2	0.081
GG	245	27.8%	37.3%	27.2%	75	26.1%	17.9%	27.0%		
IGF1R- RS2229765										
AA	149	16.9%	25.5%	16.4%	51	17.8%	17.9%	17.8%		
AG	441	50.0%	33.3%	51.0%	145	50.5%	64.3%	49.0%	2	0.888
GG	292	33.1%	41.2%	32.6%	91	31.7%	17.9%	33.2%		

The Chi-square test was used to provide information about the relationship between the variables. The test was based on the observed frequencies fi, which represents the frequencies of occurrence of the component elements of each SNP and country. The SNP variable's component elements are the country variable's genotypes (Brazil and Romania). Based on the observed frequencies, the theoretical frequency for each observed frequency is determined according to a specific calculation algorithm. Each cell with observed frequencies (fi) corresponds to a cell with theoretical values (fti). Depending on the determined Chi-square value ($\chi 2$) and the degree of freedom (df), the Chi-square test provides the actual significance threshold, denoted by p, which will be compared with the significance threshold considered in statistical research, in our case α=0.05. The decision regarding the association (dependence) or non-association (independence) of the two variables will be taken depending on the comparison between the thresholds p and α.

The tested research hypotheses were the null (H0) and alternative hypothesis (H1). The null hypothesis stands for no association between an SNP variable and its genotypes and the country variable (Brazil and Romania); the variables being independent. The alternative hypothesis (H1)

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refers to an association between an SNP variable and its genotypes and the country variable (Brazil and Romania), the variables being dependent. If p <= 0.05 (α), the null hypothesis is rejected, there is a relationship between the two variables, in the sense that the data (genotypes) of each SNP depend on the country. If p > 0.05 (α), the null hypothesis is accepted, there is no connection between the two variables, in the sense that the genotypes of each SNP do not depend on the country (they are similar).

3. Results

In group 1 (287 Romanian patients), 90.2% of patients were diagnosed with AGA, respectively 9.8% with AA. In group 2 (and 882 Brazilian patients), 94.2% of patients were diagnosed with AGA and 5.8% with AA.

Four SNPs (rs6198, rs533116, rs9282861, rs 2724719) with the related genotypes (GPR44-2, GR-ALPHA, SULT1A1, CRABP2) were statistically significant associated in Brazil and Romania (Table 2). In these cases, the null hypothesis was rejected, the data being significant from one population to the other. The non-statistically significant SNPs are shown in Table 3.

Table 2. Genes and SNPs with significant differences in Brazil and Romania.

GENA	SNP	χ^2	df	P
GR-ALPHA	RS6198	9.794	2	0.007
GPR44-2	RS533116	6.066	2	0.048
SULT1A1	RS9282861	13.368	2	0.001
CRABP2	RS12724719	16.210	2	< 0.001

Table 3. Genes and SNPs with non-significant differences in Brazil and Romania.

GENA	SNP	χ^2	df	P
CYP19A1	RS2470152	0.578	2	0.749
SRD5A1	RS39848	1.446	2	0.485
SRD5A2	RS523349	0.033	2	0.984
GPR44-1	RS545659	3.970	2	0.137
PTGFR-1	RS6686438	1.480	2	0.477
PTGFR-2	RS1328441	4.278	2	0.118
PTGFR-3	RS10782665	0.048	2	0.977
PTGES2	RS13283456	2.566	2	0.277
BTD	RS13078881	0.660	2	0.719
ACE	RS4343	5.031	2	0.081
IGF1R	RS2229765	0.238	2	0.888

The most prevalent genotype (GA) was observed in the following SPNs: rs533116 (GPR44-2 gene), rs2470152 (CYP19A1 gene), rs1328441(PTGFR-2 gene), rs4343 (ACE gene), and rs2229765 (IGF1R gene). Prostaglandin D2 receptor 2 (GPR44 or CRTH2) variants were associated with an increased GPR44 resulting in higher responsiveness to prostaglandin D2 and HF regression. The aromatase gene (CYP19A1) showed low testosterone conversion in estrogens and high conversion into DHT. Prostaglandin F receptor 2 (PTGFR-2) was mainly related to the efficacy of the treatment with prostaglandin analogue, Latanoprost. The angiotensin-converting enzyme (ACE) variants were associated with increased plasma levels of angiotensin in 49.0% of the patients with AA from Brazil and 54.8% from Romania, and insulin-like growth factor-I (IGF-I) variants were associated with lower plasma IGF-1 levels in half of the evaluated patients.

The genotype AA was more frequent for the tag SNP rs6198 (GR-ALPHA gene) and rs545659 (GPR44-1 gene) in both countries. The SNP rs6198 analysis indicates that the glucocorticoid receptor (GR or NR3C1) variants are associated with resistance or sensitivity to corticosteroids. Regarding rs545659 SPN, most subjects have an increased GPR44 leading to higher responsiveness to

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prostaglandin D2 and HF regression. Genotype AA of the GPR44-1 gene was found in 68.6% of the cases from Brazil, respectively 63.1% in AA, 75.0% of AGA cases from Romania, respectively 62.9% of AA cases.

For the tag SPNs rs9282861(SULT1A1), rs523349 (SRD5A2 gene), and rs13283456 (PTGES2 gene), the CC genotype was more prevalent. In our study, the treatment with minoxidil at regular concentration showed good results in more than half of the patients with AGA (52.9% of Brazil and 50.0% in Romania) and AA (52.6% of Brazil and 53.3% of Romania). The SULT1A1 gene can predict a good response to minoxidil treatment.

GG genotype was most frequently associated with the following SPNs rs12724719 (CRABP2 gene), rs13078881 (BTD gene) and rs6686438 (PTGFR-1 gene). SNP analysis does not indicate the necessity to supplement with retinoic acid or vitamin B as the levels were physiological. The genetic testing showed that Latanoprost might not respond positively in 60.8% of AGA in Brazilian patients and 46.4% of Romanian patients.

The gene PTGFR-3 SPN rs10782665 was commonly associated with genotype GT related to Latanoprost treatment efficacy. Regarding the SPN rs39848 (SRD5A1 gene), the more common genotype was CT. The genetic analysis showed that increased SRD5A1 activity leads to increased DHT levels and hair growth inhibition, and treatment with Dutasteride is recommended in 43.1% of Brazilian and 53.6% of Romanian in AGA patients. Figure 3 plots the p-value for each SPN. It is confirmed that the four values greater than 1.30 correspond to the SNPs seen in Table 2. The lowest nominal P-value was observed for the tag SNP RS9282861 gene SULTA1A (P = 0,001).

Regarding the type of alopecia (AA or AGA), the Chi-square test highlighted a significance threshold of p=0.02<0.05. This result shows that AGA and AA are associated with the country variable, meaning that these types significantly differ from country to country, and the null hypothesis is rejected.

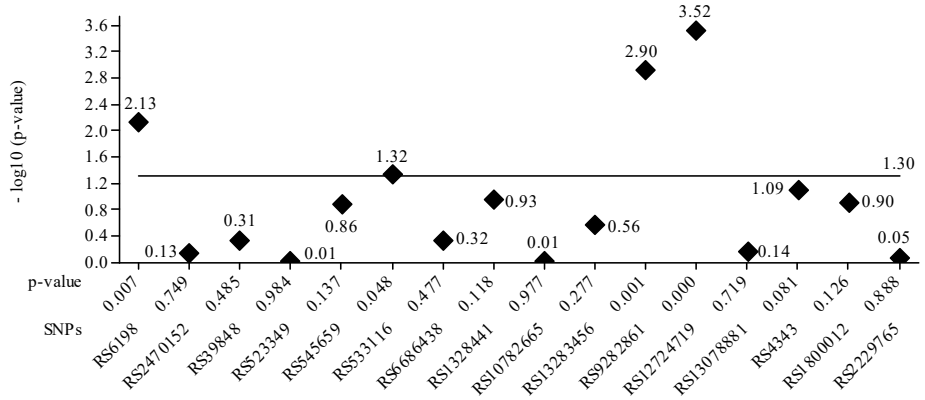

Figure 3. The value of the base 10 logarithm of the p-value is always negative because the p-value takes values between 0 and 1. For this reason, for the representation of positive values on the Oy axis, the value of the logarithm minus (- log10 (p-value)). The significance threshold considered is 0.05 - Log10 0.05 = 1.30, the constant value, represented as a horizontal line. Statistically significant values have p-value <= 0.05. For these, the -log10 values will lie above the line corresponding to p-value = 0.05, e.g., above 1.30. It is confirmed that the four values greater than 1.30 correspond to the SNPs in Table 2.

4. Discussion

The possibility of linkages between SNPs in AA and AGA has only recently been investigated in a small number of Australian [8], European (Polish [5], UK and Germany [9]), Chinese and European [10] and Korean [11] populations. Our study evaluated single-nucleotide polymorphisms (SNPs) identified from previous genome-wide AA and AGA association studies.

 doi:10.20944/preprints202308.0463.v1

4.1. Aromatase Gene (CYP19A1 Gene)

Aromatase inhibitors (AIs), which inhibit the synthesis of estrogens, cause a relative increase in the activity of 5-reductase. Aromatase is known to reduce intrafollicular testosterone [5] and DHT levels [2,6] and to catalyze the conversion of testosterone to estradiol. This increase of the aromatase can determine "pseudo male pattern androgenetic alopecia," which results in male pattern hair loss that mimics female androgenetic alopecia (FAGA) [12]. Aromatase expression varies between alopecic and non-alopecic scalp [13]. According to Yip et al., the aromatase gene (CYP19A1) may predispose women to types of non-scaring alopecia [8]. Estrogens can both prevent and modulate hair growth; hence CYP19A1 plays a crucial protective role in frontal hair lines [8]. Compared to men, women have more significant levels of CYP19A1 in their frontal and occipital follicles [14].

Fifteen menopausal women with hormone receptor-positive breast cancer who received AIs were the subjects of a study by Rossi et al [12]. After receiving AIs therapy for a year, patients began to develop frontotemporal follicle miniaturization that mimicked FAGA. Yip et al. [8] performed a gene-wide study of association between female pattern hair loss (FPHL) and the aromatase gene encoding CYP19A1. The investigators examined 61 CYP19A1 SNPs in the Australian population of 484 Caucasian women with FPHL and 471 controls. The CC genotype of rs4646 was substantially more common in FPHL (58%) than controls (48%), indicating a recessive genetic influence, even though no significant relationship was established at the allelic levels. Even than our study did not analyzed the sex of the patients with AGA, the most prevalent genotype of the CYP19A1 gene (SPN rs2470152) was GA (47.4% in Brazil and 47.7% in Romania), showing a predisposition to reduced CYP19A1 activity.

4.2. Angiotensin-Converting Enzyme (ACE Gene)

The immune-dependent disease known as AA is defined by the interaction of T cells with follicular antigens. Studies have revealed the presence of a local renin-angiotensin system in the skin, where the angiotensin-converting enzyme (ACE) is involved in autoimmunity and causes alopecia due to HF's chronic inflammation [15,16]. A few cases of non-cicatricial alopecia induced by ACE inhibitors have been identified. Lisinopril-induced alopecia in a patient of Kataria et al. [11] and enalapril-induced alopecia in a patient of Ahmad et al. [17] were reported. Hair regrowth was observed after the cessation of the treatment. A patient of Motel et al. received ACE treatment for one month, and after the medication was stopped, hair thinning was observed at a follow-up examination [18].

The serum activity of ACE was assessed in a study by Namazi et al. in 19 patients with AA and 16 healthy control participants. Although the findings were not statistically significant, AA patients had higher serum ACE levels. Moreover, in the patient group, there was no association between ACE activity and the severity or duration of the condition [19]. Fahim et al. found a significant correlation between serum ACE and disease severity despite the lower serum ACE levels in AA patients. Angiotensin I, which may also be involved in the inflammation of AA, is considered to be the cause of ACE consumption and reduced tissue levels of the enzyme [16]. In our study, 49.0% of the patients with AA from Brazil and 54.8% from Romania expressed GA genotype, resulting in a predisposition to an increased angiotensin conversion activity.

4.3. 5-Alpha-Reductase (SRD5A1, SRD5A2 Gene)

Genetics play an essential role in AGA [2]. The role of the 5-alpha-reductase (5AR) enzyme in AGA is implicated in the metabolism of testosterone to dihydrotestosterone (DHT); therefore, the effect of 5AR inhibitors in treating AGA [20]. Finasteride and dutasteride are 5AR inhibitors commonly used in AGA. Dutasteride is an inhibitor of both type I and II 5ARs and is considered to be an effective treatment for AGA [21,22]. The treatment response to dutasteride varies in each individual; however, it is unknown if the genetic factors contribute to these variations [21]. It was also observed that the response to dutasteride might be related to variations of genes involved in the metabolism of steroid hormones, such as SRD5A1. Finasteride inhibits type II 5ARs only, while

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dutasteride inhibits type I and type II 5ARs simultaneously. Accordingly, a difference in SRD5A1 could influence how well an individual responds to dutasteride when treated for AGA [22]. On the other hand, in the Korean population, the analysis of the polymorphisms of SRD5A1 and SRD5A2 genes showed that it might not be directly associated with the development of AGA [23]. Limited data is available regarding other populations. Our analysis showed that Dutasteride at standard doses is recommended in AGA in 43.1% of Brazilian and 53.6% of Romanian patients and Finasteride is recommended in 51.0% of AGA in Brazilian patients, respectively 42.9% of Romanian patients.

4.4. Glucocorticoid Receptor (GR-Alpha Gene)

Glucocorticoid receptor (GR) is known to be expressed in human hair follicles [24]. Early studies showed significant findings on glucocorticoid biochemical pathways. The GR was found to be elevated in AA patients who are "glucocorticoid resistant" and unable to bind glucocorticoids, suppressing cellular transcription [13]. Dexamethasone (DEX) is a synthetic glucocorticoid that plays a role in the inhibition of the proliferation of human hair dermal papilla (DP) cells. DEX is known to also decrease the expression of growth factors required for hair growth [25]. In addition, DEX promotes the catagen phase of the hair cycle [24]. Corticosteroids are known to be a treatment for AA. In our AA cases, tag SPN rs6198 GR-alpha showed that glucocorticoids should be effective in 73.6% of Brazilian and 67.6% of Romanian patients.

4.5. Insulin-like Growth Factor-1 (IGF-1 Gene)

The regulation of insulin-like growth factor-1 (IGF-1) by androgens is well established. IGF-1 increases HF development by controlling cellular proliferation, according to in vitro research. In organ culture, in the absence of IGF-1 or insulin, anagen HF enters the catagen phase [26]. Panchaprateep et al. reported significantly lower levels of IGF-1 and its binding proteins (IGFBP-2, IGFBP-4) in DP cells obtained from alopecic versus non-alopecic scalps [26]. We report similar results, as more than half of the patients showed predisposition to lower levels of IGF-1 levels.

4.6. Prostaglandin D, Prostaglandin E, Prostaglandin F (PTGFR-1, PTGFR-2, PTGFR-3, PTGES2, GPR44-1, GPR44-2 Gene)

One of the representative lipid mediator groups, which includes prostaglandins (PGs), is the prostanoids family. They have a variety of physiological impacts on controlling inflammatory reactions, cell proliferation, and apoptosis. Prostanoids are classified into five types as follows: PGD2, PGE2, PGF2α, PGI2. PGD2 binds to prostaglandin D2 receptors (DP1, DP2) [27], while PGE2 to prostaglandin E2 receptors (EP1, EP2, EP3, EP4) and PGF2α to the prostaglandin F2α receptor (FP) [28].

Colombe et al. investigated the expression patterns of the vital enzymes involved in the metabolism of PGs in human HF and reported that the metabolism of PGD2, PGE2, and PGF2 is expressed in human hair follicles (HF) and might be associated with hair growth and differentiation [29]. Prostaglandin D2 synthase (PTGDS) can be regulated by androgens in order to produce prostaglandin D2 (PGD2) [30]. It was reported that the expression levels of PGD2 in men with male pattern alopecia were higher than in controls [30]. Studies in mice and humans [31,32] have revealed that PGD2 inhibits the regrowth of the HF and hair development, pointing to a role for the DP2 receptor in alopecia. Increased androgen levels may induce PGD2 since androgens have been found to stimulate PTGDS [29]. Kang et al. [29] hypotheses that the expression levels of the DP2 receptor affect hair cycle progression because mRNA is highly expressed in the early catagen phase and strong staining in the outer root sheath, dermal papilla, and hair matrix was seen. They observed that in mice hair growth cycles, DP2 receptor expression is highest when the hair follicle begins to regress. Prostaglandin F2 (PTGF2) and Prostaglandin E2 (PTGE2) act synergistically in hair follicles in order to stimulate hair growth and prolong the anagen phase, which competes with PTGD2. According to a study, PTGES, the enzyme that produces PTGE2, is overexpressed in male patients with early-stage AGA [33].

 doi:10.20944/preprints202308.0463.v1

Villarreal-Villarreal et al. analyzed the expression of the PTGDR2 (GPR44 receptor), PTGDS (Prostaglandin D2 synthase) and PTGES (Prostaglandin E synthase) in 16 patients with AGA and female androgenetic alopecia (FAGA). They discovered no distinctions between the expression of these three genes in men and women; however, men with AGA had overexpressed PTGDS and PTGES relative to controls, and women did not exhibit any changes in these markers [33].

Rafati et al. [32] observed significantly important hair regrowth in AA during the treatment with latanoprost 0.005% solution. In our analysis, PTGFR-2 showed an increased likelihood of having a positive response to Latanoprost in AA in 48.4% (Brazil), respectively 48.4% (Romania), similar to PTGFR-3: 43.8% (Brazil), respectively 50.2% (Romania). In AGA, there was a 33.3%-60.7% range of recommendation for Latanoprost at regular doses.

The aromatase gene showed low conversion of testosterone in estrogens and to high conversion into DHT and Prostaglandin D2 receptor 2 (GPR44-2 or CRTH2) variants were associated with an increased GPR44 mRNA stability leading to higher responsiveness to prostaglandin D2 and HF regression. Regarding the Prostaglandin F receptors (PTGFR), they were mostly related with Latanoprost treatment efficacy known as a prostaglandin analog.

More than 67.9% of the analyzed patients had a predisposition to normal PGE2 levels. The statistical analysis observed a predisposition to normal GPR44 level in 43.4% (PTGFR-1), respectively 63.4% (PTGFR-2) of Brazil patients and 46.7% (PTGFR-1), respectively 64.1% of Romanian patients. Our SNP analysis does not indicate any treatment with prostaglandin D2 inhibitors in patients from both countries. SPN rs545659 showed that most subjects have an increased GPR44, lead to higher responsiveness to prostaglandin D2 and hair follicle regression.

4.7. Cellular Retinoic Acid-Binding Protein 2 (CRABP2 Gene)

Alopecia areata (AA) is an autoimmune disease that affect the hair follicles in the anagen phase. According to Duncan et al., vitamin A regulates the immune response and the hair cycle to slow the extension of AA [34]. Retinol, a form of vitamin A linked to Retinol binding protein 4 (RBP4) and circulates, is converted to retinoic acid by retinal dehydrogenases and transported to the nucleus by CRABP2. Retinol can be esterified or eliminated without or when RBP1/CRBP is saturated [34]. It was observed that RA induces AA, which may be attributed to an array of factors, such as dysregulated immunological function [34]. Genetic studies in mice with AA revealed increased synthesis and retinoic acid (RA) levels in the involved genes. In contrast, RA degradation genes were reduced in AA patients compared to controls. The facts were confirmed by immunohistochemistry in biopsies from patients with AA and both control mice and AA models and RA levels were also elevated in C3H/HeJ mice with AA.

In our analysis, 66.1% of AA patients from Brazil and 76.4% from Romania did not require vitamin A supplementation. In comparison, 30.8% patients with AA from Brazil and 22.8% from Romania are predisposed to reduced retinoic acid transport, and vitamin A would be recommended.

4.8. Sulfotransferase (SULT1A1 Gene)

Recent and ancient studies reported a correlation between SULT1A1 expression in the scalp and the response to minoxidil [34,35]. Topical minoxidil is the only FDA-approved treatment for AGA and most common prescribed topical treatment for female androgenetic alopecia (FAGA) [8]. Minoxidil is a pro-drug that needs to be bio-activated into minoxidil sulfate in order to stimulate hair growth by vasodilatation. Minoxidil sulfotransferase (SULT1A1), catalyzes the reaction in the hair follicle [36]. Individual differences in SULT1A1 expression in the scalp explain the varieties of clinical responses to topical minoxidil [37]. Therefore, dermatologists could achieve long-lasting persistent results of AGA treatment by using the SULT1A1 activity assay [34]. The SULT1A1 gene analysis showed that Minoxidil at regular concentrations would be highly recommended in 52.9% of AGA, 52.6% of AA in Brazil and 50% of AGA, and 53.3% of AA cases from Romania.

 doi:10.20944/preprints202308.0463.v1

4.9. Biotinidase (BTD Gene)

Biotinidase deficiency (BTD) can be categorized as primary and secondary. Primary BTD is an autosomal recessive inherited neuro-cutaneous disorder [38] while a commonly cause of acquired biotin deficiency associated with raw egg consumption. Moreover, patients treated with anticonvulsants (valproic acid), isotretinoin, or antibiotics disrupting normal gut flora may develop a biotin deficiency [39].

Acording to Georgala et al. [40], biotin (vitamin B7) might be involved in the development of AA. They reported hair regrowth in 12 of 19 patients with AA after biotin supplementation [40]. Our SNP analysis does not indicate the necessity to supplement with vitamin B in more than 90% of the cases of AA and AGA from both countries.

Our study had several limitations. First more data are needed regarding the severity, timing, personal and family history, and therapeutic options for the disease. Second, the study's population was entirely Brazilian or Romanian, which may have limited the results' applicability to a broader community. As well, the number of the studied groups of patients was unequal and the sample size can influence the research results.

5. Conclusions

The SULT1A1 gene was expressed in more than half of the patients with AA and AGA from both groups, but higher in the Romanian population than the Brazilian one. Higher responsiveness to prostaglandin D2 and hair follicle regression due to an increased GPR44 mRNA stability was found in half of the Romanian subjects with AGA, respectively three-quarters of Brazilians. Most of the studied genes showed no differences between the two populations, suggesting a common genetic background. Furthermore, the angiotensin-converting enzyme (ACE) gene variants were associated with increased plasma levels of angiotensin and insulin-like growth factor-I (IGF-I) variants were associated with lower plasma IGF-1 levels in half of the studied patients. Additionally, our analysis does not indicate the necessity to supplement with vitamin B in most of the cases of AA and AGA from both groups.

Individual genes frequently provide only a small contribution to polygenic conditions, but the identification of essential genes may reveal new and significant treatment targets. Our research indicates that AGA and AA are likely to become predicted from DNA with sufficient accuracy to support common practical uses. Further and extensive studies are necessary.

Author Contributions: Conceptualization, M.P., G.T.; methodology, M.P. and G.T.; validation, G.S.T., M.P. and V.M.C.; writing—original draft preparation, M.P. and G.S.T.; writing—review and editing, M.P. and G.S.T and V.M.C.; visualization, G.S.T and V.M.C.; supervision, G.S.T and V.M.C.; English translate and supervision, G.S.T. and M.P.; pictures and graphics, M.P. All authors have read and agreed to the published version of the manuscript.

Funding: None.

Institutional Review Board Statement: Approval for the study was obtained from the Bioethics Committee of Colentina Clinical Hospital, Bucharest, Romania, no.17/22.12.2022.

Informed Consent Statement: The informed consent was obtained for all participants. .

Data Availability Statement: Available on request.

Acknowledgments: We gratefully acknowledge Fagron Lab for supporting and analyzing the data collection. We also thank all the subjects who participated in the study. This article is part of the Ph.D. thesis of Dr. Monica Paun.

Conflicts of Interest: Dr. Paun received consulting fees from Lóreal group with no influence of the representation or interpretation of the reported research results. Prof. Dr. Tiplica received consulting fees from Genesis Biopharm Romania, Leo Pharma Romania and Antibiotice Romania. Other authors have no conflicts of interest.

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