"CAROL DAVILA" UNIVERSITY OF MEDICINE AND PHARMACY, BUCHAREST
DOCTORAL SCHOOL
MEDICINE

PHD THESIS SUMMARY

PhD supervisor:
PROF. UNIV. DR. GEORGE SORIN ȚIPLICA

PhD student:
DR. MONICA POPESCU (căs. PĂUN)

2024

"CAROL DAVILA" UNIVERSITY OF MEDICINE AND PHARMACY, BUCHAREST
DOCTORAL SCHOOL
MEDICINE

ALOPECIA AREATA: COMORBIDITIES AND THE IMPACT OF GENETIC STUDY ON THE TREATMENT

PHD THESIS SUMMARY

PhD supervisor:
PROF. UNIV. DR. GEORGE SORIN ȚIPLICA

PhD student:
DR. MONICA POPESCU (căs. PĂUN)

2024

Table of contents

Acknowledgements

The doctorate thesis was supervised by Professor Doctor George Sorin Țiplica, to whom I am incredibly appreciative of his meticulous guidance, professionalism, encouragement, and confidence. Respect and special appreciation to Professor Doctor Carmen Maria Sălăvăstru's kind suggestions, help and ideas. A special thanks to Mr. Doctor Gustavo Torres from the "Human Genome and Stem Cell" Research Center at the University of São Paulo, Brazil, for his help and support with the genetic study. At the same time, I would like to express my gratitude to the Doctoral Committee, which consists of Professor Simona Georgescu, Professor Doctor Sanda Popescu and Professor Doctor Alexandru Ulici who supported and encouraged me during the annual presentations of the Doctorate. I also thank all the participating patients from the Doctoral Studies.

Last but not least, I thank to my parents: Zhou Cun Cun and Popescu Dănut, to Doctor Luminița Stănciulescu and my family who supported, encouraged and guided me to complete and defend my Doctoral Thesis.

I. GENERAL PART

Alopecia areata (AA) is a complex autoimmune disease that affects the hair follicle and, less commonly, the retinal pigment epithelium. AA is a form of non-scarring alopecia encountered in dermatological practice. Clinically, it is characterized by the sudden appearance of round-oval alopecia plaques, most commonly located on the scalp. The population affected by AA is mainly represented by children, adolescents, young adults and it can have a negative impact on the quality of life of patients [1].

From a therapeutic point of view, various options have been described, without any curative or preventive effect. Treatment of the extensive forms of AA can be frustrating for both physician and patient due to poor therapeutic response and increased risk of disease relapse [1]. For severe cases of AA, there is currently only one medication that has been approved by the US Food and Drug Administration (FDA): baricitinib. In addition, therapeutic efficacy is difficult to estimate in the context of an incompletely elucidated pathophysiological mechanism.

A genetic component for AA has been shown by observational studies, which also showed a higher incidence in twins and first-degree relatives [2], indicating the importance of family history as a risk factor. Siblings, parents, and children of AA patients had estimated lifetime risks of 7.1%, 7.8%, and 5.7% to develop the disease, whereas the projected lifetime risk for the general population is 2% [2]. It is difficult to predict a pattern in the prevalence and development of AA in patients with a family history, indicating that other elements, such as lifestyle or environmental factors, may be required for the onset of AA [3]. It has been noted that 24.4% of family members may exhibit signs of AA [4].

At the same time, it has been proven that AA is a complex polygenic disease with hundreds of single nucleotide polymorphisms (SNPs). SNPs are used to determine a person's genetic susceptibility to developing a disease. Numerous of these polymorphisms are located in genomic parts that influence immune system phenotypes, such as antigen presentation, cytokine production, Treg and cytotoxic T cell activation and activity [5].

Genetic tests in non-cicatricial alopecia

An article published in the Journal of Investigative Dermatology in 2010, reported that individuals with AA had higher frequencies of specific single-nucleotide polymorphisms (SNPs) in their genes compared with the unaffected patients. Recently published studies have investigated the possibility of linkage between single nucleotide polymorphisms (SNPs) in a small number of subjects from the Australian [6], European (Poland [7], UK and Germany [8]), Chinese [9] and Korean [10] populations. They concluded that SPNs may contribute to the development and progression of non-scarring alopecia.

Single nucleotide polymorphisms are the most common type of genetic variation in the DNA sequence. The DNA sequence consists of a chain of four nucleotide bases such as A (Adenine), T (Thymine), C (Cytosine) or G (Guanine). SNPs can serve as biological markers in the search for genes linked to the disease [11].

TrichoTest (Fagron Laboratories, Spain) is a genetic test that analyzes 15 genes and 45 genetic variations (SNPs), the most significant that are linked to the alopecia disorders. The genetic test is based on DNA microarray technology. Being a non-invasive test, it is collected from the subject's oral mucosa. Although a particular SNP may not determine a particular condition, some SNPs may be associated with certain diseases. Trichotest evaluates SNPs of the genes associated with non-scarring alopecia [6,7,9,10,12].

Comorbidities associated with alopecia areata

Over time, alopecia areata (AA) was only thought to be an aesthetic disorder. Studies conducted recently have suggested that AA may increase the chance of developing metabolic and inflammatory comorbidities. Hyperlipidemia (19.8% vs. 6.6%), obesity (18.1% vs. 3.0%), diabetes (11.4% vs. 7.4%), and metabolic syndrome (1.4% vs. 0.3%) were all often associated to AA in research by Conic et al. with healthy controls [13].

Additionally, AA has been linked to other autoimmune conditions such vitiligo, rheumatoid arthritis, systemic lupus erythematosus, psoriasis, and Hashimoto's autoimmune thyroiditis. Positive thyroid autoantibodies (anti-thyroglobulin Antibodies and anti-thyroperoxidase Antibodies) increase the risk of a thyroid dysfunction in patients with AA [7]. Numerous retrospective studies, systematic reviews, and genetic research have indicated the possibility of shared molecular pathways between AA and its comorbidities [15,16].

Moreover, it has been noted that AA patients have higher rates of atopic dermatitis, allergic rhinitis, allergic conjunctivitis, and bronchial asthma, as well as an increase in atopic diathesis [17, 18]. Since atopic patients are known to be more likely to develop AA, the relationship is bidirectional [19]. Dust mite allergy was linked to the severity of AA, according to Zhang et al. [20]. Other research revealed that antihistamine therapy or desensitization to home dust mites could lessen the severity of alopecia in atopic patients with AA [21, 22].

Alopecia areata and vitiligo: sister diseases?

Alopecia areata (AA) and vitiligo co-occurring is a significant correlation for the present study. The HLA, IL2RA, and CTLA4 alleles directly overlap with the genes linked to vitiligo and AA, but they may also be shared by other autoimmune diseases [23]. Gill et al. [24] identified correlations between vitiligo and AA and proposed a potential shared pathophysiology for the two conditions. Melanocytes are thought to be the target of immunological attack in AA as well as the destruction caused by the immune system in vitiligo [25]. The hair follicle bulb is the source of hair follicle regeneration in both conditions; in vitiligo, it replenishes the depigmented epidermis with melanocytes, and in AA, it promotes the growth of new hairs [26]. Coordinated communication between keratinocytes and melanocytes is thought to control the regeneration process in both situations [26].

Autoimmune conditions associated with vitiligo (autoimmune thyroiditis, psoriasis, diabetes) have been observed to have higher rates among first-degree relatives, in spite of whether they suffered from vitiligo or not [27].

Furthermore, it has been documented that AA mostly affects pigmented hairs and hypopigmented hairs remains unaffected by the disease. Interesting, the regrown hairs in AA are frequently hypopigmented. In vitiligo lesions, melanocytes can be present at both levels of the infundibular and epidermis of the hair follicle. Nonetheless, the hair follicle bulb contains melanocyte precursors that can be stimulated by phototherapy to multiply, migrate, and differentiate [26, 28].

II. PERSONAL CONTRIBUTIONS

1. Hypothesis, general purpose and objectives

The general purpose of the Thesis is to analyze the results of the genetic testing of patients with AA and androgenetic alopecia (AGA) (used to obtain predictable therapeutic results) in Romanian and Brazilian patients and to evaluate AA comorbidities; vitiligo being the most important comorbidity for the present study. Within the Thesis, two Doctoral Studies presenting 12 objectives are presented.

The main objectives of the first Doctoral Study are to evaluate, analyze and compare the results of the genetic testing of 15 genes in patients clinically diagnosed with AA or androgenetic alopecia (AGA) from Romania and Brazil. The purpose of genetic testing is to identify the suitable treatment to the type of alopecia in order to achieve consistent outcomes.

In order to prove that vitiligo and AA are sister diseases, the second Doctoral Study's primary goals are to compare the sociodemographic, medical, and quality of life characteristics of Romanian patients with these two conditions. Two clinical instances are also included in this study: one involves a patient with vitiligo and AA (an uncommon coexistence), and the other has an AA patient who exhibits hypopigmented hairs that are untouched by the disease, which may provide evidence for anti-melanocyte response hair follicles in AA.

The studies were approved by the Ethics Committee of the Colentina Clinical Hospital, of Bucharest, Romania (approval number: 17/22.12.2022 for the genetic study and 18/22.12.2022 for the study of alopecia areata and vitiligo: sister diseases). For the genetic study, each patient signed an Informed Consent.

2. General research methodology

In order to respond to the purpose of the Doctoral Thesis, I conducted two studies with the objective of achieving the broad objectives specified in the paper's sub-chapter " Hypothesis, general purpose and objectives ".

The first Doctoral Study, entitled "Investigation of 15 genes in non-scarring alopecia", allowed the genetic analysis of the patient’s data with AA and AGA from Brazil and Romania who performed the genetic test used to obtain a predictable therapeutic response. The research

assessed the genetic information of 1169 individuals: 287 patients from Romania and 882 patients from Brazil. The genetic test examines the SNPs, genes, and genotypes most frequently linked to non-scarring alopecia for each patient. The project was carried out in collaboration with Dr. Gustavo Torres from the Human Genome and Stem Cell Research Department of the University of São Paulo, Brazil and the Fagron Laboratory in Spain. In the study, the objectives of analyzing and comparing genes and genotypes in patients with AA and AGA from Romania and Brazil were achieved.

In the second PhD study, "Contributions regarding the comorbidities of alopecia areata and the analysis of similarities with vitiligo," similarities between Romanian patients with AA and vitiligo in terms of socio-demographic, medical histories, and quality of life were assessed using an exploratory questionnaire. Furthermore, two unique case reports—one in which AA also had vitiligo and the other in which AA had hypopigmented hairs—were chosen from the extensive case study and included in the doctoral thesis.

3. Contributions regarding the investigation of 15 genes in non-scarring alopecia

3.1. Introduction (hypothesis and specific objectives)

Androgenetic alopecia (AGA) and alopecia areata (AA) are the most common types of non-scarring alopecia. AA is a complex autoimmune disease with a genetic substrate that targets the hair follicle in the growing anagen phase [23]. It clinically presents as round alopecia plaques and most commonly affects children and adults. Diffuse alopecia, mainly at the vertex, and retraction of the frontal hairline are the hallmarks of AGA. Patients with AGA have a shorter anagen phase of the follicular cycle, which will lead to a progressive reduction in hair length and diameter, ultimately resulting in vellus-type hairs [29]. Although the exact cause and pathogenesis of AGA remain unknown, genetic [30] and hormonal (androgen hormone) factors have been incriminated [31].

In order to evaluate and diagnose AGA and AA [7], a medical history, clinical examination, dermoscopy, and pull test should be performed. For AA, further tests such scalp biopsies are typically not required [7].

The relationships between the non-scarring alopecia types (AGA and AA) and the 45 single nucleotide polymorphisms (SNP) and 15 genes found in earlier research and linked to the alopecia types described are examined, assessed, and contrasted within the Thesis. Clinically diagnosed patients with AA or AGA from Brazil and Romania were included in the analyzed population.

The objectives of the first Doctoral Study are the following:
Objective number **A1**: Analysis and comparison of 15 genes and 45 genotypes in AA and AGA patients from Romania and Brazil.
Objective number **A2**: Comparison of treatment response in AA and AGA patients from Romania and Brazil, based on genetic results.
Objective number **A3**: Identification of the most frequent genotypes involved in AA and AGA in Romanian and Brazilian patients.
Objective number **A4**: Estimation of the prevalence of alopecia conditions (AA and AGA) of the analyzed patients.

3.2. Materials and methods

We performed a retrospective study on the associations between AA and AGA and 45 single nucleotide polymorphisms (SNPs) of 15 genes for a total of 1169 patients: 287 Romanian patients (group 1) and 882 Brazilian patients (group 2). Both groups were diagnosed with AA or AGA. The diagnosis of AGA or AA was confirmed by doctors from Romania and Brazil, based on the evolution of the disease, the clinical appearance and the dermatoscopic examination. DNA samples were collected from the oral mucosa using a swab. All genotyping assays were performed by quantitative polymerase chain reaction (qPCR).

The evaluated SNPs and associated genes were: rs9282861 (SULT1A1 gene), rs523349 (SRD5A2 gene), rs39848 (SRD5A1 gene), rs10782665 (PTGFR-3 gene), rs1328446 (SRD5A2 gene), rs132844846 (PTGFR-4 gene) gene), rs13283456 (PTGES2 gene), rs2229765 (IGF1R gene), rs6198 (GR-alpha gene), rs533116 (GPR44-2 gene), rs545659 (GPR44-1 gene), (GPR44-1 gene), (rs2471521) gene CRABP2), rs13078881 (BTD gene), rs4343 (ACE gene).
Each genetic test for alopecia displays the subject's genotype and gene and the predictability of an effective treatment (e.g.: the AA genotype of the GR-alpha gene is linked to a predisposition to normal sensitivity to topical glucocorticoids, and therefore glucocorticoids would be an effective treatment).

3.3. Results

Analyzing the genetic data of patients with AA and AGA allowed the identification and comparison of the most frequent genotypes of the Romanian and Brazilian population. Partial

results were published in the journal Medicine (MDPI) 2023 (DOI: https://doi.org/10.3390/medicina59091654). They were also presented at the Qvo Vadis National Congress? Classic and Modern, November 10-12, 2023, in Bucharest, Romania.

Genetic data were collected, analyzed, coded and entered into the Statistical Package for Social Science (IBM SPSS, NY, USA) version 23. When evaluating categorical variables (nominal or ordinal), the Chi-square test ($\chi 2$) is used to determine the association between two variables. The test is based on the observed frequencies *fi*, which represent the frequencies of occurrence of the component elements of the two variables, in our case the SNP, respectively the Country. The genotypes for Brazil and Romania, the country variables, are the component parts for the SNP variable. Based on the observed frequencies, theoretical frequencies for each observed frequency are determined according to a certain calculation algorithm. It follows that each cell with observed frequencies (*fi*) corresponds to a cell with theoretical values (*fti*).

Depending on the determined $\chi 2$ value and the number of degrees 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 $\alpha=0.05$.

The decision regarding the association (dependence) or non-association (independence) of the two variables will be taken depending on the result of the comparison between the thresholds *p* and α.

Research hypotheses:

• Null hypothesis (H0): Due to the variables' independence, there is no correlation between an SNP variable and its genotypes and the COUNTRY variable (Brazil and Romania).

• Alternative hypothesis (H1): The genotypes of an SNP variable and the COUNTRY variable (Brazil and Romania) are associated; both variables are reliant on each other.

The decision:

1. If $p <= 0.05$ (α), the null hypothesis is rejected because there is a relationship between the two variables; the genotypes of an SNP vary depending on the country.
2. If $p > 0.05$ (α), the null hypothesis is accepted and it is stated that there is no relationship between the two variables; the genotypes of an SNP vary do not depend on the country (they are similar).

The Chi-square test establishes whether or not two variables—in this case, the country (Brazil, Romania) and the SNP (SNPs with particular genotypes) have a link.

The SNPs for which the value of the significance threshold $p < 0.05$ of the study are represented by GR-ALPHA, GPR44-2, SULT1A1 and CRABP2 are dependent on the country (they are significantly different in Brazil compared to Romania). GR-ALPHA, GPR44-2, SULT1A1 and CRABP2 are genes associated with resistance or sensitivity to corticosteroids, respectively prostaglandin D2, minoxidil and retinoic acid (vitamin A).

3.3.1 Predominant genotype according to SNP

The AA genotype was the most frequent in the following genes, respectively SNP: GR-alpha-RS6198, GPR44-1-RS545659 and the AG genotype in CYP19A1- RS2470152, PTGFR-2-RS1328441, ACE-RS4343 and IGF1R- RS2229765. At the same time, CC genotypes were found more frequently in the gene, respectively SNP: SRD5A2- RS523349, PTGES2-RS13283456 and SULT1A1- RS9282861 and GG in CRABP2- RS12724719, BTD-RS13078881, GPR44-2- RS533116 and PTGFR-1- RS6686438 and for the gene SRD5A1, respectively PTGFR-3, the predominant genotypes were represented by CT, respectively GT. Furthermore, the statistical analysis revealed a higher prevalence of AA in Romania, 9.8% compared to Brazil, 5.8%. The cases of AGA were the majority, respectively 94.2% in Brazil and 90.2% in Romania.

The percentage distributions for each patient's gene are graphically displayed in the Doctoral Thesis.

3.3.2 Principal Component Analysis (PCA) – Variant AA and AGA (Romania and Brazil)

Principal component analysis (PCA) is a statistical technique used by condensing dimensions to a few principal components (PCs) that best describe the main patterns [32]. In the two analyzed cases (AA and AGA), I observed the same main components for the following SNPs: rs6198, rs2470152, rs39848, rs523349, rs545659, rs533116 and rs6686438. The mentioned SNPs correspond to the following genes: GR-alpha, CYP19A1, SRD5A1, SRD5A2, GPR44-1, GPR44-2, PTGFR-1. GR-alpha glucocorticoid receptor variants are associated with corticosteroid resistance or sensitivity, aromatase (CYP19A1) variants with low conversion of testosterone to estrogens and high conversion to dihydrotestosterone (DHT), which inhibits hair follicle growth. SRD5A1 and SRD5A2 gene variants are associated with increased activity of their activity, leading to increased levels of DHT, which can cause alopecia. Prostaglandin

D2 receptor 2 (GPR44 or CRTH2) variants are associated with increased stability of GPR44 mRNA, which causes an increased response to prostaglandin D2 and induces hair follicle regression. Last but not least, prostaglandin F receptor (PTGFR) variants are related to the effectiveness of Latanoprost (prostaglandin analog) treatment.

3.4. Discussions

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 rs2470152 (CYP19A1 gene). Prostaglandin D2 (PGD2) is known to has two receptors: GPR44 and PTGDR [33]. PDG2 receptor 2 (GPR44-2) variants have been associated with an increase in GPR44, resulting in greater responsiveness to decreased prostaglandin D2 levels, which causes reduction and miniaturization of hair follicles [34]. In the Doctoral Study, two variants of GPR44 were evaluated:

• rs533116 - associated with a higher expression of PGD2 receptors when the patient has the AA genotype [35,36].

• rs545659 - associated with increased GPR44 mRNA stability when the GG genotype is present [35,37].

The GPR44 genetic variations analyzed in the test allow the identification of a concentration and activity of GPR44 receptors. Given that PGD2 inhibitors, such as prostaquinone and thymoquinone, function by preventing PGD2 synthesis, the genetic test provides insight into the treatment's possible efficacy. In our study, PGD2 were recommended in 47.1% of AGA cases in Brazil, respectively 28.6% in Romania and 43.8% in Brazil and 37.5% in Romania for AA cases. Rossi et al. al [38] performed a double-blind study where ten patients applied topical treatment with thymoquinone, a natural PGD2 inhibitor, and reported an improvement in hair density and thickness after 3 months of treatment. Conversely, a study in men with AGA treated with setipiprant, a synthetic PGD2 inhibitor showed that the medication was well tolerated but did not demonstrate statistically significant differences in efficacy compared to placebo [39]. Regarding the rs545659 SPN analyzed in the doctoral study, most subjects showed increased GPR44 that induces hair follicle regression.

In non-scarring alopecias, the aromatase gene (CYP19A1) shows decreased conversion of testosterone to estrogens and increased to DHT. Estrogen receptors have been identified in hair follicles [40] and estradiol can influence hair follicle cycle and growth by binding to high-affinity estrogen receptors that are locally expressed [41]. Our study showed that the

application of 17-α estradiol (aromatase inducer) is recommended in 37.3% of cases in Brazil with AGA and 42.9% in Romania. On the other hand, Choe et al. conducted a study on 69 subjects with AGA of which a very high percentage (92.7%) responded favorably to 0.025% topical 17α-estradiol [42]. Surprisingly, 17-α estradiol showed a better therapeutic response in AA than in AGA (>48.0% in both groups). Plasma estradiol within normal limits can increase the activity of the hypothalamic-pituitary axis, but it can also inhibit the response under stress conditions, usually present in AA [43]. Zhang et al. found that there was a positive correlation between plasma estradiol levels in control mice, which was not present in AA mice. Studies on mice with AA showed the presence of an aberrant stress response with effects at the level of the hypothalamic-pituitary-gonadal axis [43], a fact that may also explain the increased efficacy of topical 17α-estradiol in our study.

The PTGFR (prostaglandin F receptor) gene mediates the behavior of the main cellular receptor of PGF2α and is expressed in dermal papillae [44,45]. A series of genetic variants in PGTFR that are linked to either a positive or poor therapeutic response to latanoprost are analyzed by the genetic test [46]:

• rs6686438 (PGTFR-1) and SNP rs10782665 (PGTFR-3): T allele variants are related to increased efficacy of latanoprost [46]. If the G allele is present, there is a higher probability of not achieving a favorable response to latanoprost [46].

• SNP rs1328441 (PGTFR-2): the G allele variant is associated with increased efficacy in latanoprost treatment [46]. If the A allele is present, there is an increased probability of not achieving a response to latanoprost [46].

Latanoprost is a synthetic analog of prostaglandin F2α that has a high capacity to stimulate DNA synthesis in certain cell types and to initiate the mitogenic response [47]. Blume Peytavi et al. reported that latanoprost stimulates the anagen as well as the telogen phases of hair growth and induces the appearance of new hairs in the growth phase [48].

Regarding the PTGFR-1 gene, the response to latanoprost was beneficial in AGA in more than half of the patients from Brazil (60.8%) and lower in those from Romania (46.4%). Interestingly, more than 50% of patients with AA obtained a good prediction of therapeutic response to latanoprost (GG genotype). Considering that prostaglandin F receptor 2 (PTGFR-2) has been mainly associated with the effectiveness of treatment with prostaglandin analogues such as latanoprost, following analysis of the PTGFR-2 gene in the Doctoral Study, it was observed that the therapeutic response was significantly better in Romanian patients compared to Brazilian ones, both for AGA and AA (GA genotype). Last but not least, the PTGFR-3 gene of the SPN rs10782665 was associated with the efficacy of latanoprost treatment with the better

GT genotype in patients with AGA from Romania (50.0%) compared to Brazil (33.3%). Latanoprost has been used mostly for forms of AA with gene modification, yet clinical investigations have shown a range of findings, including: efficacy at the gene level in 45% of AA patients after applying latanoprost solution 0.005% [49] or no therapeutic response [50]. In AGA, latanoprost achieved favorable results [48,51].

The function of the angiotensin-converting enzyme (ACE) is to convert the inactive form of angiotensin I into the active form, angiotensin II, which has a vasoconstrictor function [52]. The ACE gene manifests in two different ways when the genetic code is altered: ACE I and ACE D, corresponding to an insertion (I) or deletion (D) of a short DNA region within the gene [53]. ACE activity is typically lower in patients with the I allele than in patients with the D allele [53,54]. The risk allele G acts as a marker for patients carrying the D allele [54]. Patients carrying the G allele variation G2328A have elevated ACE levels, leading to increased conversion of angiotensin I to angiotensin II and thus increasing vasoconstriction [55]. Angiotensin I appear to be involved in AA inflammation and is thought to be the cause of ACE consumption and reduced tissue levels of the enzyme [56]. In the Doctoral Study, the genetic test analyzes the genetic variation of the SNP rs4343, corresponding to the ACE gene, and they were associated with increased plasma level of angiotensin in 49.0% of patients with AA from Brazil and 54.8% from Romania. Angiotensin I-induced vasoconstriction can be ameliorated by the application of minoxidil, which induces local vasodilatation.

Insulin-like growth factor I (IGF-1) has been shown to affect follicular proliferation and the hair growth cycle as well as follicular differentiation. One study reported that three patients have alopecia due to a primary cause (IGF-1 deficiency) or secondary to pituitary surgery [57]. Interestingly, in another study involving 9 patients with AGA, an increased expression of IGF-1 messenger RNA levels in the dermal papilla was associated with a favorable therapeutic response of patients to finasteride. Cefaranthine is a substance that stimulates follicular growth by increasing the production of IGF-1 [58,59]. The genetic test analyzes the presence of SNP rs2229765 in the IGF1R gene and it has been observed that patients carrying an A allele in this variation have lower plasma IGF-1 levels [60]. The Doctoral study showed that IGF-I variants were associated with lower plasma levels of IGF-1 in half of the evaluated patients, indicating the possibility of treatment with topical cefarantine, both in patients with AGA and AA.

AA genotype was more frequent for SNPs rs6198 (GR-alpha gene) and rs545659 (GPR44-1 gene) in both countries. Analysis of the rs6198 SNP indicates that variants of the glucocorticoid receptor (GR or NR3C1) are associated with corticosteroid resistance or sensitivity [61,62]. GR includes two isoforms: GRα and GRβ. [63]. GRα is the classical GR isoform that mediates

the actions of glucocorticoids, while GRβ is known to increase resistance to the pharmacological effects of corticosteroids [63]. The A3669G (rs6198) polymorphism of the GR gene encodes the GRβ isoform, being a contributing factor to corticosteroid resistance. The genetic test used in the Doctoral Study analyzes the presence of the rs6198 polymorphism, a variation with a global prevalence between 20% and 40% in the general population [64]. The presence of this polymorphism in the patient is likely to result in decreased glucocorticoid treatment efficacy because of GRβ expression. In the case of AA, the analysis showed that 73.6% of Brazilian patients, respectively 67.6% of Romanian patients, show sensitivity to topical corticotherapy. The AA genotype of the GPR44-1 gene was present in 68.6% of Brazilian cases, respectively 63.1% of AA cases, 75.0% of Romanian AGA cases and 62.9% of AA cases. A less commonly prescribed treatment is prostaglandin D2 inhibitors (Cetirizine and/or Prostaquinone); and in the current study, the genetic test recommends PGD2 inhibitors in more than half of AA cases in both countries.

For SPNs rs9282861(SULT1A1), rs523349 (SRD5A2 gene) and rs13283456 (PTGES2 gene), the CC genotype was more common. The SULT1A1 gene encodes the sulfotransferase that has the role of activating minoxidil. In the Doctoral Study, minoxidil treatment showed a prediction of a good therapeutic outcome in more than half of patients with AGA (52.9% in Brazil and 50.0% in Romania) and AA (52.6% in Brazil and 53.3% in Romania). Surprisingly, patients with AGA showed a favorable response to finasteride treatment in only 37.3% of cases in Brazil, respectively 48.1% in Romania. Interestingly, in AA, the therapeutic response to finasteride was higher than in AGA, 48.9% in Brazil, respectively 42.9% in Romania. 67.6% - 80.4% of patients with AGA and AA had normal PGES2 level. Minoxidil has the ability to increase PGE2 levels in individuals with prostaglandin deficiency [65].

The GG genotype was most frequently associated with the following SPNs: rs12724719 (CRABP2 gene), rs13078881 (BTD gene), and rs6686438 (PTGFR-1 gene). Retinoic acid can be prescribed in addition to minoxidil treatment in alopecia, in order to achieve a more potent therapeutic outcome. Cellular retinoic acid-binding protein 2 (CRABP2) is a cytoplasmic binding protein, encoded by the CRABP2 gene, with a role in the transport of retinoic acid to its intracellular receptors [66,67]. The CRABP2 gene variation rs12724719 is linked to increased blood retinoic acid levels because it reduces intracellular transport, which reduces the effectiveness of retinoic acid therapy [66]. The genetic test analyzes the presence of the homozygous A allele associated with reduced retinoic acid transporter CRABP2 [66]. Our genetic analysis revealed that retinoic acid supplementation was not necessary in 66.1% of AA cases from Brazil and Romania, as a result of the physiological levels.

Primary or secondary biotinidase may be a rare cause of non-scarring alopecia [68,69]. The BTD gene encoding biotinidase and the SNP rs13078881 corresponding to the BTD gene are analyzed in the Doctoral Study. The presence of the C allele is related to biotinidase deficiency. Genetic analysis did not indicate the need for biotin supplementation in 93% of cases from Romania and Brazil, which follows the findings of research by Georgala et al.[69] and Patel et al. [68] that have been published in the literature.

Regarding SPN rs39848 (SRD5A1 gene), the more common genotype was CT. The genetic study demonstrated that elevated DHT levels and hair growth inhibition is caused by increased SRD5A1 activity. Due to the fact that testosterone is converted to DHT by the action of 5α-reductase [70], finasteride and dutasteride (5α-reductase inhibitors) are frequently treatments used in AGA. A meta-analysis showed that dutasteride appears to be more effective than finasteride for the treatment of AGA [71]. The Doctoral Study's statistics, which are consistent with the meta-analysis, indicate that finasteride is indicated for only 37.3% of Brazilian and 48.1% of Romanian AGA patients, whereas dutasteride is advised for 43.1% of Brazilians and 53.6% of Romanians. Ghassemi et al. studied the effects of gene polymorphism and response to finasteride therapy in men with hereditary androgenetic alopecia and concluded that gene polymorphism and inheritance pattern play an important role in determining a treatment plan, dose, and duration of therapy [72].

3.4.1. Limitations of the Doctoral Study number 1

Our study had several limitations. First, more data are needed regarding the severity, onset, personal and family history, and the followed treatment. Second, the study population was from Brazil and Romania, which may have limited the applicability of the results to a larger sample. Simultaneously, the research refrained from evaluating the respondents' national origin, which could potentially be a restriction given that the populations under investigation may not be exclusively of Romanian or Brazilian descent. Additionally, the sample size may have an impact on the research findings, and the number of patients evaluated was not homogeneous.

3.5. Conclusions to the Doctoral Study number 1

GR-alpha, GPR44-2, SULT1A1 and CRABP2 genes were statistically significant in Brazil and Romania. The SULT1A1 gene, involved in evaluating the response to minoxidil treatment, showed that minoxidil is effective in half of the cases of AGA and AA. Most of the other genes

studied showed no differences between the two populations, suggesting a common genetic background.

In addition, ACE gene variants were associated with increased plasma levels of angiotensin, and insulin-like growth factor I (IGF-I) variants with lower plasma levels of IGF-1 in half of the subjects analyzed. The Doctoral Study showed that IGF-I variants were associated with lower plasma levels of IGF-1 in half of the evaluated patients, indicating the possibility of treatment with topical cefarantine, both in patients with AGA and AA.

Latanoprost is not recommended in 60.8% of AGA cases in Brazilian patients and 46.4% of Romanian patients. On the other hand, dutasteride is recommended to 43.1% of Brazilians and 53.6% of Romanians in AGA patients.

CYP19A1 gene analysis showed that 17-α estradiol could have a good therapeutic response in AA, higher than in AGA (>48.0% in both groups). The Doctoral Study does not indicate the need for biotin supplementation in more than 90% of AA and AGA cases in both groups. Additionally, statistical principal component analysis (PCA) showed that SRD5A1, SRD5A2, PTGES2, CRABP2, and BTD genes did not contribute substantially to the principal components.

Early diagnosis and recognition of the genetic pattern is important in the evaluation and management of AA. It's also critical to remember that research on SNPs' involvement in AA is ongoing, and additional studies are required to fully understand their importance.

4. Contributions on the comorbidities of alopecia areata and the comparison of vitiligo

4.1. Introduction (hypothesis and specific objectives)

Alopecia areata (AA) and vitiligo are autoimmune diseases, which can coexist; vitiligo being reported in 4.1% of patients with AA [73]. At first glance, the two diseases present a different clinical aspect: vitiligo is characterized by the appearance of hypopigmented macules, while AA is characterized by alopecic plaques, most frequently on the scalp. Both diseases are often asymptomatic [74]. However, research in the last decade shows that the immune cell populations and cytokines involved are similar and genetic risk factors and triggers are common, suggesting a common pathophysiological pathway [23, 73, 75]. It also appears that melanocyte-derived peptide antigens could act as self-antigens not only in vitiligo [76] but also in AA, and autoimmune T helper (Th) cells could also trigger a response against melanocytes hair follicles, thus determining the onset of AA [73].

At the same time, AA and vitiligo are known to be associated with thyroid dysfunction (autoimmune thyroiditis being Th1 mediated), and anti-thyroid antibodies are frequently observed exceeding the physiological level, both in patients with vitiligo and in those with AA [74]. The presence of AA or vitiligo in the patients' family history can explain the importance of the genetic factor.

Infections, pregnancy, traumatic events and the stress can be triggers for both diseases. Publications in recent years have also reported exacerbation or relapse of AA following SARS-CoV2 infection [77].

The second Doctoral Study includes 8 objectives:

Objective number **B1**: Analysis and comparison of demographic and anthropometric data of patients with AA and vitiligo.

Objective number **B2**: Evaluation and comparison of the age of onset, triggers and location of lesions of patients with AA and vitiligo.

Objective number **B3**: Assessing the presence of atopic conditions (allergies, allergic rhinitis, bronchial asthma) or other comorbidities in patients with AA and vitiligo.

Objective number **B4**: Investigating and comparing the quality of life of patients with AA and vitiligo.

Objective number **B5**: Diet study of patients with AA and vitiligo.

Objective number **B6**: Identifying factors with statistically significant association in AA and vitiligo.

Objective number **B7**: Assessing the impact of SARS-CoV2 infection in patients with AA and vitiligo.

Objective number **B8**: Determining the proportion of autoimmune diseases in family members with vitiligo and AA.

Additionally, I selected two unique clinical cases from the extensive research and I have presented them in the Doctoral Thesis. The first case was a patient diagnosed with both vitiligo and AA, which is an uncommon co-existence, and the second patient was diagnosed with AA and presented unaffected hypopigmented hairs, fact that can support the response against the melanocytes of hair follicles in AA.

4.2. Materials and methods

Two online questionnaires entitled "Vitiligo study via online questionnaire" and "Alopecia areata study via online questionnaire" were created in November and December of 2022. They

included the same set of 30 questions regarding demographics, diet, associations with other autoimmune diseases, quality of life, and the impact of SARS-CoV2 infection on disease.

Patients from the Second Department of Dermatology of the Colentina Clinical Hospital, both current and past, as well as those from online support groups for vitiligo and AA, completed the surveys between January and February of 2023. Individual phone calls were made to Colentina Clinical Hospital patients who had been diagnosed with vitiligo or AA in order to obtain their consent to fill out the questionnaire and provide a submission link. The Doctoral Study included 226 respondents in total, 185 of which had vitiligo and 77 of which had AA. The data were subsequently analyzed, processed and statistically processed.

4.3. Results

Using online questionnaires, I researched whether there is an association and what characteristics vitiligo has in common with AA. For each category of factors, including demographics, disease evolution, comorbidities, family history, triggers, disease impact on the quality of life, diet, SARS-CoV2 infection, impact on family members, and associations with other autoimmune diseases, the percentage distributions of patients with vitiligo and AA were all examined. Analyzing AA and vitiligo as well as the effects of the aforementioned types of factors was the research hypothesis. The denial of their influence on AA and vitiligo constituted the null hypothesis.

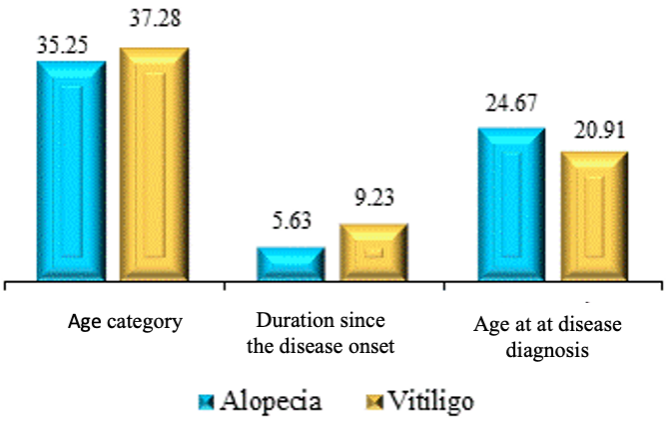
A statistically significant correlation exists if, for the categories of factors taken into consideration and the types of medical disorders (vitiligo and AA), the *p-value* is less than or equal to 0.05, and implicitly, the *p-value* value is less than 0.01. A statistically significant correlation exists if, for the categories of factors taken into consideration and the types of medical disorders (vitiligo and AA), the *p-value* is less than or equal to 0.05, and implicitly, the *p-value* value is less than 0.01.

Age group, environment, time since disease onset, age at disease diagnosis, comorbidities, genetic (family) factor, triggering factors, disease-related limitations on daily activities, infection with SARS-CoV2, and association with thyroid disease were among the factors that showed a statistically significant association. According to the questionnaire, 70% of responders were female. The majority of the patients under investigation are between the ages of 19 and 40, respectively: 7 patients with AA and 14 patients with vitiligo are between the ages of 25 and 30. Between the ages of 31 and 40, there are 15 patients with AA and 26 patients with vitiligo: 38 individuals have AA and 57 have vitiligo. Nineteen patients with vitiligo under

18 years old completed the questionnaire and only two with AA and only 1 patient with AA and 6 patients with vitiligo were over 60 years of age.

The results of the statistical analysis indicated that there is no correlation between AA and vitiligo and anthropometric parameters like height and body weight since the two significance threshold values are above 0.05 (*p-value* > 0.05). Patients with vitiligo and AA have similar body mass index data: vitiligo patients had a height of 166.06 ± 14.58 and a weight of 68.01 ± 16.65 standard deviation, while AA patients had a height of 168.09 ± 8.05 and a weight of 66.00 ± 14.92 standard deviation. According to the questionnaire, over 80% of respondents who had both vitiligo and AA were from urban areas. More than half of the patients who had both conditions were classified as non-smokers.

The mean values of three statistically significant variables, including age category, duration from disease onset, and age at disease diagnosis among AA and vitiligo, are showed in Graph No. 1. The values of the 3 factors are similar, except for the onset duration of the condition, which is longer in vitiligo (5.63 years in the case of AA and 9.23 in the case of vitiligo).



Graph no. 1: Mean values of three statistically significant factors: age category, duration and age since the disease onset in AA and vitiligo (p=0.008, respectively p=0.001, respectively 0=0.013)

The Doctoral Study found that the following comorbidities were the most common, with a statistical significance level of $p<0.001$: psoriasis (1.3% in AA and 7.5% in vitiligo patients), seasonal or food allergies (9.1% in AA and 5.9% in vitiligo patients), bronchial asthma (1% in AA and 4.3% in vitiligo), seborrheic dermatitis (9.1% in AA and 2.2% in vitiligo patients), and acne (11.7% in AA and 2.7% in vitiligo). In addition, eczema or atopic dermatitis affects 4 people (5.2% of AA patients) as opposed to vitiligo (1.6%). Remarkably, 3 of the vitiligo

patients (1.6%) experienced an AA episode. Compared to vitiligo, which affects only over 30% of individuals, over 60% of AA patients have a comorbidity.

Both vitiligo and AA share a hereditary component; a family member with AA had a diseased relative in 5.2% of cases, compared to 30.1% in vitiligo cases. Similar percentages—39.3% and 39% AA—were seen in the study among patients with vitiligo who also had a family history of thyroid disease.

According to the Doctoral research, stressful events may be a contributing factor or a recurrence of vitiligo or AA in 67.5% of AA patients and 67.7% of vitiligo patients overall. For 20 patients with AA and 84 with vitiligo, the key triggering factors were represented by stress and psychological trauma events. For 4.3% subjects with vitiligo and 7.8% of patients with AA, the pregnancy was a potential trigger. In AA and vitiligo, the majority of the patients (32.5% and 34.4%, respectively) observed the development of the disease fewer than six months after the trigger factor manifested. According to the PhD study, more than thirty percent of participants observed the disease's clinical manifestation within six months of the trigger event. A similar percentage of 64.3% of female patients with vitiligo and 61.7% of female patients with AA reported that their disease did not restrict their daily activities. On the other hand, low self-esteem and depression were more common in women than in men (71.7% in AA and 62.4% in vitiligo vs. 52.9% in AA and 45.5% in vitiligo, respectively). Based on the questionnaire, 94.8% of patients with AA and 62.0% with vitiligo are not part of a support group. Conversely, over 90% of patients did not require a change of employment. There were no significant correlations found between the two categories of medical disorders based on a gender analysis of characteristics associated to low self-esteem, depression or stigmatization caused on by illness, limitations on day-to-day activities due to illness, and the need to change jobs as a consequence of the disease. Although it occurs in less than 50% of cases, vitiligo and AA can affect patients as well as their families. The majority of family members are affected in a mild to moderate way and the factor shows statistical significance.

Diet has been correlated with both vitiligo and AA. The respondents of the questionnaire reported consuming mainly animal protein (35.1% in AA and 45.5% in vitiligo), a diet high in carbohydrates (27.3% in AA and 22.7% in vitiligo), a Mediterranean diet (16.9% in AA and 11.4% in vitiligo), and 3.9% of AA patients and 1.1% of vitiligo patients had a vegetarian or vegan diet.

Following SARS-CoV2 infection or vaccination, more than half of the patients (68.8% in AA and 87.4% in vitiligo) did not experience a relapse of their condition. However, 23.4% of AA patients and 6.6% of vitiligo patients relapsed after viral infection and 7.8% (AA) and 6%

(vitiligo) post-vaccination. The Doctoral Thesis includes a graphic representation of each result obtained in the Doctoral Study.

4.4. Discussions

Demographic data

Although vitiligo can develop at any age, most occurrences begin before the age of 20 [78], and 25% of individuals experience symptoms before the age of 10 [79]. Both sexes can be impacted but females seem to be more likely to be affected and may experience the disease earlier in life [75,78]. According to Kiprono et al., the mean age of vitiligo patients was 24 years old, and the male to female ratio was 1:1.8 [80]. Moreover, Villasante Fricke et al. did not find a gender preponderance in AA and they did find that the third and fourth decades of life are the most common times for onset [81].

Our findings in the age-stratified analysis for AA were comparable to those of Harries et al. [82]. Furthermore, the age of onset (less than 30 years old) was similar for 34.8% of respondents who were known to have vitiligo. Disease duration was between 1 and 5 years in 36.4% AA cases and over 10 years in 48.6% vitiligo. The majority of the interviewed individuals were female, which may have an impact on the findings.

According to Lu et al., there was no discernible difference in the beginning and course of the disease between Chinese citizens living in urban and rural areas [83]. A potential drawback of the study could be the fact that over 80% of patients who reside in metropolitan areas filled out our questionnaire, in contrast to the Chinese study.

Personal health record

Dai et al. reported that smokers have an increased risk of developing AA [84]. Smoking induces the release of TNF-alpha, IL-1 and IL-6. These cytokines play a role in the pathogenesis of AA by triggering a perifollicular inflammatory response. Hair follicles exhibit "immune privilege", by means of which they are protected from systemic immune attack, and the collapse of hair follicle immune privilege can lead to the onset of disease [85].

Bhandary et al. studied 46 patients with AA barbae and observed increased alcohol consumption in six patients and tobacco use in five patients [86]. Abraham et al. observed that approximately 15% of vitiligo patients were chronic alcohol users and 12% tobacco users. Interestingly, Lee et al. conducted a national study on the impact of smoking on vitiligo and observed that the risk of vitiligo was lower in smokers, in relation to the amount of tobacco

consumed [87]. The second phD Study revealed that 33.8% of patients with AA and 33% of patients with vitiligo are former or current smokers.

Comorbidities

Patients with atopic dermatitis can develop vitiligo or severe forms of AA at an early stage of the disease [88]. Publications from the dermatological archives have revealed that patients with AA are more likely to exhibit atopy. More precisely, 11% of 736 patients with AA had comorbidities such as bronchial asthma or atopic dermatitis [89].

A systematic review that included 16 studies on vitiligo stated that vitiligo patients have a significantly increased risk of atopic dermatitis compared to control patients. In addition, patients with early-onset vitiligo (<12 years) also have an increased risk of developing atopic dermatitis compared to those with late-onset vitiligo [90]. The prevalence of AA with a personal history of atopy in adults ranged from 22% to 38%, according to seven cross-sectional investigations [91].

In the current doctoral research, bronchial asthma was found in a lower proportion of atopic patients (1% in AA and 4.3% in vitiligo). Four patients with AA (5.2%) suffered from eczema or atopic dermatitis compared to 16% with vitiligo. Seasonal or food allergies, as well as allergic rhinitis, were found in less than 13% of AA patients and 7.5% of vitiligo patients. Thyroid dysfunction is one of the most common autoimmune diseases associated vitiligo [88]. Furthermore, the autoimmune etiology of AA has been supported by epidemiological studies on the association of AA with other autoimmune diseases (thyroid diseases, vitiligo, psoriasis). According to van Geel et al., there is a 15.4% correlation between vitiligo and autoimmune diseases, particularly thyroid disorders in women [92]. In a cross-sectional analysis, Han et al. examined 248,370 AA patients and observed that 0.52% and 0.54% of AA patients exhibited Graves' disease and Hashimoto's autoimmune thyroiditis, compared to 0.37% and 0.47% of control patients [93,94]. In the Doctoral research, the thyroid disfunction impacts a greater proportion of vitiligo and AA patients (47.2% vitiligo and 39.0% AA) compared to the other publications. The reason might be the small group of patients studied compared to the mentioned studies.

Triggers

More than 65% of patients experienced stressful situations, compared to 22% of control patients, according to Manolache et al.'s evaluation of the impact of stress in 45 AA patients and 32 vitiligo patients [95]. According to our research, which also revealed comparable

percentages, vitiligo was present in 67.7% of cases and AA in 67.5% of patients prior to stressful situations. Stress and traumatic psychic occurrences were the key components that contributed as the precipitating or triggering causes. Henning et al., on the other hand, carried out a study to evaluate stress in vitiligo patients. Patients with vitiligo reported feeling significantly more stressed than the individuals unaffected by the disease [96].

The pregnancy is another potential trigger factor. However, there appeared to be no significant difference in achieving a pregnancy in both the severe AA group and the control group [97]. Conversely, research from dermatological archives show that during pregnancy, the risk of AA onset or extension is low [98]. Pregnancy was identified as a potential trigger in 4.3% of vitiligo patients and 7.8% of AA patients in the Doctoral Study.

The development or recurrence of AA often develops 1-2 months post-viral infection, and AA may represent a dermatological manifestation of SARS-CoV2 virus infection [99]. At the same time, the onset and relapses of the disease were linked not only to the infection but also to the vaccines against COVID-19. Nine cases of AA were reported by Scollan et al. [100] 4-8 weeks after vaccination. In contrast, Kim et al. showed that infection with the SARS-CoV2 virus was not significantly associated with the occurrence of AA [101].

Regarding vitiligo, Schmid et al. [102] and Herzum et al. [103] publish case reports regarding the onset of vitiligo following confirmed SARS-CoV-2 infection. Subsequently, Aryanian et al. later conclude that the incidence of vitiligo cases related to the SARS-CoV-2 vaccine seems to be higher than after viral infection [104]. In the Doctoral Study, 23.4% of patients with AA and 6.6% of those with vitiligo had a relapse after the viral infection and 7.8% (AA) and 6% (vitiligo) after vaccination, but the data cannot be compared because studies evaluating the incidence of relapses after SARS-CoV-2 infection or vaccine are minimal.

Family medical history

Patients and their family members can be susceptible to develop diseases that share a similar pathophysiology and common genetic risk factors. In addition, the HLA, IL2RA, and CTLA4 alleles are shared by various autoimmune disorders and are considered common genes associated with vitiligo and AA [200].

According to Agarwal et al., 24.3% of children had a positive family history of vitiligo [105]. Kiprono et al. found that 10% of people with vitiligo had a positive family history [80]. Research that studied 3238 of vitiligo cases, found that 374 family members shared the condition, with a mean age of onset of 21.5 ± 15.0 years (median 18.5) [106]. Both vitiligo patients and their family are far more probable to be diagnosed with an autoimmune thyroid

disorder [27]. Vitiligo was also recorded in 22.6% of 133 investigated relatives of patients diagnosed with vitiligo, compared to 17.0% among control patients [27].

According to the Doctoral Study, 30.1% of the subject's family member were diagnosed with vitiligo and 5.2% with AA. Moreover, we report that the patients had as well a relative diagnosed with thyroid disease in 39% cases of AA and 39.3% cases in vitiligo.

Impact of AA and vitiligo on family members

Several studies have evaluated the quality of life of family members of patients with vitiligo. A study investigating the psychological effects of vitiligo on families in 50 households with children diagnosed with the condition and 50 control families found that the parents' quality of life deteriorated [107]. Another study that assessed the quality of life of family members of vitiligo patients in Saudi Arabia showed negative affect in 91.5% of cases. As a result, vitiligo has significantly impacted both patients' and their families' quality of life [108]. Our data varies slightly from the literature in that it shows that over 50% of family members don't seem to be impacted by the patient's condition. This reason is probably due to the fact that the respondent of the questionnaire is the patient and not the patient's family member.

4.4.1. Similarities between AA and vitiligo

The literature includes very few previous comparative investigations of AA and vitiligo. According to the authors, in 5.3%–12.5% of cases from a study with 133 patients diagnosed with generalized vitiligo had AA, which is consistent with findings from Turkey [106], Africa, China, and India [109,110].

Associations between vitiligo and AA were noted by Gill et al. [24], suggesting a potential shared pathophysiology for the two conditions. Coordinated communication between keratinocytes and melanocytes is thought to control the regeneration process in both disorders [26]. As acknowledged in the case presentations of the doctoral thesis, AA mostly affects pigmented terminal hairs and can tolerate hypopigmented ones and the regrown hair are often vellus type hairs. Furthermore, AA and vitiligo are also frequently asymptomatic conditions [8].

The Thesis noted that the average age of AA and vitiligo patients was 24.57 years for AA and 20.91 years for vitiligo, indicating that these conditions primarily affect young individuals. The second Doctoral Study suggests that stress, severe psychological events, pregnancy, and viral infections that manifest less than six months after the onset of the disease are common

triggering variables shared by the two conditions. Twenty patients and 84 vitiligo patients reported stressful experiences, while 67.5% of AA patients and 67.7% of vitiligo patients experienced stressful events and traumatic psychological events. For 4.3% of individuals with vitiligo and 7.8% of patients with AA, the pregnancy was a potential trigger. Nevertheless, following a viral infection, 7.8% of AA patients and 6% of vitiligo patients experienced relapses, as did 23.4% of AA patients and 6.6% of vitiligo patients.

A comparable percentage of the respondents to the questionnaire in both diseases were smokers or former smokers. Personal medical history revealed increased frequency of thyroid dysfunction in 39% of AA cases, respectively 47.2% of vitiligo cases. A genetic component has been linked to vitiligo and AA, as evidenced by the hereditary history of thyroid involvement in family members in present in 39.3% of vitiligo patients and 39% of AA cases.

The most common comorbidities found in AA and vitiligo are allergic rhinitis (13% in AA patients and 7.5% in vitiligo patients), seasonal or food allergies (9.1% in AA and 5.9% in patients with vitiligo) and asthma (1% in AA and 4.3% in vitiligo).

It was discovered in the Doctoral Study that the quality of life is compromised in both patient categories. Females were more affected than males regarding low self-esteem and depression (71.7% in AA and 62.4% in vitiligo, compared to men: 52.9% in AA and 45.5 % in vitiligo). In addition, vitiligo was more common in AA patients [97]. In the Doctoral Study, we found that 3 individuals (1.6%) who had vitiligo had also experienced an AA episode. One intriguing question that the Harris et al. [74] research presents is how to understand the coexistence of vitiligo and AA. The existence of shared vitiligo and AA susceptibility genes may account for the coexistence of vitiligo and AA [26].

4.4.2. Limitations of Doctoral Study number 2

First, participant self-reported survey results may contain reporting biases. Using a questionnaire to evaluate stressful circumstances may have limitations when compared to a clinical consultation, which provides a more comprehensive perspective. In addition, there are unequal numbers of groups (77 respondents with AA and 185 respondents with vitiligo), which may have an impact on the study's findings. Another restriction would be the use of a questionnaire rather than a measurable scale for assessing quality of life. Not to mention, eighty percent of the respondents were from urban areas, making it impossible to analyze the data from patients from rural areas.

4.5. Conclusions to Doctoral Study number 2

Romanian patients with AA or vitiligo share comparable demographics and characteristics such as similar triggers, common comorbidities and considerable impact on quality of life. Doctoral study number 2 confirms the relationship between AA and vitiligo and stressful events, pregnancy and viral infections that can cause disease onset or relapses. Most of the people surveyed stated the onset of the disease or relapse less than 6 months after the appearance of the precipitating factor.

Thyroid disease affects a higher percentage of AA and vitiligo patients than those reported in recent articles (39.0% AA and 47.2% vitiligo). Compared to over 30% individuals diagnosed with vitiligo, more than 60% of patients with AA displayed comorbidity. The majority of the patients who responded to the questionnaire described diseases related to atopy, including eczema, atopic dermatitis, seasonal or food allergies, bronchial asthma, and allergic rhinitis.
The genetic component is significant in both cases of vitiligo (30.1%) and AA (5.2%), including a family member affected by the disease. In comparison to recent studies, we report a higher percentage of thyroid dysfunction also in members of families affected by vitiligo or AA.

The factors with statistically significant association for AA and vitiligo were represented by: age category, environment, duration since the onset of the disease, age at the diagnosis of the disease, comorbidities, genetic factor (familial), triggering factors, limitation of daily activity due to the disease, infection with SARS-CoV2 and the association with thyroid diseases.

III. CONCLUSIONS AND PERSONAL CONTRIBUTIONS

The doctoral thesis's aim was accomplished by conducting the two analytical studies and obtaining suitable results for each of them. The aim of the Thesis included analyzing the genetic testing of patients with alopecia areata (AA) and androgenetic alopecia (AGA) in patients from Romania and Brazil, as well as evaluating the comorbidities of AA, particularly vitiligo.

The personal contributions consist in carrying out the first genetic study for patients with AA and AGA from Romania and Brazil (Doctoral Study number 1) as well as the publication of partial results in the MDPI Journal (Medicina - impact factor of 2.6) and the oral presentation of the results in the dermatological conference "Qvo vadis dermato-venerology? Classic and modern" which took place between November 10-12, 2023 in Bucharest, Romania. The study allowed the prediction of the effectiveness of the therapies frequently used in AA but also to present the less frequently prescribed treatments as 17-α estradiol, cefarantin and prostaquinone. Additionally, the most common genotypes of the genes under study as well as the statistically significant genes in Brazil and Romania were determined. The advantage of the genetic test is obtaining a predictable and optimized result for each patient diagnosed with AA, but its increased costs can prevent the implementation of the test.

In the framework of the second Doctoral Study, the comparative analysis of patients from Romania with AA and vitiligo was carried out, which allowed the determination of the similarities between the two autoimmune diseases considered "sister diseases" that present common pathophysiological pathways, despite the distinct clinical aspect. At the same time, this is among the first studies at the national level that compare demographic, anthropometric, personal and family medical history, triggering factors, diet and impact on the quality of life of patients diagnosed with AA or vitiligo. Furthermore, since certain members of the patients' families had been affected by the condition, I concluded that both disorders had a significant hereditary component. Still, the most common condition linked to both vitiligo and AA is the thyroid dysfunction.

Moreover, within the Doctoral Thesis, I was allowed to identify and characterize two clinical cases: of rare coexistence of AA with vitiligo in a Romanian patient (Subchapter 8.2.1. Clinical case no. 1: Colocalization of alopecia areata with vitiligo) and a case of unaffected hypopigmented hairs within AA (Subchapter 8.3.2. Clinical case no. 2: Alopecia areata with unaffected hypopigmented hairs).

The conclusions of the Doctoral Study

Doctoral study number 1

Regarding Objective number A1 " Analysis and comparison of 15 genes and 45 genotypes in AA and AGA patients from Romania and Brazil " of the first Doctoral Study, the conclusion is that the genes GR-alpha, GPR44-2, SULT1A1 and CRABP2 were statistically significant in Brazil and Romania. The AA genotype was more frequent for the SNP rs6198 (GR-alpha gene) and the AA case, and the analysis showed that 73.6% of the Brazilian patients, respectively 67.6% of the Romanian patients, show sensitivity to topical corticotherapy. Prostaglandin D2 receptor 2 (GPR44-2) variants cause an increased response to PGD2 and hair follicle regression and PGD2 inhibitors such as prostaquinone and thymoquinone have been recommended in over 40% of AGA and over 28% AA cases. The SULT1A1 gene, involved in evaluating response to minoxidil treatment, showed that minoxidil was effective in half of AGA and AA cases. Genetic analysis revealed the need for retinoic acid supplementation in 66.1% of AA cases from Brazil, respectively Romania, but the application of vitamin A derivatives may represent a therapeutic option for treatment-resistant AA. Most of the other genes studied showed no difference between the two populations, suggesting a common genetic background. Statistical principal component analysis (PCA) showed that SRD5A1, SRD5A2, PTGES2, CRABP2 and BTD genes do not contribute substantially to the principal components.

Regarding Objective number A2 "Comparison of treatment response in AA and AGA patients from Romania and Brazil, based on genetic results " concluded that:

1. Topical treatment with 17-α estradiol shows a favorable therapeutic response in AA, higher than in AGA (>48.0% in both groups).
2. Latanoprost (synthetic analogue of prostaglandin F), finasteride and dutasteride (5α-reductase inhibitors) show comparable efficacy in AGA.
3. IGF-I variants were associated with lower plasma IGF-1 levels in half of the evaluated patients, indicating the possibility of topical cefarantine treatment for patients with AA.
4. The ACE gene was associated with elevated plasma angiotensin level in approximately half of AA patients from Brazil and Romania

Objective number A3 entitled: " Identification of the most frequent genotypes involved in AA and AGA in Romanian and Brazilian patients" revealed that the AA genotype was the most frequent in genes, respectively SNP: GR-alpha-RS6198, GPR44-1-RS545659 and AG genotype in CYP19A1- RS2470152, PTGFR-2- RS1328441, ACE-RS4343 and IGF1R-

RS2229765. At the same time, CC genotypes were found more frequently in the gene, respectively SNP SRD5A2- RS523349, PTGES2- RS13283456 and SULT1A1- RS9282861 and GG in CRABP2- RS12724719, BTD- RS13078881, GPR44-2- RS533116 and PTGFR-1- RS6686438 and for the gene SRD5A1, respectively PTGFR-3, the predominant genotypes were represented by CT, respectively GT.

Objective number A4 entitled "Estimation of the prevalence of alopecia conditions (AA and AGA) of the analyzed patients" revealed a higher weight of AA in Romania, 9.8% compared to Brazil, 5.8%.

Doctoral study number 2

As the subject of the second doctoral study, we examined the comorbidities and similarities between vitiligo and AA, suggesting that the two conditions have a common physiopathological substrate.

Objective number B1 called "Analysis and comparison of demographic and anthropometric data of patients with AA and vitiligo" concluded that AA and vitiligo patients in Romania have similar demographic and anthropometric characteristics. Statistical analysis showed that there is no association between AA and vitiligo and body mass index (p-value > 0.05).

Objective number B2 "Evaluation and comparison of the age of onset, triggers and location of lesions of patients with AA and vitiligo" concluded that:

1. The study confirms the relationship between AA and vitiligo and triggering factors such as stressful events, pregnancy and viral infections.
2. The majority of the patients surveyed (68.8% in AA and 87.4% in vitiligo) stated that the onset of the disease or relapse occurred less than 6 months after the appearance of the precipitating factor.

Objective number B3 entitled "Assessing the presence of atopic conditions (allergies, allergic rhinitis, bronchial asthma) or other comorbidities in patients with AA and vitiligo" showed that:

1. The patients with vitiligo and AA studied present a thyroid condition in a higher percentage compared to publications in specialized journals (47.2% vitiligo and 39.0% AA).
2. The most frequently reported comorbidities in the questionnaire were from the field of atopy and are represented by bronchial asthma, allergic rhinitis, seasonal or food allergies, eczema or atopic dermatitis.

Objective number B4 called "Investigating and comparing the quality of life of patients with AA and vitiligo" showed that:

1. Female patients with AA and vitiligo were more affected than male patients with low self-esteem and depression.
2. The gender analysis of the factors related to the limitation of daily activity due to the disease and the need to change the workplace due to the disease did not show the existence of significant associations between the two types of medical conditions.

Objective number B5 objective entitled "Diet study of patients with AA and vitiligo" showed that patients with AA and vitiligo predominantly have a diet rich in animal protein and a diet rich in carbohydrates. An issue that has been partially researched and requires further investigation is the diet of patients with alopecia areata and vitiligo. In the Doctoral Study, the predominant type of diet was observed, but an extensive questionnaire is necessary in order to analyze in detail the foods consumed by the affected patients as well as relatives who live in the same environment and have a similar diet.

Object number B6 entitled "Identifying factors with statistically significant association in AA and vitiligo" showed that the following factors: age category, environment, duration since the onset of the disease, age at the diagnosis of the disease, comorbidities, the genetic (familial) factor, triggering factors, daily activity limitation due to illness, SARS-CoV2 infection, and association with thyroid disease were statistically significant in AA and vitiligo.

Objective number B7 of the second Doctoral Study called "Assessing the impact of SARS-CoV2 infection in patients with AA and vitiligo" showed that:

1. More than half of the patients did not experience disease recurrence after SARS-CoV2 infection or after vaccination.
2. However, 23.4% of AA patients and 6.6% of vitiligo patients relapsed after viral infection and 7.8% (AA) and 6% (vitiligo) post-vaccination.

Objective number B8 entitled "Determining the proportion of autoimmune diseases in family members with vitiligo and AA" concluded that:

1. The genetic factor plays an important role in both diseases
2. A family member is diagnosed with vitiligo in 30.1% of cases and AA in 5.2%
3. A family member has a personal medical history of thyroid dysfunction in over 39% of cases in both diseases.

The research within the Doctoral Thesis allowed the analysis of the proportion of the treatments with a favorable therapeutic outcome using genetic tests. Individual genes often only provide polygenic conditions, but analysis of existing genes is essential and may reveal

new and meaningful therapeutic targets. Genetic studies can provide new insights with clinical relevance and have a significant impact on the treatment of the patient diagnosed with AA.

Analyzing groups of patients with AA and vitiligo, the second Doctoral Study revealed that psychological factors and metabolic conditions may contribute to and influence the onset or progression of AA. Due to the fact that AA and vitiligo are chronic dermatological conditions and frequently encountered in current practice, it is important to highlight the similarities of the two diseases as well as the evaluation of their comorbidities because it can provide a correct medical evaluation and conduct. At the same time, the recognition of both the similarities and the differences of the two diseases can bring new elements regarding the pathogenesis of the disease, as well as the development of new treatments.

Identifying and revealing cases of colocalization of AA with vitiligo can provide information regarding the causal relationship between the two diseases. In addition, the dissemination of interesting AA cases as the ones presented in the Thesis, may be a source of inspiration for further investigations of AA progression and prognosis.

At the same time, I propose that the direction in which the research should be continued includes additional studies regarding genetic testing and the comparison of post-therapeutic clinical results of patients with AA. It might be also useful to include in the classification of AA or vitiligo the forms of coexistence of the two forms.

Further studies are needed to elucidate the interaction and causality between AA, vitiligo, and comorbidities.

References:

[1] Hsu T, Lin T, Hsu C, Jou H, Yang C. Excimer lamp as an effective alternative treatment for severe alopecia areata. Dermatologica Sinica, 33,151-153, 2015.

[2] Blaumeiser B, van der Goot I, Fimmers R, Hanneken S, Ritzmann S, Seymons K, et al. Familial aggregation of alopecia areata. Journal of the American Academy of Dermatology, 54, 627-632, 2006.

[3] Simakou T, Butcher JP, Reid S, Henriquez FL. Alopecia areata: A multifactorial autoimmune condition. Journal of Autoimmunity, 98, 74-85, 2019.

[4] Seyrafi H, Akhiani M, Abbasi H, Mirpour S, Gholamrezanezhad A. Evaluation of the profile of alopecia areata and the prevalence of thyroid function test abnormalities and serum autoantibodies in Iranian patients. BMC Dermatology, 5, 2005.

[5] Petukhova L, Duvic M, Hordinsky M, Norris D, Price V, Shimomura Y, et al. Genome-wide association study in alopecia areata implicates both innate and adaptive immunity. Nature, 466, 113-117, 2010.

[6] Yip L, Zaloumis S, Irwin D, Severi G, Hopper J, Giles G, et al. Gene-wide association study between the aromatase gene (CYP19A1) and female pattern hair loss. British Journal of Dermatology, 161, 289-294, 2009.

[7] Lintzeri DA, Constantinou A, Hillmann K, Ghoreschi K, Vogt A, Blume- Peytavi U. Alopecia areata – Current understanding and management. Journal der Deutschen Dermatologischen Gesellschaft, 20, 59-90, 2022.

[8] Redler S, Birch M, Drichel D, Dobson K, Brockschmidt F, Tazi-Ahnini R, et al. Investigation of variants of the aromatase gene (CYP19A1) in female pattern hair loss. British Journal of Dermatology, 165, 703-705, 2011.

[9] Liu F, Hamer MA, Heilmann S, Herold C, Moebus S, Hofman A, et al. Prediction of male-pattern baldness from genotypes. European Journal of Human Genetics, 24, 895-902, 2016.

[10] Seok H, Jeon HS, Park HJ, Kim SK, Choi JH, Lew B, et al. Association of HSPA1B SNP rs6457452 with Alopecia Areata in the Korean Population. Immunological Investigations, 43, 212-23, 2014.

[11] Covic M, Ștefănescu D, Sandovici I, Genetică Medicală, Editura Polirom, București, 2011.

[12] Betz RC, Petukhova L, Ripke S, Huang H, Menelaou A, Redler S, et al. Genome-wide meta-analysis in alopecia areata resolves HLA associations and reveals two new susceptibility loci. Nature Communications, 6, 5966, 2015.

[13] Conic R, Chu S, Tamashunas N, Damiani G, Bergfeld W. Prevalence of cardiac and metabolic diseases among patients with alopecia areata. Journal of the European Academy of Dermatology and Venerology, 35, e128-e129, 2021.

[14] Stochmal A, Waśkiel-Burnat A, Chrostowska S, Zaremba M, Rakowska A, Czuwara J, et al. Adiponectin as a novel biomarker of disease severity in alopecia areata. Scientific Reports, 11, 13809, 2021.

[15] Lee S, Lee H, Lee CH, Lee W. Comorbidities in alopecia areata: A systematic review and meta-analysis. Journal of the American Academy of Dermatology, 80, 466-477, 2019.

[16] Żeberkiewicz M, Rudnicka L, Malejczyk J. Immunology of alopecia areata, Central European Journal of Immunology, 45, 325-333, 2020

[17] Andersen YM, Egeberg A, Gislason GH, Skov L, Thyssen JP. Autoimmune diseases in adults with atopic dermatitis. Journal of the American Academy of Dermatology, 76, 274-280, 2017.

[18] Mohan GC, Silverberg JI. Association of Vitiligo and Alopecia Areata With Atopic Dermatitis. JAMA Dermatolology, 151, 522, 2015.
[19] Barahmani N, Schabath MB, Duvic M. History of atopy or autoimmunity increases risk of alopecia areata. Journal of the American Academy of Dermatology, 61, 581-591, 2009.
[20] Zhang X, Zhang B, Caulloo S, Chen X, Li Y, Zhao Y. Diffuse alopecia areata is associated with intense inflammatory infiltration and CD8+ T cells in hair loss regions and an increase in serum IgE level. Indian Journal of Dermatology, Venereology and Leprology, 78, 709, 2012
[21] Ohyama M, Shimizu A, Tanaka K, Amagai M. Experimental evaluation of ebastine, a second-generation anti-histamine, as a supportive medication for alopecia areata. Journal of Dermatological Science, 58, 154-157, 2010.
[22] Lee YB, Lee W. Efficacy of antihistamines in combination with topical corticosteroid and superficial cryotherapy for treatment of alopecia areata: A retrospective cohort study. Journal of the American Academy of Dermatology, 84, 1152-1154, 2021.
[23] Biran R, Zlotogorski A, Ramot Y. The genetics of alopecia areata: New approaches, new findings, new treatments. Journal of Dermatological Science, 78, 11-20, 2015.
[24] Gill L, Zarbo A, Isedeh P, et al. Comorbid autoimmune diseases in patients with vitiligo: a cross-sectional study. Journal of the American Academy of Dermatology, 74, 295–302, 2016.
[25] Mittal J, Mahajan B, Kumar S. Colocalization of vitiligo and alopecia areata: Coincidence or consequence?. International Journal of Trichology, 5, 50, 2013.
[26] Barbulescu CC, Goldstein NB, Roop DR, Norris DA, Birlea SA. Harnessing the Power of Regenerative Therapy for Vitiligo and Alopecia Areata. Journal of Investigative Dermatology, 140, 29-37, 2020.
[27] Laberge G, Mailloux CM, Gowan K, Holland P, Bennett DC, Fain PR, et al. Early disease onset and increased risk of other autoimmune diseases in familial generalized vitiligo. Pigment Cell Research, 18, 300-305, 2005.
[28] Zlotogorski A, Ramot Y, Thomaidou E, Mali A. An extraordinary colocalization of alopecia areata and vitiligo. International Journal of Trichology, 2, 108, 2010.
[29] Bergfeld WF. Androgenetic alopecia: an autosomal dominant disorder. The American Journal of Medicine, 98, 95S- 98S, 1995.
[30] Rathnayake D, Sinclair R. Male androgenetic alopecia. Expert Opinion on Pharmacotherapy, 11, 1295-1304, 2010.
[31] Michel L, Reygagne P, Benech P, Jean-Louis F, Scalvino S, Ly Ka So S, et al. Study of gene expression alteration in male androgenetic alopecia: evidence of predominant molecular signalling pathways. British Journal of Dermatology, 177, 1322-1336, 2017.
[32] Reich D, Price AL, Patterson N. Principal component analysis of genetic data. Nature Genetics, 40, 491–492, 2008.
[33] Nieves A, Garza LA. Does prostaglandin D 2 hold the cure to male pattern baldness?. Experimental Dermatology, 23, 224–227, 2014.
[34] Garza LA, Liu Y, Yang Z, Alagesan B, Lawson JA, Norberg SM, et al. Prostaglandin D 2 Inhibits Hair Growth and Is Elevated in Bald Scalp of Men with Androgenetic Alopecia. Sci Transl Med, 21,126, 2012.
[35] Campos Alberto E, MacLean E, Davidson C, Palikhe NS, Storie J, Tse C, et al. The single nucleotide polymorphism CRTh2 rs533116 is associated with allergic asthma and increased expression of CRTh2. Allergy, 67, 1357-1364, 2012.

[36] Cornejo-García JA, Perkins JR, Jurado-Escobar R, García-Martín E, Agúndez JA, Viguera E, et al. Pharmacogenomics of Prostaglandin and Leukotriene Receptors. Frontiers in Pharmacology, 7, 316, 2016.
[37] Huang J, Gao P, Mathias RA, Yao T, Chen L, Kuo M, et al. Sequence variants of the gene encoding chemoattractant receptor expressed on Th2 cells (CRTH2) are associated with asthma and differentially influence mRNA stability. Human Molecular Genetics, 13, 2691-2697, 2004.
[38] Rossi A, Priolo L, Iorio A, Vescarelli E, Gerardi M, Campo D, et al. Evaluation of a Therapeutic Alternative for Telogen Effluvium: A Pilot Study. Journal of cosmetics, dermatological sciences and applications, 3, 9-16, 2013.
[39] DuBois J, Bruce S, Stewart D, Kempers S, Harutunian C, Boodhoo T, et al. Setipiprant for Androgenetic Alopecia in Males: Results from a Randomized, Double-Blind, Placebo-Controlled Phase 2a Trial. Clinical, Cosmetic and Investigational Dermatology, 14, 1507-1517, 2021.
[40] Conrad F, Paus R. Estrogens and the hair follicle. Journal der Deutschen Dermatologischen Gesellschaft, 2, 412-423, 2004.
[41] Ohnemus U, Uenalan M, Inzunza J, Gustafsson JA, Paus R. The Hair Follicle as an Estrogen Target and Source. Endocrine Reviews, 27, 677–706, 2006.
[42] Choe SJ, Lee S, Choi J, Lee WS. Therapeutic Efficacy of a Combination Therapy of Topical 17α-Estradiol and Topical Minoxidil on Female Pattern Hair Loss: A Noncomparative, Retrospective Evaluation. Annals of Dermatology, 29, 276, 2017.
[43] Zhang X, Yu M, Yu W, Weinberg J, Shapiro J, McElwee KJ. Development of Alopecia Areata Is Associated with Higher Central and Peripheral Hypothalamic–Pituitary–Adrenal Tone in the Skin Graft Induced C3H/HeJ Mouse Model. Journal of Investigative Dermatology, 129, 1527–1538, 2009.
[44] Woodward DF, Jones RL, Narumiya S. International Union of Basic and Clinical Pharmacology. LXXXIII: Classification of Prostanoid Receptors, Updating 15 Years of Progress. Pharmacological Reviews, 63, 471–538, 2011.
[45] Colombe L, Michelet JF, Bernard BA. Prostanoid receptors in anagen human hair follicles. Experimental Dermatology, 17, 63-72, 2008.
[46] Ussa F, Fernandez I, Brion M, Carracedo A, Blazquez F, Garcia MT, et al. Association between SNPs of Metalloproteinases and Prostaglandin F2α Receptor Genes and Latanoprost Response in Open-Angle Glaucoma. Ophthalmology, 122, 1040-1048, 2015.
[47] Razi-Khosroshahi M, Sobhani S, Yousefi Km, Harooni G, Mashayekhi F, Balasi, Goodarz, J. Latanoprost in treatment of alopecia areata and androgenic alopecia: A comprehensive review. Pakistan Journal of Medical and Health Sciences, 17, 1535, 2021.
[48] Blume-Peytavi U, Lönnfors S, Hillmann K, Garcia Bartels N. A randomized double-blind placebo-controlled pilot study to assess the efficacy of a 24-week topical treatment by latanoprost 0.1% on hair growth and pigmentation in healthy volunteers with androgenetic alopecia. Journal of the American Academy of Dermatology, 66, 794–800, 2012.
[49] Coronel-Pérez I, Rodríguez-Rey E, Camacho-Martínez F. Latanoprost in the treatment of eyelash alopecia in alopecia areata universalis. European Academy of Dermatology and Venereology, 24, 481-485, 2010.
[50] Akhyani M, Jafari AR, Seyrafi H, Ghaneinezhad H, Pazouki HR, Tousi SM, et al. Latanoprost for the treatment of alopecia areata of eyelashes, 2008. Accesat pe 12 decembrie 2023: https://www.semanticscholar.org/paper/LATANOPROST-FOR-

THE-TREATMENT-OF-ALOPECIA-AREATA-OF-Akhyani-Jafari/3341a24593b3f41095dda2b3b4a32815390fb2bc
[51] Jiang S, Hao Z, Qi W, Wang Z, Zhou M, Guo N. The efficacy of topical prostaglandin analogs for hair loss: A systematic review and meta-analysis. Frontiers in Medicine, 10, 1130623, 2023.
[52] Namazi MR, Ashraf A, Handjani F, Eftekhar E, Kalafi A. Angiotensin Converting Enzyme Activity in Alopecia Areata. Enzyme Research, 2014, 1-4, 2014.
[53] Glenn KL, Du ZQ, Eisenmann JC, Rothschild MF. An alternative method for genotyping of the ACE I/D polymorphism. Molecular Biology Reports, 36,1305–1310, 2009.
[54] Eisenmann JC, Sarzynski MA, Glenn K, Rothschild M, Heelan KA. ACE I/D genotype, adiposity, and blood pressure in children. Cardiovascular Diabetology, 8, 14, 2009.
[55] Firouzabadi N, Shafiei M, Bahramali E, Ebrahimi SA, Bakhshandeh H, Tajik N. Association of angiotensin-converting enzyme (ACE) gene polymorphism with elevated serum ACE activity and major depression in an Iranian population. Psychiatry Research, 200, 336–342, 2012.
[56] Ghandi N, Fahim S, Montazer F, Tohidinik H, Naraghi Z, Abedini R, et al. Serum and tissue angiotensin-converting enzyme in patients with alopecia areata. Indian Journal of Dermatology, Venereology and Leprology, 85, 295, 2019.
[57] Trüeb RM. Further Clinical Evidence for the Effect of IGF-1 on Hair Growth and Alopecia. Skin Appendage Disorders, 4, 90–95, 2018.
[58] S. Inui and S. Itami, “Induction of insulin-like growth factor-I by cepharanthine from dermal papilla cells: A novel potential pathway for hair growth stimulation,” *J. Dermatol.*, vol. 40, no. 12, pp. 1054–1055, Dec. 2013, doi: 10.1111/1346-8138.12269.
[59] Bonafè M, Barbieri M, Marchegiani F, Olivieri F, Ragno E, Giampieri C, et al. Polymorphic Variants of Insulin-Like Growth Factor I (IGF-I) Receptor and Phosphoinositide 3-Kinase Genes Affect IGF-I Plasma Levels and Human Longevity: Cues for an Evolutionarily Conserved Mechanism of Life Span Control. The Journal of Clinical Endocrinology & Metabolism, 88, 3299-3304, 2003.
[61] Rodrigues DM, Reis RS, Dalle Molle R, Machado TD, Mucellini AB, Bortoluzzi A, et al. Decreased comfort food intake and allostatic load in adolescents carrying the A3669G variant of the glucocorticoid receptor gene. Appetite, 116, 21-28, 2017.
[62] Gasic V, Zukic B, Stankovic B, Janic D, Dokmanovic L, Lazic J, et al. Pharmacogenomic markers of glucocorticoid response in the initial phase of remission induction therapy in childhood acute lymphoblastic leukemia. Radiology and Oncology, 52, 296-306, 2018.
[63] Oakley RH, Cidlowski JA. The biology of the glucocorticoid receptor: New signaling mechanisms in health and disease. Journal of Allergy and Clinical Immunology, 132, 1033-1044, 2013.
[64] Varricchio L, Godbold J, Scott SA, Whitsett C, Da Costa L, Pospisilova D, et al. Increased frequency of the glucocorticoid receptor A3669G (rs6198) polymorphism in patients with Diamond-Blackfan anemia. Blood, 118, 473-474, 2011.
[65] Choi N, Shin S, Song S, Sung JH. Minoxidil Promotes Hair Growth through Stimulation of Growth Factor Release from Adipose-Derived Stem Cells. International Journal of Molecular Sciences, 19, 691, 2018.
[66] Manolescu DC, El-Kares R, Lakhal-Chaieb L, Montpetit A, Bhat PV, Goodyer P. Newborn serum retinoic acid level is associated with variants of genes in the retinol metabolism pathway. Pediatric Research, 67, 598–602, 2010.

[67] Zhang G, Song C, Li L, He H, Shi S, Lei C, et al. DNA methylation status of CRABP2 promoter down-regulates its expression. Gene, 676, 243-248, 2018.
[68] Patel DP, Swink SM, Castelo-Soccio L. A Review of the Use of Biotin for Hair Loss. Skin Appendage Disorders, 3, 166-169, 2017.
[69] Georgala S, Schulpis K, Papakonstantinou E, Kalogirou S, Michas T. Possible involvement of partial biotinidase deficiency in alopecia areata. Journal of the European Academy of Dermatology and Venerology, 7, 135-138, 1996.
[70] Libecco JF, Bergfeld WF. Finasteride in the treatment of alopecia. Expert Opinion on Pharmacotherapy, 5, 933-940, 2004.
[71] Zhou Z, Song S, Gao Z, Wu J, Ma J, Cui Y. The efficacy and safety of dutasteride compared with finasteride in treating men with androgenetic alopecia: a systematic review and meta-analysis. Clinical Interventions in Aging, 14, 399–406, 2019.
[72] Ghassemi M, Ghaffarpour G, Ghods S. The effect of GGC and CAG repeat polymorphisms on the androgen receptor gene in response to finasteride therapy in men with androgenetic alopecia. Journal of Research in Medical Sciences, 24, 104, 2019.
[73] Mittal J, Mahajan B, Kumar S. Colocalization of vitiligo and alopecia areata: Coincidence or consequence?. International Journal of Trichology, 5, 50, 2013.
[74] Harris JE, Rashighi M, Nguyen N, Jabbari A, Ulerio G, Clynes R, et al. Rapid skin repigmentation on oral ruxolitinib in a patient with coexistent vitiligo and alopecia areata (AA). Journal of the American Academy of Dermatology, 74, 370-371, 2016.
[75] Kridin K, Lyakhovitsky K, Onn E, Lyakhovitsky A, Ludwig R, Weinstein O, et al. Investigating the epidemiological relationship between vitiligo and psoriasis: a population-based study. Archives of Dermatological Research, 315, 395-400, 2022.
[76] Xuan L, Baohua Y, Baohua L. Alopecia areata and vitiligo as primary presentations in a young male with human immunodeficiency virus. Indian Journal of Dermatology, 59, 209, 2014.
[77] Christensen RE, Jafferany M. Association between alopecia areata and COVID-19: A systematic review. Journal of the American Academy of Dermatology International, 7, 57–61, 2022.
[78] Rodrigues M, Ezzedine K, Hamzavi I, Pandya AG, Harris JE; Vitiligo Working Group. New discoveries in the pathogenesis and classification of vitiligo. Journal of the American Academy of Dermatology, 77, 1-13, 2017.
[79] Leung AKC, Lam JM, Leong KF, Hon KL. Vitiligo: An Updated Narrative Review. Journal - Current Pediatric Reviews, 17, 76-91, 2021.
[80] Kiprono S, Chaula B. Clinical epidemiological profile of vitiligo. East African Medical Journal, 89, 278-281, 2012.
[81] Miteva M, Villasante A. Epidemiology and burden of alopecia areata: a systematic review. Clinical, Cosmetic and Investigational Dermatology, 397, 397-403, 2015.
[82] Harries M, Macbeth A, Holmes S, Chiu W, Gallardo W, Nijher M, et al. The epidemiology of alopecia areata: a population-based cohort study in UK primary care. British Journal of Dermatology, 186, 257-265, 2022.
[83] Lu T, Gao T, Wang A, Jin Y, Li Q, Li C. Vitiligo prevalence study in Shaanxi Province, China. International Journal of Dermatology, 46, 47-51, 2007.
[84] Dai Y, Yeh F, Shen Y, Tai Y, Chou Y, Chang Y, et al. Cigarette Smoking, Alcohol Consumption, and Risk of Alopecia Areata: A Population-Based Cohort Study in Taiwan. American Journal of Clinical Dermatology, 21, 901-911, 2020.

[85] Khanimov I. Association between smoking and alopecia areata: a systematic review and meta-analysis. International Journal of Dermatology, 61, 1, 2022.
[86] Girisha B, Bhandary D, Mahadevappa B. Clinico-Dermoscopic pattern of beard alopecia areata: A cross-sectional study. Indian Dermatology Online Journal, 10, 644, 2019.
[87] Lee YB, Lee JH, Lee SY, Yu DS, Han KD, Park YG. Association between vitiligo and smoking: A nationwide population-based study in Korea. Scientific Reports, 10, 6231, 2020.
[88] Tobin DJ. Alopecia areata and vitiligo – Partners in crime or a case of false alibis. Experimental Dermatology, 23,153-154, 2014.
[89] Muller SA. Alopecia Areata: An Evaluation of 736 Patients. Archives of Dermatological Research, 88, 290, 1963.
[90] Kuchabal SD. Alopecia Areata Associated with Localized Vitiligo. Case Reports in Dermatology, 2, 27-31, 2010.
[91] Kageyama R, Ito T, Hanai S, Morishita N, Nakazawa S, Fujiyama T, et al. Immunological Properties of Atopic Dermatitis-Associated Alopecia Areata. International Journal of Molecular Sciences, 22, 2618, 2021.
[92] van Geel N, Speeckaert M, Brochez L, Lambert J, Speeckaert R. Clinical profile of generalized vitiligo patients with associated autoimmune/autoinflammatory diseases. European Academy of Dermatology and Venereology, 28, 741-746, 2014.
[93] Shellow WV, Edwards JE, Koo JY: Profile of alopecia areata: a questionnaire analysis of patient and family. International Journal of Dermatology, 31,186-189, 1992.
[94] Han TY, Lee JH, Noh TK, Choi MW, Yun J, Lee KH, et al. Alopecia areata and overt thyroid diseases: A nationwide population-based study. The Journal of Dermatology, 45, 1411-1417, 2018.
[95] Manolache L, Benea V. Stress in patients with alopecia areata and vitiligo. European Academy of Dermatology and Venereology, 21, 921-928, 2007.
[96] Henning SW, Jaishankar D, Barse LW, Dellacecca ER, Lancki N, Webb K, et al. The relationship between stress and vitiligo: Evaluating perceived stress and electronic medical record data. PLoS ONE, 15, e0227909, 2020.
[97] Kim JC, Choi JW. Impact of alopecia areata on subsequent pregnancy rate: A retrospective cohort study. Australasian Journal of Dermatology, 62, e121-e123, 2021.
[98] Meachen GN, Provis FL. Case of Alopecia Areata et Totalis Cured by Pregnancy, and Relapsing with the Re-Establishment of the Menses. Journal of the Royal Society of Medicine, 5,152–154,1912.
[99] Birkett L, Singh P, Mosahebi A, Dhar S. Possible Associations Between Alopecia Areata and COVID-19 Vaccination and Infection. Aesthetic Surgery Journal, 42, NP699–NP702, 2022.
[100] Scollan ME, Breneman A, Kinariwalla N, Soliman Y, Youssef S, Bordone LA, et al. Alopecia areata after SARS-CoV-2 vaccination. Journal of the American Academy of Dermatology Case Reports, 20, 1-5, 2022.
[101] Kim J, Hong K, Yum S, Gomez REG, Chun BC. 352. COVID-19 Not a Risk Factor of Alopecia Areata: Results of a National Cohort Study in South Korea. Open Forum Infectious Diseases, 8, S280, 2021.
[102] Schmidt AF, Rubin A, Milgraum D, Wassef C. Vitiligo following COVID-19: A case report and review of pathophysiology. Journal of the American Academy of Dermatology Case Rep., 22, 47–49, 2022.

[103] Herzum A, Micalizzi C, Molle MF, Parodi A. New-onset vitiligo following COVID-19 disease. Skin Health and Disease, 2, e86, 2022.
[104] Aryanian Z, Balighi K, Hatami P, Goodarzi A, Janbakhsh A, Afshar ZM. Various aspects of the relationship between vitiligo and the COVID-19 pandemic or SARS-CoV-2 vaccines: Clinical pearls for dermatologists. Journal of Cosmetic Dermatology, 22, 1152–1156, 2023.
[105] Agarwal S, Gupta S, Ojha A, Sinha R. Childhood Vitiligo: Clinicoepidemiologic Profile of 268 Children from the Kumaun Region of Uttarakhand, India. Pediatric Dermatology, 30, 348–353, 2013.
[106] Akay BN, Bozkir M, Anadolu Y, et al. Epidemiology of vitiligo, associated autoimmune diseases and audiological abnormalities: Ankara study of 80 patients in Turkey. European Academy of Dermatology and Venereology, 24, 1144–1150, 2010.
[107] Amer A, Mchepange U, Gao X, Hong Y, Qi R, Wu Y, et al. Hidden Victims of Childhood Vitiligo: Impact on Parents' Mental Health and Quality of Life. Acta Dermato-Venereologica, 95, 322-325, 2015.
[108] Bin Saif GA, Al-Balbeesi AO, Binshabaib R, Alsaad D, Kwatra SG, Alzolibani AA, et al. Quality of Life in Family Members of Vitiligo Patients: A Questionnaire Study in Saudi Arabia. American Journal of Clinical Dermatology, 14, 489-495, 2013.
[109] Narita T, Oiso N, Fukai K, Kabashima K, Kawada A, Suzuki T. Generalized vitiligo and associated autoimmune diseases in Japanese patients and their families. Allergology International, 60, 505-508, 2011.
[110] Chen YT, Chen YJ, Hwang CY, Lin MW, Chen TJ, Chen CC, Chu SY, Lee DD, Chang YT, Liu HN. Comorbidity profiles in association with vitiligo: a nationwide population-based study in Taiwan. Journal of European Academy of Dermatology and Venereology, 29, 1362-1369, 2015.

List of published scientific papers

1. Articles published in specialized magazines:

(a) International

ISI category: Paun, M.; Torres, G.; Tiplica, G.S.; Cauni, V.M. Epidemiologic Study of Gene Distribution in Romanian and Brazilian Patients with Non-Cicatricial Alopecia. Medicina 2023 (I.F: 2.6), 59,1654. https:// doi.org/10.3390/medicina59091654

Stanciulescu E. L, **Popescu M**. Shock treatment of acute hand ischemia. Eur, Critical Emergency Medicine — Trauma and Resuscitation. 2020; 37: 58. p. 304.

Sendrea AM, Cretu S, **Popescu M**, Suru A, Salavastru CM. The importance of systemic treatment in pediatric linear morphea. Int. J. Women's Dermatology 4.4. 2018: 244.3.

Cretu S, **Popescu M**, Sendrea AM, Suru A, Salavastru CM. The use of co2 laser for facial cutaneous neurofibromas in neurofibromatosis type 1 – a case report and literature review. Int. J. Women's Dermatology 4.4. 2018: 242-243.

(b) National:

ISI category:
Sciboz OC, Paun MA, **Popescu M**, Stănciulescu S, Lascar I. The role of ngf in the regeneration of peripheral nerve injury – systematic review – implications for the provision of days of medical care after peripheral nerve injuries. Rom J Leg Med 2021 (IF: 0,363); 29(4) 352-355. http://www.rjlm.ro/system/revista/60/352-355.pdf