Dermatology Practical & Conceptual

Di-Genic Inheritance in Genodermatoses: Insights from Two Consanguineous Cases in a Reference Lebanese Center within the Middle East and North Africa (MENA) Region

Ayat Kadhi[1,2,3], Lamiaa Hamie[4,5], Edward Eid[4], Georges Nemer[1], Mazen Kurban[1,5,6]

1 Division of Genomics and Translational Biomedicine, College of Health and Life Sciences, Hamad Bin Khalifa University, Doha, Qatar
2 College of Health and Sciences, University of Doha for Science and Technology, Doha, Qatar
3 Human Genetics Department, Sidra Medicine, Doha, Qatar
4 Department of Dermatology, Medical College of Wisconsin, Milwaukee, USA
5 Department of Dermatology, Faculty of Medicine, American University of Beirut, Beirut, Lebanon
6 Department of Biochemistry and Molecular Genetics, Faculty of Medicine, American University of Beirut, Beirut, Lebanon

Key words: Digenic inheritance, Genodermatoses, Whole-exome sequencing, Microarray Gene Expression DataSets

Citation: Kadhi A, Hamie L, Eid E, Nemer G, Kurban M. Cases of Di-Genic Inheritance in Geno Dermatoses: Lessons from Consanguineous Cases in Lebanon. *Dermatol Pract Concept*. 2025;15(1):4935. DOI: https://DOI.org/10.5826/dpc.1501a4935

Accepted: November 24, 2024; **Published:** January, 2025

Funding: None.

Competing Interests: None.

Authorship: All authors have contributed significantly to this publication.

***Corresponding Authors:** Georges Nemer, Division of Genomics and Translational Biomedicine, College of Health and Life Sciences, Hamad Bin Khalifa University, Doha, Qatar. E-mail: gnemer@hbku.edu.qa

Mazen Kurban, Division of Genomics and Translational Biomedicine, College of Health and Life Sciences, Hamad Bin Khalifa University, Doha, Qatar; Department of Dermatology, Medical College of Wisconsin, Milwaukee, USA; Department of Biochemistry and Molecular Genetics, Faculty of Medicine, American University of Beirut, Beirut, Lebanon. E-mail: mk104@aub.edu.lb

ABSTRACT

Introduction: Genodermatoses refer to a group of heterogenous rare genetic diseases with cutaneous expression. Several genodermatoses present with multisystem involvement that can range from mild to life-threatening conditions leading to increased morbidity and mortality.

Objective: Given the paucity in the literature in the field of genodermatoses, especially in the Middle East and North Africa (MENA) region, and building upon the first established genodermatoses database based in Lebanon, this study aimed to decipher the genetic basis of two different types of skin-inherited diseases (androgenic alopecia and vitiligo).

Methods: We conducted a pilot study on two subjects with androgenic alopecia and vitiligo to investigate the possibility of a digenic inheritance model as a potential underlying mechanism for these conditions. Whole exome sequencing (WES) and Gene Expression Omnibus (GEO) DataSets were employed to validate the methodology and provide a foundation for future, larger-scale studies.

Results: We identified two gene variants *FOXC1(p.His484Tyr)* and *SMARCD1 (p.Arg351Cys)* responsible for androgenic alopecia and *HPS1(p.Ser566Ter)* and *ITK (p.Pro521Leu)* responsible for vitiligo. Further analysis using GEO DataSets confirmed the association between the genes involved in each each disease.

Conclusion: This study identified novel candidate disease genes and inheritance model that could explain the underlying phenotypes that could open the door for a better-guided genomic approach for personalized treatment and early diagnosis.

Introduction

Genodermatoses refer to a diverse heterogenous group of rare inherited disorders characterized by cutaneous expression and multisystem involvement, which increase patients' morbidity and mortality. Currently, there are more than 350 various conditions under genodermatoses, and they are detectable soon after birth or early in life. These conditions are mainly divided into nine subcategories: disorders with malignant potential, disorders of keratinization, genetic blistering disorders, pigmentation disorders, neurocutaneous syndromes, vascular disorders, disorders of connective tissue, X-linked dominant disorders, and ectodermal dysplasia [1-3]. Most of these disorders are monogenic, but some disorders are reported to be digenic in cases with atypical clinical presentation such as epidermolysis bullosa simplex (*KRT5* and *KRT14* genes), hypotrichosis simplex(two unlinked loci 12q21.2-q22 and 16q21-q23.1), skin melanoma (*MC1R* and *CDKN2A* genes), and generalized pustular psoriasis with hypogammaglobulinemia (*SEC6A1A* and *IL-36RA* genes) [4-7]. Herein, we will discuss androgenic alopecia, vitiligo, and albinism.

Androgenic alopecia (AGA MIM 109200) is a common, chronic, progressive hair loss disorder characterized by villus scalp hair/non-scarring alopecia in a distinctive manner. This pattern differs by sex: males typically experience vertex and bitemporal/frontal hair loss, while females experience hair loss starting from the vertex and mid-scalp, known as female pattern hair loss (FPHL) [8]. AGA affects 80% of males and 50% of females during the course of their life [9].

Vitiligo (MIM #606579) is a common chronic, acquired, idiopathic pigmentary disorder characterized by hypomelanosis of skin, hair, or mucosae. Patients exhibit well-defined white macules that can be localized or generalized, immune deficiencies, and in some cases, sensorineural hearing loss. This disease is the most common cause of depigmentation, with a worldwide prevalence of 0.5%–2% in the general population [10]. Vitiligo affects both sexes equally and can appear at any age, but most commonly occurs between the second and third decades of life [11].

Oculocutaneous albinism (OCA) (MIM #203200) is a group of rare autosomal recessive disorders characterized by hypopigmentation and ocular manifestations like decreased visual acuity, nystagmus, strabismus, and photophobia. OCA is divided into two groups: syndromic and non-syndromic disorders; the most common syndromic OCA is Hermansky-Pudlak Syndrome (HPS), which is manifested by additional bleeding diathesis and more critical systemic comorbidities such as pulmonary fibrosis and immunodeficiency[12]. The prevalence of HPS is 1–2/1,000,000 individuals worldwide.

These disorders have complicated etiologies; thus, a definitive diagnosis and treatment remains challenging. The apparent physical manifestations combined with a painful and challenging diagnosis course, leading to delayed treatment, make these diseases psychologically devastating. In this study, we used the advent of emerging tools like WES and transcriptome analysis to unravel the diseases' mechanisms, prompting a timely diagnosis which could lead to adequate targeted therapies treatment in genodermatoses.

Methods

Patient Recruitment

The recruitment of the families was done at the Genodermatoses Unit at the Department of Dermatology at the American University of Beirut Medical Center. Clinical phenotypes were provided by the referring physician. The project was reviewed and approved by the Institutional Review Board (IRB) at the American University of Beirut Medical Center (Protocol Number: DER.MK.01), and written informed consent was obtained from the participants, or from their parents if minors, to collect blood samples and pictures. The patients included in this study were unrelated, representing distinct familial backgrounds. Patient 1 was affected by androgenetic alopecia (AGA), while Patient 2 was affected by vitiligo and a family history of Hermansky-Pudlak Syndrome (HPA).

Whole Exome Sequencing and Variant Interpretation

Peripheral blood samples were collected from patients and stored at 4° C. DNA was extracted from the specimens within one hour of collection. Whole exome sequencing (WES) was conducted for the probands and family members by Macrogen Laboratory, Seoul, Korea. The FASTQ files were mapped to Human GRCh37/hg19 reference assembly using CLC Genomics Workbench (version 20.0.4). Variant calling and annotation were performed using Illumina VariantStudio software version 3.0. To identify the mutations/variants that might lead to the diseases, stringent filter was applied (Figure 1). Detailed methods for this analysis can be found elsewhere [13].

GEO DataSets

To explore the biological processes and pathways that are regulated in the diseases, we analyzed and the Gene Expression Omnibus (GEO) DataSets using Ingenuity Pathway Analysis (IPA) software [14]. We curated datasets based on: (1) studies that have skin biopsies/blood samples to conduct expression profiling by microarray or RNA sequencing; (2) studies with information about the technology used; (3) studies including normal and control groups. From that, we curated these four datasets GSE90594, GSE36169, GSE90880, and GSE75819 (Table 1).

Results

Family 1: Androgenic Alopecia

The proband (III.1, Figure 2A) is a 3-year-old female who visited the clinic due to features like adult male androgenetic alopecia, exhibiting hair loss and widening at the vertex and frontotemporal areas and had sparse eyebrows and eyelashes (Figure 2A and D). The results of the exome analysis yielded 75105 variants per sample before stringent filtering and 613 variants per sample after stringent filtering (Figure 1). After the first round of filtering, as we did not find any homozygous mutation in the patient that fit the stringent criteria, we looked for compound heterozygous mutations. Two potential variants were detected; the first variant is *FOXC1* c.1450C>T, p.H484Y, which leads to a heterozygous missense mutation with a predictive CADD score of 26.8 (Table 1 and Figure 2B and E). The variant is predicted to be likely pathogenic according to the ACMG [15] classifications and has a deleterious effect with a tree vote of 58|42 (del | benign) [16]. The variant was absent from the genomes/exomes databases in gnomad[17] , ExAC [18], 1000G [19], and 300 Lebanese in-house exomes (Table 1). The other deleterious variant, *SMARCD1* c.1051C>T, p.R351C (g.5537C>T), results in a heterozygous missense mutation with a CADD score of 33 (Table 1 and Figure 2C and F).

The analysis of the GEO datasets GSE90594 and GSE36169 of individuals affected with androgenic alopecia compared to controls showed that *FOXC1,* an upstream regulator that leads to activation, had a z-score of 0.515, p-value $3.07E^{-03}$ and z-score of 4.233, p-value 3.64E-22, respectively. In the GSE90594 dataset, under the top diseases and biofunction, hair and skin development ranked as the second top function, with a p-value of 1.78-07-$2.65E^{-65,}$ and dermatological diseases ranked as the third top diseases, with a p-value of $2.22E^{-05}$-$1.80E^{-68}$ (Table 2). For the network analysis, we found that there was a protein-protein interaction (PPI) between *FOXC1* and *SMARCD1* through *SOX2* and *YAP1* genes. We found that *FOXC1* played a role in skin formation and keratinocyte differentiation, and that both *FOXC*1 and *SMARCD1* increased the risk of skin cancer (Figure 3).

Family 2: Vitiligo and Albinism

The patient (III.1, Figure 4A) was a 12-year-old female who presented to the clinic with vitiliginous lesions accompanied by immune deficiency. Her paternal uncle, patient II.3 (Figure 4A), is a 48-year-old male diagnosed with albinism, with no immune deficiency. The exome analysis and filtering criteria is shown in Figure 2. The patient and her uncle harbored pathogenic nonsense variant *HPS1*c.1697C>A, p. Ser566Ter, that can lead to nonsense-mediated mRNA decay (NMD) (Figure 4B/D)[16] with a CADD score of 37. This variant was not present in the public databases. To explain the immune deficiency phenotype in the proband, we searched for immunoregulatory genes and found a deleterious homozygous missense variant *ITK* p. Pro521Leu with a CADD score of 29.6. (Figure 4C and E). The variant was present with a rare MAF in gnomad and 1000 Genome, and ExAC databases 0.00000796, 0.000007957, and 0.000008237, respectively (Table 1).

IPA analysis of the GEO dataset GSE90880 revealed that immune cell trafficking was ranked the top function in the top diseases and biofunction, with a p-value range of $2.15E^{-54}$ – $5.91E^{-109}$ (Table 2), confirming the relevance to the underlying trait. No significant association was detected for GSE75819. For the upstream analysis, we found that *ITK* was an upstream regulator that leads to inhibition of the trait, with a z-score: -2.08, p-value $1.19E^{-06}$ and z-score: -1.813, p-value $9.91E^{-02,}$ respectively. Finally, the network analysis showed that *ITK* and *HPS1* were upstream of the *TNF* gene (Figure 5).

Discussion

WES provides an enormous amount of information about the genetic variation in familial cases exhibiting high phenotypic variability with no clear monogenic segregation

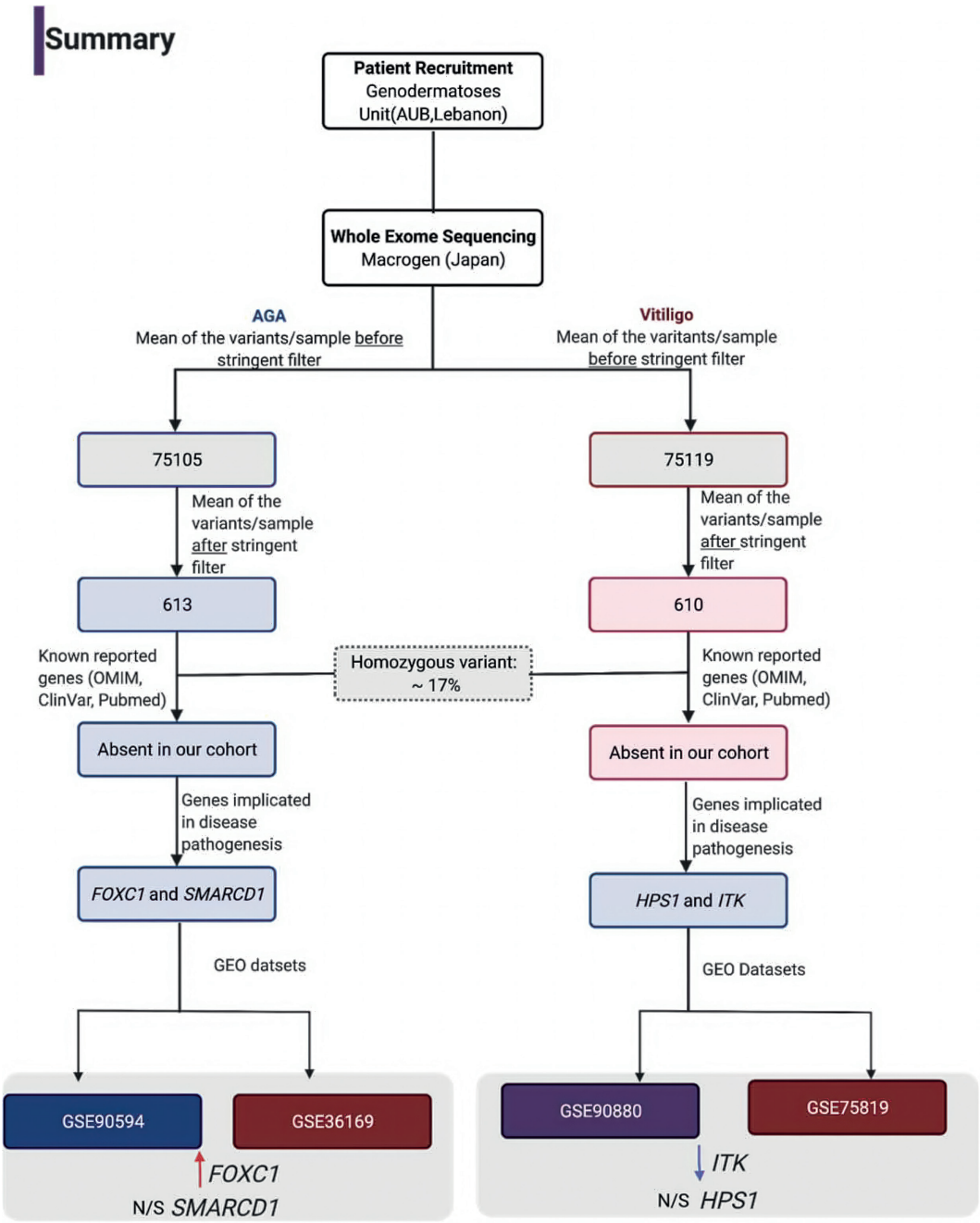

Figure 1. A Summary of methods and results. ↑: Activation ↓: Inhibition N/S: not significant.

pattern [20]. This highlights the importance of following an unbiased approach to identify disease-causing variant/genes, as demonstrated by our current families with clinically and genetically heterogeneous cases.

Transcriptional Players in AGA: FOXC1 and SMARCD1

AGA is well-known in adults but under-reported in pediatric and prepubertal children, making prevalence estimates challenging. A retrospective report identified 57 of 483 pediatric patients (13%, ages 8–19) with AGA and a single case of a 6-year-old female with AGA, all diagnosed based on clinical manifestations, strong family history, genetic predisposition, or endocrinological abnormalities [21,22]. Our patient represents the youngest case reported in the literature affected with AGA (consistent with male androgenic alopecia hair loss) with no prepubertal status or a family history of AGA, despite consanguinity originating from the granparents' generation, making it an intriguing case to further investigate.

AGA is characterized by progressive hair follicular miniaturization due to shortened anagen phase during hair cycle leading to disrupted hair growth cycles, dermal papilla size, and keratinocyte activities, which is mainly controlled by androgens through intracellular signaling of the hair follicle target cells. Androgen receptors (AR) are localized in dermal papilla cells (DPC) in a hair follicle, indicating that

Table 1. Phenotypic and Genotypic Characterization of Patients.

Clinical Features	Gene	HGVS DNA Reference	HGVS Protein Reference	CADD Score	Sift	PolyPhen	Predicted Effect (ACMG/ MutationTaster)	MAF (gnomAD)	MAF (1000 Genome)	MAF (ExAC)	MAF (Lebanese Exomes Population)
3-year-old female with features like male androgenic alopecia	*(1) FOXC1* *(2) SMARCD1*	(1) c.1450C>T (2) :c.1051C>T	(1) p.His484Tyr (2) p.Arg351Cys	(1) 26.8 (2) 33	(1) Deleterious(0) (2) Deleterious(0)	(1) Possibly damaging (0.775) (2) Possibly damaging (0.988)	(1) Uncertain significance (PM2,PP2), Likely pathogenic(PP3)/ Deletrious (58|42) (2) Likely Pathogenic (PM1/2, PP2/3)/ / Deletrious (64|36)	(1) 0/251,486 (2) 1/251,486 (0.000003976)	(1) 0/251,360 (2) 0/251,360	(1) 0 (2) 0	(1) 0/300 (2) 0/300
12-year-old male with vitiliginous lesions and immune deficiency/ 48-year-old paternal uncle affected by albinism	*(1) HPS1* *(2) ITK*	(1) c.1697C>A (2) c.1562C>T	(1) p.Ser566Ter (2) p.Pro521Leu	(1) 37 (2) 29.6	(1)- (2) Deleterious(0)	(1)- (2) Possibly damaging (1)	(1) Pathogenic (PVS1, PM2, PP3)/ Deletrious (193|7) (2) Uncertain significance (PM2) Likley Pathogenic (PP3)/Deletrious (73|27)	(1) 0/251,360 (2) 2/251,360 (0.00000796)	(1) 0/251,360 (2) 2/251,360 (0.000007957)	(1) 0 (2) 1/121,404 (0.000008237)	(1) 0/300 (2) 0/300

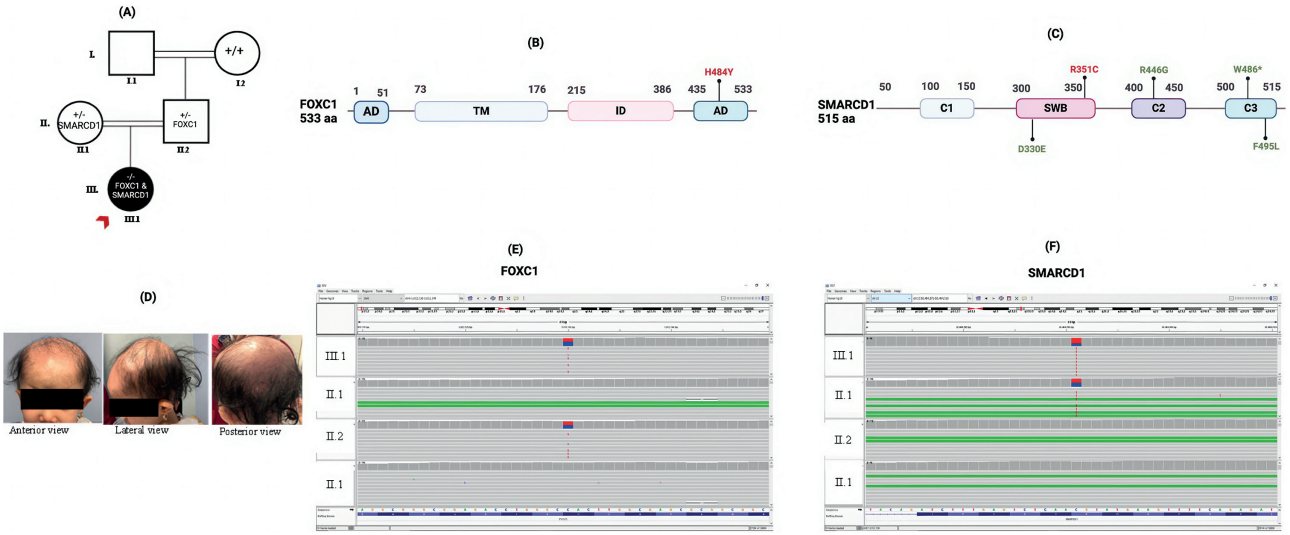

Figure 2. *FOXC1* and SMARCD1 missense mutations segregates with androgenetic alopecia. (A) The pedigree of the familial androgenetic alopecia case with one affected member (red arrow) (+/+ normal genotype, +/- heterozygous genotype). (B)Primary structure of FOXC1 (553 amino acids), two Activation Domains (AD), Forkhead DNA-binding domain (FHD), Inhibitory domain (ID), the location of the current variant is identified in red. (C)Primary structure of SMARCD1(515 amino acids): SWIB domain and coiled-coil domains (C1, C2, and C3), the location of the current variant is identified in red, green variants are related to syndrome. (D) Patients' phenotype representing male AGA hair loss pattern. (E)Integrated Genome Browser (IGV) visualization of the whole-exome sequencing results showing *FOXC1* p.His484Tyr the T > C variant change in a heterozygous form for the patient (red and blue boxes, III.1- upper panel) and heterozygous in the father (red and blue box, II.2lower panels). (F) IGV visualization of the whole-exome sequencing results showing *SMARCD1* p.Arg351Cys, the T > C variant change in the heterozygous form for the patient (red and blue box, III.1- upper panel) and heterozygous in the mother (red and blue box, II.1lower panels).

Table 2. Results of Pathway Analysis from GEO Datasets.

	Androgenic Alopecia		Vitiligo	
GSE	GSE90594	GSE36169	GSE90880	GSE75819
Platform	GPL17077	GPL96	GPL8300	GPL6884
Sample size (affected: unaffected)	14:14	10:10	8:06	15:15
PubMed ID	28403520	22440736	28129744	28852211
Upstream Regulators	FOXC1 activation (z-score: 0.515, p-value 3.07E-03) Disease and	FOXC1 activation (z-score: 4.233, p-value 3.64E-22)	ITK inhibition (z-score: -2.08, p-value 1.19E-06)	ITK inhibition (z-score: -1.813, p-value 9.91E-02)
Top diseases and biofunction	Dermatological Diseases p-value (2.22E-05-1.80E-68) Hair and Skin Development and Function p-value(1.78-07-2.65E-65)	Not Significant	Immune Cell Trafficking p-value(2.15E-54 - 5.91E-109)	Not Significant

DPC are the main target cells for androgen, which is shown in patient with AGA compared to non-AGA patients [23-27]. Androgens such as progesterone, androstenedione, and testosterone are converted into a more potent androgen, dihydrotestosterone (DHT), by the cytoplasmic 5α-reductase enzyme. DHT binds to AR in the cytoplasm, making AR-DHT complex, which translocates to the nucleus after dimerization. AR coactivators are recruited to this complex, which binds to the androgen-response element consistent with the DNA sequence. The coactivators connect AR and transcription factors and RNA polymerase, which results in gene transcription followed by protein translation, which exerts the biological activity. This multi-step molecular pathway can be implicated in AGA pathogenesis. [25,28]. As such, the genes previously reported were linked to androgen signaling pathways such as *(AR)/EDAR2*, *SRD5A1*, *SRD5A2*, *HSD17B2*, *HSD17B3*, *SHBG*, *AKR1C1*, *AKR1C2*, *AKR1C3*, and *FOXA2* [26,29-31]. Pathogenic variants in these genes were absent in our sequencing results, leading us to investigate other genes involved in AGA pathogenesis.

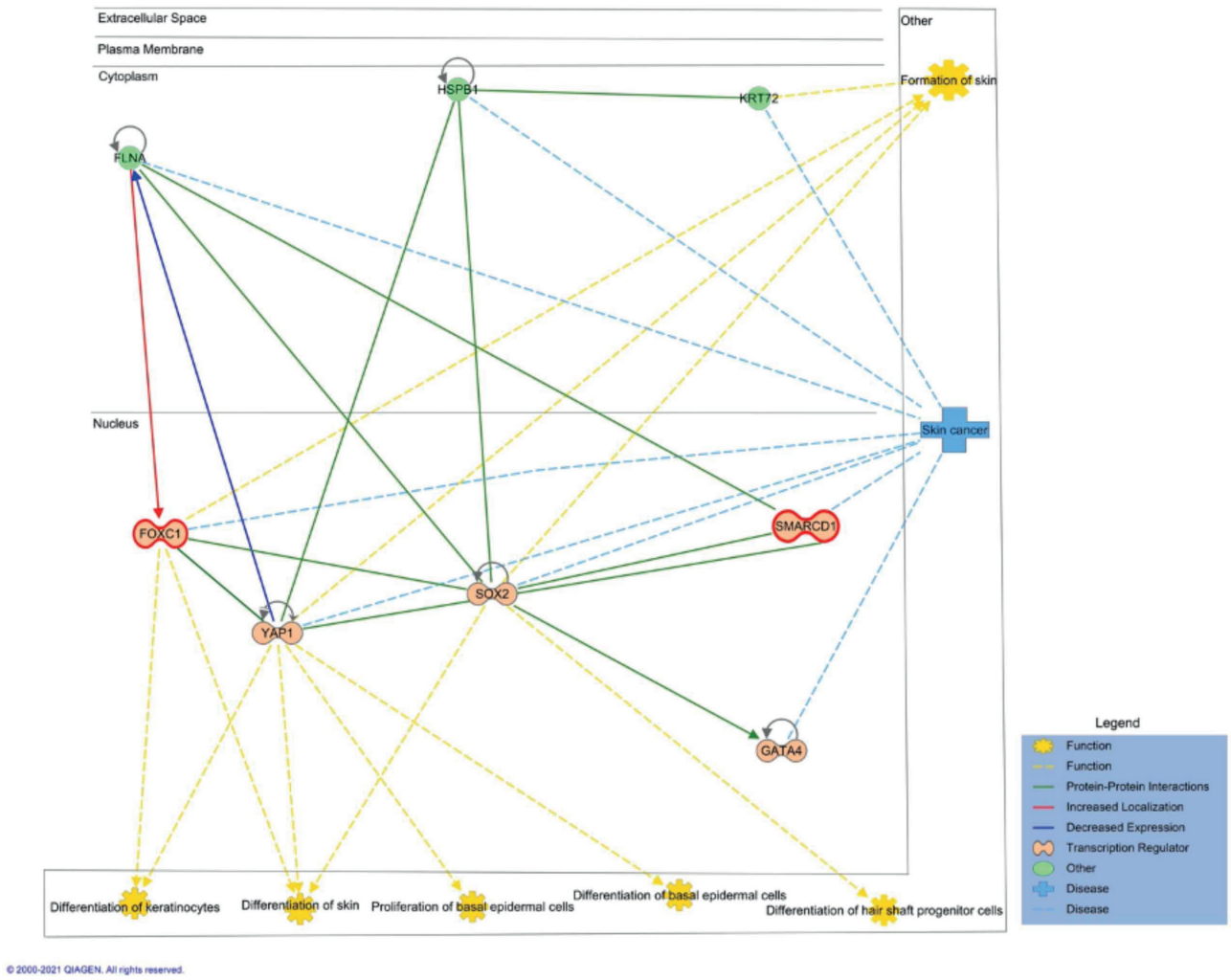

Figure 3. *FOXC1* and *SMARCD1* interactions and pathways using IPA software predictions tools.

Our analysis revealed a potential combinatorial role for FOXC1 and SMARCD1 in the phenotype (Figures 3 and 6). *SMARCD1* (SWI/SNF-related matrix-associated, actin-dependent regulator of chromatin, subfamily D, member 1) gene encodes BAF60A, a key protein in nucleosome-DNA interactions and found to have the highest induction of a strong interaction with AR. *SMARCD1* directly interacts with the coactivator groove in the AR cofactor via its FxxFF motif directly in a hormone-dependent manner and acts as a promoter for the expression of *TMPRSS2* gene [32]. The *SMARCD1* mutation lies within the SWIB domain of the protein. A previous study identified several SMARCD1 variants linked to a neurodevelopmental disorder with sparse or temporal hair deficiency as a phenotype (Figure 2B) [33].

The other player in this study is *FOXC1* (Forkhead box C1), a transcriptional factor involved in cell differentiation and embryogenesis that plays a key role in human keratinocytes terminal differentiation [34]. It has been demonstrated that *FOXC1* maintains and reinforces quiescence in self-renewing hair follicle stem cells in mice and humans leading to premature aging of these stem cells [35-37]. More importantly, a recent study showed that *FOXC1* is highly expressed in human dermal papilla cells (DPCs) at the mRNA and protein level of patients affected with AGA [38], but no previous reports identified *FOXC1* mutations with AGA. Our group previously showed a familial case of Axenfeld-Rieger Syndrome (ARS) where degenerated hair follicle cells were observed, which was explained through *FOXC1/NFATC1* genetic axis [37]. Another report showed similar digenic inheritance of a mutation in *FOXC1*, but with *PITX2* gene in ARS patient where the phenotypic severity increased due to this inheritance [39]. Our mutation is in the activation domain (Figure 2B), and GEO DataSets show that FOXC1 is highly activated in AGA patients (Table 2). This led us to hypothesize that the mutation represented a partial gain of function. The involvement of both genes in AGA pathogenesis and in silico predictions support our hypothesis. Interestingly, a recent report identified SMARCD1 as one of the top 360 FOXC1 interactors, [40] and our *in silico* analysis supports this interaction (Table 2 and Figure 3). Whether this interaction is crucial for hair follicle development remains to be explored.

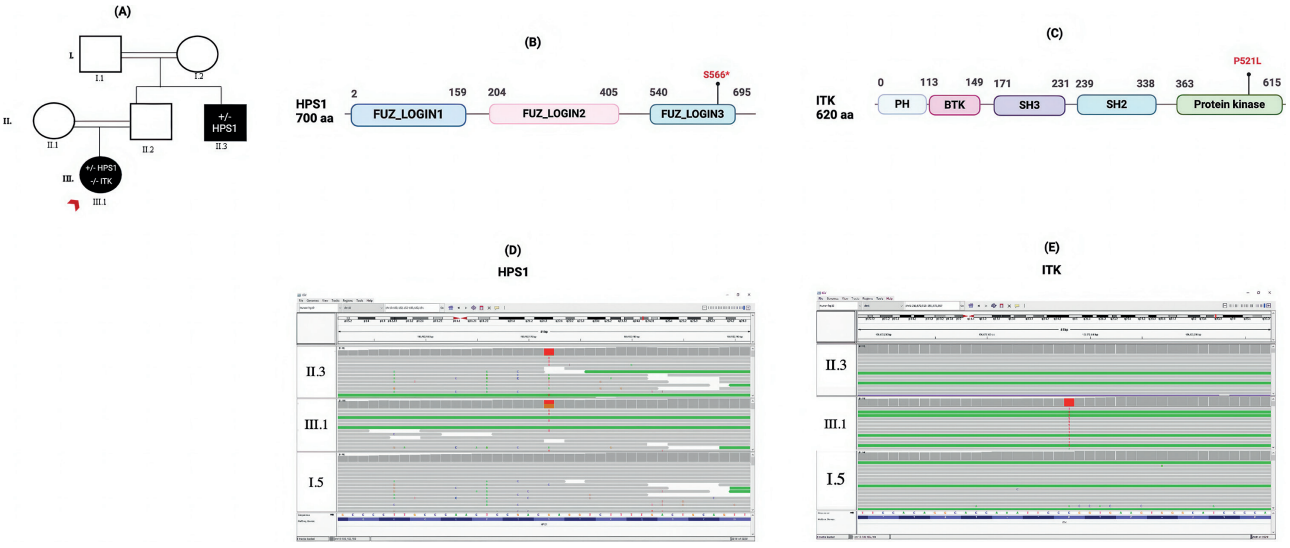

Figure 4. *HPS1* and *ITK* mutations segregate with Vitiligo and Albinism phenotypes. (A) The pedigree of the familial case with two affected members, indexed patient with red arrow (+/-heterozygous and -/- Homozygous genotype). (B) Primary structure of *HPS1* (700 amino acids) with three domains: First Longin domain of Fuzzy (FUZ) protein 1,2, and 3, the current mutation is highlighted in red. (C) Primary structure of *ITK* (620 amino acids) with Pleckstrin homology (PH) , Brutos's Tyrosine Kinase Cys-rich (BTK), Src Homology 3/2 (SH3/2), Protein Kinase Catalyic (TyrKc) domains. (D) Integrated Genome Browser (IGV)visualization of the whole-exome sequencing results showing *HPS1*; the T > G variant change in the heterozygous form for the heterozygous form for the uncle (red and green box, II.3 upper panels), the patient (red and orange box, III.1 middle panel), and a control (I.5). (E) IGV visualization of the whole-exome sequencing results showing *ITK;* p.Pro521Leu, the T > C variant change in the homozygous form for the patient (red box, III.1 lower panel) and not present in the uncle (II.2 upper panel), and a control (I.5).

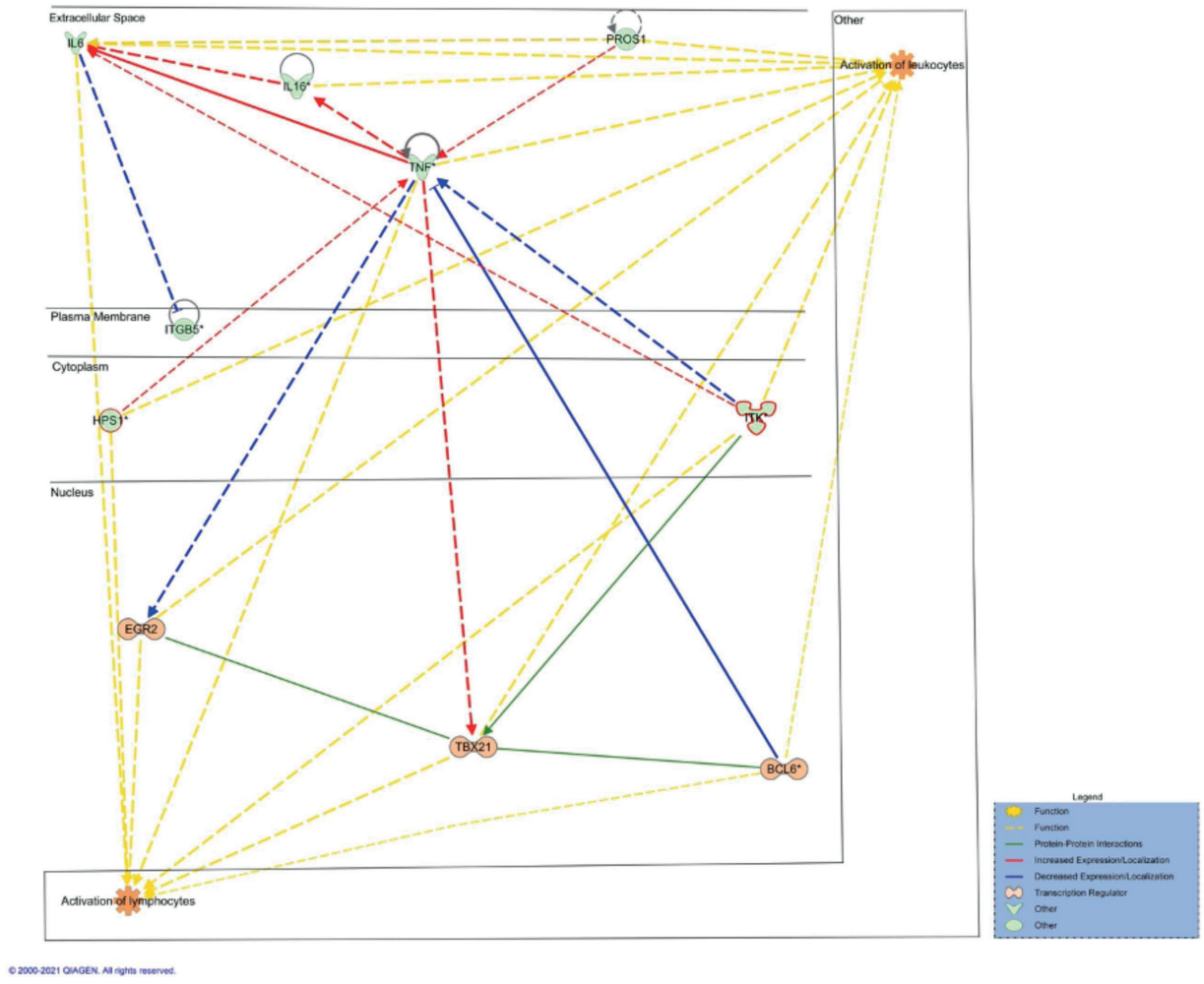

Figure 5. *ITK* and *HPS1* interactions and pathways using IPA software predictions tools.

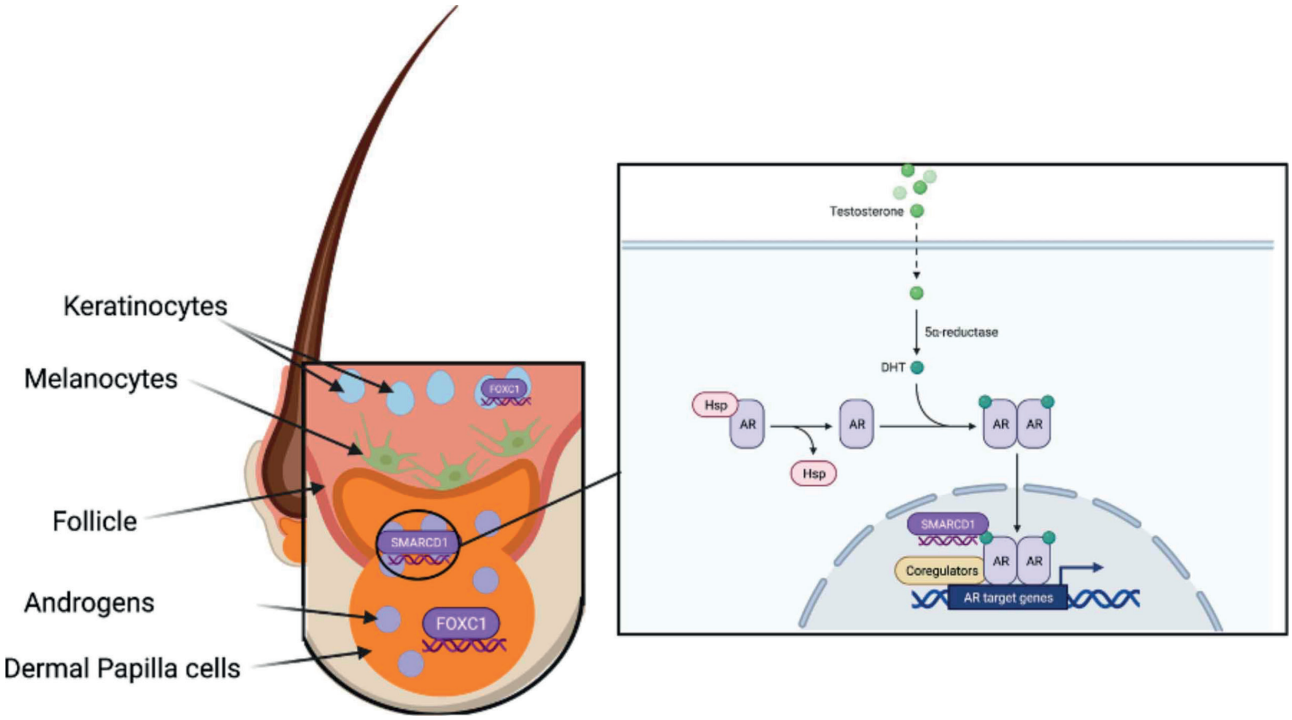

Figure 6. *FOXC1* And *SMARCD1* role in hair follicle development (anagen phase). *FOXC1* is expressed in dermal papilla cells and acts on keratinocytes differentiation while *SMARCD1* acts on AR signaling pathway.

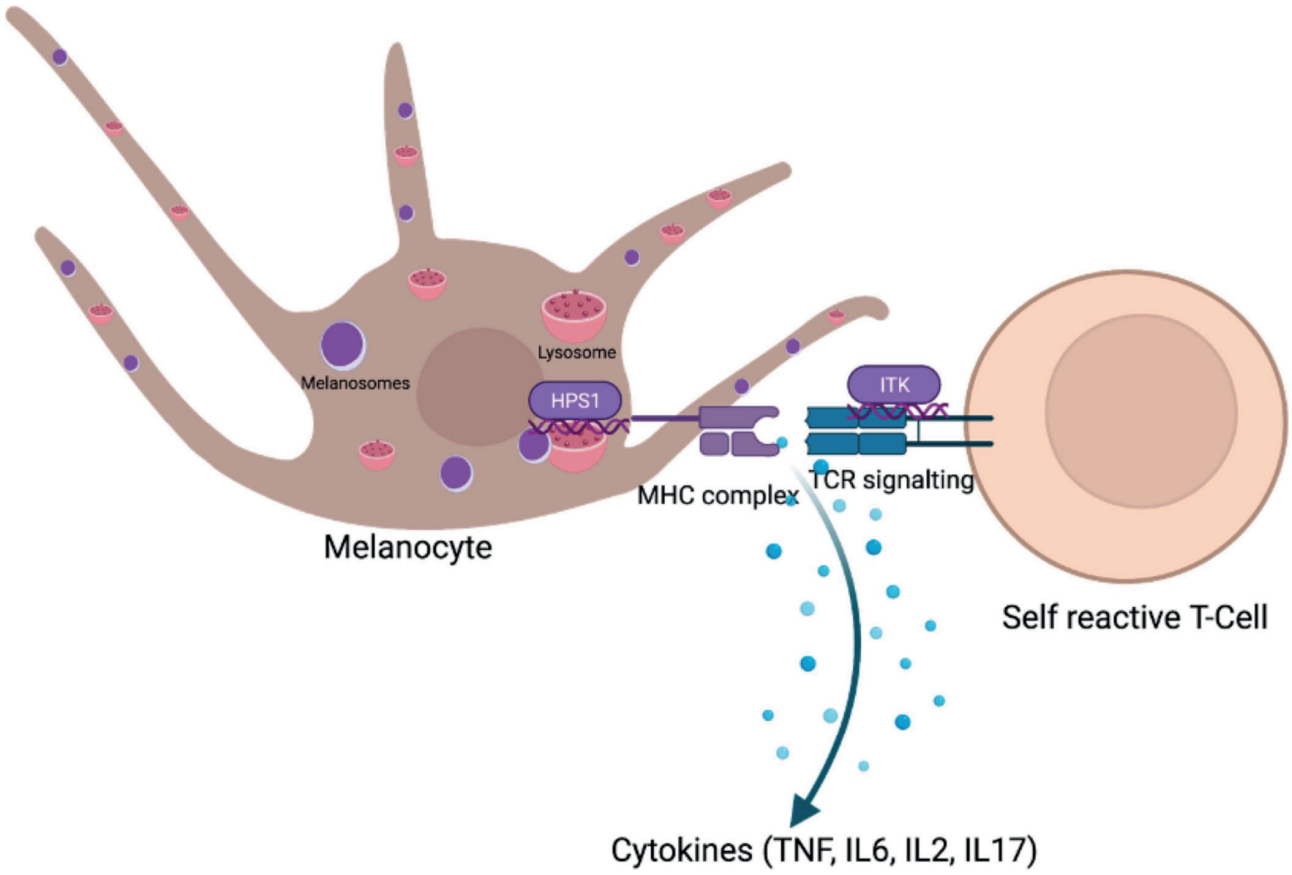

Figure 7. *HPS1* and *ITK* function in melanocyte and T-Cell. *HPS1 acts on LROS* such as melanosomes and lysosomes in a melanocyte while *ITK* plays a role in TCR signaling pathway in T-cell.

A Permissive Environment for Interaction: Trafficking a Kinase

A permissive environment is always a cordon for developing differential phenotypes within a familial case. This is the case of our second family presented with two kinds of pigmentation disorders: albinism and vitiligo, where epidermal/hair follicle melanocyte disruption is a common pathway between both disorders [41,42].

Since the hallmark of both diseases is melanocyte disruption, we looked first for the genes that encode melanocyte proteins associated with vitiligo such as *PTPN22, TYR,* and *MC1R [43]* . We then looked for specific genes associated with OCA. We did not find any pathogenic variant neither in any of the genes that encode components of the four protein complexes: adapter protein 3 (AP-3) and Biogenesis of Lysosome-related Organelles Complex 1, 2, and 3 (BLOC-1, BLOC-2, and BLOC-3) that are associate with the monogenic type, nor in the genes involved in the digenic mode of inheritance like *TYR* and *OCA2* or *SLC24A5 and OCA2* [44-47]. The only pathogenic mutation we uncovered is in the *HPS1* gene which lead to a premature stop codon and a truncated protein *HPS1* mutations which have been associated with OCA in several reports. *HPS1* (c.9C > A) mutation counted for 5% in nonconsanguineous Chinese patients, two frameshift variants in *HPS1* (c.9delC and c.1477delA) in children with OCA, and two other gene mutations in Puerto Ricans, a 16-base pair (bp) duplication in *HPS1* which accounted for 42.8% of OCA cases [48,49]. The homozygous HPS1 mutation accounts for the OCA phenotype in our family, but the heterozygous form alone does not explain the vitiligo phenotype. We hypothesized that *HPS1* could be a novel candidate gene in vitiligo since it is implicated in melanocyte activation, proliferation, and differentiation.*HPS1* is a BLOC-3 subunit which support the intracellular biogenesis and trafficking of lysosome and lysosome-related organelles (LROS) such as melanosomes, platelet dense bodies (also called delta granules), lamellar bodies of type II pneumocytes, and granule proteins of cytotoxic and suppressor T cells and natural killer (NK) cells. As such, mutations in *HPS1* leads to defective LROS trafficking and assembly [47,50]. By that, the proteins are mistargeted i.e., the proteins that are trafficked to plasma membrane are mistakenly targeted to intracellular organelles like lysosome. This disrupts normal cell-cell interactions and chemotactic detection of migrating melanocytes, affecting cell survival and proliferation. Consequently, it results in decreased melanocyte numbers in the epidermis and dermis and delayed melanocyte protein function. [51-53]. The disruption of the melanocytes occur due to factors like oxidative stress from Reactive Oxygen Species (ROS), innate immunity, or adaptive immunity and gene responsible for that are XRP1, *IFIH1, NLRP1, PTPRC* , *RERE*,CTLA4, FOXP1, *LPP, TSLP, IL12RA, GZMB,* [54-56] however none of these genes were altered in our case. Interestingly, we found a novel missense variant in *ITK* p. Pro521Leu in our indexed patient (homozygous) which was absent in the uncle (Figure 4E).

ITK (IL2 inducible T-cell kinase) belongs to TEC family kinase and is highly expressed in T cells and involved in T-cell receptor (TCR) signaling, cytokine release and differentiation regulation [57,58]. Mutations in *ITK* have been associated with benign inflammatory dermatoses that mimics cancer, eczema, lymphoproliferative disorder [59-62], and most importantly, a recent report have identified the same variant p.Pro521Leu in a patient with lymphoproliferative disorder where vitiligo was part of the phenotype [63]. Our IPA analysis revealed that ITK is inhibited in vitiligo patients (Table 2), suggesting that our mutation acts as a partial loss of function. From melanocyte disruption by immunity involvement, we propose a di-genic inheritance pattern between *HPS1* and *ITK* genes in vitiligo and found that *ITK* and *HPS1* interacts with each other through TNF (Figures 5 and 7).

We thus propose that a partial disruption of the TNF inhibition pathway imposed by the missense mutation in *ITK* combined with a partial gain of function through the truncated *HPS1* protein will lead to an exaggerated increase in TNF and other cytokines such as IL-2,IL-6, IL-17 which are frequently observed in patients with vitiligo [64]. We cannot exclude a direct interaction between the proteins that would potentially be potentiated by the loss of the C-terminal domain of *HPS1*. Despite the small sample size, this study validated the technologies and methodologies, identified challenges with software and sequencing, and established a reference center to serve the underserved MENA region. Moreover, the samples were well preserved despite the region's fluctuating temperatures, demonstrating the robustness of the protocols and paving the way for future large-scale studies.

Conclusion

In conclusion, this study unveils the culprit gene in familial cases of genodermatoses cases using WES, which is an important tool to draw genotype–phenotype correlation. Advances in the understanding of the disease mechanisms along with the changes in diagnostic patterns over time will likely contribute to the development of new therapeutic agents and better patient outcomes.

References

1. Cheraghlou S, Lim Y, Choate KA. Mosaicism in genodermatoses. *Clin Dermatol* 2020;38:408-20.DOI: 10.1016/j.clindermatol .2020.03.008.

2. Ko CJ, Atzmony L, Lim Y, et al. Review of genodermatoses with characteristic histopathology and potential diagnostic delay. *J Cutan Pathol* 2019;46:756-65. DOI: 10.1111/cup.13520.
3. Itin PH, Burger B. [Genodermatoses for practitioners--principles and concepts]. *Ther Umsch* 2010;67:483-5. DOI: 10.1024/0040-5930/a000081.
4. Yu Y, Mi Z, Fu X, et al. Digenic inheritance of KRT5 and KRT14 mutations in a family with epidermolysis bullosa simplex. *Australas J Dermatol* 2020;61:e267-e9. DOI: 10.1111/ajd.13236.
5. Basit S, Wali A, Aziz A, Muhammad N, Jelani M, Ahmad W. Digenic inheritance of an autosomal recessive hypotrichosis in two consanguineous pedigrees. *Clin Genet* 2011;79:273-81. DOI: 10.1111/j.1399-0004.2010.01455.x.
6. Box NF, Duffy DL, Chen W, et al. MC1R genotype modifies risk of melanoma in families segregating CDKN2A mutations. *Am J Hum Genet* 2001;69:765-73. DOI: 10.1086/323412. PMCID: PMC1226062.
7. Almutairi A, Amin MM, Rashwan MAM, et al. Digenic inheritance of IL-36RA and SEC61A1 mutations underlies generalized pustular psoriasis with hypogammaglobulinemia. *Clin Immunol* 2022;235:108930. DOI: 10.1016/j.clim.2022.108930.
8. Bouhanna P. Multifactorial classification of male and female androgenetic alopecia. *Dermatol Surg* 2000;26:555-61. DOI: 10.1046/j.1524-4725.2000.00009.x.
9. Piraccini BM, Alessandrini A. Androgenetic alopecia. *G Ital Dermatol Venereol* 2014;149:15-24.
10. Ezzedine K, Silverberg N. A Practical Approach to the Diagnosis and Treatment of Vitiligo in Children. *Pediatrics* 2016;138. DOI: 10.1542/peds.2015-4126.
11. Yaghoobi R, Omidian M, Bagherani N. Vitiligo: a review of the published work. *J Dermatol* 2011;38:419-31. DOI: 10.1111/j.1346-8138.2010.01139.x.
12. Okamura K, Suzuki T. Current landscape of Oculocutaneous Albinism in Japan. *Pigment Cell Melanoma Res* 2021;34:190-203. DOI: 10.1111/pcmr.12927.
13. Kadhi A, Hamie L, Tamer C, Nemer G, Kurban M. A Novel Pathogenic CDH3 Variant underlying Heredity Hypotrichosis Simplex detected by Whole-Exome Sequencing (WES)-A Case Report. *Cold Spring Harb Mol Case Stud* 2022;8. DOI: 10.1101/mcs.a006225.PMCID: PMC9528967.
14. Kramer A, Green J, Pollard J, Jr., Tugendreich S. Causal analysis approaches in Ingenuity Pathway Analysis. *Bioinformatics* 2014;30:523-30. DOI: 10.1093/bioinformatics/btt703. PMCID: PMC3928520.
15. Li MM, Datto M, Duncavage EJ, et al. Standards and Guidelines for the Interpretation and Reporting of Sequence Variants in Cancer: A Joint Consensus Recommendation of the Association for Molecular Pathology, American Society of Clinical Oncology, and College of American Pathologists. *J Mol Diagn* 2017;19:4-23. DOI: 10.1016/j.jmoldx.2016.10.002.PMCID: PMC5707196.
16. Steinhaus R, Proft S, Schuelke M, Cooper DN, Schwarz JM, Seelow D. MutationTaster2021. *Nucleic Acids Res* 2021;49:W446-W51. DOI: 10.1093/nar/gkab266.PMCID: PMC8262698.
17. Gudmundsson S, Karczewski KJ, Francioli LC, et al. Addendum: The mutational constraint spectrum quantified from variation in 141,456 humans. *Nature* 2021;597:E3-E4. DOI: 10.1038/s41586-021-03758-y.PMCID: PMC8410591.
18. Lek M, Karczewski KJ, Minikel EV, et al. Analysis of protein-coding genetic variation in 60,706 humans. *Nature* 2016;536:285-91. DOI: 10.1038/nature19057.PMCID: PMC5018207.
19. Genomes Project C, Auton A, Brooks LD, et al. A global reference for human genetic variation. *Nature* 2015;526:68-74. DOI: 10.1038/nature15393.PMCID: PMC4750478.
20. Gazzo A, Raimondi D, Daneels D, et al. Understanding mutational effects in digenic diseases. *Nucleic Acids Res* 2017;45:e140. DOI: 10.1093/nar/gkx557.PMCID: PMC5587785.
21. Gonzalez ME, Cantatore-Francis J, Orlow SJ. Androgenetic alopecia in the paediatric population: a retrospective review of 57 patients. *Br J Dermatol* 2010;163:378-85. DOI: 10.1111/j.1365-2133.2010.09777.x.
22. Vozza A, Piccolo V, Russo T, Vozza G. Familial androgenetic alopecia in siblings with normal endocrinological status. *Pediatr Dermatol* 2012;29:534-5. DOI: 10.1111/j.1525-1470.2011.01528.x.
23. Lolli F, Pallotti F, Rossi A, et al. Androgenetic alopecia: a review. Endocrine 2017;57:9-17. DOI: 10.1007/s12020-017-1280-y.
24. Randall VA. Androgens and hair growth. *Dermatol Ther* 2008;21:314-28. DOI: 10.1111/j.1529-8019.2008.00214.x.
25. Inui S, Itami S. Molecular basis of androgenetic alopecia: From androgen to paracrine mediators through dermal papilla. *J Dermatol Sci* 2011;61:1-6. DOI: 10.1016/j.jdermsci.2010.10.015.
26. Hillmer AM, Hanneken S, Ritzmann S, et al. Genetic variation in the human androgen receptor gene is the major determinant of common early-onset androgenetic alopecia. *Am J Hum Genet* 2005;77:140-8. DOI: 10.1086/431425.PMCID: PMC1226186.
27. Randall VA, Thornton MJ, Hamada K, Messenger AG. Mechanism of androgen action in cultured dermal papilla cells derived from human hair follicles with varying responses to androgens in vivo. *J Invest Dermatol* 1992;98:86S-91S. DOI: 10.1111/1523-1747.ep12462307.
28. Heinlein CA, Chang C. Androgen receptor (AR) coregulators: an overview. *Endocr Rev* 2002;23:175-200. DOI: 10.1210/edrv.23.2.0460.
29. Hillmer AM, Brockschmidt FF, Hanneken S, et al. Susceptibility variants for male-pattern baldness on chromosome 20p11. *Nat Genet* 2008;40:1279-81. DOI: 10.1038/ng.228.
30. Bamodu OA, Tzou KY, Lin CD, et al. Differential but Concerted Expression of HSD17B2, HSD17B3, SHBG and SRD5A1 Testosterone Tetrad Modulate Therapy Response and Susceptibility to Disease Relapse in Patients with Prostate Cancer. *Cancers (Basel)* 2021;13. DOI: 10.3390/cancers13143478.PMCID: PMC8303483.
31. Lewis MJ, Wiebe JP, Heathcote JG. Expression of progesterone metabolizing enzyme genes (AKR1C1, AKR1C2, AKR1C3, SRD5A1, SRD5A2) is altered in human breast carcinoma. *BMC Cancer* 2004;4:27. DOI: 10.1186/1471-2407-4-27.PMCID: PMC459223.
32. van de Wijngaart DJ, Dubbink HJ, Molier M, de Vos C, Trapman J, Jenster G. Functional screening of FxxLF-like peptide motifs identifies SMARCD1/BAF60a as an androgen receptor cofactor that modulates TMPRSS2 expression. *Mol Endocrinol* 2009;23:1776-86. DOI: 10.1210/me.2008-0280.PMCID: PMC5419170.
33. Nixon KCJ, Rousseau J, Stone MH, et al. A Syndromic Neurodevelopmental Disorder Caused by Mutations in SMARCD1, a Core SWI/SNF Subunit Needed for Context-Dependent Neuronal Gene Regulation in Flies. *Am J Hum Genet* 2019;104:596-610. DOI: 10.1016/j.ajhg.2019.02.001.PMCID: PMC6451697.
34. Bin L, Deng L, Yang H, et al. Correction: Forkhead Box C1 Regulates Human Primary Keratinocyte Terminal Differentiation. *PLoS One* 2018;13:e0191127. DOI: 10.1371/journal.pone.0191127.PMCID: PMC5755917.
35. Lay K, Kume T, Fuchs E. FOXC1 maintains the hair follicle stem cell niche and governs stem cell quiescence to preserve

long-term tissue-regenerating potential. *Proc Natl Acad Sci U S A* 2016;113:E1506-15. DOI: 10.1073/pnas.1601569113.PMCID: PMC4801248.
36. Wang L, Siegenthaler JA, Dowell RD, Yi R. Foxc1 reinforces quiescence in self-renewing hair follicle stem cells. *Science* 2016;351: 613-7. DOI: 10.1126/science.aad5440.PMCID: PMC4828140.
37. Hariri H, Kurban M, Al-Haddad C, et al. Degenerated hair follicle cells and partial loss of sebaceous and eccrine glands in a familial case of axenfeld-rieger syndrome: An emerging role for the FOXC1/NFATC1 genetic axis. *J Dermatol Sci* 2018;92: 237-44. DOI: 10.1016/j.jdermsci.2018.11.003.
38. Zhou LB, Cao Q, Ding Q, et al. Transcription factor FOXC1 positively regulates SFRP1 expression in androgenetic alopecia. *Exp Cell Res* 2021;404:112618. DOI: 10.1016/j.yexcr.2021.112618.
39. Kelberman D, Islam L, Holder SE, et al. Digenic inheritance of mutations in FOXC1 and PITX2 : correlating transcription factor function and Axenfeld-Rieger disease severity. *Hum Mutat* 2011;32:1144-52. DOI: 10.1002/humu.21550.
40. Xue H, Liu F, Ai Z, et al. FOXC1 Downregulates Nanog Expression by Recruiting HDAC2 to Its Promoter in F9 Cells Treated by Retinoic Acid. *Int J Mol Sci* 2021;22. DOI: 10.3390/ijms 22052255.PMCID: PMC7956269.
41. Njoo MD, Westerhof W. Vitiligo. Pathogenesis and treatment. *Am J Clin Dermatol* 2001;2:167-81. DOI: 10.2165/00128071 -200102030-00006.
42. Manga P, Kerr R, Ramsay M, Kromberg JG. Biology and genetics of oculocutaneous albinism and vitiligo - common pigmentation disorders in southern Africa. *S Afr Med J* 2013;103:984-8. DOI: 10.7196/samj.7046.
43. Jin Y, Andersen G, Yorgov D, et al. Genome-wide association studies of autoimmune vitiligo identify 23 new risk loci and highlight key pathways and regulatory variants. *Nat Genet* 2016;48: 1418-24. DOI: 10.1038/ng.3680.PMCID: PMC5120758.
44. Wei AH, Zang DJ, Zhang Z, Yang XM, Li W. Prenatal genotyping of four common oculocutaneous albinism genes in 51 Chinese families. *J Genet Genomics* 2015;42:279-86. DOI: 10.1016 /j.jgg.2015.05.001.
45. Sajid Z, Yousaf S, Waryah YM, et al. Genetic Causes of Oculocutaneous Albinism in Pakistani Population. *Genes (Basel)* 2021;12. DOI: 10.3390/genes12040492.PMCID: PMC8066997.
46. Marcon CR, Maia M. Albinism: epidemiology, genetics, cutaneous characterization, psychosocial factors. *An Bras Dermatol* 2019;94:503-20. DOI: 10.1016/j.abd.2019.09.023.PMCID: PMC6857599.
47. Huizing M, Helip-Wooley A, Westbroek W, Gunay-Aygun M, Gahl WA. Disorders of lysosome-related organelle biogenesis: clinical and molecular genetics. *Annu Rev Genomics Hum Genet* 2008;9:359-86.DOI:10.1146/annurev.genom.9.081307.164303. PMCID: PMC2755194.
48. Xu C, Xiang Y, Li H, et al. Genetic analysis and prenatal diagnosis of 20 Chinese families with oculocutaneous albinism. *J Clin Lab Anal* 2021;35:e23647. DOI: 10.1002/jcla.23647. PMCID: PMC7891544.
49. Santiago Borrero PJ, Rodriguez-Perez Y, Renta JY, et al. Genetic testing for oculocutaneous albinism type 1 and 2 and Hermansky-Pudlak syndrome type 1 and 3 mutations in Puerto Rico. *J Invest Dermatol* 2006;126:85-90. DOI: 10.1038/sj.jid .5700034.PMCID: PMC3560388.
50. Loredana Asztalos M, Schafernak KT, Gray J, Berry A, Paller AS, Mancini AJ. Hermansky-Pudlak syndrome: Report of two patients with updated genetic classification and management recommendations. *Pediatr Dermatol* 2017;34:638-46. DOI: 10.1111/pde.13266.
51. Nguyen T, Wei ML. Hermansky-Pudlak HPS1/pale ear gene regulates epidermal and dermal melanocyte development. *J Invest Dermatol* 2007;127:421-8. DOI: 10.1038/sj.jid.5700566.
52. Richmond B, Huizing M, Knapp J, et al. Melanocytes derived from patients with Hermansky-Pudlak Syndrome types 1, 2, and 3 have distinct defects in cargo trafficking. *J Invest Dermatol* 2005;124:420-7. DOI: 10.1111/j.0022-202X.2004.23585.x. PMCID: PMC1635963.
53. Wei ML. Hermansky-Pudlak syndrome: a disease of protein trafficking and organelle function. *Pigment Cell Res* 2006;19:19-42. DOI: 10.1111/j.1600-0749.2005.00289.x.
54. Fain PR, Gowan K, LaBerge GS, et al. A genomewide screen for generalized vitiligo: confirmation of AIS1 on chromosome 1p31 and evidence for additional susceptibility loci. *Am J Hum Genet* 2003;72:1560-4. DOI: 10.1086/375451.PMCID: PMC1180316.
55. Czajkowski R, Mecinska-Jundzill K. Current aspects of vitiligo genetics. *Postepy Dermatol Alergol* 2014;31:247-55. DOI: 10.5114/pdia.2014.43497.PMCID: PMC4171675.
56. Martina JA, Moriyama K, Bonifacino JS. BLOC-3, a protein complex containing the Hermansky-Pudlak syndrome gene products HPS1 and HPS4. *J Biol Chem* 2003;278:29376-84. DOI: 10.1074/jbc.M301294200.
57. Hu J, Sahu N, Walsh E, August A. Memory phenotype CD8+ T cells with innate function selectively develop in the absence of active Itk. *Eur J Immunol* 2007;37:2892-9. DOI: 10.1002 /eji.200737311.PMCID: PMC2770953.
58. Lechner KS, Neurath MF, Weigmann B. Role of the IL-2 inducible tyrosine kinase ITK and its inhibitors in disease pathogenesis. *J Mol Med (Berl)* 2020;98:1385-95. DOI: 10.1007/s00109-020 -01958-z.PMCID: PMC7524833.
59. Litvinov IV, Netchiporouk E, Cordeiro B, et al. The Use of Transcriptional Profiling to Improve Personalized Diagnosis and Management of Cutaneous T-cell Lymphoma (CTCL). *Clin Cancer Res* 2015;21:2820-9. DOI: 10.1158/1078-0432.CCR-14-3322.PMCID: PMC4470792.
60. Gomez-Rodriguez J, Meylan F, Handon R, et al. Itk is required for Th9 differentiation via TCR-mediated induction of IL-2 and IRF4. *Nat Commun* 2016;7:10857. DOI: 10.1038 /ncomms10857.PMCID: PMC4782063.
61. Graves PE, Siroux V, Guerra S, Klimecki WT, Martinez FD. Association of atopy and eczema with polymorphisms in T-cell immunoglobulin domain and mucin domain-IL-2-inducible T-cell kinase gene cluster in chromosome 5 q 33. *J Allergy Clin Immunol* 2005;116:650-6. DOI: 10.1016/j.jaci.2005.05.004.
62. Linka RM, Risse SL, Bienemann K, et al. Loss-of-function mutations within the IL-2 inducible kinase ITK in patients with EBV-associated lymphoproliferative diseases. *Leukemia* 2012;26:963-71. DOI: 10.1038/leu.2011.371.
63. Wallace JG, Alosaimi MF, Khayat CD, et al. ITK deficiency presenting as autoimmune lymphoproliferative syndrome. *J Allergy Clin Immunol* 2021;147:743-5 e1. DOI: 10.1016/j.jaci .2020.06.019.PMCID: PMC7779661.
64. Sushama S, Dixit N, Gautam RK, Arora P, Khurana A, Anubhuti A. Cytokine profile (IL-2, IL-6, IL-17, IL-22, and TNF-alpha) in vitiligo-New insight into pathogenesis of disease. *J Cosmet Dermatol* 2019;18:337-41. DOI: 10.1111/jocd. 12517.