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ORIGINAL PAPER

In silico analysis of gene expression data from bald frontal and haired occipital scalp to identify candidate genes in male androgenetic alopecia

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Abstract

Androgenetic alopecia (AGA) is a progressive dermatological disorder of frontal and vertex scalp hair loss leading to baldness in men. This study aimed to identify candidate genes involved in AGA through an in silico search strategy. The gene expression profile GS36169, which contains microarray gene expression data from bald frontal and haired occipital scalps of five men with AGA, was downloaded from the Gene Expression Omnibus (GEO) database. The differential gene expression analysis for all five subjects was carried out separately by PUMA package in R and identified 32 differentially expressed genes (DEGs) common to all five subjects. Gene ontology (GO) biological process and pathway- enrichment analyses of the DEGs were conducted separately for the up-regulated and down-regulated genes. ReactomeFIViz app was utilized to construct the protein functional interaction network for the DEGs. Through GO biological process and pathway analysis on the clusters of the Reactome FI network, we found that the down-regulated DEGs participate in Wnt signaling, TGF-beta signaling, and up-regulated DEGs participate in oxidative-stress- related pathways.

Keywords Androgenetic alopecia · Differential gene expression · Functional interaction network · Gene ontology · Wnt β-catenin signaling · Transforming growth factor-β signaling · Deleted in azoospermia 1 · Hair growth

Abbreviations

HBA1	Hemoglobin subunit alpha 1
HBB	Hemoglobin subunit beta
CD163	CD163 molecule
JUN	Jun proto-oncogene
PSORS1C2	Psoriasis susceptibility 1 candidate 2
PTGDS	Prostaglandin D2 synthase
LGR5	Leucine-rich repeat containing G-protein-coupled receptor 5
NPPA	Natriuretic peptide A
CORIN	Corin serine peptidase
RPS27A	Ribosomal protein S27a
EP300	E1A binding protein p300
COMP	Cartilage oligomeric matrix protein
BAMBI	BMP and activin membrane bound inhibitor
FGF18	Fibroblast growth factor 18
UBC	Ubiquitin C
SPOP	Speckle type BTB/POZ protein
DZIP1	DAZ interacting zinc finger protein 1
DAZ1	Deleted in azoospermia 1
KRT7	Keratin 7
KRT86	Keratin 86
KRT16	Keratin 16
KRT35	Keratin 35
KRT33A	Keratin 33A
KRT83	Keratin 83
KRT32	Keratin 32
KRT31	Keratin 31
KRT85	Keratin 85
KRT2	Keratin 2
EVPL	Envoplakin
TCHH	Trichohyalin
KRT75	Keratin 75

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KRT81	Keratin 81
KRT33B	Keratin 33B
LCE4A	Late cornified envelope 4A
KRTAP10-8	Keratin-associated protein 10-8
KRTAP4-7	Keratin-associated protein 4-7
KRTAP10-5	Keratin-associated protein 10-5
KRTAP2-3	Keratin-associated protein 2-3

Introduction

Androgenetic alopecia (AGA) is a progressive dermatological disorder of scalp hair loss leading to baldness with potential psychosocial ramifications [12, 31]. It is a unisex disorder and its prevalence is more common in men than women [31]. Pattern baldness is naturally experienced by men as a stressful condition which lowers their self-esteem and body image satisfaction [4]. Especially young men with adolescent AGA have worries about aging, feelings of diminished attractiveness, and helplessness [20]. The visible progression of scalp hair loss in male pattern baldness (MPB) is attributed to the conversion of large thick pigmented terminal hairs into small fine non-pigmented vellus hair through miniaturization process of hair follicles mediated by 5α-dihydrotestosterone [32].

In AGA, scalp hair loss continues in a progressive manner if left untreated [16]. Currently, topical minoxidil (Rogaine) 2–5% solutions and oral finasteride 1 mg (Propecia) are the only two synthetic drugs, approved by medical agencies, available to treat AGA with limited efficacy. In addition, new therapies like prostaglandins, latanoprost, growth factors, stem cell therapy, platelet-rich plasma therapy, scalp micro-needling, and topical androgens are practiced off-label [31]. However, an effective treatment for AGA is not available yet as the etiology of AGA is poorly understood [15]. Hence, further insight into the underlying pathogenesis is required to facilitate drug discovery and improve treatment approaches that would reverse hair loss completely.

In 2012, Luis Garza et al., carried out microarray gene expression analysis to identify differentially expressed genes (DEGs) between balding and non-balding scalp regions in Caucasian males of age 40–65 with AGA who were not treated with finasteride or minoxidil [11]. They employed analysis of variance test to rank differentially expressed genes with respect to the tissue site (haired versus bald), irrespective of the individuals, and considered the top 250 ranked genes as DEGs. In the analysis, they found prostaglandin D_2 synthase (PTGDS) as one of the top elevated genes and the subsequent work focused on the role of PTGDS and its product prostaglandin D_2 (PGD_2) in hair follicle miniaturization. However, a comprehensive bioinformatics analysis was not performed on the DEGs obtained to identify candidate genes in the balding process. In this paper, the same data of Luis Garza et al. [11] are utilized to screen DEGs in the balding site based on different methods and thresholds. Gene ontology, pathway-enrichment studies were conducted for the DEGs and Reactome functional network for the proteins of the DEGs was constructed to identify the pathways involved.

Materials and methods

Microarray data

The Gene Expression Omnibus (GEO) is an international public repository for functional genomics data supporting high-throughput microarray and next-generation sequence experimental datasets submitted by researchers around the globe [3]. The gene expression profile GSE36169, containing the raw expression dataset of haired and bald scalp samples from five Caucasian males (age 40–65 years) with AGA, submitted by Luis A. Garza and George Cotsarelis in the GEO database (https://www.ncbi.nlm.nih.gov/geo/), was downloaded for our analysis. The biopsied scalp samples were sequenced on the platform GPL96 [HG-U133A] Affymetrix Human Genome U133A Array [11].

Data preprocessing and differential gene expression analysis

The differential gene expression analysis was carried out individually for all five subjects by comparing their respective expression profile of haired and bald scalp to scrutinize inter-subject variability. Expression set object was generated from raw data using the multi-mgMOS method from PUMA package of Bioconductor [23, 29]. The DEGs were identified through Probability of Positive Log Ratio (PPLR) method implemented in the PUMA package. The PPLR values were converted into *p*-like values using the function "*p*LikeValues" to compare results from methods that provide true *p* values [23, 29]. At the outset for all five subjects, differentially expressed probes in the bald area compared to haired site complying the threshold criteria $\log_2 FC > 0.6$ and *p*-like value < 0.05 were generated separately. The probes were annotated with official gene symbols and the probes for which gene annotations were not available were discarded. In addition, for multiple probes corresponding to one gene their average expression value was considered as the gene expression value of that candidate gene [37]. Then, the DEGs which are common to five, any four out of five, any three out of five, and any two out of five subjects were sorted out. Finally, the DEGs, which were equidirectionally regulated in all five subjects, filtered with an inclusion criterion of $\log_2 FC > 1$ and *p*-like value < 0.02 were chosen and considered for further analyses.

Functional- and pathway-enrichment analyses

The gene ontology (GO) functional- and pathway-enrichment analyses were conducted by researchers to identify items in gene lists that may have relevance to the biological question being investigated [43]. GO (biological process and molecular function) and pathway analysis were done using ToppGene Suite (https://toppgene.cchmc.org/) (updated: Feb 2019), which is a one-stop portal for functional- and pathway-enrichment analysis of gene lists. The portal is based on databases such as Gene Ontology, the Kyoto Encyclopedia of Genes and Genomes (KEGG), BioCarta, BioCyc, Reactome, GenMAPP, and Molecular Signature Database (MsigDB), etc. [5]. Using the ToppFun function in the ToppGene Suite, GO and pathway-enrichment analysis for the DEGs were carried out. The default parameters such as probability density function for the *p*-value method and FDR correction were selected. Gene count > 2 and 0.01 as FDR B&H *q* value were chosen as significant cut off level for GO analysis.

Reactome protein functional interaction network

ReactomeFIViz v7.1.0 is a Cytoscape app that probes Reactome pathways and looks for disease-related pathways and network patterns utilizing the Reactome functional interaction (FI) network [41, 42] constructed, based on the popular biological pathway knowledgebase Reactome (https://reactome.org/) [6, 9]. This app enables us to construct a FI network for a set of genes, allowing us to query the FI data source, perform functional-enrichment analysis of the network nodes and also expand the network by finding genes related to the query gene set provided [41]. An expanded FI network with linker genes from the 2017 Reactome FI network version was constructed for the proteins of the DEGs. Cluster analysis of the FI network was performed. Then, GO BP and pathway-enrichment analysis for the cluster modules were carried out to identify the alopecia-related pathways in which the DEGs participate.

Results

Identification of differentially expressed genes in men with AGA

The total number of DEGs obtained for each subject in the differential gene expression analysis is given in Table 1. On comparing the DEGs mined for five subjects (Online Resource 1) and retaining only the DEGs which were equidirectionally regulated among them, we observed that 32 genes were common to all 5 subjects, 45 genes to any 4 of the 5 subjects, 69 genes to any 3 subjects, and 121 DEGs common to any 2 of the 5 subjects (Fig. 1). The list of equidirectionally regulated DEGs common to all five, to any four, and to any three subjects are given in the Online Resource 2. Finally, a total of 32 equidirectionally regulated DEGs which are differentially expressed in all five subjects fulfilling the selection criteria $\log_2$FC > 1 and *p*-like value < 0.02 were selected for further analysis (Table 2).

Table 1 Results of differential gene expression analysis between bald and haired scalp in all five subjects

Subjects	Total number of DEGs in bald scalp compared with haired scalp	Up-regulated genes	Down-regulated genes
1	174	26	148
2	67	17	50
3	153	74	79
4	165	92	73
5	153	90	63

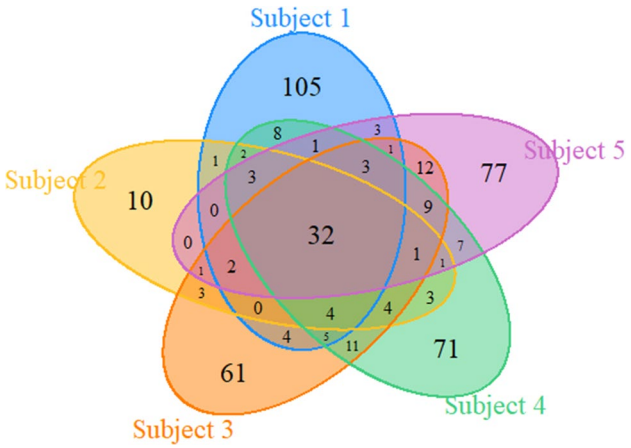

Fig. 1 Venn plot depicting the number of common DEGs equidirectionally regulated between the subjects

Elevated gene transcripts

We were intrigued to find that mRNA transcript of only four genes viz. HBA1, HBB, CORIN, and PTGDS was elevated in the bald scalp of all five men with AGA. The transcripts of the gene HBA1 and HBB contain information to make proteins, alpha-globin and beta-globin, which are components of the larger protein hemoglobin A (HbA) [25]. While we were unclear about the role of hemoglobin in scalp hair growth, GO and pathway analysis provided insights into the functions of hemoglobin (Tables 3, 4, and 5). Oxidative stress and reactive oxygen species, such as hydrogen peroxide, are presumed to be involved in hair aging and age-dependent alopecia [24, 36, 39]. Additionally, accumulation of lipid peroxides and free radicals in hair follicles which

Table 2 List of equidirectionally regulated DEGs common to all five subjects in the bald scalp compared to haired scalp

Gene symbol	$\log_2$ FC	PPLR	*p*-like value
Up-regulated genes			
HBA1	4.58	0.999027	0.001946
HBB	3.36	0.998753	0.002494
CORIN	1.69	0.992685	0.01463
PTGDS	1.54	0.99785	0.0043
Down-regulated genes			
KRT81	− 7.21	0.006004	0.012008
KRT33B	− 5.84	0.000165	0.00033
KRT83	− 5.25	0.004144	0.008289
KRTAP2-3	− 5.24	6.82E-05	0.000136
LOC100653049	− 5.05	3.21E-05	6.43E-05
KRT33A	− 4.90	5.19E-07	1.04E-06
KRTAP9-9	− 4.51	1.98E-08	3.95E-08
KRT35	− 4.49	0.0002	0.000399
KRTAP1-1	− 4.42	5.93E-06	1.19E-05
KRTAP1-3	− 4.01	9.11E-08	1.82E-07
KRT31	− 3.91	4.37E-09	8.77E-09
LY6G6F	− 3.91	0.000392	0.000785
GPRC5D	− 3.66	0.001289	0.002577
PSORS1C2	− 3.63	0.004324	0.008648
KRT86	− 3.62	0.000247	0.000493
KRT85	− 3.56	8.60E-07	1.72E-06
COMP	− 3.55	0.000795	0.00159
S100A3	− 3.45	7.22E-05	0.000144
KRTAP4-7	− 3.30	7.35E-07	1.47E-06
FGF18	− 3.26	0.010531	0.021063
KRT32	− 2.78	0.002706	0.005413
TCHH	− 2.01	0.000144	0.000289
KRT75	− 2.00	0.002279	0.004557
LGR5	− 1.99	0.003295	0.00659
DAZ1	− 1.96	0.004949	0.009898
LRRC15	− 1.65	0.004553	0.009106
KRT16	− 1.49	0.005181	0.010363
BAMBI	− 1.24	0.00232	0.00464

$\log_2$ FC, PPLR and *p*-like values are given as mean values for the DEGs expressed in multiple subjects

p-like values are defined as values between 0 and 1, where 0 identifies the highest probability of being differentially expressed, and 1 identifies the lowest probability of being differentially expressed

p-like values are used to compare results from methods that provide true p-values

p-like value = 1 – abs (2 × (PPLR − 0.5)). [Source: PUMA reference manual December 11, 2018]

PPLR probability of positive log ratio, *HBA1* hemoglobin subunit alpha 1, *HBB* hemoglobin subunit beta, *CORIN* corin serine peptidase, *PTGDS* prostaglandin D2 synthase, *KRT81* keratin 81, *KRT33B* keratin 33B, *KRT83* keratin 83, *KRTAP2-3* keratin-associated protein 2-3, *LOC100653049* keratin type 1 cuticular Ha4, *KRT33A* keratin 33A, *KRTAP9-9* keratin-associated protein 9-9, *KRT35* keratin 35, *KRTAP1-1* keratin-associated protein 1-1, *KRTAP1-3* keratin-associated protein 1-3, *KRT31* keratin 31, *LY6G6F* lymphocyte antigen 6 family member G6F, *GPRC5D* G-protein-coupled receptor class C group 5 member D, *PSORS1C2* psoriasis susceptibility 1 candidate 2, *KRT86* keratin 86, *KRT85* keratin 85, *COMP* cartilage oligomeric matrix protein, *S100A3* S100 calcium-binding protein A3, *KRTAP4-7* keratin-associated protein 4-7, *FGF18* fibroblast growth factor 18, *KRT32* keratin 32, *TCHH* trichohyalin, *KRT75* keratin 75, *LGR5* leucine-rich repeat containing G-protein-coupled receptor 5, *DAZ1* deleted in azoospermia 1, *LRRC15* leucine-rich repeat containing 15, *KRT* 16 keratin 16, *BAMBI* BMP and activin membrane bound inhibitor

induce catagen phase [39] also correlate with the BP terms enriched for HBA1, HBB genes such as cellular detoxification, response to hydrogen peroxide radicals, and MF terms such as peroxidase activity, oxidoreductase activity, antioxidant activity (Tables 2 and 4).

Prostaglandin D_2 synthase (PTGDS) and Corin serine peptidase (CORIN) are the other two elevated gene transcripts in bald scalp. PTGDS is an enzyme that catalyzes the isomerization of prostaglandin H_2 to prostaglandin D_2 which is shown to inhibit hair follicle regeneration in mice through the G protein-coupled receptor Gpr44 [28]. Corin serine peptidase is an atrial natriuretic peptide-converting enzyme and it regulates blood-pressure volume [25]. Though its expression has been detected in mice- and human-dermal papilla cells, unfortunately, its biological significance in skin and hair growth is not understood yet [44].

Down-regulated gene transcripts

The transcripts for hair keratin and keratin-associated proteins were the top down-regulated genes in the bald scalp as one would expect (Table 2). Genes belonging to the hair keratin type I family (KRT16, KRT31, KRT32, KRT33A, KRT33B, KRT35, LOC100653049), keratin type II family (KRT75, KRT81, KRT83, KRT85, KRT86) and keratin-associated protein family (KRTAP1-1, KRTAP1-3, KRTAP2-3, KRTAP4-7, KRTAP9-9) [21, 25, 34] were the common keratin genes down-regulated in all five subjects (Online Resource 1). Pathway-enrichment analysis showed that these genes participate in keratinization and developmental biology pathway (Table 5). The mRNA transcript for GPRC5D, an orphan G-protein-coupled receptor induced by retinoic acid, found in hard-keratinized structures such as hair shaft and nail is down-regulated. In hair follicles, this GPTC5D gene is found to be expressed during mid- and late-anagen, and catagen phases but not at the time of telogen and early-anagen phases [14]. However, the specific function of GPRC5D is not determined yet [25]. TCHH (trichohyalin), a large structural protein present in the inner root sheath of anagen hair follicles, which mediates keratin filamentous assembly and provides mechanical strength is also down-regulated in the gene expression [38].

Table 3 Result of GO biological process analysis of DEGs by ToppGene Suite

GO ID	Biological process term	Gene count	*q* value	Genes enriched
Up-regulated genes				
GO:0015671	Oxygen transport	2	6.43E-04	HBB, HBA1
GO:0015669	Gas transport	2	6.43E-04	HBB, HBA1
GO:0042744	Hydrogen peroxide catabolic process	2	6.43E-04	HBB, HBA1
GO:0015701	Bicarbonate transport	2	1.57E-03	HBB, HBA1
GO:0042743	Hydrogen peroxide metabolic process	2	1.57E-03	HBB, HBA1
GO:0098869	Cellular oxidant detoxification	2	3.49E-03	HBB, HBA1
GO:1990748	Cellular detoxification	2	3.49E-03	HBB, HBA1
GO:0098754	Detoxification	2	3.49E-03	HBB, HBA1
GO:0003014	Renal system process	2	5.49E-03	HBB, CORIN
GO:0042542	Response to hydrogen peroxide	2	6.56E-03	HBB, HBA1
GO:0051291	Protein heterooligomerization	2	6.56E-03	HBB, HBA1
GO:0003050	Regulation of systemic arterial blood pressure by atrial natriuretic peptide	1	6.66E-03	CORIN
GO:0030185	Nitric oxide transport	1	8.75E-03	HBB
GO:0008217	Regulation of blood pressure	2	8.75E-03	HBB, CORIN
Down-regulated genes				
GO:0008544	Epidermis development	8	1.18E-06	GPRC5D,TCHH, KRT16, LGR5, KRT31, KRT32, KRT83, KRT85
GO:0042633	Hair cycle	5	3.33E-05	GPRC5D, KRT16, LGR5, KRT33B, KRT83
GO:0042303	Molting cycle	5	3.33E-05	GPRC5D, KRT16, LGR5, KRT33B, KRT83
GO:0031424	Keratinization	3	3.27E-03	GPRC5D, TCHH, KRT16
GO:0060429	Epithelium development	8	5.77E-03	GPRC5D, TCHH, KRT16, LGR5, KRT31, KRT32, KRT83, KRT85

Table 4 Result of GO molecular function analysis of DEGs by ToppGene Suite

GO ID	Molecular function term	Gene count	*q* value	Genes enriched
Up-regulated genes				
GO:0031720	Haptoglobin binding	2	3.41E-06	HBB, HBA1
GO:0005344	Oxygen carrier activity	2	5.17E-05	HBB, HBA1
GO:0004601	Peroxidase activity	2	2.89E-04	HBB, HBA1
GO:0016684	Oxidoreductase activity, acting on peroxide as acceptor	2	2.89E-04	HBB, HBA1
GO:0019825	Oxygen binding	2	2.89E-04	HBB, HBA1
GO:0016209	Antioxidant activity	2	5.37E-04	HBB, HBA1
GO:0020037	Heme binding	2	1.37E-03	HBB, HBA1
GO:0046906	Tetrapyrrole binding	2	1.37E-03	HBB, HBA1
GO:0005506	Iron ion binding	2	1.61E-03	HBB, HBA1
GO:0004667	Prostaglandin-D synthase activity	1	1.93E-03	PTGDS
GO:0031721	Hemoglobin alpha binding	1	1.93E-03	HBB
GO:0043177	Organic acid binding	2	2.74E-03	PTGDS, HBA1
GO:0005215	Transporter activity	3	3.20E-03	PTGDS, HBB, HBA1
GO:0030492	Hemoglobin binding	1	4.04E-03	HBB
Down-regulated genes				
GO:0005198	Structural molecule activity	13	1.70E-10	KRT16, COMP, KRT75, KRT31, KRT32, KRT33A, KRT33B, KRT35, KRT81, KRT83, KRT85, KRT86, KRTAP1-3

Table 5 Result of pathway-enrichment analysis by ToppGene Suite

Reactome ID	Pathway name	Gene count	*q* value	Genes enriched
Up-regulated genes				
1270225	Erythrocytes take up oxygen and release carbon dioxide	2	6.94E-05	HBB, HBA1
1270224	Erythrocytes take up carbon dioxide and release oxygen	2	6.94E-05	HBB, HBA1
1270223	O_2/CO_2 exchange in erythrocytes	2	6.94E-05	HBB, HBA1
1269898	Scavenging of heme from plasma	2	1.22E-04	HBB, HBA1
1269897	Binding and uptake of ligands by scavenger receptors	2	5.21E-04	HBB, HBA1
1339122	Physiological factors	1	7.09E-03	CORIN
1270088	Synthesis of prostaglandins (PG) and thromboxanes (TX)	1	8.43E-03	PTGDS
Down-regulated genes				
1457790	Keratinization	17	6.49E-24	KRTAP2-3, KRTAP4-7, KRTAP9-9, TCHH, KRT16, KRT75, KRT31, KRT32, KRT33A, KRT33B, KRT35, KRT81, KRT83, KRT85, KRT86, KRTAP1-1, KRTAP1-3
1270302	Developmental biology	18	1.02E-13	KRTAP2-3, KRTAP4-7, KRTAP9-9, TCHH, KRT16, KRT75, KRT31, KRT32, KRT33A, KRT33B, KRT35, KRT81, KRT83, FGF18, KRT85, KRT86, KRTAP1-1, KRTAP1-3

In addition to above, genes such as S100A3 (S100 calcium-binding protein A3), which is known to normally express in elevated amounts in differentiating cuticular cells within the hair follicle and inferred to be involved in epithelial differentiation leading to hair shaft formation [19], FGF18 (fibroblast growth factor 18), which regulates the hair-cycle resting phase telogen [18] and induces anagen phase in the telogen stage hair follicle in mice [17], LY6G6F (lymphocyte antigen 6 family member G6F), a type I transmembrane protein of Immunoglobin superfamily that comprises cell surface proteins involved in cellular recognition and immune system [7], COMP (cartilage oligomeric matrix protein), which produces a noncollagenous extracellular matrix (ECM) protein and controls the epithelilal-mesenchymal interactions in hair follicle cycle [2], Wnt target gene LGR5 (leucine-rich repeat containing G-protein-coupled receptor 5), which actively expresses in hair follicle progenitor cells and plays a vital role in building new hair follicle during hair cycles [13, 40], and BAMBI (BMP and activin membrane bound inhibitor), a positive regulator of Wnt/β-catenin pathway, negative regulator of TGF-β, activin, and BMP pathways [22, 35] were the other hair growth-related genes that were down-regulated.

We also noted multiple probes for the gene deleted in azoospermia 1 (DAZ1) down-regulated during differential gene expression analysis in all five subjects. It is a candidate for human azoospermia factor in the Y chromosome and plays an essential role in spermatogenesis. The expression of DAZ1 gene is limited to germ cells in testis and their expression levels in scalp hair follicles are unknown [25]. Hence, further confirmative experimental studies on the expression of DAZ1 in the hair follicle are needed to understand its role in hair growth. LRRC15 (leucine-rich repeat containing 15) and PSORS1C2 (psoriasis susceptibility 1 candidate 2) whose functions are unknown, but known to express in hair follicles in mammals [1, 10] were also down-regulated. A short description of the function of all DEGs obtained and their role in hair growth and alopecia is given in Online Resource 2.

Functional interaction network

The FI network constructed by ReactomeFIViz app had 38 nodes (including 4 up-regulated, 20 down-regulated and 14 linker genes) and 82 edges (Fig. 2). The linker genes are related genes used by ReactomeFIViz app based on our query DEG to construct an expanded network that helps in identifying the pathways in which the DEGs are involved [42]. The FI network had a clustering coefficient of 0.223; network diameter of 9; network density of 0.117 and 4.316 average number of neighbors. FI cluster analysis revealed five cluster modules in the network, of which top three modules (Online Resource 5) with cut off criteria of five or more than five nodes was selected for GO BP and pathway enrichment analysis. The first cluster had 15 nodes, second had 13 nodes and the third cluster had 5 nodes (Table 6).

Biological process and pathway-enrichment analysis on the clusters (Online Resource 6 and 7) revealed that the proteins of the DEGs: BAMBI, LGR5, and FGF18 were enriched for the BP terms such as positive regulation of

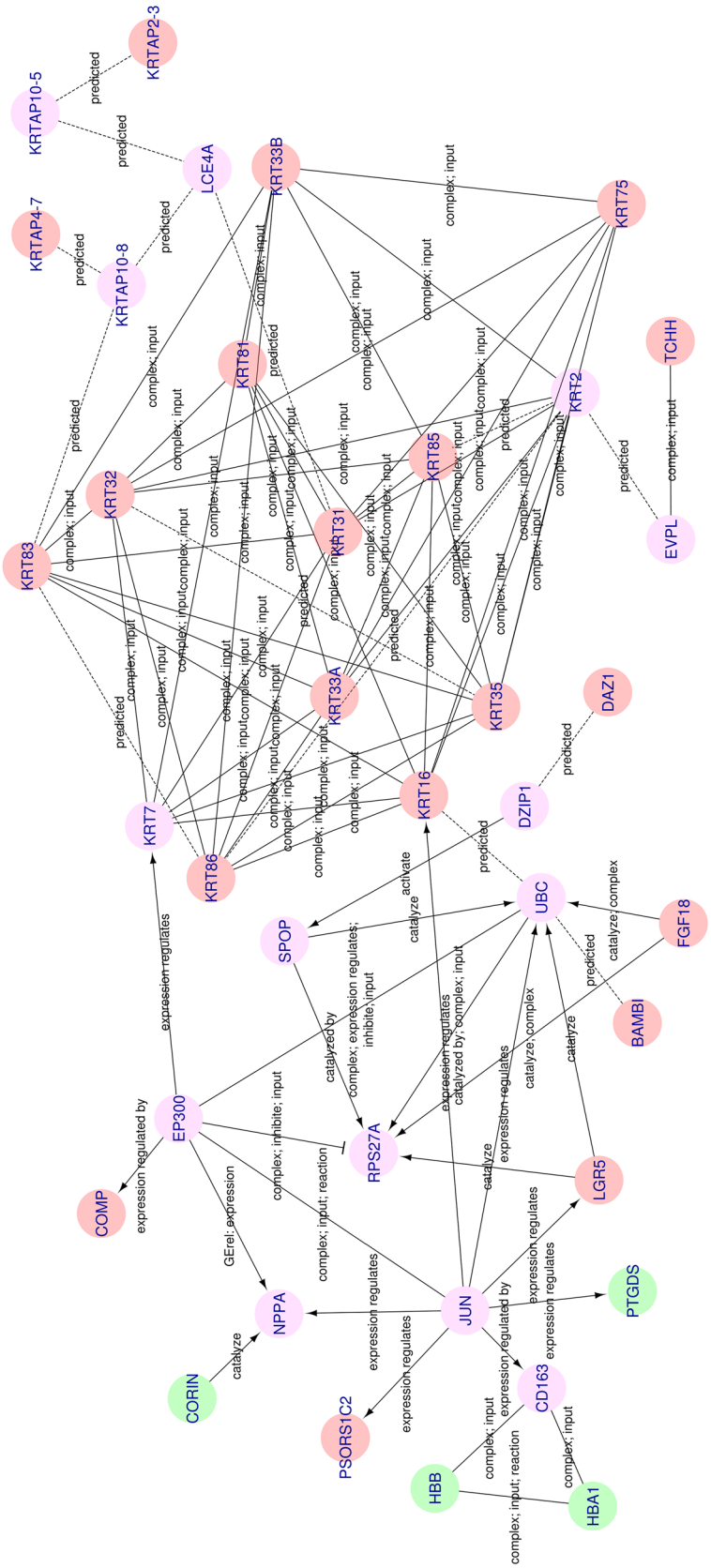

Fig. 2 Functional interaction network generated by Reactome FIViz app. The nodes are represented as circles and edges as lines. Green and red nodes indicate up-regulated and down-regulated genes, respectively. The pink nodes are linker genes used by ReactomeFIViz app to construct an expanded network that helps in identifying the pathways in which the DEGs are involved

Table 6 Cluster analysis

Cluster module	Nodes	Up-regulated nodes	Down-regulated nodes	Linker nodes
1	15	CORIN, PTGDS	BAMBI, COMP, PSORS1C2, LGR5, FGF18, DAZ1	DZIP1, EP300, JUN, NPPA, RPS27A, SPOP, UBC
2	13	–	KRT16, KRT31, KRT32, KRT33A, KRT33B, KRT35, KRT75, KRT81, KRT83, KRT85, KRT86	KRT2, KRT7
3	5	–	KRTAP4-7, KRTAP2-3	KRTAP10-5, KRTAP10-8, LCE4A

canonical Wnt-signaling pathway, negative regulation of TGF-β receptor-signaling pathway and FGF-receptor-signaling pathway. In addition Reactome pathways, such as TCF-dependent signaling in response to Wnt, signaling by TGF-β receptor complex, TGF-β signaling pathways, and FGF-receptor signaling, which are involved in normal hair follicle morphogenesis and hair cycle were enriched in pathway analysis for these DEGs [33].

Discussion

The DEGs obtained in our analysis are consistent with the original analysis reports of Garza et al. as the transcripts for hemoglobin subunits HBB, HBA1, and PTGDS were up-regulated while transcripts for hair keratin were down-regulated in the bald scalp [11]. In addition to the above, we found that the transcript for serine peptidase CORIN up-regulated, while the mRNA templates for the genes LY6G6F, PSORS1C2, S100A3, GPRC5D, COMP, FGF18, TCHH, LRRC15, DAZ1, BAMBI, and LGR5 were down-regulated in bald scalp (Table 2). Among these DEGs, studies by Garza et al. and Miao et al. have already verified the up-regulation of PTGDS and down-regulation of PSORS1C2, TCHH by quantitative real-time PCR and western blot in the bald scalps of men with AGA [11, 26].

Similar gene expression analyses between bald and haired scalp have been carried out before [8, 26, 27], but in all these studies differential gene expression analysis was performed with respect to the site and not with respect to individuals. In our analysis, we found many genes differentially expressing in the bald site of individual subjects, but only 32 DEGs were common to all the 5 subjects (Online Resources 1 and 2). Since the bald scalps from the frontal region and haired scalps from the occipital region are obtained from same individuals it is necessary to scrutinize inter-subject variability.

Generally, mRNA level and protein abundance correlation in a cell is considered to be positive in a wide range of cell types and organisms [30], but this need not be true because the mRNA levels may be affected by translational efficiency and protein stability. However, alteration in mRNA levels can be considered as an indicator of altered activity of a pathway in which the encoded gene participates. Although we have studied only the mRNA expression levels of the genes between bald and haired scalp, the obtained DEGs enriched in pathways that are necessary for hair growth. Further studies on protein expression levels of these genes and gene knockout studies may help us in identifying druggable targets for AGA.

Conclusion

In conclusion, by performing separate differential gene expression analyses with respect to subjects and tissue sites, we found 32 equidirectionally regulated DEGs common to all five subjects. Gene ontology and pathway-enrichment analysis for these DEGs revealed that the down-regulated genes are involved in keratinization, skin development, and hair cycle. Reactome FI network construction showed that the down-regulated genes LGR5 and BAMBI were involved in Wnt- and TGF-β-signaling pathways. Apart from genes coding for hair keratin and keratin-associated proteins, we also report the down-regulation of mRNA transcripts for DAZ1, a Y chromosome-linked gene, and LRRC15 of unknown biological significance in hair growth.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

Informed consent No original data are collected for this analysis, hence this study did not meet the criteria for ethical approval and formal consent is not required.

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