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Microarray analysis of androgenetic and senescent alopecia: Comparison of gene expression shows two distinct profiles

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Androgenetic alopecia (AGA) is characterized by androgenmediated miniaturization of the hair follicle in susceptible individuals. Senescent alopecia (SA) is the diffuse scalp hair thinning that is seen with advanced age even in individuals without a family history of hair loss. Differences in follicular counts, anagen/telogen percentages, and terminal/vellus hair ratios have been reported [1]. In a pilot study, older males showed nearly a two-fold decrease in levels of androgen receptors, 5-alpha reductase 1 and 2, and aromatase compared to young males with AGA [2]. However, the concept of whether SA is a definable entity distinct from AGA remains controversial.

Here, we used microarray analysis to compare gene expression profiles in AGA and SA in order to characterize novel aspects of their pathology and to identify new gene targets. The three groups of men in this study were age-matched and included: *Group 1-Controls* had no visible hair thinning. *Group 2-AGA* had male pattern hair thinning that was established to have occurred prior to age 30 and *Group 3-SA* had diffuse hair thinning that had its onset after the age of 60. RNA from scalp biopsies was isolated from each group (N = 10, pooled) and the gene expression was assessed on Affymetrix GeneChip Human U133Plus 2.0 microarrays as described [3]. Genes with fold changes of <-2 and >2 and false discovery rate (FDR) <0.05 were considered to be part of the expression profiles. A total of 1200 differentially expressed genes (DEGs) in AGA and 1360 in SA were identified compared to controls. Of these, 442 genes were unique to AGA, 602 genes were unique to SA and 758 genes were common to both AGA and SA.

Hair/skin development and function is the most significant physiological function altered in both AGA and SA, however, the DEGs in this category differed in the two diseases. Table 1 shows the 34 genes in this category that are differentially regulated in AGA that contribute to hair follicle development, morphology and cycling (BARX2, EGFR, INHBA, MSX2, OVOL1, KRTs, KRTAPs, RUNX3 and TIMP3). Many of these genes required for hair

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jdermsci.2013.06.017.

follicle homeostasis are significantly under expressed in AGA but not in SA compared to normal scalp tissue (Table 1 and Figure S1). Our data (Table 1 & Figure S1) showed that the Androgen Receptor (AR) is up regulated in AGA, but not in SA. Previous studies [4] have shown that genetic variability in AR is a prerequisite for the development of early-onset AGA. A novel AGA susceptibility locus has been identified at 17q21.31 [5]. In our dataset, the DEAD box polypeptide 5 (DDX5), a transcriptional regulator of AR [6] is down regulated in AGA and maps to this locus.

The most significant pathway altered in AGA is Notch Signaling which consists of 29 genes (Table 1) including HES1, Notch2, Notch4 and PROX1 that are known to play a role in cell fate determination [7]. The down regulated genes in this pathway in AGA include CNTN1, JAG1, NOTCH2 and PSEN1 and the genes that are up regulated include DTX3, HES and NOTCH4. The expression patterns of Notch signaling pathway genes including Notch 2 and JAG1 were validated by real-time PCR (Figure S1). Jagged1 (JAG1) gene which encodes a ligand for Notch receptor maps to chromosome 20p a susceptibility locus for male-pattern baldness [8]. A reciprocal negative feedback regulation exists between Notch and AR-dependent pathways in the prostate [9]. The activation of AR and the concomitant loss of Notch signaling may be contributing factors to hair follicle miniaturization and may serve as the mechanistic link between prostate cancer and AGA. Thus, modulating the Notch signaling pathway in AGA may lead to future therapies.

In contrast to AGA, the 15 genes unique to SA (Table 2) (CALML5, CCND1, COL7A1, CTGF, GLI2, KRT15, KRT2, MYC, NAB1, POU2F3, FOS, FYN, JUNB, ID2, PPARA) participate in skin and epidermal development, keratinocyte proliferation, differentiation and cell cycle regulation. A number of transcription (FOS, FYN, JUN, JUNB, MYC, NAB1) and growth factors (CTGF, TGFα) are significantly decreased in SA. The expression patterns of c-MYC, CTGF and TGFα were validated by real-time PCR in independent sets of AGA and SA scalp biopsies (Figure S2).

The most significant canonical pathway altered in SA is Neuregulin signaling (Table 2). Neuregulins (NRG) belong to the epidermal growth factor (EGF) family of growth factors and are ligands of the ErbB receptors. The 21 genes in this pathway that are significantly down-regulated include EGFR signaling pathway ligands (AREG, BTC, EREG, TGFα), transcription factors (EGR1, FOS, JUN, MYC) and associated genes (ADAM17, HSP90AB1, ERBB2IP, PTPN11, DCN, PSEN1, ITGA2, ITGB1). The neuregulin pathway genes that are up-regulated include kinases (PDPK1, AKT2, AKT3) and apoptotic genes (BAD, RAF, SOS1). The altered expression of neuregulin pathway genes in SA was further confirmed in independent scalp biopsies by real-time PCR (Figure S2). Although the role of neuregulins are not fully understood, previous studies have shown an important role for EGFR signaling pathway in the differentiation of the hair follicle and normal hair development [10]. Recent studies have implicated neuregulin and EGF signaling pathways with longevity and lifespan. Thus, loss of these signaling pathways may contribute to hair aging and senescent alopecia.

The differences in gene expression profiles suggest that AGA and SA may represent two independent hair disorders and that non-androgen pathways may also contribute to hair loss.

This study provides novel therapeutic targets for the prevention or treatment of two common hair disorders.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Genes and pathways altered in Androgenetic Alopecia (AGA).

Symbol	Cytoband[a]	Entrez gene name	Affymetrix ID	Fold change
Hair and skin development and function genes altered in AGA				
AR	Xq12	Androgen receptor	211110_s_at	2.46
AREG/AREGB	4q13.3	Amphiregulin	205239_at	−2.3
ARNTL2	12p12.2-p11.2	Aryl hydrocarbon receptor nuclear translocator-like 2	224204_x_at	−12.13
ATP2A2	12q24.11	ATPase, Ca+ + transporting, cardiac muscle, slow twitch 2	212362_at	−2.46
BARX2	11q25	BARX homeobox 2	210419_at	−4.92
BTC	4q13.3	Betacellulin	241412_at	−2.64
DAB2	5p13	Disabled homolog 2, mitogen-responsive phosphoprotein (Drosophila)	210757_x_at	−2
DCT	13q32	Dopachrome tautomerase	205338_s_at	2.3
DDX5	17q21	DEAD (Asp-Glu-Ala-Asp) box polypeptide 5	225886_at	−2
EGFR	7p12	Epidermal growth factor receptor	201983_s_at	2
GAB1	4q31.21	GRB2-associated binding protein 1	226002_at	−2
INHBA	7p15-p13	Inhibin, beta A	210511_s_at	3.25
IVL	1q21	Involucrin	214599_at	−2
KLK6	19q13.3	Kallikrein-related peptidase 6	204733_at	−3.48
KRT14	17q12-q21	Keratin 14	209351_at	2
KRT27	10q32.1	Keratin 27	240388_at	−4.92
KRT32	17q21.2	Keratin 32	207146_at	−2.64
MAFF	22q13.1	v-maf musculoaponeurotic fibrosarcoma oncogene homolog F (avian)	36711_at	−2
MLL	11q23	Myeloid/lymphoid or mixed-lineage leukemia (trithorax homolog, Drosophila)	1565436_s_at	3.25
MST4	Xq26.2	Serine/threonine protein kinase MST4	224407_s_at	−2.83
MSX2	5q35.2	Msh homeobox 2	205555_s_at	−2
NRAS	1p13.2	Neuroblastoma RAS viral (v-ras) oncogene homolog	202647_s_at	−2.64
OGT	Xq13	O-linked N-acetylglucosamine (GlcNAc) transferase	207564_x_at	−3.73
OVOL1	11q13	Ovo-like 1(Drosophila)	206604_at	−2
PRKCI	3q26.3	Protein kinase C, iota	213518_at	−2.3
PSEN1	14q24.3	Presenilin 1	207782_s_at	−2.14
PTPRK	6q22.2-q22.3	Protein tyrosine phosphatase, receptor type, K	233609_at	2.3
RHOB	2p24	Ras homolog gene family, member B	1553962_s_at	−3.48
RUNX3	1p36	Runt-related transcription factor 3	204198_s_at	4.59
SGK3	8q12	Serum/glucocorticoid regulated kinase family, member 3	243264_s_at	3.48
TGFBR3	1p33-p32	Transforming growth factor, beta receptor III	204731_at	3.48
TGM1	14q11.2	Transglutaminase 1	206008_at	−2.14
TIMP3	22q12.3	TIMP metallopeptidase inhibitor 3	201149_s_at	4.29
TNFRSF19	13q12.11-q12.3	Tumor necrosis factor receptor superfamily, member 19	223827_at	−2.64
Notch signaling pathway genes altered in AGA				
ANK3	10q21	Ankyrin 3, node of Ranvier	209442_x_at	2.14
CNTN1	12q11-q12	Contactin 1	227209_at	−2.46

Symbol	Cytoband[a]	Entrez gene name	Affymetrix ID	Fold change
DCP1A	3p21.1	DCP1 decapping enzyme homolog A	218508_at	2.3
DNER	2q36.3	Delta/notch-like EGF repeat containing	226281_at	2.64
ESRRG	1q41	Estrogen-related receptor gamma	207981_s_at	3.25
FAM49A	2p24.2	Family with sequence similarity 49, member A	208092_s_at	−2.14
GAS1	9q21.3-q22	Growth arrest-specific 1	204456_s_at	−2.3
GUCY1A3	4q31.1-q31.2	Guanylate cyclase 1, soluble, alpha 3	221942_s_at	2.3
HES1	3q28-q29	Hairy and enhancer of split 1	203394_s_at	3.03
HOXA5	7p15.2	Homeobox A5	213844_at	2.46
HOXC6	12q13.3	Homeobox C6	206858_s_at	2.3
JAG1	20p12.1-p11.23	Jagged 1	209098_s_at	−2.46
PNRC2	1p36.11	Proline-rich nuclear receptor coactivator 2	222406_s_at	−2.3
MLL	11q23	Myeloid/lymphoid or mixed-lineage leukemia	1565436_s_at	3.25
NOTCH2	1p13-p11	Notch 2	210756_s_at	−2.64
NOTCH4	6p21.3	Notch 4	205247_at	2
PHF20	q11.23	PHD finger protein 20	206567_s_at	2.46
PROX1	1q41	Prospero homeobox 1	207401_at	3.25
PTN	7q33	Pleiotrophin	209465_x_at	−3.25
RUNX3	1p36	Runt-related transcription factor 3	204198_s_at	4.59
SDC2	8q22-q23	Syndecan 2	212158_at	−2.64
SFRP1	8p11.21	Secreted frizzled-related protein 1	202035_s_at	3.73
SLC17A6	11p14.3	Solute carrier family 17	220551_at	4
SP4	7p15.3	Sp4 transcription factor	206663_at	2.14
SSR1	6p24.3	Signal sequence receptor, alpha	200890_s_at	−2.46
WFDC2	20q13.12	WAP four-disulfide core domain 2	203892_at	2.3
WNT2	7q31.2	Wingless-type MMTV integration site family member 2	205648_at	2.3
ZEB1	10p11.2	Zinc finger E-box binding homeobox 1	210875_s_at	−2.64
ZNF24	18q12	Zinc finger protein 24	203247_s_at	2.14

Fold changes are indicated for each gene significantly under or overexpressed ($p < 0.05$, fold change >2) in androgenetic alopecia (AGA) compared to normal scalp. Positive data indicates overexpressed and negative data indicates under-expressed in AGA scalp.

[a]Gene location obtained from National Center for Biotechnology Information public database (http://www.ncbi.nlm.nih.gov).

Table 2

Genes and pathways altered in Senescent Alopecia (SA).

Symbol	Cytoband[a]	Entrez gene name	Affymetrix ID	Fold change
Hair and skin development and function genes in SA				
CCND1	11q13	Cyclin D1	214019_at	2.14
COL7A1	3p21.1	Collagen, type VII, alpha 1	204136_at	2
CTGF	6q23.1	Connective tissue growth factor	209101_at	−3.25
EMP1	12p12.3	Epithelial membrane protein 1	201325_s_at	−2.3
FOS	14q24.3	FBJ murine osteosarcoma viral oncogene homolog	209189_at	−24.25
FYN	6q21	FYN oncogene related to SRC, FGR, YES	212486_s_at	−2
GLI2	2q14	GLI family zinc finger 2	207034_s_at	2
ID2	2p25	Inhibitor of DNA binding 2, dominant negative helix-loop-helix protein	213931_at	2.3
JUN	1p32-p31	Jun proto-oncogene	201466_s_at	−2.14
JUNB	19p13.2	Jun B proto-oncogene	201473_at	−2.64
KLK7	19q13.41	Kallikrein-related peptidase 7	205778_at	−3.03
KRT2	12q13.13	Keratin 2	207908_at	−2.46
KRT13	17q12-q21.2	Keratin 13	207935_s_at	2.46
KRT15	17q21.2	Keratin 15	204734_at	2.14
MYC	8q24.21	v-myc myelocytomatosis viral oncogene homolog (avian)	202431_s_at	−2.3
NAB1	2q32.3-q33	NGFI-A binding protein 1 (EGR1 binding protein 1)	208047_s_at	−2
POU2F3	11q23.3	POU class 2 homeobox 3	215355_at	2.14
PPARA	22q13.31	Peroxisome proliferator-activated receptor alpha	223437_at	2
PTPRK	6q22.2-q22.3	Protein tyrosine phosphatase, receptor type, K	233609_at	4.92
Neuregulin signaling in SA				
ADAM17	2p25	ADAM metallopeptidase domain 17	205746_s_at	−2.83
AKT2	q13.2	v-akt murine thymoma viral oncogene homolog 2	236664_at	3.03
AKT3	1q44	v-akt murine thymoma viral oncogene homolog 3	242876_at	2.14
AREG/AREGB	4q13.3	Amphiregulin	205239_at	−2.64
BAD	11q13.1	BCL2-associated agonist of cell death	232660_at	13
BTC	4q13.3	Betacellulin	241412_at	−2
DCN	12q21.33	Decorin	209335_at	−2.14
ERBB2IP	5q12.3	erbb2 interacting protein	222473_s_at	−2
EREG	4q13.3	epiregulin	205767_at	−4
HSP90AB1	6p12	Heat shock protein 90 kDa alpha (cytosolic), class B member 1	1557910_at	−2
ITGA2	5q11.2	Integrin, alpha 2	227314_at	−2
ITGB1	10p11.2	Integrin, beta 1	1553678_a_at	−2.14
MYC	8q24.21	v-myc myelocytomatosis viral oncogene homolog	202431_s_at	−2.3
PDPK1	16p13.3	3-Phosphoinositide dependent protein kinase-1	204524_at	2.64
PICK1	22q13.1	Protein interacting with PRKCA 1	204746_s_at	2.3
PSEN1	14q24.3	Presenilin 1	207782_s_at	−2
PTPN11	12q24	Protein tyrosine phosphatase, non-receptor type 11	209895_at	−2.64
RAF1	3p25	v-raf-1 murine leukemia viral oncogene homolog 1	1557675_at	2

Symbol	Cytoband[a]	Entrez gene name	Affymetrix ID	Fold change
SOS1	2p21	Son of sevenless homolog 1	1557354_at	3.03
SOS2	14q21	Son of sevenless homolog 2	211665_s_at	−2.46
TGFA	2p13	Transforming growth factor, alpha	205016_at	−2.14

Fold changes are indicated for each gene significantly under or overexpressed ($p < 0.05$, fold change >2) in senescent alopecia (SA) compared to normal scalp. Positive data indicates overexpressed and negative data indicates under-expressed in SA scalp.

[a]Gene location obtained from National Center for Biotechnology Information public database (http://www.ncbi.nlm.nih.gov).