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Global Expression Profile and Global Genome Methylation Signatures in Male Patients with Androgenetic Alopecia

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Editor,

Androgenetic alopecia (AGA) or male pattern baldness is the most common form of hair loss in humans occurring in more than half of men aged over 50 years. Hormonal and genetic factors trigger AGA. Although genomic studies in follicle biopsies and cell cultures have suggested the involvement of BMP2, EphrinA3, PTGDS, PGD2, and AR, no integrative analyses have been

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performed to identify novel potential drug targets for AGA. The aim of this study was to identify differentially expressed genes and methylation patterns in the balding and occipital zones of AGA patients.

Seventeen hispanic patients were included: 15 patients (aged 18 to 52 years old) with clinically and dermatoscopic confirmed diagnosis of AGA (men: II-VI Hamilton-Norwood) and 2 controls (21-22 years old). Men previously treated with finasteride or minoxidil were excluded, as were men with any chronic degenerative disease. Two 3-mm scalp biopsies were collected from each subject (affected area and occipital auto-control). RNA and DNA from scalp biopsies was isolated and quantified.

Microarray expression analyses were performed in all biopsies using Affymetrix GeneChip Human U133Plus 2.0. arrays. DNA methylation studies were performed in 10 patients (affected area and occipital auto-control) with SureSelectXT Methyl-Seq Library Prep Kit and platform HiSeq2500. Bioinformatic and statistical analyses were used to correlate findings. The altered expression of *WNT3* and *HSD17B6* genes in AGA was further confirmed in scalp biopsies by real time PCR.

We identified 53 differentially expressed genes (DEGs) ($p < 0.001$) (Fig. 1) in bald versus non-bald occipital scalp, including chromosome open reading frame and zinc finger (ZNF) genes. Twenty-eight genes were overexpressed and 25 genes were underexpressed. Genes overexpressed in bald scalp belong to gene families previously implicated in the pathophysiology of AGA such as *WNT3* and *BMP8*. *WNT3* plays an important role in cell-cell signaling during morphogenesis and is expressed in developing and mature hair follicles. Unexpectedly, we found overexpression of *WNT3* in AGA. Overexpression of *WNT3* in transgenic mouse produces a short hair phenotype due to altered differentiation of stem cells in the shaft hair¹. *HSD17B6* has not previously been associated with an alopecia phenotype. *HSD17B6* gene codes for 3 α -hydroxysteroid dehydrogenase in the human prostate which catalyzes the back-conversion of 3 α -diol to DHT². Variants of this gene are associated with hyperandrogenism and the polycystic ovary syndrome characterized by alopecia, in addition to acne, obesity, hirsutism and female infertility³. *HSD17B6* participates in different pathways including steroid hormone biosynthesis and retinol metabolism⁴. This study corroborates that genes previously associated with AGA including

PTGDS, *GSN*, *MUC7*, *INSR*, *SDHA1*, *CYB5R4* and *PCDHGB7* are overexpressed in the balding scalp of patients with AGA ($p < 0.05$)^{5–10}.

Global DNA methylation analysis showed 18 differentially methylated regions (DMRs) between the balding and the occipital biopsies of AGA patients ($p < 0.05$, percent methylation difference $\geq 20\%$). DMRs were located in 14 genes (introns and exons) such as *NINL*, *P2RY1*, *HTRA1*, *ENPP2*, *CCDC140*, *NKX6-1*, *PRMD6*, *BC035889*, *PHLDA3*, *PPP6R1*, *CCDC37*, *OPRM1*, *HOXA2* and *SFRP4* (Table 1). This last gene is interesting because its product is a known inhibitor of the Wnt pathway. Differences in methylation patterns were not observed in promoter regions or regulatory elements. No correlations between the two studies were identified.

The study confirms the importance of androgen metabolism and the Wnt pathway in the pathophysiology of AGA and suggest novel targets for treatment of AGA.

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Figure 1. Heatmap showing the differentially expressed genes. 53 genes were selected from a paired t-test in affected men comparing occipital versus affected area at $p < 0.001$. Controls are included for comparison.

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Table 1. Differentially methylated genes in AGA.

Chromosome	Position	Gene	p value	Diff. Meth.*
Chr1	201435227	PHLDA3 (exon 3/3, 2/2)	5.396201e-08	-20.82551
Chr2	223167318	CCDC140 (intron 1/1)	4.626573e-09	20.17769
Chr3	126134444	CCDC37 (intron 4/16, 3/15)	3.986833e-10	-22.50432
Chr3	152554234	P2RY1 (exon 1/1)	1.316469e-08	20.10041
Chr4	85416853	NKX6-1 (exon 2/3)	9.322602e-18	20.71495
Chr4	85417998	NKX6-1 (intron 1/2)	3.697748e-10	20.08823
Chr5	122434402	PRMD6 (intron 2/7,1/4)	9.184402e-14	20.42755
Chr6	154363174	OPRM1 (intron 1/3)	1.119863e-12	-22.09411
Chr7	27140423	HOXA2 (exon 2/2)	4.200568e-11	-25.12542
Chr7	27140432	HOXA2 (exon 2/2)	1.843863e-09	-22.43274
Chr7	27161809	BC035889 (intron 1/3)	2.868651e-13	-20.94260
Chr7	27161818	BC035889 (intron 1/3)	1.530802e-14	-22.31751
Chr7	27161827	BC035889 (intron 1/3)	5.278968e-10	-20.26263
Chr7	37955293	SFRP4 (intron 1/5)	2.828403e-09	-20.98769
Chr8	120593817	ENPP2 (intron 19/25)	1.525533e-18	20.12326
Chr10	124254714	HTRA 1 (intron 3/8)	3.665708e-08	22.82619
Chr19	55765907	PPP6R1 (intron 1/23)	2.194094e-08	-20.66135
Chr20	25478206	NINL (intron 9/23)	5.477733e-08	20.59715

*Diff. Meth. (differential methylation). Percentage of difference in methylation regarding control (negative values = hypomethylation, positive values = hypermethylation).

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