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## Similar Response Patterns to 5% Topical Minoxidil Foam in Frontal and Vertex Scalp of Men with Androgenetic Alopecia: A Microarray Analysis

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### Summary

**Background**—There are regional variations in scalp hair miniaturization seen in androgenetic alopecia (AGA). Use of topical minoxidil can lead to reversal of miniaturization in the vertex scalp. However, its effects on other scalp regions are less well studied.

**Methods**—A placebo controlled double-blinded prospective pilot study of minoxidil topical foam 5% (MTF) vs placebo was conducted in sixteen healthy men ages 18-49 with Hamilton-Norwood type IV-V thinning. The subjects were asked to apply the treatment (active drug or placebo) to the scalp twice daily for eight weeks. Stereotactic scalp photographs were taken at the baseline and final visits to monitor global hair growth. Scalp biopsies were done at the leading edge of hair loss from the frontal and vertex scalp before and after treatment with MTF and placebo and microarray analysis was done using the Affymetrix GeneChip HG U133 Plus 2.0.

**Results**—Global stereotactic photographs showed that MTF induced hair growth in both the frontal and vertex scalp of AGA patients. Regional differences in gene expression profiles were observed before treatment. However, MTF treatment induced the expression of hair keratin associated genes and decreased the expression of epidermal differentiation complex (EDC) and inflammatory genes in both scalp regions.

**Conclusions**—These data suggest that MTF is effective in the treatment of both the frontal and vertex scalp of AGA patients.

### Introduction

Androgenetic alopecia (AGA), also known as male pattern balding or hereditary thinning, is the most common type of hair loss. It has been reported that up to 50% of both men and women will manifest some degree of AGA by age 50<sup>1</sup>. The patterned hair loss in AGA seen

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both in men and women can occur as early as the teens, twenties and thirties. The pathophysiology of AGA has been extensively studied and well characterized, particularly in men<sup>2,3</sup>. The scalp regions affected by the miniaturization process<sup>4</sup> in men are the frontal hairline and the top and vertex scalp, while the temporo-occipital region is largely unaffected even in men with extensive balding<sup>5</sup>. These regional variations in patterns of scalp hair thinning may reflect differences in embryologic scalp patterning<sup>6</sup>, levels of hormonal receptors<sup>7</sup> or other factors that may influence follicular growth.

Currently there are two medications approved by the US Food and Drug Administration (FDA) for hair regrowth and reversal of miniaturization in androgenetic alopecia: topical minoxidil and oral finasteride<sup>8,9</sup>. The mechanism of action of topical minoxidil on hair regrowth is not fully understood<sup>10</sup>. Minoxidil is a potent vasodilator that acts through nitric oxide pathways and as a potassium channel opener<sup>11</sup>, but its hair re-growth properties appear to be independent of its vasodilation properties. It has been suggested that human hair follicles contain two forms of ATP-sensitive potassium channels, only one of which is sensitive to minoxidil<sup>11</sup>. Since the clinical trials of topical minoxidil in men with AGA evaluated only hair growth properties of the vertex scalp<sup>12,13</sup>, it is unclear whether topical minoxidil might be effective in other scalp regions which are also susceptible to hair miniaturization.

In this study we sought to determine whether scalp biopsies from men with AGA show variable expression of genes before and after 8 weeks of treatment with minoxidil topical foam 5% (MTF) vs placebo. Our second aim was to determine whether microarray gene expression profiles in the frontal scalp would be the same as that seen in the vertex scalp.

## Materials and Methods

A placebo controlled double-blinded prospective study was conducted. Institutional review board (IRB) approval was obtained and subjects were recruited by the Skin Study Center at Case Western Reserve University (CWRU), Cleveland OH. Sixteen healthy men ages 18-49 with Hamilton-Norwood type IV-V thinning were enrolled for this pilot study. The patients were advised to use Progain shampoo for all hair cleansing purposes during the study and apply treatment products according to the instructions provided.

Global hair photographs of AGA patients were taken before and after treatment with minoxidil to monitor hair growth. Analysis of the photographs taken with a stereotactic device has emerged as a standard technique for monitoring hair growth and volume<sup>14</sup>. At the baseline visit, stereotactic scalp photographs were taken and scalp biopsies were done at the leading edge of hair loss from the frontal and vertex scalp. The subjects were instructed to apply the treatment (active drug or placebo) as per the manufacturer's instructions "half a capful of 5% MTF or placebo topically to the affected area two times a day." Since the onset of action for 5% Rogaine has been reported to be 8 weeks, we selected this time point to identify early changes in gene expression profiles after treatment with 5% MTF (Rogaine Extra Strength for Men package insert (Pharmacia & Upjohn Consumer Healthcare —US), Rec 2/98).

A blinded evaluation of the stereotactic photographs was done to monitor global hair regrowth on a “yes or no scale” and the subjects were categorized as either responders or non-responders. For the purpose of this study, a responder was defined as a subject who showed hair growth based on stereotactic photographs after 8 weeks treatment with 5% MTF. At the final visit, repeat stereotactic scalp photographs were taken and two scalp biopsies were done contra-lateral to where the first scalp biopsies were done at visit 0. Biopsies were obtained with written consent from patients in accordance with ethical standards of IRB and with the Helsinki Declaration of 1975, as revised in 1983.

Scalp biopsy samples were snap frozen with liquid nitrogen and stored at  $-80^{\circ}\text{C}$  until used. Once all of the scalp biopsies were completed, the specimens were batch processed. RNA was extracted using the Qiagen Rneasy Tissue Mini Kit (QIAGEN, Valencia, CA). cDNA was hybridized to GeneChip HG U133 Plus 2.0 Affymetrix human 3' array chips using standard Affymetric protocols (Affymetrix, Santa Clara, CA). The data were analyzed using Affymetrix Microarray Suite 5.0 (MAS 5.0) algorithms found in their GeneChip Operating Software (GCOS). The stand alone “detection calls” and comparative “change calls” resulted from Wilcoxon signed rank statistical tests<sup>15</sup>. The differentially expressed genes in the active and placebo groups before and after treatment were compared using the multi-group Significance Analysis of Microarray (SAM) approach<sup>16</sup> with a false discovery rate (FDR) of  $<0.003$ . The difference between pre- and post-treatment groups was considered significant for a 2-fold or greater difference in average expression with  $p<0.05$  using a paired t-test.

## Results

### Enrollment and Sample Analysis

Sixteen men were enrolled for this pilot study; 10 used MTF and 6 used placebo. Three patients did not complete the study, this included 2 in the placebo group and one in the active drug group. RNA was extracted from all 13 patient samples (frontal and vertex, before and after treatment) and sent for microarray (a total of 52 samples). Of the 52 samples (36 active and 16 placebo), 5 were identified as outliers. Extreme expression values that lie outside the mean level of variation observed in the study were referred to as outliers. We used the principal component analysis (PCA) outlier detection method<sup>17</sup> for identifying outliers in this study. Of the five samples identified as outliers, 3 were from active and 2 from placebo patients. The outliers were eliminated from the data analysis, thus the final results were based on 33 active and 14 placebo samples. Four of the nine subjects who were on active drug were determined to be responders based on hair growth observed at 8 weeks using global stereotactic photographs, and the five others were deemed non-responders. The four responders had hair growth noted in both the frontal and vertex scalp. Figure 1 shows stereotactic photographs of a responder at baseline and post-treatment with MTF. Increase in hair growth and hair volume was seen in both the vertex and frontal scalp suggesting that MTF induces hair regrowth in both scalp regions.

### Vertex and Frontal Scalp before treatment

A comparison of vertex and frontal scalp before treatment identified 38 differentially expressed transcripts. As shown in Table 1, thirty three transcripts were down regulated and included both coding (DUSP1, FOS, FOSB, CYR61, HBB, EGR1, ZFP36, MS4A1, IGLI3, ATF3, PSG3, EFCAB4B, KRTAP19-1, 19-3 and 8-1) and non-coding RNAs (SNORDs, SNORAs, RNUs and Vault RNA). The five up regulated genes included MSL3L2, CD209, MUC7, SLC6A14 and ANKRD20B.

### Pre and Post Treatment with MTF – Vertex

A comparison of vertex scalp biopsy specimens after and before the use of MTF revealed the following up-regulated genes: keratin associated proteins (KRTAP7-1, KRTAP8-1, KRTAP19-1, KRTAP13-2, KRTAP19-3, KRTAP19-5) as well as small nucleolar non-coding RNAs (SNORD116-22, SNORD25, SNORA5, VTRNA1-1) (Table 2). The down-regulated genes included: the epidermal differentiation complex (EDC) genes including late cornified envelope precursors (LCE3D, LCE3E, LCE1C, LCE2A, LCE2C, LCE2D), small proline rich proteins (SPRR2B, SPRR2E, SPRR2G, SPRR2A), S100 protein (S100A7), loricrin (LOR), fillagrin (FLG2), and cornefilin (CNFN). Inflammatory genes (CCL18, IL1F7, CD177) were also down regulated. These changes were not seen in the placebo samples.

### Pre and Post Treatment with MTF - Frontal

As seen with vertex scalp, the frontal scalp showed an up regulation of keratin associated proteins (KRTAP5-9, KRTAP7-1, KRTAP8-1, KRTAP10-5, KRTAP10-7, KRTAP10-11, KRTAP19-1, KRTAP19-3, KRTAP19-5) after MTF treatment. Intriguingly, the non-coding RNA, SNORD116-22, was also up regulated in the frontal scalp after MTF treatment. Similar to the vertex scalp, the EDC genes (LCE2A, LCE2B, LCE2C, LCE3D, S100A7, SPRR2A) were down regulated (Table 3).

## Discussion

Our data showed regional differences in gene expression profiles of vertex and frontal scalp of AGA patients before treatment with minoxidil indicating baseline molecular variations in these two scalp regions. Thus, miniaturized hairs from the vertex and frontal scalp of AGA patients could be considered to bear a distinct molecular signature despite appearing identical clinically and histologically. Whether this difference is a result of embryologic patterning of the scalp or variations in extent of hair thinning or both is unclear. Early stress response genes that participate in cell proliferation and differentiation were significantly decreased in vertex compared to frontal scalp. Hair keratin associated proteins were also significantly decreased. These changes likely reflect differences in the extent of hair loss between the two scalp regions. The other significant changes were in the expression of non-coding RNAs referred to as small nucleolar RNAs (snoRNAs). SnoRNAs were discovered as the regulators of ribosomes and the protein synthesis machinery<sup>18,19</sup>. Recent studies suggest that snoRNAs may have additional roles in the alternative splicing of mRNA and as microRNAs (miRNAs) that regulate gene expression transcriptionally or post-transcriptionally.<sup>20,21</sup> The significance of our observation that snoRNAs are differentially

expressed between vertex and frontal, although intriguing, is not immediately clear as we are not aware of previous reports of snoRNAs affecting follicular physiology. It is tempting to speculate that snoRNAs play a role in the differential regulation of gene expression in different regions of the scalp and in hair growth. Indeed, cross-talk between miRNA and mRNA gene networks are reported to participate in epidermal differentiation and tissue remodeling during hair growth<sup>22</sup>.

Clinical trials have shown the efficacy of minoxidil in inducing hair growth in the vertex scalp<sup>13</sup>. However, the effects of minoxidil on other scalp regions and the mechanisms by which it induces hair growth are poorly understood. Although, regional differences in gene expression profiles were observed before treatment, the frontal and vertex scalp regions responded similarly to minoxidil treatment. This pilot study serves as a primer for future clinical trials that will have to be conducted to fully evaluate the effects of minoxidil on the frontal scalp. Genes encoding hair keratin-associated proteins were significantly up regulated after treatment in both the vertex and frontal scalp. Keratin-associated proteins are essential for the formation of a rigid and resistant hair shaft through their extensive disulfide bond cross-linking with abundant cysteine residues of hair keratins<sup>23-26</sup>. Thus, it is likely that minoxidil stimulates hair growth by inducing the expression of keratin associated proteins. The genes that were down regulated in both the frontal and vertex scalp after treatment with minoxidil included the epidermal differentiation complex (EDC) and inflammatory pathways. These pathways may potentially be involved in follicular miniaturization in AGA. The EDC genes map to chromosome 1q21 and encode the calcium-binding proteins of the S100 family, the small proline rich proteins (SPRRs) and the late cornified envelope (LCE) proteins<sup>27,28</sup> that participate in keratinocyte differentiation. EDC gene expression is regulated by the AP1 family of transcription factors (jun/fos) which are major regulators of epidermal differentiation<sup>29</sup>. In addition to EDC genes, a number of inflammatory genes were also down regulated in both the vertex and frontal scalp of AGA patients after minoxidil treatment. Previous studies<sup>30-32</sup> have implicated micro-inflammation in the pathogenesis of AGA. More recently, Garza et al<sup>33</sup> have shown a role for prostaglandin D2 in the pathogenesis of AGA. The mechanism by which minoxidil down regulates the expression of inflammatory genes is not known. However, the Jun/AP-1 transcription factors regulate inflammation in the skin<sup>34</sup> and the decreased expression of EDC and inflammatory genes may be due to the effects of minoxidil on AP1 transcription factors.

Our data suggests that despite regional baseline differences in the scalp of AGA patients, frontal and vertex scalp are responsive to MTF with a molecular pattern that is similar. After treatment with MTF, both regions of the scalp showed increased production of hair keratin-associated proteins and decreased keratinization of the epidermis and inflammatory signals which are known to be altered in AGA. Larger cohort studies are needed to correlate molecular alterations of the scalp with degree of clinical response to MTF which may potentially lead to development of response biomarkers. Additionally, further studies are needed to explain the novel finding of variable expression of non-coding RNAs of the frontal compared to the vertex scalp and to understand their potential role in follicular physiology.

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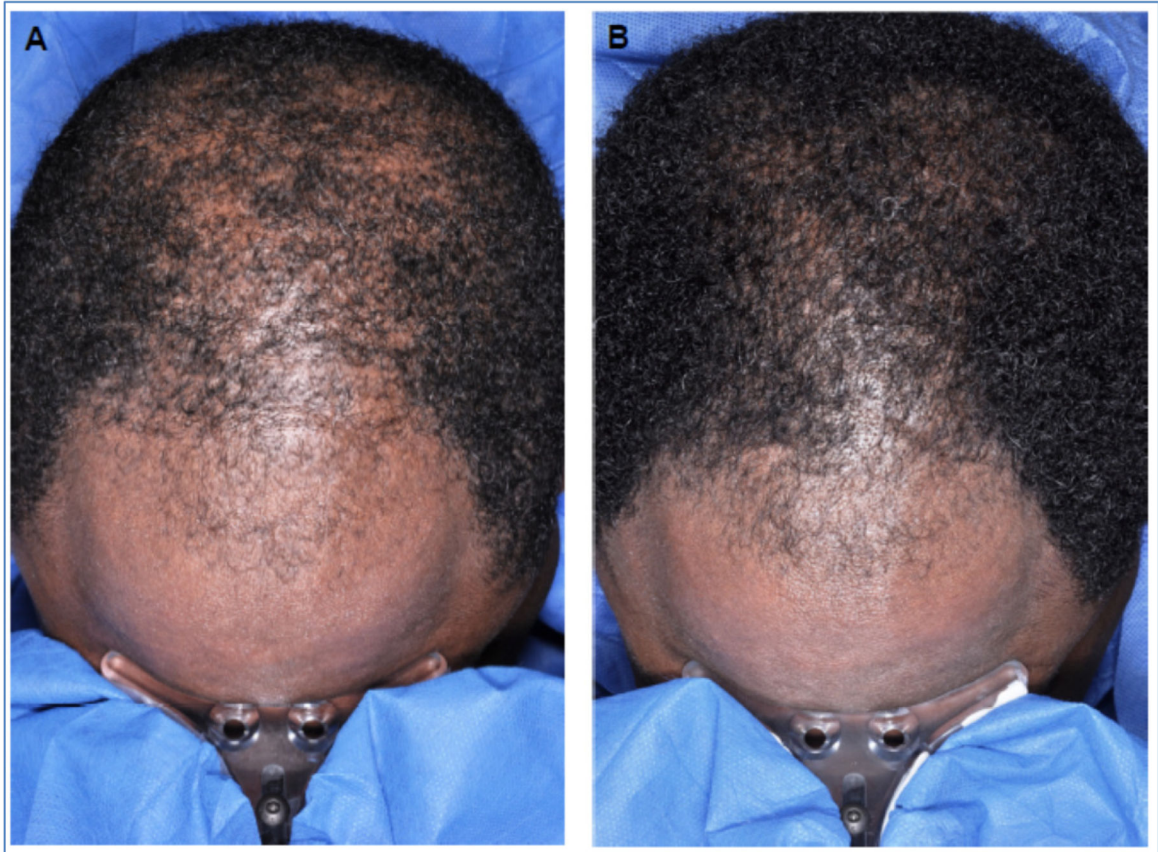
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- What's already known about this topic? There are regional variations in scalp hair miniaturization seen in AGA. Use of topical minoxidil can lead to reversal of miniaturization in the vertex scalp.
- What does this study add? Frontal scalp of AGA patients is responsive to minoxidil treatment in a manner similar to vertex scalp.
- Hair growth properties of MTF may be mediated through increased production of hair keratin-associated proteins and decreased epidermal differentiation complex and inflammatory gene expression.





**Figure 1.**

A) Base-line and B) post-treatment global photographs of the scalp of an AGA patient taken with a stereotactic device. Hair growth and increase in volume was observed after 8 weeks of 5% MTF treatment and the patient was deemed a responder.

Table 1

**Differentially expressed genes in vertex vs frontal scalp**

The Affymetrix probe IDs, Gene Symbols, Gene Description and fold-changes of the differentially expressed are shown. The down regulated genes include those that encode stress response proteins (pink highlight), keratin-associated proteins (yellow highlight) and non-coding RNA transcripts (green highlight). Five genes including non-coding RNAs were up regulated in the vertex compared to the frontal scalp.

Probe id	Gene symbol Down regulated	Fold change avg	Gene description
8115831	ATF3	-1.954440184	"Homo sapiens activating transcription factor 3 (ATF3), transcript variant 4, mRNA."
7975779	CYR61	-1.998588958	"Homo sapiens cysteine-rich, angiogenic inducer, 61 (CYR61), mRNA."
8029693	DUSP1	-2.431645062	"Homo sapiens dual specificity phosphatase 1 (DUSP1), mRNA."
7902687	EGR1	-1.895999069	"Homo sapiens early growth response 1 (EGR1), mRNA."
7946033	EFCAB4B	-1.906824031	"Homo sapiens EF-hand calcium binding domain 4B (EFCAB4B), transcript variant 1, mRNA."
8108627	FOSB	-2.327675169	"Homo sapiens FBJ murine osteosarcoma viral oncogene homolog B (FOSB), transcript variant 1, mRNA."
8005547	HBB	-1.862261909	"Homo sapiens hemoglobin, beta (HBB), mRNA."
7984259	KRTAP19-1	-1.854118595	"Homo sapiens keratin associated protein 19-1 (KRTAP19-1), mRNA."
7981730	KRTAP19-3	-2.205875048	"Homo sapiens keratin associated protein 19-3 (KRTAP19-3), mRNA."
7967028	KRTAP8-1	-2.076551532	"Homo sapiens keratin associated protein 8-1 (KRTAP8-1), mRNA."
8005553	MS4A1	-2.24590692	"Homo sapiens membrane-spanning 4-domains, subfamily A, member 1 (MS4A1), transcript variant 1, mRNA."
7948894	IGLJ3	-2.121862356	"Homo sapiens mRNA for scFv collagenase IV antibody, complete cds."
8013329	PSG3	-1.783784365	"Homo sapiens pregnancy specific beta-1-glycoprotein 3 (PSG3), mRNA."
7942594	RNU2-1	-1.991355015	"Homo sapiens RNA, U2 small nuclear 1 (RNU2-1), non-coding RNA."
7940287	RNU4-2	-2.280746608	"Homo sapiens RNA, U4 small nuclear 2 (RNU4-2), non-coding RNA."
7920873	RNU5B-1	-1.843214714	"Homo sapiens RNA, U5B small nuclear 1 (RNU5B-1), non-coding RNA."
7909610	RNU5E	-1.902622286	"Homo sapiens RNA, U5E small nuclear (RNU5E), non-coding RNA."
7960365	SCARNA5	-2.037973907	"Homo sapiens small Cajal body-specific RNA 5 (SCARNA5), non-coding RNA."
7897801	SNORD15B	-1.843219815	"Homo sapiens small nucleolar RNA, C/D box 15B (SNORD15B), non-coding RNA."
8013325	SNORD3A	-1.902175302	"Homo sapiens small nucleolar RNA, C/D box 3A (SNORD3A), non-coding RNA."
8133688	SNORD3A	-1.902175302	"Homo sapiens small nucleolar RNA, C/D box 3A (SNORD3A), non-coding RNA."
8014755	SNORD3A	-1.902175302	"Homo sapiens small nucleolar RNA, C/D box 3A (SNORD3A), non-coding RNA."

Probe id	Gene symbol Down regulated	Fold change avg	Gene description
8028652	SNORD3A	-1.902175302	"Homo sapiens small nucleolar RNA, C/D box 3A (SNORD3A), non-coding RNA."
8037231	SNORD3A	-1.902175302	"Homo sapiens small nucleolar RNA, C/D box 3A (SNORD3A), non-coding RNA."
8049297	SNORD82	-1.856869421	"Homo sapiens small nucleolar RNA, C/D box 82 (SNORD82), non-coding RNA."
8059712	SNORA14A	-1.7275943	"Homo sapiens small nucleolar RNA, H/ACA box 14A (SNORA14A), non-coding RNA."
8062490	SNORA21	-1.825872603	"Homo sapiens small nucleolar RNA, H/ACA box 21 (SNORA21), non-coding RNA."
8069822	SNORA42	-2.127392366	"Homo sapiens small nucleolar RNA, H/ACA box 42 (SNORA42), non-coding RNA."
8069831	SNORA60	-1.890606752	"Homo sapiens small nucleolar RNA, H/ACA box 60 (SNORA60), non-coding RNA."
8069863	SNORA74A	-1.867959307	"Homo sapiens small nucleolar RNA, H/ACA box 74A (SNORA74A), non-coding RNA."
8108370	VTRNA1-1	-2.631698263	"Homo sapiens vault RNA 1-1 (VTRNA1-1), non-coding RNA."
8108420	FOS	-4.412605768	"Homo sapiens v-fos FBJ murine osteosarcoma viral oncogene homolog (FOS), mRNA."
8013323	ZFP36	-1.746605695	"Homo sapiens zinc finger protein 36, C3H type, homolog (mouse) (ZFP36), mRNA."
<b>Up regulated</b>			
8059852	ANKRD20B	1.832236142	"Homo sapiens ankyrin repeat domain 20B (ANKRD20B), non-coding RNA."
8020349	CD209	1.620675753	"Homo sapiens CD209 molecule (CD209), transcript variant 2, transcribed RNA."
8169504	MSL3L2	2.23003259	"Homo sapiens male-specific lethal 3-like 2 (Drosophila) (MSL3L2), non-coding RNA."
8095504	MUC7	1.730630286	"Homo sapiens mucin 7, secreted (MUC7), transcript variant 1, mRNA."
8025301	SLC6A14	1.780898386	"Homo sapiens solute carrier family 6 (amino acid transporter), member 14 (SLC6A14), mRNA."

**Table 2**  
**Differentially expressed genes in vertex scalp after vs before treatment with minoxidil**

Epidermal differentiation complex (EDC) (yellow highlight) and inflammatory response genes (pink highlight) were down regulated after 5% MTF treatment for 8 weeks in the vertex scalp of AGA patients. Genes that were up regulated include those that encode keratin-associated proteins (yellow highlight) and non-coding RNAs (green highlight).

Probe id	Gene symbol Down regulated	Fold change avg	Gene Description
7953775	A2ML1	-1.791380784	"Homo sapiens alpha-2-macroglobulin-like 1 (A2ML1), mRNA."
7983910	AQP9	-1.685857639	"Homo sapiens aquaporin 9 (AQP9), mRNA."
7905496	Clorf46	-2.061862602	"Homo sapiens skin-specific protein (xp33) mRNA, partial cds."
7905512	Clorf68	-2.309045267	"Homo sapiens chromosome 1 open reading frame 68 (Clorf68), mRNA."
7980828	CCDC88C	-2.441701871	"Homo sapiens coiled-coil domain containing 88C (CCDC88C), mRNA."
8006594	CCL18	-2.076061673	"Homo sapiens chemokine (C-C motif) ligand 18 (pulmonary and activation-regulated) (CCL18), mRNA."
8037298	CD177	-2.10349745	"Homo sapiens CD177 molecule (CD177), mRNA."
8037179	CNFN	-1.757184518	"Homo sapiens cornifelin (CNFN), mRNA."
7915896	CYP4Z2P	-1.733354489	"Homo sapiens cytochrome P450, family 4, subfamily Z, polypeptide 2 pseudogene (CYP4Z2P), non-coding RNA."
7985555	EFTUD1	-1.851308237	"Homo sapiens elongation factor Tu GTP binding domain containing 1 (EFTUD1), transcript variant 1, mRNA."
7943562	ELMOD1	-1.876419323	"Homo sapiens ELMO/CED-12 domain containing 1 (ELMOD1), transcript variant 1, mRNA."
8034974	EPHX3	-1.673400532	"Homo sapiens epoxide hydrolase 3 (EPHX3), transcript variant 1, mRNA."
7920175	FLG2	-1.746052304	"Homo sapiens filaggrin family member 2 (FLG2), mRNA."
7952673	FLJ45950	-1.785333508	"Homo sapiens cDNA FLJ45950 fis, clone PLACE7008136."
8105331	GZMK	-1.857252097	"Homo sapiens granzyme K (granzyme 3; tryptase II) (GZMK), mRNA."
8044532	IL1F7	-1.951182337	"Homo sapiens interleukin 1 family, member 7 (zeta) (IL1F7), transcript variant 1, mRNA."
8120378	KIAA1586	-1.580414979	"Homo sapiens KIAA1586 (KIAA1586), mRNA."
7905515	KPRP	-2.395835622	"Homo sapiens keratinocyte proline-rich protein (KPRP), mRNA."
7963479	KRT2	-1.766520904	"Homo sapiens keratin 2 (KRT2), mRNA."
7905525	LCE1B	-2.186144348	"Homo sapiens late cornified envelope 1B (LCE1B), mRNA."
7920193	LCE1C	-1.711195411	"Homo sapiens late cornified envelope 1C (LCE1C), mRNA."

Probe id	Gene symbol Down regulated	Fold change avg	Gene Description
7905507	LCE2A	-2.049191721	"Homo sapiens late cornified envelope 2A (LCE2A), mRNA."
7905505	LCE2B	-2.499101451	"Homo sapiens late cornified envelope 2B (LCE2B), mRNA."
7905503	LCE2C	-3.17507158	"Homo sapiens late cornified envelope 2C (LCE2C), mRNA."
7905500	LCE2D	-2.132509503	"Homo sapiens late cornified envelope 2D (LCE2D), mRNA."
7920185	LCE3D	-2.064755474	"Homo sapiens late cornified envelope 3D (LCE3D), mRNA."
7920182	LCE3E	-1.686212956	"Homo sapiens late cornified envelope 3E (LCE3E), mRNA."
7928999	LIPN	-1.579780344	"Homo sapiens lipase, family member N (LIPN), mRNA."
8115205	LOC134466	-1.845999017	"Homo sapiens zinc finger protein 300 pseudogene (LOC134466), non-coding RNA."
8119423	LOC221442	-1.688284707	"Homo sapiens adenylate cyclase 10 pseudogene (LOC221442), non-coding RNA."
8139796	LOC441233	-1.802369111	"Homo sapiens cDNA FLJ46129 fis, clone TEST2046188."
7905563	LOR	-1.681231089	"Homo sapiens loricrin (LOR), mRNA."
7957023	LYZ	-1.766800783	"Homo sapiens lysozyme (renal amyloidosis) (LYZ), mRNA."
8059852	MSL3L2	-2.158304003	"Homo sapiens male-specific lethal 3-like 2 (Drosophila) (MSL3L2), non-coding RNA."
7937940	OR52K3P	-1.860881202	Homo sapiens clone IMAGE:110749 mRNA sequence.
7939988	OR5M3	-2.029856502	"Homo sapiens olfactory receptor, family 5, subfamily M, member 3 (OR5M3), mRNA."
8062927	PI3	-1.988287725	"Homo sapiens peptidase inhibitor 3, skin-derived (PI3), mRNA."
7903404	RNPC3	-1.721533122	"Homo sapiens RNA-binding region (RNPI, RRM) containing 3 (RNPC3), mRNA."
7920252	S100A7	-1.945756393	"Homo sapiens S100 calcium binding protein A7 (S100A7), mRNA."
8023696	SERPINB3	-1.780693185	"Homo sapiens serpin peptidase inhibitor, clade B (ovalbumin), member 3 (SERPINB3), mRNA."
8023688	SERPINB4	-2.506533137	"Homo sapiens serpin peptidase inhibitor, clade B (ovalbumin), member 4 (SERPINB4), mRNA."
8021623	SERPINB7	-1.74912009	"Homo sapiens serpin peptidase inhibitor, clade B (ovalbumin), member 7 (SERPINB7), transcript variant 1, mRNA."
8169504	SLC6A14	-1.730808765	"Homo sapiens solute carrier family 6 (amino acid transporter), member 14 (SLC6A14), mRNA."
7981980	SNORD116-16	-1.673093454	"Homo sapiens small nucleolar RNA, C/D box 116-16 (SNORD116-16), non-coding RNA."
7951030	SNORD6	-2.356015722	"Homo sapiens small nucleolar RNA, C/D box 6 (SNORD6), non-coding RNA."
7920205	SPRR2A	-1.990881958	"Homo sapiens small proline-rich protein 2A (SPRR2A), mRNA."
7920210	SPRR2B	-2.112830411	"Homo sapiens small proline-rich protein 2B (SPRR2B), mRNA."
7920201	SPRR2B	-2.116427818	"Homo sapiens small proline-rich protein 2B (SPRR2B), mRNA."

Probe id	Gene symbol Down regulated	Fold change avg	Gene Description
7920196	SPRR2D	-1.676107252	"Homo sapiens small proline-rich protein 2D (SPRR2D), mRNA."
7920214	SPRR2E	-1.944348255	"Homo sapiens small proline-rich protein 2E (SPRR2E), mRNA."
7920217	SPRR2G	-2.494933605	"Homo sapiens small proline-rich protein 2G (SPRR2G), mRNA."
8066489	WFDC12	-3.050924897	"Homo sapiens WAP four-disulfide core domain 12 (WFDC12), mRNA."
<b>Up regulated</b>			
8014230	AMAC1	1.585669668	"Homo sapiens acyl-malonyl condensing enzyme 1 (AMAC1), mRNA."
8176276	ATRX	2.298419926	"Homo sapiens alpha thalassemia/mental retardation syndrome X-linked (RAD54 homolog, S. cerevisiae) (ATRX), transcript variant 1, mRNA."
8175531	CDR1	1.66741064	"Homo sapiens cerebellar degeneration-related protein 1, 34kDa (CDR1), mRNA."
7963845	DCD	2.186042377	"Homo sapiens dermectin (DCD), mRNA."
8127396	EYS	2.220498184	"Homo sapiens eyes shut homolog (Drosophila) (EYS), transcript variant 1, mRNA."
7975779	FOS	5.135611804	"Homo sapiens v-fos FBJ murine osteosarcoma viral oncogene homolog (FOS), mRNA."
7948354	GLYATL2	1.942351062	"Homo sapiens glycine-N-acyltransferase-like 2 (GLYATL2), mRNA."
8043449	IGK@	2.385066851	"Homo sapiens immunoglobulin kappa locus, mRNA (cDNA clone MGC:22645 IMAGE:4700961), complete cds."
8043436	IGKC // IGKC	2.63060007	"Homo sapiens immunoglobulin kappa constant, mRNA (cDNA clone IMAGE:6282376)."
8069813	KRTAP13-2	2.096151572	"Homo sapiens keratin associated protein 13-2 (KRTAP13-2), nuclear gene encoding mitochondrial protein, mRNA."
8069822	KRTAP19-1	2.828617347	"Homo sapiens keratin associated protein 19-1 (KRTAP19-1), mRNA."
8069831	KRTAP19-3	3.182862681	"Homo sapiens keratin associated protein 19-3 (KRTAP19-3), mRNA."
8069838	KRTAP19-5	3.095847716	"Homo sapiens keratin associated protein 19-5 (KRTAP19-5), mRNA."
8069868	KRTAP7-1	3.61335121	"Homo sapiens keratin associated protein 7-1 (KRTAP7-1), mRNA."
8069863	KRTAP8-1	3.096976944	"Homo sapiens keratin associated protein 8-1 (KRTAP8-1), mRNA."
8013567	LOC201229	1.751220303	"Homo sapiens hypothetical protein LOC201229 (LOC201229), mRNA."
7911276	OR2T6	1.730463243	"Homo sapiens olfactory receptor, family 2, subfamily T, member 6 (OR2T6), mRNA."
7948148	OR5M10	2.250498419	"Homo sapiens olfactory receptor, family 5, subfamily M, member 10 (OR5M10), mRNA."
7933561	PARG	1.609493237	"Homo sapiens poly (ADP-ribose) glycohydrolase (PARG), mRNA."
8146957	PII5	1.551331779	"Homo sapiens peptidase inhibitor 15 (PII5), mRNA."
8136839	PIP	2.501813871	"Homo sapiens prolactin-induced protein (PIP), mRNA."
8088090	RFT1	1.652418379	"Homo sapiens RFT1 homolog (S. cerevisiae) (RFT1), mRNA."

Probe id	Gene symbol Down regulated	Fold change avg	Gene Description
8049297	SCARNA5	1.600678858	"Homo sapiens small Cajal body-specific RNA 5 (SCARNA5), non-coding RNA."
7940630	SCGB1D2	1.817550739	"Homo sapiens secretoglobin, family 1D, member 2 (SCGB1D2), mRNA."
8021081	SLC14A1	2.179329222	"Homo sapiens solute carrier family 14 (urea transporter), member 1 (Kidd blood group) (SLC14A1), transcript variant 1, mRNA."
7981992	SNORD116-22	1.810373746	"Homo sapiens small nucleolar RNA, C/D box 116-22 (SNORD116-22), non-coding RNA."
7948910	SNORD25	1.856333185	"Homo sapiens small nucleolar RNA, C/D box 25 (SNORD25), non-coding RNA."
8142685	tcag7.977	2.029854813	"Homo sapiens hypothetical protein LOC730130 (LOC730130), mRNA."
7930631	TDRD1	1.62149349	"Homo sapiens tudor domain containing 1 (TDRD1), mRNA."
7981732	VSIG6	1.985693826	Putative V-set and immunoglobulin domain-containing protein 6 gene:ENSG00000189039
8108627	VTRNA1-1	2.295648077	"Homo sapiens vault RNA 1-1 (VTRNA1-1), non-coding RNA."
8089596	WDR52	1.609278245	WD repeat protein 52 gene:ENSG00000206530
7907156	XCL1	2.275547194	"Homo sapiens chemokine (C motif) ligand 1 (XCL1), mRNA."

**Table 3**  
**Differentially expressed genes in frontal scalp after vs before treatment with minoxidil**

As seen with the vertex scalp, the epidermal differentiation complex (EDC) (yellow highlight) and inflammatory response genes (pink highlight) were down regulated in the frontal scalp after 5% MTF treatment. Genes that were up regulated include those that encode keratin-associated proteins (yellow highlight) and non-coding RNAs (green highlight).

Probe id Down regulated	Gene Symbol	Fold change avg	Gene description
7905496	Clorf46	-1.786381293	"Homo sapiens skin-specific protein (xp33) mRNA, partial cds."
7905512	Clorf68	-1.901897993	"Homo sapiens chromosome 1 open reading frame 68 (Clorf68), mRNA."
8037298	CD177	-1.90208393	"Homo sapiens CD177 molecule (CD177), mRNA."
7917942	FLJ35409	-1.784675504	"Homo sapiens cDNA FLJ35409 fis, clone SKNSH2009435."
7918620	FLJ36116	-1.804717709	"Homo sapiens cDNA FLJ36116 fis, clone TESTI2022338."
7991762	HBA1	-2.08762655	"Homo sapiens hemoglobin, alpha 1 (HBA1), mRNA."
7991766	HBA1	-2.08762655	"Homo sapiens hemoglobin, alpha 1 (HBA1), mRNA."
7981737	IGHA1 // IGHA1	-2.436976257	"Homo sapiens SNC73 protein (SNC73) mRNA, complete cds."
7981724	IGHD	-2.140677262	"Homo sapiens immunoglobulin heavy constant delta, mRNA (cDNA clone IMAGE:4855067)."
8043476	IGKC	-1.865674065	Ig kappa chain V-J region HK101 gene:ENSG00000211628
8043465	IGKC //IGKC //IGKC //IGKC //IGKC	-1.796109167	"Homo sapiens immunoglobulin kappa constant, mRNA (cDNA clone IMAGE:4692138)."
7905507	LCE2A	-1.659016222	"Homo sapiens late cornified envelope 2A (LCE2A), mRNA."
7905505	LCE2B	-2.030257255	"Homo sapiens late cornified envelope 2B (LCE2B), mRNA."
7905503	LCE2C	-2.07719648	"Homo sapiens late cornified envelope 2C (LCE2C), mRNA."
7920185	LCE3D	-1.873464294	"Homo sapiens late cornified envelope 3D (LCE3D), mRNA."
8045205	LOC150527	-1.792985994	"Homo sapiens hypothetical LOC150527 (LOC150527), transcript variant 1, non-coding RNA."
8055236	LOC150527	-1.792985994	"Homo sapiens hypothetical LOC150527 (LOC150527), transcript variant 1, non-coding RNA."
7940287	MS4A1	-1.891830913	"Homo sapiens membrane-spanning 4-domains, subfamily A, member 1 (MS4A1), transcript variant 1, mRNA."



Probe id Down regulated	Gene Symbol	Fold change avg	Gene description
8177130	NHEDC1	-1.622300855	"Homo sapiens Na+/H+ exchanger domain containing 1 (NHEDC1), transcript variant 1, mRNA."
7984259	RNU5B-1	-1.939784594	"Homo sapiens RNA, USB small nuclear 1 (RNU5B-1), non-coding RNA."
8173176	RP11-167P23.2	-1.653311406	Homo sapiens partial mRNA for XAGE-4 protein.
7920252	S100A7	-1.775381601	"Homo sapiens S100 calcium binding protein A7 (S100A7), mRNA."
7920875	SCARNA4	-2.049700515	"Homo sapiens small Cajal body-specific RNA 4 (SCARNA4), non-coding RNA."
7938329	SNORA23	-2.148712212	"Homo sapiens small nucleolar RNA, H/ACA box 23 (SNORA23), non-coding mRNA."
8047780	SNORA41	-1.805948249	"Homo sapiens small nucleolar RNA, H/ACA box 41 (SNORA41), non-coding RNA."
8096301	SPP1	-1.995863744	"Homo sapiens secreted phosphoprotein 1 (SPP1), transcript variant 1, RNA."
7920205	SPRR2A	-1.936677624	"Homo sapiens small proline-rich protein 2A (SPRR2A), mRNA."
8108627	VTRNA1-1	-2.631671674	"Homo sapiens vault RNA 1-1 (VTRNA1-1), non-coding RNA."
8066489	WFDC12	-2.638905738	"Homo sapiens WAP four-disulfide core domain 12 (WFDC12), mRNA."
<b>Up regulated</b>			
7987315	ACTC1	1.85182807	"Homo sapiens actin, alpha, cardiac muscle 1 (ACTC1), mRNA."
8042788	ACTG2	1.657203708	"Homo sapiens actin, gamma 2, smooth muscle, enteric (ACTG2), mRNA."
8173349	AWAT2	2.147338381	"Homo sapiens acyl-CoA wax alcohol acyltransferase 2 (AWAT2), mRNA."
7961075	CD69	1.794297146	"Homo sapiens CD69 molecule (CD69), transcript variant 1, mRNA."
7902687	CYR61	2.014127367	"Homo sapiens cysteine-rich, angiogenic inducer, 61 (CYR61), mRNA."
7975779	FOS	3.399003819	"Homo sapiens v-fos FBJ murine osteosarcoma viral oncogene homolog (FOS), mRNA."
8029693	FOSB	2.209993813	"Homo sapiens FBJ murine osteosarcoma viral oncogene homolog B (FOSB), transcript variant 1, mRNA."
8157727	GPR21	1.540559918	"Homo sapiens G protein-coupled receptor 21 (GPR21), mRNA."
8132843	HAUS6	1.70772238	"Homo sapiens HAUS augmin-like complex, subunit 6 (HAUS6), mRNA."
8091550	KIAA1328	1.74148094	"Homo sapiens KIAA1328 (KIAA1328), mRNA."
8069156	KRTAP10-11	1.891391247	"Homo sapiens keratin associated protein 10-11 (KRTAP10-11), mRNA."
8070782	KRTAP10-5	1.905336495	"Homo sapiens keratin associated protein 10-5 (KRTAP10-5), mRNA."
8069146	KRTAP10-7	1.68653121	"Homo sapiens keratin associated protein 10-7 (KRTAP10-7), mRNA."
8069822	KRTAP19-1	1.951151136	"Homo sapiens keratin associated protein 19-1 (KRTAP19-1), mRNA."

Probe id Down regulated	Gene Symbol	Fold change avg	Gene description
8069831	KRTAP19-3	2.979072598	"Homo sapiens keratin associated protein 19-3 (KRTAP19-3), mRNA."
8069838	KRTAP19-5	3.201872526	"Homo sapiens keratin associated protein 19-5 (KRTAP19-5), mRNA."
7942261	KRTAP5-9	1.783844649	"Homo sapiens keratin associated protein 5-9 (KRTAP5-9), mRNA."
8069868	KRTAP7-1	3.527598251	"Homo sapiens keratin associated protein 7-1 (KRTAP7-1), mRNA."
8069863	KRTAP8-1	2.554485467	"Homo sapiens keratin associated protein 8-1 (KRTAP8-1), mRNA."
8113369	SLCO4C1	1.866374928	"Homo sapiens solute carrier organic anion transporter family, member 4C1 (SLCO4C1), mRNA."
7981992	SNORD116-22	1.790164025	"Homo sapiens small nucleolar RNA, C/D box 116-22 (SNORD116-22), non-coding RNA."
7920141	TCHH	1.796011989	"Homo sapiens trichohyalin (TCHH), mRNA."
8035838	ZNF724P	1.62142221	"Homo sapiens cDNA FLJ56866 complete cds, moderately similar to Zinc finger protein 43."