Comprehensive Transcriptome Profiling of Balding and Non-Balding Scalps in Trichorhinophalangeal Syndrome Type I Patient

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Background: Trichorhinophalangeal syndrome (TRPS) patients tend to have alopecia that appears to be androgenetic, and this genetic model might give clues to the pathogenesis of hair loss or hair morphogenesis. Objective: This study was conducted to identify additional genetic evidence of TRPS and hair morphogenesis from a TRPS patient. Methods: From one TRPS type I patient, we extracted RNA and profiled whole transcriptome in non-balding and balding scalp areas using high-throughput RNA sequencing. Results: We found a total of 26,320 genes, which comprised 14,892 known genes with new isoforms and 4,883 novel genes from the non-balding and balding areas. Among these, a total of 1,242 genes showed different expression in the two scalp areas (p < 0.05 and log2 fold-change > 0). Several genes related to the skin and hair, alopecia, and the TRPS1 gene were validated by qRT-PCR. Twelve of 15 genes (KRT6C, KRTAP3-1, MKI67, GPRC5D, TYRP1, DSC1, PMEL, WIFI, SOX21, TINAG, PTGDS, and TRPS1) were down-regulated (10 genes: p < 0.01; SOX21 and PTGDS: p > 0.05), and the three other genes (HBA2, GAL, and DES) were up-regulated (p < 0.01) in the balding scalp. Many genes related to keratin and hair development were down-regulated in the balding scalp of the TRPS type I patient. In particular, the TRPS1 gene might be related to androgen metabolism and hair morphogenesis. Conclusion: Our result could suggest a novel perspective and evidence to support further study of TRPS and hair morphogenesis. (Ann Dermatol 29(5) 597∼601, 2017)

Keywords- Androgenetic alopecia, Differentially expressed gene, Transcriptome, Trichorhinophalangeal syndrome, TRPS1

INTRODUCTION

Type I trichorhinophalangeal syndrome (TRPS) presents with craniofacial dysmorphism, skeletal abnormality, and sparse scalp hairs1. TRPS patients tend to have alopecia that appears to be androgenetic, and thus, this genetic model might give clues to the pathogenesis of hair loss or hair morphogenesis, as has been found in previous studies2. Fantauzzo and Christiano1 reported that the target genes of Trps1, Wifi, Sox18, and Sox21 played an important role in vibrissa follicle morphogenesis by analyzing the gene expression profiles between wild-type and Trps1 Δgt/Δgt mutant mouse embryos to understand hair morphogenesis. This is very interesting because sparse scalp hair is a common feature of TRPS. Herein, we analyzed whole transcriptome from non-balding and balding scalp areas from the TRPS patient using high-throughput sequencing and attempted to identify important genetic information about TRPS symptoms and hair morphogenesis.
MATERIALS AND METHODS

Information of patient with TRPS type I

A 15-year-old boy visited with sparse and slowly growing scalp hairs that had been that way since his childhood. Especially, his fronto-temporal hair line regressed to the vertex and his vertex hair density and thickness decreased compared to the occiput hairs. He had the typical TRPS phenotypes, including a bulbous nose, a long philtrum, and abnormally short fingers and toes. We took tissue from the non-balding (occiput area) and balding portions (vertex area) of his scalp for genetic analysis (Supplementary Fig. 1). This study was approved by the institutional review board of Dankook University Hospital (IRB no. DKUH 2014-08-005).

RNA sequencing

We extracted total RNA from the tissues using trizol reagent, and then enriched mRNA by oligo-dT and synthesized to cDNA. We subjected the cDNA to end-repair and poly-A addition and connected it with 5' and 3' adaptors on both ends. By separating on a BluePippin 2% agarose gel (Sage Science, Beverly, MA, USA), we selected and amplified suitable fragments. The final library sizes and qualities were evaluated with an Agilent High Sensitivity DNA kit (Agilent Technologies, Santa Clara, CA, USA). Subsequently, we performed high-throughput RNA sequencing using an Illumina HiSeq2500 sequencer (Illumina, San Diego, CA, USA). Among total output reads, we mapped high-quality reads to the human reference genome (Ensembl release 72).

Differentially expressed genes and gene ontology analysis

We calculated the gene expression level based on fragments per kilobase of exon per million mapped reads (FPKM) using Cufflinks v2.1.13 from Ensembl release 72. We generated gene-level count data using HTSeq-count v0.6.1p3. Based on this, we analyzed differentially expressed genes (DEGs) using the gene TCC. We calculated normalization factors using iterative DEGES/edgeR. We filtered DEGs based on p-value < 0.05 and log2 fold change > 0. To characterize their molecular function, we analyzed gene ontology (GO) (www.geneontology.org). p-value < 0.001 was considered statistically significant.

Quantitative real-time polymerase chain reaction

We synthesized a total of 500 ng of RNA to cDNA using M-MLV reverse transcriptase (Promega, Madison, WI, USA) and an RNase inhibitor (Promega). We designed a primer pair for target genes using Primer 3 (http://bioinfo.ut.ee/primer3-0.4.0/primer3/) (Supplementary Table 1). We amplified 15 genes and a GAPDH gene as a control to normalize expression using the Eco Real-Time PCR System (Illumina). We confirmed the absence of any non-specific amplified products through melting curve analysis at 55°C ~ 95°C. All reactions were performed in triplicate and analyzed by delta-delta Ct method.

RESULTS

Dataset from RNA sequencing

We processed a total of ten billion raw reads in the filtering step and mapped 94.9% and 94.8% of the clean reads on the human reference genome (Table 1). Based on these data, we found a total of 26,320 genes, which comprised 14,892 known genes with new isoforms and 4,883 novel genes. At the transcript level, we found a total of 218,609 transcripts expressed (FPKM > 0) in either the non-balding and balding scalps.

Identifying differentially expressed genes

Based on FPKM value, we analyzed gene expression levels and identified DEGs between the non-balding and balding scalp samples. The total number of DEGs was 1,242, comprising transcripts expressed in both samples and in either sample (with p-value < 0.05 and log2 fold-change > 0) (Fig. 1). Compared to non-balding sample, up- and down-regulated genes were 636 and 606 in balding scalp; specifically, 557 genes showed sample-specific expression.

Table 1. Summary of RNA-sequencing

| Sample       | Raw reads  | Clean reads (%) | Mapped reads (%) | Properly paired (%) |
|--------------|------------|-----------------|-----------------|--------------------|
| Non-balding  | 53,351,054 | 50,338,798 (94.4)| 47,780,868 (94.9)| 36,570,528 (72.6) |
| Balding      | 54,289,736 | 51,192,244 (94.3)| 48,539,550 (94.8)| 36,789,924 (71.9) |

| Gene          | Sum | Known | Known (+ new isoforms) | Novel |
|---------------|-----|-------|------------------------|-------|
|               |     |       |                        |       |
| Non-balding   | 26,320 | 3,426 | 14,892 | 4,883 |
| Balding       | 218,609 | 150,194 | 68,415 |       |
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Fig. 1. Heat map of differently expressed genes in the non-balding and balding scalp samples. A total of 1,242 differentially expressed genes (DEGs) were identified through RNA sequencing (p-value < 0.01 and log2 fold-change > 0). The left and right columns display, respectively, the results for the non-balding and balding scalp areas. Up-regulated to down-regulated genes are indicated by red and yellow, respectively.

Fig. 2. Validation of 15 differentially expressed genes by quantitative real-time polymerase chain reaction. Three genes were up-regulated (green bar) and 12 were down-regulated (blue bar) in the non-balding scalp. Thirteen genes showed statistical significance (p-value < 0.05); the exceptions were SOX21 and PTGDS. (** p < 0.01, and *** p < 0.001).

DISCUSSION

Interestingly, the sparse hairs of TRPS patients are thin and miniaturized just as in androgenic alopecia. Therefore, we intended to find a genetic difference the between non-balding and balding scalp of a TRPS type I patient and identify a candidate genes related to hair loss or morphogenesis. Among 1,242 of DEGs, we could find lots of keratin and keratin associated genes which might be due to sampling from scalps. Two keratin-related genes (KRT6C and KRTAP3-1) were down-regulated in balding scalp. The MKI67 down-regulated in balding scalp is involved in active proliferation of cells and are reported low expression in hair follicle stem cells7. Down-regulation of MKI67 in balding scalp of TRPS type I patient in our study seemed to suggest degenerated or abnormal hair cell cycle. A key factor in TRPS pathogenesis, the TRPS1 gene was down-regulated in the balding area. Originally, TRPS1 is a transcription factor to repress its target genes via binding to GATA motif of the promoter region1. However, a recent study has revealed that TRPS1 activated the expression of target gene. Fantauzzo and Christiano1 showed Trps1 activated Wnt inhibitors and other transcription factor essential for follicle morphogenesis in mouse. Study of a TRPS
mutant mouse suggests that TRPS1 might be necessary for hair follicular formation and shows that the Wnt inhibitor and extracellular matrix protein were regulated by TRPS1 during early hair morphogenesis. Decreased TRPS1 protein can disrupt endochondral cartilage differentiation and cell interactions in hair follicle development. In addition, TRPS1 protein expression is down-regulated by androgens in human prostate cancer, and thus the TRPS1 gene might play a role in androgen metabolism in prostate cancer.

Though the correlation of TRPS1 gene and androgen metabolism has not yet been studied in the alopecia, we could expect the further study about this correlation because the male pattern baldness is associated with androgen metabolism.

WIF1 and SOX21, the target genes of TRPS1, were down-regulated in a TRPS1 Δgt/Δgt mutant mouse and in the balding scalp of a TRPS type I patient. WIF1 is a Wnt inhibitor and is expressed in dermal papilla, like TRPS1 gene. In a previous study, Wnt-related genes including WNT11 and WIF1 were up-regulated in a 120-day-old goat embryo in which secondary hair follicles and mature primary hair follicles were present, which indicates that Wnt signaling is involved in early hair follicle formation.

The SOX21 gene was shown to regulate the layered differentiation of hair follicles. Its disruption showed the human alopecia-like phenotype in a mouse with progressive hair loss. Interestingly, target gene expression in TRPS was not inversely proportional to that in Fantauzzo’s TRPS1 Δgt/Δgt mutant mouse.

We compared the gene expression patterns with those of androgenetic alopecia by Garza et al. KRT6C and GPRC5D were down-regulated and the HBA2 gene was up-regulated in balding scalp in both studies. However, PTGDS expression was not significant, unlike in a previous study. The GPRC5D gene was dramatically up-regulated in hair follicle keratinization and differentiation in the skin of an old embryo (120-day) in which secondary hair follicles had developed and primary hair follicles had matured, indicating its role in keratinization and hair follicle morphogenesis. PTGDS might be involved in androgenetic alopecia, but it is not related to hair loss in TRPS.

In conclusion, we expect our results to suggest novel perspectives and support further study to understand TRPS and hair morphogenesis.

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SUPPLEMENTARY MATERIALS

Supplementary data can be found via http://anndermatol.org/src/sm/ad-29-597-s001.pdf.

CONFLICTS OF INTEREST

The authors have nothing to disclose.

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Supplementary Fig. 1. Photograph of the trichorhinophalangeal syndrome patient. He has sparse and slowly growing scalp hairs (up), bulbous nose, long philtrum, and thin upper lip (bottom). A red circle indicates vertex area where tissue was obtained but, occiput is not appeared in this photograph.
Supplementary Table 1. Information of primer pairs used in quantitative real-time polymerase chain reaction

| No. | Symbol | DEG (RNA-seq) | Forward primer (5'-3') | Reverse primer (5'-3') | Product size |
|-----|--------|---------------|-------------------------|------------------------|--------------|
| 1   | KRT6C  | Down          | KRT6C_R_2F TCAACTTCTGAGACCTTG | KRT6C_R_2R CGTATTGGGGCTTGACCTC | 141          |
| 2   | KRTAP3-1 | Down       | KRTAP3-1_R_F TCAACACCTGTCACCCGACT | KRTAP3-1_R_R GTAGTGAATGCTGAAGCCCA | 171          |
| 3   | MKI67  | Down          | MKI67_R_2F GCCCTCTAATACCGGCTCTCA | MKI67_R_2R TGTGCCCTCACTTCACAT | 160          |
| 4   | GPRC5D | Down          | GPRC5D_R_F GCTCAATCCCCCTATTGAGTG | GPRC5D_R_R GCAGGTTGCCATATTCACAAA | 168          |
| 5   | TYRP1  | Down          | TYRP1_R_2F GCCATACGAGTAGTTGCGC | TYRP1_R_R GGAGAGGCTGTTAGCTCA | 106          |
| 6   | DSC1   | Down          | DSC1_R_F GCCAGAGGCTGAGGACATT | DSC1_R_R ACCCATATGTCCACATTCC | 188          |
| 7   | PMEL   | Down          | PMEL_R_2F CTTCTCCTGAGGCTGTC | PMEL_R_2R CCACTACTGTCCTCAAAGTCC | 160          |
| 8   | WIFI   | Down          | WIFI_R_2F GCCATTTTGTGACTGCT | WIFI_R_2R ATTTGAGAGGTTTCGCG | 189          |
| 9   | SOX21  | Down          | SOX21_R_2F CCGAGTTGAAACTGTCAC | SOX21_R_2R CCGGAAGGCGAATGTC | 155          |
| 10  | TINAG  | Down          | TINAG_R_2F AAGAGAATGAGCCTA | TINAG_R_2R AGTAGCAACAGGCTACCG | 170          |
| 11  | TRPS1  | Down          | TRPS1_R_F ATCTGGCCGACCATATTAT | TRPS1_R_R AGGCCCTACGCTCGGTAA | 165          |
| 12  | PTGDS  | -             | PTGDS_R_F AACCATGTGAGACCCGAGAC | PTGDS_R_R TCCACCACTGACACCGAGTA | 107          |
| 13  | HBA2   | Up            | HBA2_R_F TACCCGACTTGCCGACC | HBA2_R_R GCAGTGGCGTGGACCTTG | 189          |
| 14  | GAL    | Up            | GAL_R_F CTCAGGCTCCTCCCTCTC | GAL_R_R TCTTGGCGTAATGAGGTG | 148          |
| 15  | DES    | Up            | DES_R_F TATGAGACCATCGCCGCTAA | DES_R_R ATCACCGGCCATGCTGTC | 197          |
| 16  | GAPDH  | Control       | GAPDH_R_F GAGCCCCAGTCTCCATG | GAPDH_R_R GAAATCCCATACCATCTT | 120          |

DEG: differentially expressed gene.
Supplementary Table 2. DEGs and GO

| Sample     | Gene | Mod  | Unexp | DEG* | GO†   |
|------------|------|------|-------|------|-------|
|            |      |      |       |      |       |
| Non-balding| 22,449| 14,723| 3,871 | 1,242| 636 (333) |
| Balding    | 21,881| 14,466| 4,439 |      | 606 (224)  |

Unexp: unexpressed, DEG: differentially expressed gene, GO: gene ontology. *p-value < 0.05 and log2 fold change > 0. †The number with p-value < 0.001 (the number of total number).
### Supplementary Table 3. Go terms

| GO_ID   | Category     | Name                                    | Description                                                                                                                                                                                                                                                                                                                                 | GO(P-val): Control vs. Case |
|---------|--------------|-----------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------|
| GO:00   | Biological_  | Mitotic cell cycle                       | Progression through the phases of the mitotic cell cycle, the most common eukaryotic cell cycle, which canonically comprises four successive phases called G1, S, G2, and M and includes replication of the genome and the subsequent segregation of chromosomes into daughter cells. In some variant cell cycles nuclear replication or nuclear division may not be followed by cell division, or G1 and G2 phases may be absent.                                                                 | 0.000936                    |
| 00278   | process      |                                          |                                                                                                                                                                                                                                                                                                                                       |                             |
| GO:00   | Biological_  | Hair follicle development                | The process whose specific outcome is the progression of the hair follicle over time, from its formation to the mature structure. A hair follicle is a tubelike opening in the epidermis where the hair shaft develops and into which the sebaceous glands open.                                                                                                           | 0.000003                    |
| 01942   | process      |                                          |                                                                                                                                                                                                                                                                                                                                       |                             |
| GO:00   | Biological_  | Positive regulation of cytokine secretion involved in immune response | Any process that activates or increases the frequency, rate, or extent of cytokine secretion contributing to an immune response.                                                                                                                                                                                                           | 0.000934                    |
| 02741   | process      |                                          |                                                                                                                                                                                                                                                                                                                                       |                             |
| GO:00   | Biological_  | Optic cup formation involved in camera-type eye development | The developmental process pertaining to the initial formation of the optic cup, a two-walled vesicle formed from the optic vesicle.                                                                                                                                                                                                 | 0.000925                    |
| 03408   | process      |                                          |                                                                                                                                                                                                                                                                                                                                       |                             |
| GO:00   | Biological_  | Organic acid metabolic process           | The chemical reactions and pathways involving organic acids, any acidic compound containing carbon in covalent linkage.                                                                                                                                                                                                               | 0.000937                    |
| 06082   | process      |                                          |                                                                                                                                                                                                                                                                                                                                       |                             |
| GO:00   | Biological_  | Oxidative phosphorylation                | The phosphorylation of ADP to ATP that accompanies the oxidation of a metabolite through the operation of the respiratory chain. Oxidation of compounds establishes a proton gradient across the membrane, providing the energy for ATP synthesis.                                                                                                         | 0.000407                    |
| 06119   | process      |                                          |                                                                                                                                                                                                                                                                                                                                       |                             |
| GO:00   | Biological_  | Mitochondrial electron transport, NADH to ubiquinone | The transfer of electrons from NADH to ubiquinone that occurs during oxidative phosphorylation, mediated by the multisubunit enzyme known as complex I.                                                                                                                                                                                   | 0.000293                    |
| 06120   | process      |                                          |                                                                                                                                                                                                                                                                                                                                       |                             |
| GO:00   | Biological_  | DNA replication                          | The cellular metabolic process in which a cell duplicates one or more molecules of DNA. DNA replication begins when specific sequences, known as origins of replication, are recognized and bound by initiation proteins, and ends when the original DNA molecule has been completely duplicated and the copies topologically separated. The unit of replication usually corresponds to the genome of the cell, an organelle, or a virus. The template for replication can either be an existing DNA molecule or RNA.                                 | 0.000002                    |
| 06260   | process      |                                          |                                                                                                                                                                                                                                                                                                                                       |                             |
| GO:00   | Biological_  | RNA-dependent DNA replication            | A DNA replication process that uses RNA as a template for RNA-dependent DNA polymerases (e.g. reverse transcriptase) that synthesize the new strands.                                                                                                                                                                                          | 0.000003                    |
| 06278   | process      |                                          |                                                                                                                                                                                                                                                                                                                                       |                             |
| GO:00   | Biological_  | Melanin metabolic process                | The chemical reactions and pathways involving melamins, pigments largely of animal origin. High molecular weight polymers of indole quinone, they are irregular polymeric structures and are divided into three groups: allomelansins in the plant kingdom and eumelansins and phaeomelansins in the animal kingdom.                                                                 | 0.000046                    |
| 06382   | process      |                                          |                                                                                                                                                                                                                                                                                                                                       |                             |
| GO:00   | Biological_  | Lipid metabolic process                  | The chemical reactions and pathways involving lipids, compounds soluble in an organic solvent but not, or sparingly, in an aqueous solvent. Includes fatty acids; neutral fats, other fatty-acid esters, and soaps; long-chain (fatty) alcohols and waxes; sphingoids and other long-chain bases; glycolipids, phospholipids and sphingolipids; and carotenoids, polyrenols, sterols, terpenes and other isoprenoids.                                           | 0.000129                    |
| 06629   | process      |                                          |                                                                                                                                                                                                                                                                                                                                       |                             |
### Supplementary Table 3. Continued

| GO_ID   | Category               | Name                                      | Description                                                                                                                                                                                                 | GO(P-val): Control vs. Case |
|---------|------------------------|-------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------|
| GO:00   | Biological _process    | Fatty acid metabolic process              | The chemical reactions and pathways involving fatty acids, aliphatic monocarboxylic acids liberated from naturally occurring fats and oils by hydrolysis.                                                          | 0.000625                   |
| GO:00   | Biological _process    | Anion transport                           | The directed movement of anions, atoms or small molecules with a net negative charge, into, out of or within a cell, or between cells, by means of some agent such as a transporter or pore.                     | 0.000452                   |
| GO:00   | Biological _process    | Mitosis                                   | A cell cycle process comprising the steps by which the nucleus of a eukaryotic cell divides; the process involves condensation of chromosomal DNA into a highly compacted form. Canonically, mitosis produces two daughter nuclei whose chromosome complement is identical to that of the mother cell. | 0.00086                    |
| GO:00   | Biological _process    | Mitotic chromosome movement towards spindle pole | The cell cycle process in which the directed movement of chromosomes from the center of the spindle towards the spindle poles occurs. This mediates by the shortening of microtubules attached to the chromosomes, during mitosis. | 0.000924                   |
| GO:00   | biological _process    | Multicellular organismal development      | The biological process whose specific outcome is the progression of a multicellular organism over time from an initial condition (e.g. a zygote or a young adult) to a later condition (e.g. a multicellular animal or an aged adult). | 0.000008                   |
| GO:00   | Biological _process    | Neuroblast proliferation                  | The expansion of a neuroblast population by cell division. A neuroblast is any cell that will divide and give rise to a neuron.                                                                               | 0.000664                   |
| GO:00   | Biological _process    | Hindgut morphogenesis                     | The process in which the anatomical structures of the hindgut are generated and organized.                                                                                                                  | 0.000059                   |
| GO:00   | Biological _process    | Steroid metabolic process                 | The chemical reactions and pathways involving steroids, compounds with a 1,2,cyclopentanoperhydrophenanthrene nucleus.                                                                                       | 0.000641                   |
| GO:00   | Biological _process    | Epidermis development                     | The process whose specific outcome is the progression of the epidermis over time, from its formation to the mature structure. The epidermis is the outer epithelial layer of a plant or animal, it may be a single layer that produces an extracellular material (e.g. the cuticle of arthropods) or a complex stratified squamous epithelium, as in the case of many vertebrate species. | 0                   |
| GO:00   | Biological _process    | Tissue development                        | The process whose specific outcome is the progression of a tissue over time, from its formation to the mature structure.                                                                                       | 0.000424                   |
| GO:00   | Biological _process    | Positive regulation of neuron projection development | Any process that increases the rate, frequency or extent of neuron projection development. Neuron projection development is the process whose specific outcome is the progression of a neuron projection over time, from its formation to the mature structure. A neuron projection is any process extending from a neural cell, such as axons or dendrites (collectively called neurites). | 0.000573                   |
| GO:00   | Biological _process    | Phospholipid transport                    | The directed movement of phospholipids into, out of or within a cell, or between cells, by means of some agent such as a transporter or pore. Phospholipids are any lipids containing phosphoric acid as a mono- or diester. | 0.000528                   |
| GO:00   | biological _process    | Cell-cell adhesion                        | The attachment of one cell to another cell via adhesion molecules.                                                                                                                                         | 0.000316                   |
| GO:00   | Biological _process    | Phenol-containing compound metabolic process | The chemical reactions and pathways involving a phenol, any compound containing one or more hydroxyl groups directly attached to an aromatic carbon ring.                                                               | 0.00009                    |
**Supplementary Table 3.** Continued

| GO_ID   | Category               | Name                              | Description                                                                                                                                                                                                                                                                                                                                 | GO(P-val); Control vs. Case |
|---------|------------------------|-----------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------|
| GO:00   | Biological process     | Electron transport chain          | A process in which a series of electron carriers operate together to transfer electrons from donors to any of several different terminal electron acceptors to generate a transmembrane electrochemical gradient.                                                                                                                                             | 0.000037                    |
| GO:00   | Biological process     | Respiratory electron transport    | A process in which a series of electron carriers operate together to transfer electrons from donors such as NADH and FADH2 to any of several different terminal electron acceptors to generate a transmembrane electrochemical gradient.                                                                                                                                       | 0.000024                    |
| GO:00   | Biological process     | Hair follicle morphogenesis       | The process in which the anatomical structures of the hair follicle are generated and organized.                                                                                                                                                                                                                                       | 0.000274                    |
| GO:00   | Biological process     | Developmental process             | A biological process whose specific outcome is the progression of an integrated living unit: an anatomical structure (which may be a subcellular structure, cell, tissue, or organ), or organism over time from an initial condition to a later condition.                                                                                                                                       | 0.000292                    |
| GO:00   | Biological process     | T cell receptor V(D)J recombination | The process in which T cell receptor V, D, and J, or V and J gene segments, depending on the specific locus, are recombined within a single locus utilizing the conserved heptamer and nonomer recombination signal sequences (RSS).                                                                                                                                  | 0.000268                    |
| GO:00   | Biological process     | Melanin biosynthetic process      | The chemical reactions and pathways resulting in the formation of melamins, pigments largely of animal origin. High molecular weight polymers of indole quinone, they are irregular polymeric structures and are divided into three groups: allomelansins in the plant kingdom and eumelansins and phaeomelansins in the animal kingdom.                                                                 | 0.000003                    |
| GO:00   | Biological process     | Hormone metabolic process         | The chemical reactions and pathways involving any hormone, naturally occurring substances secreted by specialized cells that affects the metabolism or behavior of other cells possessing functional receptors for the hormone.                                                                                                                                         | 0.000221                    |
| GO:00   | Biological process     | Hair cycle                        | The cyclical phases of growth (anagen), regression (catagen), quiescence (telogen), and shedding (exogen) in the life of a hair; one of the collection or mass of filaments growing from the skin of an animal, and forming a covering for a part of the head or for any part or the whole of the body.                                                                 | 0                            |
| GO:00   | Biological process     | Anagen                            | The growth phase of the hair cycle. Lasts, for example, about 3 to 6 years for human scalp hair.                                                                                                                                                                                                                                         | 0.000268                    |
| GO:00   | Biological process     | Skin development                  | The process whose specific outcome is the progression of the skin over time, from its formation to the mature structure. The skin is the external membranous integument of an animal. In vertebrates the skin generally consists of two layers, an outer nonsensitive and nonvascular epidermis (cuticle or skarfskin) composed of cells which are constantly growing and multiplying in the deeper, and being thrown off in the superficial layers, as well as an inner vascular dermis (cutis, corium or true skin) composed mostly of connective tissue. | 0                            |
| GO:00   | Biological process     | Small molecule metabolic process   | The chemical reactions and pathways involving small molecules, any low molecular weight, monomeric, non-encoded molecule.                                                                                                                                                                                                             | 0.000152                    |
### Supplementary Table 3. Continued

| GO_ID   | Category           | Name                                           | Description                                                                                                                                                                                                 | GO(P-val); Control vs. Case |
|---------|--------------------|------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------|
| GO:00   | Biological         | Cellular respiration                           | The enzymatic release of energy from organic compounds (especially carbohydrates and fats) which either requires oxygen (aerobic respiration) or does not (anaerobic respiration).                              | 0.000415                   |
| 45333   | _process           | Regulation of dendrite morphogenesis           | Any process that modulates the frequency, rate or extent of dendrite morphogenesis.                                                                                                                                                 | 0.000463                   |
| GO:00   | Biological         | Anatomical structure development               | The biological process whose specific outcome is the progression of an anatomical structure from an initial condition to its mature state. This process begins with the formation of the structure and ends with the mature structure, whatever form that may be including its natural destruction. An anatomical structure is any biological entity that occupies space and is distinguished from its surroundings. Anatomical structures can be macroscopic such as a carpel, or microscopic such as an acrosome. | 0.000313                   |
| 48814   | _process           | Regulation of dendrite development             | Any process that modulates the frequency, rate or extent of dendrite development.                                                                                                                                                   | 0.00043                    |
| 48856   | _process           | Cell division                                  | The process resulting in the physical partitioning and separation of a cell into daughter cells.                                                                                                                                     | 0.000651                   |
| 51301   | _process           | Regulation of cell division                    | Any process that modulates the frequency, rate or extent of the physical partitioning and separation of a cell into daughter cells.                                                                                                     | 0.000262                   |
| 51302   | _process           | Oxidation-reduction process                    | A metabolic process that results in the removal or addition of one or more electrons to or from a substance, with or without the concomitant removal or addition of a proton or protons.                                          | 0.000023                   |
| GO:00   | Biological         | Smoothened signaling pathway involved in dorsal/ventral neural tube patterning | The series of molecular signals generated as a consequence of activation of the transmembrane protein Smoothened contributing to the dorsal/ventral pattern of the neural tube.                                              | 0.000895                   |
| 60831   | _process           | Embryonic camera-type eye formation            | The developmental process pertaining to the initial formation of a camera-type eye from unspecified neurectoderm. This process begins with the differentiation of cells that form the optic field and ends when the optic cup has attained its shape. | 0.000289                   |
| GO:00   | Cellular           | Mitochondrial inner membrane                   | The inner, i.e. lumen-facing, lipid bilayer of the mitochondrial envelope. It is highly folded to form cristae.                                                                                                                         | 0.000532                   |
| 05743   | _component         | Mitochondrial respiratory chain                | The protein complexes that form the mitochondrial electron transport system (the respiratory chain), associated with the inner mitochondrial membrane. The respiratory chain complexes transfer electrons from an electron donor to an electron acceptor and are associated with a proton pump to create a transmembrane electrochemical gradient. | 0.000077                   |
| GO:00   | Cellular           | Mitochondrial respiratory chain complex I      | A protein complex located in the mitochondrial inner membrane that forms part of the mitochondrial respiratory chain. It contains about 25 different polypeptide subunits, including NADH dehydrogenase (ubiquinone), flavin mononucleotide and several different iron-sulfur clusters containing non-heme iron. The iron undergoes oxidation-reduction between Fe(II) and Fe(III), and catalyzes proton translocation linked to the oxidation of NADH by ubiquinone. | 0.000071                   |
### Supplementary Table 3. Continued

| GO_ID      | Category      | Name                      | Description                                                                 | GO(P-val); Control vs. Case |
|------------|---------------|---------------------------|-----------------------------------------------------------------------------|-----------------------------|
| GO:00      | Cellular      | Hemoglobin complex        | An iron-containing, oxygen carrying complex. In vertebrates it is made up of  | 0.00042                     |
| 05833      | _component    |                            | two pairs of associated globin polypeptide chains, each chain carrying a    |                             |
|            |               |                           | noncovalently bound heme prosthetic group.                                  |                             |
| GO:00      | Cellular      | Cytoskeleton              | Any of the various filamentous elements that form the internal framework of  | 0                           |
| 05856      | _component    |                            | cells, and typically remain after treatment of the cells with mild detergent|                             |
|            |               |                           | to remove membrane constituents and soluble components of the cytoplasm.     |                             |
|            |               |                           | The term embraces intermediate filaments, microfilaments, microtubules, the |                             |
|            |               |                           | microtrabecular lattice, and other structures characterized by a polymeric  |                             |
|            |               |                           | filamentous nature and long-range order within the cell. The various       |                             |
|            |               |                           | elements of the cytoskeleton not only serve in the maintenance of cellular  |                             |
|            |               |                           | shape but also have roles in other cellular functions, including cellular   |                             |
|            |               |                           | movement, cell division, endocytosis, and movement of organelles.           |                             |
| GO:00      | Cellular      | Intermediate filament     | A cytoskeletal structure that forms a distinct elongated structure,          | 0                           |
| 05882      | _component    |                            | characteristically 10 nm in diameter, that occurs in the cytoplasm of    |                             |
|            |               |                           | eukaryotic cells. Intermediate filaments form a fibrous system, composed   |                             |
|            |               |                           | of chemically heterogeneous subunits and involved in mechanically         |                             |
|            |               |                           | integrating the various components of the cytoplasmic space. Intermediate  |                             |
|            |               |                           | filaments may be divided into five chemically distinct classes: Type I,    |                             |
|            |               |                           | acidic keratins; Type II, basic keratins; Type III, including desmin,      |                             |
|            |               |                           | vimentin and others; Type IV, neurofilaments and related filaments; and   |                             |
|            |               |                           | Type V, lamins.                                                           |                             |
| GO:00      | Cellular      | Protein complex           | Any macromolecular complex composed of two or more polypeptide subunits,    | 0.00038                     |
| 43234      | _component    |                            | which may or may not be identical. Protein complexes may have other       |                             |
|            |               |                           | associated non-protein prosthetic groups, such as nucleotides, metal ions  |                             |
|            |               |                           | or other small molecules.                                                  |                             |
| GO:00      | Cellular      | Keratin filament          | A filament composed of acidic and basic keratins (types I and II),         | 0                           |
| 45095      | _component    |                            | typically expressed in epithelial cells. The keratins are the most        |                             |
|            |               |                           | diverse classes of IF proteins, with a large number of keratin isoforms   |                             |
|            |               |                           | being expressed. Each type of epithelium always expresses a characteristic  |                             |
|            |               |                           | combination of type I and type II keratins.                               |                             |
| GO:00      | Cellular      | Intermediate filament     | Cytoskeletal structure made from intermediate filaments, typically         | 0                           |
| 45111      | _component    | cytoskeleton              | organized in the cytosol as an extended system that stretches from the     |                             |
|            |               |                           | nuclear envelope to the plasma membrane. Some intermediate filaments run  |                             |
|            |               |                           | parallel to the cell surface, while others traverse the cytosol; together  |                             |
|            |               |                           | they form an internal framework that helps support the shape and resilience|                             |
|            |               |                           | of the cell.                                                               |                             |
| GO:00      | Cellular      | Respiratory chain         | The protein complexes that form the electron transport system (the         | 0.000047                    |
| 70469      | _component    |                            | respiratory chain), associated with a cell membrane, usually the          |                             |
|            |               |                           | plasma membrane (in prokaryotes) or the inner mitochondrial membrane (on   |                             |
|            |               |                           | eukaryotes). The respiratory chain complexes transfer electrons from an    |                             |
|            |               |                           | electron donor to an electron acceptor and are associated with a proton    |                             |
|            |               |                           | pump to create a transmembrane electrochemical gradient.                   |                             |
| GO:00      | Cellular      | Alveolar lamellar body    | The lipid bilayer surrounding an alveolar lamellar body, a specialized     | 0.000924                    |
| 97233      | _component    | membrane                  | secretory organelle found in type II pneumocytes and involved in the      |                             |
|            |               |                           | synthesis, secretion, and reutilization of pulmonary surfactant.           |                             |
### Supplementary Table 3. Continued

| GO_ID     | Category   | Name                                      | Description                                                                                                                                                                                                                                                                                                                                 | GO(p-val): Control vs. Case |
|-----------|------------|-------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------|
| GO:00     | Molecular  | _function Microtubule motor activity      | Catalysis of movement along a microtubule, coupled to the hydrolysis of a nucleoside triphosphate (usually ATP).                                                                                                                                                                                                                             | 0.000495                   |
| 03777     |            | GO:0003777                                |                                                                                                                                                                                                                                                                                                                                           |                             |
| GO:00     | Molecular  | _function Catalytic activity              | Catalysis of a biochemical reaction at physiological temperatures. In biologically catalyzed reactions, the reactants are known as substrates, and the catalysts are naturally occurring macromolecular substances known as enzymes. Enzymes possess specific binding sites for substrates, and are usually composed wholly or largely of protein, but RNA that has catalytic activity (ribozyme) is often also regarded as enzymatic. | 0.000038                   |
| 03824     |            | GO:0003824                                |                                                                                                                                                                                                                                                                                                                                           |                             |
| GO:00     | Molecular  | _function NADH dehydrogenase activity     | Catalysis of the reaction: NADH + H+ + acceptor = NAD+ + reduced acceptor.                                                                                                                                                                                                                                                               | 0.000064                   |
| 03954     |            | GO:0003954                                |                                                                                                                                                                                                                                                                                                                                           |                             |
| GO:00     | Molecular  | _function RNA-directed DNA polymerase activity | Catalysis of the reaction: deoxynucleoside triphosphate + DNA(n) = diphosphate + DNA(n+1). Catalyzes RNA-template-directed extension of the 3' end of a DNA strand by one deoxynucleotide at a time.                                                                                   | 0.000001                   |
| 03964     |            | GO:0003964                                |                                                                                                                                                                                                                                                                                                                                           |                             |
| GO:00     | Molecular  | _function Oxidoreductase activity         | Catalysis of an oxidation-reduction (redox) reaction, a reversible chemical reaction in which the oxidation state of an atom or atoms within a molecule is altered. One substrate acts as a hydrogen or electron donor and becomes oxidized, while the other acts as hydrogen or electron acceptor and becomes reduced. | 0.000061                   |
| 04012     |            | GO:0004012                                |                                                                                                                                                                                                                                                                                                                                           |                             |
| GO:00     | Molecular  | _function Hydrogen ion transmembrane transporter activity | Catalysis of the transfer of hydrogen ions from one side of a membrane to the other.                                                                                                                                                                                                                                                      | 0.000064                   |
| 05344     |            | GO:0005344                                |                                                                                                                                                                                                                                                                                                                                           |                             |
| GO:00     | Molecular  | _function Calcium ion binding             | Interacting selectively and non-covalently with calcium ions (Ca2+).                                                                                                                                                                                                                                                                                                                                  | 0.000049                   |
| 05509     |            | GO:0005509                                |                                                                                                                                                                                                                                                                                                                                           |                             |
| GO:00     | Molecular  | _function NADH dehydrogenase (ubiquinone) activity | Catalysis of the reaction: NADH + H+ + ubiquinone = NAD+ + ubiquinol.                                                                                                                                                                                                                                                                                                                               | 0.000064                   |
| 08137     |            | GO:0008137                                |                                                                                                                                                                                                                                                                                                                                           |                             |
| GO:00     | Molecular  | _function Oxygen transporter activity      | Enables the directed movement of oxygen into, out of or within a cell, or between cells.                                                                                                                                                                                                                                                                                                           | 0.000049                   |
| 15078     |            | GO:0015078                                |                                                                                                                                                                                                                                                                                                                                           |                             |
| GO:00     | Molecular  | _function Oxidoreductase activity, acting on CH-OH group of donors | Catalysis of an oxidation-reduction (redox) reaction in which a CH-OH group act as a hydrogen or electron donor and reduces a hydrogen or electron acceptor.                                                                                                                                                                                      | 0.000061                   |
| 16491     |            | GO:0016491                                |                                                                                                                                                                                                                                                                                                                                           |                             |
| GO:00     | Molecular  | _function Oxidoreductase activity, acting on CH-CH group of donors, NAD or NADP as acceptor | Catalysis of an oxidation-reduction (redox) reaction in which a CH-CH group act as a hydrogen or electron donor and reduces NAD+ or NADP.                                                                                                                                                                                                     | 0.000073                   |
| 16614     |            | GO:0016614                                |                                                                                                                                                                                                                                                                                                                                           |                             |
| GO:00     | Molecular  | _function Oxidoreductase activity, acting on NADPH, quinone or similar compound as acceptor | Catalysis of an oxidation-reduction (redox) reaction in which NADH or NADPH acts as a hydrogen or electron donor and reduces a quinone or a similar acceptor molecule.                                                                                                                                                                           | 0.000069                   |
| 16628     |            | GO:0016628                                |                                                                                                                                                                                                                                                                                                                                           |                             |
| GO:00     | Molecular  | _function Oxygen binding                  | Interacting selectively and non-covalently with oxygen (O2).                                                                                                                                                                                                                                                                                                                                       | 0.000049                   |
| 19825     |            | GO:0019825                                |                                                                                                                                                                                                                                                                                                                                           |                             |

GO: gene ontology.