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ORIGINAL ARTICLE

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*, *WIF1*, *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 hairs[1]. 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 studies[2]. Fantauzzo and Christiano[1] reported that the target genes of Trps1, Wif1, Sox18, and Sox21 played an important role in vibrissa follicle morphogenesis by analyzing the gene expression profiles between wild-type and Trps1Δgt/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.

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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[3]. 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)[4].

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 v0.5.4p3[5]. Based on this, we analyzed differentially expressed genes (DEGs) using the gene TCC[6]. 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 (%)	Gene				Transcript		
					Sum	Known	Known (+new isoforms)	Novel	Sum	Known	Novel
Non-balding	53,351,054	50,338,798 (94.4)	47,780,868 (94.9)	36,570,528 (72.6)	26,320	3,426	14,892	4,883	218,609	150,194	68,415
Balding	54,289,736	51,192,244 (94.3)	48,539,550 (94.8)	36,789,924 (71.9)							

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.

Gene ontology analysis

To characterize the DEGs, we conducted GO analysis. A total of 1,242 DEGs were associated with 3,108 GO terms; at the cut-off of p-value <0.001, 45, 17 and 11 GO terms were associated with biological processes, molecular function, and cellular components, respectively (Supplementary Table 2, 3). The hair-related terms were follicle morphogenesis and development, the hair cycle, hair cell differentiation, and keratinization and were associated with the down-regulated *SOX21* gene.

Verifying DEGs using quantitative real-time polymerase chain reaction

We validated 15 DEGs in the non-balding and balding scalps samples using quantitative real-time polymerase chain reaction (Supplementary Table 1), preferentially selecting skin, hair, and alopecia-related genes; in addition, we validated the *WIF1*, *SOX21*, *TINAG* and *TRPS1* genes studied in TRPS1 Δgt/Δgt mutant mouse[1]. Additionally, we validated *HBA2*, *GAL*, *DES* (the up-regulated genes with the lowest p-values) and *PTGDS*, and this result was

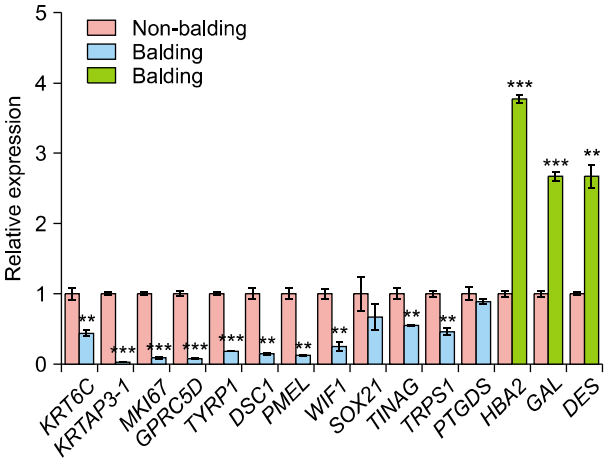

Fig. 2. Validation of 15 differentially expressed genes by quantitative real-time polymerase chain reaction. Three genes were up-regulated (green bar) and 12 were down-regulated (blue bar) in the non-balding scalp. Thirteen genes showed statistical significance (p-value <0.05); the exceptions were *SOX21*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

consistent with the RNA sequencing (Fig. 2). Thirteen of the 15 genes were statistically significant ($p<0.01$), including six genes with p-value <0.001. The *SOX21* and *PTGDS* genes were down-regulated but not significantly ($p>0.05$).

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 cells[7]. 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 region[1]. However, a recent study has revealed that *TRPS1* activated the expression of target gene. Fantauzzo and Christiano[1] 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[8]. Decreased TRPS1 protein can disrupt endochondral cartilage differentiation and cell interactions in hair follicle development[9]. 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[10]. 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[1]. *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[11]. The *SOX21* gene was shown to regulate the layered differentiation of hair follicles[12]. 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[1].

We compared the gene expression patterns with those of androgenetic alopecia by Garza et al.[13]. *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[13]. 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[11]. PTGDS might be involved in androgenetic alopecia[13], 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.

REFERENCES

1. Fantauzzo KA, Christiano AM. Trps1 activates a network of secreted Wnt inhibitors and transcription factors crucial to vibrissa follicle morphogenesis. Development 2012;139:203-214.
2. Shimomura Y, Agalliu D, Vonica A, Luria V, Wajid M, Baumer A, et al. APCDD1 is a novel Wnt inhibitor mutated in hereditary hypotrichosis simplex. Nature 2010;464:1043-1047.
3. Trapnell C, Williams BA, Pertea G, Mortazavi A, Kwan G, van Baren MJ, et al. Transcript assembly and quantification by RNA-Seq reveals unannotated transcripts and isoform switching during cell differentiation. Nat Biotechnol 2010;28:511-515.
4. Flicek P, Ahmed I, Amode MR, Barrell D, Beal K, Brent S, et al. Ensembl 2013. Nucleic Acids Res 2013;41(Database issue):D48-D55.
5. Anders S, Pyl PT, Huber W. HTSeq–a Python framework to work with high-throughput sequencing data. Bioinformatics 2015;31:166-169.
6. Sun J, Nishiyama T, Shimizu K, Kadota K. TCC: an R package for comparing tag count data with robust normalization strategies. BMC Bioinformatics 2013;14:219.
7. Morris RJ, Liu Y, Marles L, Yang Z, Trempus C, Li S, et al. Capturing and profiling adult hair follicle stem cells. Nat Biotechnol 2004;22:411-417.
8. Malik TH, Von Stechow D, Bronson RT, Shivdasani RA. Deletion of the GATA domain of TRPS1 causes an absence of facial hair and provides new insights into the bone disorder in inherited tricho-rhino-phalangeal syndromes. Mol Cell Biol 2002;22:8592-8600.
9. Gai Z, Gui T, Muragaki Y. The function of TRPS1 in the development and differentiation of bone, kidney, and hair follicles. Histol Histopathol 2011;26:915-921.
10. Chang GT, Jhamai M, van Weerden WM, Jenster G, Brinkmann AO. The TRPS1 transcription factor: androgenic regulation in prostate cancer and high expression in breast cancer. Endocr Relat Cancer 2004;11:815-822.
11. Gao Y, Wang X, Yan H, Zeng J, Ma S, Niu Y, et al. Comparative transcriptome analysis of fetal skin reveals key genes related to hair follicle morphogenesis in cashmere goats. PLoS One 2016;11:e0151118.
12. Kiso M, Tanaka S, Saba R, Matsuda S, Shimizu A, Ohyama M, et al. The disruption of Sox21-mediated hair shaft cuticle

differentiation causes cyclic alopecia in mice. Proc Natl Acad Sci U S A 2009;106:9292-9297.
13. Garza LA, Liu Y, Yang Z, Alagesan B, Lawson JA, Norberg SM, et al. Prostaglandin D2 inhibits hair growth and is elevated in bald scalp of men with androgenetic alopecia. Sci Transl Med 2012;4:126ra34.

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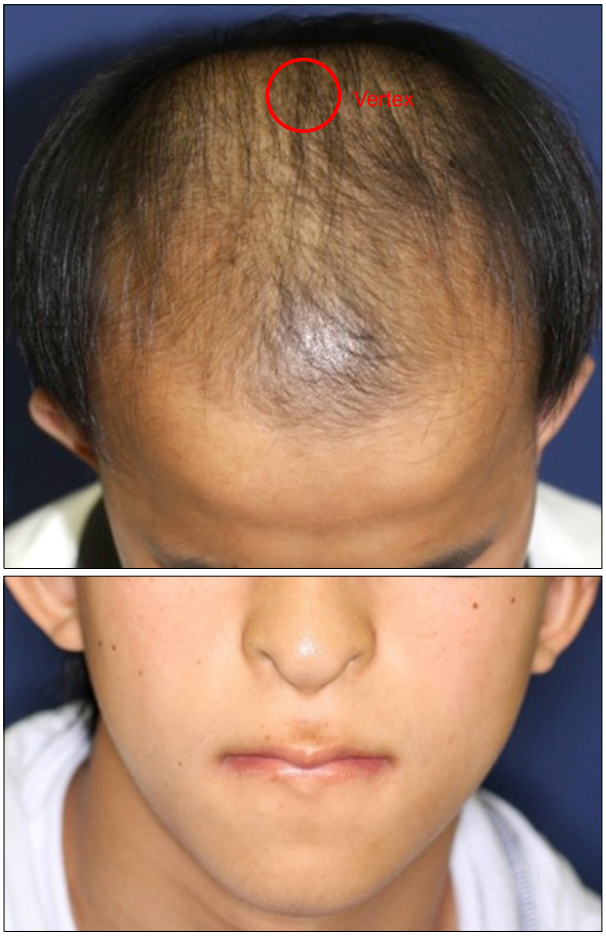

Supplementary Fig. 1. Photograph of the trichorhinophalangeal syndrome patient. He has sparse and slowly growing scalp hairs (up), bulbous nose, long philtrum, and thin upper lip (bottom). A red circle indicates vertex area where tissue was obtained but, occiput is not appeared in this photograph.

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	TCAACTTCCTGAGAGCCTTG	KRT6C_R_2R	CGTATTGGGCCTTGACCTC	141
2	*KRTAP3-1*	Down	KRTAP3-1_R_F	TCAACAACTGTCACCCGACT	KRTAP3-1_R_R	GTAGTGAGTGCTGAAGCCCA	171
3	*MKI67*	Down	MKI67_R_2F	GCCTCCTAATACGCCTCTCA	MKI67_R_2R	TTGTGCCTTCACTTCCACAT	160
4	*GPRC5D*	Down	GPRC5D_R_F	GCTCATGCCTCCAATCTAGTG	GPRC5D_R_R	GCAGGGTGTCATATTCACAAA	168
5	*TYRP1*	Down	TYRP1_R_F	GCCATAGCAGTAGTTGGCG	TYRP1_R_R	GGAGAGGCTGGTTAGCTTCA	106
6	*DSC1*	Down	DSC1_R_F	CCACAGACCTTGACGAACC	DSC1_R_R	ACCCATGTCTCGCACTTCC	188
7	*PMEL*	Down	PMEL_R_2F	CTTTCTCCGTGAGCGTGTC	PMEL_R_2R	CCACTACTGTCTCCAAAGTCCC	160
8	*WIF1*	Down	WIF1_R_2F	GGACTTTGTGTGACTCCT GGTT	WIF1_R_2R	ATTTCGACAGGGTTGTGGG	189
9	*SOX21*	Down	SOX21_R_2F	CCGAGTGGAAACTGCTCAC	SOX21_R_2R	CGGGAAGGCGAACTTGTC	155
10	*TINAG*	Down	TINAG_R_F	AAGAGAAGTGGACCTA GAGGCTT	TINAG_R_R	AGTAGCACAACGCATTCGC	170
11	*TRPS1*	Down	TRPS1_R_F	ATCTGGCGCGACCTATTTAT	TRPS1_R_R	AGCCTCTACGCCTCCGTAA	165
12	*PTGDS*	-	PTGDS_R_F	AACCAGTGTGAGACCCGAAC	PTGDS_R_R	TCCACCACTGACACGGAGTA	107
13	*HBA2*	Up	HBA2_R_F	TACTTCCCGCACTTCGACC	HBA2_R_R	GCAGTGGCTTAGGAGCTTGA	189
14	*GAL*	Up	GAL_R_F	CTCGCCTCCCTCCTCCTC	GAL_R_R	TCTTGTCGCTGAATGACCTG	148
15	*DES*	Up	DES_R_F	TATGAGACCATCGCGGCTAA	DES_R_R	ATCAGGGAATCGTTAGTGCC	197
16	*GAPDH*	Control	GAPDH_R_F	GAGCCCCAGCCTTCTCCATG	GAPDH_R_R	GAAATCCCATCACCATCTT CCAGG	120

DEG: differentially expressed gene.

Supplementary Table 2. DEGs and GO

Sample	Gene	Mod	Unexp	DEG*			GO†		
				Sum	Up (balding only)	Down (non-balding only)	Biological process	Molecular function	Cellular component
Non-balding	22,449	14,723	3,871	1,242	636 (333)	606 (224)	45 (2117)	17 (643)	11 (348)
Balding	21,881	14,466	4,439						

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:0000278	Biological_process	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
GO:0001942	Biological_process	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 tube-like opening in the epidermis where the hair shaft develops and into which the sebaceous glands open.	0.000003
GO:0002741	Biological_process	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
GO:0003408	Biological_process	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
GO:0006082	Biological_process	Organic acid metabolic process	The chemical reactions and pathways involving organic acids, any acidic compound containing carbon in covalent linkage.	0.000937
GO:0006119	Biological_process	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
GO:0006120	Biological_process	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
GO:0006260	Biological_process	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
GO:0006278	Biological_process	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
GO:0006582	Biological_process	Melanin metabolic process	The chemical reactions and pathways involving melanins, pigments largely of animal origin. High molecular weight polymers of indole quinone, they are irregular polymeric structures and are divided into three groups: allomelanins in the plant kingdom and eumelanins and phaeomelanins in the animal kingdom.	0.000046
GO:0006629	Biological_process	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 carotenes, polyprenols, sterols, terpenes and other isoprenoids.	0.000129

Supplementary Table 3. Continued

GO_ID	Category	Name	Description	GO(P-val): Control vs. Case
GO:0006631	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:0006820	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:0007067	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:0007079	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:0007275	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:0007405	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:0007442	Biological_process	Hindgut morphogenesis	The process in which the anatomical structures of the hindgut are generated and organized.	0.000059
GO:0008202	Biological_process	Steroid metabolic process	The chemical reactions and pathways involving steroids, compounds with a 1,2,cyclopentanoperhydrophenanthrene nucleus.	0.000641
GO:0008544	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:0009888	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:0010976	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:0015914	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:0016337	biological_process	Cell-cell adhesion	The attachment of one cell to another cell via adhesion molecules.	0.000316
GO:0018958	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:0022900	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:0022904	Biological_process	Respiratory electron transport chain	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:0031069	Biological_process	Hair follicle morphogenesis	The process in which the anatomical structures of the hair follicle are generated and organized.	0.000274
GO:0032502	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:0033153	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:0042438	Biological_process	Melanin biosynthetic process	The chemical reactions and pathways resulting in the formation of melanins, pigments largely of animal origin. High molecular weight polymers of indole quinone, they are irregular polymeric structures and are divided into three groups: allomelanins in the plant kingdom and eumelanins and phaeomelanins in the animal kingdom.	0.00003
GO:0042445	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:0042633	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:0042640	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:0043588	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:0044281	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:0045333	Biological_process	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
GO:0048814	Biological_process	Regulation of dendrite morphogenesis	Any process that modulates the frequency, rate or extent of dendrite morphogenesis.	0.000463
GO:0048856	Biological_process	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
GO:0050773	Biological_process	Regulation of dendrite development	Any process that modulates the frequency, rate or extent of dendrite development.	0.00043
GO:0051301	Biological_process	Cell division	The process resulting in the physical partitioning and separation of a cell into daughter cells.	0.000651
GO:0051302	Biological_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
GO:0055114	Biological_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:0060831	Biological_process	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
GO:0060900	Biological_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:0005743	Cellular_component	Mitochondrial inner membrane	The inner, i.e. lumen-facing, lipid bilayer of the mitochondrial envelope. It is highly folded to form cristae.	0.000532
GO:0005746	Cellular_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:0005747	Cellular_component	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:0005833	Cellular_component	Hemoglobin complex	An iron-containing, oxygen carrying complex. In vertebrates it is made up of two pairs of associated globin polypeptide chains, each chain carrying a noncovalently bound heme prosthetic group.	0.00042
GO:0005856	Cellular_component	Cytoskeleton	Any of the various filamentous elements that form the internal framework of 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.	0
GO:0005882	Cellular_component	Intermediate filament	A cytoskeletal structure that forms a distinct elongated structure, 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.	0
GO:0043234	Cellular_component	Protein complex	Any macromolecular complex composed of two or more polypeptide subunits, 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.	0.000038
GO:0045095	Cellular_component	Keratin filament	A filament composed of acidic and basic keratins (types I and II), 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.	0
GO:0045111	Cellular_component	Intermediate filament cytoskeleton	Cytoskeletal structure made from intermediate filaments, typically 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.	0
GO:0070469	Cellular_component	Respiratory chain	The protein complexes that form the electron transport system (the 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.	0.000047
GO:0097233	Cellular_component	Alveolar lamellar body membrane	The lipid bilayer surrounding an alveolar lamellar body, a specialized secretory organelle found in type II pneumocytes and involved in the synthesis, secretion, and reutilization of pulmonary surfactant.	0.000924

Supplementary Table 3. Continued

GO_ID	Category	Name	Description	GO(P-val): Control vs. Case
GO:0003777	Molecular_function	Microtubule motor activity	Catalysis of movement along a microtubule, coupled to the hydrolysis of a nucleoside triphosphate (usually ATP).	0.000495
GO:0003824	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
GO:0003954	Molecular_function	NADH dehydrogenase activity	Catalysis of the reaction: NADH + H+ + acceptor = NAD+ + reduced acceptor.	0.000064
GO:0003964	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
GO:0004012	Molecular_function	Phospholipid-translocating ATPase activity	Catalysis of the movement of phospholipids from one membrane bilayer leaflet to the other, driven by the hydrolysis of ATP.	0.000887
GO:0004129	Molecular_function	Cytochrome-c oxidase activity	Catalysis of the reaction: 4 ferrocytochrome c + O2 + 4 H+ = 4 ferricytochrome c + 2 H2O.	0.000089
GO:0005198	Molecular_function	Structural molecule activity	The action of a molecule that contributes to the structural integrity of a complex or assembly within or outside a cell.	0.000397
GO:0005344	Molecular_function	Oxygen transporter activity	Enables the directed movement of oxygen into, out of or within a cell, or between cells.	0.000475
GO:0005509	Molecular_function	Calcium ion binding	Interacting selectively and non-covalently with calcium ions (Ca2+).	0.000399
GO:0008137	Molecular_function	NADH dehydrogenase (ubiquinone) activity	Catalysis of the reaction: NADH + H+ + ubiquinone = NAD+ + ubiquinol.	0.000064
GO:0015078	Molecular_function	Hydrogen ion transmembrane transporter activity	Catalysis of the transfer of hydrogen ions from one side of a membrane to the other.	0.000491
GO:0016491	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
GO:0016614	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.	0.00061
GO:0016616	Molecular_function	Oxidoreductase activity, acting on the CH-OH group of donors, NAD or NADP as acceptor	Catalysis of an oxidation-reduction (redox) reaction in which a CH-OH group acts as a hydrogen or electron donor and reduces NAD+ or NADP.	0.000783
GO:0016628	Molecular_function	Oxidoreductase activity, acting on the CH-CH group of donors, NAD or NADP as acceptor	Catalysis of an oxidation-reduction (redox) reaction in which a CH-CH group acts as a hydrogen or electron donor and reduces NAD or NADP.	0.000069
GO:0016655	Molecular_function	Oxidoreductase activity, acting on NAD(P)H, 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.	0.000017
GO:0019825	Molecular_function	Oxygen binding	Interacting selectively and non-covalently with oxygen (O2).	0.00049

GO: gene ontology.