

Atopic dermatitis keratinocytes exhibit normal T_H17 cytokine responses

To the Editor:

Host response against infectious challenge is typically mediated by specialized subsets of CD4⁺ effector T_H cells. T_H1 cells producing IFN- γ mediate cellular immunity against intracellular pathogens, whereas T_H2 cells producing IL-4 and IL-13 mediate humoral immunity against parasites and helminths. T_H17 cells producing IL-17 confer early protection against pathogenic insult in epithelial surfaces by inducing neutrophil-mediated immune responses and enhanced production of antimicrobial peptides (AMPs) such as β -defensins, lipocalin, and S100 proteins.^{1,2}

Atopic dermatitis (AD) and psoriasis vulgaris are inflammatory skin diseases that share some similarities—acanthotic epidermis and abundant dermal mononuclear infiltrate—in the chronic phase. However, 1 striking difference between these 2 diseases is the high frequency of recurrent skin infections in AD³ versus an increased resistance to infections in psoriasis.⁴ AD skin is heavily colonized by toxin-producing *Staphylococcus aureus*⁵ and is more prone to cutaneous viral infections such as eczema vaccinatum and eczema herpeticum.⁶ This has been attributed to the relative deficiency of innate defense molecules, including AMPs,⁷ primarily regulated by IL-17.²

The role of T_H17 cells and IL-17 in AD is still unclear. Increased IL-17⁺ T cells have been identified in tissue sections of acute AD lesions relative to normal skin.⁸ Our previous work on chronic AD lesions also demonstrated increased IL-17 expression compared with normal skin, but the overall expression of the IL-23/T_H17 pathway is much reduced compared with psoriasis.⁹ To clarify whether AMP deficiency in AD was a result of (1)

absence of cognate IL-17 receptors, (2) defective signaling, or (3) T_H2 suppression, we obtained lesional and nonlesional skin from 18 patients with chronic AD and 15 healthy volunteers under a Rockefeller University institutional review board–approved protocol. Biopsy samples were processed for histology, RT-PCR analysis, or generation of primary keratinocyte cultures. Normal human keratinocytes were also obtained as previously described² (see this article's [Methods](#) in the Online Repository at www.jacionline.org for details).

We have previously shown that IL-17 strongly induces keratinocyte expression of innate defense molecules. To determine whether functional IL-17 receptors were essential for the epidermal response to IL-17, we cultured pooled human keratinocytes with 1 μ g/mL or 10 μ g/mL monoclonal IL-17 Receptor A blocking antibody before stimulation with IL-17. After 24 hours, keratinocytes were harvested and examined for expression of known IL-17–induced innate defense genes. [Fig 1](#) demonstrates that functional blockade of IL-17RA substantially inhibits expression of β -defensin 2 (*DEFB4*), IL-8, chemokine C-C motif ligand 20 (*CCL20*), and lipocalin2 (*LCN2*). Although all 4 genes were substantially inhibited by 10 μ g/mL IL-17RA blocking antibody, 1 μ g/mL IL-17RA blocking antibody was sufficient to suppress keratinocyte mRNA expression of *DEFB4* and IL-8, with less effective inhibition of *CCL20* and *LCN2* expression, compared with isotype-blocked keratinocytes ([Fig 1](#)). The housekeeping gene human acidic ribosomal protein was used to control for general keratinocyte gene expression effects. These results demonstrate that fully functional IL-17 receptors are necessary for IL-17–mediated barrier defense. The differential sensitivity of AMP expression to IL-17RA blockade necessitates further investigation. One possibility for residual expression of AMP despite

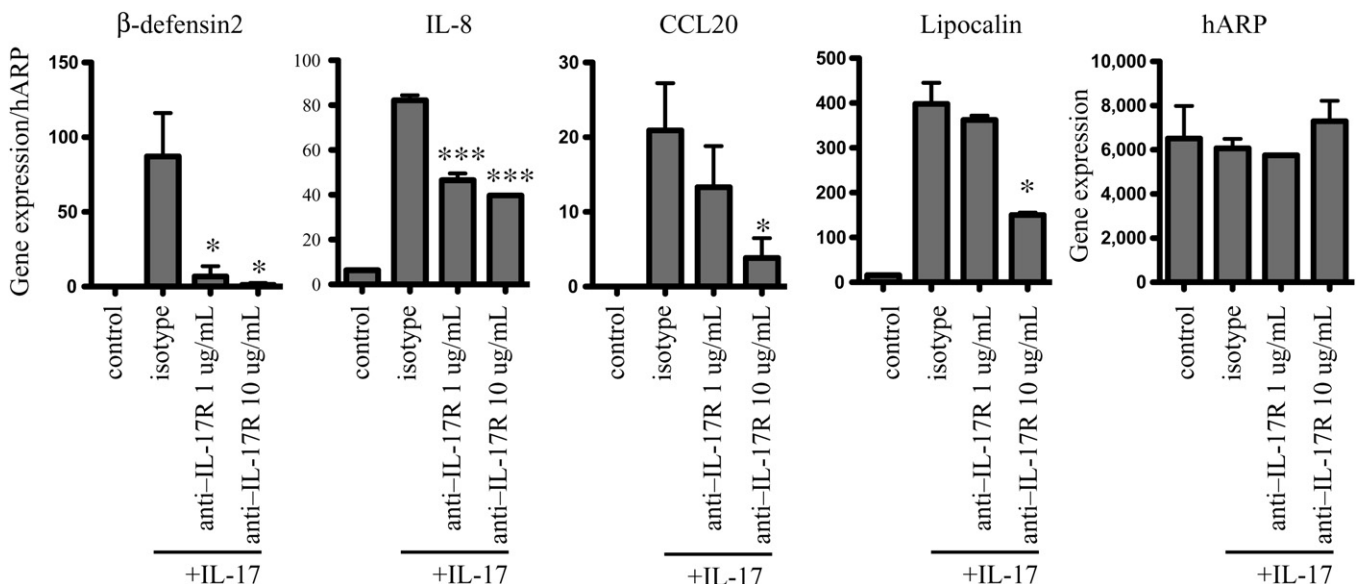


FIG 1. IL-17R blockade dampens keratinocyte expression of IL-17–induced innate defense genes. Quantitative RT-PCR analysis of β -defensin 2, IL-8, CCL20, lipocalin, and human acidic ribosomal protein (*hARP*) in keratinocytes cultured with IL-17 $\pm$ IL-17RA blocking antibody. Data normalized to *hARP* housekeeping gene and expressed as means and SEMs; * $P < .05$, *** $P < .005$ compared with IL-17 + isotype control.

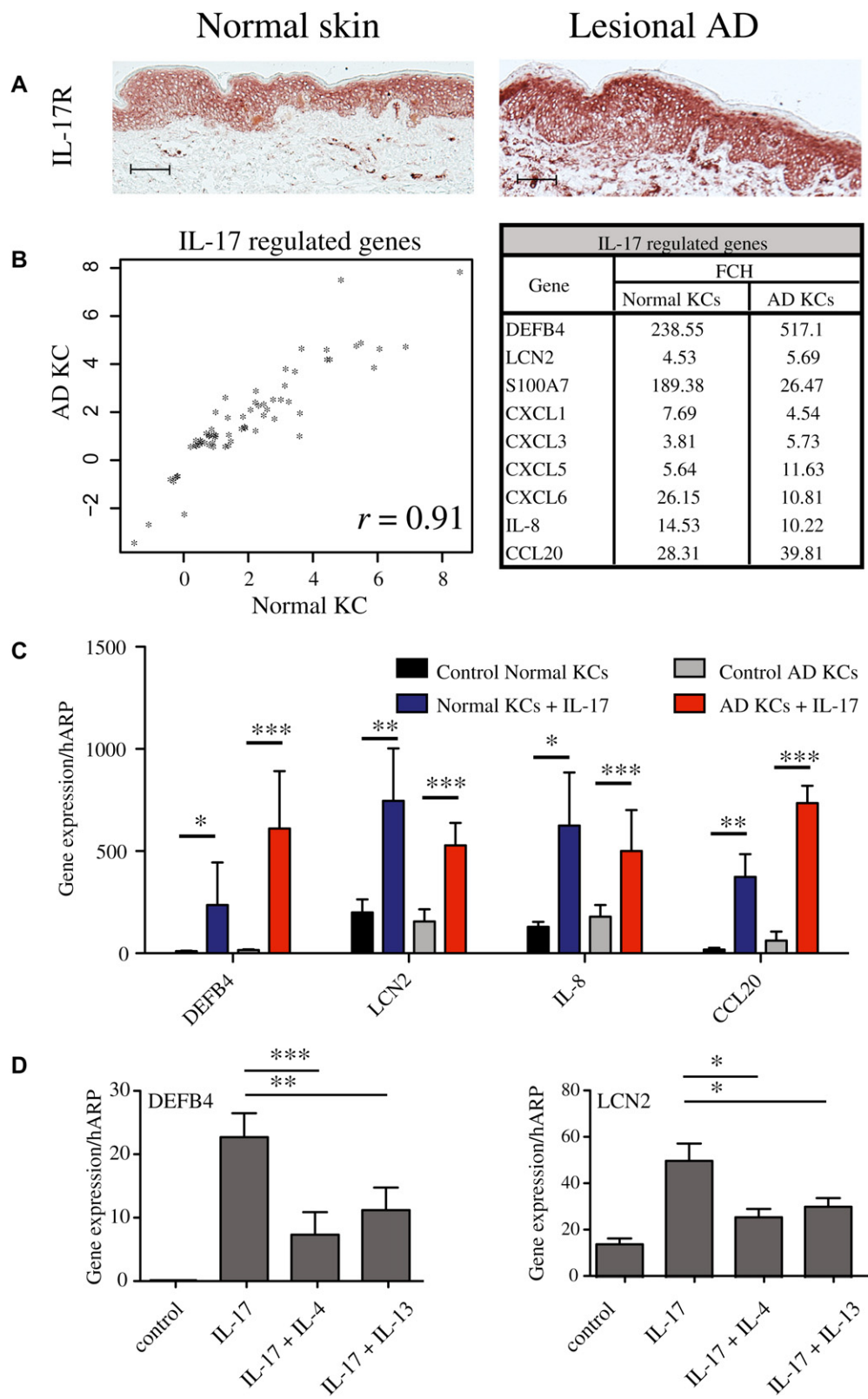


FIG 2. A, IL-17 receptors are expressed by normal and AD keratinocytes (KCs). B, Genomic analysis demonstrates linear correlation between normal and AD KCs after IL-17 stimulation. Table shows modulation of selected genes. C, RT-PCR analysis confirms upregulation of AMPs by AD KCs after IL-17 stimulation. D, T_H2 cytokines IL-4 and IL-13 suppress IL-17-induced AMP production. FCH, Fold change. **P* < .05, ***P* < .01, ****P* < .005.

IL-17RA blockade is signaling through human IL-17RC, another receptor from the IL-17R family that can bind IL-17.

We noted strong cell-surface expression of IL-17R in lesional AD keratinocytes ($n = 5$) by immunohistochemistry, with a staining pattern that is consistent with normal keratinocytes ($n = 5$; Fig 2, A). In addition to keratinocytes, scattered dermal cells were noted to express IL-17R as previously described.² Consistent with immunohistochemical findings, gene expression levels for IL-17R measured by quantitative RT-PCR were similar between AD and normal keratinocytes (see this article's Fig E1 in the Online Repository at www.jacionline.org).

After establishing surface expression of IL-17R in AD keratinocytes, we assessed whether these cells can sufficiently respond to IL-17 stimulation by genomic analysis (see Methods in the Online Repository). Genes with fold change >1.5 and false discovery rate <0.1 in IL-17-treated versus untreated AD keratinocytes were considered differentially expressed (see this article's Table E1 in the Online Repository at www.jacionline.org). The AD gene set was subsequently compared against previously published IL-17-modulated genes in normal keratinocytes.²

We found strong positive correlation between IL-17-modulated gene sets generated from AD keratinocytes and normal keratinocytes ($r = 0.91$; Fig 2, B). The close similarity between AD and normal keratinocyte gene sets was further established by the computed connectivity score¹⁰ (0.904 ; $P < 10^{-16}$), wherein a perfect agreement between the 2 gene sets would yield a value of 1, whereas a perfect disagreement would be -1 . Importantly, AD keratinocytes stimulated with IL-17 had robust upregulation of AMPs *DEFB4* (517-fold from control) and *LCN2* (5.7-fold from control), similar to normal keratinocytes (Fig 2, B). Proinflammatory neutrophil chemoattractants, *CXCL1*, 3, 5, 6, and 8, as well as $CCR6^+$ lymphocyte chemoattractant *CCL20* were likewise strongly induced in both AD and normal keratinocytes (Fig 2, B). This was verified by quantitative RT-PCR analysis showing that IL-17 significantly upregulated *DEFB4*, *LCN2*, *IL-8*, and *CCL20* expression in AD keratinocytes ($P < .005$) as it would in normal keratinocytes (Fig 2, C).

Despite increased IL-17 levels compared with normal skin^{8,9,11} and equivalent capacity to respond to cytokine stimulation, AD lesions still have minimal *DEFB4* and *LCN2* gene expression.⁹ To determine whether the T_H2 cytokine milieu within AD lesions may have an effect toward IL-17-induced AMP expression, we cultured normal keratinocytes with IL-17 (200 ng/mL) alone or in combination with IL-4 (200 ng/mL) or IL-13 (200 ng/mL) and examined for gene expression of the aforementioned AMPs. We found that both IL-4 and IL-13 can significantly antagonize IL-17 induction of *DEFB4* and *LCN2* in keratinocytes (Fig 2, D) as previously reported.¹² Thus, decreased innate defense molecule expression in AD is derived not from an inherent inability to respond to T_H17 cytokine stimulation but likely from low ligand levels, compounded by further suppressive action from the T_H2 microenvironment. Our findings have interesting implications for translational research because future therapeutics directed at increasing signaling through intact IL-17 receptors, despite low ligand expression, may potentially reverse this relative AMP deficiency in AD. One such approach might be T_H2 blockade, allowing existing IL-17 to function fully without suppression, potentially restoring AMP expression and alleviating microbial load in AD.

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UV-B-triggered induction of vitamin D3 metabolism differentially affects antimicrobial peptide expression in keratinocytes

To the Editor:

Antimicrobial peptides (AMPs) are important effectors in cutaneous innate immunity as antibiotics and signaling molecules in the defense against microbes.¹ The regulation of AMP expression and function in the skin is not completely understood. In the

METHODS

Skin samples

Lesional and nonlesional skin punch biopsies (5 mm diameter) were obtained from 18 adult patients with chronic AD and 15 healthy volunteers under a Rockefeller University institutional review board–approved protocol. Biopsy specimens were either frozen in OCT (Sakura, Torrance, Calif) and stored at -80°C for immunohistochemistry, snap-frozen in liquid nitrogen for RNA extraction, or used to generate primary keratinocyte cultures.

Keratinocyte cultures

To generate AD keratinocyte cultures, skin biopsies were incubated at 4°C overnight in dispase (Invitrogen Life Technologies). Epidermis was peeled off, then cultured in Epilife media (Cascade Biologics, Portland, Ore) with EDGS (Cascade Biologics) and penicillin-streptomycin (100 IU/mL and 0.1 mg/mL, respectively; Gibco, Carlsbad, Calif) at 37°C . Normal keratinocytes were obtained from the Yale Skin Diseases Research Center core facility as previously described.¹³ When cultured keratinocytes were 80% confluent, the culture media was supplemented with recombinant human (rh) IL-17 (200 ng/mL; R&D Systems, Minneapolis, Minn) with or without anti-IL17RA mouse mAb (clone M202; Amgen, Thousand Oaks, Calif), rhIL-4 (200 ng/mL; R&D Systems) or rhIL-13 (200 ng/mL; R&D Systems). RNA was extracted using the RNeasy Mini Kit (Qiagen) with on-column DNase digestion and used for either gene array or RT-PCR.

Immunohistochemistry

Tissue sections were stained with IL-17RA mouse mAb (clone M202; Amgen) followed by biotin-labeled horse antimouse antibody (Vector Laboratories, Burlingame, Calif). Avidin-biotin complex (Vector Laboratories) was used for amplification and developed with chromogen 3-amino-9-ethyl-carbazole (Sigma Aldrich, St Louis, Mo). Appropriate negative controls were used.

Gene array

For each Affymetrix genechip, 2 μg total RNA was reverse-transcribed, amplified, and labeled as described previously by using the BioArray High Yield RNA Transcription Labeling Kit (Enzo Biochem Inc, Farmingdale, NY).^{E1} A total of 15 μg biotinylated complementary RNA was then hybridized to the Affymetrix Human Genome U133A 2.0 Array (14,500 probe sets; Affymetrix, Santa Clara, Calif). Chips were washed, stained with streptavidin-phycoerythrin, and scanned with a Hewlett-Packard HP GeneArray Scanner (Hewlett-Packard, Palo Alto, Calif). Preprocessing and statistical analysis was conducted in R (<http://www.rproject.org/>). GeneChip CEL files were scrutinized for spatial artifacts using the Harshlight package^{E2} (<http://asterion.rockefeller.edu/Harshlight/index2.html>). Row intensities values (CEL files) were preprocessed to obtain expression values using the GCRMA algorithm.

RT-PCR

RT-PCR was performed by using EZ PCR core reagents, primers, and probes (Applied Biosystems, Foster City, Calif), as previously published.^{E1} Primers and probes used in this study were as follows: DEFB4

(Hs00175474_m1), LCN2 (Hs00194353_m1), CCL20 (Hs00171125_m1), IL-8 (forward: GCTGGCCGTGGCTCTCT; reverse: TTTAGCACTCCTTGGCAAACTG; probe: FAM-CCTTCTGTATTCTGTCAGCTCTGTGTGA-TAMRA). Data were analyzed and quantified by using the Applied Biosystems PRISM 7700 (Sequence Detection Systems, version 1.7), then normalized to the human acidic ribosomal protein housekeeping gene.

Statistical analysis

For gene array analysis, expression values in log₂-scale were obtained by GCRMA algorithm and probesets, then filtered to eliminate genes with low range intensities (values smaller than 2 in all samples) and low variation across samples ($\text{SD} < 0.1$). A moderated 2-tailed paired *t* test was performed with the Limma package from the Bioconductor project (<http://www.bioconductor.org/>). The *P* values of the moderated *t* test were adjusted for multiple hypotheses testing, controlling the false discovery rate with the Benjamini-Hochberg procedure. Results generated from AD keratinocytes were compared against those previously generated from normal keratinocytes.^{E1} The connectivity score (CS),^{E3} introduced in the context of the connectivity map, quantifies how closely upregulated or downregulated genes in IL-17–treated normal keratinocytes¹ rank in IL-17–treated AD keratinocytes. The CS was generated as follows: An enrichment score (ES)^{E4} was first calculated for both upregulated and downregulated normal KC genes. If upregulated genes in normal keratinocytes rank high in AD, the ES (up) score will be near 1; if the opposite effect happens, the value approaches -1 . An ES (down) score was likewise generated. The CS was then calculated as ES (up) minus ES (down), leading to a value between -2 and 2 followed by normalization to the unit interval. A CS value near 1 would indicate perfect agreement between normal and AD keratinocytes, 0 no agreement, and -1 a negative correlation.

The distribution of the CS under the null hypothesis was obtained by simulation (number of simulations = 10,000). For each simulation, a random permutation in the AD fold change list was produced and the connectivity score calculated (CS_n). The *P* value was obtained as 1 minus frequency of cases, where CS_n is bigger in absolute value than CS for normal KC genes.

The 2-tailed *t* test was used in the analysis of log-normalized RT-PCR data from patient samples. The 1-tailed paired *t* test was used in the analysis of log-normalized RT-PCR values from keratinocyte cultures. *P* values were designated as **P* < .05, ***P* < .01, and ****P* < .005.

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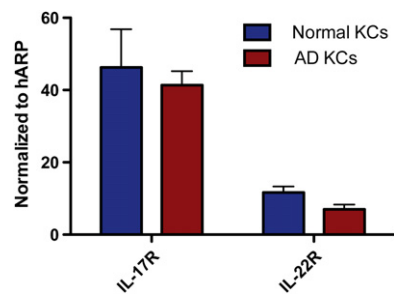


FIG E1. Atopic dermatitis (AD) keratinocytes express receptors for IL-17 and IL-22. Quantitative RT-PCR analysis for *IL-17R* and *IL-22R* in AD keratinocytes show expression levels similar to normal keratinocytes. Data normalized to hARP and expressed as mean and SEM.

Table E1: Genes modulated by IL-17 in AD Keratinocytes

Symbol	Description	FCH	p-value	FDR
DEFB4	defensin, beta 4	517.10	0.00	0.00
SERPINB4	serpin peptidase inhibitor, clade B, member 4	72.70	0.00	0.01
PDZK1IP1	PDZK1 interacting protein 1	68.16	0.00	0.00
IL1F9	interleukin 1 family, member 9	45.26	0.00	0.00
S100A12	S100 calcium binding protein A12	41.19	0.00	0.00
CCL20	chemokine (C-C motif) ligand 20	39.81	0.00	0.00
S100A7	S100 calcium binding protein A7	26.47	0.00	0.00
SPRR2C	small proline-rich protein 2C	21.36	0.00	0.00
ZC3H12A	zinc finger CCCH-type containing 12A	20.55	0.00	0.00
SAA2	serum amyloid A2	16.63	0.00	0.00
SERPINA3	serpin peptidase inhibitor, clade A, member 3	12.59	0.00	0.01
CXCL5	chemokine (C-X-C motif) ligand 5	11.63	0.00	0.02
CXCL6	chemokine (C-X-C motif) ligand 6	10.81	0.00	0.03
C3	complement component 3	10.39	0.00	0.00
IL8	interleukin 8	10.22	0.00	0.00
SLC6A14	solute carrier family 6, member 14	9.79	0.00	0.01
SAA2	serum amyloid A2	8.79	0.00	0.00
PLAT	plasminogen activator, tissue	7.98	0.00	0.02
EHF	ets homologous factor	6.84	0.00	0.00
RHCG	Rh family, C glycoprotein	6.81	0.00	0.00
SPRR2D	small proline-rich protein 2D	6.76	0.00	0.00
KYNU	kynureninase (L-kynurenine hydrolase)	6.36	0.00	0.01
CXCL2	chemokine (C-X-C motif) ligand 2	6.08	0.00	0.00
CXCL3	chemokine (C-X-C motif) ligand 3	5.73	0.00	0.00
LCN2	lipocalin 2 (oncogene 24p3)	5.69	0.00	0.01
NOD2	nucleotide-binding oligomerization domain containing 2	5.35	0.00	0.00
IL8	interleukin 8	5.32	0.00	0.01
SERPINB3	serpin peptidase inhibitor, clade B, member 3	5.13	0.00	0.00
SLC11A2	solute carrier family 11, member 2	4.82	0.00	0.00
LYN	v-src-1 Yamaguchi sarcoma viral related oncogene homolog	4.76	0.00	0.01
KYNU	kynureninase (L-kynurenine hydrolase)	4.75	0.00	0.00
CXCL1	chemokine (C-X-C motif) ligand 1	4.54	0.00	0.00
ARG1	arginase, liver	4.22	0.00	0.02
NDRG2	NDRG family member 2	4.17	0.00	0.00
IL1B	interleukin 1, beta	3.98	0.00	0.00
PI3	peptidase inhibitor 3, skin-derived (SKALP)	3.95	0.00	0.00
IL1B	interleukin 1, beta	3.89	0.00	0.00
TGM1	transglutaminase 1	3.45	0.00	0.00
SOD2	superoxide dismutase 2, mitochondrial	3.36	0.00	0.01
PI3	peptidase inhibitor 3, skin-derived (SKALP)	3.32	0.00	0.00
HPSE	heparanase	3.21	0.00	0.02
SERPINB1	serpin peptidase inhibitor, clade B, member 1	2.89	0.00	0.03
SLC11A2	solute carrier family 11, member 2	2.88	0.00	0.00
LYN	v-src-1 Yamaguchi sarcoma viral related oncogene homolog	2.85	0.00	0.01
ALDH1A3	aldehyde dehydrogenase 1 family, member A3	2.81	0.00	0.02
PBEF1	pre-B-cell colony enhancing factor 1	2.77	0.00	0.01

Symbol	Description	FCH	p-value	FDR
MGC5618	hypothetical protein MGC5618	2.70	0.00	0.01
PBEF1	pre-B-cell colony enhancing factor 1	2.67	0.00	0.00
SOD2	superoxide dismutase 2, mitochondrial	2.64	0.00	0.04
LYN	v-yes-1 Yamaguchi sarcoma viral related oncogene homolog	2.57	0.00	0.01
IL1F5	interleukin 1 family, member 5 (delta)	2.46	0.00	0.01
HSPC159	galectin-related protein	2.44	0.00	0.01
CLDN7	claudin 7	2.27	0.00	0.03
ARNTL2	aryl hydrocarbon receptor nuclear translocator-like 2	2.25	0.00	0.01
HIG2	hypoxia-inducible protein 2	2.24	0.00	0.03
ID1	inhibitor of DNA binding 1, dominant negative helix-loop-helix protein	2.16	0.00	0.00
HBEGF	heparin-binding EGF-like growth factor	2.14	0.00	0.02
FUT2	fucosyltransferase 2	2.11	0.00	0.02
BIK	BCL2-interacting killer (apoptosis-inducing)	2.06	0.00	0.00
HBEGF	heparin-binding EGF-like growth factor	2.03	0.00	0.02
MAFF	v-maf musculoaponeurotic fibrosarcoma oncogene homolog F (avian)	2.01	0.00	0.05
SLPI	secretory leukocyte peptidase inhibitor	2.00	0.00	0.00
RAB31	RAB31, member RAS oncogene family	1.97	0.00	0.02
BCL3	B-cell CLL/lymphoma 3	1.94	0.00	0.00
RAB27A	RAB27A, member RAS oncogene family	1.93	0.00	0.05
CYB5R2	cytochrome b5 reductase 2	1.89	0.00	0.00
MYO1B	myosin IB	1.86	0.00	0.00
S100A9	S100 calcium binding protein A9	1.82	0.00	0.04
CHST2	carbohydrate (N-acetylglucosamine-6-O) sulfotransferase 2	1.81	0.00	0.03
KATNB1	katanin p80 (WD repeat containing) subunit B 1	1.79	0.00	0.04
NRG1	neuregulin 1	1.79	0.00	0.05
C14orf147	chromosome 14 open reading frame 147	1.78	0.00	0.02
RAB31	RAB31, member RAS oncogene family	1.77	0.00	0.00
TFRC	transferrin receptor (p90, CD71)	1.67	0.00	0.02
ABCA11	ATP-binding cassette, sub-family A, member 11	1.67	0.00	0.02
IER3	immediate early response 3	1.62	0.00	0.03
UGCG	UDP-glucose ceramide glucosyltransferase	1.61	0.00	0.02
MREG	melanoregulin	1.61	0.00	0.03
ALAS1	aminolevulinate, delta-, synthase 1	1.61	0.00	0.04
RAB31	RAB31, member RAS oncogene family	1.60	0.00	0.03
AP1S2	adaptor-related protein complex 1, sigma 2 subunit	1.60	0.00	0.00
MYO1B	myosin IB	1.59	0.00	0.00
NMB	neuromedin B	1.59	0.00	0.00
MFHAS1	malignant fibrous histiocyteoma amplified sequence 1	1.59	0.00	0.05
IFNGR1	interferon gamma receptor 1	1.59	0.00	0.01
IFNGR1	interferon gamma receptor 1	1.59	0.00	0.01
ARHGAP17	Rho GTPase activating protein 17	1.56	0.00	0.03
TGFA	transforming growth factor, alpha	1.54	0.00	0.03
CARHSP1	calcium regulated heat stable protein 1, 24kDa	1.54	0.00	0.03
PPIF	peptidylprolyl isomerase F (cyclophilin F)	1.51	0.00	0.01
SLCO3A1	solute carrier organic anion transporter family, member 3A1	0.66	0.00	0.03
KIAA0040	KIAA0040	0.66	0.00	0.04
CEP350	centrosomal protein 350kDa	0.65	0.00	0.02

Symbol	Description	FCH	p-value	FDR
PRSS23	protease, serine, 23	0.65	0.00	0.01
F2R	coagulation factor II (thrombin) receptor	0.64	0.00	0.01
TRIM38	tripartite motif-containing 38	0.63	0.00	0.01
TIMP3	TIMP metalloproteinase inhibitor 3	0.63	0.00	0.04
DNAJB4	DnaJ homolog, subfamily B, member 4	0.63	0.00	0.02
SP110	SP110 nuclear body protein	0.63	0.00	0.04
HTRA1	HtrA serine peptidase 1	0.63	0.00	0.05
SPARC	secreted protein, acidic, cysteine-rich	0.62	0.00	0.05
ST6GALNAC2	ST6 (alpha-N-acetyl-neuraminy-2,3-beta-galactosyl-1,3)-N-acetylglucosaminide alpha-2,6-sialyltransferase 2	0.61	0.00	0.01
ORAI3	ORAI calcium release-activated calcium modulator 3	0.61	0.00	0.04
APBB2	amyloid beta (A4) precursor protein-binding, family B, member 2	0.61	0.00	0.01
FZD1	frizzled homolog 1 (Drosophila)	0.60	0.00	0.00
COL1A2	collagen, type I, alpha 2	0.60	0.00	0.01
SPATA20	spermatogenesis associated 20	0.60	0.00	0.04
DDAH2	dimethylarginine dimethylaminohydrolase 2	0.60	0.00	0.03
PDGFC	platelet derived growth factor C	0.59	0.00	0.03
ITPKB	inositol 1,4,5-trisphosphate 3-kinase B	0.59	0.00	0.03
SMARCA2	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 2	0.58	0.00	0.02
TGFB111	transforming growth factor beta 1 induced transcript 1	0.58	0.00	0.04
FLRT2	fibronectin leucine rich transmembrane protein 2	0.57	0.00	0.00
SPEG	SPEG complex locus	0.57	0.00	0.02
LHFP	lipoma HMGIC fusion partner	0.57	0.00	0.05
DAPK1	death-associated protein kinase 1	0.57	0.00	0.03
ITGB6	integrin, beta 6	0.57	0.00	0.01
ITGB6	integrin, beta 6	0.56	0.00	0.01
FN1	fibronectin 1	0.56	0.00	0.04
SLC22A18	solute carrier family 22, member 18	0.55	0.00	0.00
CLU	clusterin	0.54	0.00	0.01
CPA4	carboxypeptidase A4	0.54	0.00	0.04
HNT	neurotrimin	0.53	0.00	0.03
FLRT2	fibronectin leucine rich transmembrane protein 2	0.52	0.00	0.01
ARL4C	ADP-ribosylation factor-like 4C	0.52	0.00	0.03
COL5A2	collagen, type V, alpha 2	0.52	0.00	0.02
MMP2	matrix metalloproteinase 2	0.52	0.00	0.00
IGFBP4	insulin-like growth factor binding protein 4	0.52	0.00	0.02
SERPINE1	serpin peptidase inhibitor, clade E, member 1	0.52	0.00	0.01
HSPA12A	heat shock 70kDa protein 12A	0.51	0.00	0.01
EFNB2	ephrin-B2	0.50	0.00	0.03
POSTN	periostin, osteoblast specific factor	0.50	0.00	0.01
HSPA2	heat shock 70kDa protein 2	0.49	0.00	0.00
D4S234E	DNA segment on chromosome 4 (unique) 234 expressed sequence	0.48	0.00	0.03
DIXDC1	DIX domain containing 1	0.48	0.00	0.01
AGR2	anterior gradient homolog 2 (Xenopus laevis)	0.48	0.00	0.02
SYNC1	syncoilin, intermediate filament 1	0.48	0.00	0.02
LTBP1	latent transforming growth factor beta binding protein 1	0.47	0.00	0.01
LXN	latexin	0.46	0.00	0.04

Symbol	Description	FCH	p-value	FDR
SMARCA1	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 1	0.46	0.00	0.03
TRIM21	tripartite motif-containing 21	0.46	0.00	0.00
NEFM	neurofilament, medium polypeptide 150kDa	0.46	0.00	0.01
PRKCDBP	protein kinase C, delta binding protein	0.45	0.00	0.02
KRT15	keratin 15	0.45	0.00	0.04
PTGS1	prostaglandin-endoperoxide synthase 1	0.45	0.00	0.02
CTGF	connective tissue growth factor	0.44	0.00	0.05
LTBP1	latent transforming growth factor beta binding protein 1	0.43	0.00	0.03
COL5A2	collagen, type V, alpha 2	0.42	0.00	0.02
SLC27A3	solute carrier family 27, member 3	0.41	0.00	0.04
UCP2	uncoupling protein 2	0.40	0.00	0.02
PCDH7	protocadherin 7	0.37	0.00	0.04
NEFL	neurofilament, light polypeptide 68kDa	0.36	0.00	0.01
FLG	filaggrin	0.27	0.00	0.09
MMP9	matrix metalloproteinase 9	0.25	0.00	0.09
NID1	nidogen 1	0.22	0.00	0.04