# Association of Basophil-Expressing Genes with Effectiveness of Sublingual Immunotherapy

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Basophils were reported to be associated with allergy pathogenesis and the efficacy of allergen immunotherapy. Using a purified cedar allergen, we recently studied the effectiveness of sublingual immunotherapy for patients with Japanese cedar pollinosis. Patients were classified as high responders (HR) and nonresponders (NR), and comprehensive microarray analysis was used to examine peripheral basophils in both groups. A total of 153 genes were differentially expressed in HR and NR patients. Most of these differentially expressed genes encoded intracellular molecules, and expression levels were higher in HR patients than in NR patients. mRNA expression of the gene encoding D4, zinc, and double plant homeodomain (PHD) fingers family 2 (DPF2) was significantly correlated with copy number variation (CNV). Genetic variation in the *DPF2* gene and its expression in basophils might be associated with the efficacy of sublingual immunotherapy. (J Nippon Med Sch 2020; 87: 49–52)

**Key words:** apoptosis, copy number variation, D4, zinc, and double plant homeodomain fingers family 2, microarray analysis

## Introduction

A number of sublingual immunotherapy (SLIT) drugs for treating allergic rhinitis have recently been marketed in Japan. They are attractive because of their high efficacy and potential to provide a radical treatment. However, SLIT does not benefit some patients. We previously compared SLIT nonresponders (NR) and high responders (HR) and identified potential determinants of SLIT efficacy, namely, patients with high correlations among serum Th1/Th2 cytokines tended to respond well to SLIT<sup>1</sup>. An important source of Th1/Th2 cytokines is CD4<sup>+</sup> T cells, and taste receptors on CD4<sup>+</sup> T cells may contribute to SLIT efficacy<sup>2</sup>.

Basophils have recently served as targets for allergen immunotherapy. Basophils produce IL-4 in response to various stimuli, including IgE-dependent allergen recognition<sup>3</sup>. The proportions of basophils expressing C-type lectin receptors and FcyR II were reduced and increased, respectively, by subcutaneous immunotherapy<sup>4</sup>. Epicutaneous immunotherapy for patients with peanut allergy downregulated CD63 expression in basophils, increased peanut-specific IgG4 and IgG4/IgE ratios, and decreased Th2 cytokine production<sup>5</sup>. Herein, we used comparative microarray mRNA expression analysis of basophils collected from HR and NR patients to identify basophil-dependent mechanisms that might explain SLIT efficacy.

# Materials and Methods

## SLIT for Japanese Cedar Pollinosis (JCP)

The study design, patient recruitment, administration of cedar pollen extract, and evaluation of clinical efficacy were performed as described recently<sup>1</sup>. The present study

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included 202 adult JCP patients who presented with symptoms of allergic rhinitis from February through April for at least 3 consecutive years. The patients had positive results on a skin-test and for IgE against cedar pollen allergen. The study was registered in the University Hospital Medical Information Network Clinical Trials Registry Database (UMIN000016532) and was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines. Experimental procedures were approved by the ethical committee of all participant institutions. All patients provided informed written consent before participation.

Cedar pollen allergen was administered to all participants, as described previously<sup>1</sup>. Starting with one drop of 2 Japanese allergy units (JAU)/mL, we gradually increased the amount of allergen until it reached a concentration of 20 drops of 2,000 JAU/mL as the maintenance dose at 5 weeks, which was continued for 2 years.

Nasal symptoms of patients from February 1st through April 30th during four pollen seasons were investigated in accordance with Japanese guidelines for allergic rhinitis. The patients recorded their symptoms in an allergy diary, as previously described<sup>1</sup>. Patient quality of life was determined thrice annually—at the end of February, in the middle of March, and in the middle of April—by using the Japan Rhinoconjunctivitis Quality of Life Questionnaire No.1 (JRQLQ No1). At the end of each pollen season, SLIT efficacy was evaluated by analyzing information from the allergy diary and JRQLQ No1<sup>1</sup>.

## Cell Preparation and Microarray Analysis

Blood samples were collected twice: immediately before the start and at the end of allergen administration. Basophils were purified as CD123<sup>+</sup>CD11c<sup>low</sup>BDCA-1<sup>-</sup> BDCA-4<sup>-</sup>HLA-DR<sup>-</sup> cells from peripheral blood, as previously described<sup>1</sup>. Genomic DNA (gDNA) of the samples was extracted from blood cells.

Microarray analysis of basophils was performed as previously described<sup>6</sup>. Comparative genome-wide transcriptome analysis was performed by using the GeneChip Human Gene 1.0 ST Array Platform (Thermo Fisher Scientific, Waltham, MA); 17,743 of 33,297 probe sets were analyzed for transcripts associated with specific functionally annotated sites in the genome sequences. The results were normalized as z-scores by using the equation  $z = [v-\mu]/\sigma$ , where v is the raw value for a patient,  $\mu$  is the mean value for all HR and NR patients, and  $\sigma$  is the associated standard deviation in the population. The log2 ratios of the averaged data were calculated between the HR and NR groups.

#### Analysis of Copy Number Variation (CNV)

CNV analysis was performed as previously described<sup>2</sup>. The reaction products were analyzed with an Agilent SurePrint G3 Human CNV MicroArray Kit, 2×400K, using the Japanese male HapMap sample NA19000 and UCSC Human Genome build hg19. Probes matching single gDNA sites extracted 2,959 CNV regions. Among them, CNV regions with  $\geq$ 3 probes and a log2 ratio of  $\geq$ 0.25 were identified with ADM-2 algorithms.

#### Statistical Analysis

Data for mRNA analyses are presented in heat maps. Linear regression analysis was used to identify correlations between CNV and mRNA levels.

#### Results

Twenty-five HR and NR JCP patients were selected from patients who received 2-year SLIT, as described previously<sup>1</sup>. RNA of post-SLIT basophils of both groups was assessed in microarray analysis. The peripheral basophil population was unaffected by SLIT, and the population did not differ between HR and NR patients, as described<sup>1</sup>. However, we identified 153 genes that were differentially expressed between HR and NR patients (**Fig. 1 A**). Most of these genes were more strongly expressed in HR than in NR patients; only 12 genes had higher expression levels in NR patients. Many genes encoded intracellular molecules, such as transcription factors, enzymes, and interacting proteins. Approximately 30 small nuclear RNAs were identified (**Fig. 1B**).

We next investigated associations between gene expression levels and corresponding CNV values and observed a significant correlation in only one gene/CNV pair, namely, the D4, zinc, and double plant homeodomain (PHD) fingers family 2 (DPF2) (**Fig. 2**). Gene expression and CNV in the *DPF2* gene were significantly correlated in the HR, NR, and HR plus NR groups.

#### Discussion

The number of differentially expressed genes in basophils (153 genes) in this study was larger than that (56 genes) reported in  $CD4^+$  T cells<sup>2</sup>. We previously evaluated the significance of genes differentially expressed in  $CD4^+$  T cells of HR and NR patients by analyzing their associations with CNVs. Because CNV is generated by multiplication and deletion of DNA segments of 10<sup>3</sup> to 10<sup>6</sup> nucleotides, which frequently contain an entire gene with its regulatory region, CNVs often directly affect gene expression levels<sup>7</sup>. Expression and CNV were significantly correlated only in the *DPF2* gene. DPF2 is a ubiquitously

# A

| Log2 ratio (HR/NR) | Chromosome | Acc. No.         | Symbol       | Name   |        |      |                 |   |  |
|--------------------|------------|------------------|--------------|--|--------|------|-----------------|---|--|
| -0.446             | 1          | M77840           | RNUSF        | RNA, USF small suclear   | 0.171  | 14   | NM_172193       | KLHDC1                                  | kelch domain containing 1  |
| 0343               | 14         | NR 002980        | \$3008.450   | and endedor PNA M  | 0.121  | 14   | NNE OMISEE      | EXOCS                                   | mont comin communit  |
|                    |            | 240_992799       | 014000404    |  |        |      | 100_00000       | 200000                                  | encys compares compares 2  |
| 0,334              | 1          | NR_004389        | SNORA16B     | small nucleolar RNA, H   | 0.17   | 9    | NM_005047       | PSMD5                                   | proteasome (prosome, macropant) 205 subunt, non-A1Pase, 5  |
| -0.325             | 1          | NR_002753        | RNUSF        | RNA, USF small suclear   | 0.17   | 17   | NM_001104587    | SLFN11                                  | schlafen family member 11  |
| 0.285              | 1          | NM 017953        | ZNORITE      | ning finang HIT tang 6   | 0.169  |      | NM 054269       | METN7                                   | mediator correlex subsult 22   |
|                    |            |                  | Re-Later     | and ingle, and open  |        |      | 100,00000       | CALER (                                 |  |
| 0.28               | 8          | NR_002437        | SNORD54      | small nudeolar RNA, C  | 0.109  | . 5  | 7654_032587     | CARDS                                   | caspase recruitment domain family, member 6  |
| 0.265              | 14         | NM_001080414     | CCDCSSC      | colled-coll domain containing 88C  | 0.167  | 9    | NM_007344       | TTF1                                    | transcription termination factor, RNA polymerase I   |
| 0.256              | 3          | NM 001023570     | IOCB1        | 10 motif containing RI   | 0.166  | 17   | NM 201434       | RABIC                                   | RABIC member RAS encourse family   |
|                    |            | and another      | man and a    |  |        |      | and automation  | man a ch                                |  |
| 0.749              | 12         | NM_991130811     | PAMOA        | tanky with sequence similarity or, memoer A  | 0.100  | 10   | NM_01/853       | TANLAB                                  | Chorecolub-lake 48   |
| 0.248              | 6          | NM_001142800     | EVS          | eyes shut homolog (Drosophia)  | 0.166  | 6    | NR_002742       | SNORD52                                 | small nucleolar RNA, C   |
| 0.247              | 19         | NM 001144989     | 2NF814       | vinc finant centrin \$14   | 0.165  | 2    | NM 018079       | SEBDI                                   | \$1 RNA history domain 1   |
| 0.242              | 10         | The second       | mmelie       | die Annuelle (17   | 0.144  |      | Transient       | 1.0000000000000000000000000000000000000 | had a di onomina ri  |
| 0.242              | 19         | BC052805         | ZNF010       | Inc Inger proten 010   | 0.105  | 1    | BC036435        | LOC100132147                            | hypothetical LOC100132147  |
| 0.242              | 2          | NM_001137550     | LRRFIP1      | leucine rich repeat (in FLII) interacting protein 1  | 0.165  | 19   | NM_152604       | ZNF383                                  | zinc finger protein 383  |
| 0.238              | 1          | NR 002748        | SNORD45B     | small nucleolar RNA. C   | 0.164  | 2    | NR 003366       | ANKRD20B                                | ankyrin repeat domain 20B  |
|                    |            | 224 44444        | DOD B        | Photo  |        |      | had address     | 0.000                                   | And a second sec |
| 0.238              |            | NW_001318        | DB1          | any dronpournae branched chain transacytaise EC  | 0.104  | >    | 2634_005754     | 03BP1                                   | G1Pase activating protein (S1D domain) binding protein 1   |
| 0.236              | 1          | NM_005857        | ZMPSTE24     | zinc metallopeptidase (STE24 homolog, S. cerevisiae)   | 0.164  | 15   | NM_183376       | ARRDC4                                  | arrestin domain containing 4   |
| -0.234             | 1          | NR 003942        | SNORD76      | small nucleolar RNA. C   | 0.164  | 23   | ENST00000305570 | ZNF\$34                                 | zinc finger protein \$34   |
| 0.333              | 12         | N/R 6653333      | estoppion.   |  | 0.144  |      | NTR AAbaut      | (NORD)/                                 |  |
| 0.233              | 14         | NR_902757        | SINUKLOWA    | shaa bootkar KrvA, C   | 40,004 |      | VeV_003041      | SNURD75                                 | Sharibkeedar KAA, C  |
| 0.227              | 19         | NM_001136499     | ZNF841       | zinc finger protein 841  | 0.163  | 12   | NM_007199       | IRAK3                                   | interleukin-1 receptor-associated kinase 3   |
| 0.227              | 14         | AK(95276         | LOC283588    | hypothetical LOC283588   | 0.163  | 17   | NR 002912       | SNORA67                                 | small nucleolar RNA, H   |
| 0.721              |            | 334 416478       | NET 1        | NET 1 MENTS Must chara complex commands hands a /E campbine)   | 0.143  |      | ND4 004582      | Ch.(FB1                                 | also second sold model at our also and his first seconds 1   |
| 0.223              | 1          | 26M_9154/1       | PSL1         | NSL1, MIND REFORMER COMPLEX COMPOSER, BORNING (S. CEREMONE)  | 0.105  |      | 2006_000045     | CONTENT                                 | gracecordenia modulatory comient oncarg protein a  |
| 0.221              | 15         | NM_001040450     | FAM63B       | family with sequence similarity 63, member B   | -0.163 | 20   | NM_001037732    | DEFB128                                 | defensin, beta 128   |
| 0.219              | 13         | NR_002612        | DLEU2        | deleted in lymphocytic leukemia 2 (non-protein coding)   | 0.162  | 10   | NM_012229       | NT5C2                                   | 5'-mucleotidase, cytosolic II  |
| 0.215              | 18         | NR 002571        | SNORDISA     | small sucheolar RNA. C   | 0.162  | 2    | NR 004198       | SNORD82                                 | snall nucledar RNA. C  |
| 0.000              | 10         | State States in  | Alersen and  |  | 0.104  |      |                 |   | and the second sec   |
| 0.213              | 7          | NM_181791        | GPR141       | G proteas-coupled receptor 141   | 0.162  | 2    | NM_014622       | MRPL35                                  | matechondrait ribesomal protein L35  |
| 0.212              | 5          | NM_002538        | OCLN         | occludin   | -0.162 | 8    | NR_003041       | \$NORD13                                | small nucleolar RNA, C   |
| 0211               | 6          | NM 181817        | ORC3L        | origin recognition consiler admit 1.50e (years)  | 0.161  | 1    | NM 007259       | VPS45                                   | variable rentein sorting (5 handlog (5 correlation)  |
| 0.211              |            |                  | CAUCOL:      | ordine conductor and an a state (Acard)  | 0.101  |      | 104 001229      | 12340                                   | rational protein means at Distances (5. Concerning)  |
| 0.208              | 1          | AK125737         | LOC440570    | hypothetical LOC440570   | 0.161  | 3    | NM_134287       | DTX3L                                   | deitex 3-like (Drosophila)   |
| 0.207              | 2          | NR_002908        | SNORD20      | small nucleolar RNA, C   | 0.161  | 4    | NR_026575       | GK3P                                    | glycerol kinase 3 pseudogene   |
| 0.356              | 4          | NDJ ALEDAS       | TATALINA     | Process and store accutain 1840  | 0.16   | 13   | ND4 AMUSABLE    | \$11071                                 | \$771 supremum of (1) alida of \$701 (8 contribute)  |
| 0.000              |            | tron presents    | EPHANELOTIC- | Calculate prover 1970  | 0.10   |      | TANE OATTOALP   | 30011                                   | ourse, suppression of one and of ourse (or controlling)  |
| 0.202              | 9          | NM_017645        | HAU\$6       | HAUS augmin-like complex, subunit 6  | 0.159  | 21   | NM_006948       | HSPA13                                  | heat shock protein NikDa family, member 13   |
| 0.201              | 20         | BC632332         | PCMTD2       | protein-L-isoaspartate (D-aspartate) O-methyltransferase domain containing 2   | 0.159  | 19   | NM_138330       | ZNF675                                  | zinc finger protein 675  |
| 0.205              |            | Th/FT00000101810 | TRAINER      | terror models of the fee antick t  | 0.150  |      | 3754 001004     | 40911                                   | and that methodase lass shale family member 1  |
| 0.008              |            | 201010000071017  | 177074       | caller process provess provess, a  | 0.155  |      | 104_04040       | 740-064                                 | acyrowe symbolic angresant samy mennet i   |
| 0.199              | 11         | NR_002562        | SNORDER      | small nudeolar RNA, C  | 0.159  | . 5  | NM_183421       | FBX025                                  | F-bas protein 25   |
| 0.197              | 2          | NM_017964        | SLC30A6      | solute carrier family 30 (zinc transporter), member 6  | 0.158  | 11   | NM_173811       | HARBII                                  | harbinger transposase derived 1  |
| 0.196              | 1          | NR 003944        | SNOT THE     | and autashe PNA C  | 0.158  | 12   | NM 001666       | TEAL                                    | and and and a stars I  |
|                    |            | 244 992244       | 0140000070   | num monom nove o   | 0.150  |      | 1000_000.000    | LLOUI                                   | early encountry angles a   |
| 0.195              | 7          | NM_020725        | ATXN/L1      | atatin 7-like 1  | 0.158  | 12   | NM_003774       | GALNT4                                  | UDP-N-acetyl-alpha-D-galactosamine polypeptide N-acetylgalactosaminyltransferase 4   |
| 0.193              | 6          | NM_020466        | LYRM2        | LYR motif containing 2   | 0.158  | 9    | NM_033412       | MCART1                                  | mitochondrial carrier triple repeat 1  |
| 0.161              |            | A.K. 190621      | CUMPI        | chromosome 11 eres candica frome 34  | 0.158  | 2    | NM 000018       | PEVIL                                   | naroslasmit bionarasis factor 11   |
| 50.077             |            | A6479877         | CIUNITA      | Carolinione is synthesing mane /*  | 0.1.0  | -    | TOCOLOUNTS      | T LOUIS                                 | bet overseen on Sources more 12  |
| 0.192              | 11         | NM_001136486     | TRIM64       | tripartite motif-containing 64   | 0.157  | 1    | NM_006468       | POLR3C                                  | polymerase (RNA) III (DNA directed) polypeptide C (82kD)   |
| 0.191              | 19         | NM 021030        | ZNF14        | zinc finger protein 14   | 0.157  | 16   | NM_001142725    | ERI2                                    | exoribonuclease 2  |
| 0.10               |            | NAC ADJESS       | ATM          | alwaat in malanuma 9   | 0.157  |      | NM ALCINE       | 423478                                  | arma@la emant costables 0  |
| 0.17               |            | Mar Sourcess     | 74546        | STATUS IN LOTIONALIA -   | 0.157  |      | 104_01330       | ALAKA                                   | a mouse offen commit a   |
| 0.19               | 12         | NM_205852        | CLEC12B      | C-type lectin domain family 12, member B   | 0.157  | 10   | NM_005028       | PIP4K2A                                 | phosphatidylinositel-5-phosphate 4-kinase, type II, alpha  |
| 0.189              | 12         | NM_019012        | PLEKHAS      | pleckstrin homology domain containing, family A member 5   | 0.158  | 2    | NM_001099273    | NBEAL1                                  | neurobeachin-like 1  |
| 0.155              | 17         | NM 001128147     | NF1          | neuroffromin 1   | 0.156  | 5    | NM 009983       | 53(2)                                   | S-phase kinase-associated protein 2 (p45)  |
|                    |            | had another      | CONTRACT OF  | and the second s |        |      | 1000,000000     | Destroites                              | the state of the second process of the second s   |
| 0.155              | 1          | NM_20/300        | SEP114       | septin 14  | 0.155  | 0    | NM_183050       | BCKDHB                                  | branched chain keto acid denydrogenase 21, beta polypeptide  |
| 0.188              | 12         | NM_005653        | TFCP2        | transcription factor CP2   | -0.155 | 19   | NR_002751       | SNORD41                                 | small sucleolar RNA, C   |
| 0.155              | 13         | NM 153218        | Cilorfit     | chromosome 13 open reading frame 31  | 0.154  |      | NM 018376       | NIPSNAPIR                               | ninum homolog 38 (C. elegant)  |
| 0.107              |            | BW(233343        | WAR (TRA     | And all common de la la 12 mentes 1  | 0.151  |      | 3754 4441378    | DADAGUES                                | And another the second state of the  |
| 0.187              |            | 866397796        | FASE/3A      | same mu regence strange (), minor A  | 0.154  |      | 144_001378      | DINCILL                                 | uportes, syndparameter, determinater chain 2   |
| 0.187              | 15         | BC067846         | \$NRPA1      | small nuclear ribonucleoprotein polypeptide A'   | 0.154  | 8    | NM_001147       | ANGPT2                                  | angiopoietin 2   |
| 0.135              | 2          | NM 024093        | C2orf49      | chromosome 2 open reading frame 49   | 0.154  | 12   | NM 138337       | CLEC12A                                 | C-type lectin domain family 12 member A  |
|                    | 10         | monute of        | The set of   | mark de la mark de d   |        |      | AR 444433       | manual                                  | de familie de la composición de  |
| 0.167              | 14         | 00000000         | 0.0101       | many memory more more hand hand  | 0.174  | 10   | T-M_W/W/        | PLATV                                   | and milia house (coupped like) a   |
| 0.185              | 15         | NK_022008        | PAR5         | Prader-Will  | 0.153  | 11   | 754_016090      | XBM7                                    | XNA tending motil protein 7  |
| 0.184              | 6          | AK299076         | MCMP         | minichromosome maintenance complex component 9   | 0.153  | 13   | NM_031915       | SETDB2                                  | SET domain, bifarcated 2   |
| 0.182              | 2          | NM MISSIS        | 10036010     | vial TINA solumerase transprivated evotein 6   | 0.153  | 2    | NM 032434       | 7NF92                                   | vine finane centrin 512  |
|                    |            | had analy        | BURLION IN   | the bar of property of the bar of the second s   | 0.100  |      | ADA ADAMA       | BB (B)                                  | and any product of the second s  |
| 0.182              | 11         | NM_992645        | MKJC2A       | prospromonnae-a-samase, class 2, alpha polypeptide   | 0.153  | 12   | 7454_024004     | KPAP5                                   | KINA populerase is associated protein 3  |
| 0.131              | 6          | NM_006298        | ZNF192       | zinc finger protein 192  | 0.153  | 15   | NM_001103184    | FMN1                                    | formin 1   |
| 0.181              | 2          | NM 015475        | FAMSEA       | family with sensence similarity 98, member A   | 0.153  | 2    | NM 020923       | ZDBF2                                   | ring finger DBF-type containing 2  |
|                    |            | and an other     |              | and the report handly realized in  |        |      |                 |   | and any other states of the st |
| 0.18               | 2          | NM_016297        | PCYOXI       | prenytcysteme audase I   | 0.153  | 17   | NM_015986       | CREFS                                   | cytome receptor-use factor 3   |
| 0.179              | 7          | NM_032120        | C7orf64      | chromosome 7 open reading frame 64   | 0.152  | 11   | NR_002973       | SNORA40                                 | small nucleolar RNA, H   |
| 0.179              | 4          | NM 199324        | OTUD4        | OTU domain containing 4  | 0.152  | 9    | NR 003009       | SCARNAS                                 | small Caial body-specific RNA 8  |
| 0.170              |            | and and the      | Imout        | TIME THE ALL CONTRACTOR  | 0.1/2  | -    | 200 000000      | (20000014                               | and address of   |
| 0.179              | 0          | tring"aneota     | AD01L        | strost-me (a. occurate)  | 0.152  | 13   | 14A_000013      | 301010024                               | NUM DALKAM ANA C   |
| 0.178              | 13         | NM_001014380     | KATNALI      | katanin p60 subunit A-like 1   | 0.152  | 9    | NM_182505       | C9orf85                                 | chromosome 9 open reading frame 85   |
| -0.178             | 15         | NR_002757        | RNUSB-1      | RNA, USB small nudear 1  | 0.152  | 13   | NM_001004127    | ALG11                                   | asparagine-linked glycowlation 11, alpha-1,2-mannosyltransferase homolog (yeast)   |
| 01122              |            | NAL ASPAS        | 1993.033     | PVA LLE  | 0.163  |      | 334 013013      | VERON                                   | has death and BED.4  |
| 0.177              | 2          | 1414 018041      | PADMAG       | Alon oneng men poten 22  | 0.152  | •    | 1494 01 1915    | ALCRUD                                  | INCL OPTIME AND PLAY 0   |
| -0.175             | 17         | NR_000009        | SNORD4B      | small nucleolar RNA, C   | 0.152  | 1    | NM_012262       | H\$2ST1                                 | heparan sulfate 2-O-sulforansferase 1  |
| -0.175             | 1          | NR 004421        | RNUIA        | RNA, U1A small nuclear   | 0.151  | 4    | NM 001025595    | ARFIP1                                  | ADP-ribordation factor interacting protein 1   |
| 0.536              |            | NP ANALY         | BAR NA.      | PVA 1114 could enders  | 0.151  | - 10 | NAL ANNALS      | THEN                                    | DEVI STOR Matching country supervise bandles (P. country)  |
| -9,175             |            | NR_004421        | ANUIA        | ANN, VIN HIM BUILT   | 0.151  | 20   | 1404_001143310  | 104001                                  | wars, ourse summaries compare compared nonnoog (5. cerevens)   |
| 0.174              | 12         | NM_018448        | CAND1        | culin-associated and neddylation-dissociated 1   | 0.151  | 10   | ND4_016195      | KIF20B                                  | kinesin family member 20B  |
| 0.173              | 2          | NM_019063        | EML4         | echinodem microtubule associated protein like 4  | 0.151  | 1    | NM_005665       | EVIS                                    | ocotropic viral integration site 5   |
| 0122               |            | 48209021         | TTEAS?       | advanced translation failuring factor 4.4 Juniform 2   | 0.151  | 12   | NDA ONITACINA   | NEDDI                                   | and converse of averaged deployments in down constant 1  |
| 0.172              |            | PAD-1099421      | EAP UND      | Construction of the second statement of the second se   | 0.151  | 14   | real autority   | INSUUT                                  | neur a prevaren ven ediressen, devenjanennary devenerginanen i   |
| 0.172              | 1          | NM_005897        | IPP          | intracisternal A particle-promoted polypeptide   | 0.151  | 3    | ND4_005513      | GTF2E1                                  | general transcription factor IIE, polypeptide 1, alpha 50kDa   |
| 0.172              | 13         | NR 002574        | SNORD102     | small nucleolar RNA, C   | 0.151  | 5    | NM_001040458    | ERAP1                                   | endoplasmic reticulum aminopeptidase 1   |
| 0.172              |            | THE ADDRESS      | THE R.       | The second state term down down down.  |        |      | 334 000003      | TRATS.                                  | - for the standard back and some black   |
| 0.172              |            | PON_000208       | LNT2         | D9, mic and double PHD highers family 2  | 0.15   | 2    | 1434_090057     | ASADE                                   | radical 5-adminişti methorane doman containing 2   |
| 0.171              | 7          | NM_018224        | C7orf44      | chromosome 7 open reading frame 44   | 0.15   | 9    | NM_001042551    | \$MC2                                   | structural maintenance of chromosomes 2  |
| 0.171              | 6          | BOMILLA          | (184         | CDC.Bashinana 4  | 0.15   | 10   | NDA 005520      | TMANI                                   | Jacob ensenan biofine 1  |
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Fig. 1 Differential mRNA expression in basophils from HR and NR patients. Genome-wide transcriptome analysis of basophils from HRs and NRs was performed as described in the Methods. Log2 ratios of average expression levels in basophils between HR and NR patients after SLIT (Post) are indicated in red (HR > NR) and green (HR < NR). Genes with a difference in log2 ratio greater than 0.15 between HRs and NRs before and after treatment are listed (A). The classification and percentage of identified genes are shown (B).



Fig. 2 Correlation between expression and CNV in the *DPF2* gene. The correlations between DPF2 mRNA expression and CNV in the HR plus NR (ALL; left panel), HR (center), and NR (right) groups before SLIT were examined. Normality of the distributions was confirmed with the D'Agostino-Pearson omnibus normality test.

expressed d4-protein family member and is multifunctional<sup>89</sup>. It was reported to influence gene transcription, the noncanonical NF- $\kappa$ B pathway, chromatin organization, and protein-protein interactions via its PHD domain<sup>8</sup>. Involvement of DPF2 in apoptosis<sup>9</sup> is consistent with our previous findings, which suggested that the apoptotic pathway in basophils contributes to SLIT efficacy<sup>6</sup>. Future studies should examine the role of DPF2 in basophil function.

The significant correlation between expression and CNV in the *DPF2* gene was similar across the HR, NR, and HR plus NR groups. These results are inconsistent with our previous finding of a substantial correlation between TAS2R43 gene expression in CD4<sup>+</sup> T cells and our observation of CNV in HR but not NR patients<sup>2</sup>. The reason for these differing correlation patterns is unclear, although distinct mechanisms may be involved. The correlated and uncorrelated patterns in HR and NR patients, respectively, in the *TAS2R43* genes suggest that some factor other than its CNV regulates TAS2R43 expression in CD4<sup>+</sup> T cells in NR patients. In contrast, CNV in the *DPF2* gene, which affects its expression in basophils from both HR and NR patients, could act as a direct determinant of SLIT efficacy.

In conclusion, we identified genes differentially expressed in basophils from HR and NR patients. DPF2 expression was correlated with its CNV. Future studies should compare HR patients before and after SLIT, and functional studies of basophils could help identify the mechanisms related to SLIT efficacy.

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**Conflict of Interest:** The authors declare no conflicts of interest.

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