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 SLIT¹. An important source of Th1/Th2 cytokines is CD4⁺ T cells, and taste receptors on CD4⁺ T cells may contribute to SLIT efficacy².

Basophils have recently served as targets for allergen immunotherapy. Basophils produce IL-4 in response to various stimuli, including IgE-dependent allergen recog-

nition³. The proportions of basophils expressing C-type lectin receptors and FcγR II were reduced and increased, respectively, by subcutaneous immunotherapy⁴. Epicutaneous immunotherapy for patients with peanut allergy downregulated CD63 expression in basophils, increased peanut-specific IgG4 and IgG4/IgE ratios, and decreased Th2 cytokine production⁵. 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 recently¹. 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 previously¹. 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 described¹. 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 No1¹.

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⁺CD11c^{low}BDCA-1⁻BDCA-4⁻HLA-DR⁻ cells from peripheral blood, as previously described¹. Genomic DNA (gDNA) of the samples was extracted from blood cells.

Microarray analysis of basophils was performed as previously described⁶. 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, μ is the mean value for all HR and NR patients, and σ 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 described². 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 ≥ 3 probes and a log2 ratio of ≥ 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 previously¹. 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 described¹. However, we identified 153 genes that were differentially expressed between HR and NR patients (Fig. 1A). 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 cells². 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 10³ to 10⁶ nucleotides, which frequently contain an entire gene with its regulatory region, CNVs often directly affect gene expression levels⁷. 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
0.445	1	M7840	RNU1P	RNA, UTP small nuclear
0.343	16	NR_002980	SNORD40	small nuclear RNA, H
0.334	1	NR_004389	SNORD43	small nuclear RNA, H
0.325	1	NR_002733	RNU1P	RNA, UTP small nuclear
0.261	1	NR_017903	ZNF118	zinc finger protein 118
0.25	8	NR_002437	SNORD24	small nuclear RNA, C
0.249	14	NR_00108414	CCDC88C	coiled-coil domain containing 88C
0.256	3	NR_00102370	IQCB1	IQ motif containing 1B
0.249	12	NR_00113811	FAM60A	family with sequence similarity 60, member A
0.248	6	NR_00114280	EYS	eyes shut homolog (Drosophila)
0.247	19	NR_00114989	ZNF114	zinc finger protein 114
0.242	19	BC020305	ZNF114	zinc finger protein 114
0.242	2	NR_01137510	LRIF1P	leucine rich repeat (in FLN) interacting protein 1
0.238	1	NR_002748	SNORD43B	small nuclear RNA, C
0.238	1	NR_001918	DBT	glycylglycine branched chain transacylase E2
0.236	1	NR_001857	ZNF114	zinc finger protein 114
0.234	1	NR_003942	SNORD76	small nuclear RNA, C
0.233	12	NR_002737	SNORD9A	small nuclear RNA, C
0.227	19	NR_00113649	ZNF114	zinc finger protein 114
0.227	14	AK091278	LOC131388	hypothetical LOC131388
0.223	1	NR_015470	NLS1	NLS-1, MEND bloodstone complex component, homolog (S. cerevisiae)
0.221	15	NR_00104040	FAM81B	family with sequence similarity 81, member B
0.219	13	NR_002412	DLU2	deleted in lymphocytic leukemia 2 (non-protein coding)
0.215	18	NR_002771	SNORD58A	small nuclear RNA, C
0.213	7	NR_011796	GPR141	G protein-coupled receptor 141
0.212	1	NR_002318	OCRL	occludin
0.211	6	NR_011837	ORCL	origin recognition complex, subunit 3-like (yeast)
0.208	1	AK125737	LOC440730	hypothetical LOC440730
0.207	2	NR_002988	SNORD20	small nuclear RNA, C
0.206	4	NR_018241	TMEM184C	transmembrane protein 184C
0.202	9	NR_017645	HAUS6	HAUS augmin-like complex, subunit 6
0.201	20	BC023332	PCATD2	protein-L-asparagine (D-aspartate) O-methyltransferase domain containing 2
0.201	11	NR_0010391879	TPST2	trans-sulfolactamase domain containing 2
0.199	11	NR_002562	SNORD28	small nuclear RNA, C
0.197	2	NR_017904	SLC36A6	solute carrier family 36 (solute transporter), member 6
0.196	1	NR_003944	SNORD78	small nuclear RNA, C
0.195	7	NR_002725	ATXN7L1	ataxin 7-like 1
0.193	6	NR_004466	LYRM2	LYR motif containing 2
0.193	11	AK209833	C1orf74	chromosome 11 open reading frame 74
0.192	11	NR_00113648	TPST2	trans-sulfolactamase domain containing 2
0.191	19	NR_002730	ZNF114	zinc finger protein 114
0.19	1	NR_004833	ADAM	absent in melanoma 2
0.19	12	NR_001852	CLEC12B	C-type lectin domain family 12, member B
0.189	12	NR_019012	PLEKHA5	pleckstrin homology domain containing, family A, member 5
0.188	17	NR_001128147	NFI	neurofilament 1
0.188	7	NR_007366	SEPT14	septin 14
0.188	12	NR_004630	TPST2	trans-sulfolactamase domain containing 2
0.188	13	NR_015318	C1orf74	chromosome 13 open reading frame 74
0.187	1	NR_001792	FAM73A	family with sequence similarity 73, member A
0.187	15	BC067846	SNORP1	small nuclear ribonucleoprotein polypeptide A'
0.186	2	NR_004089	C1orf74	chromosome 2 open reading frame 74
0.185	10	BC043343	BMX1	BMX1 homolog chromosome assembly protein (yeast)
0.185	15	NR_002308	PAK3	Protein Kinase C- δ
0.184	6	AK125918	LOC440730	hypothetical LOC440730
0.182	2	NR_015335	C1orf74	chromosome 2 open reading frame 74
0.182	11	NR_002465	PRKCA	phosphoinositide 3-kinase, class 2, alpha polypeptide
0.181	6	NR_004296	ZNF192	zinc finger protein 192
0.181	2	NR_015475	FAM60A	family with sequence similarity 60, member A
0.18	2	NR_016297	PCYOX1	prolyl-4-hydroxylase oxidase 1
0.179	7	NR_002210	C1orf74	chromosome 7 open reading frame 74
0.179	4	NR_019324	OTUD1	OTU domain containing 1
0.179	6	NR_004630	TPST2	trans-sulfolactamase domain containing 2
0.178	13	NR_00104180	KATNALA	laminin β 6 subunit A-like 1
0.178	15	NR_002737	RNU1P	RNA, UTP small nuclear
0.177	5	NR_016047	RBM22	RNA binding motif protein 22
0.175	17	NR_000009	SNORD4B	small nuclear RNA, C
0.175	1	NR_004421	RNU1A	RNA, UTP small nuclear
0.175	1	NR_004421	RNU1A	RNA, UTP small nuclear
0.174	12	NR_016148	CAND1	cell-associated and mitotic-division-associated 1
0.173	2	NR_019060	EMIL4	echinoderm microtubule-associated protein-like 4
0.172	3	AB020921	EIF4A2	eukaryotic translation initiation factor 4A, isoform 2
0.172	1	NR_001897	IPP	intracellular A particle-promoted polypeptide
0.172	13	NR_002734	SNORD102	small nuclear RNA, C
0.172	11	NR_004208	DPF2	DA, zinc and double PHD fingers family 2
0.171	7	NR_018224	C1orf74	chromosome 7 open reading frame 74
0.171	5	BC061116	CELS4	CDC-like kinase 4
0.171	14	NR_017219	KLHDC1	kelch domain containing 1
0.171	14	NR_004644	EXOC5	exocyst complex component 5
0.17	9	NR_005047	PM2D5	proteasome (prosome, macropain) 20S subunit, non-ATPase, 5
0.17	17	NR_00104180	SLFN1	schistosome family member 11
0.169	9	NR_004049	MEI2L2	meiotic complex subunit 27
0.169	5	NR_012187	CARD4	cardiac recruitment domain family, member 6
0.167	9	NR_003444	TFPI1	transcription factor III, RNA polymerase I
0.166	17	NR_001454	RAB3C	RAB3C, member RAS oncogene family
0.166	16	NR_017853	TNXL4B	thioredoxin-like 4B
0.166	6	NR_002742	SNORD12	small nuclear RNA, C
0.165	2	NR_018079	SEK2	S1 RNA binding domain 1
0.165	1	BC020451	LOC1013247	hypothetical LOC1013247
0.165	19	NR_012804	ZNF183	zinc finger protein 183
0.164	2	NR_003366	ANKRD20B	ankyrin repeat domain 20B
0.164	5	NR_001754	G1BP1	GTPase activating protein (GAP domain) binding protein 1
0.164	15	NR_018376	AKRDC4	acetylcholinesterase domain containing 4
0.164	25	ENST0000030570	ZNF14	zinc finger protein 14
0.164	1	NR_003941	SNORD75	small nuclear RNA, C
0.163	12	NR_001709	RAC3	ras-related GTP-binding protein 3
0.163	17	NR_002912	SNORA87	small nuclear RNA, H
0.163	1	NR_004042	GABEB1	glucocorticoid modulatory element binding protein 1
0.163	20	NR_001037732	DEFB128	defensin, beta 128
0.162	10	NR_012229	NTSC2	N-terminus, cytosolic II
0.162	2	NR_004396	SNORD42	small nuclear RNA, C
0.162	2	NR_018622	MRPL31	mitochondrial ribosomal protein L31
0.162	8	NR_003941	ACEL1	small nuclear RNA, C
0.161	8	NR_002219	VPS46	vacuolar protein sorting 46 homolog (S. cerevisiae)
0.161	3	NR_018287	DTX1L	death domain-like 1 (Drosophila)
0.161	4	NR_006575	GKIP1	glyceral kinase 1 pseudogene
0.161	13	NR_001130912	SUGT1	SUGT1, suppressor of G2 allele of SKP1 (S. cerevisiae)
0.159	21	NR_004048	HSP41A	heat shock protein 70Da family, member 13
0.159	19	NR_018330	ZNF183	zinc finger protein 183
0.159	4	NR_004095	ACEL1	small nuclear RNA, C
0.159	8	NR_018421	FBXO25	F-box protein 25
0.158	11	NR_017811	HARDB1	hardy transposase derived 1
0.158	12	NR_001566	E2A1	early endosome antigen 1
0.158	12	NR_001774	GALNT4	UDP-N-acetyl-alpha-D-glucosamine 6-phosphate N-acetylglucosaminyltransferase 4
0.158	9	NR_013412	MCART1	mitochondrial carrier triple repeat 1
0.157	2	NR_001818	PEX31	peroxisomal biogenesis factor 13
0.157	4	NR_004095	POLR3C	polymersase (RNA) III (DNA directed) polypeptide C (3.5kD)
0.157	16	NR_01142725	ERL2	ectodermless 2
0.157	3	NR_011396	ARMC8	armadillo repeat containing 8
0.157	10	NR_001828	PJP4C2A	phosphatidylethanolamine 4-kinase, type II, alpha
0.156	2	NR_001099273	NBEAL1	neurabin-like 1
0.156	5	NR_001983	SKP2	5-phase kinase-associated protein 2 (p45)
0.155	6	NR_013050	BCKDHB	branched chain keto acid dehydrogenase E2, beta polypeptide
0.155	19	NR_001709	RAC3	small nuclear RNA, C
0.154	9	NR_018376	NIPSNAP1B	slingshot homolog 1B (C. elegans)
0.154	2	NR_018378	DYNLC12	dyslexia, cytoplasmic 1, intermediate chain 2
0.154	8	NR_001477	ANGPT2	angiopoietin 2
0.154	12	NR_018337	CLEC12A	C-type lectin domain family 12, member A
0.154	13	NR_001977	RNF6	ring finger protein (C1orf74 type) 6
0.153	11	NR_018090	RBM7	RNA binding motif protein 7
0.153	13	NR_019113	SETDB1	SET domain, bifurcated 2
0.153	2	NR_012434	ZNF122	zinc finger protein 122
0.153	12	NR_014004	RPA93	RNA polymerase II associated protein 3
0.153	15	NR_00103184	FBN1	fibronectin 1
0.153	2	NR_001823	ZDBF2	zinc finger, DBF-type containing 2
0.153	17	NR_015986	CELF3	cytoskeleton receptor-like factor 3
0.152	11	NR_002973	SNORA40	small nuclear RNA, H
0.152	9	NR_001099	SCARNA8	small Cajal body-specific RNA 8
0.152	9	NR_000019	SNORD14	small nuclear RNA, C
0.152	9	NR_012505	CtBP1	chromosome 9 open reading frame 85
0.152	13	NR_00104127	ALG11	asparagine-linked glycosylation 11, alpha-1,2-mannosyltransferase homolog (yeast)
0.152	4	NR_017912	HEKDC6	hect domain and RLD 6
0.152	1	NR_012362	HIST1T1	histone H1-like 2-O-methyltransferase 1
0.151	4	NR_00102198	ARFIP1	ADP-ribosylation factor interacting protein 1
0.151	20	NR_01147316	DON1	DON1, MEND bloodstone complex component, homolog (S. cerevisiae)
0.151	10	NR_016139	KIF20B	kinesin family member 20B
0.151	1	NR_005665	EVF1	ectopic viral integration site 5
0.151	12	NR_0113175	NEDD1	neural precursor cell expressed, developmentally down-regulated 1
0.151	3	NR_001513	GTP1B1	general transcription factor III β , polypeptide 1, alpha 5kDa
0.151	5	NR_001040438	ERAP1	endoplasmic reticulum aminopeptidase 1
0.15	2	NR_001857	RSAD2	radical 5-adenosyl methionine domain containing 2
0.15	9	NR_00404251	SMC2	structural maintenance of chromosomes 2
0.15	18	NR_001070	LMAN1	lectin, mannose-binding 1

B

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^{8,9}. It was reported to influence gene transcription, the noncanonical NF- κ B pathway, chromatin organization, and protein-protein interactions via its PHD domain⁸. Involvement of *DPF2* in apoptosis⁹ is consistent with our previous findings, which suggested that the apoptotic pathway in basophils contributes to SLIT efficacy⁶. 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⁺ T cells and our observation of CNV in HR but not NR patients². 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⁺ 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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