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Letter to the Editor

Involvement of taste receptors in the effectiveness of sublingual immunotherapy

Dear Editor,

Japanese cedar pollinosis (JCP) is a specific seasonal allergic disease which affects ~30% of the Japanese population, between February and April, every year.[1] Apart from a series of symptom-reliever medications, allergen-specific immunotherapy (AIT) is one of the most effective treatments for JCP. After several years of relying on the application of subcutaneous immunotherapy (SCIT) with standardized Japanese cedar pollen extract (since the 1960s), the use of sublingual immunotherapy (SLIT) was approved in 2014.[2] In addition to the numerous clinical and scientific evidences pertaining to its effectiveness and safety on JCP including the results of randomized, placebo-controlled, double-blind studies,[3,4] SLIT is also easier to administer and safer than SCIT, in which a systemic allergen injection is required and severe side effects include fatal anaphylaxis. However, the underlying mechanisms through which SLIT and SCIT exhibit their efficacy have not been fully elucidated.

Despite the usefulness of SLIT, it has been reported that approximately 30% of JCP patients do not respond to this therapy.[4,5] Dividing patients into high-responder (HR) and non-responder (NR) groups could be helpful in understanding the mechanisms of SLIT. We recently performed a clinical study of SLIT with cedar pollen extract on 193 adult patients with JCP.[5] Among 142 patients who completed 2 years of SLIT, 102 (72%) showed more than 1 level of improvement in the severity score. Regardless of whether they improved due to a placebo effect, we selected the top 33 HR patients in order of improvement rank.[5] The bottom 34 NR patients were also selected, and their serum factors were comparatively analyzed before and after SLIT. Although the HR and NR groups were not distinguishable by any single parameter, they could be clearly separated by processing the parameters with an ensemble algorithm, Adaptive Boosting.[5] We also analyzed the population of peripheral blood $CD4^+$ T cells, basophils, conventional dendritic cells, and plasmacytoid dendritic cells.[5] Although there were no significant differences in these populations, between the HR and NR groups, $CD4^+$ T cells are implicated in the effect of AIT.[6] In addition, by using cluster analysis for all serum parameters, we found that the presence of specific cytokines for Th1 and Th2 cell subsets was strongly correlated with HR but not NR patients in our previous study.[5] Therefore, comparative genome-wide transcriptome analyses with $CD4^+$ T cell mRNA, isolated from the HR and NR patients, were performed herein. After the exclusion of samples of cypress pollen-specific IgE-positive patients and samples in which the RNA or DNA was damaged, 25 samples each in the HR and NR groups underwent microarray analyses. We identified 56 genes, differentially expressed between the HR and NR patients, based on the log2 ratio of their averages (Fig. 1). Among these, 5 genes encoded taste receptors, 4 of which tended to increase in the HR group but not in the NR group, after SLIT. Consistently, the expression of TAS2R13, 43 and 50 in $CD4^+$ T cells could be retrieved by BioGPS (http://biogps.org/) (Supplementary Figs. 1–3). Among them, we confirmed the cell surface expression of TAS2R43 on $CD4^+$ T cells (Supplementary Fig. 4). SLIT-induced increasing tendency was also observed for several small nuclear RNAs and microRNAs especially in the HR group. The results of one-way two-class ANOVA of the log2 ratios suggested that the pre-treatment expression level distributions of those genes were biased between the HR and NR groups.

To identify gene expression-related and germline gDNA structural variations, a genome-wide copy number variation (CNV) analysis was performed. Several CNV regions relating to differential mRNA expression between the HR and NR groups were identified (Supplementary Table 1, Supplementary Fig. 5). Figure 2A shows one such CNV region on chromosome 12 that contains several *TAS2R* genes. Deletion-type CNVs in this region, in a Japanese population, have also been reported previously.[7] Genome-wide CNV and mRNA association analysis indicated a significant correlation between the CNV and mRNA expression level for the *TAS2R43* gene in the HR group, but not the NR group, both before and after SLIT (Fig. 2B, C). Taste receptors are G-protein-coupled receptors located on the tongue,[8] and are often expressed by airway smooth muscle cells and mast cells.[9,10] Deshpande *et al.* consistently showed that TAS2R agonists such as saccharin, chloroquine, and denatonium (DN) induced the relaxation of isolated human airway smooth muscle cells.[9] Ekoff *et al.* demonstrated that IgE-mediated mast cell degranulation was suppressed by TAS2R agonists.[10] To ascertain the functional role of TAS2R in $CD4^+$ T cells, the effects of TAS2R agonists, e.g., DN and phenylthiocarbamide (PTC), on Th2 cytokine expression were examined. Stimulation through T cell receptors and CD28 strongly induced interleukin (IL)-4, IL-5, and IL-13 mRNA expression in $CD4^+$ T cells (Supplementary Fig. 6A), though their enhanced levels were much different among donors. Interestingly, the expression of IL-4 but not IL-5 or IL-13 was slightly but significantly augmented by the addition of DN and PTC (Supplementary Fig. 6B). Although mechanisms underlying the differential contribution of TAS2R to each cytokine remain to be further elucidated, these findings suggest that the difference in taste receptor expression may affect $CD4^+$ T-cell responsiveness, and consequently, SLIT efficacy.

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Log2 ratio				Gene				
HR/NR		Post/Pre						
Pre	Post	HR	NR	Type	Chromosome	Acc. No.	Symbol	Name
-0.105	-0.023	0.086	0.004	Transmembrane	12	BC095518	TAS2R13	taste receptor, type 2, member 13
-0.157	-0.066	0.094	0.003	Transmembrane	12	BC112100	TAS2R50	taste receptor, type 2, member 50
-0.104	-0.045	0.061	0.003	Transmembrane	12	BC117421	TAS2R31	taste receptor, type 2, member 31
-0.136	-0.071	0.065	0	Transmembrane	12	NM_176887	TAS2R46	taste receptor, type 2, member 46
0.089	0.141	0.07	0.019	Transmembrane	12	BC117423	TAS2R43	taste receptor, type 2, member 43
0.066	0.105	0.041	0.001	Transmembrane	5	AK290697	OCLN	occludin
0.108	0.065	-0.158	-0.115	Transmembrane	6	BC032350	HLA-DRA	major histocompatibility complex, class II, DR alpha
0.131	0.028	-0.042	0.061	Transmembrane	13	BC128146	TPTE2	transmembrane phosphoinositide 3-phosphatase and tensin homolog 2
-0.105	0.073	0.142	-0.036	Transmembrane	19	AF146747	CD177	CD177 molecule
0.138	0.11	0.006	0.034	Protease	11	BC048255	CTSW	cathepsin W
-0.022	0.168	0.006	-0.184	Zinc finger protein	19	AK299106	ZNF578	zinc finger protein 578
0.116	0.12	0.01	0.007	Small nuclear ribonucleoprotein	15	JX629742	SNRPN	small nuclear ribonucleoprotein polypeptide N
0.098	0.118	0.165	0.145	Small nuclear ribonucleoprotein	15	AF400492	SNRPN	small nuclear ribonucleoprotein polypeptide N
0.032	0.146	0.201	0.088	Small nuclear ribonucleoprotein	15	AF400501	SNRPN	small nuclear ribonucleoprotein polypeptide N
-0.101	0.012	0.01	-0.102	Small nuclear RNA	1	NR_002753	RNU5F-1	RNA, U5F small nuclear 1
0.031	0.147	0.2	0.084	Small nuclear RNA	15	NR_003297	SNORD115-5	small nucleolar RNA, C/D box 115-5
0.123	0.087	0.019	0.056	Small nuclear RNA	15	NR_003300	SNORD115-8	small nucleolar RNA, C/D box 115-8
0.023	0.12	0.095	-0.002	Small nuclear RNA	15	NR_003309	SNORD115-17	small nucleolar RNA, C/D box 115-17
0.008	0.164	0.173	0.016	Small nuclear RNA	15	NR_003312	SNORD115-20	small nucleolar RNA, C/D box 115-20
0.175	0.06	-0.019	0.096	Small nuclear RNA	15	NR_003342	SNORD115-25	small nucleolar RNA, C/D box 115-25
0.057	0.122	0.053	-0.012	Small nuclear RNA	15	NR_003346	SNORD115-31	small nucleolar RNA, C/D box 115-31
0.124	0.096	-0.023	0.005	Small nuclear RNA	15	NR_003350	SNORD115-35	small nucleolar RNA, C/D box 115-35
-0.05	0.1	0.233	0.082	Small nuclear RNA	15	NR_003356	SNORD115-41	small nucleolar RNA, C/D box 115-41
-0.155	0.072	0.293	0.066	microRNA	4	NR_029511	MIR95	microRNA 95
-0.107	-0.012	0.055	-0.04	microRNA	5	NR_030741	MIR9-2	microRNA 9-2
-0.122	0.114	0.152	-0.083	microRNA	9	NR_029836	MIR101-2	microRNA 101-2
0.01	0.102	0.038	-0.053	microRNA	14	ENST00000459389	MIR376C	microRNA 376c
0.044	0.154	0.119	0.009	microRNA	19	NR_030218	MIR519A1	microRNA 519a-1
0.11	-0.144	-0.046	0.208	microRNA	19	NR_030198	MIR520C	microRNA 520c
0.119	0.056	0.171	0.234	Mitochondrial RNA	M	ENST00000387419	MT-TD	mitochondrially encoded tRNA aspartic acid
0.106	0.054	0.13	0.182	Mitochondrial RNA	M	ENST00000387429	MT-TG	mitochondrially encoded tRNA glycine
0.103	0.04	0.1	0.163	Mitochondrial RNA	M	ENST00000387456	MT-TL2	mitochondrially encoded tRNA leucine 2 (CUN)
-0.165	0.011	0.059	-0.117	Other functional protein	1	NR_002771	DLEU2L	deleted in lymphocytic leukemia 2-like
-0.046	0.108	0.084	-0.07	Other functional protein	1	NM_001373	DNAH14	dynein, axonemal, heavy chain 14
-0.135	0.009	0.124	-0.021	Other functional protein	5	BC005383	CETN3	centrin, EF-hand protein, 3
0.106	0.031	-0.08	-0.006	Other functional protein	10	AF335324	DDIT4	DNA-damage-inducible transcript 4
0.184	0.059	-0.039	0.086	Other functional protein	10	ENST00000475252	LOC100652732	coiled-coil and C2 domain-containing protein 2A-like
-0.004	-0.12	-0.008	0.108	Other functional protein	16	BC027892	HBZ	hemoglobin, zeta
-0.051	0.173	0.158	-0.065	Other functional protein	22	ENST00000494065	DEPDC5	DEP domain containing 5
-0.122	0.05	0.194	0.022	Pseudogene	1	ENST00000411054	RNU5A-5P	RNA, U5A small nuclear 5, pseudogene
0.14	-0.017	-0.025	0.133	Pseudogene	1	ENST00000365393	RNA5SP22	RNA, 5S ribosomal pseudogene 22
0.064	-0.157	0.003	0.224	Pseudogene	1	NR_036753	ZNF847P	zinc finger protein 847, pseudogene
-0.062	-0.104	-0.026	0.016	Pseudogene	3	ENST00000363036	RNU5B-2P	RNA, U5B small nuclear 2, pseudogene
0.102	0.035	-0.112	-0.045	Pseudogene	3	X58062	SNORD13P3	small nucleolar RNA, C/D box 13 pseudogene 3
-0.219	-0.123	-0.053	-0.149	Pseudogene	4	ENST00000516533	RNA5SP164	RNA, 5S ribosomal pseudogene 164
-0.244	-0.042	0.081	-0.121	Pseudogene	5	ENST00000362452	RNA5SP183	RNA, 5S ribosomal pseudogene 183
0.206	0.021	-0.137	0.049	Pseudogene	5	ENST00000410376	RNA5SP198	RNA, 5S ribosomal pseudogene 198
0.049	0.11	-0.018	-0.078	Pseudogene	10	ENST00000516765	RNU5B-6P	RNA, U5B small nuclear 6, pseudogene
0.061	0.11	0.102	0.054	Pseudogene	11	ENST00000364986	RNA5SP331	RNA, 5S ribosomal pseudogene 331
0.143	0.005	-0.102	0.036	Pseudogene	13	ENST00000384727	RNY3P9	RNA, Ro-associated Y3 pseudogene 9
0.041	0.108	0.025	-0.043	Pseudogene	15	ENST00000516720	RNU3P3	RNA, U3 small nucleolar pseudogene 3
0.117	0.019	-0.063	0.034	Unknown	8	AF119907	PRO2949	uncharacterized protein PRO2949
0.079	0.105	0.077	0.051	Unknown	9	OTTHUMT00000037121	OTTHUMG00000013331	NULL
0.023	0.132	0.041	-0.068	Unknown	9	OTTHUMT00000051887	IFNA10	NULL
0.129	0.022	-0.027	0.08	Unknown	15	OTTHUMT00000418283	OTTHUMG00000172395	NULL
0.112	0.125	0.125	0.112	Unknown	17	OTTHUMT00000446612	OTTHUMG00000179479	NULL

Fig. 1. Differential mRNA expression in $CD4^+$ T cells, between HR and NR patients. A genome-wide transcriptome analysis was performed for $CD4^+$ T cells from the HR and NR groups, as described in the Supplementary Methods. Log2 ratios of the average HR and NR levels in the $CD4^+$ T cells before (pre) and after (post) SLIT and those of the average pre- and post-treatment levels in the HR and NR groups are indicated. The genes in which the difference of the log2 ratio between the two groups was >0.1 before and after treatment are listed.

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