

Transcriptome analysis reveals two distinct endotypes and putative immune pathways in tonsils from children with periodic fever, aphthous stomatitis, pharyngitis, and cervical adenitis syndrome

To the Editor,

Periodic fever, aphthous stomatitis, pharyngitis, and cervical adenitis syndrome (PFAPA) is one of the most common periodic fevers in children. Almost all patients exhibit three frequent symptoms (tonsillitis, cervical adenitis, and aphthous stomatitis), but 2/3 of patients also have gastrointestinal symptoms, arthralgia, myalgia, skin rashes, and/or neurological symptoms. Interestingly, some patients repeatedly develop the same symptoms, suggesting the existence of different phenotypes in PFAPA.¹ However, the pathophysiologic mechanisms underlying these symptoms remain unknown. High serum IL-1 β levels² and exaggerated proinflammatory cytokine production by patients' monocytes upon lipopolysaccharide stimulation³ suggest that some kinds of innate immune responses are involved in PFAPA flares. Because systemic corticosteroids—but not any antibiotics—rapidly improve these symptoms, such innate immune responses are likely to be just triggers of steroid-sensitive acquired immune response(s) rather than directly inducing inflammatory reactions in PFAPA flares.⁴ However, it remains largely unknown what types of innate immune stimuli or immune pathways may be involved.

The high rate of tonsillitis during febrile episodes and almost complete resolution of such flares after tonsillectomy⁵ suggest that the tonsils play critical roles in the pathogenesis of PFAPA.⁶ Hoping to clarify what type(s) of innate immune response(s) trigger the PFAPA pathogenesis, we tried to identify the involved immune pathways using the mRNA expression profiles of innate immunity-related genes in the tonsils of children with PFAPA. Detailed information regarding the subjects and methods is described in Table S1 and the Subjects and Methods section in this article's Appendix S1 section.

Primary component analysis (PCA) of the expression levels of 52 innate immunity-related genes indicated that there were no extreme outliers in the microarray data obtained from the tonsils of 15 patients enrolled in this study (Figure S1). Unbiased hierarchical clustering analysis revealed that the patients could be classified into 2 distinct subtypes (tentatively named Endotype 1 and Endotype 2), which was in agreement with the PCA results (Figure 1A). One distinct gene cluster was identified as being characteristic of each endotype, which was also confirmed by volcano plot (Figure S2).

Endotype 1 was characterized by overexpression of 11 innate immunity-related genes, including 6 toll-like receptors (TLRs) and 3 inflammasome-related genes (Figure 1B-2: differences in 3/11 expression levels were statistically significant by volcano plot). In order to clarify whether or not such a different gene expression profile found in the clustering analysis was dependent on the cellular composition of the tonsils, we compared the gene expression levels of the leukocyte-specific markers between the endotypes. We found that the mRNA expression of two monocyte/macrophage-specific marker molecules, CD14 and CD68, was significantly elevated in Endotype 1 (Table S3). Those elevated expression levels (CD14 and/or CD68) correlated significantly with the expression levels of TLR2, TLR4, TLR8, and nod-like receptor family, caspase recruitment domain-containing 4 (NLRC4) (Figure 1C). Specific markers for B cells, dendritic cells, mast cells, and natural killer cells were also elevated in Endotype 1 (Table S3); however, the expression levels of the above 4 genes did not correlate with these cell type-specific markers (data not shown). In addition, the gene expression levels of monocyte chemotactic protein-1 (MCP-1) and macrophage migration inhibiting factor (MIF)—but not lymphocyte-, neutrophil-, or eosinophil-attracting chemokines—were found to correlate significantly with the expression levels of CD14 and CD68 (Figure 1D and data not shown). These results suggest that the overexpression of Endotype 1-specific genes is at least in part due to the increased number of monocytes/macrophages recruited into the tonsils of these children.

Upstream regulator analysis indicated that activation of TLRs, interferon (IFN)- γ production and decrease in 1,25-dihydroxyvitamin D are the putative immune pathways for the induction of these Endotype 1-specific genes (Figure S3), some of which is inconsistent with previous studies.⁷⁻⁹

In contrast, Endotype 2 was characterized by overexpression of 7 innate immunity-related genes, including TLR3 and 5 inflammasome-related genes (Figure 1B-1: differences in 5/7 expression levels were statistically significant). No cell compositions were increased in Endotype 2 when compared with Endotype 1 (Table S3). Also, no significant correlations were seen between the expression levels of any of the leukocyte-specific markers and the expression levels of these innate-related genes (data not shown). These two findings suggest that the overexpression of Endotype 2-specific genes was not

TABLE 1 Clinical characteristics and phenotype data of two endotypes

Characteristics	n	Endotype 1	n	Endotype 2	P value ^a	Coef ^b	P value ^c
Age at onset of symptoms (months)	8	32 (8-72)	7	36 (9-92)	.63	-	-
Gender (% male)	8	35.0 (4/8)	7	42.9 (3/7)	>.99	-	-
Interval of fever (weeks)	8	4 (3-4)	7	4 (3-4)	.28	.3	.21
Duration of febrile episode (days)	8	3.5 (2-5)	7	5 (4-6)	.02 [*]	-2	<.01
Maximum temperature (degrees C)	8	39 (38-41)	7	40 (39-42)	.14	-1.24	.04 [*]
Associated symptoms							
Aphthous stomatitis (%)	8	50.0 (4/8)	7	57.1(4/7)	>.99	-.04	.88
Cervical lymphadenitis (%)	8	62.5 (5/8)	7	71.4 (5/7)	>.99	-.09	.74
Pharyngitis/tonsillitis (%)	8	100 (8/8)	7	100 (7/7)	-	-	-
Tonsil symptoms:							
White coat (white spot) (%)	7	42.9 (3/7)	6	83.3 (5/6)	.27	-.36	.13
Swelling (%)	7	71.4 (5/7)	6	50 (3/6)	.59	.36	.17
Redness (%)	7	28.6 (2/7)	6	66.7 (4/6)	.29	-.38	.12
Arthralgia (%)	8	12.5 (1/8)	7	28.6 (2/7)	.57	-.16	.47
Abdominal pain (%)	8	12.5 (1/8)	7	42.9 (3/7)	.28	-.48	.02 [*]
Headache (%)	8	37.5 (3/8)	7	71.4 (5/7)	.32	-.51	.02 [*]
Total number of associated symptoms (no.)	8	3 (2-8)	7	5 (4-8)	.12	-1.99	.04 [*]
Family history (%)	8	37.5 (3/8)	7	71.4 (5/7)	.32	-	-
Response to treatment (% effectiveness)							
Corticosteroids	7	100 (8/8)	7	100 (7/7)	-	-	-
Shortening of the interval between fever attacks after using corticosteroids	6	100 (6/6)	7	85.7 (6/7)	>.99	.14	.38
Cimetidine	3	0 (0/3)	5	20.0 (1/5)	>.99	-.2	.48
Antibiotics	8	0 (8/8)	7	0 (7/7)	-	-	-
Age at operation (months)	8	69 (48-93)	7	111 (54-170)	.11	-35.8	.06
Duration between operation and last febrile episode (days)	8	30 (10-60)	7	30 (20-60)	.49	-6.79	.45
Duration between operation and last corticosteroid use (weeks)	5	8 (4-24)	6	14 (3-24)	.78	-.2	.97
Time from disease onset to operation (months)	8	37.5 (18-57)	7	39 (18-147)	.63	.31	.23
Response to tonsillectomy (% effectiveness)	8	100 (8/8)	7	100 (7/7)	-	-	-
Bacterial mass in histopathological examination (%)	8	37.5 (3/8)	3	14.3 (1/7)	.57	.23	.35

Note: After univariate analysis, multivariate linear regression was performed, adjusting for possible differences in background characteristics (age at onset, gender, and family history). Data are expressed as medians (ranges) or percentages (positive number/total number).

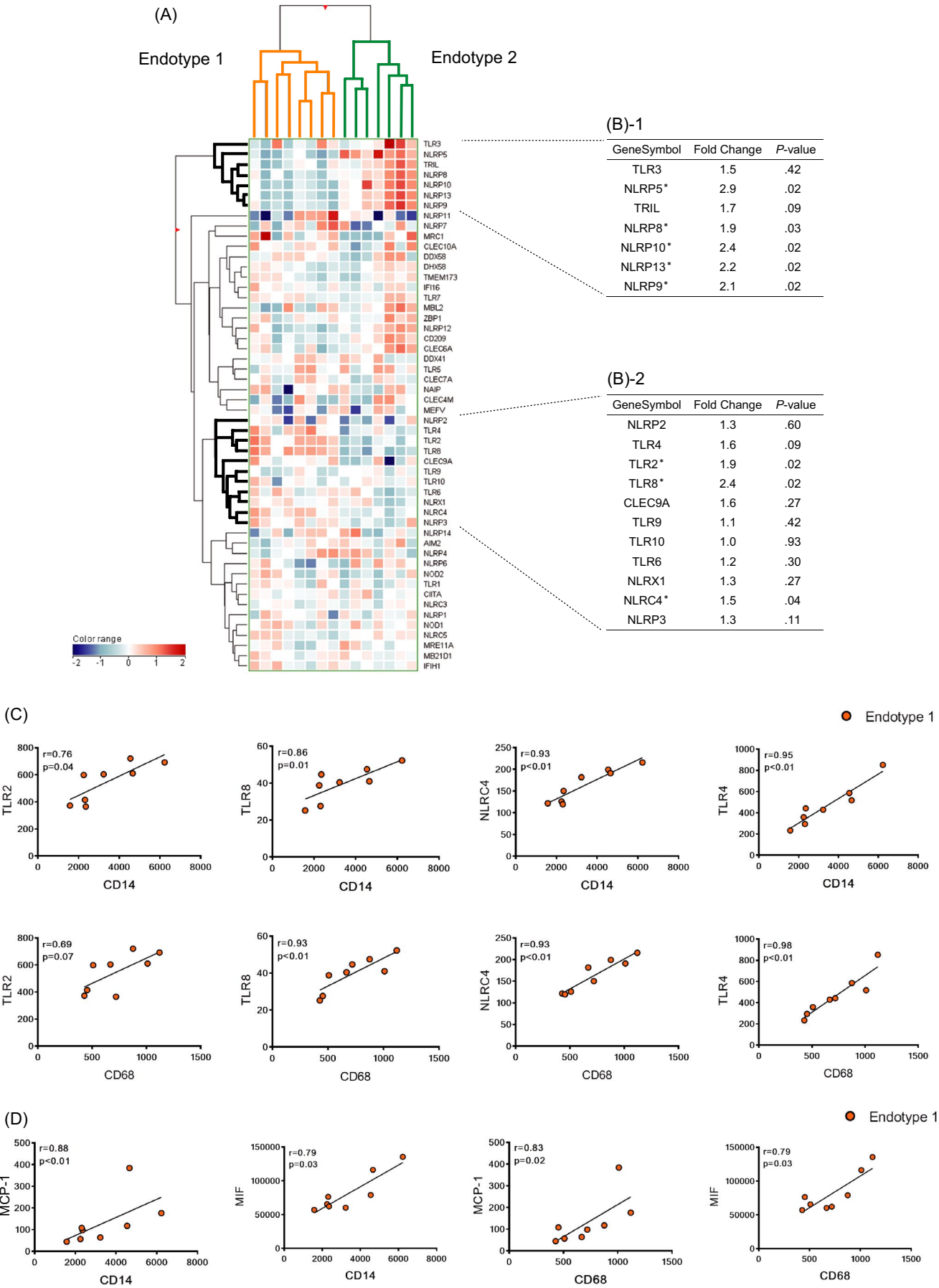
^aMann-Whitney *U* test, Fisher's exact test.

^bPartial regression coefficient.

^cMultivariate linear regression.

**P* < .05.

FIGURE 1 Transcriptome analysis of innate immunity-related genes in tonsils from children with PFAPA syndrome. A, Unbiased hierarchical cluster analysis based on microarray expression data of innate immunity-related genes in PFAPA tonsils. B, Detailed information of the genes in each Endotype-specific gene cluster (B-1 for Endotype 2 and B-2 for Endotype 1). Gene symbols, fold changes, and *P* values are shown for Endotype 1 and Endotype 2. C, Correlations between mRNA expression levels of monocyte/macrophage cell markers (CD14 and CD68) and mRNA expression levels of innate immunity-related genes (TLR2, 4, 8, and NLRP4) in Endotype 1 are shown. D, Correlations between mRNA expression levels of monocyte/macrophage cell markers (CD14 and CD68) and mRNA expression levels of macrophage chemotactic factors (MIF, MCP-1) in Endotype 1 are shown. TLR, toll-like receptor; NLRP, NLR family, pyrin domain-containing; TRIL, TLR4 interactor with leucine-rich repeats; CLEC9A, C-type lectin domain family 9, member A; NLRX1, NLR family member X1; NLRP4, NLR family, caspase recruitment domain-containing 4, MIF, macrophage inhibitory factor; MCP-1, monocyte chemotactic protein-1



due to any changes in the cellular component of the tonsils in these patients. Upstream regulator analysis did not identify any significantly related signaling pathways for these genes in Endotype 2, presumably because the number of genes was small, and/or the functions of these genes have never been sufficiently analyzed.

To investigate whether these endotypes identified by the gene expression profiles of the tonsils link to the clinical manifestations, we next compared the clinical symptoms seen with each PFAPA endotype (Table 1). Multivariate regression analysis revealed that the length of febrile episodes, maximum body temperature, frequency of abdominal pain, frequency of headache, and total number of associated symptoms were significantly different between Endotype 1 and Endotype 2, which supports the earlier suggestion that different phenotypes may exist in PFAPA.¹ Prostaglandin E2 is well known to be involved in pain accompanied by fever. Thus, we analyzed the gene expression levels of three genes (cyclooxygenase-1, cyclooxygenase-2, and prostaglandin-E-synthase) responsible for the synthesis of PGE2, and found that none of them differed between Endotypes 1 and 2 (data not shown).

This study has some limitations. All tonsillectomies were performed at least 3 weeks after febrile episodes. Tonsils obtained during febrile episodes might provide much more direct information about the pathogenesis of PFAPA, but that is ethically impossible. The sample size is small (N = 15), but tonsillar cells for this rare disease cannot be easily obtained. Therefore, we thought that it is important to report even this limited evidence. Finally, we did not perform any functional analyses of the genes identified in this study; that warrants further investigation.

In summary, we identified two distinct innate immune endotypes in tonsils from children with PFAPA, and those endotypes were significantly linked to the clinical phenotypes. Upstream regulator analysis indicated that activation of TLRs, IFN- γ production and decrease in 1,25-dihydroxyvitamin D are the putative immune pathways for the pathogenesis of PFAPA presumably through induction of both innate and acquired immune responses and their interaction.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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KEYWORDS

endotype, innate immune response, PFAPA syndrome, phenotype, tonsil

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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High fractional exhaled nitric oxide levels may predict short-term worsening of respiratory oscillometry in asthma

To the Editor,

High fractional exhaled nitric oxide (FeNO) levels, reflecting uncontrolled airway inflammation, are regarded as a risk factor for an upcoming asthma exacerbation.¹ A previous study found that persistently high FeNO (>40 ppb) was associated with a rapid decline in FEV1, even in controlled asthma patients, in a 3-year prospective study.² However, little is known about the effect of high FeNO levels on short-term course of lung function. Oscillometry, also known as the forced oscillation technique, is a noninvasive method with which to measure respiratory mechanics and is useful for the evaluation or management of asthma.³ The aim of this study was to assess whether high FeNO levels (>35 ppb) would predict the short-term worsening of lung function, especially oscillometric parameters.

This was a multicenter, prospective observational study. It was approved by the institutional review board of Shizuoka General Hospital and written informed consent was obtained from all subjects prior to the study. The study subjects included adult asthma patients who had routine check-ups at each institution from April 2015 to December 2016. Patients received standard treatment, including inhaled corticosteroid (ICS) and other medications, according to the Global Initiative for Asthma (GINA) report⁴ and the medications were not changed for at least 3 months prior to the study. Patients were excluded from the study if they had an exacerbation upon enrollment. Subjects who could not perform oscillometry and spirometry were also excluded.

On the examination days (baseline and after 3 months), eligible subjects underwent the Asthma Control Test (ACT), FeNO, oscillometry, and spirometry, in that order. The patients were classified into high or low FeNO groups (FeNO $\geq$ 35 ppb or <35 ppb) based on the baseline FeNO levels according to the previous study.⁵ After a 3-month period of observation without changes in medications, the changes in parameters were compared between the two groups. The

primary outcomes were the changes in oscillometric parameters. Secondary outcomes included the changes in ACT scores, FEV1, and FeNO levels. The correlations between baseline FeNO and the other parameters were evaluated.

One hundred and forty-three patients were screened and 46 patients were excluded upon entry because of asthma exacerbation, a change in medications, or difficulty in taking measurements. The clinical characteristics of the patients at baseline are shown in Table S1. Of the 97 subjects who completed the study, 28 (28.9%) patients had FeNO levels $\geq$ 35 ppb. There was no difference in the demographic, ACT, and lung function data between high and low FeNO groups at baseline except for FeNO levels (Table S2). Overall, median FeNO levels significantly decreased (29.0 ppb to 24.0 ppb; $P = .008$). However, there was no change in the ACT scores or FEV1. A comparison between the groups revealed that FeNO decreased significantly in the high FeNO group but not in the low FeNO group (Table S2). There was no difference in the ACT score and lung function data between high and low FeNO groups after 3 months except for FeNO levels (Table S2). Respiratory system resistance at 5 Hz (R5), the difference between respiratory system resistance at 5 Hz and 20 Hz (R5-R20), respiratory system reactance at 5 Hz (X5), resonant frequency (Fres), and area under the reactance (AX) significantly worsened in the high FeNO group but not in the low FeNO group (Figure 1 and Table S2). In the high FeNO group, there were no differences in changes in FEV1 and oscillometric parameters between patients who had FeNO <35 ppb after 3 months and those who had $\geq$ 35 ppb after 3 months. There was no significant change in the ACT scores, FEV1, or R20 in any group. In the high FeNO group, baseline FeNO levels correlated with the changes in R5, R20, and R5-R20, but not with changes in X5, Fres, and AX (Figure 2). Furthermore, a trend toward a negative association between changes in FeNO levels and the changes in R5 and R20 was found (p -value between 0.05

359-363 Appendix S1

Transcriptome analysis reveals two distinct endotypes and putative immune pathways in tonsils from children with periodic fever, aphthous stomatitis, pharyngitis, and cervical adenitis syndrome

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SUBJECTS AND METHODS

Subjects

This study enrolled 15 children (7 boys and 8 girls) who were brought to the division of Otorhinolaryngology of National Center for Child Health and Development from March 2015 to April 2018 and who fulfilled the original criteria for PFAPA syndrome proposed by Thomas ^{s1} and/or Padeh ^{s2 s3}. Febrile episodes completely disappeared after tonsillectomy in all participants in this study, suggesting that the tonsils played critical roles in these patients.

Exclusion criteria

All patients who received systemic glucocorticoids for at least 3 weeks prior to the tonsillectomy were excluded from this study.

Ethics

This study was approved by the Ethics Committee of the National Research Institute for Child Health and Development (#906), and written informed consent was obtained from all guardians of the participants.

Tonsillar cells

All tonsillectomies were performed at least 3 weeks after febrile episodes. Tonsillar tissues were minced with surgical knives, and cells were collected and filtered with a cell strainer (Corning, Corning CA). Collected cells were stored in a -80°C deep freezer until use.

Total RNA extraction

Total RNA was extracted from the tonsillar cells using an RNeasy Micro kit (Qiagen, Valencia, CA), and the quality of the RNA was evaluated on an Agilent Bioanalyzer with RNA 6000 Nano kit (Agilent Technologies, Santa Clara, CA).

Transcriptome analysis

Gene expression analysis was performed using the total RNAs extracted from the tonsillar cells and Agilent SurePrint G3 Human GE 8 x 60k microarrays (Agilent Technologies), according to the manufacturer's instructions.

Raw data obtained from the scanner were normalized and analyzed with GeneSpring software version 12.5 (Agilent Technologies).

Innate immunity-related genes

In the present study, we selected 52 innate immunity-related genes, including toll-like receptors (TLRs), nod-like receptors (NLRs), retinoic acid-inducible gene (RIG)-like receptors, C-type lectin-like receptors and DNA sensors (Table E2).

Post-transcriptome analysis

Transcriptome data were further analyzed using BaseSpace software (Illumina, San Diego, CA) and Ingenuity Pathway analysis (IPA[®], QIAGEN, Redwood City, CA) software according to the manufacturers' instructions to search for possible biological processes, pathways and networks. Because we used only innate immunity genes in this study, Gene Ontology and Canonical pathway analyses were not performed.

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TABLE S1. Description of demographic data of PFAPA cases

Characteristics	Values
Number	15
Age at onset of symptoms (months)	36.0 (8-92)
Gender (% male)	46.7 (7/15)
Interval of fever (weeks)	4.0 (2.5-4.0)
Duration of febrile episode (day)	5.0 (2.5-6.0)
Maximum temperature (degrees)	39 (38-42)
Associated symptoms	
Aphthous stomatitis (%)	53.3 (8/15)
Cervical lymphadenitis (%)	66.7 (10/15)
Pharyngitis/tonsillitis (%)	100 (15/15)
Tonsil symptoms: White coat (white spot) (%)	86.7 (8/13)
Swelling (%)	86.7 (8/13)
Redness (%)	41.2 (6/13)
Arthralgia (%)	20.0 (3/15)
Abdominal pain (%)	26.7 (4/15)
Headache (%)	53.3 (8/15)
Total number of associated symptoms (no.)	4.0 (2-8)
Family history (%)	53.3 (8/15)
Response to treatment (% effectiveness)	
Corticosteroids	100 (15/15)
Shortening the interval between fever attacks after using corticosteroids	92.3 (12/13)
Cimetidine	12.5 (1/8)
Antibiotics	0 (15/15)
Age at operation (months)	75 (48-170)
Duration between operation and last febrile episode (days)	30 (10-60)
Duration between operation and last corticosteroid use (weeks)	12 (3-24)
Time from disease onset to operation (months)	37.5 (18-147)
Response to tonsillectomy (% effectiveness)	100 (15/15)
Bacterial mass in histopathological examination (%)	26.7 (4/15)

Data are expressed as medians (ranges) or percentages (positive number/total number).

TABLE S2. List of innate immunity-related genes used in this study

Accession Number	Gene symbol	Description
A_23_P10873	TLR1	toll-like receptor 1
A_23_P92499	TLR2	toll-like receptor 2
A_23_P29922	TLR3	toll-like receptor 3
A_32_P66881	TLR4	toll-like receptor 4
A_33_P3263423	TLR5	toll-like receptor 5
A_23_P256561	TLR6	toll-like receptor 6
A_23_P85240	TLR7	toll-like receptor 7
A_23_P73837	TLR8	toll-like receptor 8
A_33_P3380807	TLR9	toll-like receptor 9
A_33_P3383970	TLR10	toll-like receptor 10
A_24_P183664	TRIL	TLR4 interactor with leucine-rich repeats
A_24_P129277	NOD1	nucleotide-binding oligomerization domain containing 1
A_23_P420863	NOD2	nucleotide-binding oligomerization domain containing 2
A_23_P89550	NLRP1	NLR family, pyrin domain containing 1
A_24_P213161	NLRP2	NLR family, pyrin domain containing 2
A_23_P9883	NLRP3	NLR family, pyrin domain containing 3
A_33_P3406047	NLRP4	NLR family, pyrin domain containing 4
A_23_P4962	NLRP5	NLR family, pyrin domain containing 5
A_23_P311632	NLRP6	NLR family, pyrin domain containing 6
A_23_P16384	NLRP7	NLR family, pyrin domain containing 7
A_23_P351866	NLRP8	NLR family, pyrin domain containing 8
A_33_P3356170	NLRP9	NLR family, pyrin domain containing 9
A_23_P403488	NLRP10	NLR family, pyrin domain containing 10
A_32_P27479	NLRP11	NLR family, pyrin domain containing 11
A_23_P101434	NLRP12	NLR family, pyrin domain containing 12
A_23_P399834	NLRP13	NLR family, pyrin domain containing 13
A_23_P47579	NLRP14	NLR family, pyrin domain containing 14
A_23_P110473	NAIP	NLR family, apoptosis inhibitory protein
A_24_P244356	NLRX1	NLR family member X1
A_23_P340019	NLRC3	NLR family, CARD domain containing 3
A_23_P119835	NLRC4	NLR family, CARD domain containing 4
A_23_P26583	NLRC5	NLR family, CARD domain containing 5
A_23_P140967	MEFV	Mediterranean fever
A_32_P44394	AIM2	absent in melanoma 2
A_33_P3418170	DDX58	DEAD (Asp-Glu-Ala-Asp) box polypeptide 58
A_23_P38346	DHX58	DEXH (Asp-Glu-X-His) box polypeptide 58
A_24_P305345	CD209	CD209 molecule
A_23_P208482	CLEC4M	C-type lectin domain family 4, member M
A_33_P3295328	CLEC6A	C-type lectin domain family 6, member A
A_24_P227415	CLEC7A	C-type lectin domain family 7, member A
A_33_P3319022	CLEC9A	C-type lectin domain family 9, member A
A_23_P141505	CLEC10A	C-type lectin domain family 10, member A
A_23_P35529	MBL2	mannose-binding lectin (protein C) 2, soluble
A_23_P12746	MRC1	mannose receptor, C type 1
A_32_P209960	CIITA	class II, major histocompatibility complex, transactivator
A_23_P122116	DDX41	DEAD (Asp-Glu-Ala-Asp) box polypeptide 41
A_23_P160025	IFI16	interferon, gamma-inducible protein 16
A_23_P68155	IFIH1	interferon induced with helicase C domain 1
A_32_P1173	MB21D1	Mab-21 domain containing 1
A_23_P150189	MRE11A	MRE11 meiotic recombination 11 homolog A (S. cerevisiae)
A_23_P61371	TMEM173	transmembrane protein 173
A_23_P259141	ZBP1	Z-DNA binding protein 1

TABLE S3. Expression values[†] of cell type-specific genes in two endotypes

Cell type	Marker gene	Endotype 1 (n=8)		Endotype 2 (n=7)		P value
		Median	Range	Median	Range	
B cell	CD19	79942	64660-93583	64351	28227-89707	0.03*
DC	pDC	512	322-782	260	208-384	<0.01*
	CD11c	1096	572-1391	625	377-722	0.04*
	mDC	398	286-899	208	113-602	0.03*
Monocyte/ Macrophage	CD14	2792	1577-6228	1355	806-2630	0.01*
	CD68	694	428-4420	436	260 -877	0.07
T cell	CD4	6962	5635-10606	7179	3028-7684	0.34
	CD8A	4460	2619-6401	2418	1108-6010	0.19
	CD8B	2214	1580-3845	1951	1184-3089	0.28
Neutrophil	MPO	10	5-16	7	4-11	0.28
Eosinophil	PRSS33	5	3-8	6	4-9	0.12
Mast cell	TPSAB1	59	74-425	129	20-188	0.04*
NK cell	CD16	702	6131925	551	180-776	0.09
	CD56	48	25-91	21	12-49	0.02*

DC, dendritic cell; pDC, plasmacytoid dendritic cell; mDC, myeloid dendritic cell; NK, natural killer cell.

P value; Mann-Whitney U Test, * $P < 0.05$

[†]Gene expression analysis was performed using Agilent SurePrint G3 Human GE 8 x 60k microarrays (Agilent Technologies). Numbers represent microarray raw data analyzed with GeneSpring software version 12.5 (Agilent Technologies).

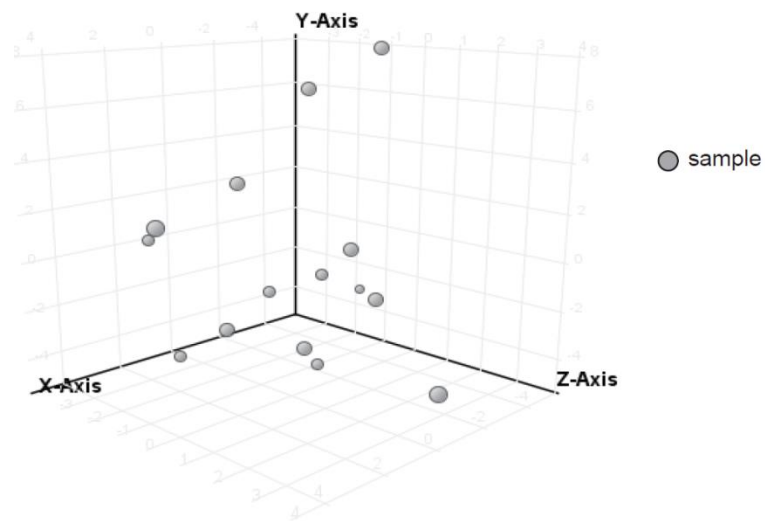


FIGURE S1. Principal component analysis of gene expression profiles of 15 tonsils from children with PFAPA. Each circle indicates a single PFAPA case. Three components are presented: PC1 on the X-axis (8.05%), PC2 on the Y-axis (14.62%), PC3 on the Z-axis (27.53%).

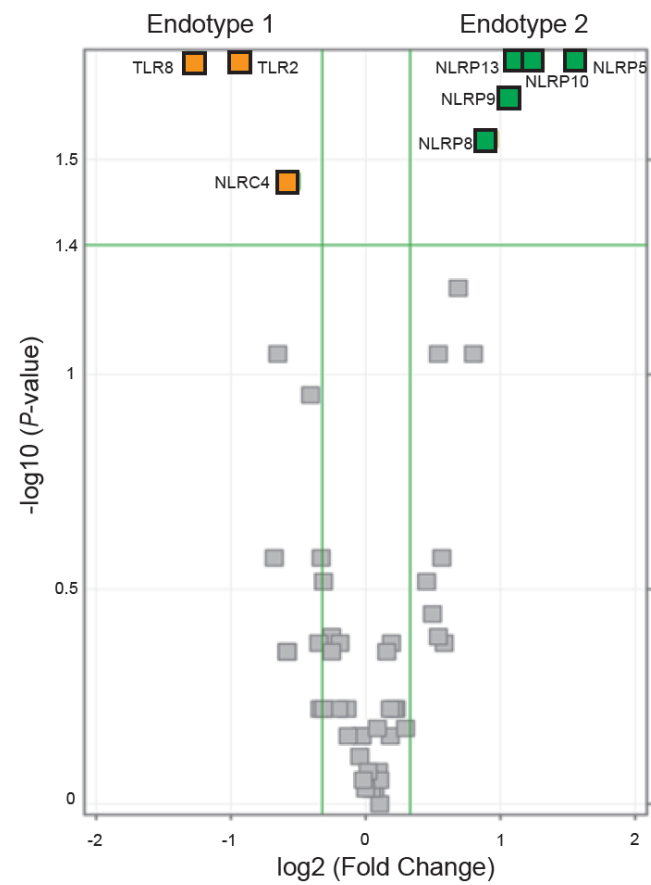


FIGURE S2. Volcano plot analysis demonstrating different expression genes contained in the cluster specific for each of the two endotypes. Each plot shows the relative expression of each innate immunity-related gene. Genes specific for Endotype 1 and Endotype 2 with fold change >1.4 and P-value <0.05 are highlighted in yellow and green, respectively.

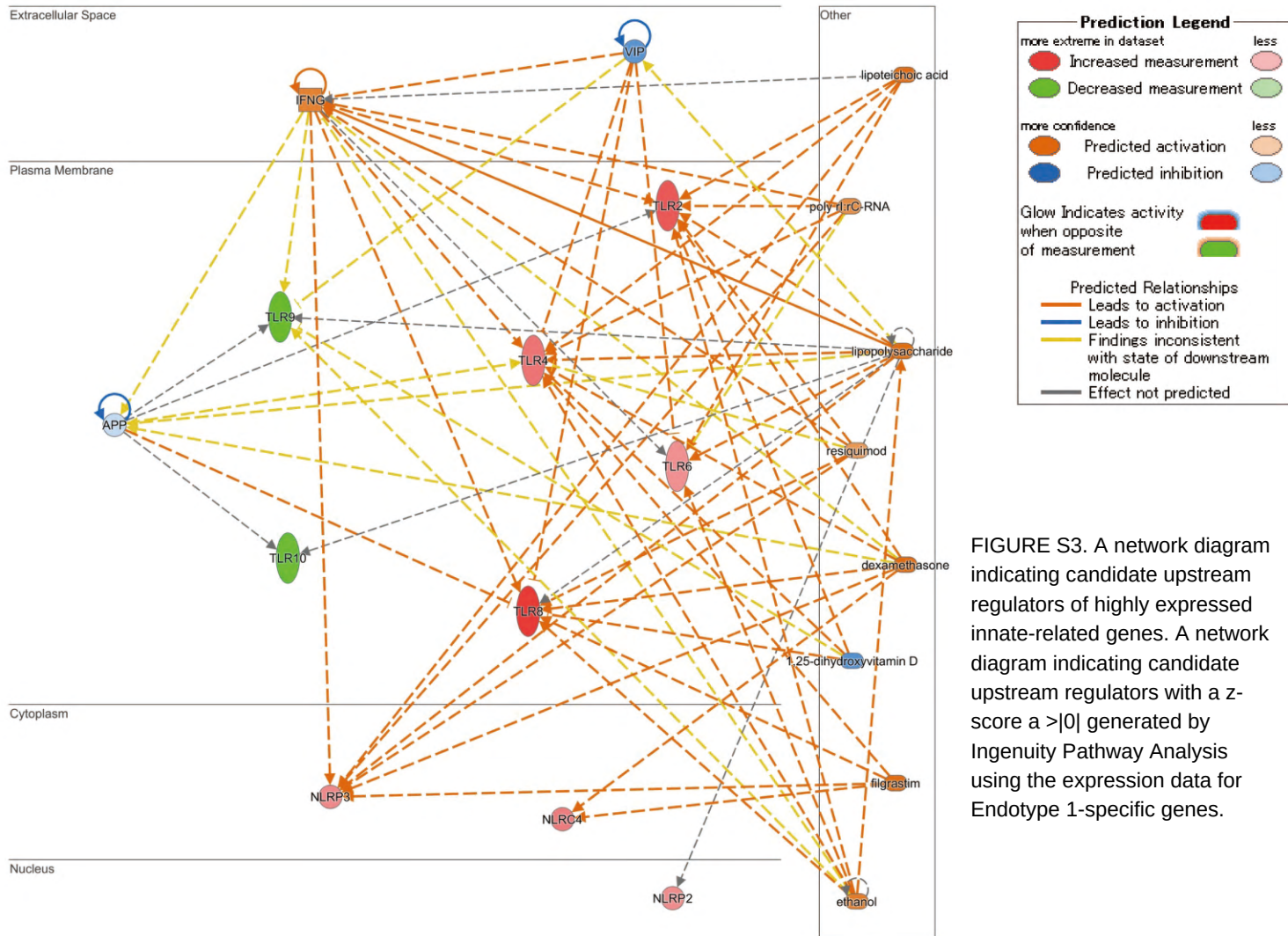


FIGURE S3. A network diagram indicating candidate upstream regulators of highly expressed innate-related genes. A network diagram indicating candidate upstream regulators with a z-score a >|0| generated by Ingenuity Pathway Analysis using the expression data for Endotype 1-specific genes.