

Distinct gene expression patterns of peripheral blood cells in hyper-IgE syndrome

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Summary

Hyper-immunoglobulin E (IgE) syndrome (HIES) is one of the primary immunodeficiency syndromes. Although the cytokine dysregulation is suggested to play a role in its pathophysiology, the causative gene has not yet been identified. To investigate the pathophysiology and candidate genes involved in this disease, we performed microarray analysis of unstimulated peripheral CD4⁺ T cells and CD14⁺ cells, as well as peripheral blood mononuclear cells (PBMNC) stimulated with *Staphylococcus aureus* isolated from HIES patients and healthy controls. By microarray analysis, 38 genes showed over 2-fold differences between the HIES patients and healthy controls in purified CD14⁺ cells, although only small differences in the gene expression profiles were observed between the two groups in purified CD4⁺ T cells. *RGC32* expression levels showed the greatest difference between the two groups, and were significantly elevated in HIES compared with those in severe atopic dermatitis or healthy controls using real-time PCR. A significantly larger number of lysosome-related genes were up-regulated, and significantly larger number of genes related to cell growth and maintenance were down-regulated in HIES. After the stimulation of PBMNC with *Staphylococcus aureus*, 51 genes showed over 3-fold differences between HIES patients and healthy controls. A significantly large number of immunoglobulin-related genes were up-regulated in HIES. The distinct patterns of gene expression profiles and *RGC32* expression levels will be useful for understanding the pathophysiology and for diagnosis of HIES, respectively.

Keywords: hyper-immunoglobulin E syndrome, microarray/ genomics/ proteomics, immunodeficiency–primary

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Introduction

Hyper-immunoglobulin E (IgE) syndrome (HIES) is a rare primary immunodeficiency, characterized by severe recurrent staphylococcal infections, eczema, and markedly elevated levels of IgE [1,2]. Pneumatocoles, dental and skeletal abnormalities and neutrophil defects are sometimes observed in patients with HIES [3,4]. Since the causative gene of HIES has not been identified, the diagnosis of HIES is sometimes difficult because of the similarity of symptoms to severe atopic dermatitis, particularly during early infancy.

Previous studies suggested that there might be a Th1/Th2 imbalance, especially a defect in Th1 induction [5–9], which may account for the high serum levels of IgE and eosino-

philia in HIES. In other reports, mitogen-induced secretion of IL-4 and IFN- γ by PBMNC was not different between patients with HIES and healthy controls [10,11]. Susceptibilities to bacterial and fungal infections in HIES indicate possible defects in phagocytes and T cells, while bone and dental abnormalities suggest certain defects in monocyte-lineage cells such as osteoclasts and osteoblasts.

Thus, to assess the pathogenesis and pathophysiology of the HIES, we purified CD4⁺ T cells and CD14⁺ cells from HIES patients and analysed the whole gene expression with microarray. HIES showed characteristic gene expression patterns compared with controls. Analysis of distinct gene expression patterns would be useful in the understanding of the pathophysiology as well as in the diagnosis of HIES.

Methods

Patients

Eight patients (median age 16.5 years, range 5–34 years; 3 males and 5 females) with HIES were enrolled in this study. The patients' characteristics are shown in Table 1. The samples obtained from patients 4 and 7 (Table 1) were used in the microarray analysis. As the controls, peripheral blood was obtained from 17 age-matched patients with severe atopic dermatitis (median age 8 years, range 1.0–34 years; 11 males and 6 females) and 15 healthy donors (median age 11 years, range 3–20 years, 8 males and 7 females). All the atopic patients had typical skin manifestation with increased serum levels of IgE (range 183–19504 IU/ml, median 3438 IU/ml) with or without eosinophilia (range 308–2018/μl, median 860/μl), and did not have the recurrent infection, lung cyst, abnormal facies, or bone abnormalities. The median values (ranges) of points of HIES diagnosis scoring system [12] were 25 (18–31) in HIES patients, and 16 (4–25) in atopic dermatitis patients ($P < 0.01$, by Mann–Whitney's test). Informed consent was obtained from all patients and healthy donors. This study was approved by Regional Committee of Ethics for Human research at Faculty of Medicine of Kyushu University.

Isolation of CD4⁺ T cells and CD14⁺ cells

PBMNC were separated immediately using density-gradient centrifugation using LSM (Cappel-ICN Immunobiologicals, Costa Mesa, CA, USA). CD4⁺ T cells were purified using cell sorter (EPICS ALTRA; Beckman Coulter, Hialeah, FL, USA) after staining with phycoerythrin (PE)-conjugated anti-CD4 antibody (Beckman Coulter). CD14⁺ cells were isolated using CD14 MicroBeads (Miltenyi Biotec GmbH, Bergisch Gladbach, Germany). The purity of CD4⁺ T cells or CD14⁺ cells was higher than 97%.

PBMNC stimulation with *Staphylococcus aureus*

PBMNC were incubated in 48 well-culture plates at the concentration of 1×10^6 cells/ml. Heat killed *Staphylococcus aureus* (frozen at the concentration of 1×10^9 CFU/ml) was added at the concentration of 1×10^7 CFU/ml. After 6 h, the cells were harvested, washed twice with phosphate buffered saline, and used for total RNA extraction.

RNA extraction, amplification and labelling

Total RNA was extracted using Isogen (Nippon Gene, Tokyo, Japan). For the microarray analysis of purified CD4⁺ T cells or CD14⁺ cells, linear amplification of RNA was carried out using an Amino Allyl Message Amp aRNA Kit (Ambion, Austin, TX, USA). Briefly, double-stranded complementary DNA (cDNA) was synthesized from total RNA using oligo

Table 1. Clinical characteristics of the patients with HIES.

Patient	Age	Gender	Eczematous rash	Skin abscesses	Lymphadenitis	Pneumonia	Lung cyst	Characteristic facies	Retained primary teeth	Fractures with minor trauma	Articular over-extension	Scoliosis	Serum IgE level (IU/ml)	<i>St. aureus</i> -specific IgE (U/ml)*	Eosinophil count (cells/μl)
1	5y	Female	+	+	+	+	+	+	+	+	+	+	40100	2260	2088
2	9y	Male	+	+	+	+	+	+	+	+	+	+	27180	1675	9520
3	11y	Male	+	+	+	+	+	+	+	+	+	+	17420	1331	1884
4	16y	Female	+	+	+	+	+	+	+	+	+	+	37780	2219	1198
5	17y	Female	+	+	+	+	+	+	+	+	+	+	16000	460	3663
6	18y	Female	+	+	+	+	+	+	+	+	+	+	24807	2305	1064
7	18y	Female	+	+	+	+	+	+	+	+	+	+	18300	716	18300
8	34y	Male	+	+	+	+	+	+	+	+	+	+	28100	14530	1480

*Normal range: < 400 U/ml.

dT primer with a T7 RNA polymerase promoter site added to the 3' end. Then, *in vitro* transcription was performed in the presence of amino allyl UTP to produce multiple copies of amino allyl labelled complementary RNA (cRNA). Amino allyl labelled cRNA was purified, and then 15 µg of cRNA was reacted with N-hydroxy succinimide esters of Cy3 (Amersham Pharmacia Biotech, Piscataway, NJ, USA) for cRNA from the purified cells, and Cy5 (Amersham Pharmacia Biotech) for that from 5 control PBMNC, according to the protocol of Hitachi Software Engineering (Yokohama, Japan).

For the gene expression analysis of PBMNC before and after the stimulation with *Staphylococcus aureus*, cDNA microarray was applied. After the linear amplification using RiboAmp RNA Amplification Kit (Arcturus Bioscience Mountain View, CA, USA), FluoroLink Cy3-dUTP- or FluoroLink Cy5-dUTP-labelled cDNA was synthesized using RNA Fluorescence Labeling Core Kit (M-MLV version) ver.2.0 (Takara Bio, Tokyo Japan), according to the manufacturer's instructions.

Microarray analysis

Microarray analysis for the purified CD4⁺ T cells and CD14⁺ cells was performed using an AceGene Human Oligo Chip 30K (Hitachi Software Engineering) that contains approximately 30 000 genes. Each gene expression level of CD4⁺ T cells and CD14⁺ cells from the patients or controls was determined by comparison with that from the standard sample. The arrays were scanned by FLA-8000 (Fuji Photo Film, Tokyo, Japan), and changed to the numerical values by ArrayVision (Amersham Biosciences). The numerical data were normalized using LOWESS method. In the microarray analysis of CD4⁺ T cells, data from 2 HIES patients and those from 2 healthy controls were compared. In the analysis of CD14⁺ cells, data from 2 HIES patients were compared with those from 2 healthy controls and one with severe atopic dermatitis to find out genes that were expressed specifically in HIES. Genes that were consistently up-regulated or consistently down-regulated in both HIES patients compared with all controls, and that showed over 2.0-fold differences by the comparison between the two groups in the mean expression levels were selected.

For the microarray analysis of PBMNC before and after the stimulation with *Staphylococcus aureus*, Agilent Human 1 cDNA Microarray (Agilent Technology, Palo Alto, CA, USA) was used, which contains 13 767 genes. The changes of the gene expression were analysed by direct comparison of the samples before and after the stimulation with *Staphylococcus aureus* in two patients with HIES, 2 healthy controls. The data were scanned by FLA-8000 (Fuji Photo Film), and changed to the numerical values by ArrayVision (Amersham Biosciences). The ratios of each gene expression level before and after the stimulation were obtained and normalized by being divided by the median value in each plate. Genes that

were expressed consistently higher or consistently lower in both HIES patients compared with all controls, and that showed over 3.0-fold differences by the comparison between the two groups in the mean expression levels were selected.

The data with low signal-to-noise ratios ($S/N < 3$) were not used for further analysis. The data were analysed using Gene Spring software (Silicon Genetics, Redwood City, CA, USA).

Real-time PCR

An Assay-on-Demand Gene Expression Product Hs.00204129_m1 (Applied Biosystems) was used for primers and probes for *response gene to complement 32* (RGC32). A Pre-Developed TaqMan Assay Reagent Human ACTB (β -actin) (Applied Biosystems) was used for the internal control. TaqMan assay was performed according to the manufacturer's instructions. In brief, PCR primer set and TaqMan probe for the target gene were added in the TaqMan Universal PCR Master Mix (Applied Biosystems) at the final volume of 25 µl. PCR condition was as follows: 50°C for 2 min and 95°C for 10 min, followed by 50 cycles of amplification at 94°C for 15 s and 60°C for 1 min. PCR amplification and the detection of the amounts of PCR products were performed by ABI PRISM 7700 Sequence Detector (Applied Biosystems). The gene expression levels were described by the relative ratios of the levels of target genes to those of the internal control (β -actin gene).

Statistical analyses

Chi-square test was used to analyse the deviated distribution of genes in each Gene Ontology into the two groups. One-way ANOVA test was used for the comparison of gene expression levels in three groups, and comparison of the two groups among 3 was performed with Scheffe's test. The differences were considered to be significant when the P -value < 0.05.

Results

To evaluate the gene expression profiles of circulating CD4⁺ T cells and CD14⁺ cells (monocytes) from patients with HIES, we extracted mRNAs from purified cells and performed microarray analysis, together with those from non-HIES controls. Six up-regulated and 4 down-regulated genes in HIES showed over 2-fold differences compared with controls in CD4⁺ T cells (Table 2), while 33 up-regulated and 5 down-regulated genes in HIES showed over 2-fold differences compared with controls in CD14⁺ cells (Table 3). The expression levels of major Th1- or Th2-related genes in CD4⁺ T cells and CD14⁺ cells did not show more than 2-fold differences between HIES patients and controls (Tables 2 and 3). In addition, there were only small differences in the expression levels of most cytokine-related genes between

Table 2. Microarray analysis of purified CD4⁺ T cells between HIES and healthy controls.

Gene name	Synonyms	GeneBank	*Fold difference
HIES > Control			
Haemoglobin, delta	HBD	NM_000519	2.4 (3.0, 2.0)
Chromosome 6 open reading frame 111	C6orf111	AF161424	2.3 (2.5, 2.1)
Haemoglobin, alpha 2	HBA2	AF281258	2.3 (2.5, 2.1)
No name		XM_031045	2.2 (2.2, 2.2)
Dynein, cytoplasmic, light intermediate polypeptide 2	DNCLI2	NM_006141	2.2 (2.8, 1.7)
Topoisomerase-related function protein 4-2		AF089897	2.0 (2.2, 1.9)
HIES < Control			
Polymerase (RNA) II (DNA directed) polypeptide D	POLR2D	BC010427	1/3.7 (1/3.8, 1/3.6)
No name		AC026785.3.13728-33112.2	1/2.3 (1/2.7, 1/1.9)
Sarcoglycan, gamma (35 kDa dystrophin-associated glycoprotein)	SGCG	NM_000231	1/2.3 (1/2.7, 1/1.9)
No name		U82695.2.1.167460.8	1/2.0 (1/2.6, 1/1.6)

*The difference of mean gene expression levels between 2 HIES patients and 2 healthy controls in microarray analysis is given. Values in parentheses are the fold differences of the gene expression in each HIES patient compared with the mean expression value in healthy controls. Genes that showed more than 2 fold expressional differences between HIES and healthy controls were selected.

HIES patients and controls (data, not shown). Among genes with over 2-fold differences in the expression between HIES patients and controls, there was no gene with a functional relevance to the abnormalities of bone and neutrophils, and increased serum levels of IgE, which might directly relate to the pathogenesis of HIES.

The expression levels of most up-regulated or down-regulated genes in CD14⁺ cells that showed over 2-fold differences between HIES patients and controls were confirmed by real-time quantitative PCR. The expression levels of *RGC32*, with the highest expression among 33 up-regulated genes in HIES in CD14⁺ cells by microarray analysis (Table 3), were significantly increased in PBMC of HIES patients, compared with those in patients with atopic dermatitis or controls (Fig. 1). We were not able to analyse the expression levels of *DNCL1* (dynein, cytoplasmic, light polypeptide 1) gene, with the lowest expression among 5 down-regulated genes in HIES in CD14⁺ cells by microarray analysis (Table 3), because the primers and probe were unable to be set to obtain precise and reproducible results due to the presence of many cDNA-type pseudogenes.

We compared the genes expressed in CD4⁺ T cells or CD14⁺ cells between HIES patients and controls, according to Gene Ontology Classifications from Gene Ontology Consortium to assess the differences in functionally related gene groups. At first, the genes that showed consistent expressional differences between HIES patients and controls in the two individual experiments were selected (HIES > normal: 1507 genes, HIES < control: 1116 genes). Among them, statistical analysis was performed in each gene group by the χ^2 test (Table 4). In unstimulated CD4⁺ T cells, there were no gene groups which showed significant expressional differences between HIES patients and controls (data not shown). In unstimulated CD14⁺ cells, a significantly larger number of lysosome-related genes was up-regulated (26 (1.73%) of 1507 total up-regulated genes, $P < 0.05$), and a significantly

larger number of genes related to the cell growth and maintenance was down-regulated (246 (22.0%) of 1116 total down-regulated genes, $P < 0.05$) in HIES (Table 4).

Recurrent staphylococcal infection is one of the characteristics of patients with HIES. We stimulated PBMC with heat-killed *Staphylococcus aureus*, and analysed the alteration of mRNA expression levels in HIES patients and healthy controls. Table 5 shows the genes with over 3.0-fold differences in the expression between HIES patients and controls before and after *Staphylococcus aureus* stimulation. Among

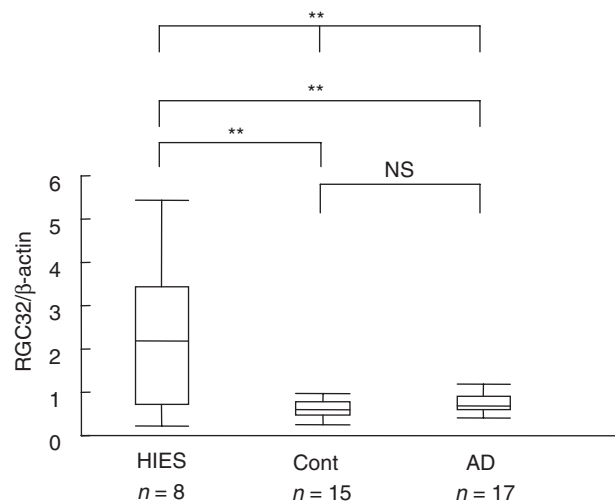


Fig. 1. Comparison of *RGC32* expression in patients with HIES, severe atopic dermatitis, and healthy controls. *RGC32* expression in PBMC was analysed by real-time PCR. HIES: patients with HIES, Cont: healthy controls, AD: patients with severe atopic dermatitis, NS: not significant. Whiskers indicate the ranges, boxes the 25th to 75th percentiles and horizontal bars inside boxes the median values. Three groups were compared using One-way ANOVA test and each two groups were compared using Scheffe's test. ** $P < 0.01$.

Table 3. Microarray analysis of purified CD14⁺ cells between HIES and healthy controls.

Gene name	Synonyms	GeneBank	*Fold difference
HIES > Control			
Response gene to complement 32	RGC32	NM_014059	3.8 (4.2, 3.5)
Homeo box A1	HOXA1	NM_005522	3.8 (3.9, 3.7)
Cathepsin L	CTSL	NM_001912	3.2 (2.1, 4.8)
C-type lectin, superfamily member 2 (activation-induced)	CLECSF2	NM_005127	3.1 (1.8, 5.3)
Epstein-Barr virus induced gene 2	EBI2	NM_004951	2.9 (2.8, 2.9)
Fatty acid binding protein 5 (psoriasis-associated)	FABP5	NM_001444	2.8 (1.6, 5.0)
Myristoylated alanine-rich protein kinase C substrate	MARCKS	XM_047795	2.6 (2.2, 3.2)
Interleukin 1 receptor, type II	IL1R2	NM_004633	2.6 (3.4, 1.9)
Adaptor-related protein complex 3, sigma 1 subunit	AP3S	XM_003926	2.4 (2.6, 2.3)
CD3D antigen, delta polypeptide (TiT3 complex)	CD3D	M12727	2.4 (1.6, 3.7)
Carboxypeptidase, vitellogenic-like	CPVL	AF106704	2.4 (3.2, 1.8)
Receptor-interacting serine-threonine kinase 2	RIPK2	NM_003821	2.3 (3.0, 1.8)
Hypothetical protein FLJ20186	FLJ20186	NM_017702	2.3 (1.5, 3.7)
Protein tyrosine phosphatase, receptor type, E	PTPRE	NM_006504	2.3 (2.5, 2.1)
X Kell blood group precursor-related, Y-linked	XKRY	NM_004677	2.3 (3.1, 1.6)
Toll-like receptor 1	TLR1	NM_003263	2.3 (1.8, 2.8)
Spermidine/spermine N1-acetyltransferase	SAT	BC002503	2.2 (2.7, 1.9)
KIAA0431 protein	KIAA0431	NM_015251	2.2 (2.0, 2.4)
Vimentin	VIM	NM_003380	2.2 (2.8, 1.8)
Pleckstrin homology, Sec7 and coiled-coil domains, binding protein	PSCDBP	NM_004288	2.2 (2.3, 2.0)
Recombining binding protein suppressor of hairless (Drosophila)	RBPSUH	NM_015874	2.2 (1.6, 2.9)
Proteasome (prosome, macropain) 26S subunit, non-ATPase, 12	PSMD12	AB003103	2.2 (2.4, 1.9)
Multiple ankyrin repeats, single KH-domain (MASK) homolog	MASK	NM_024668	2.2 (2.5, 1.9)
Ubiquitin-conjugating enzyme E2B (RAD6 homolog)	UBE2B	NM_003337	2.2 (1.6, 2.8)
Syndecan 2	SDC2	AK025488	2.2 (1.6, 2.8)
Sialyltransferase 10 (alpha-2,3-sialyltransferase VI)	SIAT10	XM_044802	2.1 (1.8, 2.6)
Rearranged L-myc fusion sequence	RLF	NM_012421	2.1 (2.2, 2.0)
RAB6 interacting protein 1	RAB6IP1	AK025499	2.1 (2.5, 1.8)
No name		XM_008071	2.1 (2.0, 2.2)
CDC-like kinase 1	CLK1	NM_004071	2.1 (2.5, 1.7)
Hypothetical protein FLJ22965	FLJ22965	NM_022101	2.1 (2.2, 1.9)
Hypothetical protein CL25022	CL25022	AF131802	2.0 (1.8, 2.3)
Opioid growth factor receptor-like 1	OGFRL1	NM_024576	2.0 (2.2, 1.8)
HIES < Control			
Dynein, cytoplasmic, light polypeptide 1	DNCL1	NM_003746	1/2.9 (1/1.9, 1/4.4)
Calmodulin regulated spectrin-associated protein 1	CAMSAP1	NM_018627	1/2.4 (1/1.7, 1/3.2)
Placenta-specific 8	PLAC8	NM_016619	1/2.1 (1/1.6, 1/2.8)
Vacuolar protein sorting 35 (yeast)	VPS35	NM_018206	1/2.0 (1/1.6, 1/2.6)
Butyrophilin, subfamily 3, member A1	BTN3A1	NM_007048	1/2.0 (1/1.5, 1/2.8)

*The difference of mean gene expression levels between 2 HIES patients and 3 controls (2 healthy donors and a atopy patient) in microarray analysis is given. Values in parentheses are the fold differences of the gene expression in each HIES patient compared with the mean expression value in controls. Genes that showed more than 2 fold expressional differences between HIES and healthy controls were selected.

Table 4. Comparison of gene expression according to the Gene Ontology groups.

	HIES > Control	HIES < Control	P-value*
CD14 ⁺ cells	(1507 genes)	(1116 genes)	
Lysosome related genes (total 137 genes)	26 (1.73%)	8 (0.72%)	< 0.05
Genes related to the cell growth and maintenance (total 2572 genes)	254 (16.9%)	246 (22.0%)	< 0.01
MNC stimulated with <i>Staphylococcus aureus</i>	(1194 genes)	(1331 genes)	
Immunoglobulin-related genes (total 54 genes)	29 (2.43%)	4 (0.30%)	< 0.01

* χ^2 test.

Table 5. Microarray analysis of the change of gene expression after the stimulation with *Staphylococcus aureus*.

Gene name	Synonyms	Gene bank	Fold increase of gene expression*		Fold difference** HIES/Control
			HIES	Control	
HIES > Control					
Ubinuclein 1	UBN1	AF108460	3.5	1/1.4	4.9 (5.0, 4.9)
Rho guanine nucleotide exchange factor (GEF) 1	ARHGEF1	Y09160	2.7	1/1.7	4.7 (2.7, 8.3)
Hydroxymethylbilane synthase	HMBS	X04808	3.4	1/1.3	4.3 (1.4, 13)
Bromodomain containing 3	BRD3	D26362	2.5	1/1.6	4.0 (3.7, 4.4)
Incyte EST			2.9	1/1.3	3.9 (2.8, 5.2)
KIAA0582 protein	KIAA0582	AK000856	1.3	1/3.0	3.8 (3.4, 4.2)
A kinase (PRKA) anchor protein (yotiao) 9	AKAP9	NM_005751	2.1	1/1.8	3.7 (4.6, 2.9)
Haloacid dehalogenase-like hydrolase domain	HDHD1A	M86934	2.6	1/1.4	3.7 (1.3, 11)
KIAA0555 gene product		AL137976	5.1	1.4	3.7 (2.3, 5.8)
Ral GEF with PH domain and SH3 binding motif 2	RALGPS2	AK001106	1.6	1/2.2	3.6 (3.2, 3.9)
Tripartite motif-containing 2	TRIM2	AB011089	1.5	1/2.4	3.5 (2.6, 4.7)
Serine (or cysteine) proteinase inhibitor, member 1	SERPINI1	NM_005025	2.1	1/1.6	3.5 (7.9, 1.5)
Cysteine conjugate-beta lyase; cytoplasmic	CCBL1	X82224	1.4	1/2.4	3.4 (3.3, 3.5)
Homeo box A7	HOXA7	AJ005814	3.2	1.0	3.3 (3.7, 3.0)
Scaffold attachment factor B2	SAFB2	D50928	3.3	1.0	3.3 (4.5, 2.4)
5'-nucleotidase, ecto (CD73)	NT5E	X55740	1.4	1/2.3	3.3 (3.7, 2.9)
Mad4 homolog		AL040187	1.4	1/2.3	3.3 (3.3, 3.3)
Aldo-keto reductase family 1, member C1	AKR1C1	M86609	5.8	1.8	3.3 (2.5, 4.3)
Hypothetical protein FLJ13213	FLJ13213	AK000867	2.6	1/1.3	3.3 (4.5, 2.4)
Human FLI1 gene for ERGB transcription factor		AB012624	2.0	1/1.6	3.3 (3.1, 3.4)
Myosin XVb, pseudogene	MYO15B	AK026339	1.3	1/2.5	3.2 (3.3, 3.2)
Adducin 2 (beta)	ADD2	S81079	1.6	1/2.0	3.2 (2.5, 4.2)
Zinc finger protein 236	ZNF236	AF085244	1.7	1/1.9	3.2 (4.1, 2.5)
Yippee-like 1 (Drosophila)	YPEL1	AW006162	3.1	1.0	3.2 (2.0, 5.1)
Incyte EST			1.1	1/3.0	3.2 (3.3, 3.0)
NIMA (never in mitosis gene a)-related kinase 1	NEK1	AL050385	3.3	1.1	3.1 (5.0, 2.0)
Protocadherin 9	PCDH9	AF169692	1.7	1/1.8	3.1 (4.2, 2.3)
Poly (ADP-ribose) polymerase family, member 2	ADPRTL2	AK001980	2.1	1/1.4	3.0 (3.2, 2.8)
Calcium/calmodulin-dependent protein kinase (CaM kinase) II delta	CAMK2D	AF071569	1.5	1/2.0	3.0 (1.7, 5.3)
HIES < Control					
Solute carrier organic anion transporter family, member 1A2	SLCO1A2	U21943	1.8	24	1/14 (1/29, 1/6.7)
Normal mucosa of oesophagus specific 1	NMES1	AK026298	4.6	37	1/8.1 (1/10, 1/6.3)
Syndecan 2	SDC2	J04621	4.1	23	1/5.6 (1/12, 1/2.7)
Interleukin 17	IL17	U32659	1/1.3	4.1	1/5.5 (1/8.0, 1/3.7)
TBC1 domain family, member 15	TBC1D15	AK022147	1/2.0	2.2	1/4.5 (1/6.9, 1/2.9)
Syndecan 4 (amphiglycan, ryudocan)	SDC4	D13292	1.0	4.6	1/4.4 (1/5.2, 1/3.7)
Recombining binding protein suppressor of hairless (Drosophila)	RBPSUH	AW968632	1/2.3	1.7	1/4.0 (1/4.8, 1/3.3)
Humour necrosis factor, alpha-induced protein 6	TNFAIP6	M31165	5.5	22	1/3.9 (1/3.0, 1/5.2)
Hypothetical protein FLJ10199	FLJ10199	AI216532	1.0	3.8	1/3.9 (1/6.2, 1/2.4)
Small inducible cytokine subfamily B (Cys-X-Cys), member 11	CXCL11	AF030514	1.5	5.6	1/3.8 (1/2.7, 1/5.4)
Cathepsin L	CTSL	AI041851	5.5	21	1/3.8 (1/7.7, 1/1.9)
Enhancer of rudimentary homolog (Drosophila)	ERH	U66871	1/1.9	1.9	1/3.6 (1/3.0, 1/4.3)
Tryptophanyl-tRNA synthetase	WARS	X59892	1/1.7	2.1	1/3.6 (1/3.8, 1/3.4)
Epithelial membrane protein 1	EMP1	Y07909	1.2	4.4	1/3.6 (1/5.6, 1/2.3)
NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 5, 13 kDa	NDUFA5	U53468	1/3.7	1/1.1	1/3.4 (1/4.1, 1/2.9)
Hect domain and RLD 5	HERC5	AB027289	7.5	25	1/3.3 (1/1.2, 1/8.7)
Feline leukaemia virus subgroup C cellular receptor	FLVCR	AK001419	1/2.8	1.2	1/3.3 (1/5.2, 1/2.0)
FK506 binding protein 5	FKBP5	U42031	1/2.3	1.3	1/3.1 (1/4.5, 1/2.2)
Deleted in lymphocytic leukaemia, 2	DLEU2	AW978447	1/2.8	1.1	1/3.1 (1/2.1, 1/4.6)
Cathepsin L2	CTSL2	AB001928	4.2	13	1/3.0 (1/5.7, 1/1.6)
Methionine adenosyltransferase II, alpha	MAT2A	F07456	1/2.4	1.3	1/3.0 (1/1.8, 1/5.1)
v-maf musculoaponeurotic fibrosarcoma oncogene homolog (avian)	MAF	AF055376	1.0	2.9	1/3.0 (1/3.7, 1/2.5)

*Fold increase of the gene expression after stimulation with *Staphylococcus aureus*. **Fold difference of the change of the gene expression levels after stimulation with *Staphylococcus aureus* between two HIS patients and two healthy controls. Values in parentheses are the fold differences of the gene expression in each HIS patient compared with the mean expression value in healthy controls. Genes that showed more than 3 fold expressional differences between HIES and healthy controls were selected.

cytokine-related genes, the relative expression levels of *IL-17* and *small inducible cytokine subfamily B (Cys-X-Cys), member 11 (CXCL11)* genes after stimulation were decreased to 1/5.5 and 1/3.8, respectively, in HIES (Table 5). Analysis of the differences in functionally related gene groups in stimulated PBMC from HIES patients and controls according to Gene Ontology Classifications revealed that a significantly larger number of immunoglobulin-related genes were up-regulated in HIES (29 of 1194 total up-regulated genes: 2.43%, $P < 0.05$ χ^2 test, Table 4).

These results showed unique patterns of the gene expression in unstimulated and stimulated conditions in peripheral blood cells from HIES patients.

Discussion

HIES is a multisystem disorder with the manifestations of recurrent infections especially with *Staphylococcus aureus*, characteristic facies, hyperextensibility of joints, multiple bone fractures, scoliosis, and delayed shedding of the primary teeth [4]. Therefore, it is likely that HIES is caused by a molecule which affects various biological functions rather than only by a Th1/Th2-related molecule. Chehimi *et al.* [8] analysed 375 cytokine and chemokine gene expression by microarray, and found that the expression of some chemokine genes was decreased in PBMC of HIES after the stimulation with PHA, which may account for the decreased responsiveness against microorganisms. Because of the complex multisystem nature of this disease, we thought more comprehensive analysis of gene expression profiles in purified populations without and with the stimulation of *Staphylococcus aureus* would be necessary.

It is worthy of note that there were no remarkable differences in the whole gene expression levels of CD4⁺ T cells containing Th1/Th2 cells between HIES patients and controls in an unstimulated condition. On the other hand, in unstimulated CD14⁺ cells, 33 genes were up-regulated in HIES and unique gene expression patterns were observed in HIES (Tables 3 and 4). By quantitative PCR, the expression levels of *RGC32* that was the most up-regulated gene in microarray analysis, were significantly elevated in HIES compared with healthy controls and atopic dermatitis (Fig. 1). Although *RGC32* appears to be a cell cycle regulatory factor that mediates cell proliferation [13], it was not clear why the expression of *RGC32* was most up-regulated in CD14⁺ cells in HIES and how it was related to the pathogenesis or pathophysiology of HIES. Nevertheless, the analysis of expression levels of *RGC32* would be helpful in the diagnosis of HIES. The down-regulation of a significantly large number of genes related to the cell growth and maintenance (Table 4) might contribute to the pathophysiology of HIES such as a decreased responsiveness against microorganisms.

After the stimulation with *Staphylococcus aureus*, decreased expression levels of *IL-17* and *CXCL11* genes were observed in PBMC from patients with HIES (Table 5). *IL-*

17, produced by T cells, is a proinflammatory cytokine that induces production of *IL-1 β* and *TNF- α* by macrophages [14], and *IL-6*, *IL-8* and *GM-CSF* by stromal cells [15]. It induces migration and functional maturation of neutrophils [16–18] and proinflammatory cytokine production by keratinocytes [19]. In addition, it is involved in the osteoclastogenesis [20]. *CXCL11* gene is located on chromosome 4q21, where HIES is linked. The chemokine, *CXCL11* is produced by monocytes and keratinocytes after the stimulation with *IFN- γ* , and is also involved in the inflammatory response by inducing a chemotaxis of monocytes and activated T cells [21]. Osteoblasts express CXC chemokine receptor 3, the receptor of *CXCL11* [22]. It is possible that a decreased expression of *IL-17* and *CXCL11* genes, of which translated products have pleiotropic biological activities, may play a role in the pathogenesis or pathophysiology of HIES.

One of the remarkable findings was up-regulation of immunoglobulin-related genes after the stimulation with *Staphylococcus aureus* (Table 4). It is reported that an increase of IgE levels was observed even in the cord blood of HIES [23], suggesting the primary dysregulation of IgE production. Our result suggested that the dysregulation was more generalized in immunoglobulin-related genes, and more prominent with the staphylococcal stimulation.

The number of patients used for the microarray analysis was low in this study, and it is possible that other genes or gene groups have more important meanings than those we picked up in the pathophysiology of HIES. Further genetic analysis based on the unique gene expression profile would clarify the pathophysiology and causative genetic abnormality of HIES, and help to develop a new diagnostic method in the near future.

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