

# Elevated granulocyte colony-stimulating factor levels predict treatment failure in patients with Kawasaki disease

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**Background:** Kawasaki disease (KD) is an acute vasculitis in young children, frequently associated with coronary artery aneurysms. The intravenous infusion of high-dose IgG (IVIG) effectively reduces the systemic inflammation and the incidence of coronary artery lesions, although the precise underlying mechanisms are unknown.

**Objective:** We performed expression profiling of whole blood cells to investigate the mechanisms underlying the effect of IVIG and to identify biomarkers associated with unresponsiveness to IVIG.

**Methods:** We compared the transcript abundance among pre-IVIG and post-IVIG patients and febrile control patients. Then we analyzed the mRNA levels and the protein levels among the different cohort of patients with KD who were either responsive or nonresponsive to the initial IVIG.

**Results:** A total of 298 transcripts were overrepresented or underrepresented in the pre-IVIG patients compared with post-IVIG patients and febrile controls, of which 15 transcripts were differentially expressed in nonresponsive patients with KD compared with responsive patients before IVIG. The protein levels of polycythemia rubra vera 1, which was one of the most variably expressed transcripts in pre-IVIG patients, and the serum granulocyte colony-stimulating factor levels were significantly higher in nonresponsive patients than in responsive patients before the initial IVIG administration.

**Conclusion:** These findings suggest that the variable gene expression profiles were correlated to the responses of patients with KD to IVIG administration. Polycythemia rubra vera 1 and granulocyte colony-stimulating factor levels may be good biomarkers for predicting response to IVIG in patients with KD. (*J Allergy Clin Immunol* ■■■■;■■■:■■■-■■■.)

**Key words:** Kawasaki disease, vasculitis, neutrophil, PRV-1, G-CSF, microarray, inflammation, IVIG, biomarker

## Abbreviations used

CAL: Coronary artery lesion  
CR1: Complement component 3b/4b receptor 1  
GAS7: Growth arrest-specific 7  
G-CSF: Granulocyte colony-stimulating factor  
ITGAM:  $\alpha$ -M integrin  
IVIG: Intravenous infusion of high-dose IgG  
KD: Kawasaki disease  
MFI: Mean fluorescence intensity  
PRV: Polycythemia rubra vera

Kawasaki disease (KD) is an acute systemic vasculitis in infants and young children.<sup>1</sup> It preferentially affects coronary arteries and is the leading cause of acquired heart disease in childhood in developed countries.<sup>2</sup> The intravenous infusion of high-dose IgG (IVIG) effectively reduces the systemic inflammation and the incidence of coronary artery lesions (CALs).<sup>3</sup> However, the precise underlying mechanisms of the effect of IVIG are unknown, and about 15% of patients with KD are nonresponsive to IVIG and develop CALs more frequently than responsive patients.<sup>4</sup> Many researchers have attempted to identify risk factors associated with nonresponsiveness to IVIG.<sup>5-9</sup> Among them, the patients' age, the white blood cell count, and the serum levels of aspartate aminotransferase and C-reactive protein were frequently shown to be useful in determining a risk classification instrument. However, because these factors were statistically determined by using available laboratory test data, the precise mechanism underlying the relationships between these factors and the clinical outcome of the patients remains uncertain.

In a previous study, we performed expression profiling of purified PBMCs and monocytes obtained before and after IVIG from patients with acute KD.<sup>10</sup> The results demonstrated that IVIG influenced the gene expressions in a broad functional range in both PBMCs and monocytes, favoring downregulation. This finding is consistent with the suppressive effects of IVIG that are clinically observed in acute patients. In this study, we performed expression profiling of whole blood cells, including neutrophils, to extend further our understanding of the mechanisms underlying the effect of IVIG on patients with KD, in the hope of eventually identifying biomarkers associated with the unresponsiveness to IVIG. Our results suggested that the protein levels of polycythemia rubra vera (PRV)-1, one of the most enriched transcripts in pre-IVIG patients, and granulocyte colony-stimulating factor (G-CSF) were significantly elevated in nonresponsive patients.

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TABLE I. Demographic data of patients

|                                         | First-array patients      |                           |                          | Second-array patients     |                           |                           |                          |
|-----------------------------------------|---------------------------|---------------------------|--------------------------|---------------------------|---------------------------|---------------------------|--------------------------|
|                                         | KD-pre                    | KD-post                   | Control                  | Responsive-pre            | Responsive-post           | Nonresponsive-pre         | Nonresponsive-post       |
| Donors (n)                              | 4                         | 4                         | 4                        | 6                         | 6                         | 4                         | 4                        |
| Age (mo after birth)                    | 8-54<br>(median, 13)      |                           | 12-39<br>(median, 20)    | 39-60<br>(median, 56)     |                           | 12-38<br>(median, 33)     |                          |
| Sex (male, female)                      | 2, 2                      |                           | 2, 2                     | 2, 4                      |                           | 2, 2                      |                          |
| Cardiac involvement (positive patients) | 0                         | 0                         |                          | 0                         | 0                         | 0                         | 0                        |
| Blood drawn (d after onset)             | 3-7<br>(median, 6.5)      | 8-10<br>(median, 8.5)     | 3-7<br>(median, 5)       | 4-7<br>(median, 4.5)      | 7-9<br>(median, 8)        | 3-5<br>(median, 4)        | 6-8<br>(median, 7)       |
| % Neutrophil                            | 49-86<br>(median, 53.5)   | 14-67<br>(median, 36)     | 36-70<br>(median, 52.3)  | 80-86<br>(median, 83)     | 18-73<br>(median, 35)     | 81-91<br>(median, 84)     | 59-77<br>(median, 69)    |
| <i>P</i> value*                         |                           | .07                       | .56                      |                           | .03                       | .67                       | .07                      |
| % Monocyte                              | 4-16<br>(median, 7.1)     | 5-10<br>(median, 8.5)     | 2-13<br>(median, 7.5)    | 2.4-7.0<br>(median, 3.7)  | 7-10<br>(median, 9)       | 1.3-10.4<br>(median, 5.0) | 3-14<br>(median, 4.0)    |
| <i>P</i> value                          |                           | .99                       | .77                      |                           | .03                       | .92                       | .99                      |
| % Lymphocyte                            | 10-44<br>(median, 30)     | 21-67<br>(median, 47)     | 27-63<br>(median, 30.3)  | 10-14<br>(median, 11)     | 19-62<br>(median, 46)     | 6-14<br>(median, 8)       | 13-36<br>(median, 19)    |
| <i>P</i> value                          |                           | .07                       | .56                      |                           | .03                       | .14                       | .07                      |
| C-reactive protein (mg/dL)              | 6.4-14.5<br>(median, 8.4) | 0.8-12.1<br>(median, 3.3) | 0.3-4.1<br>(median, 1.4) | 7.8-16.1<br>(median, 9.9) | 3.5-14.2<br>(median, 7.0) | 5-19<br>(median, 8.8)     | 1.7-21<br>(median, 12.3) |
| <i>P</i> value                          |                           | .07                       | .08                      |                           | .2                        | .67                       | .47                      |

\**P* values between KD-post vs KD-pre, or control vs KD-pre in the first-array patients, and responsive-pre vs nonresponsive-pre, or responsive-pre vs responsive-post in the second-array patients.

## METHODS

### Patients

The patients with KD ( $n = 7$ ) were treated at Chiba University Hospital or Chiba Kaihin Municipal Hospital between May 2005 and June 2007. All the patients fulfilled the Criteria for Diagnostic Guidelines for Kawasaki Disease (5th revision) published by the Kawasaki Disease Research Committee in Japan and were initially treated with IVIG (2.0 g/kg for 1-2 days) and oral aspirin (30 mg/kg daily).<sup>11</sup> Informed consent was obtained from the parents according to the guidelines of each medical center. Clinical data including age, sex, duration of illness, laboratory findings, response to IVIG, and coronary artery involvement were documented and are summarized in Table I and this article's Table E1 in the Online Repository at [www.jacionline.org](http://www.jacionline.org). Abnormal cardiac function was monitored by using 2-dimensional echocardiography, and the presence of CALs was diagnosed according to the Japanese Ministry of Health criteria. Two patients developed coronary aneurysms at 1 month after the onset of their diseases. Nonresponsiveness to IVIG was defined as a persistent or recrudescence fever (body temperature higher than 37.5°C) at 24 hours after the completion of the IVIG treatment.

Venous blood was drawn from each patient before IVIG treatment (3-7 days after the onset of fever) and within 2 days after the completion of IVIG. In addition, sera obtained from 8 nonresponsive patients with KD treated at Chiba University Hospital before 2005 were used in an ELISA. These patients were initially treated using IVIG (2.0 g/kg for 1-2 days). Four of the 8 patients developed coronary aneurysms at 1 month after disease onset. Control blood samples were obtained from 15 patients who had been febrile (body temperature higher than 38°C) for at least 3 days (Tables I and E1). The clinical diagnoses of these control patients were pneumonia ( $n = 7$ ), acute upper respiratory infection ( $n = 3$ ), toxic shock syndrome-like disease ( $n = 2$ ), infectious mononucleosis ( $n = 1$ ), cervical lymphadenitis ( $n = 1$ ), and meningitis ( $n = 1$ ).

### Extraction of RNA and reduction of globin mRNA content

Venous blood samples (2.5 mL) were collected in PAXgene Blood RNA Tubes (BD Biosciences, San Jose, Calif), and RNA was purified by using the PAXgene kit (Qiagen, Valencia, Calif) according to the manufacturer's instructions. The quality and the quantity of RNA were examined using the

RNA 6000 Nano LabChip kit (Agilent Technologies, Santa Clara, Calif). The total RNA samples were further processed to reduce the  $\alpha$ -globin and  $\beta$ -globin mRNA content according to the Globin Reduction Protocol (Affymetrix, Santa Clara, Calif).<sup>12</sup> Briefly, total RNA was hybridized with Globin Reduction Oligo Mix (Affymetrix) and digested by using RNase H at 37°C for 10 minutes. The digested total RNA was then purified by using an RNeasy Micro kit (Qiagen) and quantified.

### RNA amplification and GeneChip expression analysis

Gene expression profiles were examined by using the Human Genome U133 Plus 2.0 array (GeneChip; Affymetrix) according to the manufacturer's protocols. Briefly, double-stranded cDNA were synthesized from 2  $\mu$ g total RNA by using the One-cycle cDNA Synthesis kit (Affymetrix). The cDNA was subjected to *in vitro* transcription by using the IVT Labeling kit (Affymetrix) and hybridized with a U133 Plus 2.0 array. The fluorescence intensity of each transcript was quantified by using a Affymetrix GeneChip Scanner 3000 (Affymetrix), and the expression value was determined by using the GeneChip Operating Software (Affymetrix). The results of the microarray analysis can be found on our web site at <http://www.nch.go.jp/imal/GeneChip/KAWASAKI2.htm>.

The data were further analyzed with GeneSpring GX version 7.3 (Agilent Technologies). Each array was normalized (mean centered) to the median intensity array. To minimize the influence of background noise, only probes reliably detected in at least 2 out of all the samples were included in the analysis. A 1-way ANOVA analysis and unsupervised hierarchical clustering using Pearson correlation were performed with GeneSpring GX version 7.3 software.

### Quantitative real-time PCR

The PCR primers were designed based on sequences from GenBank. The primer sequences were as follows: *haptoglobin* forward primer, 5'-TCGGCATGTCTAAGTACCAGGAA-3'; *haptoglobin* reverse primer, 5'-AGGTCGTGAACGGCAAAGG-3'; *prv-1* forward primer, 5'-GGCCCAACC TTCCAGCTT-3'; *prv-1* reverse primer, 5'-CTTCTCACGCGCAGAGAAG A-3'; *growth arrest-specific 7 (gas7)* forward primer, 5'-GCAGCTGCGGC ATGAAA-3'; *gas7* reverse primer, 5'-TGGCCGGGTCCACTTTT-3';  $\alpha$ -M

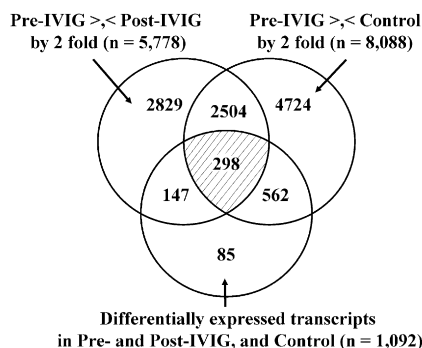


**FIG 1.** Variably expressed transcripts in the white blood cells obtained from the first cohort of patients with KD and febrile controls (Table I). Expression values of 1092 transcripts were significantly different among the 3 groups (1-way ANOVA,  $P < .05$ ). Most variably expressed ( $>2.0\times$  or  $<0.5\times$ ) transcripts ( $n = 298$ ) in pre-IVIG patients compared with post-IVIG patients and controls were selected.

*integrin (itgam)* forward primer, 5'-CAGACTTGTGAGACCCTGAACTA CA-3'; and *itgam* reverse primer, 5'-CGAAAGCAGACAATGGCGTT-3'. PCR was performed by using the ABI 7700 sequence detector system (PE Applied Biosystem, Foster City, Calif) in a 25- $\mu$ L reaction mixture containing iTaq SYBR Green Supermix with ROX (Bio-Rad Laboratories, Hercules, Calif). Samples were subjected to 40 cycles of amplification at 95°C for 15 seconds for denaturing, and at 60°C for 1 minute for annealing-extension. The expression of each target cDNA relative to glyceraldehyde-3-phosphate dehydrogenase was calculated for each sample by using a comparative cycle-threshold method described by the manufacturer (PE Applied Biosystems).

## Flow cytometry

Cell surface molecules were measured by using mAbs in conjunction with 2-color immunofluorescence staining following a standard protocol. The mAbs used were anti-CD177 (PRV-1) in combination with fluorescein isothiocyanate-conjugated antimouse IgG<sub>1</sub> and phycoerythrin-conjugated anti-CD16b (BD Biosciences), or phycoerythrin-conjugated anticomplement component 3b/4b receptor 1 (CR1) in combination with fluorescein isothiocyanate-conjugated anti-CD16b (Beckman Coulter, Fullerton, Calif), respectively.

## Measurement of serum G-CSF levels

An ELISA was performed to quantify the G-CSF concentrations in the sera. mAbs against human G-CSF, BVD13-3A5 (Beckman Coulter) as a capture antibody, and BVD11-37G10 (Beckman Coulter) as a detection antibody were used. The protein concentration was calculated using Microplate Manager III software (Bio-Rad Laboratories).

## Statistical analysis

For the GeneChip microarray data, a nonparametric Mann-Whitney  $U$  test was performed by using the GeneSpring GX software version 7.3. For the real-time PCR, the flow cytometry, and the ELISA data, a 1-factor ANOVA and the Bonferroni/Dunn  $F$  test as a post hoc test were used to compare responsive and nonresponsive patients with KD and febrile control patients. A value of  $P < .05$  was considered statistically significant.

## RESULTS

### Gene expression profiles of responsive patients with KD before and after IVIG

We first examined the gene expression profiles of patients with KD ( $n = 4$ ) before and after IVIG therapy and age-matched febrile controls ( $n = 4$ ). The demographic and laboratory data of the patients are summarized in Table I. Expression values of 1092

transcripts were significantly different among the 3 groups by a 1-factor ANOVA. To focus on the most variably expressed genes in the pre-IVIG patients, we selected 298 out of 1092 transcripts whose expression values in pre-IVIG patients were more than double or less than half of those in post-IVIG patients and in febrile controls (Fig 1; see this article's Table E2 in the Online Repository at [www.jacionline.org](http://www.jacionline.org)). Among the 298 transcripts, 193 genes (225 transcripts) or 64 genes (67 transcripts) were more or less abundant in the pre-IVIG compared with the post-IVIG patients and control patients, respectively. Six genes (6 transcripts) were more abundant in control patients than in pre-IVIG and post-IVIG patients. The top 20 transcripts whose expression values were higher in the pre-IVIG patients compared with those in the post-IVIG patients are listed in Table II.

In an article examining the transcriptional program of terminal granulocytic differentiation, Theilgaard-Monch et al<sup>13</sup> reported that 6700 genes were differentially expressed among the highly purified bone marrow granulocyte precursors—that is, promyelocytes, myelocytes, and bands. According to their gene list, 154 of the 298 genes (52%) that showed variable expression values in our study were expressed during the granulocytic differentiation (Table E2). Interestingly, among the 225 more abundant transcripts in the pre-IVIG patients, 107 (48%) were specifically expressed in myelocytes or bands, whereas only 4 transcripts (2%) were specific to promyelocytes. On the other hand, among the 67 less abundant transcripts in the pre-IVIG patients, only 4 transcripts (6%) were specifically expressed in myelocytes or bands, whereas 37 (55%) were specific to promyelocytes.

### Gene expression profiles of nonresponsive patients with KD

We next questioned how these 298 transcripts were expressed in patients who were nonresponsive to IVIG. Because nonresponsiveness to IVIG is frequently associated with high percentages of neutrophils in patients with KD, we selected patients who had similar percentages of neutrophils before IVIG for a second microarray analysis. They included 6 responsive and 4 nonresponsive patients with KD, and blood samples were collected before and after IVIG (Table I). By an unsupervised hierarchical clustering of these 20 samples, the expression profiles of the 298 transcripts that were variably expressed in the first cohort of patients were significantly different between pre-IVIG and post-IVIG patients (data not shown). However, we were unable to discriminate between the responsive and the nonresponsive pre-IVIG patients in this clustering analysis. Thus, we directly compared the expression values of these 298 transcripts between responsive and nonresponsive patients before IVIG and found that 15 genes were differentially expressed between the 2 groups (Table III).

### Elevated PRV-1 expression in nonresponsive patients with KD

The microarray analysis suggested that 15 genes were differentially expressed between responsive and nonresponsive patients with KD even before the initiation of IVIG. Among them, the biological functions of haptoglobin, CD177, and ITGAM genes are well characterized and are likely to be associated with the pathophysiological conditions of patients with KD. On the other hand, the transcripts of *gas7* gene had been enriched in PBMCs and purified monocytes obtained from pre-IVIG patients in our previous studies.<sup>10</sup> Thus, we selected these 4 genes and measured

**TABLE II.** The top 20 most variably changed transcripts in pre-IVIG patients

| Probe ID     | Gene symbol | Gene name                                    | Fold change pre-IVIG/post-IVIG | Fold change pre-IVIG/control |
|--------------|-------------|----------------------------------------------|--------------------------------|------------------------------|
| 219669_at    | CD177       | Polycythemia rubra vera 1                    | 18.6                           | 6.5                          |
| 1557924_s_at | ALPL        | Alkaline phosphatase, liver/bone/kidney      | 9.1                            | 6.3                          |
| 217552_x_at  | CR1         | Complement component (3b/4b) receptor 1      | 7.5                            | 11.9                         |
| 237618_at    | —           | Transcribed sequences                        | 6.8                            | 3.3                          |
| 206548_at    | FLJ23556    | Hypothetical protein FLJ23556                | 6.5                            | 3.0                          |
| 244889_at    | —           | LOC388210 mRNA                               | 6.2                            | 8.0                          |
| 231886_at    | —           | cDNA DKFZp434B2016                           | 6.1                            | 3.4                          |
| 232465_at    | —           | cDNA FLJ11687                                | 5.9                            | 3.0                          |
| 227250_at    | KREMEN1     | Kringle containing transmembrane protein 1   | 5.9                            | 5.8                          |
| 215783_s_at  | ALPL        | Alkaline phosphatase, liver/bone/kidney      | 5.5                            | 5.2                          |
| 237568_at    | —           | Transcribed sequences                        | 5.3                            | 2.6                          |
| 228648_at    | LRG1        | Leucine-rich $\alpha$ -2-glycoprotein 1      | 5.0                            | 2.9                          |
| 218660_at    | DYSF        | Dysferlin, limb girdle muscular dystrophy 2B | 4.8                            | 3.3                          |
| 240156_at    | —           | Transcribed sequences                        | 4.8                            | 4.3                          |
| 216782_at    | —           | cDNA: FLJ23026                               | 4.8                            | 3.2                          |
| 204713_s_at  | F5          | Coagulation factor V                         | 4.6                            | 5.0                          |
| 227055_at    | METTL7B     | Integrin, $\alpha$ 7                         | 4.6                            | 3.5                          |
| 236592_at    | —           | Transcribed sequences                        | 4.5                            | 3.6                          |
| 228758_at    | BCL6        | B-cell/lymphoma 6                            | 4.5                            | 2.9                          |
| 229296_at    | —           | LOC389793 mRNA                               | 4.4                            | 6.5                          |

**TABLE III.** Differentially expressed transcripts between responsive and nonresponsive patients before IVIG

| Probe ID     | Gene symbol | Gene name                                                                 | Fold change nonresponder/responder |
|--------------|-------------|---------------------------------------------------------------------------|------------------------------------|
| 208470_s_at  | HP          | Haptoglobin                                                               | 4.44                               |
| 239701_at    | —           | Transcribed sequence                                                      | 2.21                               |
| 219669_at    | CD177       | Polycythemia rubra vera 1                                                 | 2.08                               |
| 224818_at    | SORT1       | Sortilin 1                                                                | 1.96                               |
| 225499_at    | —           | Clone CDABP0105                                                           | 1.87                               |
| 232500_at    | C20orf74    | Chromosome 20 open reading frame 74                                       | 1.66                               |
| 211974_x_at  | RBPJ        | Recombination signal binding protein for immunoglobulin $\kappa$ J region | 1.66                               |
| 202191_s_at  | GAS7        | Growth arrest-specific 7                                                  | 1.59                               |
| 205786_s_at  | ITGAM       | Integrin, $\alpha$ M (complement component 3 receptor 3 subunit)          | 1.47                               |
| 226080_at    | SSH2        | Slingshot homolog 2                                                       | 1.42                               |
| 229295_at    | LOC150166   | Hypothetical protein LOC150166                                            | 1.26                               |
| 212516_at    | CENTD2      | Centaurin, delta 2                                                        | 0.75                               |
| 216841_s_at  | SOD2        | Superoxide dismutase 2                                                    | 0.70                               |
| 1558972_s_at | C6orf190    | Chromosome 6 open reading frame 190                                       | 0.51                               |
| 226682_at    | LOC283666   | Hypothetical protein LOC283666                                            | 0.42                               |

mRNA levels using a real-time PCR in additional responsive ( $n = 18$ ) and nonresponsive ( $n = 8$ ) patients with KD who had similar percentages of neutrophils before IVIG administration (Table E1). Four responsive and 2 nonresponsive patients examined by the microarray were included in this analysis. The results indicated that mRNA levels of all 4 genes were decreased after IVIG in both responsive and nonresponsive patients. Before IVIG administration, the transcripts of *hp* and *prv-1* genes were more abundant in nonresponsive patients than in responsive patients, confirming the microarray results (Fig 2, A). However, the transcripts of *gas7* and *intam* genes were not significantly different between the 2 groups.

Because *prv-1* gene showed the most variable transcript abundance in the pre-IVIG patients in the first microarray analysis and the levels of PRV-1 mRNA were significantly higher in nonresponsive patients than in responsive patients, we next examined the protein levels of PRV-1 by immunostaining and flow cytometry. CR1 was also measured as a control. We examined 26 responsive patients with KD, 14 nonresponsive patients with KD, and 12 age-matched febrile control patients (Table E1). The mean fluorescence intensity (MFI) of PRV-1 and CR1 on the neutrophils

was significantly elevated in pre-IVIG patients compared with those in febrile controls (mean  $\pm$  SEM,  $104.2 \pm 7.8$  vs  $52.8 \pm 8.7$ ,  $P = .002$ ; and  $328.7 \pm 28.0$  vs  $177.1 \pm 40.9$ ,  $P = .007$ , respectively), and these levels decreased after IVIG ( $104.2 \pm 7.8$  vs  $67.0 \pm 5.9$ ,  $P < .0001$ ; and  $328.7 \pm 28.0$  vs  $247.0 \pm 29.6$ ,  $P = .02$ , respectively; Fig 2, B). Importantly, MFI of PRV-1 on neutrophils was markedly increased in nonresponsive pre-IVIG patients compared with that in responsive pre-IVIG patients ( $138.3 \pm 16.0$  vs  $87.2 \pm 6.7$ ;  $P = .0004$ ). MFI of CR1 was not significantly different between the 2 groups.

### Increased serum G-CSF levels in nonresponsive patients with KD

PRV-1 is known to be highly expressed in neutrophils from patients with polycythemia rubra vera.<sup>14</sup> Increased neutrophil PRV-1 mRNA levels are also associated with increased neutrophil production induced by acute bacterial infection and injection of recombinant G-CSF.<sup>15</sup> We speculated that elevated PRV-1 staining in nonresponsive pre-IVIG patients with KD may be caused

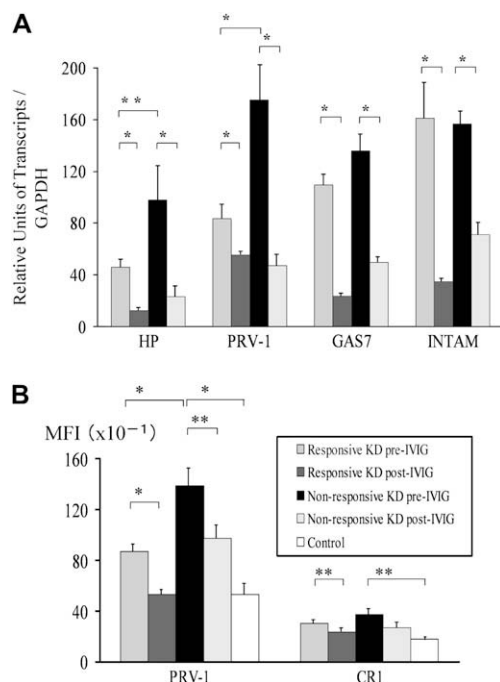


**FIG 2. A**, mRNA levels in responsive and nonresponsive patients before and after IVIG administration. Real-time RT-PCR results are presented as relative units of each transcript compared with GAPDH. Error bars indicate SEM. \* $P = .001$ , \*\* $P = .01$  compared between pre- and post-IVIG patients (Wilcoxon signed-rank test), and between responsive and nonresponsive patients (Mann-Whitney  $U$  test). **B**, Immunostaining of PRV-1 and CR1 on neutrophils. Blood samples were double-immunostained and analyzed by flow cytometry. The results are expressed as MFI, and error bars indicate SEM. \* $P = .001$ , \*\* $P = .01$  compared between pre-IVIG and post-IVIG patients (Wilcoxon signed-rank test), and among responsive and nonresponsive pre-IVIG patients and febrile controls (Bonferroni/Dunn  $F$  test). HP, Haptoglobin.

by an elevation of serum G-CSF levels in these patients. Therefore, we measured the serum levels of G-CSF in patients with KD before and after IVIG. In addition to our patient group, we examined 8 nonresponsive pre-IVIG patients with KD who were treated at Chiba University Hospital between 2004 and 2005, 4 of whom developed CAL 1 month after the onset of their illnesses.

Fig 3 shows that the serum G-CSF levels in nonresponsive pre-IVIG patients with KD ( $n = 24$ ) were markedly elevated compared with those in responsive pre-IVIG patients ( $n = 35$ ;  $592.6 \pm 97.8$  pg/mL vs  $183.0 \pm 40.8$  pg/mL;  $P < .0001$ ). After IVIG, the G-CSF levels were decreased in both responsive and nonresponsive patients, but the levels were still higher in nonresponsive patients than in responsive patients ( $158.2 \pm 43.9$  pg/mL vs  $52.8 \pm 15.0$  pg/mL;  $P < .0001$ ). Importantly, patients who developed CAL (closed symbols) had significantly higher pre-IVIG levels of G-CSF than patients without coronary aneurysms ( $918.4 \pm 264.9$  pg/mL vs  $273.1 \pm 39.5$  pg/mL;  $P < .0001$ ).

## DISCUSSION

An increased white blood cell count is a hallmark of active KD and has been suggested to be a risk factor for the development of CAL by several investigators.<sup>6,9</sup> The production of active oxygen species by neutrophils has been postulated to contribute to endothelial cell damage in KD.<sup>16</sup> Recently, Takahashi et al<sup>17</sup> reported that neutrophils had infiltrated a coronary aneurysm in pathological specimens obtained from a patient who died early during the

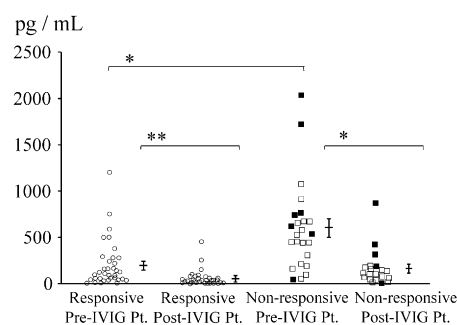


**FIG 3.** Elevated serum G-CSF levels in nonresponsive pre-IVIG patients. Concentration of G-CSF was measured by ELISA in responsive and nonresponsive patients before and after IVIG. Solid and open squares indicate patients with and without CAL, respectively. The bar indicates mean  $\pm$  SEM for each group. \* $P = .001$ , \*\* $P = .01$  compared using the Bonferroni/Dunn  $F$  test as a post hoc test.

acute phase of KD. In this study, in the first cohort of patients, we demonstrated that a group of 298 transcripts were overrepresented in the patients with KD before IVIG compared with the age-matched febrile controls and post-IVIG patients with KD, and 154 of 298 transcripts were known to be expressed in the late granulocyte differentiation.<sup>13</sup>

In the second cohort of KD patients who had similar percentages of neutrophils before IVIG (Table I), the pre-IVIG patients also showed a distinct gene expression profiles of these granulocyte-derived transcripts from the post-IVIG patients after an unsupervised hierarchical clustering (data not shown). However, because this analysis failed to distinguish responsive and nonresponsive patients before the initiation of IVIG, we directly compared the gene expression values before IVIG between responsive and nonresponsive patients and found that 15 transcripts were differentially expressed. Among them, it is reported that haptoglobin and PRV-1 were mainly expressed in myelocytes, whereas sortilin 1, ITGAM, sling-shot homolog 2, centaurin delta 2, and superoxide dismutase 2 were mainly expressed in bands in the bone marrow.<sup>13</sup> Thus, these results are consistent with the clinical observations and suggest that stimulation of granulocyte differentiation and recruitment from the bone marrow may be associated with the unresponsiveness of a patient with KD against IVIG therapy.

In the next step, we examined the microarray results by real-time PCR and flow cytometry in our third cohort of patients with KD. Among the 4 transcripts examined, the mRNA levels of haptoglobin and PRV-1 but not of GAS7 or INTAM were significantly higher in nonresponsive patients than those in responsive patients. The reason the transcripts of GAS7 and INTAM showed inconsistent results between microarray and real-time PCR analysis remains to be investigated. In our previous microarray studies, the *gas7* and *intam* genes but not the *hp* and *prv-1* genes were constitutively expressed by the resting neutrophils as well as the monocytes and the lymphocytes<sup>18</sup> (supplemental data in <http://www.nch.go.jp/imal/GeneChip/public.htm>). Therefore, the expression by these resting cells might affect the expression profiles of the *gas7* and *intam* genes differently from the *hp* and *prv-1* genes during the acute phase of KD. The *hp* and *prv-1* genes are both expressed mainly in myelocytes at steady state, but the transcription of these genes is extremely upregulated during an emergency situation such as acute bacterial infection.<sup>15,19,20</sup> Cytokines such as IL-6 and G-CSF stimulate haptoglobin synthesis during granulocyte differentiation through the induction of CCAAT/enhancer-binding protein- $\beta$  in

granulocyte progenitors.<sup>19,20</sup> On the other hand, PRV-1, a surrogate marker of a patient with polycythemia rubra vera, is also induced in severe sepsis and in healthy volunteers injected with recombinant G-CSF.<sup>15</sup> Accordingly, in our patients with KD, the serum levels of G-CSF were positively correlated with the intensity of PRV-1 staining (data not shown) and higher in nonresponsive patients than in responsive patients both before and after IVIG. These findings suggest that G-CSF may be involved in the proliferation and the recruitment from the bone marrow of the granulocyte precursors in the acute phase of KD. More importantly, among the nonresponsive patients, the serum G-CSF levels were higher in the CAL(+) patients than in CAL(−) patients. Elevated G-CSF levels in patients with KD and their association with CAL have been reported previously by other investigators.<sup>21–23</sup> Because vascular endothelial and smooth muscle cells are known to produce G-CSF under inflammatory conditions,<sup>24–26</sup> it is of interest to examine whether IVIG works directly on vascular endothelial and smooth muscle cells to reduce the production of G-CSF or indirectly on tissue macrophages and hepatocytes to reduce the production of proinflammatory cytokines, such as IL-1 $\beta$ , which stimulates G-CSF production by vascular endothelial cells.

The prevention of CAL is one of the most important end points in the treatment of patients with KD. Because CAL often accompanies IVIG nonresponsiveness, many studies have attempted to identify risk factors associated with IVIG nonresponsiveness by using patients' demographic and laboratory test data.<sup>5–9</sup> Our finding that the PRV-1 protein levels differed significantly between the responsive and nonresponsive patients with KD before the initiation of IVIG may be interesting in this point. Because the measurement of PRV-1 using flow cytometry is simple and fast, this method may be of clinical relevance during the initial IVIG administration. It would be interesting to determine whether the measurement of PRV-1 levels by flow cytometry is useful in predicting a patient's response against initial IVIG administration so as to prepare more carefully for the possible nonresponsiveness in a high-risk patient.

In conclusion, using a DNA microarray technology, we found that a group of transcripts related to the late differentiating granulocytes was overrepresented in patients with acute KD and decreased after IVIG in responsive patients, but not in nonresponsive patients. The protein levels of PRV-1 on neutrophils and G-CSF concentrations were significantly higher in nonresponsive patients than in responsive patients. These findings suggest that the G-CSF stimulation of granulocytes may be an important risk factor in the pathogenesis of KD.

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**Clinical implications:** These findings suggest that the elevated serum levels of G-CSF and the PRV-1 expression on circulating granulocytes are useful biomarkers for nonresponsiveness to IVIG in patients with KD.

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**TABLE E1.** Demographic data of patients (2)

|                                                | Responsive patients<br>with KD |                      |                      | Nonresponsive patients<br>with KD |                      |                      | Febrile controls     |
|------------------------------------------------|--------------------------------|----------------------|----------------------|-----------------------------------|----------------------|----------------------|----------------------|
|                                                | q-PCR                          | FACS                 | ELISA                | q-PCR                             | FACS                 | ELISA                | FACS                 |
| Donors (n)                                     | 18                             | 26                   | 35                   | 8                                 | 14                   | 24                   | 12                   |
| Samples (n)                                    | 36                             | 52                   | 68                   | 16                                | 28                   | 44                   | 12                   |
| Age<br>(mo after birth)                        | 8-66<br>(median, 39)           | 2-44<br>(median, 14) | 3-82<br>(median, 26) | 5-32<br>(median, 16)              | 3-38<br>(median, 13) | 3-95<br>(median, 21) | 1-39<br>(median, 12) |
| Sex<br>(male, female)                          | 9, 9                           | 13, 13               | 17, 18               | 5, 3                              | 8, 6                 | 13, 11               | 6, 6                 |
| Cardiac<br>involvement                         | 0                              | 0                    | 0                    | 0                                 | 2                    | 7                    |                      |
| Blood drawn<br>pre-IVIG<br>(days after onset)  | 4-6<br>(median, 5)             | 3 -6<br>(median, 4)  | 3-8<br>(median, 5)   | 3 -6<br>(median, 5)               | 3-6<br>(median, 4)   | 3 -6<br>(median, 4)  | 3-7<br>(median, 4)   |
| Blood drawn<br>post-IVIG<br>(days after onset) | 6-10<br>(median, 7)            | 6-10<br>(median, 8)  | 6-12<br>(median, 7)  | 6 -9<br>(median, 7)               | 6-10<br>(median, 7)  | 6-10<br>(median, 7)  |                      |
| % Neutrophil<br>(pre-IVIG)                     | 52-93<br>(median, 74)          |                      |                      | 59-92<br>(median, 76)             |                      |                      |                      |
| <i>P</i> value*                                |                                |                      |                      | .81*                              |                      |                      |                      |
| % Monocyte<br>(pre-IVIG)                       | 2- 10<br>(median, 6)           |                      |                      | 1-8<br>(median, 4)                |                      |                      |                      |
| <i>P</i> value                                 |                                |                      |                      | .12*                              |                      |                      |                      |
| % Lymphocyte<br>(pre-IVIG)                     | 1-42<br>(median, 18)           |                      |                      | 5-31<br>(median, 19)              |                      |                      |                      |
| <i>P</i> value                                 |                                |                      |                      | .98*                              |                      |                      |                      |
| C-reactive protein<br>(mg/dL)<br>(pre-IVIG)    | 4.4-18.0<br>(median, 9.0)      |                      |                      | 4.6 -10.9<br>(median, 8.1)        |                      |                      |                      |
| <i>P</i> value                                 |                                |                      |                      | .74*                              |                      |                      |                      |

FACS, Fluorescence-activated cell sorting; *q-PCR*, quantitative real-time PCR.\**P* values between responsive patients with KD and nonresponsive patients with KD.

**TABLE E2.** The 298 most variably expressed transcripts in pre-IVIG patients

| Probe ID     | Gene symbol | Gene name                                                            | Fold change (pre-IVIG/<br>post-IVIG) | Fold change (pre-IVIG/<br>febrile control) | Expression in granulocytes |           |      |
|--------------|-------------|----------------------------------------------------------------------|--------------------------------------|--------------------------------------------|----------------------------|-----------|------|
|              |             |                                                                      |                                      |                                            | Promyelocyte               | Myelocyte | Band |
| 219669_at    | CD177       | Polycythemia rubra vera 1                                            | 18.6                                 | 6.5                                        |                            | +         |      |
| 1557924_s_at | ALPL        | Alkaline phosphatase, liver                                          | 9.1                                  | 6.3                                        |                            |           | +    |
| 217552_x_at  | CR1         | Complement component (3b/<br>4b) receptor 1                          | 7.5                                  | 11.9                                       |                            |           | +    |
| 237618_at    | —           | Transcribed sequences                                                | 6.8                                  | 3.3                                        |                            |           |      |
| 206548_at    | FLJ23556    | Hypothetical protein FLJ23556                                        | 6.5                                  | 3.0                                        |                            |           |      |
| 244889_at    | —           | LOC388210 mRNA                                                       | 6.2                                  | 8.0                                        |                            |           |      |
| 231886_at    | —           | cDNA DKFZp434B2016                                                   | 6.1                                  | 3.4                                        |                            |           |      |
| 232465_at    | —           | cDNA FLJ11687                                                        | 5.9                                  | 3.0                                        |                            |           |      |
| 227250_at    | KREMEN1     | Kringle containing<br>transmembrane protein 1                        | 5.9                                  | 5.8                                        |                            |           | +    |
| 215783_s_at  | ALPL        | Alkaline phosphatase, liver                                          | 5.5                                  | 5.2                                        |                            |           | +    |
| 237568_at    | —           | Transcribed sequences                                                | 5.3                                  | 2.6                                        |                            |           |      |
| 228648_at    | LRG1        | Leucine-rich $\alpha$ 2-glycoprotein 1                               | 5.0                                  | 2.9                                        |                            |           |      |
| 218660_at    | DYSF        | Dysferlin, limb girdle muscular<br>dystrophy 2B                      | 4.8                                  | 3.3                                        |                            | +         | +    |
| 240156_at    | —           | Transcribed sequences                                                | 4.8                                  | 4.3                                        |                            |           |      |
| 216782_at    | —           | cDNA: FLJ23026                                                       | 4.8                                  | 3.2                                        |                            |           |      |
| 204713_s_at  | F5          | Coagulation factor V                                                 | 4.6                                  | 5.0                                        |                            | +         | +    |
| 227055_at    | METTL7B     | Integrin, $\alpha$ 7                                                 | 4.6                                  | 3.5                                        |                            |           |      |
| 236592_at    | —           | Transcribed sequences                                                | 4.5                                  | 3.6                                        |                            |           |      |
| 228758_at    | BCL6        | B-cell/lymphoma 6                                                    | 4.5                                  | 2.9                                        |                            | +         | +    |
| 229296_at    | —           | LOC389793 mRNA                                                       | 4.4                                  | 6.5                                        |                            |           |      |
| 41386_i_at   | JMJD3       | Jumonji domain containing 3                                          | 4.4                                  | 3.0                                        |                            |           |      |
| 227062_at    | TncRNA      | cDNA DKFZp686L01105                                                  | 4.4                                  | 4.7                                        |                            |           |      |
| 1568830_at   | IRAK3       | IL-1 receptor-associated<br>kinase 3                                 | 4.4                                  | 3.6                                        |                            |           |      |
| 205931_s_at  | CREB5       | cAMP responsive element<br>binding protein 5                         | 4.3                                  | 2.4                                        |                            |           | +    |
| 1566959_at   | —           | cDNA DKFZp564H023                                                    | 4.3                                  | 2.6                                        |                            |           |      |
| 208470_s_at  | HP          | Haptoglobin-related protein                                          | 4.2                                  | 6.0                                        |                            | +         |      |
| 1558011_at   | —           | LOC389297                                                            | 4.1                                  | 2.4                                        |                            |           |      |
| 244218_at    | —           | Transcribed sequences                                                | 4.1                                  | 2.6                                        |                            |           |      |
| 236439_at    | —           | A40138 glycogen<br>phosphorylase                                     | 4.1                                  | 2.6                                        |                            |           |      |
| 205312_at    | SPI1        | Spleen focus forming virus<br>proviral integration oncogene<br>sp1   | 4.0                                  | 2.5                                        |                            | +         | +    |
| 1553723_at   | GPR97       | G protein-coupled receptor 97                                        | 4.0                                  | 4.1                                        |                            | +         | +    |
| 208540_x_at  | —           | LOC729659                                                            | 4.0                                  | 6.7                                        |                            |           |      |
| 218978_s_at  | SLC25A37    | Solute carrier family 25,<br>member 37                               | 4.0                                  | 2.4                                        |                            |           | +    |
| 236495_at    | —           | Neuronal thread protein                                              | 4.0                                  | 2.7                                        |                            |           |      |
| 1570194_x_at | —           | Clone IMAGE:4480721                                                  | 3.9                                  | 2.9                                        |                            |           |      |
| 207890_s_at  | MMP25       | Matrix metalloproteinase 25                                          | 3.8                                  | 3.0                                        |                            | +         | +    |
| 215990_s_at  | BCL6        | B-cell CLL/lymphoma 6                                                | 3.8                                  | 2.1                                        |                            | +         | +    |
| 202191_s_at  | GAS7        | Growth arrest-specific 7                                             | 3.8                                  | 3.6                                        |                            |           |      |
| 217966_s_at  | FAM129A     | Chromosome 1 open reading<br>frame 24                                | 3.8                                  | 2.9                                        |                            |           | +    |
| 239102_s_at  | —           | Transcribed sequences                                                | 3.8                                  | 3.2                                        |                            |           |      |
| 1565598_at   | —           | Clone IMAGE:4275461                                                  | 3.8                                  | 2.5                                        |                            |           |      |
| 211133_x_at  | LILRB3      | Leukocyte immunoglobulin-<br>like receptor, subfamily B,<br>member 3 | 3.7                                  | 2.2                                        |                            |           | +    |
| 202205_at    | VASP        | Vasodilator-stimulated<br>phosphoprotein                             | 3.7                                  | 2.7                                        |                            | +         | +    |
| 220404_at    | GPR97       | G protein-coupled receptor 97                                        | 3.7                                  | 2.4                                        |                            | +         | +    |
| 226872_at    | RFX2        | Regulatory factor X, 2                                               | 3.7                                  | 3.7                                        |                            |           |      |
| 218880_at    | FOSL2       | FOS-like antigen 2                                                   | 3.7                                  | 2.3                                        |                            | +         | +    |
| 206697_s_at  | HP          | Haptoglobin                                                          | 3.6                                  | 6.3                                        |                            | +         |      |
| 226364_at    | HIP1        | Huntingtin interacting protein 1                                     | 3.6                                  | 4.0                                        |                            |           |      |
| 204750_s_at  | DSC2        | Desmocollin 2                                                        | 3.6                                  | 3.5                                        |                            | +         | +    |

(Continued)

TABLE E2. (Continued)

| Probe ID     | Gene symbol | Gene name                                                                                    | Fold change (pre-IVIG/<br>post-IVIG) | Fold change (pre-IVIG/<br>febrile control) | Expression in granulocytes |           |      |
|--------------|-------------|----------------------------------------------------------------------------------------------|--------------------------------------|--------------------------------------------|----------------------------|-----------|------|
|              |             |                                                                                              |                                      |                                            | Promyelocyte               | Myelocyte | Band |
| 233816_at    | —           | ZA32P mRNA                                                                                   | 3.6                                  | 2.6                                        |                            |           |      |
| 207704_s_at  | GAS7        | Growth arrest-specific 7                                                                     | 3.5                                  | 2.5                                        |                            |           |      |
| 239555_at    | —           | Transcribed sequences                                                                        | 3.4                                  | 2.7                                        |                            |           |      |
| 220023_at    | APOB48R     | Apolipoprotein B48 receptor                                                                  | 3.4                                  | 2.3                                        |                            | +         | +    |
| 203591_s_at  | CSF3R       | Colony stimulating factor 3<br>receptor (granulocyte)                                        | 3.4                                  | 2.4                                        |                            |           | +    |
| 201392_s_at  | IGF2R       | Insulin-like growth factor 2<br>receptor                                                     | 3.4                                  | 2.8                                        |                            |           | +    |
| 210706_s_at  | RNF24       | Ring finger protein 24                                                                       | 3.4                                  | 2.1                                        |                            |           | +    |
| 237310_at    | —           | Transcribed sequences                                                                        | 3.4                                  | 2.6                                        |                            |           |      |
| 1559391_s_at | —           | Partial mRNA; ID EE2-8E                                                                      | 3.4                                  | 2.7                                        |                            |           |      |
| 1556067_a_at | JMJD3       | Jumonji domain containing 3                                                                  | 3.4                                  | 2.4                                        |                            |           |      |
| 204669_s_at  | RNF24       | Ring finger protein 24                                                                       | 3.3                                  | 2.3                                        |                            |           | +    |
| 200601_at    | ACTN4       | α-Actinin                                                                                    | 3.3                                  | 2.5                                        |                            |           |      |
| 58780_s_at   | FLJ10357    | Hypothetical protein FLJ10357                                                                | 3.3                                  | 3.0                                        |                            |           | +    |
| 211067_s_at  | GAS7        | Growth arrest-specific 7                                                                     | 3.3                                  | 2.3                                        |                            |           |      |
| 239274_at    | —           | cDNA clone HTCCHG09                                                                          | 3.3                                  | 3.0                                        |                            |           |      |
| 243748_at    | —           | Transcribed sequences                                                                        | 3.3                                  | 2.0                                        |                            |           |      |
| 229373_at    | —           | Transcribed sequences                                                                        | 3.3                                  | 2.9                                        |                            |           |      |
| 205425_at    | HIP1        | Huntingtin interacting protein 1                                                             | 3.3                                  | 3.7                                        |                            |           |      |
| 1556066_at   | JMJD3       | Jumonji domain containing 3                                                                  | 3.3                                  | 2.7                                        |                            |           |      |
| 213146_at    | JMJD3       | Jumonji domain containing 3                                                                  | 3.3                                  | 2.2                                        |                            |           |      |
| 222757_s_at  | ZAK         | Sterile α motif and leucine<br>zipper containing kinase<br>AZK                               | 3.3                                  | 2.3                                        | +                          |           |      |
| 225239_at    | —           | CDNA FLJ26120                                                                                | 3.3                                  | 3.8                                        |                            |           |      |
| 211661_x_at  | PTAFR       | Platelet activating factor<br>receptor                                                       | 3.2                                  | 3.2                                        |                            |           | +    |
| 210423_s_at  | SLC11A1     | Solute carrier family 11<br>(proton-coupled divalent<br>metal ion transporters),<br>member 1 | 3.2                                  | 2.1                                        |                            |           | +    |
| 1561167_at   | —           | cDNA clone YA75A09                                                                           | 3.2                                  | 2.5                                        |                            |           |      |
| 230590_at    | —           | Transcribed sequences                                                                        | 3.2                                  | 3.6                                        |                            |           |      |
| 241041_at    | —           | Transcribed sequences                                                                        | 3.2                                  | 3.3                                        |                            |           |      |
| 239701_at    | —           | Retinoblastoma binding<br>protein 7                                                          | 3.2                                  | 2.2                                        | +                          |           |      |
| 211135_x_at  | LILRB3      | Leukocyte immunoglobulin-<br>like receptor, subfamily B,<br>member 3                         | 3.2                                  | 2.1                                        |                            |           | +    |
| 232174_at    | —           | cDNA DKFZp686H2244                                                                           | 3.1                                  | 2.6                                        |                            |           |      |
| 212762_s_at  | TCF7L2      | T-cell specific transcription<br>factor 7-like 2 (T-cell<br>specific, HMG-box)               | 3.1                                  | 2.2                                        |                            |           | +    |
| 232726_at    | —           | CDNA: FLJ21303                                                                               | 3.1                                  | 4.4                                        |                            |           |      |
| 225372_at    | C10orf54    | PP2135 protein                                                                               | 3.1                                  | 2.6                                        |                            |           | +    |
| 1557688_at   | —           | cDNA DKFZp666E118                                                                            | 3.1                                  | 2.1                                        |                            |           |      |
| 216841_s_at  | SOD2        | Superoxide dismutase 2,<br>mitochondrial                                                     | 3.1                                  | 2.5                                        |                            |           | +    |
| 244548_at    | —           | cDNA clone YP77A07                                                                           | 3.1                                  | 2.3                                        |                            |           |      |
| 242106_at    | —           | Transcribed sequences                                                                        | 3.1                                  | 2.8                                        |                            |           |      |
| 232522_at    | —           | cDNA FLJ26983                                                                                | 3.1                                  | 2.5                                        |                            |           |      |
| 203509_at    | SORL1       | Sortilin 1                                                                                   | 3.1                                  | 2.9                                        |                            | +         | +    |
| 229452_at    | TMEM88      | cDNA 2600017H02                                                                              | 3.0                                  | 3.8                                        |                            |           |      |
| 1562289_at   | —           | cDNA DKFZp434N0220                                                                           | 3.0                                  | 2.1                                        |                            |           |      |
| 1553297_a_at | CSF3R       | Colony stimulating factor 3<br>receptor (granulocyte)                                        | 3.0                                  | 2.5                                        |                            |           | +    |
| 206472_s_at  | TLE3        | Transducin-like enhancer of<br>split 3 (E(sp1) homolog,<br><i>Drosophila</i> )               | 3.0                                  | 2.1                                        |                            |           | +    |
| 225234_at    | CBL         | Cas-Br-M (murine) ecotropic<br>retroviral transforming<br>sequence                           | 3.0                                  | 2.5                                        |                            | +         | +    |

(Continued)

TABLE E2. (Continued)

| Probe ID     | Gene symbol | Gene name                                          | Fold change (pre-IVIG/<br>post-IVIG) | Fold change (pre-IVIG/<br>febrile control) | Expression in granulocytes |           |      |
|--------------|-------------|----------------------------------------------------|--------------------------------------|--------------------------------------------|----------------------------|-----------|------|
|              |             |                                                    |                                      |                                            | Promyelocyte               | Myelocyte | Band |
| 239600_at    | —           | cDNA FLJ26461                                      | 3.0                                  | 2.2                                        |                            |           |      |
| 208488_s_at  | CR1         | Complement component (3b/<br>4b) receptor 1        | 3.0                                  | 3.6                                        |                            |           | +    |
| 206244_at    | CR1         | Complement component (3b/<br>4b) receptor 1        | 3.0                                  | 3.3                                        |                            |           | +    |
| 225937_at    | —           | Clone IMAGE:4815142                                | 3.0                                  | 2.6                                        |                            |           |      |
| 221753_at    | SSH1        | Slingshot 1                                        | 3.0                                  | 3.0                                        |                            |           | +    |
| 217024_x_at  | SIRPA       | SHP substrate-1                                    | 3.0                                  | 3.5                                        |                            |           |      |
| 200808_s_at  | ZYX         | Zyxin                                              | 3.0                                  | 3.5                                        |                            | +         | +    |
| 210569_s_at  | SIGLEC9     | Sialic acid binding Ig-like<br>lectin 9            | 3.0                                  | 2.5                                        |                            |           | +    |
| 200919_at    | PHC2        | Polyhomeotic-like 2                                | 3.0                                  | 2.1                                        |                            |           | +    |
| 209179_s_at  | LENG4       | Leukocyte receptor cluster<br>member 4             | 3.0                                  | 2.6                                        | +                          |           |      |
| 217967_s_at  | FAM129A     | Chromosome 1 open reading<br>frame 24              | 2.9                                  | 2.4                                        |                            |           | +    |
| 229295_at    | LOC150166   | cDNA FLJ32274                                      | 2.9                                  | 2.2                                        |                            |           |      |
| 205418_at    | FES         | Feline sarcoma oncogene                            | 2.9                                  | 2.4                                        |                            |           |      |
| 209791_at    | PADI2       | Peptidyl arginine deiminase,<br>type II            | 2.9                                  | 2.1                                        |                            | +         | +    |
| 240638_at    | —           | Transcribed sequences                              | 2.9                                  | 2.1                                        |                            |           |      |
| 215706_x_at  | ZYX         | Zyxin                                              | 2.9                                  | 3.2                                        |                            | +         | +    |
| 221541_at    | CRISPLD2    | DKFZp434B044                                       | 2.9                                  | 2.3                                        |                            |           | +    |
| 1552667_a_at | SH2D3C      | Src homology region-domain<br>containing 3C        | 2.9                                  | 2.1                                        |                            |           | +    |
| 239296_at    | —           | Transcribed sequences                              | 2.8                                  | 2.1                                        |                            |           |      |
| 223553_s_at  | DOK3        | Dok-like protein                                   | 2.8                                  | 3.0                                        |                            |           | +    |
| 211160_x_at  | ACTN1       | Actinin, $\alpha$ 1                                | 2.8                                  | 2.9                                        |                            |           |      |
| 220326_s_at  | FLJ10357    | Hypothetical protein FLJ10357                      | 2.8                                  | 2.4                                        |                            |           | +    |
| 238135_at    | —           | Angiotensin II receptor-<br>associated protein     | 2.8                                  | 3.1                                        |                            |           | +    |
| 232744_x_at  | —           | cDNA clone IMAGE:4617157                           | 2.8                                  | 2.4                                        |                            |           |      |
| 244579_at    | —           | Transcribed sequences                              | 2.8                                  | 2.4                                        |                            |           |      |
| 202888_s_at  | ANPEP       | Alanyl (membrane)<br>aminopeptidase (CD13)         | 2.8                                  | 5.3                                        |                            |           | +    |
| 1570151_at   | —           | Clone IMAGE:4340670                                | 2.8                                  | 2.3                                        |                            |           |      |
| 224846_at    | SHKBP1      | Hypothetical protein<br>BC007653                   | 2.7                                  | 3.3                                        |                            |           | +    |
| 243182_at    | —           | Transcribed sequences                              | 2.7                                  | 3.1                                        |                            |           |      |
| 213446_s_at  | IQGAP1      | IQ motif containing GTPase<br>activating protein 1 | 2.7                                  | 2.4                                        |                            | +         | +    |
| 225954_s_at  | MIDN        | Midnolin                                           | 2.7                                  | 2.6                                        |                            | +         | +    |
| 241722_x_at  | —           | Thromboxane A2 receptor<br>isoform $\beta$         | 2.7                                  | 2.0                                        |                            |           |      |
| 203628_at    | IGF1R       | Insulin-like growth factor<br>1 receptor           | 2.7                                  | 3.1                                        |                            | +         | +    |
| 1554899_s_at | FCER1G      | Fc fragment of IgE receptor                        | 2.7                                  | 2.7                                        |                            |           | +    |
| 203167_at    | TIMP2       | Tissue inhibitor of<br>metalloproteinase 2         | 2.7                                  | 2.4                                        |                            | +         | +    |
| 227069_at    | —           | Clone IMAGE:4815142                                | 2.7                                  | 2.1                                        |                            |           |      |
| 202192_s_at  | GAS7        | Growth arrest-specific 7                           | 2.7                                  | 2.9                                        |                            |           |      |
| 204714_s_at  | F5          | Coagulation factor V                               | 2.7                                  | 3.6                                        |                            | +         | +    |
| 206420_at    | IGSF6       | Immunoglobulin superfamily,<br>member 6            | 2.7                                  | 2.3                                        |                            |           | +    |
| 1569385_s_at | KIAA1546    | KIAA1546 protein                                   | 2.7                                  | 2.5                                        |                            |           |      |
| 230707_at    | SORL1       | Sortilin 1                                         | 2.7                                  | 2.5                                        |                            | +         | +    |
| 216537_s_at  | SIGLEC7     | Sialic acid binding Ig-like<br>lectin 7            | 2.7                                  | 2.1                                        |                            |           | +    |
| 234989_at    | TncRNA      | cDNA clone GKCEOD05                                | 2.7                                  | 4.9                                        |                            |           |      |
| 225899_x_at  | —           | LOC388312                                          | 2.7                                  | 3.7                                        |                            |           |      |
| 1568780_at   | —           | LOC389622                                          | 2.6                                  | 2.2                                        |                            |           |      |

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TABLE E2. (Continued)

| Probe ID    | Gene symbol | Gene name                                                                                               | Fold change (pre-IVIG/<br>post-IVIG) | Fold change (pre-IVIG/<br>febrile control) | Expression in granulocytes |           |      |
|-------------|-------------|---------------------------------------------------------------------------------------------------------|--------------------------------------|--------------------------------------------|----------------------------|-----------|------|
|             |             |                                                                                                         |                                      |                                            | Promyelocyte               | Myelocyte | Band |
| 235703_at   | PLB1        | cDNA clone CS0DE013YE22                                                                                 | 2.6                                  | 2.5                                        |                            |           |      |
| 226064_s_at | DGAT2       | Diacylglycerol O-acyltransferase homolog 2                                                              | 2.6                                  | 4.1                                        |                            |           | +    |
| 206278_at   | PTAFR       | Platelet-activating factor receptor                                                                     | 2.6                                  | 3.4                                        |                            |           | +    |
| 1558965_at  | PHF21A      | BRAF35/HDAC2 complex (80 kd)                                                                            | 2.6                                  | 2.3                                        |                            |           | +    |
| 236934_at   | —           | cDNA FLJ34654                                                                                           | 2.6                                  | 6.5                                        |                            |           |      |
| 236345_at   | —           | Thromboxane A synthase 1                                                                                | 2.6                                  | 2.4                                        |                            |           | +    |
| 224909_s_at | PREX1       | Phosphatidylinositol 3,4,5-trisphosphate-dependent ras-related C3 botulinum toxin substrate exchanger 1 | 2.6                                  | 2.0                                        |                            |           | +    |
| 208928_at   | POR         | P450 (cytochrome) oxidoreductase                                                                        | 2.6                                  | 2.0                                        |                            | +         | +    |
| 211576_s_at | SLC19A1     | Solute carrier family 19 (folate transporter), member 1                                                 | 2.6                                  | 2.2                                        |                            |           | +    |
| 219259_at   | SEMA4A      | Hypothetical protein FLJ12287                                                                           | 2.5                                  | 3.6                                        |                            |           |      |
| 219994_at   | APBB1IP     | Amyloid $\beta$ (A4) precursor protein-binding, family B, member 1 interacting protein                  | 2.5                                  | 2.2                                        |                            | +         | +    |
| 244358_at   | —           | PM0-BT0340-231199-001-h11 BT0340                                                                        | 2.5                                  | 2.3                                        |                            |           |      |
| 205179_s_at | ADAM8       | A disintegrin and metalloproteinase domain 8                                                            | 2.5                                  | 2.5                                        |                            | +         | +    |
| 210210_at   | MPZL1       | Myelin protein zero-like 1                                                                              | 2.5                                  | 2.3                                        |                            |           | +    |
| 202896_s_at | SIRPA       | SHP substrate-1                                                                                         | 2.5                                  | 2.5                                        |                            |           |      |
| 1558397_at  | —           | Clone FCBBF3007597                                                                                      | 2.5                                  | 2.9                                        |                            |           |      |
| 1569512_at  | —           | Clone IMAGE:4294265                                                                                     | 2.5                                  | 3.3                                        |                            |           |      |
| 228918_at   | —           | cDNA FLJ32207                                                                                           | 2.5                                  | 2.4                                        |                            |           |      |
| 205786_s_at | ITGAM       | Integrin, $\alpha$ M (CD11b)                                                                            | 2.5                                  | 2.6                                        |                            | +         | +    |
| 205285_s_at | FYB         | FYN binding protein (FYB-120/130)                                                                       | 2.5                                  | 2.1                                        |                            | +         | +    |
| 240652_at   | —           | cDNA clone IMAGE:111702                                                                                 | 2.5                                  | 2.2                                        |                            |           |      |
| 221006_s_at | SNX27       | Sorting nexin family member 27                                                                          | 2.4                                  | 2.1                                        |                            | +         | +    |
| 203501_at   | PGCP        | Plasma glutamate carboxypeptidase                                                                       | 2.4                                  | 2.6                                        |                            |           | +    |
| 238642_at   | —           | Hypothetical protein LOC338692                                                                          | 2.4                                  | 2.1                                        |                            |           | +    |
| 213812_s_at | CAMKK2      | Calcium/calmodulin-dependent Protein kinase kinase 2, $\beta$                                           | 2.4                                  | 2.1                                        |                            | +         | +    |
| 244357_at   | —           | Transcribed sequences                                                                                   | 2.4                                  | 2.9                                        |                            |           |      |
| 231205_at   | —           | Transcribed sequences                                                                                   | 2.4                                  | 2.8                                        |                            |           |      |
| 224920_x_at | MYADM       | Myeloid-associated differentiation marker                                                               | 2.4                                  | 2.7                                        |                            |           |      |
| 200766_at   | CTSD        | Cathepsin D (lysosomal aspartyl protease)                                                               | 2.4                                  | 2.0                                        |                            |           |      |
| 229389_at   | ATG16L2     | Transcribed sequences                                                                                   | 2.4                                  | 2.1                                        |                            |           |      |
| 222688_at   | PHCA        | Phytoceramide, alkaline                                                                                 | 2.4                                  | 2.1                                        |                            | +         |      |
| 240498_at   | —           | Transcribed sequences                                                                                   | 2.4                                  | 2.7                                        |                            |           |      |
| 226673_at   | SH2D3C      | SH2 domain containing 3C                                                                                | 2.4                                  | 2.1                                        |                            |           | +    |
| 227129_x_at | FLJ45340    | Hypothetical LOC340346                                                                                  | 2.4                                  | 3.2                                        |                            |           |      |
| 238999_at   | —           | Transcribed sequences                                                                                   | 2.4                                  | 2.1                                        |                            |           |      |
| 229010_at   | CBL         | Cas-Br-M (murine) ecotropic retroviral transforming sequence                                            | 2.4                                  | 2.9                                        |                            | +         | +    |
| 1558877_at  | —           | cDNA clone IMAGE:143896                                                                                 | 2.4                                  | 2.5                                        |                            |           |      |
| 212516_at   | CENTD2      | Centaurin, delta 2                                                                                      | 2.4                                  | 2.1                                        |                            |           | +    |
| 225001_at   | RAB3D       | D2-2                                                                                                    | 2.4                                  | 3.2                                        |                            |           |      |
| 241913_at   | —           | Hypothetical protein FLJ20234                                                                           | 2.4                                  | 2.7                                        |                            |           |      |

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TABLE E2. (Continued)

| Probe ID     | Gene symbol | Gene name                                                                                         | Fold change (pre-IVIG/<br>post-IVIG) | Fold change (pre-IVIG/<br>febrile control) | Expression in granulocytes |           |      |
|--------------|-------------|---------------------------------------------------------------------------------------------------|--------------------------------------|--------------------------------------------|----------------------------|-----------|------|
|              |             |                                                                                                   |                                      |                                            | Promyelocyte               | Myelocyte | Band |
| 214746_s_at  | EZI         | Likely ortholog of mouse zinc finger protein EZI                                                  | 2.3                                  | 2.3                                        |                            |           | +    |
| 1552274_at   | PXK         | PX domain containing serine/threonine kinase                                                      | 2.3                                  | 2.4                                        |                            |           |      |
| 207224_s_at  | SIGLEC7     | Sialic acid binding Ig-like lectin 7                                                              | 2.3                                  | 2.2                                        |                            |           | +    |
| 201118_at    | PGD         | Phosphogluconate dehydrogenase                                                                    | 2.3                                  | 2.9                                        |                            |           |      |
| 211974_x_at  | RBPJ        | Recombining binding protein suppressor of hairless                                                | 2.3                                  | 2.2                                        |                            |           |      |
| 201702_s_at  | PPP1R10     | Protein phosphatase 1, regulatory subunit 10                                                      | 2.3                                  | 2.4                                        |                            |           |      |
| 224818_at    | SORT1       | Sortilin 1                                                                                        | 2.3                                  | 2.2                                        |                            | +         |      |
| 1557797_a_at | —           | cDNA clone IMAGE:2952955                                                                          | 2.3                                  | 2.0                                        |                            |           |      |
| 236338_at    | —           | Transcribed sequences                                                                             | 2.3                                  | 5.9                                        |                            |           |      |
| 225673_at    | MYADM       | Myeloid-associated differentiation marker                                                         | 2.3                                  | 2.6                                        |                            |           |      |
| 208594_x_at  | LILRA6      | Leukocyte immunoglobulin-like receptor, subfamily B, member 6                                     | 2.3                                  | 2.2                                        |                            |           |      |
| 214366_s_at  | ALOX5       | Arachidonate 5-lipoxygenase                                                                       | 2.3                                  | 2.3                                        |                            | +         | +    |
| 202897_at    | SIRPA       | Src homology region-2 domain phosphatase substrate-1                                              | 2.3                                  | 2.0                                        |                            |           |      |
| 239809_at    | —           | Transcribed sequences                                                                             | 2.3                                  | 3.4                                        |                            |           |      |
| 217489_s_at  | IL6R        | IL-6 receptor                                                                                     | 2.2                                  | 2.8                                        |                            | +         | +    |
| 243099_at    | NFAM1       | Nuclear factor of activated T cells activation molecule 1                                         | 2.2                                  | 2.2                                        |                            |           | +    |
| 224991_at    | CMIP        | c-Maf-inducing protein                                                                            | 2.2                                  | 2.2                                        |                            | +         | +    |
| 32091_at     | —           | KIAA0446 gene product                                                                             | 2.2                                  | 2.2                                        |                            |           | +    |
| 232500_at    | C20orf74    | Chromosome 20 open reading frame 74                                                               | 2.2                                  | 2.2                                        |                            |           |      |
| 203175_at    | RHOG        | ras homolog gene family, member G (p G)                                                           | 2.2                                  | 2.7                                        |                            |           |      |
| 213926_s_at  | HRB         | HIV-1 Rev binding protein                                                                         | 2.2                                  | 2.0                                        |                            | +         |      |
| 208637_x_at  | ACTN1       | Actinin, $\alpha$ 1                                                                               | 2.2                                  | 2.5                                        |                            |           |      |
| 240271_at    | —           | cDNA clone DKFZp566P1724                                                                          | 2.2                                  | 2.1                                        |                            |           |      |
| 201389_at    | ITGA5       | Integrin, $\alpha$ 5 (fibronectin receptor, $\alpha$ polypeptide)                                 | 2.2                                  | 2.7                                        |                            |           | +    |
| 211299_s_at  | FLOT2       | Flotillin 2                                                                                       | 2.1                                  | 2.1                                        |                            | +         | +    |
| 225530_at    | MOBK12A     | MOB1, Mps One Binder kinase activator-like 2A (yeast)                                             | 2.1                                  | 2.0                                        |                            |           |      |
| 200678_x_at  | GRN         | Granulin                                                                                          | 2.1                                  | 2.0                                        |                            |           |      |
| 1554952_s_at | NALP12      | NACHT, leucine rich repeat and PYD containing 12                                                  | 2.1                                  | 2.7                                        |                            |           | +    |
| 204789_at    | FMNL1       | Formin-like 1                                                                                     | 2.1                                  | 2.3                                        |                            | +         | +    |
| 1559502_s_at | LRRC25      | Leucine-rich repeat containing 25                                                                 | 2.1                                  | 2.4                                        |                            |           |      |
| 212152_x_at  | ARID1A      | AT rich interactive domain 1A                                                                     | 2.1                                  | 2.0                                        |                            |           |      |
| 226076_s_at  | MBD6        | Methyl-CpG binding domain protein 6                                                               | 2.1                                  | 2.6                                        |                            |           | +    |
| 204445_s_at  | ALOX5       | Arachidonate 5-lipoxygenase                                                                       | 2.1                                  | 2.0                                        |                            | +         | +    |
| 212136_at    | ATP2B4      | ATPase, Ca++ transporting, plasma membrane 4                                                      | 2.1                                  | 2.1                                        | +                          |           |      |
| 212014_x_at  | CD44        | CD44 antigen                                                                                      | 2.1                                  | 2.1                                        |                            | +         | +    |
| 225499_at    | —           | cDNA: FLJ22541                                                                                    | 2.1                                  | 2.1                                        |                            |           |      |
| 205645_at    | REPS2       | V-rat simian leukemia viral oncogene homolog binding protein 1 associated Eps domain containing 2 | 2.1                                  | 2.2                                        |                            |           | +    |
| 223014_at    | UBE2R2      | Ubiquitin-conjugating enzyme E2R 2                                                                | 2.1                                  | 2.1                                        |                            | +         | +    |
| 213400_s_at  | TBL1X       | Transducin ( $\beta$ )-like 1X-linked                                                             | 2.1                                  | 2.5                                        |                            |           | +    |

(Continued)

TABLE E2. (Continued)

| Probe ID    | Gene symbol | Gene name                                                                                              | Fold change (pre-IVIG/<br>post-IVIG) | Fold change (pre-IVIG/<br>febrile control) | Expression in granulocytes |           |      |
|-------------|-------------|--------------------------------------------------------------------------------------------------------|--------------------------------------|--------------------------------------------|----------------------------|-----------|------|
|             |             |                                                                                                        |                                      |                                            | Promyelocyte               | Myelocyte | Band |
| 227184_at   | —           | Hypothetical protein FLJ13213                                                                          | 2.1                                  | 2.2                                        |                            |           | +    |
| 225282_at   | SMAP1L      | Hypothetical protein<br>AL133206                                                                       | 2.0                                  | 2.3                                        |                            |           | +    |
| 218023_s_at | FAM53C      | Chromosome 5 open reading<br>frame 6                                                                   | 2.0                                  | 2.0                                        |                            |           | +    |
| 228460_at   | ZNF319      | Zinc finger protein 319                                                                                | 2.0                                  | 2.4                                        |                            |           |      |
| 226080_at   | SSH2        | Slingshot 2                                                                                            | 2.0                                  | 2.2                                        |                            |           | +    |
| 234196_at   | —           | cDNA: FLJ21377                                                                                         | 2.0                                  | 2.4                                        |                            |           |      |
| 222413_s_at | MLL3        | Myeloid/lymphoid or mixed-<br>lineage leukemia 3                                                       | 2.0                                  | 2.2                                        |                            |           |      |
| 240862_at   | RASGRP4     | RAS guanyl releasing protein 4                                                                         | 2.0                                  | 2.8                                        |                            | +         | +    |
| 200986_at   | SERPING1    | Serine proteinase inhibitor,<br>clade G (C1 inhibitor)                                                 | 4.7                                  | 0.5                                        | +                          |           |      |
| 238439_at   | ANKRD22     | Clone IMAGE:2456964                                                                                    | 3.7                                  | 0.5                                        |                            |           |      |
| 235139_at   | GNGT2       | Guanine nucleotide binding<br>protein (G protein), $\gamma$ -<br>transducing activity<br>polypeptide 2 | 3.5                                  | 0.5                                        |                            |           |      |
| 238581_at   | GBP5        | Guanylate binding protein 5                                                                            | 2.7                                  | 0.4                                        |                            |           | +    |
| 239196_at   | ANKRD22     | Clone IMAGE:2456964                                                                                    | 2.7                                  | 0.4                                        |                            |           |      |
| 231426_at   | —           | Chromosome 9 open reading<br>frame 71                                                                  | 2.3                                  | 0.4                                        |                            |           |      |
| 208716_s_at | TMCO1       | Putative membrane protein                                                                              | 0.5                                  | 0.3                                        |                            |           |      |
| 226682_at   | LOC283666   | Hypothetical protein<br>LOC283666                                                                      | 0.5                                  | 0.3                                        |                            |           |      |
| 213677_s_at | PMS1        | PMS1 postmeiotic segregation<br>increased 1                                                            | 0.5                                  | 0.3                                        | +                          |           |      |
| 201398_s_at | TRAM1       | Translocation associated<br>membrane protein 1                                                         | 0.5                                  | 0.3                                        | +                          |           |      |
| 211469_s_at | CXCR6       | Chemokine (C-X-C motif)<br>receptor 6                                                                  | 0.5                                  | 0.1                                        |                            |           |      |
| 239835_at   | KBTBD8      | T-cell activation kelch repeat<br>protein                                                              | 0.5                                  | 0.3                                        | +                          |           |      |
| 208838_at   | CAND1       | TATA-binding protein–<br>interacting protein                                                           | 0.5                                  | 0.3                                        | +                          |           |      |
| 221253_s_at | TXNDC5      | Thioredoxin domain<br>containing 5                                                                     | 0.5                                  | 0.3                                        | +                          |           |      |
| 202503_s_at | KIAA0101    | KIAA0101 gene product                                                                                  | 0.5                                  | 0.2                                        | +                          |           |      |
| 218738_s_at | RNF138      | Ring finger protein 138                                                                                | 0.5                                  | 0.3                                        |                            |           |      |
| 219600_s_at | TMEM50B     | Chromosome 21 open reading<br>frame 4                                                                  | 0.5                                  | 0.2                                        | +                          |           |      |
| 220547_s_at | FAM35A      | Hypothetical protein MGC5560                                                                           | 0.5                                  | 0.3                                        |                            |           |      |
| 224740_at   | —           | CDNA clone IMAGE:5270526                                                                               | 0.5                                  | 0.3                                        |                            |           |      |
| 206666_at   | GZMK        | Granzyme K                                                                                             | 0.5                                  | 0.1                                        | +                          |           |      |
| 203494_s_at | CEP57       | Translokin (KIAA0092)                                                                                  | 0.5                                  | 0.3                                        | +                          |           |      |
| 202558_s_at | STCH        | Stress 70 protein chaperone,<br>microsome-associated,<br>60kDa                                         | 0.5                                  | 0.3                                        | +                          |           |      |
| 205692_s_at | CD38        | CD38 antigen                                                                                           | 0.5                                  | 0.2                                        | +                          |           |      |
| 234863_x_at | FBXO5       | F-box only protein 5                                                                                   | 0.5                                  | 0.2                                        | +                          |           |      |
| 202780_at   | OXCT1       | 3-Oxoacid CoA transferase                                                                              | 0.5                                  | 0.3                                        | +                          |           |      |
| 227418_at   | KIAA1826    | KIAA1826 protein                                                                                       | 0.5                                  | 0.3                                        | +                          |           |      |
| 222500_at   | PP1L1       | Peptidylprolyl isomerase-like 1                                                                        | 0.4                                  | 0.2                                        | +                          |           |      |
| 202502_at   | ACADM       | Acyl-coenzyme A<br>dehydrogenase                                                                       | 0.4                                  | 0.3                                        | +                          |           |      |
| 208309_s_at | MALT1       | Mucosa associated lymphoid<br>tissue lymphoma<br>translocation gene 1                                  | 0.4                                  | 0.3                                        | +                          |           |      |
| 223167_s_at | USP25       | Ubiquitin specific protease 25                                                                         | 0.4                                  | 0.2                                        |                            |           |      |
| 201764_at   | —           | Hypothetical protein MGC5576                                                                           | 0.4                                  | 0.3                                        | +                          |           |      |
| 219276_x_at | C9orf82     | Chromosome 9 open reading<br>frame 82                                                                  | 0.4                                  | 0.3                                        |                            |           |      |

(Continued)

TABLE E2. (Continued)

| Probe ID     | Gene symbol | Gene name                                                      | Fold change (pre-IVIG/<br>post-IVIG) | Fold change (pre-IVIG/<br>febrile control) | Expression in granulocytes |           |      |
|--------------|-------------|----------------------------------------------------------------|--------------------------------------|--------------------------------------------|----------------------------|-----------|------|
|              |             |                                                                |                                      |                                            | Promyelocyte               | Myelocyte | Band |
| 217779_s_at  | PNRC2       | Proline-rich nuclear receptor<br>coactivator 2                 | 0.4                                  | 0.3                                        | +                          |           |      |
| 223270_at    | CTDSPL2     | Hypothetical protein HSPC129                                   | 0.4                                  | 0.2                                        | +                          |           |      |
| 201889_at    | FAM3C       | Family with sequence<br>similarity 3, member C                 | 0.4                                  | 0.3                                        | +                          |           |      |
| 212721_at    | SFRS12      | Splicing factor, arginine/serine-<br>rich 12                   | 0.4                                  | 0.3                                        | +                          |           |      |
| 222550_at    | ARMC1       | Armado repeat containing<br>protein                            | 0.4                                  | 0.3                                        | +                          |           |      |
| 236280_at    | —           | Transcribed sequences                                          | 0.4                                  | 0.3                                        |                            |           |      |
| 210116_at    | SH2D1A      | SH2 domain protein 1A,<br>Duncan disease                       | 0.4                                  | 0.3                                        |                            | +         | +    |
| 208079_s_at  | AURKA       | STK15, serine/threonine kinase<br>15                           | 0.4                                  | 0.2                                        | +                          |           |      |
| 224661_at    | PIGY        | Hypothetical protein<br>MGC14156                               | 0.4                                  | 0.2                                        |                            |           |      |
| 218889_at    | NOC3L       | AD24 protein                                                   | 0.4                                  | 0.2                                        |                            |           |      |
| 202950_at    | CRYZ        | Crystallin, zeta (quinone<br>reductase)                        | 0.4                                  | 0.2                                        | +                          |           |      |
| 214093_s_at  | FUBP1       | Far upstream element (FUSE)<br>binding protein 1               | 0.4                                  | 0.3                                        |                            |           |      |
| 207165_at    | HMMR        | Hyaluronan-mediated motility<br>receptor                       | 0.4                                  | 0.1                                        | +                          |           |      |
| 201663_s_at  | SMC4        | SMC4 structural maintenance<br>of chromosomes 4-like 1         | 0.4                                  | 0.2                                        | +                          |           |      |
| 221122_at    | HRASLS2     | HRAS-like suppressor 2                                         | 0.4                                  | 0.1                                        |                            | +         |      |
| 224628_at    | C2orf30     | XTP3-transactivated protein B                                  | 0.4                                  | 0.2                                        | +                          |           |      |
| 203362_s_at  | MAD2L1      | MAD2 mitotic arrest deficient-<br>like 1                       | 0.4                                  | 0.2                                        | +                          |           |      |
| 222572_at    | PPM2C       | Protein phosphatase 2C                                         | 0.4                                  | 0.2                                        |                            |           |      |
| 235170_at    | ZNF92       | Zinc finger protein 92 (HTF12)                                 | 0.4                                  | 0.2                                        |                            |           |      |
| 222631_at    | PI4K2B      | cDNA clone IMAGE:2295962                                       | 0.4                                  | 0.2                                        |                            |           |      |
| 228032_s_at  | —           | cDNA FLJ36663                                                  | 0.4                                  | 0.2                                        |                            |           |      |
| 228323_at    | CASC5       | AF15q14 protein                                                | 0.4                                  | 0.1                                        | +                          |           |      |
| 1558972_s_at | C6orf190    | cDNA clone IMAGE:5764019                                       | 0.4                                  | 0.2                                        |                            |           |      |
| 226254_s_at  | KIAA1430    | KIAA1430 protein                                               | 0.4                                  | 0.2                                        | +                          |           |      |
| 212592_at    | IGJ         | IgJ polypeptide                                                | 0.4                                  | 0.2                                        | +                          |           |      |
| 227674_at    | ZNF585A     | Zinc finger protein 585A                                       | 0.4                                  | 0.1                                        |                            |           |      |
| 203593_at    | CD2AP       | CD2-associated protein                                         | 0.3                                  | 0.1                                        | +                          |           |      |
| 206641_at    | TNFRSF17    | TNF receptor superfamily,<br>member 17                         | 0.3                                  | 0.1                                        |                            |           |      |
| 211211_x_at  | SH2D1A      | SH2 domain protein 1A                                          | 0.3                                  | 0.1                                        |                            | +         | +    |
| 230165_at    | SGOL2       | Tripin                                                         | 0.3                                  | 0.1                                        |                            |           |      |
| 231576_at    | ETNK1       | Clone IMAGE:4798349                                            | 0.3                                  | 0.1                                        |                            |           |      |
| 223262_s_at  | FGFR1OP2    | DKFZp564O1863 protein                                          | 0.3                                  | 0.2                                        |                            |           |      |
| 201890_at    | RRM2        | cDNA clone IMAGE:3905920                                       | 0.3                                  | 0.1                                        | +                          |           |      |
| 202557_at    | STCH        | Stress 70 protein chaperone,<br>microsome-associated,<br>60kDa | 0.3                                  | 0.1                                        | +                          |           |      |
| 222502_s_at  | UFM1        | Hypothetical protein BM-002                                    | 0.3                                  | 0.1                                        |                            |           |      |
| 228377_at    | KLHL14      | KIAA1384 protein                                               | 0.3                                  | 0.1                                        |                            |           |      |
| 213599_at    | OIP5        | Opa-interacting protein 5                                      | 0.3                                  | 0.1                                        | +                          |           |      |
| 235117_at    | CHAC2       | Clone MGC:61554<br>IMAGE:6174351                               | 0.3                                  | 0.1                                        |                            |           |      |
| 211210_x_at  | SH2D1A      | SH2 domain protein 1A                                          | 0.2                                  | 0.1                                        |                            | +         | +    |
| 222805_at    | MANEA       | Mannosidase, endo- $\alpha$                                    | 0.2                                  | 0.1                                        |                            |           |      |
| 219918_s_at  | ASPM        | asp (Abnormal spindle)-like,<br>microcephaly associated        | 0.2                                  | 0.1                                        | +                          |           |      |