therapy (high-dose intravenous gammaglobulin therapy), resulting in the prevention of the development of Kawasaki disease. Moreover, severe cases are associated with decreased immune function, particularly T cell-mediated immunity, a factor related to vulnerability to infection. In any event, the reported patients and ours had common clinical features that favored the establishment of infection.

Wong et al. explained that the breakdown of the microbiological barrier function of epithelium due to burns was associated with the entry of infectious antigens, and exotoxins or superantigens caused Kawasaki disease [33]. Unlike other antigens, superantigens refer to antigens that are bound to self-MHC class II molecules via TcR, and are recognized, not by the CDR formed in the V region of a particular TcR, but via particular regions other than the CDR of a particular Vbeta element. Thus, superantigens activate large numbers of T cells, inducing overproduction of cytokines. Bacterial superantigens collectively refer to exotoxins produced by *S. aureus* and streptococci, and cause toxic shock syndrome (TSS) and scarlet fever [29]. Although more than 50% of individuals over 5 years of age carry anti-TSST-1 antibodies, TSS patients have been reported to have low titers of anti-TSST-1 antibodies [30]. Some studies have reported little or no involvement of superantigen-producing bacteria in Kawasaki disease [14,18], or the development of Kawasaki disease after cellulitis due to TSST-1-positive staphylococci [19]. The present patients also showed an increase in anti-TSST-1 antibody titer in the convalescent stage; moreover, the patients suddenly developed symptoms of Kawasaki disease in the absence of signs of infection and with a normal general condition. The superantigen activity of exotoxins produced by *S. aureus* has been considered to induce abnormal activation of cell-mediated immunity even in the stage of colonization [4,6]; therefore, it is very likely that the infection secondary to burns directly or indirectly caused Kawasaki disease.

TSS was first described in 1978 by Todd et al. [27] It is an acute toxic disease that manifests itself with high fever, muscle pain, vomiting, and diarrhea, followed by hypotension, diffuse rashes and peeling of the skin, and multi-organ failure, presenting clinical features similar to those of Kawasaki disease. Since Frame et al. [7] first described seven patients with TSS following burns in 1985, many studies have reported its development in patients under 6 years of age after burns [6,7,25], and this is well known in the field of Plastic and Reconstructive Surgery. However, in some patients with incomplete forms of TSS, Kawasaki disease should be suspected [21,25]. Reported patients with TSS or neonatal TSS-like exanthematous disease, which develops by a mechanism similar to that of TSS, included some who developed coronary artery abnormalities [17]. The possibility cannot be excluded that instead of Kawasaki disease, TSS was diagnosed because of the detection of *S. aureus* or MRSA in wound areas.

Regarding differences between Kawasaki disease and TSS, blood pressure reduction, central nervous symptoms, such as convulsion, digestive symptoms, elevations of CK, BUN, and Cr, and thrombocytopenia in peripheral blood occur more frequently in TSS [1,14]. However, all these findings are nonspecific, and precise distinction of the two diseases is difficult. Edwards-Jones et al. reported that the incidence of TSS after burns was about 2.5% [5]. Thus, TSS should be initially suspected when distinction is difficult. However, in the field of Pediatrics, the early treatment of Kawasaki or analogous diseases has been increasingly applied when the condition meets the diagnostic criteria of Kawasaki disease, and no other disease is apparent, which is also based on a report [3] that high-dose intravenous gammaglobulin therapy is effective for TSS because they contain anti-TSS-1 antibodies.

4. Conclusions

We encountered four patients who developed mimicking Kawasaki disease after sustaining burns. Kawasaki disease is not sufficiently understood because of the few cases reported in the field of Plastic and Reconstructive Surgery. In the future, the differential diagnosis of fever and polymorphous exanthem following infection of burns and other wounds should include Kawasaki disease. We need further understanding of the importance of local management, and consider the period of susceptibility to Kawasaki disease, the depth of burns, and their treatment after the development of Kawasaki disease.

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Focused Microarray Analysis of Peripheral Mononuclear Blood Cells from Churg–Strauss Syndrome Patients

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Abstract

DNA diagnostics are useful but are hampered by difficult ethical issues. Moreover, it cannot provide enough information on the environmental factors that are important for pathogenesis of certain diseases. However, this is not a problem for RNA diagnostics, which evaluate the expression of the gene in question. We here report a novel RNA diagnostics tool that can be employed with peripheral blood mononuclear cells (PBMCs). To establish this tool, we identified 290 genes that are highly expressed in normal PBMCs but not in TIG-1, a normal human fibroblast cell. These genes were entitled *PREP* after predominantly expressed in PBMC and included 50 uncharacterized genes. We then conducted *PREP* gene-focused microarray analysis on PBMCs from seven cases of Churg–Strauss syndrome (CSS), which is a small-vessel necrotizing vasculitis. We found that *PREP135* (coactosin-like protein), *PREP77* (prosaposin), *PREP191* (cathepsin D), *PREP234* (*c*-fgr), and *PREP136* (lysozyme) were very highly up-regulated in all seven CSS patients. Another 28 genes were also up-regulated, albeit more moderately, and three were down-regulated in all CSS patients. The nature of these up- and down-regulated genes suggest that the immune systems of the patients are activated in response to invading microorganisms. These observations indicate that focused microarray analysis of PBMCs may be a practical, useful, and low-cost bedside diagnostics tool.

Keywords: focused microarray; RNA diagnosis; PMBC; allergic granulomatosis angiitis; Churg–Strauss syndrome

1. Introduction

The advent of array technology and the subsequent development of high-density oligonucleotide arrays[1] have been enormously helpful in improving our understanding of the genome-wide transcriptional profiles of many biological systems in both basic and applied research.[2] Array technology has also been extremely useful for discovering and developing diagnostic gene markers for disease subcategories, disease prognosis, and treatment outcome; this has paved the way for effective pharmaceutical drug discovery, the development of novel strategies for molecular (DNA, RNA) diagnostics, and the design of personalized drug regimens.[2]

Oligonucleotide microarrays, which were initially designed to analyze genome-wide gene expression levels, have turned out to be particularly useful in DNA diagnostics as they can be used for many different

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applications, including discovering polymorphisms and genotyping patients by identifying inheritable genetic markers.[3] With regard to the latter applications, such DNA diagnostics have greatly improved our understanding of and ability to detect the causative mutations of various diseases. However, this technology is hampered by major ethical and privacy issues since the genome sequence of a person carries unchangeable private information whose discovery can affect the life of not only that person, but also to some extent their family members. The need to protect patient privacy upon DNA diagnostics testing and the practical problems this constitutes also imposes extra costs on the testing procedure. Moreover, many kinds of diseases are caused not only by the DNA polymorphism, but also by environmental status. These environmental factors can be reflected in the RNA status, but DNA diagnostics usually cannot provide enough information on the environments. Thus, the activity of the disease can be detected by RNA or protein level but not by DNA level.

These problems are not faced by RNA diagnostics, in which the genome-wide mRNA levels are monitored by oligonucleotide (or cDNA) microarrays. Ethical issues of the nature described above do not apply here because the information obtained from RNA diagnostics does not necessarily relate to the DNA sequence. Instead, this technology monitors the dynamic changes in gene activities, namely, the increased or decreased transcriptional (mRNA) levels in the samples provided by the individual. The transcriptional levels would vary in the same individual depending on the health of the individual. In this sense, DNA diagnostics is a static test of the genome, whereas RNA diagnostics is a dynamic test of the genome.

However, it is not possible to monitor all 44 000 kinds of mRNA species that are transcribed from the human genome for many samples at the same time. A novel way to circumvent this problem is to develop a 'focused array' in which a limited number of mRNAs are tested in a low-density array. In the present study, we developed a focused oligonucleotide (or cDNA) array for use with patient peripheral blood mononuclear cells (PBMCs). For this, we selected the genes that are predominantly expressed in normal PBMCs, as determined by stepwise subtractive hybridization[4] and by genome-wide cDNA microarray analysis. From the 290 'PBMC-focused' genes we identified, we can prepare the PBMC-focused cDNA array. We examined the expression levels of these *PREP* genes to analyze the PBMC RNAs obtained from patients suffering the autoimmune disease Churg–Strauss syndrome (CSS), which is an alternative name of allergic granulomatosis angiitis, because the autoimmune response of CSS patients is expected to disturb the expression levels of immune-related genes in PBMC. Indeed, we identified several genes whose expressions are markedly up- or down-regulated in all CSS patients tested. These observations suggest that this low-cost RNA diagnostics test is useful, practical, and can be used at the bedside.

2. Patients, Materials and methods

2.1. *Human subjects: patients and healthy controls*

Blood was obtained from eight healthy volunteers (four males and four females; aged 25–49 years) for the cDNA library preparation. Blood was also obtained from seven cases of CSS patients whose profiles are shown in Supplementary Table S1 and 18 healthy controls (six males and 11 females; aged 25–86 years) for focused microarray analysis. CSS patients were diagnosed according to the diagnostic criteria of the American College of Rheumatology.[5] This study was reviewed and approved by the Internal Review Board of the Research Institute for Microbial Diseases, Osaka University. In accordance with the requirements of the Board, a written informed consent was obtained from each participant before venous blood samples were obtained. Serum samples were consecutively obtained regardless of the patient's symptom, active, or inactive phase.

2.2. *Preparation of RNA*

The RNA of the PBMCs obtained from healthy volunteers was prepared as described previously.[6] Briefly, heparinized venous blood (10 mL) was mixed with an equal volume of 2% dextran/saline solution and incubated at room temperature for 30 min to precipitate the red blood cells. Total RNA was extracted from the PBMC pellet by adding guanidine–thiocyanate solution and the samples were used for cDNA library preparation and subtractive hybridization.[7] Total RNA was also prepared by acid guanidinium–phenol–chloroform extraction for the DNA microarray, northern blot, and RT–PCR analyses. In some experiments, total RNA was synthesized using Ribo Max kit (Promega, Madison, WI) from the PBMC cDNA library. Total RNA or mRNA from human fibroblast TIG-1 cells was prepared as described previously.[7] Ex*Taq* DNA polymerase for RT–PCR was purchased from TaKaRa Co. Ltd. (Otsu, Japan). Probe labeling and detection for northern blots were performed by using the Gene Images Random-Prime Labelling and Detection System (GE Healthcare Bio-Sciences Corp., Piscataway, NJ).

2.3. *Preparation of the subtracted cDNA library and stepwise subtraction*

Poly(A)$^+$ RNA was purified from total RNA by oligo(dT) cellulose chromatography. A cDNA library

with eight million independent clones was constructed from the PBMC mRNAs by using the linker-primer method and the pAP3neo vector as described previously.[7] The poly(A)$^+$ RNAs from exponentially growing TIG-1 cells that had been incubated with 10% serum in tissue culture plates were also purified and biotinylated by using photobiotin. After converting the cDNA library to a single-stranded form by transfection with an f1 helper phage, we hybridized it with the biotinylated mRNAs and subtracted it by biotin–avidin interactions.[7] The unhybridized clones were converted to the double-stranded form and used to transform competent *Escherichia coli* cells. This generated a first-stage subtracted cDNA library of 11 million independent clones.

We then prepared plasmid DNA from ~800 randomly selected cDNA clones and numbered and digested an aliquot of each plasmid DNA with *Sma*I and *Not*I restriction enzymes to prepare 10 sheets of Southern blots, each of which included 80 clones arranged in order. We then purified the cDNA inserts of clones 1–20 on 1% agarose gels by digesting them with *Eco*RI and *Not*I; these inserts were labeled with fluorescent dye and then used as probes for northern analysis with PBMC and TIG-1 RNAs to determine which clones contained *PREP* genes (data not shown). The DNA sequences of the *PREP* clones from the 5′ end of the cDNA inserts were determined by the dideoxy-chain termination reaction using an automatic DNA sequencer (Licor 4000L; Lincoln, NE). After these analyses, we selected the next 20 unhybridized clones on the Southern blot (from 21 onwards) for the next round of cDNA insert preparation, fluorescent labeling, and northern analysis. This procedure was repeated until we finished testing all 800 unhybridized clones. The *PREP* genes identified in the preceding step were then converted to RNA, biotinylated with photobiotin, and used to subtract the first-stage subtracted cDNA library. The second-stage subtracted cDNA library was then analyzed as described above and subtracted again. This process was repeated three times as described previously.[4]

After the clones whose transcription was conspicuously up-regulated in PBMCs as compared with TIG-1 cells were sequenced, the DNA sequences were used to search the EST database by using the BLAST algorithm (http://www.ncbi.nlm.nih.gov/BLAST/).

2.4. *Human cDNA microarray analysis for identification of PREP genes*

The total RNAs (500 ng) from the normal human PBMCs were pooled, as were the TIG-1 cell RNAs, after which both pools were reverse-transcribed by using oligo-dT primers containing the T7 RNA polymerase promoter sequence. The cDNAs were then subjected to *in vitro* transcription using T7 RNA polymerase to label the complementary RNAs (cRNAs) with cyanine 3 (Cy3)-CTP or cyanine 5 (Cy5)-CTP (Amersham Pharmacia Biotech, Piscataway, NJ). The Cy-labeled cRNAs from the normal human PBMCs (1 μg) were then mixed with the same amount of reverse color Cy-labeled TIG-1 cell-derived cRNAs. Hybridizations, rinsing, scanning, and gene analysis on the Agilent's all human cDNA microarray (Hu44K) were conducted according to the manufacturer's protocol (G2940BA; Agilent Technologies, Inc., Palo Alto, CA). Fluorophore reversal (dye swap) duplicates were used in two-color DNA microarray experiments. The 399 genes that showed the highest level of up- or down-regulation were selected and subjected to RT–PCR analysis using the normal human PBMC and TIG-1 RNAs (Fig. 1) with relevant oligonucleotide primers (Supplementary Table S2). Of these, 122 genes were identified as *PREP* genes, 33 of which were already identified as *PREP* genes by stepwise subtraction.[4]

2.5. *Individual microarray analysis on CSS patients*

The quality of the RNA samples obtained from PBMCs of each CSS patient was examined by using the RNA 6000 Nano LabChip Kit (p/n 5065-4476) on the Agilent 2100 Bioanalyzer. To conduct the individual cDNA microarray (Hu44K) analysis on CSS patients and normal volunteers, we generated fluorescently labeled cRNA by *in vitro* transcription with T7 RNA polymerase in the presence of Cy5-CTP or Cy3-CTP using a Low RNA Input Fluorescent Linear Amplification Kit (Agilent Technologies, Inc.) as described above. The Cy5-labled cRNA of each patient was then mixed with the Cy3-labled cRNA of normal volunteers to probe the cDNA microarray. *PREP* gene expression analysis was then conducted by using the Gene Spring software 7.3.1 (Agilent Technologies, Inc.) by setting appropriate parameters to select 33 or 3 *PREP* genes whose expressions are up-regulated (>1.2-fold change) or down-regulated (<1.0-fold change) in all seven CSS patients as compared with healthy volunteers. We also conducted similar analysis to select nine conspicuously up-regulated *PREP* genes (>2.0-fold change).

3. Results

3.1. *Isolation of putative PBMC-specific genes*

To isolate putative human PBMC-specific genes, we first prepared mRNAs from the PBMCs of healthy volunteers and generated a human PBMC cDNA library with 80 million independent clones by using the linker-primer method described previously.[7] We also prepared mRNAs from exponentially growing normal

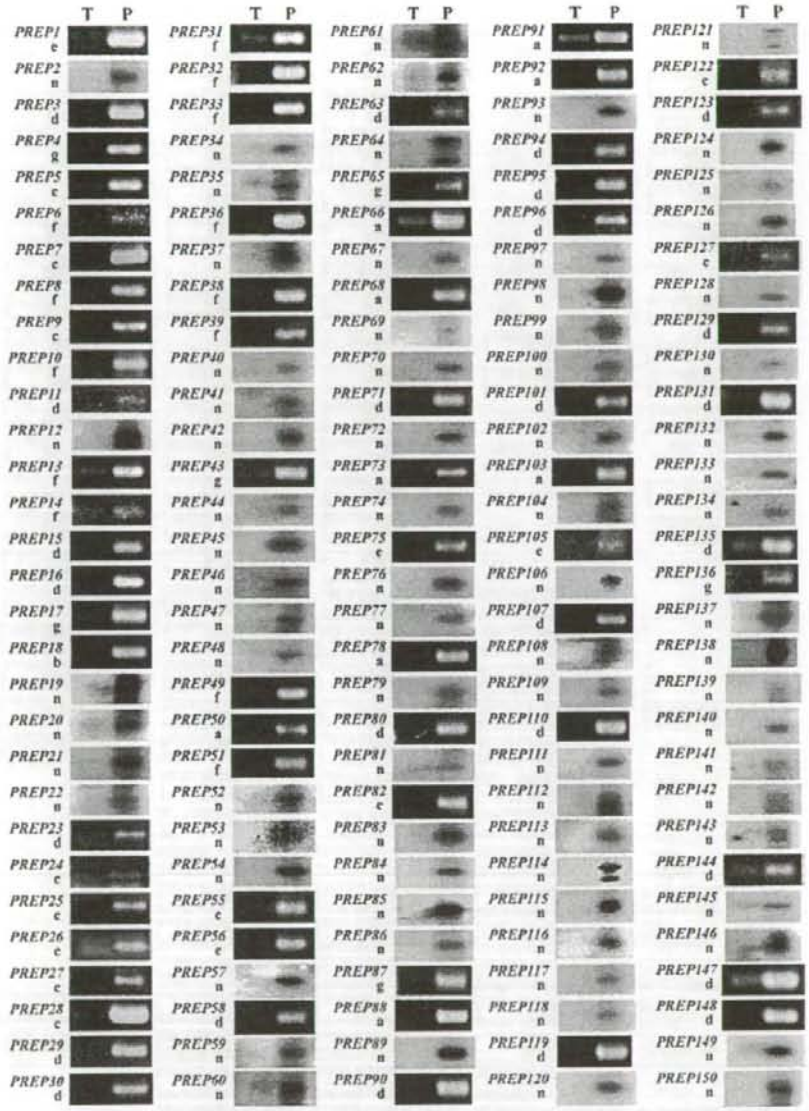

Figure 1. Identification of *PREP* genes. Individual *PREP* cDNA clones were subjected to northern blot or RT–PCR analysis to compare their gene expression levels in the PBMCs of healthy volunteers (right lanes, denoted as P) and human fibroblast TIG-1 cells (left lanes, denoted as T). The names of the *PREP* genes are shown in Table 1. The RT–PCR data for *GAPDH* and the northern blot data for β-actin are also shown as loading controls. The annealing temperature and amplification cycles used in the RT–PCRs are denoted to the left of each image by a–h: a, 50°C and 35 cycles, b, 50°C and 30 cycles, c, 55°C and 35 cycles, d, 55°C and 40 cycles, e, 55°C and 45 cycles, f, 55°C and 30 cycles, and g, 50°C and 40 cycles, respectively. The northern blots are denoted as n.

human fibroblast TIG-1 cells, which are control cells that express non-PBMC transcripts. These mRNAs were biotinylated with photobiotin[7] and used to subtract the PBMC cDNA library as described previously[4] to remove the housekeeping and non-PBMC-specific genes. Briefly, after converting the PBMC cDNA library to a single-stranded form by transfection with f1 helper phage, we hybridized it with the

Figure 1. Continued

biotinylated TIG-1 mRNAs and subtracted it by biotin–avidin interactions. The unhybridized clones were converted to the double-stranded form, which was then used to transform competent *E. coli* cells, thereby generating a subtracted cDNA library.

To analyze this subtracted cDNA library, we prepared plasmid DNA from several hundred randomly selected and numbered cDNA clones. An aliquot of each plasmid DNA was then digested with *Eco*RI and *Not*I and their cDNA inserts were purified on 1% agarose gels and fluorescently labeled. These probes were then used in northern analysis to identify those genes whose transcript levels were much higher in PBMCs than in TIG-1 cells, namely, those genes that

showed almost a plus/minus type difference. These genes were named *PREP* after predominantly expressed in PBMC. As shown in Fig. 1, each northern sheet only included two lanes, one each for the RNAs extracted from PBMCs and TIG-1. The DNA sequences of these candidate *PREP* genes were determined from the 5′ end of the cDNA inserts by the dideoxy-chain termination reaction and were used to search the EST database by employing the BLAST algorithm (http://www.ncbi.nlm.nih.gov/BLAST/). For those candidate *PREP* genes whose northern blot signals were weak or undetectable, we examined whether these candidate genes were indeed up-regulated by RT–PCR using oligonucleotide primers based on the DNA sequences of these genes (Supplementary Table S2) (Fig. 1). These procedures were repeated until almost all subtracted cDNAs were tested, as described previously.[4] The end result was the isolation of 197 *PREP* genes.

To reduce the possibility of missing important PBMC-specific pathogenic genes by the stepwise subtraction method, we also performed in parallel a genome-wide cDNA microarray analysis by using Agilent's Hu44K array with the same pooled RNA samples obtained from the PBMCs and TIG-1. The 399 genes that showed the greatest up-regulation in PBMCs compared with TIG-1 were then tested by RT–PCR. This resulted in the identification of 126 *PREP* genes, 33 of which were the same as those obtained by the stepwise subtraction analysis. The candidate *PREP* genes were tested by northern and/or RT–PCR analyses to confirm their PBMC-specifc expression (Fig. 1). We did not test those genes that showed lower up-regulation in PBMCs than the 399 genes because the efficiency of detecting a *PREP* was conspicuously lower in the top 151–400 microarray genes than in the top 1–150 genes. Thus, it appears that while the stepwise subtraction method failed to identify 93 *PREP* genes, the DNA microarray analysis failed to detect 164 *PREP* genes. This disparity is mainly due to the differently sized drivers or probes used for hybridization; cDNA subtraction uses kilobase-order biotinylated mRNA as the driver for subtraction, while DNA microarrays are probed by 60-base oligonucleotides. These data together indicate that we identified 290 *PREP* genes (Supplementary Table S3).

3.2. *Characterization of the unknown* PREP *genes*

Of the 290 *PREP* genes that we identified, *PREP1–PREP50* are uncharacterized genes. Homology and motif search of the gene products (Prep1–Prep50 proteins) using the Motif Scan algorithm (http://myhits.isb-sib.ch/cgi-bin/motif_scan) revealed the following notable motifs in *PREP1–PREP11* (Fig. 2),

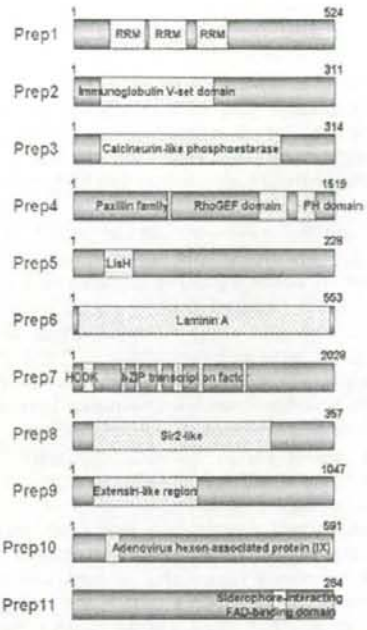

Figure 2. Schematic depiction of the uncharacterized gene products Prep1-Prep11. Numbers indicate the amino acids of the protein. RRM, RNA recognition motif; GEF, Guanine nucleotide exchange factor; PH, pleckstrin homology; LisH, LIS1 (lissencephaly) homology; bZIP, basic region/leucine zipper; Sir2, sirtuin 2; FAD, flavin adenine dinucleotide.

which may suggest their putative physiological functions. The other uncharacterized *PREP* genes did not have any notable motifs.

PREP1 encodes an RNA-binding protein because it harbors three RNA recognition motifs.[8] *PREP2* encodes a protein that harbors a V-set Ig-like domain that is responsible for binding to sialic acid.[9] A specialized subgroup of the Ig superfamily, called sialic acid-binding immunoglobulin-like lectins (Siglecs), can recognize sialylated glycoconjugates and play a role in cell–cell recognition and intracellular signaling. At least 11 related genes have been identified in the human genome, all of which encode type 1 membrane proteins that carry an N-terminal sialic acid-binding V-set Ig domain and varying numbers of C2-set Ig domains.[9] Thus, Prep2 may be a Siglec-type protein. Prep3 protein belongs to the large calcineurin-like phosphoesterase superfamily. Members of this superfamily include the polymerase-associated B-subunits and all carry an active site harboring two divalent metal ions for catalysis.[10] This suggests that Prep3 protein may participate in cell growth by regulating DNA replication. *PREP4*

encodes a novel member of the paxillin family proteins that bear the RhoGEF and PH domains.[11] Paxillin is a multi-domain adaptor that recruits associated proteins to focal adhesions, where it plays a pivotal role in transducing cell signaling, thereby eliciting changes in cell migration and gene expression.[12] Given that the RhoGEF and PH domains both are also found in these cell signaling molecules, Prep4 protein appears to function in the signal transduction of growth signals. *PREP5* encodes a protein with a LisH (LIS1 homology) motif, which is found in many signaling proteins of the WD-40 family and is believed to help regulate microtubule dynamics by mediating dimerization or binding to cytoplasmic dynein heavy chain.[13] Prep6 protein is homologous to laminin A, which is one of the 15 laminin isoforms that are the major component of basement membranes.[14] *PREP7* encodes a basic-leucine zipper (bZip) transcription factor[15] with a DNA-binding motif called AT-hook.[16] *PREP8* encodes a protein that is weakly homologous to Sir2, an NAD-dependent deacetylase that links metabolism with longevity in yeast, flies, and worms.[17] Prep9 protein harbors a region that is weakly homologous to extensin, a hydroxyproline-rich cell wall structural glycoprotein of higher plants.[18] The N-terminal region of Prep10 protein bears a domain that has homology to the adenovirus hexon-associated protein IX.[19] Prep11 protein carries in its C-terminus a domain with homology to the flavin adenine dinucleotide (FAD)-binding domain of siderophore-interacting protein which may be involved in iron chelation and iron utilization.[20]

3.3. *Expression pattern of PREP1–PREP11 and proto-oncogene PREP genes in PBMCs*

To determine whether the uncharacterized *PREP1–PREP11* genes are expressed in particular human blood cells, we performed RT–PCR on multiple tissue cDNA (MTC) panels from Clontech (Palo Alto, CA). As shown in Fig. 3, *PREP3*, *PREP4*, and *PREP6* mRNAs were only detected in resting CD14+ cells (monocytes; M), whereas *PREP1* mRNAs were predominantly found in monocytes but were also faintly expressed in resting CD19+ cells (B cells). In addition, *PREP11* showed a strong signal in monocytes and B cells and a weak signal in T-suppressor/cytotoxic cells and activated CD4+ T cells. *PREP2* was strongly detected in monocytes, T-helper/inducer, and B cells, and weakly in T-suppressor/cytotoxic and activated CD4+ cells. *PREP7* was strongly expressed in all mononuclear cells (B cells, T cells, and monocytes), weakly expressed in activated CD8+ T cells, and faintly expressed in activated CD4+, CD19+, and mononuclear cells. *PREP10* was detected strongly in

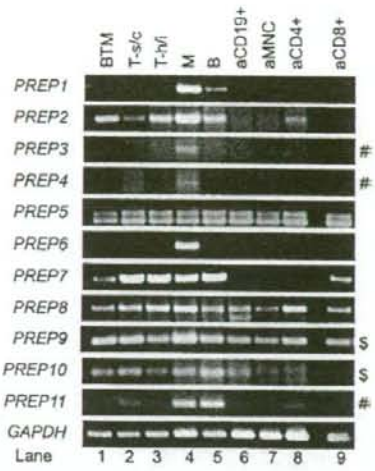

Figure 3. Determination by RT–PCR of the human blood cells that express *PREP1–PREP11*. RT–PCR was performed using a multiple tissue cDNA panel for human blood fractions (MTC, Clontech). *GAPDH* was also amplified as a loading control. PCR amplifications involved 40 cycles at 55°C except as indicated to the right of the panels: 35 cycles at 55°C (#) or 35 cycles at 58°C ($). Lane 1, mononuclear cells (B cells, T cells, and monocytes). Lane 2, resting CD8+ cells (T-suppressor/cytotoxic cells). Lane 3, resting CD4+ cells (T-helper/inducer). Lane 4, resting CD14+ cells (monocytes). Lane 5, resting CD19+ cells (B cells). Lane 6, activated CD19+ cells. Lane 7, activated mononuclear cells (aMNC). Lane 8, activated CD4+ cells. Lane 9, activated CD8+ cells.

all resting mononuclear cells, but only faintly in the other cell types examined. The other *PREP* genes were ubiquitously expressed at almost equal levels in all cell types examined and indeed could serve as a loading control like GAPDH.

Unexpectedly, we found six proto-oncogenes were *PREP* genes, namely, c-*ets*, c-*src*, c-*yes*, c-*fgr*, c-*fos*, and c-*vav*. We initially asked whether the augmented expression of these proto-oncogenes is due to infection-induced T-cell or macrophage stimulation in one of the PBMC donors. To test this, we examined the expression of these proto-oncogenes by RT–PCR on the MTC panel described above, since the sample RNAs used to generate the panel is not the same as our RNAs (Fig. 4). To our surprise, we found all cell types examined strongly expressed c-*ets*, c-*src*, and c-*yes*. All mononuclear cells (B cells, T cells, and monocytes) and activated CD4+ and CD19+ cells, but not activated CD8+ and mononuclear cells, also expressed c-*vav*. In contrast, c-*fgr* was only detected in T-suppressor/cytotoxic cells, while c-*fos* was only detected in resting T-suppressor/cytotoxic cells, B cells, and mononuclear cells. Thus, the expression of these proto-oncogenes is a commonly observed phenomenon.

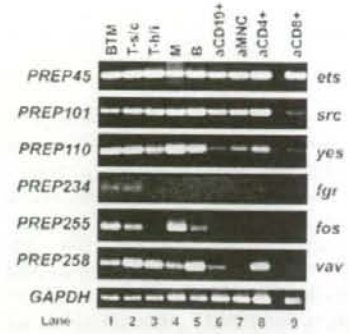

Figure 4. Expression profiles of the indicated proto-oncogenes in human blood fractions. RT–PCR was performed using the multiple tissue cDNA panel (MTC, Clontech). *GAPDH* was also amplified as a loading control. PCR amplifications involved 35 cycles at 55°C for all samples. Lane 1, mononuclear cells (B, T cells and monocytes). Lane 2, resting CD8+ cells (T-suppressor/cytotoxic cells). Lane 3, resting CD4+ cells (T-helper/inducer). Lane 4, resting CD14+ cells (monocytes). Lane 5, resting CD19+ cells (B cells). Lane 6, activated CD19+ cells. Lane 7, activated mononuclear cells (aMNC). Lane 8, activated CD4+ cells. Lane 9, activated CD8+ cells.

3.4. *Focused PREP analysis of CSS PBMCs*

We next examined whether the *PREP* genes can serve in a focused array that can be used to diagnostically. For this purpose, we used the array to determine if CSS patients can be characterized by a particular PREP mRNA expression pattern. CSS is a rare autoimmune disease (ca. 2.4 per million), namely, a small-vessel necrotizing vasculitis that is typically characterized by asthma, lung infiltrates, extravascular necrotizing granulomas, and hypereosinophilia.[21] This disease was chosen because diagnostic gene markers for this disease have not yet been reported. Moreover, it is likely that the symptoms of CSS patients will be reflected in abnormal gene expressions in their PBMCs. Indeed, we found with the aid of expression profiling analysis (GeneSpring) that, compared with normal volunteers, 33 *PREP* genes are consistently up-regulated in the PBMCs of all seven cases of CSS patients tested (Fig. 5A, uppermost panel). Of these, *PREP135*, *PREP77*, *PREP191*, *PREP234*, and *PREP136*, which are highlighted by larger arrows in Fig. 5A, showed the most conspicuous up-regulation in all CSS patients (>2.5-fold change).

PREP135 encodes the coactosin-like protein (CLP), which is a small, evolutionarily conserved F-actin-binding protein that can also bind to 5-lipoxygenase (5LO) and regulate its activity.[22] *PREP77* encodes prosaposin, the precursor of the sphingolipid activator proteins (saposin A, B, C, and D) that are required for the enzymatic hydrolysis of sphingolipids by specific lysosomal hydrolases.[23] *PREP191*, which encodes a lysosomal aspartyl peptidase cathepsin D (CTSD), is known to be involved in prosaposin

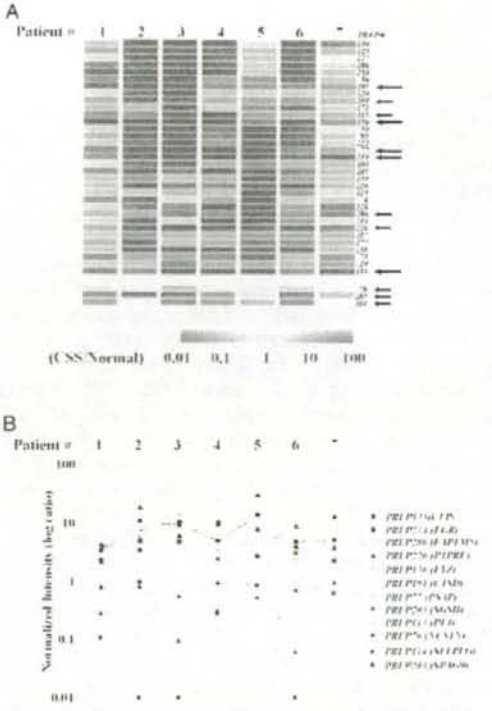

Figure 5. Expression profiles of *PREP* genes in CSS patient PBMCs. Agilent's whole human genome DNA microarray was employed for this analysis. (**A**) Presentation of mosaic tiles for the 33 and 3 *PREP* genes whose expressions are up-regulated (>1.2-fold change) or down-regulated (<1.0-fold change) in all seven CSS patients as compared with healthy volunteers. The gene names are indicated on the right of the panels. The *PREP* genes were selected by a filter and their expressions in all seven cases of CSS patients were grouped together by using GeneSpring software 7.3 (Agilent Technologies, Inc.). In other words, their raw expression values were normalized according to the manufacturer's instructions by conducting a ratio calculation of the two channels. This was followed by a per-gene normalization to ensure all ratios for each gene were normalized to 1. The nine conspicuously up-regulated *PREP* genes (>2.0-fold change) are denoted by arrows; the five large arrows signify more enhanced up-regulation (>2.5-fold change). Tile colors indicate the mean relative transcript level; green corresponds to a log10 ratio of 0.01 (down-regulation), red corresponds to a log10 ratio of 100 (up-regulation), and yellow indicates unchanged (bottom-most panel) when CSS and normal PBMCs are compared (bottom-most panel). (**B**) Graphical presentation of the nine CSS-up-regulated and three CSS-down-regulated *PREP* genes (denoted by arrows in Fig. 5A). Mean values were plotted for the genes that showed a significant ($P < 0.05$) difference in expression. The ordinate scale indicates relative log ratio values.

proteolysis.[24] Thus, the enhanced expression of *PREP77* and *PREP191* suggests sphingolipid levels are increased in CSS. This is consistent with the pivotal role sphingolipids are known to play as proinflammatory factors.[25] Thus, sphingolipids and related enzymes may not only be diagnostic marker for CSS, they may also be novel therapeutic targets in CSS (see Discussion).

PREP234 encodes Gardner–Rasheed feline sarcoma viral (*v*-fgr) oncogene homolog (*c*-fgr: FGR), which is known to play an important role in lipopolysaccharide (LPS)-induced macrophage activation.[26] Indeed, Fgr-deficient mice fail to develop lung eosinophilia in response to repeated challenge with aerosolized ovalbumin (OVA)[27]. Src-family tyrosine kinases are required to support the accumulation of polymorphonuclear leukocytes (PMN) along with adherent platelets at the site of vascular injury.[28] *PREP136* encodes lysozyme, which is one of the genes responsible for autosomal dominant hereditary amyloidosis.[29] Indeed, there are several case reports of amyloidosis associated with CSS that occurs together with a destructive inflammatory or granulomatous reaction to amyloid.[30] Several case reports have also described granulomatous angiitis in patients with sporadic, amyloid beta peptide (Abeta)-related cerebral amyloid angiopathy and Abeta-related angiitis with development of amyloid-associated inflammation.[31]

There are also four other less conspicuously CSS-up-regulated *PREP* genes (2.0–2.5-fold change). Of these, *PREP226* encodes a receptor (membrane)-type variant of the protein tyrosine phosphatase (PTPepsilonM) family protein that negatively regulates insulin receptor (IR) signaling by dephosphorylating IR, thereby suppressing the phosphorylation of IR downstream enzymes such as Akt, extracellular regulated kinase (ERK), and glycogen synthase kinase 3 (GSK3).[32] Notably, the cytosolic isoform of PTPepsilon (PTPepsilonC) selectively inhibits interleukin-6 (IL-6)- and IL-10-induced Janus kinases (JAK)-signal transducer and activator of transcription 3 (STAT3) signaling in blood cells.[33]

Another of the four less conspicuously up-regulated *PREP* genes, *PREP113*, encodes platelet factor 4 (PF4; CXC chemokine ligand 4), which is a specific marker for megakaryocyte cells and that induces the differentiation of monocytes into macrophages during the inflammatory process.[34] *PREP286* encodes lysosomal-associated multi-spanning membrane protein-5 (LAPTM5). While the function of LAPTM5 is unknown, it may participate in host defense in collaboration with other lysosome-related genes (*PREP77*, *PREP191*, and *PREP136*) in the blood cells of CSS patients. These results suggest that the regulation of cell signaling in blood cells from CSS patients is abnormal. The last of the four less conspicuously up-regulated *PREP* genes is *PREP203*, which encodes the spliceosomal protein thioredoxin-like 4B (TXNL4B). However, its physiological significance in the pathogenesis of CSS remains unclear.

We also identified three *PREP* genes that, compared with normal volunteers, are down-regulated in the PBMCs of all seven CSS patients (Fig. 5A, middle panel). *PREP76* encodes nicastrin;[35] its down-regulation may disturb the phagocytic response of macrophages to inflammatory cytokines since gamma-secretase catalyzes the intramembrane cleavage of its protein substrates and targets phagocytosis-related proteins of macrophages.[36] *PREP114* encodes selectin P ligand (SELPLG), which stimulates T lymphocytes, and plays a critical role in the tethering of these cells to activated platelets or endothelia expressing P-selectin.[37] *PREP283* encodes sperm-associated antigen 9 (SPAG9), which has also been identified as a serum target of the autoantibody produced in systemic sclerosis (SSc).[38] Thus, the reduced expression of these three genes could also serve as diagnostic markers of CSS. Unfortunately, we could not correlate the expression levels of these genes to the profiles of tested CSS patients (Supplementary Table S1).

3.5. *Expression pattern of CSS-up-regulated PREP genes in PBMCs*

To examine whether these 12 CSS-up- or -down-regulated *PREP* genes are expressed in particular human blood cells, we performed RT–PCR on MTC panels (Fig. 6). *PREP76*, *PREP135*, and *PREP203* were expressed by nearly all cells, and were the only genes that were expressed by activated CD19+ and activated CD8+ cells. Most of the 12 *PREP* genes were expressed by resting CD14+ cells (monocyte; M) and T-helper/inducer (T-h/i) cells, albeit with different expression levels. All genes except *PREP283* were expressed by T-suppressor/cytotoxic (T-s/c) cells. *PREP76*, *PREP113*, *PREP114*, *PREP135*, and *PREP203* were expressed at varying expression levels by activated mononuclear cells (aMNC). All genes except *PREP114*, *PREP135*, and *PREP283* were expressed by B cells, albeit comparatively weakly. Thus, many of the CSS-up-regulated or -down-regulated *PREP* genes are expressed by T cells or monocytes. Unfortunately, we could not perform the same analysis on CSS patients because of the low numbers of PBMCs available.

4. Discussion

4.1. *Isolation of genes that are predominantly expressed in human PBMCs*

Here we show for the first time (to our knowledge) that RNA diagnostics can be performed by using a

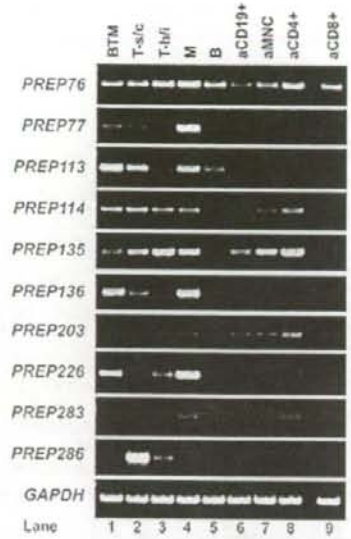

Figure 6. Expression of CSS-up-regulated or -down-regulated *PREP* genes by various human blood cells. RT–PCR was performed with the MTC. The *GAPDH* expression profile served as a loading control. All PCR amplifications involved 35 cycles at 55°C. We could not detect an amplified band for *PREP119* even though we tested three different primer sets (Supplementary Table S1). Lane 1, mononuclear cells (B, T cells and monocytes). Lane 2, resting CD8+ cells (T-suppressor/cytotoxic cells). Lane 3, resting CD4+ cells (T-helper/inducer). Lane 4, resting CD14+ cells (monocytes). Lane 5, resting CD19+ cells (B cells). Lane 6, activated CD19+ cells. Lane 7, activated mononuclear cells (aMNC). Lane 8, activated CD4+ cells. Lane 9, activated CD8+ cells.

PBMC-specific focused array. Such RNA diagnostics methods have the advantage over DNA diagnostics in that ethical problems concerning the genetic information of individuals are avoided. To select the PBMC-specific genes for use in RNA diagnostics, we utilized a technique that we developed previously, namely, stepwise subtraction.[4] In this technique, all genes expressed by a particular cell, tissue, or whole organism are used to generate a cDNA library. This library is then systematically subtracted in a stepwise manner by using the biotinylated mRNAs from a control population to isolate essentially all those genes that are up-regulated relative to the control population expression profile. This technique has proven to be useful for isolating the protein-coding genes that are specifically expressed during cancer metastasis, meiosis, and spermatogenesis, and in patients with autoimmune diseases.[4,39–41] It has also been used to identify putative non-coding RNAs that cannot be identified by microarray screening.[42] By using this technique along with high-density oligonucleotide microarray analysis, we identified 290 *PREP* genes that are predominantly expressed in human PBMCs as compared with fibroblast (TIG-1) cells (Fig. 1 and Table 1). These genes could be used to generate a PBMC-specific focused array that could be used to characterize the abnormal expression profiles in the PBMCs of patients with a particular disease. The abnormal profiles could then serve as a diagnostic tool for other patients presenting with this disease.

The 290 *PREP* genes we identified include 50 uncharacterized genes, 11 of which possess notable amino acid motifs (Fig. 2). Two of these, *PREP1* and *PREP6*, were almost exclusively expressed in monocytes (Fig. 6). Of the 240 *PREP* genes that have been characterized previously, 11, eight, eight, seven, six, five, five, nine, and 36 are HLA genes, CD antigen genes, ribosomal protein genes, interferon (IFN)-related genes, S100 calcium-binding protein-related genes, interleukin (IL)-related genes, platelet-related genes, signal transduction-related genes, and immunity-related genes, respectively (Supplementary Table S4). We did not identify any other immunity-related genes as *PREP* genes by these techniques, probably because their expression levels are lower than the *PREP* genes.

4.2. *PBMC-specific array analysis with CSS patients*

To explore the usefulness of the PBMC-specific focused array analysis in RNA diagnostics, we tested the PBMCs of seven cases of CSS patients.[21] The precise pathogenetic mechanisms of CSS remain elusive, in part because the rarity of this disease. We found that compared with normal volunteers, 22 *PREP* genes were up-regulated by >1.5-fold in the PBMCs of all seven CSS patients (Fig. 5). Of these, *PREP135*, *PREP77*, *PREP191*, *PREP234*, *PREP136*, *PREP203*, and *PREP113* were the most conspicuously up-regulated in CSS patients. How these genes contribute to the pathogenesis of CSS is suggested by the known functions of their products, as follows. *PREP77* (prosaposin) and *PREP135* (CLP) may indicate the enhanced immune responses that cause vascular inflammation; *PREP203* (TXNL4B) and *PREP234* (*c*-fgr) may reflect the augmented intracellular signaling that occurs after an infection causes inflammation; and the increased expression of the lysosome-related genes *PREP77*, *PREP136*, *PREP191*, and *PREP286* may indicate the mounting of an effective defense against invading microorganisms by the lysosomes in blood cells. Thus, our *PREP* gene-focused analysis of CSS patients has helped to identify genes that may be putative diagnostic and/or therapeutic targets.

There are few molecular tools that can be used to aid the diagnosis of CSS. The main tool that is commonly employed is the detection of antineutrophil cytoplasmic autoantibodies (ANCA), which can be

detected by an immunofluorescent assay (IFA) on ethanol-fixed neutrophils. ANCA are associated with systemic necrotizing vasculitis and glomerulonephritis diseases such as CSS, Wegener's granulomatosis (WG), and microscopic polyarteritis (MPA).[43] Two different ANCA immunostaining patterns are observed, namely, a diffuse cytoplasmic staining pattern (c-ANCA) that recognizes proteinase 3, and a perinuclear/nuclear pattern (p-ANCA) that commonly recognizes myeloperoxidase (MPO), which is a neutrophil granule protein that helps generate oxygen radicals and is associated with the antimicrobial properties of neutrophils. The c-ANCA pattern is most frequently observed in WG patients, whereas the p-ANCA pattern is detected in CSS and MPA patients.[44] Notably, ANCAs against alternative antigens such as cathepsin G, which is a major protease released by activated neutrophils, are also occasionally observed.

Since antibodies against cathepsin D (*PREP191*), PTPRE (*PREP226*), FGR (*PREP234*), PF4 (*PREP113*), CLP (*PREP135*), and prosaposin (*PREP77*) are currently commercially available, it may be worthwhile to use these antibodies in IFAs on ethanol-fixed blood cell samples to determine whether these antibodies have a relationship with the ANCA-staining patterns in CSS and whether they can help confirm the diagnosis in ANCA-negative CSS patients. Unfortunately, these experiments will have to be performed in the future because the CSS blood samples we obtained were used up during RNA preparation. Moreover, as CSS occurs only very rarely (only 2.4 patients per 1 million people in Japan), it will take some time to accumulate enough patients for such a study.

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Table S1. Profiles of Churg-Strauss syndrome patients tested in this study.

Patient profile	Case 1 #1	Case 1 #2	Case 2	Case 3	Case 4	Case 5 #1	Case5 #2
Age	33	33	63	54	72	83	83
Gender	Female	Female	Female	Female	Female	Male	Male
Duration of disease	3 months	5 months	1 year	1 month	4 months	2 years and 3 months	2 years and 6 months
Clinical feature	Asthma, mononeuritis multiplexus at both lower extremities, fever >39°C, pulmonary infiltration, pericardial effusion.	Impairment of ventrical muscle (EF decreased from 0.55 at adminstration to 0.34).	Asthma, mononeuritis multiplexus, high fever, abdominal pain, gait disturbance.	Atsthma, purpura at lower extremities, mononeuritis multiplexus, crescent forming glomerular nephritis.	Asthma, mononeuritis multiplexus, edema at lower extemities, pulmonary infiltration.	Asthma, high fever, mononeuritis multiplexus, pulmonary infiltration, gait disturbance were recorded at onset. Only severe numbness at both extremities was remained.	Numbness at both extremities.
Disease activity	Very active phase	Two months after treatment	Remission phase	Active phase	Four months after treatment	Remission phase	Remission phase
WBC (*/mm^3)	16,500	9,600	5,400	12,700	4,300	4,500	5,600
Lympocytes	495	1,497	324	1,016	1,010	1,755	1,904
Eosinophils	1,485	854	0	6,414	60	90	168
CRP (mg/dl)	4.8	0.6	0.1	3.6	0.1	0.13	0.08
IgE (IU/ml)	8,700	59	390	3,530	18	ND	ND
MPO-ANCA (U/ml)	640	36	604→ <10	640	<10	<10	<10
Treatment	Sample was obtained after PSL 60mg/d for 14 days, PSL 50 mg/d for 2 months,	mPSL 500mg IV x 3 times , PSL 30mg/d, azatioprine 50mg/d	mPSL 500mg x 3 IV, PSL 60mg/d, IVIG 20g for 5 days	PSL 30mg/d for 1 month	PSL 40mg/d, EDX 40mg/d were administrated at aother hospital. PSL 10mg/d, EDX 50mg/d	PSL 1mg/d	PSL 1mg/d

All patients except Case 5 were inpatients when their blood samples were examined. Disease activity was described by physician's gross evaluation.
ND: not determined., mPSL: methyl prednisolone, PSL: prednosolone, EF: ejection fruction, EDX: cyclophosphamide, IVIG: intraveneous (high-dose) immunoglobulin therapy

Table S1. List of primers used for RT-PCR.

Gene name	Forward Primer (5'-...-3')	Reverse primer (5'-...-3')
PREP1	ATTCCCAATCCCACCGCAATG	CATCAACAAAAATCCCGTGAGTGGA
PREP3	AAGGCCAAAACGGTGCCTTCA	GTTCCAGCGATCTCCTGCCTCA
PREP4	CATCGGGGACAGCGGACTCTGC	GAGCAGGCTCCCGGCGAAAGG
PREP5	GAACAGACCAGGCGGAAA	AGCAACCTATTCCGCACTTC
PREP6	TGCCCCACACATGAATGACCAC	CGCCGGAAAAATTTTGGCTCGT
PREP7	GATCCGCAGACCGTGTGGTATGA	TGCCGCAGCCAGGTTACACAT
PREP8	GCAGGGCTGTTTGTTTCCAAAG	GCGCCCAGCAAAATAAACACA
PREP9	GCCACAAGTCCTCAAGACCA	TTGCGTTGTCCCATTTTACA
PREP10	CAGACTGGGTGACAGAGCAA	TCAAGTGATCCTCCCACCTC
PREP11	TGGTGGCCACAAAGGACTGTGA	GCTGGTGTCGGATTCCCTGAGA
PREP13	TGCCACGACTAGGGGAAAAAGA	GGTCCCAAACTCACACCCACA
PREP14	TCCGGTCCAGGTCTCTGACTTC	GGGGGAGGGAGTAGAAAAGCAAG
PREP15	TCTAAGGGTAAATTACACATCTCTCAA	GCATCAACCTATAACTTGGTATAAACT
PREP16	TTCACTGGGGAGGTTTTCAG	AGCACATGGTGGGGTAGAAG
PREP17	GACAACAGATCTATGAGTGTTGACCT	CCAGGTTTTCAGAGAGTGAAGC
PREP18	TCTGAGTTTCCAGCCTCGTT	ACGTTCCAAGCATCGTCCTA
PREP23	CTTCAGCCGCCTCGTCGTCAGC	TCAGCCACAGCCGGAAGGAGACC
PREP24	GGGCGCTCCTCAGTGTGTTTGGG	GAAGAGGCCGCACCACACCAGAG
PREP25	GGACCCATGTACCCAGGGACCAG	CCTGAGCGGGCAAGAGGAAAGC
PREP26	AGTACGCCAAGCCACACTTCCAC	TCCAGTCCACTATGCCTCGCTGC
PREP27	TAATTGCGGTTTTTGCCATT	CCCCCAAATATGAAGCACTTT
PREP28	CAGCGGCGTGCAGACCTCTAAA	TTGTTTCCCCGGTGAATACCATCC
PREP29	GCACCGGCAGTGATTGGAACA	AGGTGTCCGGGGATGCTTTGA
PREP30	TTGGCCTCTCAGACCCACTTGC	TGGAATGGCCGACATGAGGAA
PREP31	GGGCCGAGCAATACCTGAAGA	TCCAGGCATACTCAGGGCTCA
PREP32	GCACACACTCCTGTTTCCCTCTG	AAGGCAGCATTCGCCAAGAAG
PREP33	CTACGTGGACGACACGCAGTTC	TGTCACTGCTTGCAGCCTGAG
PREP36	AGCCCCACCTCTCTGGAACAA	TACGCCTACGACGGCAAGGAT
PREP38	AGCAGACGAAGCCATTGCAGA	GCAGAAGAACCTGGGCTGTGA
PREP39	GGTCTGCAGTTGGTTGGTGGA	TGTTCGGCTGCCAATCATCTT
PREP43	TGACGTGTGAGCAGAATGCCTTA	ATGTTCCCCCTTTTCCCCTCA
PREP49	CAAGAAGAAGCCACTGAAACAG	GTACAACAGCTCCAAGACAAC
PREP50	ACGAGAAGAAGCCCACCTGGA	AACGTCAAAGTGGTCGCCAGA
PREP51	CTGCCCAGGAGAAGATGAGCA	TCACGTTTCAGGTTTGCTGGAA
PREP55	GGCTGGTTTCAAACTCCTGA	ATGTGGTTCTGGGGTCATGT
PREP56	TGAAAGTAGAGAGATCTGCCCTGT	CAGCTGGTGGCTTGAGAGA
PREP58	CCACCTTCTTCTCCTGCATC	GCACACACGTAGTAGGCATGG
PREP63	TTCCCCACTTCTTGCAGTCT	CATTTCCCTGATGGTTGATG
PREP65	CAGGGGAAGATTGGTCCTTA	GCTGCTCATAGTCAGAAGAGCAT
PREP66	CACATCCCACCACTCTCCTT	TCTAGGCACCCTCAGGAAGA
PREP68	ATCTGTGAGCCCAAGTCCAG	CGAGAGCTACCACCAGCACT
PREP71	AGGCTGACCAGGGAACCTAT	CATGCAGCACAATGGTTTTC
PREP73	GCCACCATTGTCTCTGTTCA	GACCGTGTGACTCCTGCATA
PREP75	CTGCTTGCTGTGACCACTGT	GGAAGGCTAGCTCCATGTCA
*PREP76**	GCCTGTCTCAGATTGGGATT	AAAGGAAAAAGACAAAAACAAAAA
*PREP77**	CCAGAGCTGGACATGACTGA	GCTTCGAGCACATTTTGTCA
PREP78	ATCTCCGCTGTGACTCCACT	TGGATGGAGCATGATCTGG
PREP80	TCCACCACACTGCAAACAAT	CACTGGATTGGCCATCTTCT
PREP82	ACTATGCGGGCAAGTGCTAT	GTAGGCCACTTCCTCCTCGT
PREP87	TGCCATGTTCAGTTAAAGTGC	TGTAAATGGCTGAACAAATCCA
PREP88	GACTGCTGTCTGCCCTCTCT	TTTGGGATGAGGAAAGGAAA
PREP90	ACTGCAGCTGGAGAGAAAGG	ATTACAGCGGGGGCTTAGTT
PREP91	TCTCAATTTCCATTCCCTTCA	TTTAAATCAACAAACTCATCTTCCTC
PREP92	CGCTCAACTCCCAGAAGAAG	TCCAAAACTTGGGCATCAAT
PREP94	TTGCCAAAGATATGCCATCA	CGCTGTTGCTTTCAGTACCA
PREP95	AGGAAAGTTGCCGCCTTATT	CCATGGATGCAAATGAATGA
PREP96	AAGCCAAGCGAAGCGATGAGC	CGGGTGGCATCGGGTCATATT
PREP101	GAATACCTGGAGGGCAACAA	AGCTGCTCTCGGAGCTGTAG
PREP103	TGATCTTGGCTCACTGCAAG	TTTGGGAGGGTCTCAAGAAG
PREP105	TGGACTTAGTGCCTGTCTGG	GCACAGTGTTTACTAAAGGCACA
PREP107	CGGGACATTGAGAAGAGCAT	GGTGAATCTCGGGGCTATTT
PREP110	CTGCCTTTTCTTTCCAGCAC	GCAGAGGGAATGGCATACAT
*PREP113**	CTGCTGTTCCTGGGGTTG	ATATAGCAAATGCACACACGTAGG
*PREP114**	AAGGGCAGACCTTTCTTTGG	GGGATATTGGTCCGAGGTTT
PREP119	GCCACGAAGATCCTACCAAA	GCTTGGCCCCTACATCATTA
PREP122	GGAGCCCGTCGGTAATTT	AAACGGAAAAGATTCCCAAAA
PREP123	TTTCAAGCGGTTACAAGAGGA	TTTCAAGCGGTTACAAGAGGA

PREP127	AGGAGCAGATCCAGGAGCA	TCTTAAGCTAAGTTGCCAGATATTTA
PREP129	CCGTAGACACTGCCAGAACA	ACATGGGGACAAAATTTCCA
PREP131	CAGCTGGAACGCAACATAGA	CCACAGCCAAGACAGTTTGA
PREP135	ATGACGCTCATGGAAACCTCT	CAGTCTGAGCCGGACCTAAC
*PREP135**	TGCAGCCTTCATAAGCACAC	TTCTTCCCCAAAACGAAATG
PREP136	GATTACAGGCGTGAGCCACT	TTTACATCATGCATAACTTCACATTT
*PREP136**	CAAAACCCCAGGAGCAGTTA	ACACGGTGAAACCCTGTCTC
PREP144	CCGGGAGTCCATCTCCTT	GTGACTCTGATGGCCCAGTT
PREP147	CTTCATCCGGCGACTAGC	AAAAGAGGTTTAGTCAAAAAGACCAA
PREP148	TGGAGGGAACCTGCTGTATC	TCCCCGTTTTACAGGTTCTG
PREP152	GGTCTCAAACATCTCCCATGA	AACATCTTCGGGGCTGAGT
PREP154	AACCAACCACAAGCACACAG	ACCATGGTTTTGCCATCTTC
PREP155	CACCGTAGGCCTGTCTAACC	CACAGAGTCAGCAGCAGCA
PREP156	TGTGGGACACAAAGCACCT	CACTCTTCAGCCGTGAGG
PREP158	CATGTCACCTTAGGTAGTAGGAAGAA	TTACAGCAGATGCCTAGGATATTA
PREP160	ACACTCCAGCTTCTGCATCA	GTCGAAAAGGGAATTCAGTCA
PREP161	CCAAACACTTGTTGCCATTG	AAGGTACCCGCTGACAGAGA
PREP163	CAAAGTCAAGACGACGACCA	AAGGACAGCTCACCAAAGGA
PREP164	GTCCTCACGGTGCCTTTTT	TGCAGTTTAGACACAGCCAAA
PREP169	GGGATTCGTGTCTGGCTCT	TTTGATGACAGGCTTGGGTA
PREP174	CCTCCTGCTGGCTGAACCT	CTTGAGGCTGGAAGTCACCT
PREP180	GGAGGCCTGTAGAAATTGGA	TTGCAGGCTTTTCAACACTG
PREP189	AGCATGCTGACCAGGCAGAAA	TGGTGGATGGCCTTGTCTTCA
PREP190	ACGCCTGTAATCCCAGTGAC	CTCCTGACCTCAGGTGATCC
PREP191	CCCTGGGTTCAGAAATGCT	AAGGGGAGGACAACAGAGGT
*PREP191**	TCTGTTTGGTGGGGGTAGAG	CGGAGGCCAACAACTGTATT
*PREP191**	CAAGGGTTCTCTGTCCTACCTG	TTGAGCGTGTAGTCCTCTGG
PREP196	GGTTGGAGCTGGAGGGGATGGAG	TGCCAGGGGTGACAGGCGTCC
PREP197	GGCTGTAGACAAGGCCAGGGCTG	TCCCGCAGCAAAGCCCAGACAG
PREP198	GCCCCTTCCATGCAGTGTGACAG	TGGACACAGACACTTCCCCAGCC
PREP199	GCCTCCCATGTGCAGACAGTGTG	GCCATCTTCACGACATCCCCTGC
PREP200	TCCTGCGTGCGCGTGTGAGTG	GTGATGTGCGGGCCTCCCTGTC
PREP201	GCTGCAGCTGATGCCTTTA	CACAAGTGTGTGTTCAGGTTCC
PREP202	CCTTCCCACGACAAAAACAA	CCAAATGCTATCCGCAGAGT
PREP203	ACCACCTCTCCAACAGAAGC	CAGGCAGGTCCAGAGTTCAT
*PREP203**	TCCTACTGCCCAAGCTGACT	AGGCTTGCAGGGAGTAGACA
PREP204	CTGACAACACAGGAGCCAAA	GCAAACAAATACTTTTTAATGGGTTTA
PREP205	TTAGTGACGGGGTTCAGGAC	GGCCCAAAGAAACAGTTGAA
PREP206	TGATTTCCCTGGAGGTTCTG	AGGAGAATTTGTGGCACAGC
PREP207	CCCTTGAACTTTGAACCTGTG	AAACCCCTTACAAAAGGCAGT
PREP208	GGATCTGTGACCCCAGCCATGA	TCAGCAGCAGAATGCCCAGAGTC
PREP209	TTCAGGTTGATTGATGTGGGACAGC	TCGGAAAACACCCTCGCACAA
PREP210	GGAGGCATGTGCTGTGCTGTCA	TGTCCACACACCAGCCCAACA
PREP211	ATCGTGGGAGAAAATGCAAC	GCAGACAAAACTGGGTGGAT
PREP212	GATTCTCTTCCGTCGCAGAGTTTCG	TGGTGGCCAGCCTCTTATCTCTCTC
PREP213	TTGCCAGCACCTACCCTGCTCA	GCATGCAAAATTGAAAACCCCATGT
PREP214	CGATCACGCTCATTTACCTGCAATG	CGTCCTGGGAAAAACCTTCTCTGG
PREP215	TCTACCAGGAACGGCAGGAATGC	ACAGATGATGAGCCCCAGCATGA
PREP216	TGGGCAAGCTGTTTCGACCAA	TCCCTTTGCACTCCCCTTCCA
PREP217	ATGCCACCAGTCCCCATTTCT	TGCAAATGCATGAAGGGCTCTC
PREP218	TGCCGTCCCAATGTGACCACT	AAAATCGCAGGGCCTGGTTCA
PREP219	AAATTTCAATCAGGGTTTTTAGAT	TTTCCATACAATCAGAAACAGTAAAA
PREP220	TCTCTCCCAGGCCACAAGACA	TGGTGAAGAACTCAAGCTGAAGAGG
PREP221	TCGTGTGCGGAACCTGCAAAG	CCACTGGCCACCAACAGTTTCC
PREP222	CTCACGCTGGGCAATGTGTTTG	TGGCCCCAGAACTCCCTTCAA
PREP223	AGCATCCTTGGCCGGAAAGTGT	ATGGGGCAAGGTTGCCAGAGA
PREP224	GCAGACATGGGGACCATGAAG	TCTCAGAGCCCAGAAGCCTGA
PREP225	TTACTGGAAAGTTGGCCAGCAGAGG	TGCAAGTCTGCCCCAATGAAGG
PREP226	TGATCGCACCCTCATGTTGCAG	GGGCTGTTTGGGGGCTGTTTT
*PREP226**	ACCAGCCATCGGAGTTACAC	TTTTTCCCCGTCAGTCAATC
PREP227	CTTGTCAACCTCTGGGGTATT	CCTCTCTTGTGACAGGATCAA
PREP228	TCTGGCCTCCGCTGCATACAA	CCCCACTGCTGATGGCATTGA
PREP229	TTGGAAACCTGGGGTGCCTTG	CCATGGGGAGGTTTTGGCTGA
PREP230	TGCCCTCAGGCGTGTCTAGGAA	TGTGTGTCCTTCACGGCAGCA
PREP231	TCTCCCGCCAAGCTGGGATAAG	TGAAGTTCCAGGACCCCAGATGC
PREP232	AAGCAGCCTCCTTGGAGAAAACCTG	CCCGTTGTTTCAGCCTTTCCTCTG
PREP233	AAGCACCAAGAGGCTGCCAGTG	CCATGGCTCTGCCAGGAATCTG
PREP234	GGTGAATGACGGGCTGTGCAA	TGGGGATTGGCAAGGACTGGT
PREP235	GCCCAAAGCATCCCATGGCTA	CCTCCTGCTCATGCCTGTCCAT
PREP236	CTGTCTGCCATGGACCAACAAGC	GCAGCAGGCTGGATTCTTTGGTG
PREP237	TGTGACAATCAAAGGGCGACCA	GACAGGGATTCCTTGGCAACATGA

PREP238	GGGCTTTGTCATTCCCAGAGG	AATCGGCCCTTTTTGGACCTT
PREP239	CCTGGGCAGCCTTCCTGAGAA	CCCACCCCTTGGGGATTCTTG
PREP240	ACTGAAAAAGCCCCAGAGCCACA	TGGGCCAGCAACAACCACAAA
PREP241	AGTTCGGAAGGGGCATTTTGACAC	GGCAAGGCTGGGTTTTGGTGA
PREP242	CCATGCCCGGCTAATTTTTGT	ACAGCCGTGCTTTTCACATCAA
PREP243	CCGTTGCGAATCGATCAGTGG	CCATCAAGGGAGCCTTCCGTTC
PREP244	GGCGTGTGTTTCTGGGCAGACT	TTTTGCCAGTGCAGGGGAGGT
PREP245	CAAAGCGGCCAAGAGCAAATCC	CCTCAACTTGCCACCCCACAGA
PREP246	GCAGGCTCCCTGCCCATAAAAC	ATTCTCGTGAGAGGGGAGGACAGC
PREP247	CAAGCATTTGGCTGGGCTGTG	CCGGTTGTAGCCCAATACCAGCA
PREP248	TTATCCGTCACTCGCCGAGGTTC	TCAAACATGCAACAACGCCACTG
PREP249	TTCGTGGCCCATGTGGAAAGC	GGAATGAGGTCCCCCAAGTTGC
PREP250	TGGCTGCCCTTAACCATCGAGA	TGGGCAAGCCAAGGGTTCTGA
PREP251	TCGCTTCCTGAACTCCGTTCACTC	CCAATTCACCAAGTCAGCCCGTTC
PREP252	TGGGGACACCCAACCACGTTT	GCTGTGAGGCTTCAGGCCAAGA
PREP253	AGCCAACGCCTCCCTGTGAAA	AGGGGAATTGAGGGAGGGCAAG
PREP254	ACCACGATGACCTCCCCCATCT	TCACAGCCTTTCGTGGCAAGC
PREP255	CTGGCGTTGTGAAGACCATGAC	CACAGCCTGGTGTGTTTCACG
PREP256	TCGGTGTCGGAATGTCACAGGAC	TCCACGAGGATCCTGCAACCA
PREP257	TGCAGCCTCACCTCTGAGAAAACC	AGAAGGGACAGGAACTGCGGAGAG
PREP258	GGCCGACATGGGCAAGATTTC	CCACGTAGTTGGCAGGGAACCA
PREP259	ATGTACGGGCCTGCATCGTGA	CACCGAGGCCCAGAGTTTGATG
PREP260	GCTCAAGCGCCTGCAGAACAA	CCACGTTGCCTCTGTCCTGCTT
PREP261	AGCACTGCTGGGACACCTTTG	CTCCCGGGTTCACGCTACTCT
PREP262	CTGGCAGAAGGTTTGCGGAATG	ATGAGGTCACCCCGTTGGAAGG
PREP263	TCTGCACAGCCTGCTCATCACC	GGTCTGAGCATCCAAGGCTCTGTCT
PREP264	GCAGCGACAACTTCGAGGGCTA	CACGAGTCCTCGTCGCCAAATG
PREP265	CGCTCTACCCGGTTCAAGCAT	TAACACGGCCTCCTTCCCAGT
PREP266	GCCCAGGTGCAAAGAGGTCAGA	AAATCCTGCGCATCCGTCCAT
PREP267	TCATGTTGGCCAGGCTGGTCTC	TGCATCAGACTGGGGGCATGT
PREP268	TTCCTACCATGCCCACCAGCA	GCGCACAGCACTTGCGAATCT
PREP269	TCAACGGGGCCAAGATGAAGC	GCTGCATGCCACCCTCATATCC
PREP270	AGTCATGGCGGCAGTTCCACA	GGCTGACTGGGGTTCATTGGTG
PREP271	TCAAGGAGGAACCGGAGCATCA	GCAGCACCCATCTCAGACCACA
PREP272	TACCTGTCGCCGAAGTCCCAGA	GCCGGTGTTTGCAAAGGGAAG
PREP273	TGACATTGCCCCCATGCAAAC	GCTTCCTCTTGCCAGGCCCTTT
PREP274	AGGAGATGATGGCCCAGAAGC	GCTGCACAGCAGCAAAACTCA
PREP275	TGCCGTCATCACTATGGCAGGA	TCGCACGTTGTCGAGGAAGGA
PREP276	TTCCCTTCGGACACTGCTGCTG	ATCCGCTGCCGTAGCCTCAAA
PREP277	GAAGCCGGGAGCTGAACAAACC	AGACCGCAGCATTGCACTCCA
PREP278	ACTCAGGGCAGAGTTCTGAAT	TTTGTCTGGGGCTTAACTTCT
PREP279	GAAGAAACAATGCAAGACATACA	TTCTGAAAACAGTAAGCAGAAAAGA
PREP280	CTGGCGTTGTGAAGACCATGAC	CACAGCCTGGTGTGTTTCACG
PREP281	TCCTCCCACCACACACACATGC	CATGGCCACGTGCTTTCTCTCG
PREP282	GTTCTTATTCCTTTCCTGGAGCAG	TGAGCAAAATGAGCCCGACACA
PREP283	TGCTGTTCAACCTGGCAACATCC	TGGAATGATGAGGCCGTCCAA
PREP284	CCGTGCCCGCCTATTTCAATG	TTCAAAACGCCCCAGCAGGTT
PREP285	TGACATCTCGGTGGTTAGCA	TTTCACAGATACAGAACATAAATCCA
PREP286	TTTGCTTTGGTGGCCCCTGTG	TGAATGGATGGCCAGCGACAG
*PREP286**	GGAGCTGCCTCATAATCTGC	ATGGTTTGGAGGCTAACGTG
PREP287	TCAAGAATGCCCAGCCCCCTA	TTTGCCTTCACACGCACGATTG
PREP288	CATCTCCCGCGGATGCTTTTC	CACCCTCACAAGCTCTGGCACA
PREP289	CCCGCAAAAGGCTACCACGAA	TCACGTCACACCCACGTCCACT
PREP290	TCAAGAATGCCCAGCCCCCTA	TTTGCCTTCACACGCACGATTG

* These primers are used only for MTC analysis (Fig. 6).

Table S3. List of *PREP* genes. Of 290 *PREP* genes, 168, 93 or 29 genes were identified by stepwise subtraction (SS) alone (denoted by s at *Origin* section), by DNA microarray (DM) alone (denoted by d) or by both techniques (denoted by s/d), respectively.

PREP#	*Accession No.*	*Sequence description*	*Origin*
PREP1	NM_019027	RNA-binding protein (FLJ20273)	d
PREP2	NM_022153	chromosome 10 open reading frame 54 (C10orf54)	s
PREP3	NM_018340	hypothetical protein (FLJ11151)	d
PREP4	NM_018071	hypothetical protein (FLJ10357)	s
PREP5	NM_017896	chromosome 20 open reading frame 11 (C20orf11)	s
PREP6	NM_024829	hypothetical protein (FLJ22662)	d
PREP7	AB040942	mRNA for KIAA1509 protein, partial cds	d
PREP8	BX355535	FLJ22586	s
PREP9	BC011905	cDNA clone IMAGE:4121832	s
PREP10	AB002384	mRNA for KIAA0386 gene, partial cds	d
PREP11	NM_004848	chromosome 1 open reading frame 38 (C1orf38)	d
PREP12	NM_018121	chromosome 10 open reading frame 6 (C10orf6)	s
PREP13	NM_020141	chromosome 1 open reading frame 119 (C1orf119)	s
PREP14	BC105283	cDNA clone IMAGE:4067106	s
PREP15	BC045174	cDNA clone IMAGE:5273245	s
PREP16	XM_498893	LOC440853	s
PREP17	NM_015054	KIAA0701 protein (KIAA0701)	s
PREP18	NR_001459	chromosome 14 open reading frame 62 (C14orf62)	s
PREP19	AK026539	FLJ22886	s
PREP20	NM_173473	chromosome 10 open reading frame 104 (C10orf104)	s
PREP21	AL050204	cDNA DKFZp586F1223	s
PREP22	XM_935088	similar to protein immuno-reactive with anti-PTH polyclonal antibodies, transcript variant 4 (LOC645784)	s
PREP23	NM_014732	KIAA0513	s
PREP24	BC112359	cDNA clone IMAGE:40032542	s
PREP25	AK025515	FLJ21862, highly similar to AF052101 Homo sapiens clone 23872	s
PREP26	NM_017439	hypothetical protein (LOC54103)	s
PREP27	AL512690	cDNA DKFZp761L0916	s
PREP28	NM_018169	hypothetical protein (FLJ10652)	d
PREP29	AB029488	C11orf21 mRNA, complete cds	d
PREP30	AK097080	cDNA FLJ39761, clone SPLEN1000083	d
PREP31	NM_006817	endoplasmic reticulum protein 29 (ERP29)	s
PREP32	AK096577	sortilin-related receptor, L(DLR class) A repeats-containing (SORL1)	s
PREP33	D38525	major histocompatibility complex, class I, A (HLA-A)	s
PREP34	NM_021109	thymosin, beta 4, X-linked (TMSB4X)	s
PREP35	BC070140	ADP-ribosylation-like factor 6 interacting protein 6 (ARL6IP6)	s
PREP36	AB088110	HLA-B gene for major histocompatibility complex, class I, B (HLA-B)	s
PREP37	AB208814	cysteine-tRNA ligase isoform b variant protein	s
PREP38	NM_052862	RCSD domain containing 1 (RCSD1)	s
PREP39	AK091882	sterile alpha motif domain containing 3 (SAMD3)	s
PREP40	NM_004872	transmembrane protein 59 (TMEM59)	s
PREP41	NM_032349	nudix (nucleoside diphosphate linked moiety X)-type motif 16-like 1 (NUDT16L1)	s
PREP42	BC041056	ribonuclease T2 (RNASET2)	s
PREP43	BC015929	nuclear receptor subfamily 1, group D, member 2 (NR1D2)	s
PREP44	DQ147772	apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like 3G (APOBEC3G)	s
PREP45	NM_005238	v-ets erythroblastosis virus E26 oncogene homolog 1 (avian) (ETS1)	s
PREP46	NM_002121	major histocompatibility complex, class II, DP beta 1 (HLA-DPB1)	s/d
PREP47	AF004230	monocyte/macrophage Ig-related receptor MIR-7 (MIRcl-7)	s
PREP48	AY645741	major histocompatibility complex, class I, E (HLA-E)	s
PREP49	CR598431	GRB2-related adaptor protein 2 (GRAP2)	s
PREP50	AK022481	PIAS4: Protein inhibitor of activated STAT, 4 (PIAS4)	s
PREP51	NM_004779	CCR4-NOT transcription complex, subunit 8 (CNOT8)	s
PREP52	AK056420	ATPase type 13A1 (ATP13A1)	s
PREP53	NM_015013	amine oxidase (flavin containing) domain 2 (AOF2), transcript variant 2	s
PREP54	NM_019613	WDR45-like (WDR45L)	s
PREP55	NM_015553	phosphoinositide-binding protein PIP3-E (PIP3-E)	s
PREP56	NM_000397	cytochrome b-245, beta polypeptide (chronic granulomatous disease) (CYBB)	s
PREP57	NM_152280	synaptotagmin XI (SYT11)	s
PREP58	NM_138373	myeloid-associated differentiation marker (MYADM)	s
PREP59	NM_002357	MAX dimerization protein 1 (MXD1)	s
PREP60	AL833186	solute carrier family 25, member 37 (SLC25A37)	s
PREP61	AK097266	Apolipoprotein L, 6 (APOL6)	s

PREP62	BC032312	transforming growth factor beta regulator 1 (TBRG1)	s
PREP63	AK022008	Notch homolog 2 (Drosophila) (NOTCH2)	s
PREP64	NM_016025	DORA reverse strand protein 1 (DREV1)	s
PREP65	NM_016576	guanosine monophosphate reductase 2 (GMPR2)	s
PREP66	NM_005475	lymphocyte adaptor protein (LNK)	s
PREP67	NM_030935	TSC22 domain family, member 4 (TSC22D4)	s
PREP68	NM_006291	tumor necrosis factor, alpha-induced protein 2 (TNFAIP2)	s
PREP69	M15007	T-cell receptor germline gamma-chain (TCRGC1)	s
PREP70	BC007673	lymphocyte cytosolic protein 1 (L-plastin) (LCP1)	s/d
PREP71	NM_153206	adhesion molecule, interacts with CXADR antigen 1 (AMICA1)	s/d
PREP72	NM_002107	H3 histone, family 3A (H3F3A)	s
PREP73	NM_012455	pleckstrin and Sec7 domain containing 4 (PSD4)	s
PREP74	NM_153374	LysM, putative peptidoglycan-binding, domain containing 2 (LYSMD2)	s
PREP75	NM_006332	interferon, gamma-inducible protein 30 (IFI30)	s
PREP76	NM_015331	nicastrin (NCSTN)	s
PREP77	NM_002778	prosaposin (variant Gaucher disease and variant metachromatic leukodystrophy) (PSAP)	s
PREP78	AY148100	colony stimulating factor 3 receptor (granulocyte) (CSF3R)	s
PREP79	NM_004840	Rac/Cdc42 guanine nucleotide exchange factor (GEF) 6 (ARHGEF6)	s
PREP80	NM_201623	C-type lectin domain family 12, member A (CLEC12A)	s
PREP81	NM_001556	inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase beta (IKBKB)	s
PREP82	NM_017859	uridine kinase-like 1 (URKL1)	s
PREP83	NM_004877	glia maturation factor, gamma (GMFG)	s
PREP84	NM_002923	regulator of G-protein signalling 2, 24kDa (RGS2)	s
PREP85	NM_002095	general transcription factor IIE, polypeptide 2, beta 34kDa (GTF2E2)	s
PREP86	NM_000634	interleukin 8 receptor, alpha (IL8RA)	s
PREP87	NM_003340	ubiquitin-conjugating enzyme E2D 3 (UBC4/5 homolog, yeast) (UBE2D3)	s
PREP88	NM_004084	defensin, alpha 1 (DEFA1)	s
PREP89	NM_000655	selectin L (lymphocyte adhesion molecule 1) (SELL)	s
PREP90	NM_004131	granzyme B (granzyme 2, cytotoxic T-lymphocyte-associated serine esterase 1) (GZMB)	s/d
PREP91	NM_003564	transgelin 2 (TAGLN2)	s
PREP92	NM_003730	ribonuclease T2 (RNASET2)	s
PREP93	NM_000558	hemoglobin, alpha 2 (HBA2)	s
PREP94	NM_002432	myeloid cell nuclear differentiation antigen (MNDA)	s/d
PREP95	NM_014381	mutL homolog 3 (E. coli) (MLH3)	s
PREP96	NM_002661	phospholipase C, gamma 2 (phosphatidylinositol-specific) (PLCG2)	d
PREP97	NM_018434	ring finger protein 130 (RNF130)	s
PREP98	AK090873	paraspeckle component 1 (PSPC1)	s
PREP99	NM_023018	NAD kinase (NADK)	s
PREP100	NM_002872	ras-related C3 botulinum toxin substrate 2 (rho family, small GTP binding protein Rac2) (RAC2)	s
PREP101	NM_004383	c-src tyrosine kinase (CSK)	s
PREP102	NM_000518	hemoglobin, beta (HBB)	s/d
PREP103	NM_019034	ras homolog gene family, member F (in filopodia) (ARHF)	s
PREP104	NM_001928	complement factor D (adipsin) (CFD)	s/d
PREP105	NM_007162	transcription factor EB (TFEB)	s
PREP106	NM_002201	interferon stimulated exonuclease gene 20kDa (ISG20)	s/d
PREP107	NM_001157	annexin A11 (ANXA11)	s
PREP108	NM_006363	Sec23 homolog B (S. cerevisiae) (SEC23B)	s
PREP109	NM_003870	IQ motif containing GTPase activating protein 1 (IQGAP1)	s
PREP110	NM_002350	v-yes-1 Yamaguchi sarcoma viral related oncogene homolog (LYN)	s/d
PREP111	CR626993	fibrinogen-like protein 2 (FGL2)	s
PREP112	AY323826	interleukin 10 receptor, beta (IL10RB)	s
PREP113	NM_002619	platelet factor 4 (chemokine (C-X-C motif) ligand 4) (PF4)	s/d
PREP114	NM_003006	selectin P ligand (SELPLG)	s
PREP115	NM_001800	cyclin-dependent kinase inhibitor 2D (p19, inhibits CDK4) (CDKN2D)	s
PREP116	NM_001000	ribosomal protein L39 (RPL39)	s
PREP117	NM_005620	S100 calcium binding protein A11 (calgizzarin) (S100A11)	s
PREP118	NM_175709	chromobox homolog 7 (CBX7)	s
PREP119	NM_001803	CD52 antigen (CAMPATH-1 antigen) (CD52)	s/d
PREP120	NM_001287	chloride channel 7 (CLCN7)	s
PREP121	NM_015254	kinesin family member 13B (KIF13B)	s
PREP122	NM_005194	CCAAT/enhancer binding protein (C/EBP), beta (CEBPB)	s
PREP123	NM_003337	ubiquitin-conjugating enzyme E2B (RAD6 homolog) (UBE2B)	s
PREP124	NM_006472	thioredoxin interacting protein (TXNIP)	s
PREP125	NM_000987	ribosomal protein L26 (RPL26)	s
PREP126	NM_002727	proteoglycan 1, secretory granule (PRG1)	s/d
PREP127	NM_005385	natural killer-tumor recognition sequence (NKTR)	s
PREP128	NM_002704	pro-platelet basic protein (chemokine (C-X-C motif) ligand 7) (PPBP)	s/d