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Biomarkers in Systemic Juvenile Idiopathic Arthritis: A comparison with biomarkers in Cryopyrin Associated Periodic Syndromes

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Abstract

Purpose of review—This review summarizes biomarkers in Systemic Juvenile Idiopathic Arthritis (sJIA). Broadly, the markers are classified under protein, cellular, gene expression and genetic markers. We also compare the biomarkers in sJIA to biomarkers in cryopyrin associated periodic syndromes (CAPS).

Recent findings—Recent publications showing the similarity of clinical response of sJIA and CAPS to anti IL1 therapies prompted a comparison at the biomarker level.

Summary—sJIA traditionally is classified under the umbrella of juvenile idiopathic arthritis. At the clinical phenotypic level, sJIA has several features that are more similar to those seen in Cryopyrin Associated Periodic Syndromes (CAPS). In this review, we summarize biomarkers in sJIA and CAPS and draw upon the various similarities and differences between the two families of diseases. The main difference between sJIA and CAPS biomarkers are genetic markers with CAPS being a family of monogenic diseases with mutations in *NLRP3*. There have been a small number of publications describing cellular biomarkers in sJIA with no such studies described for CAPS. Many of the protein markers characteristic of sJIA are also seen to characterize CAPS. The gene expression data in both sJIA and CAPS show a strong upregulation of innate immunity pathways. In addition, we describe a strong similarity between sJIA and CAPS at the gene expression level where several genes that form a part of the erythropoiesis signature are upregulated in both sJIA and CAPS.

Keywords

Biomarkers; Cellular Markers; Cryopyrin Associated Periodic Syndromes; Macrophage Activation Syndrome; Systemic Juvenile Idiopathic Arthritis

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Introduction

Systemic juvenile idiopathic arthritis (sJIA) is classified as a subtype of Juvenile Idiopathic Arthritis (JIA). The emerging consensus in pediatric rheumatology is that sJIA is not a “classic” autoimmune disease, and therefore should be viewed as a distinct autoinflammatory syndrome. Indeed, many clinical features of sJIA like fever, skin rashes and multisystem involvement are similar to those seen in autoinflammatory syndromes including Familial Mediterranean Fever (FMF) and Cryopyrin-Associated periodic syndromes (CAPS), a family of syndromes that includes MWS, NOMID and FCAS. As in autoinflammatory syndromes, patients with sJIA are at risk for amyloidosis. The most beneficial therapeutic strategies in sJIA are aimed at neutralization of IL1 and IL6 rather than TNF α , similar to CAPS.

There is a considerable body of work studying biomarkers in sJIA and CAPS. In this review, we focus on protein, cellular, mRNA and DNA markers in sJIA and similarities and differences in the molecular markers that have characterized these diseases. Is there a corresponding correlation between biomarkers in sJIA vs biomarkers in CAPS?

Serum biomarkers

Since sJIA is a systemic disease, one could infer that there might be molecular markers in circulation that either associate with disease severity/activity and/or disease prognosis. There have been several publications describing biomarkers in circulation. Some of the serum markers reported for sJIA are proteins involved in innate immunity like IL6[1], IL18[2] as well as those associated with neutrophil activation. We describe below key protein biomarkers in serum which appear to correlate with disease activity.

Ferritin

In many inflammatory diseases such as rheumatoid arthritis or lupus, ferritin levels rise moderately in parallel with other inflammatory acute phase reactants. In sJIA, however, ferritin levels may be extremely high and exceed 1,000 ng/mL (normal range 7-140 ng/mL) [3;4] and may be helpful in establishing the diagnosis of sJIA. The reasons for extreme hyperferritinemia in sJIA are not clear. The fact that many patients with active sJIA without macrophage activation syndrome (MAS) may still have ferritin levels exceeding 1,000 ng/ml suggests that there may be a distinct inflammatory pathway unique to this disease. Consistent with this idea, ferritin has been shown to have both pro- and anti-inflammatory properties and may serve as an important immunomodulator although the exact pathogenic role of ferritin in these pathways still needs to be elucidated. When complicated by MAS, levels of ferritin are strikingly high [2]. Interestingly in contrast to sJIA, no increase in ferritin levels was noted in a study of acute phase response in FMF [5;6]. In addition, there are no reports of increased ferritin levels in CAPS.

S100A8, S100A9, and S100A12

S100A8 (alias: MRP8), S100A9 (alias: MRP14) and S100A12 are typically secreted during activation of neutrophils and monocytes. S100A8/A9 form a complex that can serve as an endogenous TLR (toll-like receptor) agonist and trigger TLR4 signalling pathways [7]

leading to production of proinflammatory cytokines including IL1- β . S100A12 can also activate human monocytes via TLR4 [8;9]. Strikingly high levels of the calcium-binding proteins S100A8/A9 and S100A12 are characteristic of active sJIA and appear to distinguish sJIA from many other febrile illnesses, including systemic infection, various forms of leukemia, and Kawasaki disease [10;11]. Highly elevated S100 proteins are a feature that may be shared with other auto-inflammatory syndromes such as FMF and CAPS [12].

S100A8/9 serum concentrations correlate closely in response to drug treatment and disease activity and therefore might be an additional measurement for monitoring anti-inflammatory treatment of individual patients with sJIA. During clinically inactive disease, S100A8/9 serum concentrations are reported to be one of the first predictive biomarkers indicating subclinical disease activity. Serum levels of S100A8-S100A9 above 740 ng/ml predicted diseases flares with 92% sensitivity and 88% specificity [13].

Interleukin 18

IL18 is a unique cytokine in the IL1 family, and is constitutively present in keratinocytes, epithelial cells and blood monocytes. IL18 induces production of IFN γ by NK cells and T cells as well as TNF α and chemokine secretion by macrophages. The most commonly used ELISA for circulating IL18 detects about 100 pg/mL in the serum of healthy humans, whereas in diseases characterized by systemic inflammation, the levels are elevated, several fold. CAPS is associated with elevated function of the NLRP3 inflammasome which in turn results in the activation of caspase-1, cascading to increased levels of IL18 [14]. Levels of IL18 in CINCA patients, the most severe form of CAPS, are about three fold higher than in healthy subjects (unpublished data). In sJIA, serum IL18 levels are elevated out of proportion compared with other cytokines in distinct contrast to CAPS as well as other diseases such as rheumatoid arthritis, sepsis etc. Particularly when complicated by MAS, levels of circulating IL18 can be elevated several more than tenfold. Although levels of free IL18 correlate strongly with underlying disease activity in sJIA and decrease with clinical remission, in many sJIA patients it remains elevated even during remission which may be predictive of upcoming flare of sJIA [15]. In particular, the S100 proteins and the IL18 protein markers seem to correlate particularly well with disease status of active vs inactive disease [16]. A list of protein markers observed in sJIA and CAPS is shown in Table 1[2-4;10-12;17-22].

Cellular Biomarkers in sJIA

The analysis of cellular biomarkers in sJIA patients, such as leukocyte subset composition and activation or differentiation state of these subsets is scarce. Macaubas *et al.* [23;24] provided recent reports on lymphocyte subsets comprising monocytes, dendritic cells, NK cells, α/β and γ/δ T cells, and B cells in quiescent and active disease stages in sJIA patients. The authors reported that the relative abundance of T and B cells amongst the mononuclear cells appears lower in flaring sJIA patients compared to age-matched healthy controls.

Two other studies assessed the frequency of Th1 and Th17 T cells in sJIA patients. While Omoyinmi *et al.* [25] found an increased frequency of circulating Th1 and Th17 cells in sJIA patients compared to age-matched controls and regardless of their sJIA status, Lasieglie

et al. [26] did not find a difference in the frequency of Th17 cells in sJIA patients. In contrast, the latter study found that Th17 cells were more abundant in CAPS patients prior to treatment with an IL1 antagonist.

Cells of the myeloid lineage appear to be the prominent players in sJIA, and Macaubas *et al.* [23] performed a more in depth analysis of monocyte subsets. While the abundance of CD14+ monocytes was significantly higher in patients experiencing flare vs healthy controls of quiescent sJIA individuals, the relative abundance of “conventional” CD14++ monocytes or “inflammatory” CD14+ CD16+ monocytes was not different between disease state and healthy controls. However, monocytes from sJIA patients, irrespective of disease state, expressed significantly higher levels of CD16 and CD14 than healthy controls. This finding was replicated in a subsequent study which assessed the polarization state of the monocyte populations in sJIA patients with quiescent and active disease, respectively [24]. CD16 is believed to be expressed by monocytes or macrophages which have a more inflammatory M1 phenotype while CD14 is upregulated on monocytes which appear to have a more anti-inflammatory M2 gene expression profile [27]. The same study by Macaubas *et al.* [24] presented a more refined analysis for monocyte/macrophage surface markers indicative for M1 or M2 polarization. They reported increased expression of the prototypic M1 markers CD40 and CD80 on CD14++CD16– and CD14+CD16+ monocyte population in patients with active disease compared to blood monocytes from quiescent disease state or healthy controls. Intriguingly, 90% of the monocyte population with increased M1 markers in active disease also expressed the CD163 and CD209 surface markers which are associated with an M2 phenotype. Thereby, monocytes from patients in an active disease state appear to have a mixed phenotype reflecting an inflammatory state and at the same time the induction of counterbalancing anti-inflammatory pathways. Another recent study on cellular biomarkers within the RAPPORT trial assessing Rilonacept in sJIA reported a positive correlation between the expression of the M2-specific transcription factor KLF-4 with active disease [28], supporting the previous notion of an in-part anti-inflammatory phenotype of blood monocytes in active sJIA.

The surprising finding from the cellular studies focusing on the monocyte as one of the innate effector cells with a potential major involvement in the pathophysiology of sJIA is that the expression of typical activation markers, such as CD86 and HLA-DR were not different between disease states or increased over the control cohort. Though stimulated production of intracellular pro-IL1 β is clearly higher in sJIA samples compared to healthy controls, the release of mature IL1 β seems to be lower. This might be related to the partial polarization of the sJIA monocytes towards an anti-inflammatory M2 transcriptional program. Thus, monocytes may constitute a regulatory cell type in sJIA counteracting the action of inflammatory mediators potentially secreted by other cell types, such as neutrophils, lymphocytes or endothelial cells. It remains to be seen whether this peculiar monocyte phenotype reflects a physiological response to inflammation orchestrated by a different cell type or an intrinsic physiological or genetic defect.

Gene expression profiling

There are several publications that describe the gene expression profiling analysis of sJIA patients, usually in PBMCs [4;29-31]. In general, a majority of the differentially expressed genes in active sJIA appear to be upregulated rather than downregulated when compared to either healthy controls or inactive disease [31]. There is substantial evidence that IL1 plays a major role via the disruption of innate immunity in systemic JIA [32;33]. Genes involved in innate immune activation like the components of the IL1, IL18 and TLR signaling pathways are seen to be upregulated in sJIA relative to healthy controls. In addition, some genes comprising the IL1 inflammasome are also upregulated and there is also upregulation of several neutrophil-related transcripts [31;34]. This concept is further substantiated by the fact that the response to anti IL1 β , anti IL6 and anti-IL1R therapies is relatively high in sJIA [34-36]. A list of genes differentially expressed in sJIA (from [29]) can be found in the supplementary material [29].

Comparison of the gene expression profiles of the various forms of JIA in PBMCs relative to healthy controls [30] showed the overexpression of several pathways, with sJIA having the maximum number of dysregulated genes, and therefore, pathways. Chief among these pathways were the IL10 signaling and innate immunity pathways. However, natural killer (NK) cell, T cell, and antigen-presentation pathways were downregulated in sJIA.

To determine what aspects of the gene expression changes in sJIA versus controls are unique to sJIA, Allantaz et al. compared differentially expressed genes in sJIA and in infectious diseases like those caused by *S. Aureus*, *S. Pneumoniae* and other infections using SLE and PAPA syndrome as negative controls. The comparison of the gene expression profiles shows that about 88 transcripts [29] were significantly and specifically dysregulated in sJIA. Applying more stringent cutoffs led to a list of 12 genes which were specific to sJIA and could distinguish, with high sensitivity) sJIA patients from the others. Six of these genes have no known function, while the others are involved in various processes relating to transport, nuclear mRNA splicing, etc.

A comparison of the upregulated genes in sJIA with those in other inflammatory diseases shows the highest similarity between sJIA and CINCA (or NOMID) relative to SLE, polyarticular JIA and Kawasaki disease (KD). Ogilvie *et al.* [31] report that of the ~285 genes that are upregulated in sJIA, about 35 genes are seen to be upregulated in CINCA as well, followed by sJIA and KD with ~17 overlapping genes followed by much weaker overlap with SLE or polyarticular JIA. Conversely, analysis of the gene expression profiles of CAPS indicates a list of about 60 genes that are also upregulated in sJIA [37]. A list of differentially expressed genes in CAPS relative to healthy controls (from [38]) can be found in the supplementary material.

Comparison of differentially expressed genes in CAPS [38;39] to those in sJIA identified a list of common genes shown in Table 2 in more detail. One striking feature of this list of genes is that several of these genes are seen in immature cells. For example, ALAS2, a gene which is highly upregulated in both sJIA and CAPS is an enzyme critical to heme synthesis and is very active in immature cells. Other examples include HB1, HB2, HBG and AQP9.

Hematopoietic stem cells in the bone marrow give rise to two major progenitor cell lineages, myeloid and lymphoid progenitors (Figure 1). Hinze *et al.* [37] have noted that unusual cell populations such as early myeloid progenitors distinguish sJIA from other types of JIA and other febrile illnesses. They developed a 67-gene signature for erythropoiesis (supplementary material from [37]). Our comparison of the list of genes shown in Table 2 shows that 17 of the 67 genes are seen to be highly upregulated in CAPS while in sJIA, the enrichment of this erythropoietic signature is much higher (it is to be noted that this signature was characterized based on earlier work on the gene expression profiles in sJIA [4], hence the high enrichment of genes differentially expressed in sJIA is to be expected). This gene signature enrichment could be a reflection of the severity of the inflammatory processes in these diseases resulting in relatively higher populations of the immature and monomyelocytoid cells in both sJIA and CAPS PBMCs.

Genetic markers

There have been several studies in sJIA cohorts that attempt to find genetic risk factors for sJIA. Associations between gene variants encoding inflammasome-related proteins and sJIA have been reported in *IL6*, *IL18* and the *IL1* family of genes; most of these associations reached borderline significance [40]. Although in other JIA subtypes, many risk loci have been identified, the results in sJIA have been disappointing. In a recent genome wide association meta-analysis only a weak association was found with the 3 Mb interval that contains a range of genes involved in both innate and adaptive immunity including *BTNL2*.

However, the general consensus in the field is that sJIA is a polygenic disease and it is likely that further research could uncover alleles in additional genes that may contribute to inherited risk factors in sJIA. In CAPS, however, unlike sJIA, there is a clear link to gain of function mutations in *NLRP3* [41] which lead to constitutive IL1b secretion.

Macrophage activation syndrome (MAS), a life threatening complication of sJIA, is caused by excessive activation and expansion of T cells and CD163+ hemophagocytic macrophages. Due to their highly activated status, these cells shed off some of their receptors including *CD163* and *IL2Ra* chains. As a result, in MAS, serum levels of soluble *CD163* and soluble *IL2Ra* chains appear to reflect the degree of activation and expansion of T cells and macrophages, respectively, and may serve as biological markers that can help with the diagnosis and assessment of treatment responses [18]. Another potential MAS biomarker is neopterin, a catabolic product of guanosine triphosphate that is produced by IFN stimulated macrophages. One recent report suggested that highly increased levels of neopterin could distinguish patients with MAS from the patients with active sJIA without MAS features [19;19]. Another recent report described significantly elevated levels of follistatin-like protein 1 in MAS compared to a conventional sJIA flare[42]. Rapidly increasing serum ferritin is a feature that raises the suspicion for MAS, but a significant proportion of sJIA patients without MAS features may have highly elevated levels of ferritin as well [4]. When the diagnosis of MAS is established, however, serum ferritin levels are traditionally used to gauge the response to treatment. There have been association studies in MAS patients where variants in perforin and MUNC genes appear to associate with the propensity to develop MAS [43;44].

Another intriguing aspect of sJIA is the biphasic course of the disease. Typically, sJIA begins with a highly-inflammatory febrile phase that in the majority of patients converts over time to a febrile chronic arthritic phase. It has been recently suggested that while the systemic phase of the disease is indeed driven mainly by innate immune mechanisms, during the chronic arthritic stage, abnormalities in adaptive immunity may contribute as well. One example of such abnormalities is the emergence of autoreactive Th17 T cells [45]. Therefore, one important direction of future research in sJIA is the identification of biomarkers that would distinguish the two phases.

One of the challenges in diseases like sJIA is that the disease activity is still scored based on a mixture of objective (like presence of fever and a number of active joints) and subjective measures (physician's assessment score like ACR or DAS). In order to assess disease activity in a standardized manner, targeted studies aimed at the discovery of molecular markers predictive of disease state will simplify disease assessment and could lead to further discovery of markers predictive of disease flares, MAS as well as the ability to taper treatment for the patient when there is confidence that the patient has inactive disease. One or more of the markers described in this review could indeed be part of the biomarker solution that can assist the physician to determine when and how to initiate or stop treatment of sJIA in a patient.

Conclusion

A survey of biomarkers in sJIA and a comparison with known markers in CAPS shows that in addition to shared clinical phenotypes, there are common molecular markers as well. In particular, protein markers like IL18, S100 proteins are upregulated in both sJIA and CAPS. Gene expression profiling shows an upregulation of innate immunity pathways which points to similar inflammatory processes driving both disease types. For example, there is a good correspondence between the elevated level of total IL18 protein in serum and elevated transcript levels of IL18. Similarly, we observe elevated levels of S100A8/9 and S100A12 both at the gene and the transcript level in sJIA and CAPS patients. Interestingly, upon comparison of the differentially regulated genes in the microarray data, an erythropoietic signature is shared between CAPS and sJIA, although to different extents. Cellular biomarker research on immune cells, in contrast, may provide more insight into disease pathophysiology of sJIA by assessing the physiological characteristics of blood leukocytes. There is no work published to date on cellular markers in CAPS. We can also point to some distinct differences as well, notably the much higher incidence of macrophage activation syndrome in sJIA, which is a much rarer event for CAPS patients, resulting in higher levels of ferritin as well as other markers like CD163, in sJIA. Further research into the similarities and differences in the molecular background between sJIA and CAPS could potentially help clinicians design optimal treatment strategies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Key Points

- Serum biomarkers, such as ferritin, IL18, and S100 proteins are specifically correlated with macrophage activation syndrome (MAS), systemic juvenile idiopathic arthritis (sJIA), or cryopyrin associated periodic syndrome (CAPS), respectively.
- The polarization status of peripheral blood monocytes in sJIA patients appears to correlate with disease activity, suggesting in part, an anti-inflammatory role.
- Transcriptional profiling of peripheral blood mononuclear cells revealed common dysregulated transcripts and pathways, but also distinct differences between sJIA and CAPS patients.
- MAS is a clinical complication of sJIA and other autoinflammatory or autoimmune diseases which is characterized by a distinct serum biomarker profile.

Figure 1. Unusual cell populations in peripheral circulation in active sJIA

Hematopoietic stem cells in the bone marrow give rise to two major progenitor cell lineages, namely myeloid and lymphoid progenitors. Some of the signatures distinguishing sJIA from other febrile illnesses reflect the appearance of early myeloid progenitors (red circle) in peripheral circulation (see text).

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Table 1

A list of protein markers in SJIA and CAPS. Original table.

Biomarker	SJIA	SJIA/MAS	FMF & CAPS
S100A8/A9	↑↑↑ ¹		↑↑↑ [11]
S100A12	↑↑↑		↑↑↑ [11]
IL6	↑		↑
IL18	↑↑↑ ²		↑
Ferritin	↑↑	↑↑↑	↑ in FMF
sIL2Rα		↑↑↑	
sCD163		↑↑↑	
Neopterin		↑↑↑	

¹ During clinical remission, increased serum levels predict disease flare

² More elevated in a subgroup of patients with active disease

Table 2

List of genes differentially expressed in both SJIA [21] and CAPS [30]. Table is from reference 19.

Gene	Probeset	Fold Change	p-value
FCGR1A	216950_s_at	2.74	3.50E-05
FCGR1B	214511_x_at	2.8	0.00014
S100A12	205863_at	2.23	0.000149
SLC4A1	205592_at	14.96	1.00E-06
TNS1	221748_s_at	4.17	1.00E-06
TPM1	206116_s_at	3.25	1.00E-06
BCL2L1	206665_s_at	3.26	5.50E-05
SNCA	204466_s_at	6.9	1.00E-06
ADIPOR1	217748_at	2.47	7.20E-05
AMY1A	208498_s_at	-2.17	0.000128
AQP9	205568_at	3.63	8.10E-05
C5orf4	220751_s_at	4.58	1.00E-06
CA1	205950_s_at	11.64	1.00E-06
CLU	208792_s_at	5.25	1.00E-06
FECH	203116_s_at	2.48	0.000558
GMPR	204187_at	8.1	1.00E-06
KCNJ15	210119_at	7.27	6.10E-05
NP	201695_s_at	2.43	1.00E-06
SEC31A	215009_s_at	-2.38	1.00E-06
SLC25A37	221920_s_at	4.64	2.00E-06
XK	206698_at	6.4	1.00E-06
CREBZF	202978_s_at	-2.13	1.00E-06
CSDA	201161_s_at	2.53	0.000182
FOXO3	204131_s_at	2.46	2.00E-06
MAGOH	210093_s_at	-2.27	6.00E-06
TRMT11	218877_s_at	-2.04	1.00E-06
HIST1H1C	209398_at	2.48	3.00E-06
HIST2H2AA3/HIST2H2AA4	214290_s_at	3.32	1.00E-06
CTSA	200661_at	2.13	2.00E-06
RABGGTB	209181_s_at	-2.44	1.00E-06
SELENBP1	214433_s_at	11.5	1.00E-06
ALAS2	211560_s_at	124.07	1.00E-06
ANK1	208353_x_at	2.59	1.00E-06
EPB49	204505_s_at	4.33	1.00E-06
ERAF	219672_at	9.81	1.00E-06
GLRX5	221932_s_at	2.86	5.10E-05
HBA1	211745_x_at	3.23	1.90E-05

Gene	Probeset	Fold Change	p-value
HBB	209116_x_at	4.4	6.00E-06
HBD	206834_at	23.87	1.00E-06
HBG1/HBG2	204848_x_at	6.02	0.000137
MPP1	202974_at	3.41	1.00E-06
NFE2	209930_s_at	4.32	1.00E-06
CIRBP	200810_s_at	-2.22	1.00E-06
DYSF	218660_at	3.47	2.00E-06
KIAA0329	204308_s_at	2.92	1.00E-06
KRT1	205900_at	4.34	0.00095
LOC92482	213224_s_at	-2.08	1.00E-06
LRRN3	209840_s_at	-2.44	0.001134
PDZK1IP1	219630_at	4.45	1.00E-06
RNF10	207801_s_at	2.47	2.80E-05
SMOX	210357_s_at	4.04	1.00E-06
TMEM158	213338_at	4.21	1.00E-06
TRIM58	215047_at	4.61	2.50E-05
TSPAN5	209890_at	2.64	9.00E-06
UBXD1	220757_s_at	2.02	0.000405

Table 3

List of genes that comprise the erythropoietic signature [29]. Genes in this signature which are differentially expressed in SJIA and CAPS are shown with the respective fold changes. Original table.

Gene Symbol	Gene Title	CAPS[30]	SJIA[21]
----	237299_at		3.3
----	239210_at		4.3
ADIPOR1	adiponectin receptor 1	2.47	9.3
ALAS2	aminolevulinate, delta-, synthase 2	124.07	593.4
ALS2CR2	amyotrophic lateral sclerosis 2 (juvenile) chromosome region, candidate 2		13.9
ANXA3	annexin A3		15.4
BCL2L1	BCL2-like 1	3.26	6.4 ^I
BNIP3L	BCL2/adenovirus E1B 19kDa interacting protein 3-like		6.1
BPGM	2,3-bisphosphoglycerate mutase		8.5
C16orf35	chromosome 16 open reading frame 35		5.6
C19orf22	chromosome 19 open reading frame 22		4.0 ^I
C19orf22	chromosome 19 open reading frame 22		
C20orf108	chromosome 20 open reading frame 108		5.3 ^I
CA1	carbonic anhydrase I	11.64	75.6
CDC34	cell division cycle 34 homolog (S. cerevisiae)		3.8
CEACAM1	carcinoembryonic antigen-related cell adhesion molecule 1 (biliary glycoprotein)		8.1
CR1/CR1L	complement component (3b/4b) receptor 1 (Knops blood group) /// complement component (3b/4b) receptor 1-like		4.5
CSDA	cold shock domain protein A	2.53	4.9 ^I
E2F2	E2F transcription factor 2		3.8
ELL2	elongation factor, RNA polymerase II, 2		
EPB42	erythrocyte membrane protein band 4.2		142.5
EPB49	erythrocyte membrane protein band 4.9 (dematin)	4.33	11.5
ERAF	erythroid associated factor	9.81	30
FGFR1OP2	FGFR1 oncogene partner 2		
FHDC1	FH2 domain containing 1		
FKBP8	FK506 binding protein 8, 38kDa		
GLRX5	glutaredoxin 5	2.86	
GLUL	glutamate-ammonia ligase (glutamine synthetase)		
GMPR	guanosine monophosphate reductase	8.1	18.8
GSPT1	G1 to S phase transition 1		6.3
GYPA	glycophorin A (MNS blood group)		8.3 ^I
GYPC	glycophorin C (Gerbich blood group)		5.4
HBB/HBD	hemoglobin, beta /// hemoglobin, delta	4.4 ^I	36.4

Gene Symbol	Gene Title	CAPS[30]	SJIA[21]
HBG1/HBG2	hemoglobin, gamma A /// hemoglobin, gamma G	6.02	49.0 ^I
HBM	hemoglobin, mu		161.6
HBQ1	hemoglobin, theta 1		
HIST1H1C	histone cluster 1, H1c	2.48	4.1
JAZF1	JAZF zinc finger 1		
KLF1	Kruppel-like factor 1 (erythroid)		6.7
LOC284422	similar to HSPC323		
MKRN1	makorin ring finger protein 1		3.8
MPP1	membrane protein, palmitoylated 1, 55kDa		7.9
MYL4	myosin, light chain 4, alkali; atrial, embryonic		5.0 ^I
NEDD4L	neural precursor cell expressed, developmentally down-regulated 4-like		
NUDT4/NUDT4P1	nudix (nucleoside diphosphate linked moiety X)-type motif 4 /// nudix (nucleoside diphosphate linked moiety X)-type motif 4 pseudogene 1		6.6
OR2W3	olfactory receptor, family 2, subfamily W, member 3		13.4
OSBP2	oxysterol binding protein 2		10.9
PCGF5	polycomb group ring finger 5		
PINK1	PTEN induced putative kinase 1		
PLEK2	pleckstrin 2		
RIOK3	RIO kinase 3 (yeast)		3.8 ^I
RNF10	ring finger protein 10	2.47	5.4
SEC14L1	SEC14-like 1 (S. cerevisiae)		
SELENBP1	selenium binding protein 1	11.5	101.4
SESN3	sestrin 3		7.6 ^I
SIAH2	seven in absentia homolog 2 (Drosophila)		9.0
SLC22A4	solute carrier family 22 (organic cation/ergothioneine transporter), member 4		
SLC25A37	solute carrier family 25, member 37	4.64	
SLC25A39	solute carrier family 25, member 39		
SLC4A1	solute carrier family 4, anion exchanger, member 1 (erythrocyte membrane protein band 3, Diego blood group)	14.96	64.2
SLC6A8	solute carrier family 6 (neurotransmitter transporter, creatine), member 8		9.2
SNCA	synuclein, alpha (non A4 component of amyloid precursor)	6.9	30.9
TMCC2	transmembrane and coiled-coil domain family 2		
TMOD1	tropomodulin 1		9.9 ^I
TRIM10	tripartite motif-containing 10		
UBXN6	UBX domain protein 6		
YOD1	YOD1 OTU deubiquinating enzyme 1 homolog (S. cerevisiae)		

^I Where more than one probeset maps to the same gene, the lower fold change is shown.