Transcriptomic analysis of patients with tetralogy of Fallot reveals the effect of chronic hypoxia on myocardial gene expression

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Objectives: In cyanotic patients undergoing repair of heart defects, chronic hypoxia is thought to lead to greater susceptibility to ischemia and reoxygenation injury. We sought to find an explanation to such a hypothesis by investigating the cardiac gene expression in patients with tetralogy of Fallot undergoing cardiac surgery.

Methods: The myocardial gene profile was investigated in right ventricular biopsy specimens obtained from 20 patients with a diagnosis of cyanotic (n = 11) or acyanotic (n = 9) tetralogy of Fallot undergoing surgical repair. Oligonucleotide microarray analyses were performed on the samples, and the array results were validated with Western blotting and enzyme-linked immunosorbent assay.

Results: Data revealed 795 differentially expressed genes in cyanotic versus acyanotic hearts, with 198 upregulated and 597 downregulated. Growth/morphogenesis, remodeling, and apoptosis emerged as dominant functional themes for the upregulated genes and included the apoptotic gene *TRAIL* (tumor necrosis factor—related apoptosis-inducing ligand), the remodeling factor *OPN* (osteopontin), and the mitochondrial function gene *COX11* (cytochrome-c oxidase 11). In contrast, transcription, mitogen-activated protein kinase signaling, and contractile machinery were the dominant functional classes for the downregulated genes, which included the calcium-handling gene *NCX1* (sodium-calcium exchanger). Protein levels of COX11, NCX1, OPN, and LYZ (lysozyme) in the myocardium followed the same pattern obtained by means of transcriptomics. The TRAIL level did not change in myocardium but increased in circulating blood of cyanotic patients, suggesting the myocardium as a possible source. Additionally, our data showed increased protein expression of apoptosis markers in cyanotic myocardium.

Conclusions: Chronic hypoxia in cyanotic children with tetralogy of Fallot induced the expression of genes associated with apoptosis and remodeling and reduced the expression of genes associated with myocardium contractility and function. (J Thorac Cardiovasc Surg 2010;140:337-45)

Supplemental material is available online.

Cyanotic pediatric patients undergoing cardiac surgery are exposed to a chronic hypoxic state that can reduce their antioxidant reserve capacity, leading to a greater susceptibility to the oxidative stress of ischemia and reperfusion at the time of surgical correction. Our group has shown that cyanotic children have worse myocardial reperfusion injury and clinical outcomes compared with acyanotic children after

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similar periods of ischemic cardioplegic arrest.⁴ We have also shown that reintroduction of high oxygen levels to cyanotic patients undergoing cardiopulmonary bypass (CPB) leads to myocardial damage before ischemic cardioplegic arrest.⁵ Our results suggest that the injury seen after cardioplegic arrest might in part be due to CPB-induced reoxygenation injury, and this has also been demonstrated in previous studies.⁶

Several recent studies have used microarray technology to examine the global myocardial stress response during cardiac surgery, focusing on the human congenitally malformed hearts. Table 18 Kaynak and colleagues examined gene expression profiles in patients with tetralogy of Fallot (TOF), ventricular septal defect, and right ventricular hypertrophy (RVH) compared with profiles seen in those with normal hearts. Another investigation examined gene expression changes in patients with cyanotic TOF in comparison with those seen in patients with normal hearts and showed that the upregulation of genes encoding vascular endothelial growth factor (VEGF) and extracellular matrix proteins are the key events contributing to RVH and stunted angiogenesis in patients with TOF. Konstantinov and associates examined the gene expression profiles in children undergoing

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Abbreviations and Acronyms

BAX = Bcl-2-associated X protein CPB = cardiopulmonary bypass

ELISA = enzyme-linked immunosorbent assay ERK = extracellular signal-regulated kinase

FDR = false discovery rate

MAPK = mitogen-activated protein kinase RVH = right ventricular hypertrophy

TOF = tetralogy of Fallot

VEGF = vascular endothelial growth factor

cardiac surgery for right heart obstructive lesions, including TOF, and showed that neonatal myocardium has a unique pattern of gene expression dominated by genes with cardio-protective, antihypertrophic, and antiproliferative properties, reflecting a stress-induced protective program.

However, to our knowledge, no previous genome-wide investigation has been made to determine the global gene expression profiles associated with chronic hypoxia in patients with TOF. The aim of this study was therefore to investigate the global ventricular myocardial gene expression profiles of cyanotic and acyanotic patients with a diagnosis of TOF undergoing corrective cardiac surgery.

MATERIALS AND METHODS

Twenty patients with a diagnosis of cyanotic (n = 11) or acyanotic (n = 9) TOF undergoing surgical repair at the Bristol Royal Hospital for Children were studied. All patients were in stable condition without preoperative respiratory or inotropic support. Preoperative characteristics in the 2 groups are summarized in Table 1. Patients with cyanotic TOF presented with several episodes of spells and repeated saturation measurements of less than 90% (mean, $79.6\% \pm 7.5\%$), whereas patients with acyanotic TOF did not have a history of cyanotic spells and presented with saturations of greater than 90% (mean, $94.2\% \pm 3.5\%$). Preoperative echocardiographic data showed significant gradient across the right ventricular outflow tract in both groups. The study was approved by the hospital research ethics committee, and parental informed consent was gained for all patients. Intraoperative anesthetic and operative techniques were standardized, as previously reported. After the operation, all patients were admitted to the pediatric intensive care unit and were managed according to unit protocols. $^{4.5}$

Postoperative inotropic support was considered to be either minimal (dopamine $\leq 5~kg^{-1} \cdot min^{-1}$ for ≤ 24 hours) or significant (dopamine $> 5~\mu g \cdot kg^{-1} \cdot min^{-1}$ with or without other inotropic agents, such as adrenaline, noradrenaline, or milrinone). Decisions regarding inotropic support and ventilation were based on hemodynamic status (eg, low mixed venous saturation and high lactic acidosis) and clinical judgment. $^{4.5}$

Cardiac Muscle Biopsy Specimens

Ventricular biopsy specimens (10 mg net weight) were collected from the apex of the right ventricle by using a ''Trucut'' needle 4 immediately after institution of CPB. Each specimen was immediately put in RNA Later solution (Qiagen, Crawley, United Kingdom) and kept overnight at 4° C. The next day, RNA Later solution was removed, and biopsy specimens were kept at -80° C until RNA extraction.

The concentration of total RNA samples was assessed by means of spectrophotometry (Nanodrop, Wilmington, Del) and was further analyzed for

TABLE 1. Baseline patients' characteristics and clinical outcomes

	Cyanotic patients	Acyanotic patients	P
	(n = 11)	(n=9)	value
Age (mo)	10.6 ± 5.5	9.5 ± 2.3	.1
Weight (kg)	8.04 ± 1.7	8.01 ± 1.0	.7
RVOT velocity (m/s)	4.6 ± 0.4	4.4 ± 0.7	.2
RV wall indexed (mm/m ²)	16.6 ± 3.0	17.1 ± 1.9	.1
VSD size (mm)	9.9 ± 1.3	9.8 ± 1.6	.1
Use of preoperative β -blockers	4	1	.05
Preoperative O ₂ saturation (%)	79.6 ± 7.5	94.2 ± 3.5	.01
Crossclamp time (min)	78.0 ± 15.6	73.0 ± 20.7	.1
CPB time (min)	132.0 ± 29.0	90.2 ± 17.7	.05
In-hospital mortality	0	0	
Inotropic support	1/10	7/2	.01
(minimal/significant)			
Inotropic duration (h)	54 (42–136)	22 (12-29)	.03
Total μ g dopamine/kg ($\times 10^3$)	16.3 (12.2–36.3)	5.2 (1.9-7.3)	.01
Postoperative ventilation time (h)	42 (24–77)	18 (5-33)	.01
Postoperative ICU stay (h)	87 (49–122)	40 (26–48)	.001
Postoperative hospital stay (d)	11 (9–12)	7 (5–9)	.05

Data are presented as means \pm standard deviations or medians (interquartile ranges). *RVOT*, Right ventricular outflow tract; *RV*, right ventricular; *VSD*, Ventricular septal defect; *ICU*, intensive care unit.

integrity with a Bioanalyzer 2100 with RNA 6000 Nano Assay (Agilent Technologies, Stockport, United Kingdom).

Gene Microarrays: Data Analysis

Ventricular total RNAs (1 μ g) from individual patients were processed as previously described. ¹¹ Raw data (CEL files) were uploaded into ArrayStar software version 2.1 (DNASTAR, Inc, Madison, Wis) for normalization and statistical analysis. The robust multichip analysis algorithm was used for background correction, quantile normalization, and median polish summarization. The statistical analysis was carried out with ArrayStar software. A Student's t test and Benjamini and Hochberg multiple testing corrections for false discovery rate (FDR) were used. The FDR-adjusted P value was set to less than .05, and transcripts were filtered on the basis of 1.8-fold or greater difference.

Functional Annotation and Network Analysis

Differentially expressed genes were analyzed according to predefined pathways and functional categories annotated by Kyoto Encyclopedia of Genes and Genomes and Gene Ontology by using the Database for Annotation, Visualization, and Integrated Discovery bioinformatics resource. 12 For an overrepresented Gene Ontology or Kyoto Encyclopedia of Genes and Genomes pathway, cutoff *P* values of .01 and .05 have been selected for the downregulated and upregulated genes, respectively. For Tables E1 and E2, all gene annotations were checked by using online tools and databases: Entrez Gene (www.ncbi.nlm.nih.gov/sites/entrez). The software PathwayStudio (Ariadne, Rockville, Md) and the ResNet database were used to explore the networks of interactions in which the physiologically regulated genes are potentially involved.

Immunohistochemistry and Western Blotting

Right ventricular specimens were fixed in 4% paraformaldehyde, washed in PBS, and embedded in paraffin, and 4- μ m sections were obtained. Immunohistochemistry was performed with the ABC-Kit from DakoCytomation (Glostrup, Denmark). Photos were taken at $40 \times$ magnification.

Western blotting was performed as previously described.¹³ Statistical analysis (unpaired *t* test) was carried out with Instat 3 software (GraphPad Software, Inc, La Jolla, Calif), and a *P* value was calculated for comparison.

Enzyme-Linked Immunosorbent Assay Protein Analysis

Blood was collected from all patients before surgical intervention, and plasma was separated and stored at -80° C. Human TRAIL levels were measured in plasma samples by using the Diaclone enzyme-linked immunosorbent assay (ELISA) kit. Each sample was performed in duplicate. Statistical analysis (unpaired t test) was carried out with Instat 3 software, and a P value was calculated for comparison.

Statistical Analysis

For the clinical data, continuous variables were summarized by using means and standard deviations (or medians and interquartiles range if the distribution was skewed), and categorical data were summarized as numbers and percentages. Differences between groups were compared by using the χ^2 test for categorical variables and the t or Wilcoxon rank sum tests, as appropriate, for continuous variables.

RESULTS

Clinical data are summarized in Table 1. The 2 groups were comparable in terms of age, weight, and crossclamp time. There were no deaths and no major morbidity in both groups. One patient in the cyanotic group required a pacemaker but regained sinus rhythm at follow-up. One patient in the acyanotic group required another run of CPB to resect more muscle from the right ventricular outflow tract after the first procedure. Echocardiographic analysis at discharge did not show any significant residual gradient across the right ventricular outflow tract (mean velocity <3 m/s in both groups) or residual ventricular septal defects. CPB time was longer in the cyanotic group, who required more extensive transannular patching and reconstruction of the pulmonary artery compared with the acyanotic patients. Cyanotic patients had longer inotropic support, ventilation time, and intensive care and hospital stays compared with the acyanotic group.

Gene Microarray Analysis

Analysis of differentially expressed genes. We have used the Affymetrix GeneChip Human Genome U133 Plus 2.0 Array in this study because it represents the most comprehensive whole human genome expression array, providing coverage of more than 47,000 well-substantiated human genes (www.affymetrix.com). Of the genes examined, 795 were identified as differentially expressed in cyanotic versus acyanotic heart biopsy specimens (P < .05, t test followed by Benjamini and Hochberg FDR correction), with 198 (25%) upregulated and 597 (75%) downregulated by more than 1.8-fold (Figure 1, A, and Tables E1 and E2). Genes identified as upregulated or downregulated beyond 1.8-fold unmasked overrepresented biologic process in each gene set (Figure 1, B and C). Myocardial contractility and function, as identified by means of

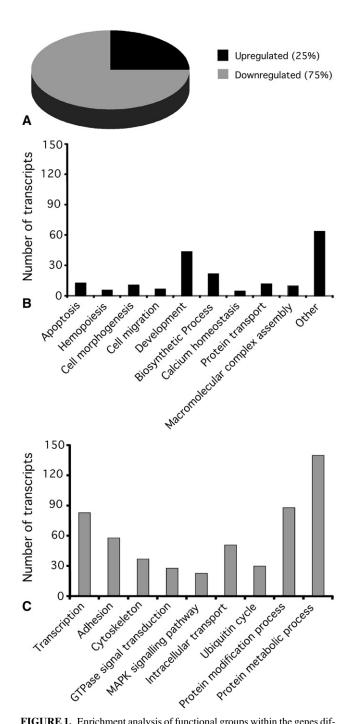


FIGURE 1. Enrichment analysis of functional groups within the genes differentially expressed in cyanotic compared with acyanotic patients. A, Pie chart of the regulated genes in cyanotic compared with acyanotic patients (\geq 1.8-fold). Three quarters of the genes were downregulated and a quarter were upregulated. B and C, Selected GO annotations of genes upregulated (B) and downregulated (C) in cyanotic patients. The histograms show the distribution of these annotations.

cell adhesion, cytoskeletal organization, transcription regulation, guanosine triphosphatase signal transduction, mitogen-activated protein kinase (MAPK) signaling, and

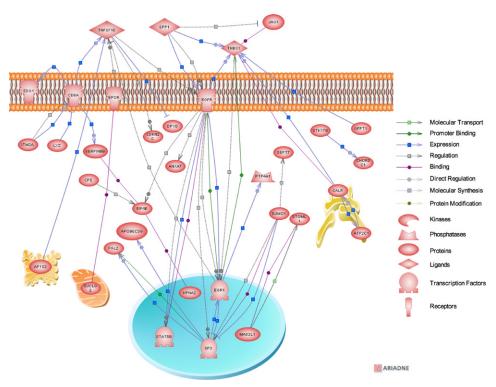


FIGURE 2. Biological association network of the significantly upregulated genes (>1.8-fold) of cyanotic versus acyanotic patient comparisons. The association between the network entities were based on available PubMed citations. Each node in the network map is linked to an html page showing the protein annotation based on available public databases. *Small square boxes* connecting 2 nodes are linked to an html page showing the type of effect (positive or negative) and the supporting Medline references.

intracellular transport, were downregulated in cyanotic hearts (Figure 1, *C*). Upregulated transcripts functionally overrepresented apoptosis, cell movement, morphogenesis, and development (Figure 1, *B*). Collectively, these changes indicate genetic remodeling of the cyanotic myocardium in response to chronic hypoxia.

Pathway analysis. Using Pathway Studio software (Ariadne), we further analyzed the differentially expressed genes (cyanotic vs acyanotic). The analysis looked for the direct pathways connecting genes. For the upregulated genes, the resulting biological association networks are presented in Figure 2. Interestingly, the upregulated genes spanned almost all the cellular organelles. Analysis of downregulated genes associated with the ontological "regulation of transcription" and "MAPK signaling pathway" classes were composed of 83 and 23 downregulated genes, respectively. Of these, 38 and 18 genes, respectively, integrated into direct biological association networks (Figure 3), whereas 45 and 5 genes did not possess interactions.

Cyanosis increases growth, morphogenesis, and remodeling processes and apoptosis signaling. Increased expression of growth, morphogenesis, and remodeling signaling genes emerged as a dominant functional theme for the upregulated genes. This was evident by the increased expression levels of the growth signaling factors *FGF7*, *TGFBR2*, *EGFR*, and insulin-like growth factor binding pro-

tein 7 and the remodeling factors *SPP1 (OPN)* and *ACTL6A* (Table E1 and Figure 2).

Another dominant functional theme for the upregulated genes was apoptosis. Indeed, our data showed increased expression levels of genes involved in apoptosis signaling, such as *TNFSF10 (TRAIL)*, B-cell chronic lymphocytic leukemia/lymphoma 10 (BCL10), *STAT5B*, *GULP1 (CED6)*, and *STK17B (DRAK2*; Table E1 and Figure 2).

Cyanosis reduces transcription, MAPK signaling, and contractile machinery. One of the dominant functional classes for the downregulated genes was transcription. The transcription factors that showed a reduction of expression levels included *JUN*, *JUND*, *ATF2*, *STAT3*, *GATA4*, *EPAS1*, *NCOA2*, *ARNT*, and *SP1* (Table E2 and Figure 3, A).

Reduction of MAPK signaling genes emerged as another important functional theme for the downregulated genes. This was evident by the decreased expression levels of MAPK1 (ERK), MAPK14 (SAPK2), MAP3K2, MAP3K3, MAP4K4, AKT2, TAOK1, SOS1, and SOS2 (Table E2 and Figure 3, B). In addition, our data exhibited a downregulation in the contractile machinery (cytoskeleton and cell-adhesion clusters). The downregulated transcripts included troponin T type 2, myosin XVIIIB, ARP2 actinrelated protein 2 and nebulin-related anchoring protein (Figure E1).

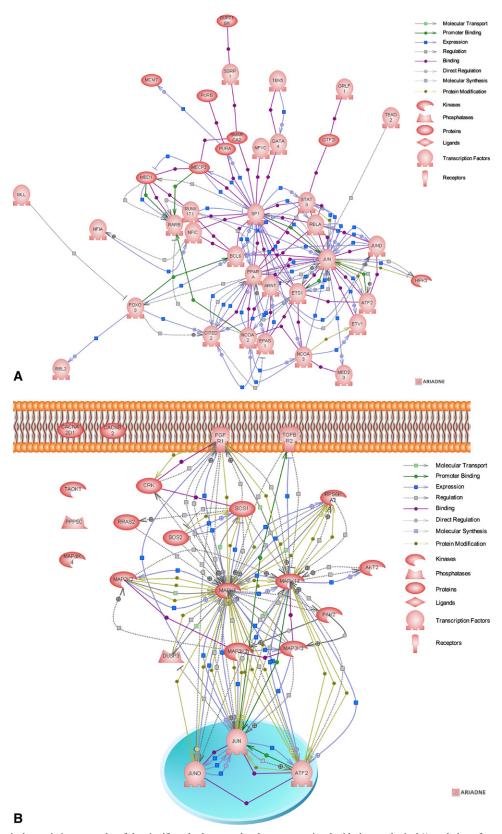


FIGURE 3. Biological association networks of the significantly downregulated genes associated with the ontological "regulation of transcription" (A) and "MAPK signaling pathway" (B) classes. Each node in network maps is linked to an html page showing the protein annotation based on available public databases. *Small square boxes* connecting 2 nodes are linked to an html page showing the type of effect (positive or negative) and the supporting Medline references.

Validation of Differential Expression of Selected Gene Products

Despite being greatly informative, changes in mRNA levels are insufficient to predict protein expression levels. We therefore went onto assessing the protein levels of the identified genes in the myocardium and blood of cyanotic and acyanotic children using immunohistochemistry, Western blotting, and ELISA. By using immunohistochemistry, we showed COX11, NCX1, OPN, TRAIL, and LYZ protein immunostaining in the myocardium of cyanotic and acyanotic children (Figures E2, C and D; E3, A and B, E4, C and D; and E5, A and B). The strongest signals observed were those of COX11 and LYZ. Subsequently, we semiquantitatively assessed protein level changes in the myocardium of cyanotic and acyanotic children by using Western blotting. COX11, OPN, and LYZ showed significant increases in cyanotic samples compared with levels seen in acyanotic samples (Figures E2, E and F; E5, C and D; and E6, C and D), which is similar to that observed at the mRNA level. NCX1 protein levels showed a significant decrease caused by cyanosis (Figure E3, C and D), resembling that obtained at the transcriptomic level. In contrast, TRAIL protein levels showed no alteration in the myocardium of the 2 groups of patients (Figure E4, A and B). Because TRAIL is a soluble protein that could be released in blood, we assessed TRAIL plasma levels by means of ELISA. Our data indicated a significant 42% increase of TRAIL plasma levels in cyanotic compared with acyanotic children (Figure E4, E).

Functional Validation

To functionally validate our transcriptomics-derived hypothesis, we examined protein expression levels of markers of hypoxia, apoptosis, and MAPK signaling activation in myocardial tissue. Western blotting of hypoxia-inducible factor 1 alpha showed a significant upregulation in cyanotic compared with acyanotic tissue, confirming the hypoxic state this patient group's experiences (Figure E7, A). Additionally, we showed a significant increase in cleaved caspase-3 (activated form) and Bcl-2-associated X protein (BAX) protein expression in cyanotic compared with acyanotic myocardium (Figure E7, B and C). Caspase-3 is a critical apoptosis executioner, and BAX is a well-documented apoptotic activator. Increased levels of cleaved caspase-3 and BAX in cyanotic myocardium indicate an increased apoptotic activity in the hearts of these patients. Furthermore, we showed a significant decrease in phospho-SAPK (activated form) expression levels in cyanotic compared with acyanotic myocardium (Figure E7, D). We also detected a tendency toward a decrease in phosphorylated extracellular signal-regulated kinase (ERK) levels in cyanotic myocardium, although it was not significant (data not shown).

DISCUSSION

The results of this microarray-based gene expression profiling study seem to confirm the existence of a reprogramming response that is most evident in the chronically hypoxic cyanotic myocardium. Growth/morphogenesis, remodeling, and apoptosis emerged as dominant functional themes for the upregulated genes (Figures 1 and 2). In contrast, transcription, MAPK signaling, and contractile machinery (cytoskeleton and adhesion) were the dominant functional classes for the downregulated genes (Figures 1 and 3).

Previous studies have shown RVH in patients with TOF compared with that seen in healthy control subjects. 7,10 These studies did not differentiate between cyanotic and acyanotic TOF. Our clinical data did not indicate a higher RVH in cyanotic patients, suggesting that the observed transcriptomic changes are mainly related to cyanosis. In our investigation the expression level changes of extracellular matrix factors (remodeling markers) in cyanotic compared with acyanotic patients went in both directions. Indeed, levels of FBLN1 and SPP1, for example, increased in cyanotic patients, whereas levels of COL3A1, COL4A1, COL4A2, COL6A1, COLA6A2, and COL12A1 decreased (Figure E8). Increased levels of collagen I and III have been shown to be associated with RV remodeling. 10 It is tempting to speculate that the right ventricle remodels differently in cyanotic and acyanotic patients, with more injury-related reprogramming occurring in cyanotic patients. However, in the absence of gene expression data from age-matched healthy control subjects, we are unable to validate such a hypothesis.

Our data showed increased gene expression levels of the growth-signaling factors *FGF7*, *TGFBR2*, EGFR, and insulin-like growth factor binding protein 7 (Table E1 and Figure 2). These gene expression changes could be attributed to a myocardial survival program in an attempt at protecting itself from hypoxia-related cell damage. It is documented that growth factors mediate the survival response in many cell types, ¹⁴ and the activation of growth factor signaling is cardioprotective. ¹⁵ A previous study showed an increased expression level of VEGF in the right ventricle when comparing patients with TOF with healthy control subjects. ¹⁰ Our findings showed no changes in VEGF expression levels in cyanotic compared with acyanotic patients.

Additionally, our data showed increased apoptosis in cyanotic myocardium and increased expression of apoptosis signaling genes, such as TNFSF10 (TRAIL), B-cell chronic lymphocytic leukemia/lymphoma 10 (BCL10), STAT5B, GULP1 (CED6), and STK17B (DRAK2; (Table E1 and Figure 2). The protein encoded by BCL10 (CARMEN) contains a caspase recruitment domain and has been shown to induce apoptosis and to activate nuclear factor κB . The protein encoded by STAT5B, a member of the signal transducer and activator of transcription family of transcription factors, has been shown to be involved in diverse biological

processes, including apoptosis.¹⁷ GULP1 is an evolutionarily conserved adaptor protein required for efficient engulfment of apoptotic cells by phagocytes.¹⁸ *DRAK2* is a member of the death-associated protein kinase family capable of inducing apoptosis on overexpression in cell culture.¹⁹ The activation of apoptosis signaling illustrates how the chronic hypoxic stress seems to promote an injury program within cyanotic patients' myocardium. This program might explain the worst reoxygenation injury that cyanotic patients experience during and after corrective heart surgery.⁴

Transcription, MAPK signaling, and contractile machinery (cytoskeleton and adhesion) were the important functional classes for the downregulated genes (Figures 1 and 3). Three of the identified downregulated transcription factors, JUND, JUN, and ATF2, are players in both transcription and MAPK signaling direct association networks (Figure 3). Other downregulated transcription factors included STA3, GATA4, EPAS1, ARNT, NCOA2, and SP1. The reduction of transcription represents part of the injury-related program initiated by chronic hypoxia. Indeed, fully functional transcription machinery is crucial for myocardial function and survival, and a decrease in transcription factor levels would have a repercussion on cardiac function and survival.

Similarly, the reduction of MAPK signaling is another component of the injury-related program triggered by chronic hypoxia. Indeed, the MAPK pathway is crucial for myocyte survival and function. Decreased expression levels of key players of this pathway (eg, activated SAPK) could have a serious effect on the myocardium. Examples of the MAPK pathway players that showed decreased expression levels are MAPK1 (ERK), MAPK14 (SAPK2), MAP3K2, MAP3K3, MAP4K4, AKT2, TAOK1, SOS1, and SOS2 (Table E2 and Figure 3, B). MAPKs phosphorylate a number of known transcription factors to alter their transactivating activities, thus presumably influencing gene expression to elicit the cellular response.²⁰ In cardiac myocytes members of the ERK MAPK family have been implicated in survival signaling in response to ischemia and reperfusion, oxidative stress, and hypoxia.²¹ Other studies have also shown that the MAPK/ERK kinase and phosphoinositide 3-kinase-protein kinase Akt/protein kinase B pathways might protect against apoptosis.14

In addition, our study showed a downregulation in the contractile machinery (cytoskeleton and cell adhesion clusters). The downregulated transcripts belonging to this grouping included troponin T type 2, myosin XVIIIB, ARP2 actin-related protein 2, and nebulin-related anchoring protein. The reduction of contractile machinery factors seems to represent another part of the injury-related program initiated by cyanosis. Such alteration of contractility factor levels could explain the susceptibility of cyanotic patients to reoxygenation injury (Figure E1).

COX11

Cytochrome-c oxidase 11 (COX11) showed important upregulation in cyanotic patients at the mRNA and protein levels. The COX11 protein is a constituent of the inner mitochondrial membrane and might be involved in biosynthesis of heme A. The upregulation of COX11 in our study suggests that the cyanosis state might trigger a compensation mechanism through COX11 and copper transport to palliate the lack of oxygen.

NCX1

The sodium-calcium (Na⁺-Ca²⁺) exchanger (NCX1, SLC8A1) showed an important downregulation in cyanotic patients at both the mRNA and protein levels. NCX1 is a membrane protein that is considered to play an important role in the Ca²⁺ handling of cardiac myocytes. The observed NCX1 downregulation could decrease myocyte calcium handling capacity, leading to mechanical dysfunction.

TRAIL

TNFSF10 (TRAIL) showed also important upregulation in cyanotic patients. TRAIL is a cytokine that belongs to the tumor necrosis factor ligand family. The binding of this protein to its receptors has been shown to trigger the activation of MAPK8/c-Jun NH2-terminal kinase, caspase-8, and caspase-3, thus inducing apoptosis. The observed TRAIL increase in the circulation of cyanotic patients could emanate from the myocardium. TRAIL increases in the circulation of cyanotic patients could induce apoptosis in organs and tissues expressing TRAIL receptors. The activity of this protein might be modulated by binding to decoy receptors that cannot induce apoptosis. These decoy receptors, such as osteoprotegerin, present great potential for use as a therapy to reduce any TRAIL induced-apoptosis.

SPP1

SPP1, also known as osteopontin (OPN), was upregulated by 2-fold in cyanotic patients at the mRNA and protein levels. OPN is expressed in the heart at low levels under normal conditions. Although the exact role of OPN in cardiomyocyte function is unknown, there is evidence that cardiomyocytes are a prominent source of OPN in vivo and that induction of OPN expression is strongly associated with ventricular remodeling. Additionally, increase of OPN expression in the myocardium coincides with the development of heart failure. Our data showed an increase in OPN expression at the mRNA and protein levels in cyanotic children, suggesting a remodeling program initiated by chronic hypoxia in the myocardium.

LYZ

LYZ (lysozyme) showed the highest upregulation (5:1) in cyanotic patients. LYZ, the natural substrate of which is

bacterial cell wall peptidoglycan, is one of the antimicrobial agents found in human milk and is also present in the spleen, lung, kidney, white blood cells, plasma, saliva, and tears. Here we show protein expression of the LYZ gene product in the ventricular myocardium and increased levels in cyanotic compared with acyanotic patients. The biological significance of this increase is not known, and further studies would be required to uncover it.

Limitations

Even though there was no difference between the 2 groups in terms of right ventricular wall thickness, one cannot completely exclude the possibility that RVH could be a potential confounder in our findings.

As expected, there was a difference in the 2 groups regarding the preoperative use of β -blockers, and this might be a potential confounding factor because it might influence the course of RVH and remodeling.

We did not study normal or nondiseased right ventricular myocardium; however, our focus was on the gene expression differences between cyanotic and acyanotic patients with TOF.

Finally, our investigation can be interpreted as a descriptive study that generates more hypotheses. However, it is our belief that this will provide a better understanding of the mechanisms associated with chronic hypoxia and help develop interventions aimed at improving the clinical outcome in this high-risk group of patients.

CONCLUSIONS

Overall, the transcriptional profile in the cyanotic group was characterized by increased expression level of genes with literature-validated apoptosis and growth/morphogenesis/remodeling properties. Furthermore, it showed decreased expression levels of genes with cardiac function, cell survival, and cytoprotective properties. The molecular signatures identified suggest a reprogramming response in the cyanotic myocardium activated by the chronic hypoxia imposed by the structural congenital heart disease.

CLINICAL IMPLICATIONS

In this study patients in both groups (cyanotic and acyanotic TOF) exhibited a significant hypertrophy of the right ventricle caused by pressure overload. In cyanotic patients the higher degree of right ventricular obstruction and the smaller size of the pulmonary annulus and main pulmonary artery were responsible for the preoperative chronic hypoxic state. We therefore believe that these 2 groups of patients constitute the best possible model to study the influence of cyanosis on gene expression. Our analysis identified several genes deregulated in the cyanotic heart that might be responsible for the susceptibility of cyanotic children to ischemia and reoxygenation injury during and after surgical

intervention. Indeed, our previous data^{4,5} showed that cyanotic patients have significant perioperative myocardial cell damage compared with acyanotic patients undergoing cardiac surgery. This susceptibility to ischemia and reperfusion damage can be explained by the impairment of factors crucial to cardiac function, induction of apoptotic pathways, and alteration of signal transduction pathways seen in cyanotic patients in this study. This information might have a significant effect in improving surgical strategies in cyanotic patients with TOF undergoing corrective cardiac surgery.

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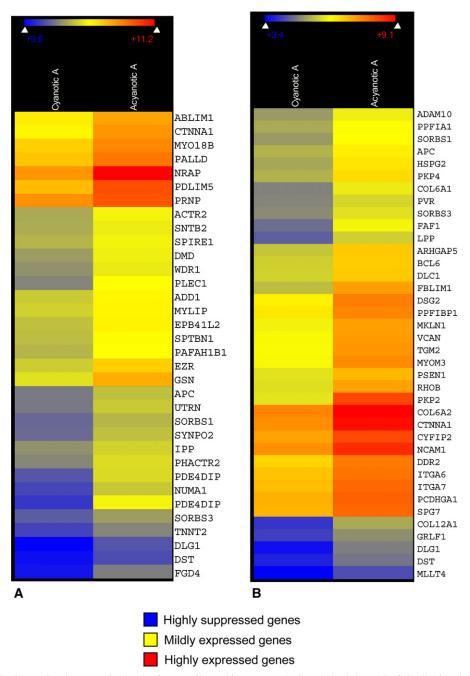


FIGURE E1. Heat Map illustrating the expression levels of contractile machinery genes. A, Cytoskeletal cluster. B, Cell adhesion cluster. Hierarchical clustering has been performed with ArrayStar software. A 3-color scale was used to illustrate expression level differences, with blue indicating low expression values, yellow indicating intermediately expressed genes, and red representing highly expressed genes.

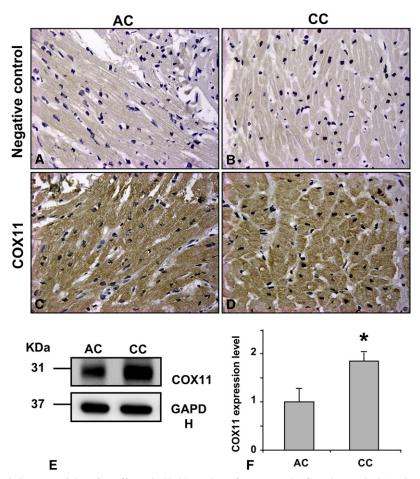


FIGURE E2. COX 11 protein immunostaining of paraffin-embedded heart tissue from acyanotic (C) and cyanotic (D) patients using COX11-specific antibody. 3,3'-Diaminobenzidine (DAB) staining (brown) reveals expression of COX11 in both tissues but not in control tissue (A and B). E and F, COX11 protein expression level in myocardium of acyanotic (AC) and cyanotic (CC) patients. Biopsy specimens were lysed to isolate protein content and Western blotting analysis was performed, probing for COX11 and glyceraldehyde-3-phosphate dehydrogenase (GAPDH). COX11 was significantly upregulated in cyanotic biopsy specimens compared with that seen in acyanotic specimens. COX11 bands were normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) levels. Data are presented as means \pm standard errors of the mean. *P < .05 (n = 6).

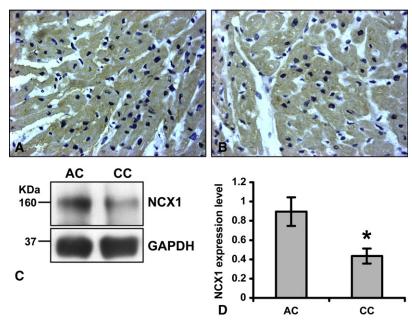


FIGURE E3. A and B, NCX1 protein immunostaining of paraffin-embedded heart tissue from acyanotic (A) and cyanotic (B) patients using NCX1-specific antibody. 3,3'-Diaminobenzidine (DAB) staining (brown) reveals expression of NCX1 in both tissues. C and D, NCX1 protein expression level in myocardium of acyanotic (AC) and cyanotic (CC) patients. Biopsy specimens were lysed to isolate protein content, and Western blotting analysis was performed, probing for NCX1 and glyceraldehude-3-phosphate dehydrogenase (GAPDH). NCX1 was significantly downregulated in cyanotic biopsy specimens compared with acyanotic specimens. NCX1 bands were normalized to GAPDH levels. Data are presented as means \pm standard errors of the mean. *P < .05 (n = 4).

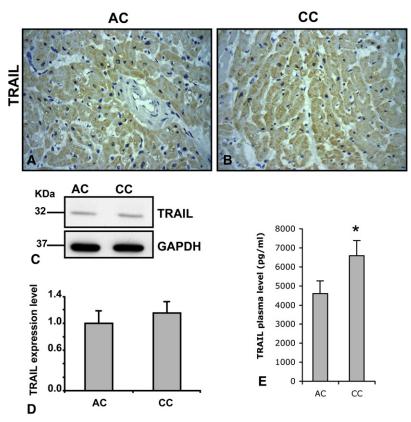


FIGURE E4. TRAIL protein immunostaining of paraffin-embedded heart tissue from acyanotic (A) and cyanotic (B) patients using TRAIL-specific antibody. 3,3'-Diaminobenzidine (DAB) staining (brown) reveals expression of TRAIL in both tissues. C and D, TRAIL protein expression level in myocardium of acyanotic (AC) and cyanotic (AC) patients. Biopsy specimens were lysed to isolate protein content, and Western blotting analysis was performed, probing for TRAIL and glyceraldehude-3-phosphate dehydrogenase (GAPDH). TRAIL was not upregulated in cyanotic biopsy specimens compared with acyanotic specimens. TRAIL bands were normalized to GAPDH levels. Data are presented as means \pm standard errors of the mean (n = 6). E, Plasma levels of TRAIL in cyanotic (AC) and acyanotic (AC) children. Solid bars represent median protein concentration in preoperative plasma samples (in picograms per milliliter). A significantly enhanced TRAIL protein expression was observed in cyanotic samples. Data are presented as means \pm standard errors of the mean. *P < .05 (n = 9-11).

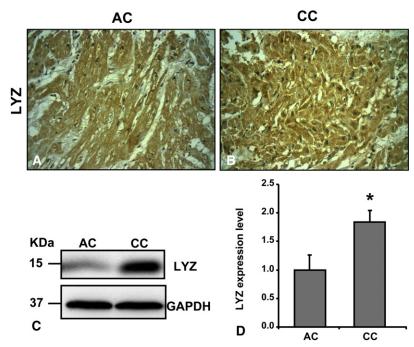


FIGURE E5. LYZ protein immunostaining of paraffin-embedded heart tissue from acyanotic (A) and cyanotic (B) patients using LYZ-specific antibody. 3,3'-Diaminobenzidine (DAB) staining (brown) reveals expression of LYZ in both tissues. C and D, LYZ protein expression level in myocardium of acyanotic (AC) and cyanotic (CC) patients. Biopsy specimens were lysed to isolate protein content, and Western blotting analysis was performed, probing for LYZ and glyceraldehude-3-phosphate dehydrogenase (GAPDH). LYZ was significantly upregulated in cyanotic biopsy specimens compared with acyanotic specimens. LYZ bands were normalized to GAPDH levels. Data are presented as means \pm standard errors of the mean. * $P \le .05$ (n = 6).

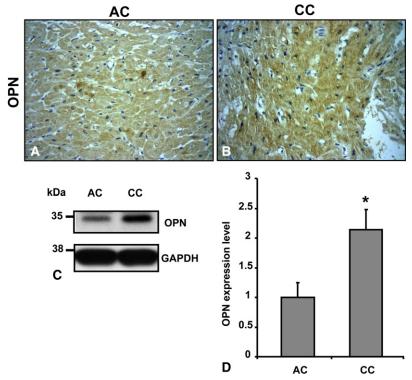


FIGURE E6. OPN protein immunostaining of paraffin-embedded heart tissue from acyanotic (A) and cyanotic (B) patients using OPN-specific antibody. 3,3'-Diaminobenzidine (DAB) staining (brown) reveals expression of OPN in both tissues. C and D, OPN protein expression level in myocardium of acyanotic (AC) and cyanotic (AC) and cyanotic (AC) patients. Biopsy specimens were lysed to isolate protein content, and Western blotting analysis was performed, probing for OPN and glyceraldehude-3-phosphate dehydrogenase (AC). OPN was significantly upregulated in cyanotic biopsy specimens compared with acyanotic specimens. OPN bands were normalized to GAPDH levels. Data are presented as means \pm standard errors of the mean. \pm 0.05 (\pm 0.05 (\pm 0.05).

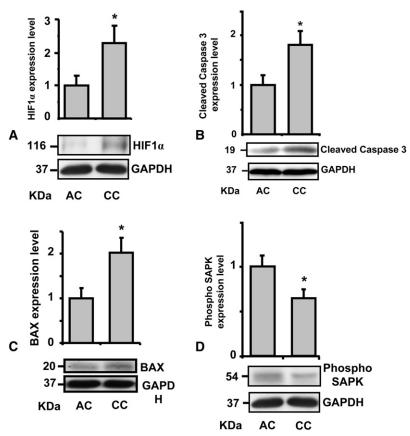


FIGURE E7. Functional validation of hypoxia-inducible factor 1 alpha, cleaved caspase-3, BAX, and phospho-SAPK protein levels in myocardium of acyanotic (AC) and cyanotic (CC) patients. Biopsy specimens were lysed to isolate protein content, and Western blotting analysis was performed, probing for HIF1α (A), cleaved caspase-3 (B), BAX (C), phospho-SAPK (D) and glyceraldehude-3-phosphate dehydrogenase (GAPDH). HIF1α, cleaved caspase-3, and BAX were significantly upregulated in cyanotic compared with acyanotic biopsy specimens. Phospho-SAPK was significantly downregulated in cyanotic compared with acyanotic biopsy specimens. HIF1 α , cleaved caspase-3, BAX, and phospho-SAPK bands were normalized to GAPDH levels. Data are presented as means \pm standard errors of the mean. *P < .05 (n = 6).

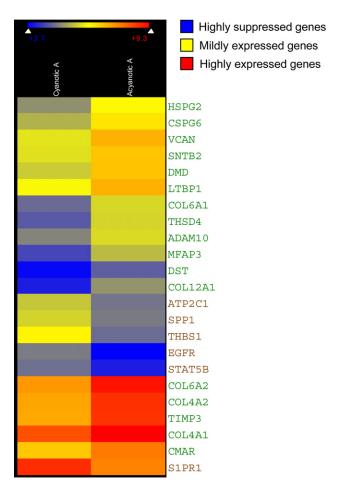


FIGURE E8. Heat Map illustrating the expression levels of the genes implicated in extracellular matrix. *Gene symbols in brown* represent transcripts that were upregulated in cyanotic patients, whereas *gene symbols in green* represent genes downregulated by means of cyanosis. Hierarchical clustering has been performed with ArrayStar software. A 3-color scale was used to illustrate expression level differences, with blue indicating low expression values, yellow indicating intermediately expressed genes, and red representing highly expressed genes.

TABLE E1. Genes exhibiting 1.8-fold or greater expression change in ventricular biopsy specimens of cyanotic versus acyanotic children (upregulated genes)

Gene title	Symbol	Fold	P value	Gene ID
Lysozyme	LYZ	5.103 up	.0112	4069
Protein phosphatase 1, catalytic subunit, beta isoform	PPP1CB	3.849 up	.00559	5500
Early growth response 1	EGR1	3.498 up	.0213	1958
RAP2C, member of RAS oncogene family	RAP2C	3.364 up	.0063	57826
Thrombospondin 1	THBS1	3.285 up	.0265	7057
Nipsnap homolog 3A	NIPSNAP3A	3.055 up	.0229	25934
Ubiquitin B	UBB	3.008 up	.00998	7314
NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 5, 13 kd	NDUFA5	2.984 up	.0187	4698
EF-hand calcium binding domain 2	EFCAB2	2.946 up	.0214	84288
Lactamase, beta 2	LACTB2	2.815 up	.0139	51110
Hypothetical protein DKFZp564O0523	DKFZP564O0523	2.769 up	.0101	84060
Beta-1,3-N-acetylgalactosaminyltransferase 1	B3GALNT1	2.748 up	.0355	8706
Actin-binding rho-activating protein	ABRA	2.711 up	.0112	137735
Eukaryotic translation initiation factor 4E	EIF4E	2.706 up	.0133	1977
KIAA0776	KIAA0776	2.694 up	.0372	23376
CDNA FLJ32626 fis, clone SYNOV1000045		2.692 up	.0448	
CDNA clone IMAGE:4820809		2.685 up	.0113	
COBW domain containing 1	CBWD1	2.681 up	.0212	150472
Zinc finger E-box binding homeobox 2	ZEB2	2.671 up	.0129	9839
Proteasome maturation protein	POMP	2.639 up	.0107	51371
Integral membrane protein 2A	ITM2A	2.631 up	.016	9452
Succinate-CoA ligase, GDP-forming, beta subunit	SUCLG2	2.629 up	.0443	8801
Ubiquitin-specific peptidase 28	USP28	2.612 up	.0124	57646
Lysophospholipase I	LYPLA1	2.583 up	.0109	10434
KIAA0372	KIAA0372	2.579 up	.0139	9652
Epidermal growth factor receptor (v-erb-b oncogene)	EGFR	2.553 up	.0102	1956
Family with sequence similarity 76, member B	FAM76B	2.552 up	.0473	143684
FYVE, RhoGEF and PH domain containing 4	FGD4	2.543 up	.0211	121512
Hypothetical protein FLJ13611	FLJ13611	2.534 up	.011	80006
COX11 homolog, cytochrome c oxidase assembly protein	COX11	2.530 up	.00736	1353
Hypothetical LOC401397	LOC401397	2.484 up	.0167	401397
Kelch-like 20	KLHL20	2.435 up	.0142	27252
FCF1 small subunit (SSU) processome component homolog	FCF1	2.431 up	.0163	93487
Calmodulin 3 (phosphorylase kinase, delta)	CALM3	2.431 up	.0205	808
Family with sequence similarity 73, member A	FAM73A	2.431 up	.0228	374986
CDNA clone IMAGE:5299642		2.407 up	.0432	
Proteasome (prosome, macropain) 26S subunit, ATPase, 2	PSMC2	2.368 up	.0135	5701
Tumor necrosis factor (ligand) superfamily, member 10	TNFSF10	2.363 up	.0456	8743
CDNA clone IMAGE:5267328		2.361 up	.016	
Fibroblast growth factor 7	FGF7	2.338 up	.0381	2252
EGF-containing fibulin-like extracellular matrix protein 1	EFEMP1	2.311 up	.0422	2202
Cytochrome P450, family 51, subfamily A, polypeptide 1	CYP51A1	2.276 up	.0113	1595
Protein phosphatase 1B (formerly 2C), magnesium-dependent, beta isoform	PPM1B	2.264 up	.00902	5495
Lumican	LUM	2.260 up	.025	4060
Serpin peptidase inhibitor, clade B (ovalbumin), member 9	SERPINB9	2.260 up	.0391	5272
Amyotrophic lateral sclerosis 2 (juvenile)	ALS2	2.256 up	.0112	57679
Rab geranylgeranyltransferase, beta subunit	RABGGTB	2.255 up	.00808	5876
Ubiquitin-conjugating enzyme E2D 1 (UBC4/5 homolog, yeast)	UBE2D1	2.243 up	.0281	7321
Hypothetical protein LOC386597	LOC386597	2.233 up	.0107	386597
Tubulin, epsilon 1	TUBE1	2.213 up	.0182	51175
COMM domain containing 8	COMMD8	2.204 up	.016	54951
Erythropoietin receptor	EPOR	2.190 up	.016	2057

TABLE E1. Continued

Gene title	Symbol	Fold	P value	Gene ID
Adaptor-related protein complex 1, sigma 2 subunit	AP1S2	2.186 up	.0454	8905
Striatin, calmodulin binding protein 3	STRN3	2.186 up	.0243	29966
Phosphatase and actin regulator 2	PHACTR2	2.181 up	.0203	9749
Zinc-binding alcohol dehydrogenase, domain containing 1	ZADH1	2.162 up	.0266	145482
Tetraspanin 12	TSPAN12	2.159 up	.036	23554
Transcribed locus		2.157 up	.0121	
Ankyrin 1, erythrocytic	ANK1	2.148 up	.0298	286
Adenosine kinase	ADK	2.143 up	.0113	132
Chromosome 18 open reading frame 55	C18orf55	2.140 up	.0169	29090
Solute carrier family 22, member 25	UST6	2.130 up	.0242	387601
Glycine amidinotransferase (L-arginine:glycine amidinotransferase)	GATM	2.130 up	.0343	2628
Arginyltransferase 1	ATE1	2.130 up	.0291	11101
Serum deprivation response	SDPR	2.128 up	.0391	8436
(phosphatidylserine-binding protein)		•		
Calponin 3, acidic	CNN3	2.125 up	.0407	1266
Hypothetical protein LOC285708	LOC285708	2.110 up	.0154	285708
71 1		2.106 up	.0368	
Chromosome 10 open reading frame 110	C10orf110	2.102 up	.0482	55853
Stomatin (EPB72)–like 1	STOML1	2.099 up	.011	9399
Heterogeneous nuclear ribonucleoprotein H1 (H)	HNRPH1	2.092 up	.0254	3187
Solute carrier family 2 (facilitated glucose transporter),	SLC2A13	2.092 up	.0237	114134
member 13	~~~~~			
Pallidin homolog (mouse)	PLDN	2.087 up	.0138	26258
Trinucleotide repeat containing 6B	TNRC6B	2.079 up	.0357	23112
Ubiquitin-conjugating enzyme E2D 1	UBE2D1	2.075 up	.0342	7321
(UBC4/5 homolog, yeast)	*			
Nuclear factor of activated T cells, cytoplasmic,	NFATC2IP	2.075 up	.0136	84901
calcineurin-dependent 2 interacting protein	.,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,	2.072 up	.0120	0.501
Syntaxin-binding protein 3	STXBP3	2.073 up	.0115	6814
Transcription elongation factor B (SIII), polypeptide 1	TCEB1	2.071 up	.0243	6921
(15 kd, elongin C)				
EF-hand domain family, member A2	EFHA2	2.064 up	.0142	286097
Lyrm7 homolog (mouse)	LYRM7	2.052 up	.0481	90624
ARP3 actin-related protein 3 homolog (yeast)	ACTR3	2.048 up	.0298	10096
Short coiled-coil protein	SCOC	2.047 up	.0213	60592
Ribosomal protein L15///similar to ribosomal protein L15	RPL15	2.047 up	.0212	728088
Hypothetical LOC441642	LOC441642	2.046 up	.0159	441642
CDNA clone IMAGE:4794011	200111012	2.043 up	.0237	111012
Homo sapiens, clone IMAGE:4480721, mRNA		2.043 up	.0448	
Chromosome 8 open reading frame 4	C8orf4	2.042 up	.0388	56892
Polymerase (RNA) II (DNA directed) polypeptide K, 7.0 kd	POLR2K	2.042 up	.0176	5440
PRO1268 protein	PRO1268	2.040 up	.0237	29006
Sarcolemma-associated protein	SLMAP	2.038 up	.0169	7871
Glyceraldehyde-3-phosphate dehydrogenase–like 19///similar	GAPDHL19///	2.036 up	.0153	442262///
to glyceraldehyde-3-phosphate dehydrogenase (GAPDH)		2.030 up	.0133	
Eukaryotic translation initiation factor 4E	<i>LOC732268</i> <i>EIF4E</i>	2.022	.0264	732268 1977
WD repeat and SOCS box–containing 2		2.033 up		
	WSB2	2.031 up 2.022 up	.0371 .00678	55884
CDNA FLJ32691 fis, clone TESTI2000221	COMMD10	•		51207
COMM domain containing 10	COMMD10	2.020 up	.0196	51397
Zinc finger protein, X-linked	ZFX	2.014 up	.0112	7543
Ribosomal protein L23a pseudogene 7	RPL23AP7	2.011 up	.0106	118433
SH3 domain–binding glutamic acid–rich protein like	SH3BGRL	2.004 up	.0214	6451
p21 (CDKN1A)–activated kinase 2	PAK2	2.002 up	.0114	5062
Copine IV	CPNE4	2.000 up	.0172	131034

TABLE E1. Continued

Gene title	Symbol	Fold	P value	Gene ID
NLR family, pyrin domain containing 1	NLRP1	1.999 up	.0303	22861
X-prolyl aminopeptidase (aminopeptidase P) 3, putative	XPNPEP3	1.998 up	.0379	63929
Ribosomal protein L37a	RPL37A	1.997 up	.035	6168
Protein tyrosine phosphatase type IVA, member 1	PTP4A1	1.989 up	.0175	7803
CDNA FLJ30565 fis, clone BRAWH2005008		1.988 up	.0169	
Basic leucine zipper nuclear factor 1 (JEM-1)	BLZF1	1.987 up	.0405	8548
Karyopherin alpha 2 (RAG cohort 1, importin alpha 1)	KPNA2	1.986 up	.0242	3838
Transcribed locus		1.982 up	.0437	
Insulin-like growth factor binding protein 7	IGFBP7	1.981 up	.0404	3490
Centrosomal protein 170 kd	CEP170	1.977 up	.0458	9859
B-cell chronic lymphocytic leukemia/lymphoma 10	BCL10	1.964 up	.0237	8915
Poly(A) binding protein interacting protein 1	PAIP1	1.962 up	.0347	10605
Mediterranean fever	MEFV	1.957 up	.0137	4210
Similar to 60S ribosomal protein L35	LOC643653	1.957 up	.0222	643653
Annexin A7	ANXA7	1.955 up	.012	310
Folliculin	FLCN	1.952 up	.0255	201163
Septin 7	SEPT7	1.948 up	.0483	989
TM2 domain containing 1	TM2D1	1.945 up	.0157	83941
CD8a molecule	CD8A	1.942 up	.0186	925
Secreted phosphoprotein 1 (osteopontin, bone sialoprotein I,	SPP1	1.941 up	.0115	6696
early T-lymphocyte activation 1)				
Selenoprotein P, plasma, 1	SEPP1	1.937 up	.0206	6414
Transmembrane protein 123	TMEM123	1.935 up	.011	114908
CDNA clone IMAGE:5273964		1.935 up	.0483	
Src kinase–associated phosphoprotein 2	SKAP2	1.934 up	.0406	8935
Cyclin-dependent kinase inhibitor 2C (p18, inhibits CDK4)	CDKN2C	1.933 up	.0125	1031
		1.927 up	.024	
Transcribed locus		1.926 up	.0221	
Spermidine/spermine N1-acetyltransferase 1	SAT1	1.925 up	.0162	6303
THAP domain containing 5	THAP5	1.924 up	.0113	168451
Actin-like 6A	ACTL6A	1.923 up	.0182	86
SMT3 suppressor of mif two 3 homolog 1 (S cerevisiae)	SUMO1	1.922 up	.00972	7341
MAD2 mitotic arrest deficient-like 1 (yeast)	MAD2L1	1.922 up	.0114	4085
MANSC domain containing 1	MANSC1	1.921 up	.0232	54682
Endothelial differentiation, sphingolipid	EDG1	1.915 up	.0242	1901
G protein–coupled receptor, 1 Wingless-type MMTV integration site family, member 6	WNT6	1.914 up	.0387	7475
Carboxypeptidase E	CPE	1.914 up 1.914 up	.0236	1363
** *	CFE	1.914 up 1.909 up	.0315	1303
Transcribed locus Interleukin 33	IL33	1.909 up 1.901 up	.0456	90865
	OMA1	1.899 up	.0263	
OMA1 homolog, zinc metallopeptidase (<i>S cerevisiae</i>)				115209
Clare 114 tymer rejection entiren	CALR	1.898 up	.0443	811
Clone 114 tumor rejection antigen	\$1.612.42	1.895 up	.0405	6559
Solute carrier family 12	SLC12A2	1.893 up	.0158	6558
(sodium/potassium/chloride transporters), member 2		1.893 up	.0217	
EF-hand domain family, member A1	EFHA1	1.892 up	.0243	221154
Ribosomal protein L38	RPL38	1.890 up	.0253	6169
Homo sapiens, clone IMAGE:5440917, mRNA	M LJU	1.889 up	.0233	0109
Yip1 domain family, member 4	YIPF4	1.887 up	.0374	84272
Guanine nucleotide binding protein (G protein),	GNG10///	1.884 up	.0224	2790///
gamma 10///hypothetical protein LOC552891	LOC552891	1.00 + up	.0224	552891
Peroxisomal biogenesis factor 7	PEX7	1.881 up	.0135	5191
Signal transducer and activator of transcription 5B	STAT5B	1.881 up	.0133	6777

TABLE E1. Continued

Gene title	Symbol	Fold	P value	Gene ID
Inhibitor of DNA binding 2, dominant negative	ID2///ID2B	1.875 up	.0448	3398///84099
helix-loop-helix protein///inhibitor of DNA binding				
2B, dominant negative helix-loop-helix protein				
Eukaryotic translation initiation factor 1A, X-linked	EIF1AX	1.866 up	.0317	1964
OTU domain containing 6B	OTUD6B	1.865 up	.0325	51633
RAB12, member RAS oncogene family	RAB12	1.864 up	.024	201475
Late cornified envelope 1E	LCE1E	1.862 up	.00469	353135
RNA binding motif (RNP1, RRM) protein 3	RBM3	1.862 up	.0125	5935
Thiopurine S-methyltransferase	TPMT	1.858 up	.0232	7172
Mitochondrial ribosomal protein L50	MRPL50	1.856 up	.0389	54534
Hydroxysteroid dehydrogenase like 2	HSDL2	1.853 up	.0352	84263
CDNA clone IMAGE:4830861		1.849 up	.0181	
Methylenetetrahydrofolate dehydrogenase	MTHFD2L	1.847 up	.0293	441024
(NADP ⁺ dependent) 2–like				
Jagged 1 (Alagille syndrome)	JAG1	1.847 up	.0319	182
		1.847 up	.0111	
Myozenin 2	MYOZ2	1.843 up	.032	51778
Family with sequence similarity 115, member A	FAM115A	1.842 up	.0368	9747
Cysteine and histidine-rich domain	CHORDC1	1.841 up	.0109	26973
(CHORD)–containing 1				
ATPase, Ca ⁺⁺ transporting, type 2C, member 1	ATP2C1	1.841 up	.0435	27032
Copine IV	CPNE4	1.841 up	.0149	131034
NLR family, pyrin domain containing 1	NLRP1	1.834 up	.0241	22861
Basic transcription factor 3	BTF3	1.834 up	.0104	689
Golgi transport 1 homolog B (S cerevisiae)	GOLT1B	1.833 up	.0312	51026
Transcribed locus		1.832 up	.0171	
Hypothetical protein FLJ22222	FLJ22222	1.829 up	.0115	79701
Dihydropyrimidine dehydrogenase	DPYD	1.826 up	.0395	1806
Chromosome 5 open reading frame 33	C5orf33	1.825 up	.0109	133686
Epidermal growth factor receptor (erythroblastic	EGFR	1.824 up	.0152	1956
leukemia viral (v-erb-b) oncogene homolog, avian)				
Jerky homolog–like (mouse)	JRKL	1.824 up	.0387	8690
Sphingomyelin synthase 1	SGMS1	1.824 up	.0109	259230
Zinc finger protein 654	ZNF654	1.823 up	.0367	55279
Transcribed locus (serine/threonine kinase 17B)	STK17B	1.819 up	.0236	
(apoptosis-inducing)		•		
Gap junction protein, beta 4, 30.3 kd	GJB4	1.814 up	.032	127534
Sp3 transcription factor	SP3	1.813 up	.0124	6670
Leucine-rich repeat containing 25	LRRC25	1.813 up	.028	126364
Transmembrane and tetratricopeptide repeat containing 3	TMTC3	1.813 up	.0209	160418
Nucleosome assembly protein 1-like 1	NAP1L1	1.812 up	.0266	4673
Fibrinogen-like 2	FGL2	1.812 up	.0191	10875
Zinc finger protein 552	ZNF552	1.808 up	.0125	79818
Hypothetical gene supported by AK023501	FLJ13439	1.808 up	.0206	399876
Full-length insert cDNA clone ZD82B02		1.807 up	.0197	
Mesenchymal stem cell protein DSC96		1.805 up	.034	
Apolipoprotein B mRNA editing enzyme, catalytic	APOBEC3G	1.803 up	.0176	60489
polypeptide–like 3G		•		
Eukaryotic translation initiation factor 1A,	EIF1AP1///EIF1AX	1.803 up	.0298	1964///280661
X-linked///eukaryotic translation initiation		-		
factor 1A pseudogene 1				
GULP, engulfment adaptor PTB domain containing 1	GULP1	1.801 up	.0437	51454

TABLE E2. Genes exhibiting 1.8-fold or greater expression change in ventricular biopsy specimens of cyanotic versus acyanotic children (downregulated genes)

Gene title	Symbol	Fold	P value	Gene ID
Oxoglutarate (alpha-ketoglutarate)	OGDH	6.227 down	.0218	4967
dehydrogenase (lipoamide)				
Nebulin-related anchoring protein	NRAP	5.878 down	.0154	4892
Plakophilin 2	PKP2	5.058 down	.0124	5318
Mitofusin 2	MFN2	4.453 down	.0216	9927
Glucosamine (N-acetyl)-6-sulfatase	GNS	4.439 down	.0136	2799
AHNAK nucleoprotein	AHNAK	4.417 down	.011	79026
Solute carrier family 8 (sodium/calcium exchanger), member 1	SLC8A1	4.284 down	.00929	6546
Coatomer protein complex, subunit alpha	COPA	4.221 down	.0222	1314
Cardiomyopathy-associated 5	CMYA5	4.185 down	.0118	202333
Phosphodiesterase 4D–interacting protein (myomegalin)	PDE4DIP	3.875 down	.0114	9659
Nuclear mitotic apparatus protein 1	NUMA1	3.768 down	.0116	4926
SET domain containing 5	SETD5	3.652 down	.0124	55209
Forkhead box O3	FOXO3	3.606 down	.0271	2309
Solute carrier family 39 (zinc transporter), member 14	SLC39A14	3.589 down	.0199	23516
Plectin 1, intermediate filament binding protein 500 kd	PLEC1	3.542 down	.0228	5339
Zinc finger protein 36, C3H type–like 2	ZFP36L2	3.495 down	.0347	678
Homeodomain interacting protein kinase 3	HIPK3	3.485 down	.0121	10114
Chromosome 19 open reading frame 6	C19orf6	3.457 down	.0113	91304
Filamin binding LIM protein 1	FBLIM1	3.429 down	.0314	54751
Interleukin 6 signal transducer (gp130,	IL6ST	3.397 down	.0114	3572
oncostatin M receptor)				
YTH domain family, member 3	YTHDF3	3.396 down	.0146	253943
DEAD (Asp-Glu-Ala-Asp) box polypeptide 42	DDX42	3.385 down	.0124	11325
AF4/FMR2 family, member 4	AFF4	3.372 down	.0136	27125
DEAH (Asp-Glu-Ala-His) box polypeptide 9	DHX9	3.350 down	.011	1660
WD repeat domain 68	WDR68	3.246 down	.0131	10238
ATPase type 13A3	ATP13A3	3.204 down	.00672	79572
Sec61 alpha 1 subunit (S cerevisiae)	SEC61A1	3.183 down	.0175	29927
Chaperone, ABC1 activity of bc1 complex homolog	CABC1	3.182 down	.0219	56997
Signal-induced proliferation-associated 1 like 2	SIPA1L2	3.164 down	.0183	57568
Gelsolin (amyloidosis, Finnish type)	GSN	3.155 down	.0125	2934
A kinase (PRKA) anchor protein 6	AKAP6	3.123 down	.00579	9472
Peroxisome proliferator-activated receptor alpha	PPARA	3.107 down	.03	5465
PDZ and LIM domain 5	PDLIM5	3.101 down	.0125	10611
Tetratricopeptide repeat domain 3	TTC3	3.088 down	.0109	7267
Sp1 transcription factor	SP1	3.080 down	.0146	6667
Trafficking protein, kinesin binding 1	TRAK1	3.074 down	.0224	22906
Inositol polyphosphate-5-phosphatase, 40 kd	INPP5A	3.072 down	.0145	3632
Phosphodiesterase 3A, cGMP-inhibited	PDE3A	3.069 down	.00601	5139
Suppressor of Ty 16 homolog (S cerevisiae)	SUPT16H	3.057 down	.0187	11198
Nucleoporin 62 kd	NUP62	3.054 down	.0161	23636
Proteasome (prosome, macropain) activator subunit 4	PSME4	3.047 down	.0113	23198
FYVE, RhoGEF and PH domain containing 4	FGD4	3.043 down	.00671	121512
ELKS/RAB6-interacting/CAST family member 1	ERC1	3.033 down	.0219	23085
PRP6 pre-mRNA processing factor 6 homolog	PRPF6	3.029 down	.0125	24148
Collagen, type VI, alpha 1	COL6A1	3.000 down	.00664	1291
Myotubularin related protein 1	MTMR1	2.983 down	.00996	8776
CDNA FLJ38472 fis, clone FEBRA2022148		2.981 down	.0437	
Transformation/transcription domain-associated protein	TRRAP	2.973 down	.0136	8295
Ubiquitination factor E4B (UFD2 homolog, yeast)	UBE4B	2.970 down	.0116	10277
Myeloid cell leukemia sequence 1 (BCL2-related)	MCL1	2.967 down	.0218	4170
ATPase, Ca ⁺⁺ transporting, plasma membrane 4	ATP2B4	2.957 down	.0121	493

TABLE E2. Continued

Gene title	Symbol	Fold	P value	Gene ID
Filamin A interacting protein 1	FILIP1	2.950 down	.0066	27145
Ribosomal protein S23	RPS23	2.945 down	.0156	6228
Carboxypeptidase D	CPD	2.937 down	.0137	1362
F-box and WD repeat domain containing 11	FBXW11	2.925 down	.0112	23291
LAG1 homolog, ceramide synthase 6	LASS6	2.914 down	.0366	253782
SWI/SNF related, matrix associated, actin dependent	SMARCA2	2.905 down	.0111	6595
regulator of chromatin, subfamily a, member 2				
Sorbin and SH3 domain containing 1	SORBS1	2.894 down	.0176	10580
Guanine nucleotide binding protein (G protein), alpha 13	GNA13	2.865 down	.0241	10672
SCY1-like 2 (S cerevisiae)	SCYL2	2.852 down	.0119	55681
Fas (TNFRSF6) associated factor 1	FAF1	2.819 down	.0139	11124
Ezrin	EZR	2.818 down	.0295	7430
Zinc finger protein 672	ZNF672	2.814 down	.0144	79894
GATA binding protein 4	GATA4	2.805 down	.0212	2626
Phosphodiesterase 1C, calmodulin-dependent 70 kd	PDE1C	2.803 down	.0109	5137
Fibroblast growth factor receptor 1	FGFR1	2.800 down	.0132	2260
Muscleblind-like 2 (Drosophila)	MBNL2	2.780 down	.0244	10150
Ubiquitin protein ligase E3 component n-recognin 1	UBR1	2.771 down	.0253	197131
Sodium channel, voltage-gated, type VII, alpha	SCN7A	2.769 down	.0113	6332
Collagen, type VI, alpha 2	COL6A2	2.742 down	.0112	1292
Myelin basic protein	MBP	2.742 down	.0103	4155
Eukaryotic translation initiation factor 4 gamma, 1	EIF4G1	2.740 down	.0125	1981
Exportin 7	XPO7	2.736 down	.0112	23039
Endothelial PAS domain protein 1	EPAS1	2.725 down	.0232	2034
MutS homolog 6 (E coli)	MSH6	2.724 down	.0129	2956
Transforming growth factor, beta receptor II (70/80 kd)	TGFBR2	2.715 down	.0124	7048
Gamma-aminobutyric acid (GABA) A receptor, beta 1	GABRB1	2.710 down	.0311	2560
Pleckstrin homology-like domain, family B, member 2	PHLDB2	2.706 down	.0174	90102
Catenin (cadherin-associated protein), alpha 1, 102 kd	CTNNA1	2.703 down	.0228	1495
Carboxyl ester lipase (bile salt–stimulated lipase)	CEL	2.700 down	.0449	1056
Prune homolog	PRUNE	2.678 down	.0233	58497
BCL2-like 1	BCL2L1	2.674 down	.0329	598
Solute carrier family 16, member 1 (monocarboxylic acid transporter 1)	SLC16A1	2.672 down	.0128	6566
CDNA FLJ38048 fis, clone CTONG2014264///CDNA FLJ39067 fis, clone NT2RP7014910		2.668 down	.0154	
ADP-ribosylation factor guanine nucleotide-exchange factor 2	ARFGEF2	2.654 down	.0168	10564
Chromosome 9 open reading frame 5	C9orf5	2.650 down	.01	23731
Zinc finger, MYND domain containing 11	ZMYND11	2.635 down	.011	10771
Ras homolog gene family, member B	RHOB	2.634 down	.0488	388
Kringle containing transmembrane protein 1	KREMEN1	2.620 down	.0448	83999
F-box protein 38	FBXO38	2.617 down	.0125	81545
Secretory carrier membrane protein 1	SCAMP1	2.616 down	.0123	9522
Putative homeodomain transcription factor 2	PHTF2	2.605 down	.0178	57157
Spastic paraplegia 7	SPG7	2.598 down	.0451	6687
Membrane protein, palmitoylated 5 (MAGUK p55	MPP5	2.595 down	.0103	64398
subfamily member 5)				
BCL2-associated transcription factor 1	BCLAF1	2.585 down	.0138	9774
Nuclear receptor coactivator 2	NCOA2	2.580 down	.0116	10499
Myomesin family, member 3	MYOM3	2.579 down	.0346	127294
Forkhead box N3	FOXN3	2.573 down	.0118	1112
Blood vessel epicardial substance	BVES	2.570 down	.0295	11149
Family with sequence similarity 62 (C2 domain containing) member B	FAM62B	2.569 down	.0134	57488

TABLE E2. Continued

Gene title	Symbol	Fold	P value	Gene ID
Procollagen-proline, 2-oxoglutarate 4-dioxygenase	P4HB	2.564 down	.0384	5034
(proline 4-hydroxylase), beta polypeptide				
Ubiquitin-specific peptidase 2	USP2	2.544 down	.00576	9099
Zinc finger, FYVE domain containing 1	ZFYVE1	2.540 down	.0149	53349
General transcription factor II, i	GTF2I	2.539 down	.0135	2969
Son of sevenless homolog 2	SOS2	2.539 down	.0211	6655
Thrombospondin, type I, domain containing 4	THSD4	2.536 down	.0177	79875
Jumonji domain containing 1C	JMJD1C	2.533 down	.0098	221037
Ring finger protein 38	RNF38	2.532 down	.00664	152006
Muscleblind-like	MBNL1	2.517 down	.0418	4154
Coiled-coil domain containing 93	CCDC93	2.515 down	.0102	54520
Cleft lip and palate associated transmembrane protein 1	CLPTM1	2.505 down	.0301	1209
HECT domain containing 1	HECTD1	2.502 down	.0133	25831
Transmembrane protein 168	TMEM168	2.500 down	.00924	64418
ST6 beta-galactosamide alpha-2,6-sialyltranferase 1	ST6GAL1	2.495 down	.0306	6480
Glutamate-ammonia ligase (glutamine synthetase)	GLUL	2.493 down	.0239	2752
WD repeat domain 1	WDR1	2.490 down	.0212	9948
UBX domain containing 7	UBXD7	2.483 down	.0233	26043
Dihydrolipoamide branched chain transacylase E2	DBT	2.482 down	.0161	1629
Zinc finger, BED-type containing 1	ZBED1	2.478 down	.0168	9189
Solute carrier family 29 (nucleoside transporters), member 1	SLC29A1	2.476 down	.0163	2030
Mitogen-activated protein kinase 14	MAPK14	2.472 down	.0205	1432
GRB2-associated binding protein 1	GAB1	2.465 down	.00951	2549
Coiled-coil domain containing 22	CCDC22	2.461 down	.0137	28952
Latent transforming growth factor beta binding protein 3	LTBP3	2.459 down	.0169	4054
Replication initiator 1	REPIN1	2.455 down	.0256	29803
Utrophin	UTRN	2.452 down	.0111	7402
Signal recognition particle receptor ("docking protein")	SRPR	2.451 down	.0181	6734
Forkhead box J3	FOXJ3	2.450 down	.0142	22887
Collagen, type XII, alpha 1	COL12A1	2.446 down	.0109	1303
TIMP metallopeptidase inhibitor 3 (Sorsby fundus	TIMP3	2.445 down	.0338	7078
dystrophy, pseudoinflammatory)				
Sperm associated antigen 9	SPAG9	2.445 down	.0109	9043
Zinc finger protein 395	ZNF395	2.441 down	.0264	55893
A kinase (PRKA) anchor protein 1	AKAP1	2.437 down	.0226	8165
Activating transcription factor 2	ATF2	2.434 down	.0145	1386
Collagen, type IV, alpha 2	COL4A2	2.431 down	.0277	1284
Myelin basic protein	MBP	2.427 down	.00494	4155
Golgi associated PDZ and coiled-coil motif containing	GOPC	2.424 down	.00952	57120
Microfibrillar-associated protein 3	MFAP3	2.423 down	.0171	4238
UBX domain containing 2	UBXD2	2.420 down	.0136	23190
Heparan sulfate proteoglycan 2	HSPG2	2.415 down	.0109	3339
Desmoglein 2	DSG2	2.411 down	.0203	1829
Exocyst complex component 4	EXOC4	2.410 down	.019	60412
Chloride intracellular channel 4	CLIC4	2.410 down 2.410 down	.0207	25932
RAN binding protein 2	RANBP2	2.405 down	.0116	5903
RAN binding protein 2 Ring finger and CCCH-type zinc finger domains 2	RC3H2	2.405 down 2.405 down	.0110	54542
ATP-binding cassette, sub-family C (CFTR/MRP),	ABCC9	2.403 down 2.402 down	.0066	10060
member 9	ABCC9		.0000	10000
LSM14B, SCD6 homolog B (S cerevisiae)	LSM14B	2.396 down	.0118	149986
Synaptopodin 2	SYNPO2	2.395 down	.0246	171024
Tripeptidyl peptidase I	TPP1	2.391 down	.0215	1200
Sema domain, transmembrane domain (TM), and cytoplasmic domain, (semaphorin) 6D	SEMA6D	2.386 down	.0442	80031
Discs, large homolog 1 (Drosophila)	DLG1	2.382 down	.0197	1739

TABLE E2. Continued

Gene title	Symbol	Fold	P value	Gene ID
Obscurin-like 1	OBSL1	2.381 down	.0441	23363
Ubiquitination factor E4B (UFD2 homolog, yeast)	UBE4B	2.377 down	.0108	10277
LIM domain containing preferred translocation partner in lipoma	LPP	2.372 down	.0134	4026
Plakophilin 4	PKP4	2.366 down	.00687	8502
Dual specificity phosphatase 3 (vaccinia virus phosphatase VH1-related)	DUSP3	2.361 down	.0233	1845
Phosphatase and actin regulator 2	PHACTR2	2.361 down	.0158	9749
DEAD (Asp-Glu-Ala-Asp) box polypeptide 3, X-linked	DDX3X	2.357 down	.0208	1654
Protein tyrosine phosphatase, nonreceptor type 11 (Noonan syndrome 1)	PTPN11	2.351 down	.0112	5781
Chromosome 6 open reading frame 106	C6orf106	2.348 down	.012	64771
Rho GTPase activating protein 5	ARHGAP5	2.348 down	.0103	394
Runt-related transcription factor 1; translocated to, 1	RUNX1T1	2.348 down	.0119	862
(cyclin D-related)				
Phosphoinositide-3-kinase, class 2, alpha polypeptide	PIK3C2A	2.347 down	.0169	5286
Phospholipase D1, phosphatidylcholine-specific	PLD1	2.347 down	.0256	5337
Dystrophin (muscular dystrophy, Duchenne and Becker types)	DMD	2.344 down	.00618	1756
Ankyrin 2, neuronal	ANK2	2.343 down	.00798	287
Janus kinase 1 (a protein tyrosine kinase)	JAK1	2.340 down	.0227	3716
Lamin A/C	LMNA	2.335 down	.0134	4000
Bromodomain containing 4	BRD4	2.334 down	.039	23476
Ubiquitin specific peptidase 42	USP42	2.321 down	.0166	84132
Methyl CpG binding protein 2 (Rett syndrome)	MECP2	2.320 down	.0479	4204
Transglutaminase 2 (C polypeptide, protein-glutamine-gamma-glutamyltransferase)	TGM2	2.319 down	.0206	7052
Ankylosis, progressive homolog (mouse)	ANKH	2.312 down	.0205	56172
Muskelin 1, intracellular mediator containing kelch motifs	MKLN1	2.311 down	.0249	4289
Lysosomal-associated membrane protein 1	LAMP1	2.311 down	.0274	3916
CUG triplet repeat, RNA binding protein 1	CUGBP1	2.309 down	.0112	10658
Secretory carrier membrane protein 1	SCAMP1	2.308 down	.0113	9522
WD repeat domain 68	WDR68	2.307 down	.0172	10238
Ribosomal protein S6 kinase, 90 kd, polypeptide 2	RPS6KA2	2.304 down	.0354	6196
Junctional adhesion molecule 3	JAM3	2.303 down	.0158	83700
Bromodomain containing 2	BRD2	2.294 down	.022	6046
Phosphatidylinositol 4-kinase, catalytic, beta	PI4KB	2.292 down	.0128	5298
Jun D proto-oncogene	JUND	2.289 down	.0242	3727
Microtubule associated serine/threonine kinase family member 4	MAST4	2.285 down	.0448	375449
Utrophin	UTRN	2.284 down	.011	7402
Chromosome 11 open reading frame 30	C11orf30	2.284 down	.0433	56946
RAB6A, member RAS oncogene family	RAB6A	2.283 down	.0134	5870
Chromosome 4 open reading frame 18	C4orf18	2.283 down	.0142	51313
Protein tyrosine phosphatase, non-receptor type 11 (Noonan syndrome 1)	PTPN11	2.283 down	.00661	5781
Upstream binding transcription factor, RNA polymerase I	UBTF	2.281 down	.0101	7343
Collagen, type VI, alpha 1	COL6A1	2.280 down	.0169	1291
Calcium channel, voltage-dependent, beta 2 subunit	CACNB2	2.280 down	.0449	783
Corin, serine peptidase	CORIN	2.277 down	.0284	10699
Spire homolog 1 (<i>Drosophila</i>)	SPIRE1	2.276 down	.0311	56907
Collagen, type VI, alpha 1	COL6A1	2.273 down	.00933	1291
Angiopoietin-like 2	ANGPTL2	2.272 down	.0125	23452
Sortilin 1	SORT1	2.271 down	.0214	6272
Vacuolar protein sorting 35 homolog (S cerevisiae)	VPS35	2.269 down	.0219	55737
ADAM metallopeptidase domain 9 (meltrin gamma)	ADAM9	2.269 down 2.269 down	.016	8754
Palladin, cytoskeletal associated protein	PALLD	2.266 down	.010	23022

TABLE E2. Continued

Gene title	Symbol	Fold	P value	Gene ID
Tumor necrosis factor receptor superfamily, member 19	TNFRSF19	2.258 down	.0218	55504
Zinc finger protein, X-linked///zinc finger protein, Y-linked	ZFX///ZFY	2.256 down	.0216	7543///7544
Oxoglutarate (alpha-ketoglutarate) dehydrogenase (lipoamide)	OGDH	2.251 down	.0124	4967
CDV3 homolog (mouse)	CDV3	2.251 down	.0111	55573
Meningioma expressed antigen 5 (hyaluronidase)	MGEA5	2.245 down	.0113	10724
Solute carrier family 7 (cationic amino acid transporter,	SLC7A6	2.245 down	.0455	9057
y+ system), member 6				
Component of oligomeric Golgi complex 3	COG3	2.241 down	.0125	83548
Protein tyrosine phosphatase, non-receptor type 21	PTPN21	2.239 down	.0199	11099
Obscurin-like 1	OBSL1	2.238 down	.0324	23363
Phosphorylase kinase, gamma 1 (muscle)	PHKG1	2.236 down	.0239	5260
LanC lantibiotic synthetase component C-like 1 (bacterial)	LANCL1	2.234 down	.0183	10314
Obscurin-like 1	OBSL1	2.229 down	.0282	23363
Ubiquitin specific peptidase 47	USP47	2.227 down	.00285	55031
Structural maintenance of chromosomes 3	SMC3	2.226 down	.0134	9126
Ankyrin repeat and KH domain containing 1	ANKHD1	2.225 down	.0259	404734
Kelch repeat and BTB (POZ) domain containing 2	KBTBD2	2.225 down	.0116	25948
Arginine-glutamic acid dipeptide (RE) repeats	RERE	2.224 down	.0448	473
Nuclear factor of activated T-cells, cytoplasmic,	NFATC2IP	2.223 down	.0151	84901
calcineurin-dependent 2 interacting protein				
Spire homolog 1 (Drosophila)	SPIRE1	2.217 down	.0146	56907
Protein phosphatase 1, regulatory (inhibitor) subunit 3B	PPP1R3B	2.217 down	.0234	79660
Versican	VCAN	2.212 down	.0487	1462
Mitogen-activated protein kinase 14	MAPK14	2.210 down	.0377	1432
ARP2 actin-related protein 2 homolog (yeast)	ACTR2	2.205 down	.0088	10097
Sorbin and SH3 domain containing 1	SORBS1	2.205 down	.0112	10580
Son of sevenless homolog 2 (Drosophila)	SOS2	2.204 down	.0404	6655
Mitogen-activated protein kinase 14	MAPK14	2.201 down	.0111	1432
Glucosaminyl (N-acetyl) transferase 2, I-branching enzyme (I blood group)	GCNT2	2.194 down	.0255	2651
MRNA; cDNA DKFZp451B209 (from clone DKFZp451B209)		2.193 down	.028	
ARP2 actin-related protein 2 homolog (yeast)	ACTR2	2.193 down	.0167	10097
Syntaxin 16	STX16	2.192 down	.0145	8675
Non-POU domain containing, octamer-binding	NONO	2.190 down	.0255	4841
Jumonji, AT rich interactive domain 1B	JARID1B	2.189 down	.0274	10765
Replication protein A1, 70 kd	RPA1	2.185 down	.0103	6117
Sorting nexin 13	SNX13	2.183 down	.0124	23161
Neural cell adhesion molecule 1	NCAM1	2.177 down	.0119	4684
Ubiquitin protein ligase E3B	UBE3B	2.177 down	.012	89910
Endoplasmic reticulum aminopeptidase 2	ERAP2	2.172 down	.0346	64167
Mitogen-activated protein kinase kinase kinase kinase 5	MAP4K5	2.171 down	.0111	11183
Chromosome 6 open reading frame 166	C6orf166	2.168 down	.0171	55122
Prickle homolog 1 (Drosophila)	PRICKLE1	2.168 down	.0424	144165
Erythrocyte membrane protein band 4.1-like 2	EPB41L2	2.168 down	.0116	2037
Platelet-activating factor acetylhydrolase, isoform Ib, alpha subunit 45 kd	PAFAH1B1	2.165 down	.0262	5048
Chromosome 1 open reading frame 55	C1orf55	2.164 down	.0169	163859
v-erb-b2 erythroblastic leukemia viral oncogene homolog 2,	ERBB2	2.163 down	.0132	2064
neuro/glioblastoma derived oncogene homolog (avian)				
DnaJ (Hsp40) homolog, subfamily C, member 14	DNAJC14	2.163 down	.0253	85406
Family with sequence similarity 115, member A///family	FAM115A///	2.162 down	.011	653199///9747
with sequence similarity 115, member B	FAM115B			
Trans-Golgi network protein 2	TGOLN2	2.160 down	.0212	10618
Presenilin 1 (Alzheimer disease 3)	PSEN1	2.160 down	.0142	5663

TABLE E2. Continued

Gene title	Symbol	Fold	P value	Gene ID
Cbp/p300-interacting transactivator, with Glu/Asp-rich	CITED2	2.160 down	.0323	10370
carboxy-terminal domain, 2				
Ubiquitin-like modifier activating enzyme 1	UBA1	2.159 down	.0234	7317
Phosphatidylinositol-4-phosphate 5-kinase, type I, alpha	PIP5K1A	2.157 down	.0366	8394
B-cell chronic lymphocytic leukemia/lymphoma 6 (zinc	BCL6	2.156 down	.0463	604
finger protein 51)				
Zinc finger protein 317	ZNF317	2.155 down	.0206	57693
Sorting nexin 13	SNX13	2.153 down	.0236	23161
SUMO1/sentrin/SMT3 specific peptidase 3	SENP3	2.150 down	.0127	26168
Structural maintenance of chromosomes 3	SMC3	2.149 down	.0115	9126
Methionine adenosyltransferase II, alpha	MAT2A	2.148 down	.0187	4144
Polymerase (DNA-directed), delta interacting protein 3	POLDIP3	2.145 down	.0133	84271
Receptor interacting protein kinase 5	RIPK5	2.139 down	.012	25778
Retinoic acid receptor, beta	RARB	2.137 down	.0307	5915
O-linked N-acetylglucosamine (GlcNAc) transferase	OGT	2.136 down	.0154	8473
(UDP-N-acetylglucosamine:polypeptide-N-acetylglucosaminyl				
transferase)	DDEIDD1	2.424.1	0.1.2	0.40
PTPRF interacting protein, binding protein 1 (liprin beta 1)	PPFIBP1	2.136 down	.012	8496
Trinucleotide repeat containing 6C	TNRC6C	2.136 down	.0124	57690
FRY-like	FRYL	2.134 down	.0128	285527
Ankyrin repeat and FYVE domain containing 1	ANKFY1	2.134 down	.0125	51479
DIP2 disco-interacting protein 2 homolog C (<i>Drosophila</i>)	DIP2C	2.134 down	.0113	22982
ARP2 actin-related protein 2 homolog (yeast)	ACTR2	2.133 down	.015	10097
Deleted in liver cancer 1	DLC1	2.132 down	.0206	10395
ets variant gene 1	ETV1	2.131 down	.0239	2115
Translocated promoter region (to activated MET oncogene)	TPR	2.131 down	.0243	7175
Signal transducer and activator of transcription 3	STAT3	2.130 down	.0187	6774
(acute-phase response factor)	G 4 D 2 O	2.420.1	0015	
Calcium binding protein 39	CAB39	2.129 down	.0217	51719
Glutamine and serine rich 1	QSER1	2.129 down	.0119	79832
Actin binding LIM protein 1	ABLIM1	2.127 down	.017	3983
Zinc finger protein 36, C3H type-like 2	ZFP36L2	2.125 down	.0394	678
Junctional adhesion molecule 3	JAM3	2.122 down	.00921	83700
Golgi autoantigen, golgin subfamily a, 2	GOLGA2	2.121 down	.0272	2801
Nuclear factor I/A	NFIA	2.121 down	.0102	4774
Cholinergic receptor, muscarinic 2	CHRM2	2.120 down	.0355	1129
Ankyrin 3, node of Ranvier (ankyrin G)	ANK3	2.120 down	.0365	288
Neuroblastoma breakpoint family, member 1	NBPF1	2.117 down	.0114	149013
FRY-like	FRYL	2.115 down	.0254	285527
UDP-N-acetyl-alpha-D-galactosamine:polypeptide	GALNT2	2.113 down	.0158	2590
N-acetylgalactosaminyltransferase 2 (GalNAc-T2)	MADEO	2 112 1	0276	2011
MAP/microtubule affinity-regulating kinase 2	MARK2	2.113 down	.0376	2011
Transmembrane 9 superfamily protein member 4	TM9SF4	2.113 down	.0212	9777
Nucleobindin 1	NUCB1	2.109 down	.0258	4924
Rho GTPase activating protein 26	ARHGAP26	2.109 down	.0228	23092
Dynactin 1 (p150, glued homolog, <i>Drosophila</i>)	DCTN1	2.109 down	.0157	1639
Ubiquitin specific peptidase 10	USP10	2.106 down	.0209	9100
Squamous cell carcinoma antigen recognized by T cells 3	SART3	2.105 down	.0113	9733
Importin 8	IPO8	2.104 down	.0137	10526
Hepatocyte growth factor-regulated tyrosine kinase substrate	HGS	2.103 down	.0206	9146
Solute carrier family 8 (sodium/calcium exchanger), member 1	SLC8A1	2.102 down	.0122	6546
Protein tyrosine phosphatase, non-receptor type 12	PTPN12	2.102 down	.041	5782
Chloride intracellular channel 4	CLIC4	2.100 down	.026	25932
FK506 binding protein 15, 133 kd	FKBP15	2.099 down	.0249	23307
Insulin-like growth factor binding protein 5	IGFBP5	2.097 down	.0263	3488

TABLE E2. Continued

Gene title	Symbol	Fold	P value	Gene ID
ATPase, H ⁺ transporting, lysosomal 70 kd, V1	ATP6V1A	2.097 down	.0143	523
subunit A				
Neighbor of BRCA1 gene 1///similar to	LOC727732///	2.096 down	.0126	4077///727732
neighbor of BRCA1 gene 1	NBR1			
TEA domain family member 2	TEAD2	2.096 down	.0399	8463
Cytokine-like nuclear factor n-pac	N-PAC	2.095 down	.0225	84656
CCR4-NOT transcription complex, subunit 1	CNOT1	2.093 down	.0122	23019
Myosin XVIIIB	MYO18B	2.090 down	.0218	84700
Leucine-rich repeats and immunoglobulin-like domains 1	LRIG1	2.090 down	.0134	26018
Zinc finger protein 514	ZNF514	2.090 down	.0335	84874
Apolipoprotein E	APOE	2.088 down	.0284	348
Minichromosome maintenance complex component 7	MCM7	2.086 down	.0258	4176
Arsenate resistance protein 2	ARS2	2.082 down	.0209	51593
N-acetyltransferase 11	NAT11	2.081 down	.0317	79829
GM2 ganglioside activator	GM2A	2.079 down	.0372	2760
Jun D proto-oncogene	JUND	2.079 down	.0218	3727
Mitogen-activated protein kinase kinase kinase 2	MAP3K2	2.077 down	.00667	10746
Karyopherin alpha 1 (importin alpha 5)	KPNA1	2.076 down	.0169	3836
YY1 transcription factor	<i>YY1</i>	2.075 down	.048	7528
KDEL (Lys-Asp-Glu-Leu) endoplasmic reticulum	KDELR1	2.074 down	.0175	10945
protein retention receptor 1				
Prolyl endopeptidase-like	PREPL	2.072 down	.0113	9581
O-linked N-acetylglucosamine (GlcNAc) transferase	OGT	2.072 down	.0136	8473
(UDP-N-acetylglucosamine:polypeptide-N-acetylglucosaminyl				
transferase)				
Insulin-like growth factor 2 receptor	IGF2R	2.072 down	.0102	3482
Zinc finger protein 275	ZNF275	2.070 down	.0462	10838
Zinc finger, MYM-type 2	ZMYM2	2.069 down	.0255	7750
GTPase activating protein (SH3 domain) binding protein 2	G3BP2	2.069 down	.0134	9908
CDNA FLJ33081 fis, clone TRACH2000321		2.069 down	.0287	
KIAA0430	KIAA0430	2.068 down	.0136	9665
ets variant gene 1	ETV1	2.068 down	.0125	2115
Leucine rich repeat (in FLII) interacting protein 2	LRRFIP2	2.067 down	.0162	9209
Endothelial differentiation, lysophosphatidic acid	EDG7	2.066 down	.0437	23566
G-protein-coupled receptor, 7				
Obscurin-like 1	OBSL1	2.066 down	.0243	23363
Retinoblastoma-like 2 (p130)	RBL2	2.065 down	.0175	5934
Ubiquitin-conjugating enzyme E2G 2 (UBC7	UBE2G2	2.065 down	.0125	7327
homolog, yeast)				
Calcium channel, voltage-dependent, alpha	CACNA2D1	2.062 down	.0237	781
2/delta subunit 1				
Uridine-cytidine kinase 1-like 1	UCKL1	2.062 down	.0344	54963
Bromodomain containing 2	BRD2	2.059 down	.0473	6046
Tripartite motif-containing 56	TRIM56	2.059 down	.0129	81844
AF4/FMR2 family, member 4	AFF4	2.059 down	.0442	27125
Ubiquitously transcribed tetratricopeptide repeat,	UTX	2.058 down	.0299	7403
X chromosome				
Spectrin, beta, nonerythrocytic 1	SPTBN1	2.057 down	.017	6711
Striatin, calmodulin binding protein	STRN	2.057 down	.0113	6801
Uridine-cytidine kinase 1	UCK1	2.056 down	.0281	83549
Solute carrier family 2 (facilitated glucose transporter),	SLC2A11	2.055 down	.0197	66035
member 11				
Hypothetical LOC552889	LOC552889	2.053 down	.0136	552889
Peroxisome proliferator-activated receptor alpha	PPARA	2.051 down	.0151	5465
1 Cloxisolile profilerator-activated receptor alpha	1 1 /111/1		.0151	

TABLE E2. Continued

Gene title	Symbol	Fold	P value	Gene ID
Outer dense fiber of sperm tails 2	ODF2	2.049 down	.0114	4957
O-linked N-acetylglucosamine (GlcNAc) transferase	OGT	2.048 down	.016	8473
(UDP-N-acetylglucosamine:polypeptide-N-acetylglucosaminyl				
transferase)				
Janus kinase 1 (a protein tyrosine kinase)	JAK1	2.047 down	.0312	3716
Structure specific recognition protein 1	SSRP1	2.047 down	.0211	6749
Glutamyl-prolyl-tRNA synthetase	<i>EPRS</i>	2.047 down	.0149	2058
Karyopherin alpha 4 (importin alpha 3)	KPNA4	2.045 down	.0126	3840
MAD1 mitotic arrest deficient-like 1 (yeast)	MAD1L1	2.045 down	.0331	8379
Zinc finger CCCH-type containing 11A	ZC3H11A	2.045 down	.0113	9877
Ubiquitin specific peptidase 25	USP25	2.044 down	.0103	29761
Tribbles homolog 2 (Drosophila)	TRIB2	2.044 down	.00966	28951
Adenomatous polyposis coli	APC	2.043 down	.0111	324
Centrosomal protein 68 kd	CEP68	2.040 down	.0174	23177
Protein phosphatase 1, regulatory (inhibitor) subunit 12B	PPP1R12B	2.040 down	.012	4660
v-crk sarcoma virus CT10 oncogene homolog (avian)	CRK	2.037 down	.022	1398
Protein phosphatase 2, regulatory subunit B', delta isoform	PPP2R5D	2.034 down	.0319	5528
GATA zinc finger domain containing 1	GATAD1	2.033 down	.0295	57798
Dipeptidyl-peptidase 8	DPP8	2.031 down	.0442	54878
Mitochondrial tumor suppressor 1	MTUS1	2.030 down	.0258	57509
Ubiquitination factor E4B (UFD2 homolog, yeast)	UBE4B	2.028 down	.0118	10277
Fusion (involved in t(12;16) in malignant liposarcoma)	FUS	2.027 down	.0394	2521
Hippocalcin-like 1	HPCAL1	2.027 down	.0449	3241
ATP-binding cassette, sub-family C (CFTR/MRP), member 9	ABCC9	2.027 down	.0125	10060
Protein tyrosine phosphatase, non-receptor type 21	PTPN21	2.025 down	.0234	11099
Ubiquitin specific peptidase 34	USP34	2.024 down	.0159	9736
DEAD (Asp-Glu-Ala-Asp) box polypeptide 3, X-linked	DDX3X	2.024 down	.0203	1654
Phosphatidylinositol binding clathrin assembly protein	PICALM	2.023 down	.0158	8301
Transportin 3	TNPO3	2.022 down	.0166	23534
Non-POU domain containing, octamer-binding	NONO	2.022 down	.0192	4841
ORM1-like 3 (S cerevisiae)	ORMDL3	2.021 down	.014	94103
Mitogen-activated protein kinase kinase kinase kinase 4	MAP4K4	2.021 down	.0371	9448
Tribbles homolog 1 (Drosophila)	TRIB1	2.021 down	.0206	10221
WD repeat domain 6	WDR6	2.020 down	.0135	11180
Aspartate beta-hydroxylase	ASPH	2.020 down	.00586	444
Zinc finger protein 45	ZNF45	2.019 down	.0125	7596
SET domain containing 5	SETD5	2.019 down	.0266	55209
Eukaryotic translation initiation factor 4E nuclear import factor 1	EIF4ENIF1	2.017 down	.0142	56478
High-density lipoprotein binding protein (vigilin)	HDLBP	2.013 down	.0162	3069
Mannosyl (alpha-1,3-)-glycoprotein beta-1,	MGAT4B	2.012 down	.0175	11282
4-N-acetylglucosaminyltransferase, isozyme B				
AT rich interactive domain 1A (SWI-like)	ARID1A	2.012 down	.0426	8289
Alkylglycerone phosphate synthase	AGPS	2.011 down	.00981	8540
MLCK protein	MLCK	2.010 down	.0158	91807
FYVE, RhoGEF and PH domain containing 6	FGD6	2.009 down	.0163	55785
Cytoplasmic FMR1 interacting protein 2	CYFIP2	2.007 down	.0392	26999
Acyl-CoA thioesterase 11	ACOT11	2.003 down	.0417	26027
Ubiquitin specific peptidase 11	USP11	2.001 down	.0306	8237
Zinc finger protein 395///F-box protein 16	FBXO16///	2.000 down	.035	157574///
•	ZNF395			55893
Adrenergic, alpha-1A-, receptor	ADRA1A	1.998 down	.0404	148
M-phase phosphoprotein 9	MPHOSPH9	1.996 down	.0239	10198
Nudix (nucleoside diphosphate linked moiety X)-type motif 21	NUDT21	1.995 down	.0167	11051
Kruppel-like factor 3 (basic)	KLF3	1.993 down	.0169	51274
Synovial sarcoma translocation, chromosome 18	SS18	1.992 down	.0185	6760

TABLE E2. Continued

Gene title	Symbol	Fold	P value	Gene ID
Exocyst complex component 5	EXOC5	1.991 down	.016	10640
Rabaptin, RAB GTPase binding effector protein 1	RABEP1	1.990 down	.0482	9135
PRP4 pre-mRNA processing factor 4 homolog (yeast)	PRPF4	1.990 down	.0206	9128
CDNA FLJ10151 fis, clone HEMBA1003402		1.986 down	.0136	
CDNA clone IMAGE:5263531		1.986 down	.0114	
SH3-domain GRB2-like endophilin B2	SH3GLB2	1.985 down	.0301	56904
Serologically defined colon cancer antigen 1	SDCCAG1	1.985 down	.0111	9147
Zinc finger protein 398	ZNF398	1.981 down	.0365	57541
Cytochrome b reductase 1	CYBRD1	1.980 down	.0237	79901
Transformation/transcription domain-associated protein	TRRAP	1.977 down	.0235	8295
SON DNA binding protein	SON	1.975 down	.0125	6651
Nucleoporin 133 kd	NUP133	1.975 down	.0187	55746
Discoidin domain receptor family, member 2	DDR2	1.973 down	.0112	4921
A kinase (PRKA) anchor protein 8-like	AKAP8L	1.971 down	.0291	26993
Lanosterol synthase (2,3-oxidosqualene-lanosterol cyclase)	LSS	1.971 down	.0347	4047
dpy-19-like 1 (C elegans)	DPY19L1	1.968 down	.0174	23333
Dystonin	DST	1.967 down	.00579	667
Splicing factor, arginine/serine-rich 8	SFRS8	1.967 down	.0239	6433
(suppressor-of-white-apricot homolog, <i>Drosophila</i>)				
NGFI-A binding protein 1 (EGR1 binding protein 1)	NAB1	1.965 down	.0132	4664
Exocyst complex component 7	EXOC7	1.963 down	.0278	23265
Syntrophin, beta 2 (dystrophin-associated protein A1, 59 kd,	SNTB2	1.962 down	.0196	6645
basic component 2)				
SEC63 homolog (S cerevisiae)	SEC63	1.961 down	.0142	11231
SHC (Src homology 2 domain containing) transforming	SHC1	1.960 down	.0129	6464
protein 1	22202			
Engulfment and cell motility 2	ELMO2	1.960 down	.021	63916
Chromosome 1 open reading frame 25	Clorf25	1.959 down	.0309	81627
Glutamate-ammonia ligase (glutamine synthetase)	GLUL GLUL	1.958 down	.0355	2752
MYST histone acetyltransferase (monocytic leukemia) 4	MYST4	1.957 down	.0229	23522
FOS-like antigen 2	FOSL2	1.957 down	.0326	2355
KIAA0692	KIAA0692	1.957 down	.025	23141
Potassium channel tetramerization domain containing 20	KCTD20	1.955 down	.028	222658
WD repeat domain 26	WDR26	1.955 down	.0125	80232
Senataxin	SETX	1.953 down	.0121	23064
Trinucleotide repeat containing 6B	TNRC6B	1.953 down	.00655	23112
Transmembrane 9 superfamily member 1	TM9SF1	1.951 down	.0301	10548
Family with sequence similarity 108, member B1	FAM108B1	1.948 down	.0404	51104
			.0137	55754
Transmembrane protein 30A CTD (carboxy-terminal domain, RNA polymerase II,	TMEM30A	1.948 down	.0238	
	CTDSPL2	1.948 down	.0258	51496
polypeptide A) small phosphatase like 2	ZNE24	1.047.4	011	7570
Zinc finger protein 24	ZNF24	1.947 down	.011	7572
HLA-B associated transcript 3	BAT3	1.947 down	.0299	7917
Chromosome 21 open reading frame 33	C21orf33	1.946 down	.0321	8209
Protein kinase C substrate 80K-H	PRKCSH	1.945 down	.0205	5589
Pleckstrin homology, Sec7 and coiled-coil domains 3	PSCD3	1.943 down	.0134	9265
BCL2-antagonist of cell death	BAD	1.941 down	.0326	572
A kinase (PRKA) anchor protein 10	AKAP10	1.941 down	.0355	11216
Sorbin and SH3 domain containing 3	SORBS3	1.939 down	.0162	10174
v-akt murine thymoma viral oncogene homolog 2	AKT2	1.939 down	.035	208
Myeloid/lymphoid or mixed-lineage leukemia	MLL	1.939 down	.0264	4297
(trithorax homolog, Drosophila)				
Integrin, alpha 7	ITGA7	1.938 down	.0256	3679
Ankyrin repeat and KH domain containing	ANKHD1///	1.938 down	.0401	404734///
1///ANKHD1-EIF4EBP3	ANKHD1-			54882
	EIF4EBP3			

TABLE E2. Continued

TABLE E2. Continued				
Gene title	Symbol	Fold	P value	Gene ID
CCR4-NOT transcription complex, subunit 4	CNOT4	1.937 down	.0261	4850
CDNA FLJ38461 fis, clone FEBRA2020977		1.935 down	.0168	
Membrane-associated ring finger (C3HC4) 6	7-Mar	1.935 down	.00973	10299
Zinc finger protein 540	ZNF540	1.934 down	.024	163255
Solute carrier family 30 (zinc transporter), member 5	SLC30A5	1.934 down	.00814	64924
Ral guanine nucleotide dissociation stimulator	RALGDS	1.934 down	.0212	5900
Mitogen-activated protein kinase 1	MAPK1	1.933 down	.0124	5594
Small G protein signaling modulator 3	SGSM3	1.932 down	.0354	27352
Intracisternal A particle-promoted polypeptide	IPP	1.932 down	.0337	3652
Mediator complex subunit 23	MED23	1.931 down	.0242	9439
Bone morphogenetic protein receptor, type II	BMPR2	1.929 down	.0111	659
(serine/threonine kinase)				
MOCO sulphurase C-terminal domain containing 2	MOSC2	1.928 down	.00932	54996
Myeloid/lymphoid or mixed-lineage leukemia	MLL	1.927 down	.0172	4297
(trithorax homolog, <i>Drosophila</i>)				
Troponin T type 2 (cardiac)	TNNT2	1.926 down	.0352	7139
ATPase, H ⁺ transporting, lysosomal V0 subunit a1	ATP6V0A1	1.925 down	.0372	535
RAD23 homolog A (S cerevisiae)	RAD23A	1.924 down	.0205	5886
Nuclear receptor coactivator 3	NCOA3	1.924 down	.0496	8202
Myelin basic protein	MBP	1.924 down	.0167	4155
Solute carrier family 33 (acetyl-CoA transporter),	SLC33A1	1.923 down	.0112	9197
member 1		1.525 down		
T-box 5	TBX5	1.923 down	.0203	6910
DEAH (Asp-Glu-Ala-His) box polypeptide 9	DHX9	1.923 down	.0112	1660
Intraflagellar transport 122 homolog (Chlamydomonas)	IFT122	1.922 down	.0247	55764
Calnexin	CANX	1.921 down	.0103	821
Rab and DnaJ domain containing	RBJ	1.921 down	.03	51277
Hydroxyacyl-coenzyme A dehydrogenase/3-ketoacyl-	HADHA	1.921 down	.0375	3030
coenzyme A thiolase/enoyl-coenzyme A hydratase				
(trifunctional protein), alpha subunit				
Dystonin	DST	1.919 down	.0112	667
Phosphoinositide-3-kinase, class 2, alpha polypeptide	PIK3C2A	1.919 down	.0216	5286
ADAM metallopeptidase domain 10	ADAM10	1.918 down	.0433	102
Fibronectin type III domain containing 3A	FNDC3A	1.918 down	.00672	22862
Spire homolog 1 (Drosophila)	SPIRE1	1.917 down	.0109	56907
RAS and EF-hand domain containing	RASEF	1.917 down	.0401	158158
Phosphodiesterase 1C, calmodulin-dependent 70 kd	PDE1C	1.916 down	.0136	5137
Ring finger protein 19A	RNF19A	1.916 down	.0112	25897
alkB, alkylation repair homolog 5 (E coli)	ALKBH5	1.916 down	.0208	54890
zer-1 homolog (C elegans)	ZER1	1.915 down	.0125	10444
Obscurin, cytoskeletal calmodulin and titin-interacting	OBSCN	1.914 down	.0387	84033
RhoGEF				
Zinc finger with KRAB and SCAN domains 1	ZKSCAN1	1.914 down	.0142	7586
Chloride intracellular channel 5	CLIC5	1.913 down	.0391	53405
WW domain containing adaptor with coiled-coil	WAC	1.913 down	.00669	51322
Wolfram syndrome 1 (wolframin)	WFS1	1.912 down	.0492	7466
Son of sevenless homolog 1 (Drosophila)	SOS1	1.911 down	.0448	6654
Cytoskeleton associated protein 5	CKAP5	1.909 down	.0109	9793
Cullin 4B	CUL4B	1.909 down	.0204	8450
Paired related homeobox 1	PRRX1	1.908 down	.0262	5396
Minichromosome maintenance complex component 3	MCM3AP	1.907 down	.0424	8888
associated protein	WICWIJAI	1.707 down	.0724	0000
Sp1 transcription factor	SP1	1.906 down	.0327	6667
	NFYC	1.900 down 1.904 down		
Nuclear transcription factor Y, gamma	IVFIC	1.904 down	.0239	4802

TABLE E2. Continued

Gene title	Symbol	Fold	P value	Gene ID
v-rel reticuloendotheliosis viral oncogene homolog A,	RELA	1.904 down	.0498	5970
nuclear factor of kappa light polypeptide gene				
enhancer in B-cells 3, p65 (avian)				
Chromosome 5 open reading frame 22	C5orf22	1.903 down	.0126	55322
MYST histone acetyltransferase (monocytic leukemia) 4	MYST4	1.903 down	.0135	23522
Iroquois homeobox 5	IRX5	1.903 down	.0313	10265
Epoxide hydrolase 1, microsomal (xenobiotic)	EPHX1	1.903 down	.0158	2052
Microtubule associated monoxygenase, calponin and	LOC731210	1.903 down	.0487	57553//
LIM domain containing 3///similar to Protein MICAL-3	///MICAL3			731210
Putative homeodomain transcription factor 2	PHTF2	1.902 down	.04	57157
Mitogen-activated protein kinase kinase kinase 3	MAP3K3	1.901 down	.0458	4215
Zinc finger protein 227	ZNF227	1.900 down	.0262	7770
Membrane associated guanylate kinase, WW	MAGI2	1.900 down	.035	9863
and PDZ domain containing 2				
Bicaudal D homolog 2 (Drosophila)	BICD2	1.900 down	.0232	23299
Adducin 1 (alpha)	ADD1	1.900 down	.0136	118
Plexin B1	PLXNB1	1.900 down	.034	5364
Adenylate cyclase 9	ADCY9	1.899 down	.0163	115
Zinc finger CCCH-type containing 7A	ZC3H7A	1.899 down	.0118	29066
TOX high mobility group box family member	LOC285412	1.899 down	.0263	285412//
4///similar to Epidermal Langerhans cell protein LCP1	///TOX4			9878
Nucleoporin 98 kd	<i>NUP</i> 98	1.898 down	.0212	4928
Platelet derived growth factor C	PDGFC	1.897 down	.0461	56034
Block of proliferation 1///similar to block of proliferation 1	BOP1///	1.897 down	.0448	23246//
	LOC727967			727967
Obscurin, cytoskeletal calmodulin and titin-interacting RhoGEF	OBSCN	1.896 down	.0392	84033
guanine nucleotide binding protein (G protein), beta 5	GNB5	1.894 down	.0396	10681
Protein tyrosine phosphatase, nonreceptor type 21	PTPN21	1.894 down	.0278	11099
Adaptor-related protein complex 3, delta 1 subunit	AP3D1	1.890 down	.0112	8943
v-akt murine thymoma viral oncogene homolog 2	AKT2	1.889 down	.0174	208
Solute carrier family 25 (mitochondrial carrier; adenine nucleotide translocator), member 6	SLC25A6	1.889 down	.0291	293
Zinc finger, DHHC-type containing 5	ZDHHC5	1.888 down	.0136	25921
Latent transforming growth factor beta binding protein 1	LTBP1	1.888 down	.0126	4052
Rho GTPase activating protein 26	ARHGAP26	1.887 down	.0245	23092
ATPase, class I, type 8B, member 2	ATP8B2	1.885 down	.0109	57198
Tuberous sclerosis 2	TSC2	1.885 down	.0281	7249
ATPase, class VI, type 11A	ATP11A	1.884 down	.0107	23250
RIO kinase 3 (yeast)	RIOK3	1.883 down	.0238	8780
Golgi apparatus protein 1	GLG1	1.882 down	.0234	2734
Nicotinamide nucleotide transhydrogenase	NNT	1.880 down	.0106	23530
Chromosome 19 open reading frame 6	C190rf6	1.879 down	.0125	91304
Bromodomain containing 2	BRD2	1.879 down	.0228	6046
Purine-rich element binding protein B	PURB	1.879 down	.0281	5814
Glutathione reductase	GSR	1.879 down	.00928	2936
ADP-ribosylation factor guanine nucleotide-exchange factor 1 (brefeldin A-inhibited)	ARFGEF1	1.877 down	.00671	10565
ADP-ribosylation factor interacting protein 1 (arfaptin 1)	ARFIP1	1.877 down	.0116	27236
Rhotekin	RTKN	1.876 down	.0109	6242
Jumonji domain containing 3, histone lysine demethylase	JMJD3	1.875 down	.0102	23135
Zinc finger and SCAN domain containing 18	ZSCAN18	1.874 down	.0223	65982
Kelch repeat and BTB (POZ) domain containing 4	KBTBD4	1.873 down	.044	55709
PRP6 pre-mRNA processing factor 6 homolog (S cerevisiae)	PRPF6	1.872 down	.0132	24148
CCR4-NOT transcription complex, subunit 3	CNOT3	1.871 down	.0226	4849

TABLE E2. Continued

Gene title	Symbol	Fold	P value	Gene ID
Zinc finger, RAN-binding domain containing 1	ZRANB1	1.870 down	.0289	54764
Glucosidase, alpha; neutral AB	GANAB	1.870 down	.0149	23193
Protein tyrosine phosphatase, receptor type,	PPFIA1	1.870 down	.0281	8500
f polypeptide (PTPRF),				
interacting protein (liprin), alpha 1				
Vitamin K epoxide reductase complex, subunit 1-like 1	VKORC1L1	1.869 down	.0114	154807
Ring finger and CCCH-type zinc finger domains 2	RC3H2	1.869 down	.0112	54542
F-box and leucine-rich repeat protein 15	FBXL15	1.869 down	.0287	79176
Zinc finger, FYVE domain containing 9	ZFYVE9	1.869 down	.0129	9372
Transcribed locus		1.867 down	.0217	
Trinucleotide repeat containing 6B	TNRC6B	1.867 down	.0128	23112
Anterior pharynx defective 1 homolog A (C elegans)	APH1A	1.867 down	.0317	51107
Protein phosphatase 5, catalytic subunit	PPP5C	1.866 down	.0448	5536
Zinc finger protein 289, ID1 regulated	ZNF289	1.866 down	.0462	84364
UDP-N-acetyl-alpha-D-galactosamine:polypeptide	GALNT2	1.865 down	.0191	2590
N-acetylgalactosaminyltransferase 2 (GalNAc-T2)				
Solute carrier family 25, member 30	SLC25A30	1.864 down	.0372	253512
Kelch-like 23 (Drosophila)	KLHL23	1.864 down	.0281	151230
RAB3 GTPase activating protein subunit 2	RAB3GAP2	1.862 down	.0129	25782
(non-catalytic)				
Ring finger and CCCH-type zinc finger domains 2	RC3H2	1.861 down	.0128	54542
Phospholipase C, beta 4	PLCB4	1.859 down	.016	5332
Glucosidase, beta (bile acid) 2	GBA2	1.858 down	.0134	57704
Cyclin-dependent kinase inhibitor 1C (p57, Kip2)	CDKN1C	1.858 down	.0266	1028
WWC family member 3	WWC3	1.857 down	.0109	55841
Tripeptidyl peptidase I	TPP1	1.856 down	.0294	1200
CD99 molecule-like 2	CD99L2	1.855 down	.0203	83692
Zinc finger protein 655	ZNF655	1.853 down	.0226	79027
Actin filament associated protein 1-like 1	AFAP1L1	1.853 down	.016	134265
Eukaryotic translation initiation factor 4B	EIF4B	1.852 down	.0112	1975
p21 (CDKN1A)-activated kinase 2	PAK2	1.852 down	.0125	5062
Poliovirus receptor	PVR	1.852 down	.00712	5817
RAB3 GTPase activating protein subunit 1 (catalytic)	RAB3GAP1	1.852 down	.0274	22930
Interleukin enhancer binding factor 3, 90 kd	ILF3	1.851 down	.0116	3609
Chromosome 6 open reading frame 106	C6orf106	1.850 down	.03	64771
Purine-rich element binding protein A	PURA	1.850 down	.0384	5813
Wolf-Hirschhorn syndrome candidate 1-like 1	WHSC1L1	1.849 down	.0167	54904
Fem-1 homolog b (C elegans)	FEM1B	1.849 down	.0214	10116
Jun oncogene	JUN	1.848 down	.0243	3725
NDRG family member 2	NDRG2	1.848 down	.0415	57447
Protocadherin gamma subfamily A, 1	PCDHGA1	1.847 down	.0112	9708
Solute carrier family 6 (neurotransmitter transporter, creatine), member 8	SLC6A8	1.847 down	.0266	6535
Upstream binding transcription factor, RNA polymerase I	UBTF	1.847 down	.0227	7343
Zer-1 homolog (<i>C elegans</i>)	ZER1	1.846 down	.0166	10444
Prion protein (p27-30) (Creutzfeldt-Jakob disease,	PRNP	1.845 down	.0168	5621
Gerstmann-Strausler-Scheinker syndrome, fatal familial insomnia)				
General transcription factor IIIC, polypeptide 1, alpha 220 kd	GTF3C1	1.844 down	.0274	2975
Pumilio homolog 1 (<i>Drosophila</i>) Splicing factor, argining/sering rich 1 (splicing factor 2)	PUM1	1.843 down	.0257	9698 6426
Splicing factor, arginine/serine-rich 1 (splicing factor 2, alternate splicing factor)	SFRS1	1.842 down	.0158	6426
Kelch repeat and BTB (POZ) domain containing 2	KBTBD2	1.842 down	.0124	25948
Translocase of inner mitochondrial membrane 44 homolog (yeast)	TIMM44	1.841 down	.0241	10469
1-Acylglycerol-3-phosphate O-acyltransferase 3	AGPAT3	1.841 down	.011	56894

TABLE E2. Continued

Gene title	Symbol	Fold	P value	Gene ID
Ezrin	EZR	1.840 down	.0279	7430
Down syndrome critical region gene 3	DSCR3	1.840 down	.0355	10311
v-ets erythroblastosis virus E26 oncogene homolog 1 (avian)	ETS1	1.839 down	.0347	2113
Ubiquitin-conjugating enzyme E2S///similar	LOC731049	1.839 down	.049	27338///
to ubiquitin-conjugating enzyme E2S	///UBE2S			731049
(Ubiquitin-conjugating enzyme E2-24 kd)				
(Ubiquitin-protein ligase)				
(Ubiquitin carrier protein) (E2-EPF5)				
Solute carrier family 6 (neurotransmitter transporter,	SLC6A8	1.837 down	.0172	6535
creatine), member 8				
Minichromosome maintenance complex component	MCM3AP	1.835 down	.017	8888
3 associated protein				
Glucocorticoid receptor DNA binding factor 1	GRLF1	1.834 down	.0218	2909
Aryl hydrocarbon receptor nuclear translocator	ARNT	1.832 down	.0179	405
Integrin, alpha 6	ITGA6	1.832 down	.0394	3655
La ribonucleoprotein domain family, member 5	LARP5	1.831 down	.0238	23185
Heat shock transcription factor 1	HSF1	1.831 down	.0255	3297
DCP1 decapping enzyme homolog A (S cerevisiae)	DCP1A	1.829 down	.0142	55802
Sterile alpha motif domain containing 4A	SAMD4A	1.829 down	.0121	23034
Desmin	DES	1.829 down	.0484	1674
KIAA1826	KIAA1826	1.828 down	.0423	84437
Microtubule associated serine/threonine kinase family member 4	MAST4	1.828 down	.0448	375449
Nuclear factor I/C (CCAAT-binding transcription factor)	NFIC	1.828 down	.0119	4782
Heat shock 70 kd protein 4	HSPA4	1.828 down	.0167	3308
Forkhead box O3	FOXO3	1.827 down	.024	2309
Core-binding factor, beta subunit	CBFB	1.825 down	.0121	865
Mitogen-activated protein kinase kinase 2	MAP2K2	1.822 down	.0295	5605
RRN3 RNA polymerase I transcription factor homolog	LOC653390///	1.822 down	.0307	54700///
(S cerevisiae)///RRN3 RNA polymerase I transcription	LOC730092			653390///
factor homolog (S cerevisiae) pseudogene	///RRN3			730092
GCN1 general control of amino-acid synthesis	GCN1L1	1.822 down	.0242	10985
1-like 1 (yeast)				
Cullin 5	CUL5	1.821 down	.0119	8065
CDNA FLJ34585 fis, clone KIDNE2008758		1.821 down	.0142	
Related RAS viral (r-ras) oncogene homolog 2	RRAS2	1.820 down	.0206	22800
MAX dimerization protein 4	MXD4	1.820 down	.0161	10608
Transglutaminase 2 (C polypeptide,	TGM2	1.820 down	.0419	7052
protein-glutamine-gamma-glutamyltransferase)	GOT 44.1	1.020.1	0016	1202
Collagen, type IV, alpha 1	COL4A1	1.820 down	.0216	1282
Polymerase I and transcript release factor	PTRF	1.819 down	.0286	284119
Methyl-CpG binding domain protein 1	MBD1	1.819 down	.0245	4152
BTG family, member 2	BTG2	1.819 down	.0337	7832
G protein–coupled receptor kinase 5	GRK5	1.819 down	.0452	2869
Protein associated with topoisomerase II homolog 1 (yeast)	PATL1	1.818 down	.0147	219988
Interleukin enhancer binding factor 3, 90 kd	ILF3	1.818 down	.0328	3609
Ras homolog gene family, member J	RHOJ	1.817 down	.0278	57381
Chromosome 10 open reading frame 18	C10orf18	1.817 down 1.817 down	.0136	54906
NMDA receptor regulated 2 Transportation factor 25 (horizobalist learn helist)	NARG2 TCF25		.0424	79664
Transcription factor 25 (basic helix-loop-helix) Spastic paraplegia 7 (pure and complicated autosomal recessive)		1.817 down	.0142	22980
	SPG7 HNPPM	1.817 down	.0368	6687 4670
Heterogeneous nuclear ribonucleoprotein M	HNRPM FIGN	1.815 down	.0206	4670 55137
Fidgetin Chromosome 5 open reading frame 41	FIGN C5 orf41	1.814 down	.0484 .049	55137 153222
	C5orf41	1.814 down		57154
SMAD specific E3 ubiquitin protein ligase 1	SMURF1 CIRBP	1.812 down	.0237	
Cold inducible RNA binding protein	CINDF	1.812 down	.04	1153

TABLE E2. Continued

Gene title	Symbol	Fold	P value	Gene ID
SLAIN motif family, member 2	SLAIN2	1.812 down	.0309	57606
Required for meiotic nuclear division 5 homolog	RMND5A	1.811 down	.0383	64795
A (S cerevisiae)				
SH3 domain protein D19	SH3D19	1.811 down	.0445	152503
Pericentriolar material 1	PCM1	1.810 down	.00823	5108
Quaking homolog, KH domain RNA binding (mouse)	QKI	1.810 down	.0266	9444
Insulin-degrading enzyme	IDE	1.809 down	.0192	3416
zinc finger, HIT type 4	ZNHIT4	1.808 down	.018	83444
T-box 5	TBX5	1.808 down	.0128	6910
Myeloid/lymphoid or mixed-lineage leukemia	MLLT4	1.807 down	.0283	4301
(trithorax homolog, Drosophila); translocated to, 4				
Myosin regulatory light chain interacting protein	MYLIP	1.807 down	.031	29116
Phosphate cytidylyltransferase 1, choline, alpha	PCYT1A	1.806 down	.0208	5130
RNA binding motif protein 8A	RBM8A	1.805 down	.0339	9939
Protein kinase C and casein kinase substrate in neurons 2	PACSIN2	1.804 down	.0284	11252
Polymerase (RNA) II (DNA directed) polypeptide E, 25 kd	POLR2E	1.804 down	.0356	5434
Stromal antigen 2	STAG2	1.804 down	.0203	10735
Chromosome 1 open reading frame 71	C1orf71	1.803 down	.0187	163882
Transmembrane protein 127	TMEM127	1.803 down	.0244	55654
Valyl-tRNA synthetase	VARS	1.802 down	.0291	7407
prosaposin (variant Gaucher disease and variant	PSAP	1.801 down	.0324	5660
metachromatic leukodystrophy)				
Zinc finger protein 12	ZNF12	1.800 down	.0384	7559