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Integrated Genomic Analyses in Bronchopulmonary Dysplasia

Namasivayam Ambalavanan, MD[1], **C. Michael Cotten, MD**[2], **Grier P. Page, PhD**[3], **Waldemar A. Carlo, MD**[1], **Jeffrey C. Murray, MD**[4], **Soumyaroop Bhattacharya, MS, MEd**[5], **Thomas J. Mariani, PhD**[5], **Alain C. Cuna, MD**[6], **Ona M. Faye-Petersen, MD**[7], **David Kelly, MD**[7], and **Rosemary D. Higgins, MD**[8,*] **on behalf of the Genomics and Cytokine Subcommittees of the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development Neonatal Research Network**

[1]Department of Pediatrics, University of Alabama at Birmingham, Birmingham, AL

[2]Department of Pediatrics, Duke University, Durham, NC

[3]RTI International, Atlanta, GA

[4]Department of Pediatrics, University of Iowa, Iowa City, IA

[5]Division of Neonatology and Program in Pediatric Molecular and Personalized Medicine, University of Rochester, Rochester, NY

[6]Department of Pediatrics, University of Missouri-Kansas City School of Medicine, Kansas City, MO

[7]Department of Pathology, University of Alabama at Birmingham, Birmingham, AL

[8]*Eunice Kennedy Shriver* National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD

Abstract

Objective—To identify single nucleotide polymorphisms (SNPs) and pathways associated with bronchopulmonary dysplasia (BPD) because O_2 requirement at 36 weeks' post-menstrual age risk is strongly influenced by heritable factors.

Study design—A genome-wide scan was conducted on 1.2 million genotyped SNPs, and an additional 7 million imputed SNPs, using a DNA repository of extremely low birth weight infants. Genome-wide association and gene set analysis was performed for BPD or death, severe BPD or

Corresponding author/Reprint requests: Namasivayam Ambalavanan, MD, 176F Suite 9380, Women and Infants Center, 619 South 20th St., University of Alabama at Birmingham, Birmingham, AL 35249, Tel (205) 934-4680 Fax (205) 934-3100 nambalavanan@peds.uab.edu.

*List of members of the Genomics and Cytokine Subcommittees of the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development Neonatal Research Network is available at www.jpeds.com (Appendix).

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death, and severe BPD in survivors. Specific targets were validated using gene expression in BPD lung tissue and in mouse models.

Results—Of 751 infants analyzed, 428 developed BPD or died. No SNPs achieved genome-wide significance ($p<10^{-8}$) although multiple SNPs in adenosine deaminase (ADARB2), CD44, and other genes were just below $p<10^{-6}$. Of approximately 8000 pathways, 75 were significant at False Discovery Rate (FDR) <0.1 and p<0.001 for BPD/death, 95 for severe BPD/death, and 90 for severe BPD in survivors. The pathway with lowest FDR was miR-219 targets (p=1.41E-08, FDR 9.5E-05) for BPD/death and Phosphorous Oxygen Lyase Activity (includes adenylate and guanylate cyclases) for both severe BPD/death (p=5.68E-08, FDR 0.00019) and severe BPD in survivors (p=3.91E-08, FDR 0.00013). Gene expression analysis confirmed significantly increased miR-219 and CD44 in BPD.

Conclusions—Pathway analyses confirmed involvement of known pathways of lung development and repair (CD44, Phosphorus Oxygen Lyase Activity) and indicated novel molecules and pathways (ADARB2, Targets of miR-219) involved in genetic predisposition to BPD.

Keywords

Bronchopulmonary dysplasia; Infant; premature; Infant mortality; Single nucleotide polymorphisms

Bronchopulmonary dysplasia (BPD) is common in extremely preterm infants, and genetic factors may account for much of the variance in risk for BPD.[1] Targeted candidate gene analyses suggest single nucleotide polymorphisms (SNPs) in certain cytokines, surfactant proteins, and related molecules[2, 3] but not others[4] are associated with BPD. Hadchouel et al[5] identified the SPOCK2 gene as associated with BPD in a genome-wide association study (GWAS) that evaluated the entire genome in an unbiased manner. However, Wang et al[6] did not find SNPs associated with BPD in a GWAS.

Most complex diseases (such as BPD) involve gene-environment interactions and interactions among different loci. However, conventional single marker analysis does not explicitly look for interactions among different genes in the same biological pathway that have a multiplicative or a threshold effect.[7] Most GWAS that focus on analysis of single markers lack the power to identify the small contribution of most genetic variants.[8] Pathway-based approaches, which consider multiple contributing factors in the same biological pathway, complement the single marker approach and provide understanding of GWAS data in many diseases.[9]

In this study, we utilized a GWAS combined with pathway-based approaches to increase our understanding of the role of genetics in BPD susceptibility, and integrated these results with gene expression comparing BPD with controls, and a newborn mouse model of hyperoxia exposure simulating BPD. We hypothesized that SNPs in biological pathways involved in lung development and injury will be enriched in infants who develop BPD or die. The combined outcome of BPD or death was used because death is a competing outcome for BPD i.e., infants who die early cannot develop BPD even though they may be at the highest risk of BPD.

METHODS

Patients included were a subset of infants enrolled in the Eunice Kennedy Shriver NICHD Neonatal Research Network's Cytokines study that enrolled infants 401–1000 g at birth, < 72 h age, and free of major congenital anomalies.[10] The study was approved by institutional review boards (IRBs) at participating centers, and written informed consent was obtained from parent(s). Additional IRB review allowed the GWA genotyping results with a limited phenotype data to be included in the NHGRI Database of Genotypes and Phenotypes (DbGaP).

DNA was extracted from the earliest age blood spot collected on filter paper. Whole genome amplification was used for samples that did not provide adequate genomic DNA. Genotyping was done on the Illumina HumanOmni1-Quad_v1-0_B BeadChip.

BPD was defined by supplemental O_2 at 36 weeks' postmenstrual age. Severe BPD was defined as therapy with O_2>21% for at least 28 days plus use of ≥30% O_2 and/or positive pressure (ventilation or nasal continuous positive airway pressure) at 36 weeks' post-menstrual age.[11] Death was defined as in-hospital death prior to discharge.

Ancestry was classified as Black (African-American), White (non-Hispanic Caucasians), Hispanic (Hispanic Caucasian), and others including Asian and multi-racial using GWASTools[12] to generate eigenvalues for the entire dataset.

Imputation was run using beagle 3.3.1. 769,757 SNPs were used for imputation with 7,500,443 SNPs being imputed.[13]

Analysis of SNPs was done using two complementary methods: a standard GWA analysis followed by a pathway analysis.

SNPs were analyzed using PLINK[14] using logistic regression under an additive model. Three models were run: BPD or death vs. survival without BPD, Severe BPD or death vs. survival without severe BPD, Severe BPD in survivors vs. survivors without severe BPD. The regression model included covariates for GA, small for GA, sex, Apgar at 5 min < 5, antenatal steroids, and the race ethnicity Eigenvalues 1–4. The top 10 SNPs (by lowest p-value) for each of the 3 models were mapped to genes.

We assigned genes to pathways (gene sets) using the Molecular Signatures Database (MSigDB) (http://www.broadinstitute.org/gsea/msigdb/collections.jsp). SNPs were assigned to gene(s) based on being exonic, intronic, untranslated region, or within 20 kb of the ends of the gene model. Pathways were analyzed using Gene Set Enrichment Analysis **(**GSEA).[15]

Gene expression values for individual members of pathways considered most important were extracted from an existing dataset describing genome-wide expression in lung tissue obtained from BPD cases or controls and assessed for differential expression.[16] Two selected molecules (miR-219 and CD44) were further evaluated by TaqMan Gene Expression assays (Life Technologies, Grand Island, NY) from RNA isolated using Qiagen RNeasy FFPE kit (Qiagen, Valencia, CA) from paraffin-embedded formalin-fixed samples

of lungs collected at autopsy from extremely preterm infants (24–28 weeks gestation) who died soon after birth, term stillborn infants, and preterm infants who died due to BPD at term corrected age (36–44 weeks post-menstrual age) (n=4/group).

Three molecules (miR-219, ADARB2, and CD44) were selected for further evaluation in a mouse model. Gene expression was evaluated at different points during alveolar septation and hyperoxia exposure, using samples from studies approved by the UAB Institutional Animal Care and Use Committee.[17],[18] RNA was isolated from lung homogenates for real-time RT-PCR using specific primers.[19]

RESULTS

The GWAS cohort included 834 infants whose DNA samples were successfully genotyped. 172 (20%) samples required whole genome amplification. 751 infants met inclusion criteria with adequate information on BPD phenotype and genotyping (> 97% call rate). Characteristics of the study cohort are listed in Table I (available at www.jpeds.com). As expected, infants who developed outcomes of interest (BPD/death; severe BPD/death; Severe BPD in survivors) were more immature, of lower birth weight, more likely to be male, mechanically ventilated, and ventilated for a longer duration as compared with those who did not develop these outcomes.

GWA analysis

None of the SNPs were significant at the genome-wide significance level ($p<10^{-8}$). The analysis for top ten SNPs for BPD/death (Table II) identified 4 SNPs in adenosine deaminase (ADARB2), two SNPs in CD44, one in NSMC4A, one in WDR45L, and two associated with no known gene. Similarly, the top ten SNPs for severe BPD/death were 4 SNPs in ADARB2, one in CD44, one in NSMC4A, one in NUAK1, one in KCNH7, and two associated with no known gene (Table II). The analysis for severe BPD in survivors also found ADARB2, CD44, NUAK1, KCNH7, WDR45B, in addition to GRIP1 and GALNTL6 (Table II). Most of these SNPs had p-values of 10^{-6} to 10^{-7}.

Pathway /Gene Set Enrichment Analysis

Of the approximately 7650 gene sets evaluated, 75 were significant at a False Discovery Rate (FDR) of <0.1 (suggesting about 10% of the pathways are false positives) and p <0.001 for the BPD or death vs. no BPD comparison. 95 pathways were significant for severe BPD or death, and 90 for severe BPD in survivors.

a. Pathways associated with BPD or death vs. survivors without BPD (Table III; available at www.jpeds.com): 77 pathways were identified with a FDR <0.1 (75 significant at p<0.001). Of these 77 pathways, only 3 were shared with Severe BPD or death, or Severe BPD in survivors (MORF_BRCA1, MOREAUX_MULTIPLE_MYELOMA_BY_TACI_UP, and PACHER_TARGETS_OF_IGF1_AND_IGF2_UP) (Figure 1).The top pathway was MIR-219 (http://www.broadinstitute.org/gsea/msigdb/cards/GACAATC,MIR-219.html), which includes 143 genes.

b. Pathways associated with severe BPD or death vs. survivors without severe BPD (Table IV; available at www.jpeds.com): 123 pathways were identified with a FDR <0.1, of which 95 were significant at p<0.001. Of these 123 pathways, 3 were shared with those involved in BPD or death. 108 of these pathways (which included the 3 shared with BPD or death) were shared with those involved with severe BPD in survivors, including the top 43 pathways, indicating significant overlap in the models for these outcomes. The top pathway associated with severe BPD or death (and survivors with severe BPD) was Phosphorus Oxygen Lyase Activity (http://www.broadinstitute.org/gsea/msigdb/cards/PHOSPHORUS_OXYGEN_LYASE_ACTIVITY.html), which includes ten genes consisting of adenylate cyclases and guanylate cyclases.

c. Pathways associated with Severe BPD in survivors (Table V; available at www.jpeds.com): 142 pathways were identified with a FDR <0.1, of which 90 were significant at p<0.001. 108 of these 142 pathways (including the top 43) were also associated with Severe BPD or death.

d. Pathways associated with BPD or death by race (Table VI; available at www.jpeds.com): Of the 77 pathways identified at FDR<0.1 in all infants, 20 were noted in Black infants, 13 in Hispanic infants, and 24 in White infants for the same FDR threshold. Importantly, there was little overlap in the major pathways between these racial/ethnic groups. For example, targets of miR-219, which was the top pathway for all infants (FDR 9.52E-05, p=1.41E-08), was ranked 415th (FDR 0.29, p=0.018) for Black infants, 2597th (FDR 0.34, p=0.13) for Hispanic infants (but with FDR 5.92 E-43, p=7.48E-44 for severe BPD in survivors for the same cohort of Hispanic infants), and 1477th (FDR 0.25, p=0.055) for White infants (but with FDR 2.68E-44, p=2.66E-45 for severe BPD in survivors for the same cohort of White infants).

Evaluation of individual SNPs and pathways/gene sets using gene expression dataset

Gene expression for six of the nine genes with the lowest single SNP p-values could be assessed by a total of 20 probe sets present in the data set.[16] Two (NUAK1 and GRIP1) of these six genes were significantly dysregulated in BPD lung tissue, with lower expression in BPD when compared with controls. In addition to these significant genes, 2 probe sets for CD44 demonstrated a trend for increased expression in BPD lungs (p<0.01) (Table VII; available at www.jpeds.com).

We selected four pathways for further evaluation using data from the lung tissue gene expression data set.[16] These pathways were (1) miR-219 pathway, the top pathway for BPD/death, (2) PACHER_TARGETS_OF_IGF1_AND_IGF2_UP, one of the three pathways shared among all three outcomes, as IGF1 is important in lung development[20] and is increased in BPD,[21] (3) Phosphorus Oxygen Lyase Pathway, the top pathway associated with severe BPD/death as well as severe BPD in survivors, and (4) Cell Cycle: G2/M DNA Damage Checkpoint Regulation canonical pathway, previously appreciated as the top pathway in the BPD gene expression dataset[16] but not specifically evaluated in this study (as

it is not defined in MSigDB), but with overlap with MORF_BRCA1, a pathway shared among all three outcomes.

1. MiR-219 Pathway (Table VIII; available at www.jpeds.com): Gene expression for all 143 genes in this pathway was assessed. 32 of 143 (22%) of pathway genes were dysregulated in BPD lung tissue (vs. 7 expected at random, $p < 0.0001$). Fourteen genes had increased expression in BPD lung and 19 genes had decreased expression. Interestingly, independent probe sets for MAPT had increased (1 probe set) or decreased (3 probe sets) expression. Likewise, THRB had increased (1 probe set) or decreased (1 probe set) expression. These observations might suggest alternative splicing.
2. Targets of IGF1 and IGF2 Pathway (Table IX; available at www.jpeds.com): Gene expression for 34 of the 36 genes in this pathway was assessed using 78 probe sets. Two of the 34 genes had significantly increased expression in BPD; IGF1 (fold change>2, $p<0.01$) and SFMBT2 (fold change >1.07, $p<0.05$). Four independent probe sets demonstrated significance for IGF1.
3. Phosphorus Oxygen Lyase Activity Pathway: Gene expression for all 10 genes was assessed. ADCY8 had significantly reduced expression (fold change=0.59, $p=0.0041$) in BPD.
4. Cell Cycle Pathway (Table X; available at www.jpeds.com): Gene expression for all 23 genes was assessed using 61 probe sets. 35% of all pathway genes (8 of 23) were dysregulated in BPD, with increased expression. Many of these observations were demonstrated by multiple probe sets (15 probe sets different). Brca1 was increased by 1.21 fold in one probe set, with a $p= 0.07$, and by 1.3 fold in another, with $p=0.09$.

Evaluation of miR-219 and CD44 in mouse models and in human lung

Expression of miR-219 and CD44 decreased over the course of alveolar septation as they were reduced on postnatal day 14 and 42 compared with day 1. Exposure to hyperoxia was associated with increased miR-219 and CD44 on day 14. ADARB2 transcripts were not detected in the lung in significant amounts (detected at more than 35 cycles of qPCR).

Expression of miR-219 and CD44 were both increased in human BPD lung compared with preterm and term lung (Figure 2).

DISCUSSION

BPD has a strong genetic component, but conventional single-marker approaches have not successfully explained more than a small fraction of the heritability of BPD. In this exploratory analysis, we identified biological pathways that contribute to the heritability of BPD using gene set analysis. Our analysis suggests involvement of known pathways (e.g. phosphorus oxygen lyase activity) and molecules (e.g. CD44) involved in lung development and repair. In addition, we identified novel pathways (e.g. targets of miR-219) and molecules (e.g. ADARB2, CD44) that may be involved in genetic predisposition to BPD or death. We validated this survey of gene sets associated with BPD in extremely preterm

infants using a gene expression dataset from an independent population and evaluated selected molecules in a newborn mouse model and by gene expression in autopsy lung samples of BPD lung compared with normal preterm and term lung. Our results also indicate that severe BPD or death are associated with pathways distinct from mild/moderate BPD, suggesting that they have a different pathophysiologic basis, and that much variation is present in genetic predisposition to BPD by race/ethnicity.

To date, analysis of the pathways affected in BPD has relied on two GWAS[5, 6] and a genome-wide transcriptional profiling study.[16] The GWAS by Hadchouel et al.[5] identified SPOCK2 gene as associated with BPD, but the GWAS by Wang et al[6] did not identify any SNPs associated with BPD at a $p<5 \times 10^{-8}$ and pathway analyses were also not informative. Bhattacharya et al[16] analyzed RNA from lung tissue obtained at autopsy from 11 BPD cases and 17 age-matched controls without BPD. 159 genes were differentially expressed in BPD, and pathway analysis confirmed previously known (e.g. DNA damage regulation of cell cycle) and novel (e.g. B-cell development) pathways.

In the present study, we identified multiple pathways associated with BPD/death, severe BPD/death, and severe BPD in survivors. Notably, the overlap in pathways between any BPD/death and severe BPD/death (or severe BPD in survivors) was limited to only 3 pathways, a small fraction of the total number of pathways associated with each outcome. This suggests that the pathways associated with any BPD/death but not with severe BPD/death are those associated with mild or moderate BPD. This suggests that the difference in clinical phenotype between mild and moderate BPD versus severe BPD is also manifest at the genomic level. Similarly, the 105 pathways in the large overlap between severe BPD/death and severe BPD in survivors, especially the top 43 pathways, are probably pathways associated with severe BPD. The 15 pathways in severe BPD/death that do not overlap with severe BPD in survivors may be those associated with death. These results suggest that distinct biologic pathways are involved in the pathogenesis of mild/moderate BPD as compared with severe BPD or death and indicate that they do not represent a continuum in lung disease severity. A detailed evaluation of the specific pathways involved may shed light on the possible differences in pathogenesis.

The pathway “Targets of MicroRNA GACAATC, MIR-219” was the top pathway for BPD/death. Many members of this pathway are transcription factors. Other members include the alpha-type platelet-derived growth factor receptor (PDGFRA) important in lung alveolar septation.[22] miR-219 is involved in resolution of acute inflammation[23] which may be relevant to BPD. Not all targets of miR-219 were dysregulated in BPD lung, perhaps because most genes are regulated by multiple miRNA as well as by other factors (transcription factors, lncRNA, DNA methylation etc). A preliminary evaluation of highly conserved targets of miR-219 in hyperoxia-vs. air-exposed mice using publicly-available datasets (e.g. GSE25293) found that all targets were reduced with hyperoxia (data not shown). Our findings that miR-219 in the murine newborn lung reduced over the course of alveolar septation and increased during hyperoxia, and was increased in the human BPD lung suggests that this miRNA may regulate normal lung development and injury response.

The more important clinical outcomes are probably those related to severe BPD or death, as most infants with mild/moderate BPD improve over time. The top pathway associated with severe BPD/death and in survivors with severe BPD was Phosphorus Oxygen Lyase Activity. The second pathway was Cyclase Activity, which shares considerable overlap (10 of 11 genes) with Phosphorus Oxygen Lyase Activity. Cyclic AMP produced by adenylate cyclase is important in lung development.[24] Cyclic GMP produced by guanylate cyclase mediates nitric oxide signaling, and guanylate cyclase is involved in lung injury and development.[25] These results suggest that modulation of the cGMP and cAMP pathways may be specifically relevant to severe BPD, and perhaps less important in mild/moderate BPD.

A major finding was that of the top ten SNPs in the model for BPD/death, four were SNPs associated with ADARB2 and two were SNPs associated with CD44. These genes were also highly represented in the models for severe BPD/death and severe BPD in survivors. ADARB2 is RNA-editing deaminase 2,[26] a double-stranded RNA adenosine deaminase expressed mostly in the brain.[27] It is unclear at the current time why there is a strong association of ADARB2 with BPD/death. CD44 is a hyaluronic acid cell surface receptor important in leukocyte trafficking and involved in lung injury. In mouse models, CD44 is protective during hyperoxia-induced lung injury[28]. However, severe lung fibrosis is promoted by CD44 in adult mice, indicating that CD44 may also have detrimental effects.[29] We observed in the murine newborn lung that CD44 decreased over the course of alveolar septation and increased during hyperoxia, and was increased in human BPD lung, suggesting a role of this molecule in neonatal lung development and injury. The role of ADARB2 and CD44 in the pathophysiology of BPD requires further study.

Our study did not confirm findings of previous GWA studies[5, 6] or all pathways of the gene expression study,[16] perhaps due to different methods (pathways vs. single gene; genetic predisposition via SNPs vs. gene expression in established disease that may mask signals of early initiating events) or the populations being studied. For example, the population studied by Wang et al[6] was mainly of Mexican Hispanic origin, and our study was about 54% Black and 45% White. We observed marked differences in pathways by race/ethnicity. The large differences in pathways by race/ethnicity suggest that although the clinical phenotype of BPD may be similar, the underlying genetic predisposition may differ significantly. This may be considered anticipated, as ancestry-specific associations contribute to chronic lung diseases such as asthma[30] and emphysema.[31] This also suggests that potential therapies may need to be specifically targeted at pathways that are found to be involved, and therefore suggests a role of “personalized genomics” in BPD.

The results of this study provide complementary information to conventional single-marker analysis, help fill in the ‘missing heritability”, and provide useful information to guide mechanistic studies based on pathway inhibition/augmentation. Future studies will need to validate the gene set analysis, perhaps by analysis of gene expression and epigenetic data to determine if similar pathways are involved. In addition, sequencing methods may help identify individuals who might be genetically predisposed to severe lung disease, such as those with mutations in SFTPB, ABCA3, FOXF1 or NKX2-1.Finally, translational studies

are required to identify “druggable” mechanistic pathways and evaluate drug development strategies targeting these pathways.

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Abbreviations

ADARB2	adenosine deaminase
FDR	False Discovery Rate
SNP	Single Nucleotide Polymorphism

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Appendix

The following investigators, in addition to those listed as authors, are members of the Genomics and Cytokine Subcommittees of the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development Neonatal Research Network:

Abhik Das (DCC PI) and Grier Page (DCC Statistician) had full access to all the data in the study and take responsibility for the integrity of the data and accuracy of the data analysis. NRN Steering Committee Chair: Alan H. Jobe, MD PhD (University of Cincinnati).

Genomics Subcommittee: C. Michael Cotten, MD MHS (chair); Jeffrey C. Murray, MD (vice chair); Edward F. Bell, MD; Ronald N. Goldberg, MD; Kurt Schibler, MD; Beena G. Sood, MD MS; David K. Stevenson, MD; Barbara J. Stoll, MD; Krisa P. Van Meurs, MD; Abhik Das, PhD; Rosemary D. Higgins, MD; Karen J. Johnson, RN; Kristin M. Zaterka-Baxter, RN BSN. Cytokines Subcommittee: Waldemar A. Carlo, MD (chair); Richard A. Ehrenkranz, MD; Ronald N. Goldberg, MD; Seetha Shankaran, MD; Barbara J. Stoll, MD; Jon E. Tyson, MD MPH; Abhik Das, PhD; Rosemary D. Higgins, MD.

Case Western Reserve University, Rainbow Babies & Children's Hospital (U10 HD21364, M01 RR80) – Michele C. Walsh, MD MS; Avroy A. Fanaroff, MD; Nancy S. Newman, RN; Bonnie S. Siner, RN.

Cincinnati Children's Hospital Medical Center, University Hospital, and Good Samaritan Hospital (U10 HD27853, M01 RR8084) – Kurt Schibler, MD; Edward F. Donovan, MD; Vivek Narendran, MD MRCP; Barbara Alexander, RN; Cathy Grisby, BSN CCRC; Jody Hessling, RN; Marcia Worley Mersmann, RN CCRC; Holly L. Mincey, RN BSN.

Duke University School of Medicine University Hospital, Alamance Regional Medical Center, and Durham Regional Hospital (M01 RR30, U10 HD40492) – Ronald N. Goldberg, MD; Kathy J. Auten, MSHS.

Emory University, Children's Healthcare of Atlanta, Grady Memorial Hospital, and Emory Crawford Long Hospital (U10 HD27851, M01 RR39) – Barbara J. Stoll, MD; Ellen C. Hale, RN BS CCRC.

Eunice Kennedy Shriver National Institute of Child Health and Human Development – Linda L. Wright, MD; Sumner J. Yaffe, MD; Elizabeth M. McClure, Med; Stephanie Wilson Archer, MA.

RTI International (U10 HD36790) – Abhik Das, PhD; Scott A. McDonald, BS; Kristin M. Zaterka-Baxter, RN BSN; W. Kenneth Poole, PhD; Betty K. Hastings; Jeanette O'Donnell Auman, BS.

Stanford University, Lucile Packard Children's Hospital (U10 HD27880, M01 RR70) – Krisa P. Van Meurs, MD; David K. Stevenson, MD; M. Bethany Ball, BS CCRC.

University of Alabama at Birmingham Health System and Children's Hospital of Alabama (U10 HD34216, M01 RR32) – Namasivayam Ambalavanan, MD; Monica V. Collins, RN BSN MaEd; Shirley S. Cosby, RN BSN.

University of California – San Diego Medical Center and Sharp Mary Birch Hospital for Women (U10 HD40461) – Neil N. Finer, MD; Maynard R. Rasmussen, MD; David Kaegi, MD; Kathy Arnell, RNC; Clarence Demetrio, RN; Wade Rich, BSHS RRT.

University of Iowa Children's Hospital and Mercy Medical Center (U10 HD53109, M01 RR59, UL1 TR442) – Edward F. Bell, MD; Karen J. Johnson, RN.

University of Miami Holtz Children's Hospital (U10 HD21397, M01 RR16587) – Shahnaz Duara, MD; Charles R. Bauer, MD; Ruth Everett-Thomas, RN MSN.

University of Tennessee (U10 HD21415) – Sheldon B. Korones, MD; Henrietta S. Bada, MD; Tina Hudson, RN BSN.

University of Texas Southwestern Medical Center at Dallas, Parkland Health & Hospital System, and Children's Medical Center Dallas (U10 HD40689, M01 RR633) – Pablo J. Sánchez, MD; Abbot R. Laptook, MD; Walid A. Salhab, MD; Susie Madison, RN; Nancy A. Miller, RN; Gaynelle Hensley, RN; Alicia Guzman.

University of Texas Health Science Center at Houston Medical School, Children's Memorial Hermann Hospital, and Lyndon B. Johnson General Hospital (U10 HD21373) – Jon E. Tyson, MD MPH; Kathleen A. Kennedy, MD MPH; Esther G. Akpa, RN BSN; Patty A. Cluff, RN; Claudia I. Franco, RNC MSN; Anna E. Lis, RN BSN; Georgia E. McDavid, RN; Patti Pierce Tate, RCP.

Wayne State University, Hutzel Women's Hospital, and Children's Hospital of Michigan (U10 HD21385) – Seetha Shankaran, MD; Beena G. Sood, MD MS; G. Ganesh Konduri, MD; Rebecca Bara, RN BSN; Geraldine Muran, RN BSN.

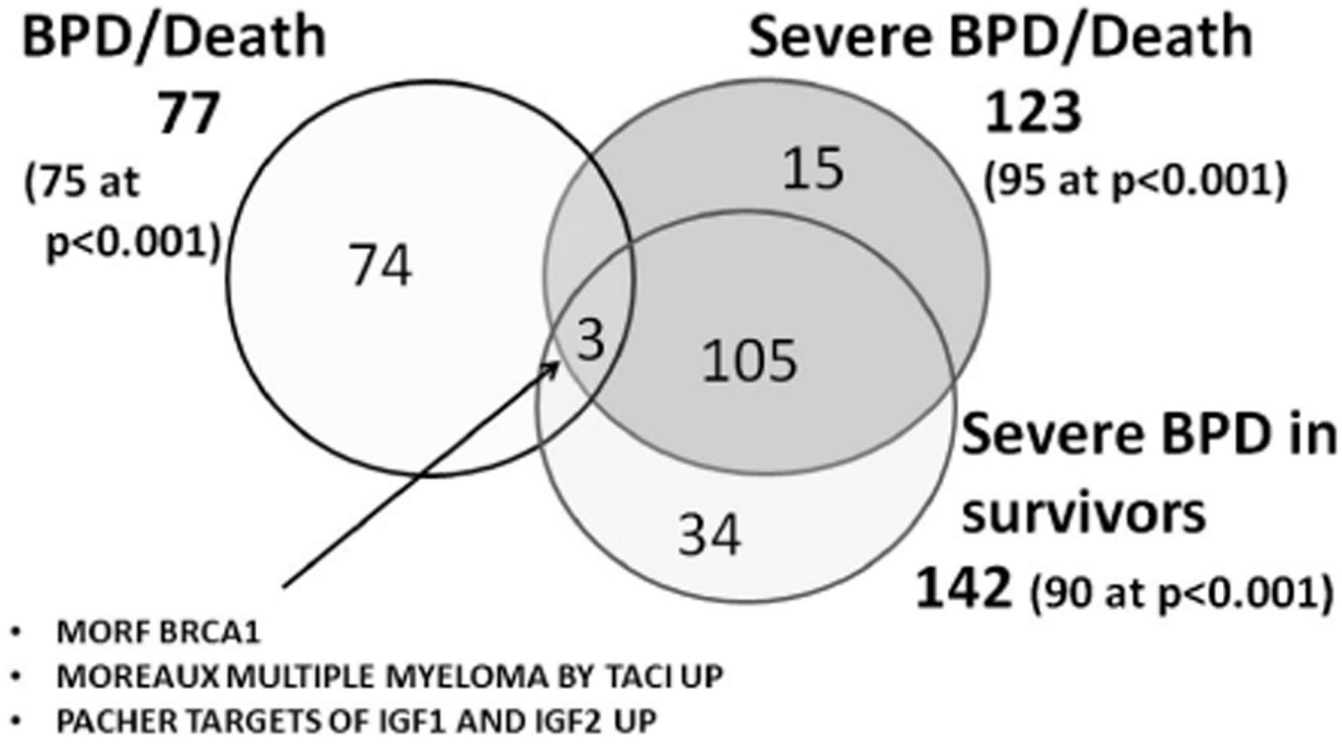

Figure 1.

Pathways at FDR<0.1. Venn diagram indicating number of pathways significant at FDR<0.1 and overlap for outcomes of BPD/death, severe BPD/death, and severe BPD in survivors.

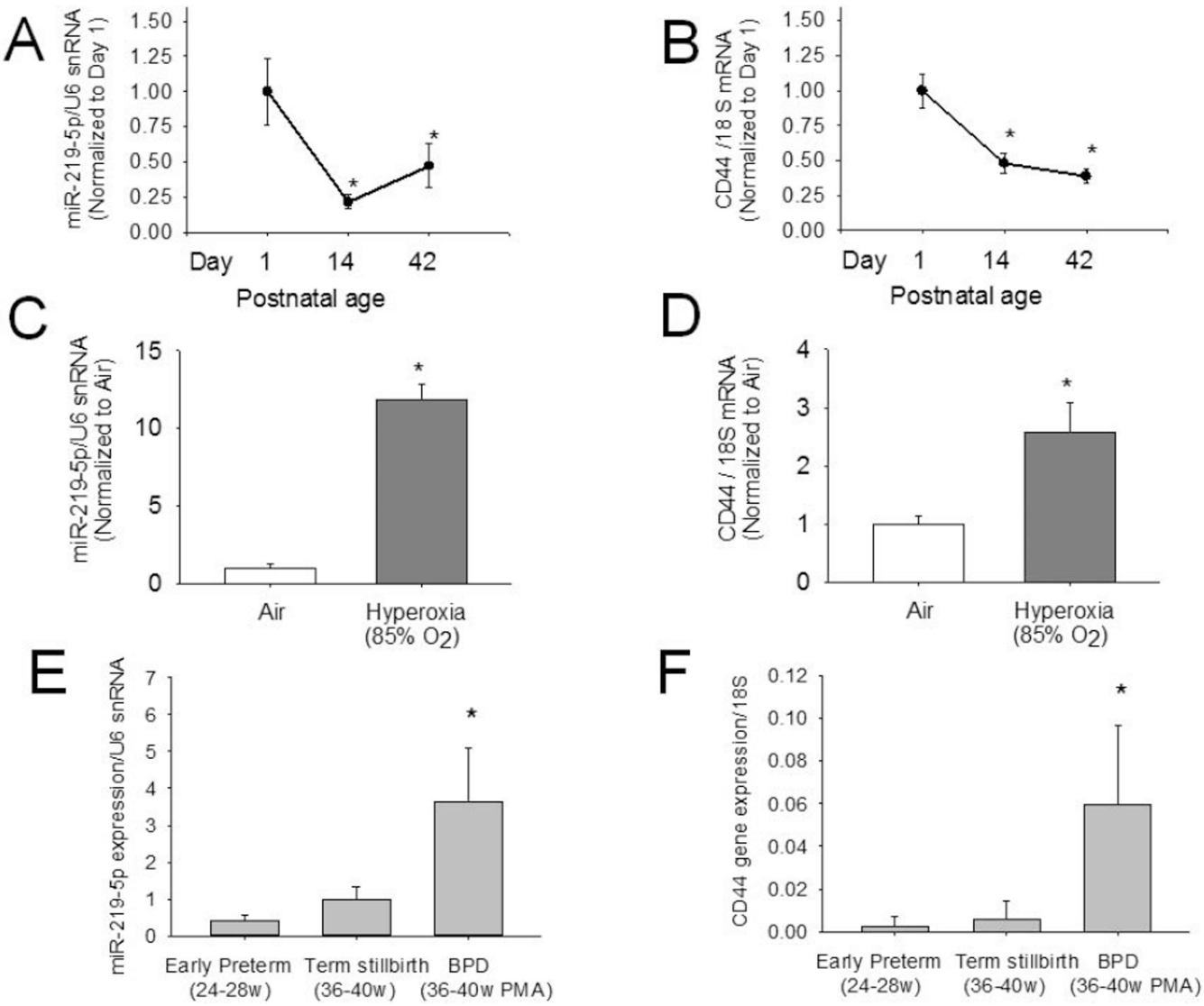

Figure 2.

Evaluation of miR-219 and CD44 in a newborn mouse model (Panels A-D) and in human lung (Panels E-F). Lung miR-219 (Panel A) and CD44 mRNA (Panel B) decreased during alveolar septation, with expression on postnatal days 14 and 42 significantly less as compared with day 1; *p<0.05. Lung miR-219 (Panel C) and CD44 mRNA (Panel D) were also increased on postnatal day 14 during hyperoxia exposure (*p<0.05 compared with air). Lung miR-219 (Panel E) and CD44 mRNA (Panel F) were increased in human lungs with BPD as compared to early preterm or term stillbirth lungs (Mean±SEM; n=4/group)

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Table 1

Characteristics of enrolled infants

Variable	Entire population	BPD or Death vs. Survival without BPD		Severe BPD or Death vs. Survival without severe BPD		Severe BPD in survivors vs. Survivors without severe BPD	
		BPD or Death	Survival without BPD	Severe BPD or Death	Survival without severe BPD	Severe BPD in survivors	Survival without severe BPD
Sample size	**751**	**428**	**322**	**243**	**469**	**102**	**469**
Birth weight in grams (mean, SD)	758 (140)	723 (135)	804 (133)	688 (129)	787 (132)	707 (121)	787 (132)
Gestational age in weeks (mean, SD, Range)	25.8 (1.96) (20–33)	25.3 (1.9) (20–33)	26.5 (1.9) (20–32)	24.9 (1.8) (21–33)	26.1 (1.9) (22–32)	25.4 (1.8) (21–33)	26.1 (1.9) (22–32)
Multiple gestation (%)	137 (18.2)	84 (19.6)	53 (16.5)	43 (17.7)	86 (18.3)	11 (10.8)	86 (18.3)
Antenatal steroids (%)	545 (73)	301 (71)	244 (76)	167 (69)	348 (74)	78 (77)	348 (74)
SGA (%)	105 (14.0)	46 (19.6)	59 (18.3)	26 (10.7)	70 (14.9)	15 (14.7)	70 (14.9)
Race: White	339 (45.1)	186 (43.5)	153 (47.5)	98 (40.3)	219 (46.7)	40 (39.2)	219 (46.7)
Race: Black	404 (53.8)	236 (55.1)	167 (51.9)	142 (58.4)	245 (52.2)	61 (59.8)	245 (52.2)
Race: Other	8 (1)	6 (1.4)	2 (0.6)	3 (1.2)	5 (1.1)	1 (1)	5 (1.1)
Ethnicity: Hispanic	156 (20.8)	82 (19.2)	74 (23.0)	49 (20.2)	97 (20.7)	20 (19.6)	97 (20.7)
Male sex	354 (47.1)	227 (53.0)	127 (39.4)	128 (52.7)	210 (44.8)	53 (52)	210 (44.8)
Apgar score at 5 minutes (mean, SD)	6.6 (1.8)	6.4 (1.9)	6.8 (1.6)	6.1 (1.9)	6.7 (1.9)	6.2 (2.1)	6.7 (1.7)
Cesarean delivery Yes (%)	430 (57.3)	229 (53.5)	201 (62.4)	120 (49.4)	284 (60.6)	59 (57.8)	284 (60.6)
Any Mechanical ventilation (%)	697 (92.8)	426 (99.5)	270 (83.9)	243 (100)	422 (90)	102 (100)	422 (90)

Variable	Entire population	BPD or Death vs. Survival without BPD		Severe BPD or Death vs. Survival without severe BPD		Severe BPD in survivors vs. Survivors without severe BPD	
		BPD or Death	Survival without BPD	Severe BPD or Death	Survival without severe BPD	Severe BPD in survivors	Survival without severe BPD
Days of assisted ventilation (Days, SD)	26.9 (27.5)	37.5 (29.8)	12.8 (15.2)	41.7 (35)	20.7 (19.6)	62.2 (31.6)	20.7 (19.6)

TABLE 2

Important single nucleotide polymorphisms (SNPs) and associated gene (using the NCBI database of SNPs (dbSNP; http://www.ncbi.nlm.nih.gov/snp/) and from UCSC Genome browser at http://genome.ucsc.edu), in relation to p-value for outcomes

Chromosome	SNP	Gene	p value
BPD or Death vs. Survival without BPD			
10	rs59582957	ADARB2 (adenosine deaminase)	7.18E-07
10	chr10:123726948	NSMC4A (non-SMC element 4 homolog A (S.	7.08E-07
10	rs17119652	No gene	1.38E-06
10	chr10:1488099	ADARB2	6.69E-07
10	chr10:1488186	ADARB2	6.42E-07
10	chr10:1488126	ADARB2	4.71E-07
11	chr11:35165510	CD44	8.60E-07
11	chr11:35167447	CD44	1.72E-06
12	rs1504316	No gene	6.30E-07
17	rs8082435	WDR45L (WD repeat domain 45B)	0.008752
Severe BPD or death vs. survival without severe BPD			
10	chr10:1488126	ADARB2	4.71E-07
10	chr10:1488186	ADARB2	6.42E-07
10	chr10:1488099	ADARB2	6.69E-07
10	chr10:123726948	NSMC4A	7.08E-07
10	rs59582957	ADARB2	7.18E-07
11	chr11:35165510	CD44	8.60E-07
12	rs1427793	NUAK1 (NUAK family SNF1-like kinase 1)	1.09E-06
10	rs57481375	No gene	1.10E-06
10	rs17119652	No gene	1.38E-06
4	rs2653829	KCNH7 (Potassium voltage-gated channel	1.48E-06
Severe BPD in survivors vs. Survivors without severe BPD			
11	chr11:35165510	CD44	4.64E-07
12	rs1504316	GRIP1 (glutamate receptor interacting protein 1)	6.30E-07
10	rs17119652	No gene	8.89E-07
11	chr11:35167447	CD44	9.04E-07
17	rs8082435	WDR45B (WD repeat domain 45B)	9.97E-07
4	rs2653829	KCNH7	1.01E-06
10	chr10:1488126	ADARB2	1.72E-06
4	rs2610201	GALNTL6 (UDP-N-acetyl-alpha-D-	1.91E-06
4	rs2653824	GALNTL6	2.04E-06
12	rs1427793	NUAK1	2.06E-06

Table 3

Biological pathways associated with BPD or death, as compared to survivors without BPD. Pathways from the annotated gene sets of the molecular signatures database at the Broad Institute (http://www.broadinstitute.org/gsea/msigdb/index.jsp) are listed in order of increasing false discovery rate (FDR). Only pathways with FDR ≤0.1 are shown.

Pathway Names	P values	FDR
GACAATC,MIR-219	1.41E-08	9.52E-05
NUCLEAR_UBIQUITIN_LIGASE_COMPLEX	2.16E-07	0.00073
YRCCAKNNGNCGC_UNKNOWN	7.11E-07	0.0016
V$E2F_Q2	9.88E-07	0.001668
NEURON_PROJECTION	1.37E-05	0.015893
V$GABP_B	1.41E-05	0.015893
TOMLINS_METASTASIS_DN	1.66E-05	0.015965
JAEGER_METASTASIS_UP	3.34E-05	0.018801
NUCLEOBASENUCLEOSIDENUCLEOTIDE_KINASE_ACTIVITY	2.74E-05	0.018801
RUIZ_TNC_TARGETS_DN	2.99E-05	0.018801
SUGAR_BINDING	2.71E-05	0.018801
V$E2F_Q4	3.27E-05	0.018801
CARBOHYDRATE_BINDING	4.87E-05	0.024581
UBIQUITIN_LIGASE_COMPLEX	5.10E-05	0.024581
V$E2F_Q6	5.64E-05	0.025396
LIPID_KINASE_ACTIVITY	6.62E-05	0.027946
V$FREAC7_01	7.88E-05	0.031284
CAGCTTT,MIR-320	0.000106	0.033841
DAZARD_RESPONSE_TO_UV_NHEK_UP	0.000101	0.033841
HALMOS_CEBPA_TARGETS_DN	9.54E-05	0.033841
MOREAUX_MULTIPLE_MYELOMA_BY_TACI_UP	0.00011	0.033841
TAGHAVI_NEOPLASTIC_TRANSFORMATION	0.000107	0.033841
COLLER_MYC_TARGETS_UP	0.000116	0.034047
V$AML_Q6	0.000138	0.038786
REACTOME_CALCITONIN_LIKE_LIGAND_RECEPTORS	0.00017	0.045858
AMIT_EGF_RESPONSE_480_HELA	0.000182	0.04725
BIOCARTA_CTCF_PATHWAY	0.000253	0.047743
BIOCARTA_RAC1_PATHWAY	0.0002	0.047743
CTCTATG,MIR-368	0.000226	0.047743
GNF2_SPINK1	0.000219	0.047743
IIZUKA_LIVER_CANCER_PROGRESSION_L0_L1_UP	0.000242	0.047743

Pathway Names	P values	FDR
IVANOVA_HEMATOPOIESIS_LATE_PROGENITOR	0.000263	0.047743
module_320	0.000223	0.047743
MUELLER_COMMON_TARGETS_OF_AML_FUSIONS_DN	0.000246	0.047743
REACTOME_ACTIVATION_OF_NMDA_RECEP	0.000263	0.047743
TOR_UPON_GLUTAMATE_BINDING_AND_POSTSYNAPTIC_EVENTS		
RESPONSE_TO_STEROID_HORMONE_STIMULUS	0.000269	0.047743
SCGGAAGY_V$ELK1_02	0.000241	0.047743
V$E47_01	0.000207	0.047743
CELLULAR_BIOSYNTHETIC_PROCESS	0.000294	0.048217
DAZARD_RESPONSE_TO_UV_SCC_DN	0.000296	0.048217
REGULATION_OF_CELLULAR_PROTEIN_METABOLIC_PROCESS	0.0003	0.048217
V$ZF5_01	0.000283	0.048217
GAGACTG,MIR-452	0.000334	0.050215
KAPOSI_LIVER_CANCER_POOR_SURVIVAL_UP	0.000342	0.050215
MORF_BRCA1	0.00034	0.050215
PACHER_TARGETS_OF_IGF1_AND_IGF2_UP	0.000341	0.050215
chr4q26	0.000363	0.052097
BIOCARTA_MCM_PATHWAY	0.000395	0.054476
GGCACAT,MIR-455	0.000391	0.054476
BEGUM_TARGETS_OF_PAX3_FOXO1_FUSION_UP	0.000423	0.056037
SENESE_HDAC1_AND_HDAC2_TARGETS_DN	0.000415	0.056037
CTCNANGTGNY_UNKNOWN	0.000445	0.056717
REGULATION_OF_PROTEIN_METABOLIC_PROCESS	0.000437	0.056717
BIOCARTA_RAS_PATHWAY	0.000475	0.057752
chr10q23	0.000468	0.057752
module_321	0.00048	0.057752
V$NRF2_01	0.000488	0.057752
KENNY_CTNNB1_TARGETS_DN	0.000518	0.060337
chr2q13	0.000568	0.064501
REGULATION_OF_CELL_CYCLE	0.000573	0.064501
GNF2_SERPINI2	0.000604	0.066831
V$T3R_Q6	0.00066	0.071888
ACCGAGC,MIR-423	0.000686	0.073179
HELLER_HDAC_TARGETS_UP	0.000694	0.073179
LEE_DIFFERENTIATING_T_LYMPHOCYTE	0.000712	0.073942

Pathway Names	P values	FDR
CHESLER_BRAIN_HIGHEST_EXPRESSION	0.00079	0.080012
MATSUDA_NATURAL_KILLER_DIFFERENTIATION	0.000794	0.080012
MICROTUBULE_MOTOR_ACTIVITY	0.000807	0.080139
AGGGCCA,MIR-328	0.000836	0.081055
HOFMANN_CELL_LYMPHOMA_DN	0.000841	0.081055
CYTOSKELETAL_PART	0.000873	0.082976
CYTOSKELETON	0.00092	0.085062
OHM_EMBRYONIC_CARCINOMA_UP	0.000909	0.085062
CAGCCTC,MIR-485-5P	0.000934	0.085164
PROTEIN_UBIQUITINATION	0.000992	0.089296
chr9q22	0.001106	0.096946
FERREIRA_EWINGS_SARCOMA_UNSTABLE_VS_STABLE_DN	0.001105	0.096946

Table 4

Biological pathways associated with severe BPD or death, as compared to survivors without severe BPD. Pathways from the annotated gene sets of the molecular signatures database at the Broad Institute (http://www.broadinstitute.org/gsea/msigdb/index.jsp) are listed in order of increasing false discovery rate (FDR). Only pathways with FDR ≤0.1 are shown.

Pathway Names	P values	FDR
PHOSPHORUS_OXYGEN_LYASE_ACTIVITY	5.68E-08	0.000192
CYCLASE_ACTIVITY	4.57E-08	0.000192
GNF2_PRDX2	2.16E-07	0.000487
MORF_RAP1A	6.18E-07	0.001043
KUMAMOTO_RESPONSE_TO_NUTLIN_3A_UP	1.87E-06	0.002526
MITOCHONDRION	2.73E-06	0.003076
SHEDDEN_LUNG_CANCER_POOR_SURVIVAL_A6	3.48E-06	0.003357
VALK_AML_CLUSTER_2	5.04E-06	0.003783
GLUCOSE_CATABOLIC_PROCESS	4.73E-06	0.003783
REACTOME_TRANSMISSION_ACROSS_CHEMICAL_SYNAPSES	6.68E-06	0.00435
BIOCARTA_CCR5_PATHWAY	7.09E-06	0.00435
GRAHAM_CML_DIVIDING_VS_NORMAL_QUIESCENT_UP	1.09E-05	0.006114
MORF_BRCA1	1.35E-05	0.006995
MAP_KINASE_ACTIVITY	3.16E-05	0.009928
REACTOME_NOREPINEPHRINE_NEUROTRANSMITTER_RELEASE_CYCLE	3.12E-05	0.009928
KCCGNSWTTT_UNKNOWN	3.24E-05	0.009928
RESPONSE_TO_ENDOGENOUS_STIMULUS	2.73E-05	0.009928
DELAYED_RECTIFIER_POTASSIUM_CHANNEL_ACTIVITY	2.79E-05	0.009928
module_428	2.35E-05	0.009928
MORF_ATRX	2.86E-05	0.009928
TAGCTTT,MIR-9	3.21E-05	0.009928
REACTOME_CLASS_C3_METABOTROPIC_GLUTAMATE_PHEROMONE_RECEPTORS	2.80E-05	0.009928
RESPONSE_TO_DNA_DAMAGE_STIMULUS	3.54E-05	0.010382
TOMLINS_PROSTATE_CANCER_UP	4.63E-05	0.012494
MCCLUNG_DELTA_FOSB_TARGETS_2WK	4.48E-05	0.012494
TRANSCRIPTION_COACTIVATOR_ACTIVITY	5.68E-05	0.014751
RODRIGUES_NTN1_AND_DCC_TARGETS	6.01E-05	0.015023
V$CREB_02	6.67E-05	0.016079
GCM_GSPT1	7.38E-05	0.017173
FERRARI_RESPONSE_TO_FENRETINIDE_DN	8.00E-05	0.017994
ATAAGCT,MIR-21	8.47E-05	0.018436

Pathway Names	P values	FDR
NAKAMURA_CANCER_MICROENVIRONMENT_DN	9.69E-05	0.020443
LEE_INTRATHYMIC_T_PROGENITOR	0.00011	0.022509
TCCCCAC,MIR-491	0.000128	0.025452
CYTOSOLIC_PART	0.000133	0.025637
WINTER_HYPOXIA_UP	0.000169	0.029972
TRANSCRIPTION_ACTIVATOR_ACTIVITY	0.000162	0.029972
MOREAUX_MULTIPLE_MYELOMA_BY_TACI_UP	0.000165	0.029972
NELSON_RESPONSE_TO_ANDROGEN_UP	0.000179	0.030963
PATTERSON_DOCETAXEL_RESISTANCE	0.000187	0.031433
MORF_PPP5C	0.000191	0.031433
DNA_REPAIR	0.000198	0.031826
V$NFY_Q6_01	0.000206	0.032314
XU_HGF_TARGETS_INDUCED_BY_AKT1_48HR_UP	0.000218	0.033408
WATANABE_RECTAL_CANCER_RADIOTHERAPY_RESPONSIVE_DN	0.000245	0.035718
MORF_CCNF	0.000241	0.035718
REGULATION_OF_LIPID_METABOLIC_PROCESS	0.000249	0.035718
WHITE_NEUROBLASTOMA_WITH_1P36.3_DELETION	0.000258	0.036278
REGULATION_OF_SMALL_GTPASE_MEDIATED_SIGNAL_TRANSDUCTION	0.000338	0.043476
LAMELLIPODIUM	0.000344	0.043476
REACTOME_GLUTAMATE_NEUROTRANSMITTER_RELEASE_CYCLE	0.000331	0.043476
TONKS_TARGETS_OF_RUNX1_RUNX1T1_FUSION_SUSTAINED_IN_GRANULOCYTE_UP	0.000325	0.043476
V$IRF_Q6	0.000318	0.043476
V$CRX_Q4	0.000348	0.043476
PENG_LEUCINE_DEPRIVATION_UP	0.000363	0.044551
chr6q24	0.000386	0.046488
INDUCTION_OF_APOPTOSIS_BY_INTRACELLULAR_SIGNALS	0.000409	0.048445
module_471	0.000425	0.049432
PUJANA_CHEK2_PCC_NETWORK	0.000442	0.050452
V$CREB_Q4_01	0.00047	0.050452
PERINUCLEAR_REGION_OF_CYTOPLASM	0.000486	0.050452
GAZDA_DIAMOND_BLACKFAN_ANEMIA_ERYTHROID_DN	0.000471	0.050452
MATTIOLI_MULTIPLE_MYELOMA_WITH_14Q32_TRANSLOCATIONS	0.000482	0.050452
PROTEIN_HOMODIMERIZATION_ACTIVITY	0.000464	0.050452

Pathway Names	P values	FDR
GRAHAM_CML_DIVIDING_VS_NORMAL_DIVIDING_DN	0.000465	0.050452
LIU_TARGETS_OF_VMYB_VS_CMYB_UP	0.000498	0.050942
REACTOME_IONOTROPIC_ACTIVITY_OF_KAINATE_RECEPTORS	0.000527	0.053129
PYEON_CANCER_HEAD_AND_NECK_VS_CERVICAL_DN	0.000553	0.054085
ZHANG_PROLIFERATING_VS_QUIESCENT	0.000548	0.054085
EXTERNAL_SIDE_OF_PLASMA_MEMBRANE	0.000604	0.058227
GNF2_APEX1	0.00062	0.058953
ZUCCHI_METASTASIS_DN	0.000638	0.059768
SNIJDERS_AMPLIFIED_IN_HEAD_AND_NECK_TUMORS	0.000651	0.060177
V$USF_C	0.000708	0.062681
RNTCANNRNNYNATTW_UNKNOWN	0.000689	0.062681
DEPHOSPHORYLATION	0.000715	0.062681
chr5q34	0.000713	0.062681
RHEIN_ALL_GLUCOCORTICOID_THERAPY_DN	0.000727	0.062882
CELLULAR_CARBOHYDRATE_CATABOLIC_PROCESS	0.000743	0.063492
REGULATION_OF_RAS_PROTEIN_SIGNAL_TRANSDUCTION	0.000757	0.06388
RICKMAN_HEAD_AND_NECK_CANCER_D	0.000767	0.06388
module_441	0.00079	0.064239
REGULATION_OF_RAS_GTPASE_ACTIVITY	0.000815	0.064239
CHIBA_RESPONSE_TO_TSA_UP	0.000816	0.064239
SEIDEN_ONCOGENESIS_BY_MET	0.000811	0.064239
MORI_MATURE_B_LYMPHOCYTE_DN	0.000818	0.064239
ION_TRANSMEMBRANE_TRANSPORTER_ACTIVITY	0.000836	0.064863
TIEN_INTESTINE_PROBIOTICS_24HR_UP	0.000879	0.067459
SENGUPTA_NASOPHARYNGEAL_CARCINOMA_WITH_LMP1_UP	0.000902	0.068386
REGULATION_OF_CATABOLIC_PROCESS	0.000926	0.069418
TSAI_DNAJB4_TARGETS_DN	0.000946	0.069432
DNA_DAMAGE_RESPONSESIGNAL_TRANSDUCTION_RESULTING_IN_INDUCTION_OF_APOPTOSIS	0.000939	0.069432
KEGG_DRUG_METABOLISM_OTHER_ENZYMES	0.00099	0.070374
ENK_UV_RESPONSE_EPIDERMIS_UP	0.000973	0.070374
NUCLEOBASENUCLEOSIDENUCLEOTIDE_AND_NUCLEIC_ACID_TRANSMEMBRANE_TRANSPORTER_ACTIVITY	0.000986	0.070374

Pathway Names	P values	FDR
KEGG_LIMONENE_AND_PINENE_DEGRADATION	0.001016	0.07146
SAGIV_CD24_TARGETS_UP	0.001083	0.075354
NUCLEOBASENUCLEOSIDENUCLEOTIDE_AND_NUCLEIC_ACID_TRANSPORT	0.001155	0.076817
JISON_SICKLE_CELL_DISEASE_UP	0.001119	0.076817
module_528	0.001156	0.076817
ONDER_CDH1_TARGETS_1_UP	0.001145	0.076817
TAKEDA_TARGETS_OF_NUP98_HOXA9_FUSION_16D_UP	0.001161	0.076817
SARRIO_EPITHELIAL_MESENCHYMAL_TRANSITION_UP	0.001194	0.078262
chr6p	0.001286	0.083098
ZIRN_TRETINOIN_RESPONSE_DN	0.001293	0.083098
PACHER_TARGETS_OF_IGF1_AND_IGF2_UP	0.001389	0.087629
PAPASPYRIDONOS_UNSTABLE_ATEROSCLEROTIC_PLAQUE_DN	0.001384	0.087629
REACTOME_GLYCOGEN_BREAKDOWN_GLYCOGENOLYSIS	0.001428	0.089249
GGTAACC,MIR-409-5P	0.001467	0.090596
EXTRACELLULAR_SPACE	0.001476	0.090596
ZHAN_MULTIPLE_MYELOMA_CD2_DN	0.001539	0.093601
REGULATION_OF_GTPASE_ACTIVITY	0.00161	0.096446
REACTOME_ACTIVATION_OF_CHAPERONES_BY_IRE1_ALPHA	0.001615	0.096446
TGAGATT,MIR-216	0.001668	0.096635
OKUMURA_INFLAMMATORY_RESPONSE_LPS	0.001654	0.096635
V$IRF1_Q6	0.001668	0.096635
MOOTHA_HUMAN_MITODB_6_2002	0.001675	0.096635
TGAYRTCA_V$ATF3_Q6	0.001731	0.098992
LOPES_METHYLATED_IN_COLON_CANCER_UP	0.0018	0.099652
BASSO_HAIRY_CELL_LEUKEMIA_DN	0.001801	0.099652
HONRADO_BREAST_CANCER_BRCA1_VS_BRCA2	0.001772	0.099652
MORF_PSMF1	0.001769	0.099652

Table 5

Biological pathways associated with severe BPD in survivors, as compared to survivors without BPD. Pathways from the annotated gene sets of the molecular signatures database at the Broad Institute (http://www.broadinstitute.org/gsea/msigdb/index.jsp) are listed in order of increasing false discovery rate (FDR). Only pathways with FDR <0.1 are shown.

Pathway Names	P values	FDR
PHOSPHORUS_OXYGEN_LYASE_ACTIVITY	3.91E-08	0.000132037
CYCLASE_ACTIVITY	2.55E-08	0.000132037
KUMAMOTO_RESPONSE_TO_NUTLIN_3A_UP	1.22E-06	0.002374878
GNF2_PRDX2	1.41E-06	0.002374878
SHEDDEN_LUNG_CANCER_POOR_SURVIVAL_A6	2.69E-06	0.003636936
MORF_RAP1A	4.01E-06	0.004511149
GRAHAM_CML_DIVIDING_VS_NORMAL_QUIESCENT_UP	5.99E-06	0.005773791
TOMLINS_PROSTATE_CANCER_UP	6.89E-06	0.005811578
VALK_AML_CLUSTER_2	9.42E-06	0.006927232
GLUCOSE_CATABOLIC_PROCESS	1.03E-05	0.006927232
MAP_KINASE_ACTIVITY	1.57E-05	0.008805869
MITOCHONDRION	1.49E-05	0.008805869
V$CREB_02	2.13E-05	0.011048338
REACTOME_TRANSMISSION_ACROSS_CHEMICAL_SYNAPSES	2.51E-05	0.012111145
MORF_BRCA1	5.17E-05	0.018918766
ATAAGCT,MIR-21	6.17E-05	0.018918766
RODRIGUES_NTN1_AND_DCC_TARGETS	5.41E-05	0.018918766
TCCCCAC,MIR-491	5.87E-05	0.018918766
REACTOME_NOREPINEPHRINE_NEUROTRANSMITTER_RELEASE_CYCLE	5.27E-05	0.018918766
NAKAMURA_CANCER_MICROENVIRONMENT_DN	6.01E-05	0.018918766
KCCGNSWTTT_UNKNOWN	5.54E-05	0.018918766
BIOCARTA_CCR5_PATHWAY	5.95E-05	0.018918766
RESPONSE_TO_ENDOGENOUS_STIMULUS	7.72E-05	0.020857418
TRANSCRIPTION_COACTIVATOR_ACTIVITY	7.32E-05	0.020857418
XU_HGF_TARGETS_INDUCED_BY_AKT1_48HR_UP	7.59E-05	0.020857418
DELAYED_RECTIFIER_POTASSIUM_CHANNEL_ACTIVITY	8.90E-05	0.022241771
RESPONSE_TO_DNA_DAMAGE_STIMULUS	8.76E-05	0.022241771
FERRARI_RESPONSE_TO_FENRETINIDE_DN	9.70E-05	0.02337315
module_428	0.000103	0.023984694
MORF_ATRX	0.000113	0.025358723
GCM_GSPT1	0.000122	0.026655373

Pathway Names	P values	FDR
WINTER_HYPOXIA_UP	0.000139	0.029219502
TRANSCRIPTION_ACTIVATOR_ACTIVITY	0.00015	0.030772585
NELSON_RESPONSE_TO_ANDROGEN_UP	0.000176	0.034427242
WATANABE_RECTAL_CANCER_RADIOTHERAPY_RESPONSIVE_DN	0.000179	0.034427242
TAGCTTT,MIR-9	0.000189	0.034900695
EXTERNAL_SIDE_OF_PLASMA_MEMBRANE	0.000196	0.034900695
MCCLUNG_DELTA_FOSB_TARGETS_2WK	0.000192	0.034900695
REGULATION_OF_SMALL_GTPASE_MEDIATED_SIGNALTRANSDUCTION	0.000216	0.036492426
PUJANA_CHEK2_PCC_NETWORK	0.000216	0.036492426
WHITE_NEUROBLASTOMA_WITH_1P36.3_DELETION	0.000228	0.037550752
CYTOSOLIC_PART	0.000242	0.038012621
chr6q24	0.000241	0.038012621
BENPORATH_ES_CORE_NINE_CORRELATED	0.000249	0.038221556
SENGUPTA_NASOPHARYNGEAL_CARCINOMA_WITH_LMP1_UP	0.000265	0.039765851
SAGIV_CD24_TARGETS_UP	0.000315	0.04177443
KEGG_DRUG_METABOLISM_OTHER_ENZYMES	0.00031	0.04177443
RHEIN_ALL_GLUCOCORTICOID_THERAPY_DN	0.000309	0.04177443
REACTOME_CLASS_C3_METABOTROPIC_GLUTAMATE_PHEROMONE_RECEPTORS	0.000306	0.04177443
chr3p25	0.000316	0.04177443
V$CREB_Q4_01	0.000306	0.04177443
LAMELLIPODIUM	0.000338	0.043890934
ZUCCHI_METASTASIS_DN	0.000372	0.047406175
MOREAUX_MULTIPLE_MYELOMA_BY_TACI_UP	0.000383	0.047933234
REACTOME_STEROID_HORMONE_BIOSYNTHESIS	0.000393	0.048235755
chr3p14	0.00041	0.048235755
LEE_INTRATHYMIC_T_PROGENITOR	0.000404	0.048235755
SARRIO_EPITHELIAL_MESENCHYMAL_TRANSITION_UP	0.000414	0.048235755
module_441	0.000431	0.049268618
GNF2_APEX1	0.00044	0.049520971
PERINUCLEAR_REGION_OF_CYTOPLASM	0.000448	0.049520971
V$USF_C	0.000482	0.052423429
MORF_CCNF	0.000495	0.053037318
V$NFY_Q6_01	0.000536	0.056482951
REGULATION_OF_RAS_PROTEIN_SIGNAL_TRANSDUCTION	0.000597	0.057783747
LINDGREN_BLADDER_CANCER_CLUSTER_2B	0.000599	0.057783747
LOPES_METHYLATED_IN_COLON_CANCER_UP	0.000594	0.057783747

Pathway Names	P values	FDR
BASSO_HAIRY_CELL_LEUKEMIA_DN	0.00057	0.057783747
GAZDA_DIAMOND_BLACKFAN_ANEMIA_ERYTHROID_DN	0.000561	0.057783747
REACTOME_GLUTAMATE_NEUROTRANSMITTER_RELEASE_CYCLE	0.000584	0.057783747
TSAI_DNAJB4_TARGETS_DN	0.000646	0.058786732
PYEON_CANCER_HEAD_AND_NECK_VS_CERVICAL_DN	0.000669	0.058786732
TONKS_TARGETS_OF_RUNX1_RUNX1T1_FUSION_SUSTAINED_IN_GRANULOCYTE_UP	0.000661	0.058786732
GGTAACC,MIR-409-5P	0.000671	0.058786732
REGULATION_OF_CATABOLIC_PROCESS	0.000647	0.058786732
NUCLEOBASENUCLEOSIDENUCLEOTIDE_AND_NUCLEIC_ACID_TRANSPORT	0.000645	0.058786732
CELLULAR_CARBOHYDRATE_CATABOLIC_PROCESS	0.000663	0.058786732
MCBRYAN_PUBERTAL_BREAST_5_6WK_UP	0.000698	0.059610192
RICKMAN_HEAD_AND_NECK_CANCER_D	0.000696	0.059610192
PATTERSON_DOCETAXEL_RESISTANCE	0.000719	0.060660099
DNA_REPAIR	0.000744	0.061980193
REGULATION_OF_RAS_GTPASE_ACTIVITY	0.000769	0.063320275
SNIJDERS_AMPLIFIED_IN_HEAD_AND_NECK_TUMORS	0.000804	0.065381496
MYLLYKANGAS_AMPLIFICATION_HOT_SPOT_25	0.000831	0.066760855
REGULATION_OF_GTPASE_ACTIVITY	0.000917	0.071987568
CHIARETTI_T_ALL_REFRACTORY_TO_THERAPY	0.000917	0.071987568
module_471	0.000932	0.072274597
MAYBURD_RESPONSE_TO_L663536_UP	0.001005	0.074582373
V$IRF_Q6	0.000987	0.074582373
TGAGATT,MIR-216	0.000994	0.074582373
MATTIOLI_MULTIPLE_MYELOMA_WITH_14Q32_TRANSLOCATIONS	0.001002	0.074582373
REACTOME_IONOTROPIC_ACTIVITY_OF_KAINATE_RECEPTORS	0.001081	0.077418566
REACTOME_GLYCOGEN_BREAKDOWN_GLYCOGENOLYSIS	0.00109	0.077418566
CCCNNGGGAR_V$OLF1_01	0.001076	0.077418566
RNTCANNRNNYNATTW_UNKNOWN	0.001062	0.077418566
HENDRICKS_SMARCA4_TARGETS_UP	0.001129	0.079382915
MORF_PPP5C	0.001202	0.081971096
CHIBA_RESPONSE_TO_TSA_UP	0.001195	0.081971096
GTGTGAG,MIR-342	0.001186	0.081971096
PUJANA_BRCA1_PCC_NETWORK	0.001284	0.086662282
PENG_LEUCINE_DEPRIVATION_UP	0.00132	0.087158417

Pathway Names	P values	FDR
ZHANG_RESPONSE_TO_IKK_INHIBITOR_AND_TNF_UP	0.001309	0.087158417
VICENT_METASTASIS_UP	0.00133	0.087158417
GNF2_ANP32B	0.001384	0.088728588
DEPHOSPHORYLATION	0.001389	0.088728588
V$IRF2_01	0.001393	0.088728588
HASINA_NOL7_TARGETS_DN	0.001413	0.089142284
INDUCTION_OF_APOPTOSIS_BY_INTRACELLULAR_SIGNALS	0.001441	0.090064396
OKUMURA_INFLAMMATORY_RESPONSE_LPS	0.001493	0.092456662
NUCLEAR_UBIQUITIN_LIGASE_COMPLEX	0.001523	0.093434244
TGAYRTCA_V$ATF3_Q6	0.001538	0.093522358
FERREIRA_EWINGS_SARCOMA_UNSTABLE_VS_STABLE_UP	0.001631	0.09598751
REGULATION_OF_LIPID_METABOLIC_PROCESS	0.001594	0.09598751
V$PAX3_01	0.001635	0.09598751
BLUM_RESPONSE_TO_SALIRASIB_UP	0.00165	0.09598751
chr6p	0.001628	0.09598751
ENK_UV_RESPONSE_EPIDERMIS_UP	0.001673	0.096498142
BIOCARTA_LONGEVITY_PATHWAY	0.001722	0.097653706
chr5q34	0.001707	0.097653706
NUCLEOLUS	0.00174	0.097890086
PACHER_TARGETS_OF_IGF1_AND_IGF2_UP	0.001832	0.098235946
AXON	0.001769	0.098235946
TIEN_INTESTINE_PROBIOTICS_24HR_UP	0.001796	0.098235946
JISON_SICKLE_CELL_DISEASE_UP	0.00181	0.098235946
CTGCAGY_UNKNOWN	0.001848	0.098235946
REACTOME_ACTIVATION_OF_CHAPERONES_BY_IRE1_ALPHA	0.001839	0.098235946
KEGG_LIMONENE_AND_PINENE_DEGRADATION	0.001808	0.098235946
REACTOME_DOWN_STREAM_SIGNAL_TRANSDUCTION	0.002053	0.099165696
NUCLEOBASENUCLEOSIDENUCLEOTIDE_AND_NUCLEIC_ACID_TRANSMEMBRANE_TRANSPORTER_ACTIVITY	0.001938	0.099165696
REACTOME_PYRUVATE_METABOLISM	0.001953	0.099165696
FUNG_IL2_SIGNALING_1	0.002071	0.099165696
PAPASPYRIDONOS_UNSTABLE_ATEROSCLEROTIC_PLAQUE_DN	0.002003	0.099165696
ZHANG_PROLIFERATING_VS_QUIESCENT	0.001967	0.099165696
CAAGGAT,MIR-362	0.001995	0.099165696
PENG_RAPAMYCIN_RESPONSE_UP	0.002047	0.099165696
V$CRX_Q4	0.002058	0.099165696

Pathway Names	P values	FDR
EXTRACELLULAR_SPACE	0.001993	0.099165696
CARBOHYDRATE_CATABOLIC_PROCESS	0.001922	0.099165696
TTCCGTT,MIR-191	0.002022	0.099165696
HONRADO_BREAST_CANCER_BRCA1_VS_BRCA2	0.001971	0.099165696
SEIDEN_ONCOGENESIS_BY_MET	0.001986	0.099165696
module_318	0.002096	0.099652829

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Table 6

Biological pathways associated with BPD or death classified by race, as compared to survivors without BPD. Pathways from the annotated gene sets of the molecular signatures database at the Broad Institute (http://www.broadinstitute.org/gsea/msigdb/index.jsp) are listed in order of increasing false discovery rate (FDR). Only the top 12 pathways are shown for All infants, White infants, and Black infants.

All infants			**White infants**			**Black infants**		
Pathway	**P value**	**FDR**	**Pathway**	**P value**	**FDR**	**Pathway**	**P value**	**FDR**
GACAATC, MIR-219	1.41E-08	9.52E-05	module_320	1.82E-49	1.23E-45	RODRIGUES_THYROID_CARCINOMA_DN	7.80E-07	0.005262
NUCLEAR_UBIQUITIN_LIGASE_COMPLEX	2.16E-07	0.00073	GNF2_BUB1	2.29E-41	7.73E-38	TOMLINS_METASTASIS_DN	4.91E-06	0.007876
YRCCAKNNGNCGC_UNKNOWN	7.11E-07	0.0016	GNF2_TTK	1.03E-31	2.32E-28	ATAAGCT,MIR-21	5.14E-06	0.007876
V$E2F_Q2	9.88E-07	0.001668	GNF2_SMC2L1	1.38E-31	2.33E-28	KEGG_CELL_ADHESION_MOLECULES_CAMS	2.39E-06	0.007876
NEURON_PROJECTION	1.37E-05	0.015893	GNF2_HMMR	4.80E-24	6.48E-21	module_349	5.83E-06	0.007876
V$GABP_B	1.41E-05	0.015893	GNF2_ESPL1	8.12E-23	9.14E-20	BEGUM_TARGETS_OF_PAX3_FOXO1_FUSION_UP	8.48E-06	0.008177
TOMLINS_METASTASISDN	1.66E-05	0.015965	GNF2_CENPE	1.23E-22		CHEOK_RESPONSE_TO_MERCAPTOPURINE_AND_HD_MTX_UP	8.15E-06	0.008177
NUCLEOBASENUCLEOSIDENUCLEOTIDE_KINASE_ACTIVITY	2.74E-05	0.018801	GNF2_CDC20	5.47E-22		AMIT_EGF_RESPONSE_480_HELA	1.00E-05	0.008437

All infants			White infants			Black infants		
Pathway	**P value**	**FDR**	**Pathway**	**P value**	**FDR**	**Pathway**	**P value**	**FDR**
V$E2F_Q4	3.27E-05	0.018801	module_244	1.14E-21	8.55E-19	CELLULAR_MACROMOLECULE_METABOLIC_PROCESS	1.98E-05	0.014845
RUIZ_TNC_TARGETS_DN	2.99E-05	0.018801	GNF2_CKS1B	3.48E-20	2.35E-17	KENNY_CTNNB1_TARGETS_DN	2.26E-05	0.015232
JAEGER_METASTASIS_UP	3.34E-05	0.018801	MORI_LARGE_PRE_BII_LYMPHOCYTE_UP	2.86E-19	1.76E-16	PROTEIN_UBIQUITINATION	2.94E-05	0.017542
SUGAR_BINDING	2.71E-05	0.018801	GNF2_CKS2	7.66E-19	4.31E-16	CELLULAR_PROTEIN_METABOLIC_PROCESS	3.27E-05	0.017542

Table 7

Six of the nine genes (represented by 20 probesets) identified as having the top 10 SNPs are on HG-U133 plus array for gene expression. Two genes (NUAK1 and GRIP1) show significantly reduced expression in BPD lung tissue at $p<0.05$ and CD44 shows a trend towards increased expression in BPD, but none of the identified SNPs were found to have a more than two-fold change in expression level.

Gene Symbol	Gene Description	Entrez Gene Id	HU-133plus2 ProbesetID	Adj P-value	Fold Change	Log Fold Change
NUAK1	"NUAK family, SNF1-like kinase, 1"	9891	204589_at	0.0061	0.601971	−0.73224
GRIP1	glutamate receptor interacting protein 1	23426	235957_at	0.0227	0.82473	−0.27801
CD44	CD44 molecule (Indian blood group)	960	204489_s_at	0.0638	1.225241	0.293066
CD44	CD44 molecule (Indian blood group)	960	210916_s_at	0.1006	1.341149	0.423469
CD44	CD44 molecule (Indian blood group)	960	209835_x_at	0.1152	1.251629	0.323807
CD44	CD44 molecule (Indian blood group)	960	204490_s_at	0.1160	1.259262	0.332579
CD44	CD44 molecule (Indian blood group)	960	212014_x_at	0.1312	1.238713	0.308843
CD44	CD44 molecule	960	1557905_s_at	0.1570	1.29494	0.37288
	(Indian blood group)		t			5
CD44	CD44 molecule (Indian blood group)	960	212063_at	0.2456	1.082907	0.11491
GALNTL6	UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyl transferase-like 6	442117	1555273_at	0.3811	1.041958	0.059298
KCNH7	"potassium voltage-gated channel, subfamily H (eagrelated), member 7"	90134	224099_at	0.4570	1.057103	0.080117
ADARB2	"adenosine deaminase, RNA-specific, B2"	105	237437_s_at	0.5247	1.023965	0.034166
CD44	CD44 molecule (Indian blood group)	960	229221_at	0.5401	1.114431	0.156307

Gene Symbol	Gene Description	Entrez Gene Id	HU-133plus2 ProbesetID	Adj P-value	Fold Change	Log Fold Change
CD44	CD44 molecule (Indian blood group)	960	1565868_at	0.6141	0.928276	−0.10737
CD44	CD44 molecule (Indian blood group)	960	217523_at	0.6336	1.055196	0.077511
ADARB2	“adenosine	105	220648_at	0.6381	1.04929	0.06941
	deaminase, RNA-specific, B2”					4
GRIP1	glutamate receptor interacting protein 1	23426	214018_at	0.6900	1.016749	0.023963
CD44	CD44 molecule (Indian blood group)	960	234418_x_at	0.7458	1.055799	0.078335
KCNH7	“potassium voltage-gated channel, subfamily H (eag-related), member 7”	90134	1555316_a_at	0.7512	1.012446	0.017845
CD44	CD44 molecule (Indian blood group)	960	234411_x_at	0.8856	1.01904	0.02721

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Table 8

Targets of miR-219. Table showing the 32 genes (represented by 42 probe sets) of the 143 unique genes (represented by 515 probe sets) in the miR-219 pathway that were significant by t-test in the gene expression data set.

Gene Symbol	Gene Description	Entrez Gene Id	HU-133plus2 ProbesetID	Adj P-value	Fold Change	Log Fold Change
AKAP13	A kinase (PRKA) anchor protein 13	11214	243450_at	0.007055	0.806146	−0.31089
BTBD7	BTB (POZ) domain containing 7	55727	220297_at	0.017654	0.761286	−0.39349
BTBD7	BTB (POZ) domain containing 7	55727	1556000_s_at	0.043006	0.902493	−0.14801
CAMK2G	calcium/calmodulin-dependent prote…	818	212669_at	0.032278	0.875267	−0.19221
CBFA2T3	core-binding factor, runt domain, …	863	208056_s_at	0.015365	0.711408	−0.49125
CC2D1A	coiled-coil and C2 domain containi…	54862	221888_at	0.028829	0.922347	−0.11662
CD164	CD164 molecule, sialomucin	8763	208654_s_at	0.026803	1.14101	0.190312
CELF2//CUGBP2		10659	1554569_a_at	0.025722	1.451321	0.537367
CHD7	chromodomain	55636	218829_s_at	0.03446	0.86567	−0.2081
	helicase DNA binding …			3	9	
CHD7	chromodomain helicase DNA binding …	55636	226123_at	0.037899	0.881078	−0.18266
CPEB3	cytoplasmic polyadenylation elemen…	22849	237508_at	0.026069	0.909195	−0.13734
CXXC5	CXXC finger 5	51523	236516_at	0.019538	1.065213	0.091142
ELK1	ELK1, member of ETS oncogene family	2002	203617_x_at	0.028357	1.163519	0.218495
ELMOD2	ELMO/CED-12 domain containing 2	255520	1553928_at	0.026066	1.263172	0.337051

Gene Symbol	Gene Description	Entrez Gene Id	HU-133plus2 ProbesetID	Adj P-value	Fold Change	Log Fold Change
FMNL2	formin-like 2	114793	230663_at	0.048782	1.318197	0.398566
GTPBP1	GTP binding protein 1	9567	219357_at	0.048337	1.129973	0.176288
HAS3	hyaluronan synthase 3	3038	228179_at	0.02455	0.832614	−0.26428
ING3	inhibitor of growth family, member 3	54556	205070_at	0.031469	0.811354	−0.3016
INPP5J//PIB5PA		27124	213651_at	0.046666	0.883715	−0.17835
KCNH8	potassium voltage-gated channel, s…	131096	1552742_at	0.025073	0.526414	−0.92573
MAPT	microtubule-associated protein tau	4137	203929_s_at	0.003632	0.697501	−0.51973
MAPT	microtubule-associated protein tau	4137	206401_s_at	0.020414	0.794843	−0.33126
MAPT	microtubule-associated protein tau	4137	225379_at	0.045822	0.76185	−0.39242
MTAP	methylthioadenosine phosphorylase	4507	204956_at	0.01869	1.162458	0.217178
NEK6	NIMA (never in mitosis gene a)-rel…	10783	237761_at	0.048777	1.417647	0.503498
PHACTR2	phosphatase and actin regulator 2	9749	204047_s_at	0.029784	0.759421	−0.39703
PHF19	PHD finger protein 19	26147	227212_s_at	0.020779	1.493804	0.578991
PHF19	PHD finger protein 19	26147	227211_at	0.025393	1.46813	0.55398
RECK	reversion-inducing-cysteine-rich p…	8434	1558116_x_at	0.026168	1.264182	0.338204
RECK	reversion-inducing-cysteine-rich p…	8434	216156_at	0.037335	1.162564	0.21731
RNF6	ring finger protein (C3H2C3 type) 6	6049	210931_at	0.040623	1.494437	0.579602

Gene Symbol	Gene Description	Entrez Gene Id	HU-133plus2 ProbesetID	Adj P-value	Fold Change	Log Fold Change
SDK1	sidekick homolog 1 (chicken)	221935	229407_at	0.005004	0.692523	−0.53007
SH3D19	-	152503	243636_s_at	0.017455	0.638183	−0.64796
SH3D19	-	152503	237157_at	0.021708	0.619348	−0.69118
SLC31A1	solute carrier family 31 (copper t…	1317	203971_at	0.049064	1.504041	0.588844
SNRK	SNF related kinase	54861	237942_at	0.022275	0.763608	−0.3891
SNRK	SNF related kinase	54861	209481_at	0.029946	0.868858	−0.20281
SOX6	SRY (sex determining region Y)-box 6	55553	243255_at	0.042059	0.890386	−0.1675
TACC1	transforming, acidic coiled-coil c…	6867	234010_at	0.002504	0.704959	−0.50439
THRB	thyroid hormone receptor, beta (er…	7068	228716_at	0.021753	0.806933	−0.30948
THRB	thyroid hormone receptor, beta (er…	7068	235927_at	0.029745	1.2889	0.36614

Table 9

Targets of IGF-1 and IGF-2. Table showing the 2 genes (represented by 5 probe sets) of the 34 unique genes (represented by 78 probe sets) out of the 36 listed in the IGF-1 and IGF-2 pathway that were significant by t-test in the gene expression data set.

Gene Symbol	Gene Description	Entrez Gene Id	ProbesetID (HU-133+)	Adj P-value	Fold Change	Log Fold Change
IGF1	insulin-like growth factor 1 (somatomed…	3479	209542_x_at	0.0089	2.035383	1.0253
IGF1	insulin-like growth factor 1 (somatomed…	3479	211577_s_at	0.009588	1.990326	0.993005
IGF1	insulin-like growth factor 1 (somatomed…	3479	209540_at	0.013246	1.940347	0.956315
IGF1	insulin-like growth factor 1 (somatomed…	3479	209541_at	0.014723	1.875297	0.907119
SFMBT2	Scm-like with four mbt domains 2	57713	232938_at	0.049014	1.075057	0.104413

Table 10

Cell Cycle: G2/M DNA Damage Checkpoint Regulation canonical pathway. Table showing the 8 genes (represented by 15 probe sets) of the 23 unique genes (represented by 61 probe sets) of the cell cycle pathway that were significant by t-test in the gene expression data set.

Molecule Name	ProbesetID (HU-133+)	Gene Symbol	Gene Title	Entrez Gene	Adj P-value	Fold Change
Cdc2	231534_at	CDC2	Cell division cycle 2, G1 to S and G2 to M	983	0.00291	2.282306
Cdc2	203214_x_at	CDC2	cell division cycle 2, G1 to S and G2 to M	983	0.003788	2.055242
Cdc2	210559_s_at	CDC2	cell division cycle 2, G1 to S and G2 to M	983	0.004146	2.102061
CKS2	204170_s_at	CKS2	CDC28 protein kinase regulatory subunit 2	1164	0.004267	1.522256
Chk1	205393_s_at	CHEK1	CHK1 checkpoint homolog (S. pombe)	1111	0.005303	1.723429
Chk1	205394_at	CHEK1	CHK1 checkpoint homolog (S. pombe)	1111	0.009462	1.73522
MDM2	237891_at	MDM2	Mdm2, transformed 3T3 cell double minute 2, p53 binding protein (mouse)	4193	0.010258	1.447509
Cdc2	203213_at	CDC2	cell division cycle 2, G1 to S and G2 to M	983	0.013499	1.861818

Molecule Name	ProbesetID (HU-133+)	Gene Symbol	Gene Title	Entrez Gene	Adj P-value	Fold Change
p19Arf	209644_x_at	CDKN2A	cyclin-dependent kinase inhibitor 2A (melanoma, p16, inhibits CDK4)	1029	0.015326	1.424757
Chk1	238075_at	CHEK1	CHK1 checkpoint homolog (S. pombe)	1111	0.025811	1.552763
Plk1	202240_at	PLK1	polo-like kinase 1 (Drosophila)	5347	0.026476	1.455546
14-3-3σ	33323_r_at	SFN	stratifin	2810	0.029624	2.786214
14-3-3σ	33322_i_at	SFN	stratifin	2810	0.033602	2.215685
ATR	209903_s_at	ATR	ataxia telangiectasia and Rad3 related	545	0.040315	1.171942
Chk2	210416_s_at	CHEK2	CHK2 checkpoint homolog (S. pombe)	11200	0.048167	1.2259