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Neonatal Salivary Analysis Reveals Global Developmental Gene Expression Changes in the Premature Infant

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BACKGROUND: There is an important need to develop noninvasive biomarkers to detect disease in premature neonates. Our objective was to determine if salivary genomic analysis provides novel information about neonatal expression of developmental genes.

METHODS: Saliva (50–200 μL) was prospectively collected from 5 premature infants at 5 time points: before, starting, and advancing enteral nutrition; at the introduction of oral feeds; and at advanced oral feeds. Salivary RNA was extracted, amplified, and hybridized onto whole-genomic microarrays.

RESULTS: Bioinformatics analyses identified 9286 gene transcripts with statistically significant gene expression changes across individuals over time. Of these genes, 3522 (37.9%) were downregulated, and 5764 (62.1%) were upregulated. Gene expression changes were highly associated with developmental pathways. Significantly downregulated expression was seen in embryonic development, connective tissue development and function, hematologic system development and function, and survival of the organism ($10^{-14} < P < 10^{-3}$). Conversely, genes associated with behavior, nervous system development, tissue development, organ development, and digestive system development were significantly upregulated ($10^{-11} < P < 10^{-2}$).

CONCLUSIONS: Comparative genomic salivary analyses provide robust, comprehensive, real-time information regarding nearly all organs and tissues in the developing preterm infant. This innovative and noninvasive technique represents a new approach for monitoring health, disease, and development in this vulnerable patient population. By comparing these data in healthy infants with data from infants who develop medical complications, we expect to identify new biomarkers that will ultimately improve newborn care.

An estimated 540 000 infants are born prematurely in the US each year, producing $12.6 billion in annual healthcare costs. This neonatal population has unique and often severe medical sequelae, a consequence of the disruption of typical organ and tissue development *(1)*. In particular, medical complications affecting the nervous system (i.e., developmental delay, cerebral palsy) *(2, 3)* and the gastrointestinal system (i.e., short gut syndrome following necrotizing enterocolitis) *(4, 5)* can cause lifelong morbidities. The ability to determine predisposing risk factors for these complications remains limited for neonates *(6)*. Therefore, there is an important need to develop noninvasive biomarkers to detect disease early to enable the initiation of treatment *(7)*.

Saliva is a body fluid that can be obtained noninvasively and repeatedly. Filtered and processed from blood in the salivary glands, saliva has been described as the "mirror of the body" and the "perfect medium to be explored for health and disease surveillance" *(8)*. It is a rich source of nucleic acids, and recent technological advances allow stabilization of salivary RNA for downstream genomic applications *(9)*. Although genomic microarray analysis of adult saliva has been performed *(10)*, no study to date has applied this technology to premature neonates.

We hypothesized that comparative microarray analyses of salivary RNA obtained serially from premature infants could provide novel information regarding their development and health, particularly with regard to their developing gastrointestinal and nervous systems. This discovery-driven approach could lead to a better understanding of the typical and abnormal developmental processes that occur in the premature infant and would potentially provide novel noninvasive biomarkers for assessment and diagnosis of this patient population, thus facilitating earlier treatment.

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Table 1. Clinical information regarding study individuals.

Infant no.	Sex	Gestational age at birth, weeks	Birth weight, g	Medications (during study)	Medical complications	Nutrition
1	Male	29 0/7	1389	Ampicillin	Respiratory distress syndrome (surfactant administration)	Formula
				Gentamicin	Hyperbilirubinemia	
				Caffeine	Apnea	
					Anemia	
2	Female, fraternal twin	28 3/7	942	Ampicillin	Respiratory distress syndrome (CPAP)[a]	Breast milk
				Gentamicin	Hyperbilirubinemia	
				Caffeine	Apnea	
				Vitamin E	Anemia	
				Iron		
3	Male, fraternal twin	28 3/7	1123	Ampicillin	Respiratory distress syndrome (CPAP)	Breast milk
				Gentamicin	Hyperbilirubinemia	
				Caffeine	Apnea	
				Vitamin E	Anemia	
				Iron	Retinopathy of prematurity (stage 2, zone 2)	
4	Female, identical twin from a set of quadruplets	32 0/7	1683	Ampicillin	Respiratory distress syndrome (CPAP)	Formula
				Gentamicin	Hyperbilirubinemia	
				Caffeine	Apnea	
				Zantac	Anemia	
5	Female, identical twin from a set of quadruplets	32 0/7	1379	Ampicillin	Respiratory distress syndrome (CPAP)	Formula
				Gentamicin	Hyperbilirubinemia	
				Caffeine	Apnea	
					Anemia	

[a] CPAP, continuous positive airway pressure.

Materials and Methods

STUDY INDIVIDUALS

This study was approved by the Tufts Medical Center Institutional Review Board. Five infants born between 28 and 32 weeks' gestation without known genetic diseases or congenital anomalies and admitted to the Tufts Medical Center Neonatal Intensive Care Unit were enrolled in the study with parental informed consent. Demographic and relevant clinical information regarding the infants are shown in Table 1. Of note, this data set includes 1 set of fraternal twins and 1 set of identical twins from a set of quadruplets. Every attempt was made to control for medical complications and drug exposure among the study individuals (Table 1).

SALIVA ACQUISITION

Approximately 50–200 μL of saliva was collected from each enrolled neonate by means of a 1-mL syringe attached to low-wall suction. Saliva samples were collected at the following time points: (*a*) before enteral nutrition (baseline), (*b*) at the start of enteral nutrition, (*c*) during the advancement of enteral nutrition, (*d*) at the start of oral feeding, and (*e*) at full or mostly full oral feeding. Saliva was collected before a scheduled feed to avoid milk contamination and was stabilized in 1 mL of RNAProtect Saliva™ Reagent (Qiagen) within 1 min of acquisition. Samples were briefly vortex-mixed, placed immediately on ice, and then stored at 4 °C for 48–96 h before RNA extraction.

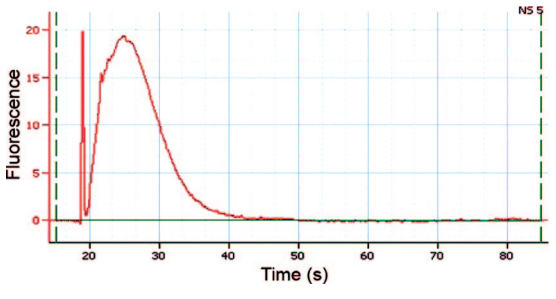

Fig. 1. Amplified neonatal salivary RNA as assessed by the Agilent Bioanalyzer.

Time in seconds is on the *x* axis, and fluorescence is on the *y* axis. The area under the curve represents the quantity of RNA extracted from a saliva sample (849.11 ng/μL). NS, neonatal saliva.

SALIVARY RNA EXTRACTION, AMPLIFICATION, AND HYBRIDIZATION

Total RNA was extracted with the RNeasy Protect Saliva Mini Kit (Qiagen) per the manufacturer's instructions. For each sample, DNase treatment was carried out on column during RNA extraction. Eluted RNA was stored at −80 °C before amplification with the WT-Ovation™ Pico RNA Amplification System (NuGEN Technologies). The quantity and quality of amplified RNA were assessed with an Agilent Bioanalyzer 2100 (Agilent Technologies) (Fig. 1) before fragmentation and biotinylation with the FL-Ovation™ cDNA Biotin Module V2 (NuGEN Technologies). For each sample from each infant at each time point, a standard 5 μg of amplified and labeled RNA was hybridized on an Affymetrix GeneChip® Human Genome U133 Plus 2.0 whole-genomic microarray (n = 25). After hybridization, each array was washed and stained in the GeneChip Fluidics Station 400 (Affymetrix). Arrays were then scanned with the GeneArray Scanner (Affymetrix), and data were initially analyzed with the GeneChip Microarray Suite 5.0 (Affymetrix).

BIOINFORMATICS AND COMPUTATIONAL ANALYSES

All calculations were done in R version 2.8.1 *(11)*, Bioconductor version 2.3 *(12)*, and lme4 *(13)*. Probe sets were summarized, and the RMA algorithm in the Bioconductor affy package was used with default settings to normalize arrays *(14)*. For each probe set, we determined the influence of increasing postnatal age by fitting 2 statistical models. The first model fit a random individual infant effect. The second model fit a fixed linear age effect and a random individual infant effect. ANOVA was used with the likelihood ratio test to compare the 2 models. *P* values were adjusted with the Benjamini–Hochberg procedure *(15)*. Probe sets were identified as significantly differentially expressed for age when the false-discovery rate (FDR)[5] *P* value was <0.05. *t*-scores were calculated to differentiate between those genes with significantly greater or lesser gene expression over time. To evaluate whether any single infant skewed the data, we also performed ANOVAs that compared individual and age interactions and calculated FDR *P* values.

To determine the effect of feeding status due to gestational age on gene expression changes, we again compared 2 models. The first model fit age with a random individual infant effect; the second model accounted for whether the infant was orally fed (yes/no) and whether the infant was being fed via a nasogastric tube (yes/no). Again, ANOVA was performed with the likelihood ratio test to compare the 2 models; *P* values were adjusted with the Benjamini–Hochberg procedure *(15)*.

INDIVIDUALS FROM MULTIPLE GESTATIONS

All 5 infants in this data set were treated as individuals. To determine if the genetically related infants (1 set of fraternal twins; 1 set of identical twins from a set of quadruplets) could have led to biases caused by underestimating the variance between individuals, we performed an additional 3 regression analyses of expression for each probe set as a function of postnatal age: (*a*) for all 5 infants, (*b*) for the 2 sets of related infants, and (*c*) for the identical twins only. The random individual-infant effect was intentionally omitted so that any differences in infant groups would be reflected in the analysis. Mean square errors (MSEs) were then calculated. To adjust for variation between probe sets, we took the natural logarithm of the MSE from each of the additional 3 analyses, and for each gene subtracted the mean of log MSE over each of the 3 regressions.

DATA ANALYSES

Significantly up- or downregulated gene transcripts were analyzed with the Ingenuity® software package (Ingenuity Systems) to assess gene–gene relationships, associated network functions, and physiological developmental systems. The top 5 significantly upregulated and downregulated categories of physiological-system development and function were analyzed further. For each of these categories, individual functions within a category that had ≥20 genes were assessed (Tables 2 and 3), and we reviewed all individual genes with EntrezGene to better understand the relevant physiological processes (see Tables 1 and 2 in the Data Supplement

[5] Nonstandard abbreviations: FDR, false-discovery rate; MSE, mean square error.

Table 2. Upregulated gene expression categories and functions.

Category	Function (functional annotation)	No. of genes	*P*
Behavior	Behavior	147	1.2×10^{-11}
	Learning (learning)	76	7.9×10^{-7} to 4.4×10^{-3}
	Cognition (cognition)	66	1.5×10^{-6} to 1.5×10^{-2}
	Psychological process	68	9.2×10^{-6}
	Feeding (feeding, feeding of organism)	80	1.2×10^{-5} to 8.7×10^{-3}
	Locomotion	63	1.9×10^{-5}
	Memory	32	2.0×10^{-4}
Nervous system development and function	Neurotransmission (cells, normal cells, neurons, synapse)	141	1.8×10^{-9} to 1.4×10^{-2}
	Synaptic transmission (cells, normal cells)	108	2.2×10^{-9} to 1.7×10^{-2}
	Differentiation (neurons)	70	4.9×10^{-7}
	Synaptogenesis	21	5.0×10^{-7}
	Neurological process (cells, eukaryotic cells, normal cells, tissue, neurons, synapse, nervous tissue, nerves, brain, axons, cell lines, neuroglia)	395	5.1×10^{-6} to 7.4×10^{-3}
	Development (nervous system, neurons, central nervous system, nerves, dentate gyrus, brain, Schwann cells, olfactory receptor neurons, nervous tissue, forebrain, olfactory bulb, astrocytes, trigeminal ganglion neurons)	319	1.1×10^{-4} to 1.7×10^{-2}
	Memory	32	2.0×10^{-4}
	Branching (dendrites, neurites)	34	3.2×10^{-4} to 3.3×10^{-4}
	Neurogenesis	101	9.6×10^{-4}
	Growth (neurites)	76	9.7×10^{-4}
	Quantity (neurons, trigeminal ganglion neurons)	47	1.3×10^{-3} to 1.1×10^{-2}
	Migration (Schwann cells, neurons)	45	1.4×10^{-3} to 1.1×10^{-2}
	Outgrowth (neurons)	67	1.8×10^{-3}
	Myelination (myelination, nerves, normal cells)	33	2.2×10^{-3} to 1.6×10^{-2}
	Survival (trigeminal ganglion neurons, spinal cord cells, neurons, dorsal root ganglion cells, ganglion cells)	61	2.7×10^{-3} to 1.2×10^{-2}
	Proliferation (neurons, neuronal progenitor cells)	29	4.9×10^{-3} to 5.9×10^{-3}
Tissue development	Development (tissue, cartilage tissue, nerves, placode, muscle, metanephros, epithelial tissue, fetal membranes, connective tissue, nervous tissue, allantois, bone marrow, olfactory bulb)	394	4.6×10^{-7} to 1.7×10^{-2}
	Developmental process (tissue, nerves, cartilage tissue, muscle, epithelial tissue, placode, nervous tissue, atrioventricular canal cushion, flexor muscle)	363	4.5×10^{-5} to 1.7×10^{-2}
	Formation (cortical bone, mammary placode 3, connective tissue, bone tissue, trabecular bone)	125	4.9×10^{-3} to 9.6×10^{-3}
Organ development	Development (organ, testis, gonad, dentate gyrus, brain, ovarian follicle, heart, prostate gland, metanephros, kidney, ovary, forebrain, lung)	454	6.0×10^{-6} to 1.6×10^{-2}
	Organogenesis	139	7.5×10^{-6}
	Morphogenesis	41	2.4×10^{-3}
Digestive system development and function	Feeding (feeding, feeding of organism)	80	1.2×10^{-5} to 8.7×10^{-3}

Table 3. Downregulated gene expression categories and functions.

Category	Function (functional annotation)	No. of genes	*P*
Embryonic development	Disease (embryonic cell lines)	86	7.2×10^{-14}
	Infectious disorder (embryonic cell lines)	84	3.9×10^{-13}
	Apoptosis (embryonic cell lines)	30	1.2×10^{-3}
	Cell death (embryonic cells lines)	37	9.3×10^{-3}
Connective tissue development and function	Proliferation (fibroblast cell lines, fibroblasts)	97	4.8×10^{-8} to 2.6×10^{-6}
	Growth (fibroblast cell lines, fibroblasts)	75	3.8×10^{-6} to 1.3×10^{-4}
	G_1 phase (arrest in G_1 phase of fibroblast cell lines, G_1 phase of fibroblast cell lines)	26	7.5×10^{-4} to 1.2×10^{-3}
Hematologic system development and function	Differentiation (blood cells, leukocytes, T lymphocytes, lymphocytes)	272	1.0×10^{-7} to 7.3×10^{-4}
	Hematopoiesis	116	1.4×10^{-7}
	Hematologic process	129	2.4×10^{-6}
	Quantity (lymphocytes, mononuclear leukocytes, blood cells, leukocytes, B and T lymphocytes)	438	5.4×10^{-6} to 2.2×10^{-3}
	Development (blood cells, leukocytes, lymphocytes, T lymphocytes)	196	2.0×10^{-4} to 9.2×10^{-4}
	Proliferation (leukocytes, lymphocytes, T lymphocytes)	356	6.4×10^{-4} to 1.7×10^{-3}
Hematopoiesis	Differentiation (leukocytes, T lymphocytes, lymphocytes)	178	1.1×10^{-7} to 7.3×10^{-4}
	Hematopoiesis	116	1.3×10^{-7}
	Development (leukocytes, lymphocytes, T lymphocytes)	135	9.2×10^{-4}
Organismal survival	Death (mammalia, animal)	503	1.4×10^{-6} to 1.2×10^{-3}

that accompanies the online version of this article at http://www.clinchem.org/content/vol56/issue3).

Results

For each infant, we analyzed 5 gene expression arrays from each of the 5 previously described time points (25 arrays total). Twenty-four days of postnatal age separated the youngest and oldest infants at the time of the first saliva collection. At the time of acquisition of the final saliva sample, when the infants were successfully orally feeding, only 2 postnatal days separated these infants (Fig. 2). Bioinformatics analyses revealed that gene expression was more significantly affected by postnatal age than by feeding status. There were no statistically significant changes in gene expression (FDR *P* value <0.05) according to feeding status. Conversely, of the 54 675 probes on the array, 9286 gene transcripts (17%) showed significant changes in gene expression over time (i.e., FDR *P* value <0.05). Calcu-

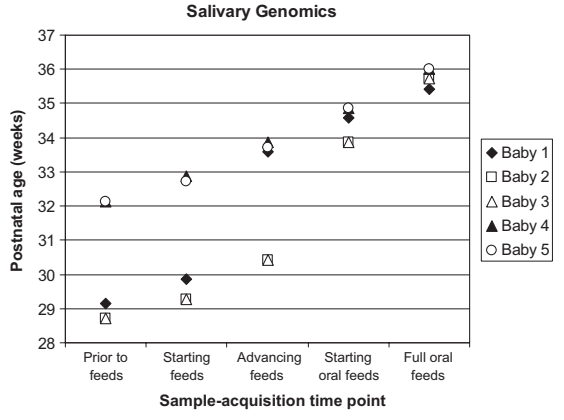

Fig. 2. Scatter plot depicting postnatal age of infants at time of sample acquisition.

Feeding milestone is on the *x* axis; postnatal age in weeks is on the *y* axis.

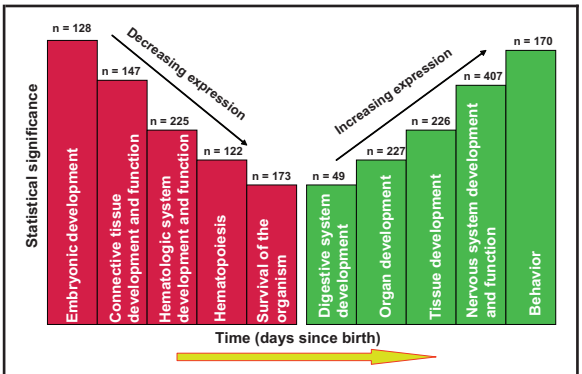

Fig. 3. Schematic depiction of the 5 most significantly upregulated and downregulated physiological developmental systems ($10^{-14} < P < 10^{-2}$) within this data set.

Days since birth (*x* axis) represents real-time changes in gene expression occurring with increasing postnatal age. Although all categories were highly significant, the height of a column represents the degree of significance within a group (i.e., the higher the column, the more statistically significant the system). Values at the top of each column represent the number of genes within the category. Certain genes were significant in >1 category. Note that the figure is not drawn to scale.

lated *t*-scores for the gene transcripts revealed that 3522 genes (37.9%) were significantly downregulated and 5764 genes (62.1%) were significantly upregulated. FDR *P* values for all interactions comparing infant and age were >0.20, suggesting that our results were not driven by any single infant.

SIGNIFICANTLY UPREGULATED GENE TRANSCRIPTS OVER TIME

The top 5 upregulated physiological systems that were significantly affected by postnatal age were behavior ($10^{-11} < P < 10^{-2}$) and development of the nervous system ($10^{-9} < P < 10^{-7}$), tissues ($10^{-7} < P < 10^{-2}$), organs ($10^{-6} < P < 10^{-2}$), and the digestive system ($10^{-5} < P < 10^{-2}$) (Fig. 3). Functional descriptions within each category, the number of genes within a specific function, and the respective *P* values are shown in Table 2. Table 1 in the online Data Supplement provides a complete list of upregulated genes and their functional descriptions for each category. Individual genes may be listed in >1 category.

SIGNIFICANTLY DOWNREGULATED GENE TRANSCRIPTS OVER TIME

The top 5 downregulated physiological systems were embryonic development ($10^{-14} < P < 10^{-3}$), connective tissue development and function ($10^{-8} < P < 10^{-3}$), hematologic system development and function ($10^{-7} < P < 10^{-3}$), hematopoiesis ($10^{-7} < P < 10^{-4}$), and survival of the organism ($10^{-7} < P < 10^{-3}$) (Fig. 3). Functional descriptions within each category, the number of genes within a function, and the respective *P* values are shown in Table 3. The complete list of downregulated genes and their functional descriptions for each category are provided in Table 2 in the online Data Supplement. Individual genes may be listed in >1 category.

ANALYSIS OF INFANTS FROM MULTIPLE GESTATIONS

The natural logarithm of MSE varied from −6.3 to 2.3 across probe sets, with quartiles at −1.8 and −0.5 (see Fig. 1 in the online Data Supplement). The mean ln MSE value for all 5 infants after subtracting the mean across the cases was 0.015 (A); the mean for the 2 sets of twins was −0.011 (B); and the mean for the identical twins was −0.003 (C). The differences were small and were not in the hypothesized order (i.e., C < B < A). Thus, it is unlikely that our results are biased by including infants that are genetically related to each other.

Discussion

Our work is the first to demonstrate the potential diagnostic and clinical utility of transcriptional analysis of saliva from premature neonates. It lays the foundation for prospective clinical studies that develop hypotheses regarding abnormal gene expression in neonatal pathophysiology. We have demonstrated that comparative genomic analyses of salivary mRNA transcripts obtained from premature neonates during the first weeks of postnatal life provide novel dynamic information regarding nearly all developing organs and tissues.

Despite the limited quantities of saliva obtained for analysis in this study, the assay we have used provides a comprehensive genomic analysis of both neonatal salivary cells and supernatant. This approach is novel compared with that described in previously published reports on the cell-free adult salivary transcriptome obtained from supernatant *(10)*. Although the cellular source of the salivary samples is currently unknown, it likely contributes to the RNA pool in these genomic analyses. Future studies will be required to elucidate the cell source and the respective contributions of RNA from both the supernatant and cellular layers.

We identified 9286 genes with significant changes in gene expression occurring over time. Although the achievement of oral feeding and advancing postnatal age are inherently linked, our analysis revealed that advancing postnatal age, rather than attainment of a feeding milestone, produced significant changes in gene ex-

pression. The convergence of the postnatal ages of the infants with the acquisition of successful oral-feeding skills likely contributed to this finding (Fig. 2). Although there were 2 sets of genetically related infants within this data set, our additional statistical analysis suggested that no bias was introduced into our analysis by their inclusion.

Although a comprehensive physiological and functional analysis of the complete gene list is beyond the scope of this report, the data demonstrate important changes in pathways associated with neurodevelopment and digestion. The upregulation of transcripts involved in digestive system development revealed genes encoding enzymes necessary for the proper processing of enteral nutrition, neuronal genes regulating satiety and food consumption, and structural genes associated with proper dentition formation. Examples of these upregulated genes include *LALBA*[6] (lactalbumin, alpha-), which encodes a principal milk protein that enables lactose production; *CCKAR* (cholecystokinin A receptor), which encodes a major physiological mediator of pancreatic enzyme secretion and smooth muscle contraction of the gallbladder and stomach; *HCRTR2* [hypocretin (orexin) receptor 2], which encodes a protein involved in stimulation of food intake; *MCHR1* (melanin-concentrating hormone receptor 1), which encodes a protein involved in neuronal regulation of food consumption; and *DMP1* (dentin matrix acidic phosphoprotein 1), which encodes an extracellular matrix protein crucial for proper mineralization of bone and dentin (see Table 2 in the online Data Supplement).

Within the category of nervous system development and function, nearly every functional aspect of the developing brain and of the peripheral and central nervous systems was represented in the salivary gene list, including neuronal development, myelination, synaptic formation, and neurogenesis. These findings also coincide with the major "burst" of active brain growth that occurs in the last half of human gestation *(16)*. Interestingly, genes associated with cranial nerve V (the trigeminal nerve) function were specifically highlighted by this analysis. Although primarily involved in facial sensation, the trigeminal nerve has associated motor functions that include biting, chewing, and swallowing. One of the most important and difficult neurologic tasks facing the premature neonate is the successful coordination of sucking and swallowing to facilitate oral feeding. In most neonatal intensive care units, the determination of an infant's readiness to feed is largely subjective. We speculate that salivary monitoring of gene expression data related to trigeminal nerve development may provide clear and objective evidence of a premature infant's ability to successfully feed orally.

We acknowledge that our work represents an early proof-of-principle study. These findings will need to be validated by an independent cohort and with independent technologies, such as reverse-transcription PCR. As with any study of human individuals, each infant in this cohort had a unique clinical course; however, every attempt was made to control for similar drug exposure and outcome. It is unlikely that the small clinical between-infant variation observed in this population contributed to the findings of this study. Rather, a major strength of our salivary genomic analysis is the strong clinical correlation between the identified significantly upregulated and downregulated physiological systems and the expected neonatal physiology. The most significantly downregulated physiological system was embryonic development. Over time, genes involved in neurogenesis during embryonic development, such as *AES* (amino-terminal enhancer of split), and genes involved in the arrangement of 3-dimensional tissue structure and angiogenesis, such as *CEACAM1* [carcinoembryonic antigen-related cell adhesion molecule 1 (biliary glycoprotein)], were actively suppressed as the infants matured. Simultaneously, genes involved in lung development, including *SFTPB* (surfactant protein B), and tissue development, such as *FREM2* (FRAS1 related extracellular matrix protein 2), which is required for maintaining skin epithelium, were upregulated over time and were detectable in neonatal saliva. These findings highlight the important normophysiological processes that occurred in multiple developing organs within this population. Thus, transcripts identified in the saliva of these relatively healthy premature neonates can serve as a comparison transcriptome for gestationally age-matched infants who have severe neonatal sequelae involving the lungs (i.e., bronchopulmonary dysplasia), the gastrointestinal system (i.e., necrotizing enterocolitis), the eyes (i.e., retinopathy of prematurity), and the immune system (i.e., sepsis).

Although we acknowledge that serial microarray analyses of neonatal saliva may be cost-prohibitive for many research centers, the technique described here for saliva acquisition, stabilization, and RNA extraction is feasible, reproducible, and cost-effective (approximately $11/sample). Alternative downstream applications, including reverse-transcription PCR for

[6] Human genes: *LALBA*, lactalbumin, alpha-; *CCKAR*, cholecystokinin A receptor; *HCRTR2*, hypocretin (orexin) receptor 2; *MCHR1*, melanin-concentrating hormone receptor 1; *DMP1*, dentin matrix acidic phosphoprotein 1; *AES*, amino terminal enhancer of split; *CEACAM1*, carcinoembryonic antigen-related cell adhesion molecule 1 (biliary glycoprotein); *SFTPB*, surfactant protein B; *FREM2*, FRAS1 related extracellular matrix protein 2.

specific genes of interest identified in this report, could be used for large-scale international studies.

In summary, salivary genomic analyses provide a noninvasive means of assessing developmental progression in the premature neonate. This technique provides a large amount of data from a single sample. In particular, we demonstrated the dynamic nature of genes expressed as part of the neurodevelopmental and digestive systems in the first few weeks of postnatal life. By comparing these data from healthy preterm infants to data from infants who develop medical complications, we expect to develop new noninvasive biomarkers that will ultimately improve newborn care.

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