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High correlations in gene expression between paired umbilical cord blood and neonatal blood of healthy newborns on Guthrie cards

Jaime Slaughter¹, Changshuai Wei¹, Steven J. Korzeniewski^{2,3}, Qing Lu¹, John S. Beck⁴, Sok Kean Khoo⁴, Ariel Brovont¹, Joel Maurer⁵, Denny Martin⁵, Madeleine Lenski¹, and Nigel Paneth^{1,6}

¹Department of Epidemiology and Biostatistics, College of Human Medicine, Michigan State University, East Lansing, MI

²Department of Obstetrics & Gynecology, Wayne State University School of Medicine, Detroit, MI

³Perinatology Research Branch, National Institute of Child Health and Human Development, National Institutes of Health, Department of Health and Human Services, Bethesda, Maryland, Detroit, Michigan

⁴Laboratory of Microarray Technology, Van Andel Institute, Grand Rapids, MI

⁵Department of Obstetrics, Gynecology, and Reproductive Biology, College of Human Medicine, Michigan State University, East Lansing, MI

⁶Department of Pediatrics and Human Development, College of Human Medicine, Michigan State University, East Lansing, MI

Abstract

Objective—To examine the correlation in genes expressed in paired umbilical cord blood (UCB) and newborn blood (NB).

Methods—Total mRNA and mRNA of three gene sets (inflammatory, hypoxia, and thyroidal response) was assessed using microarray in UCB and NB spotted on Guthrie cards from 7 mother/infant pairs.

Results—The average gene expression correlation between paired UCB and NB samples was 0.941 when all expressed genes were considered, and 0.949 for three selected gene sets.

Conclusion—The high correlation of UCB and NB gene expression suggest that either source may be useful for examining gene expression in the perinatal period.

Corresponding Author: Nigel Paneth, MD MPH, 909 Fee Road Room 218, Michigan State University, East Lansing, MI 48824, Fax: 517-432-7730, Phone: 517-290-5062, paneth@msu.edu.

DECLARATION OF INTERESTS

The authors have no conflicts of interests.

Keywords

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INTRODUCTION

Umbilical cord blood has been commonly used in perinatal studies for the purpose of determining a variety of exposures at birth, including heavy metals [1], infections and inflammation [2], and asphyxiating conditions [3]. This blood can be difficult to obtain and is rarely available for large population studies. Neonatal blood, on the other hand, is routinely collected on Guthrie cards during the first several days of life for use in newborn screening programs [4]. Most programs consume only a small portion of the available blood; the remainder is often archived under various conditions and time periods. In the US, 14 newborn screening programs retain newborn blood for 21 years or more, constituting a unique resource for the retrospective study of diseases of perinatal origin [5].

Little is known about the utility of this resource for large-scale investigations of gene expression. While some studies have used archived newborn blood spots to investigate human and viral DNA or proteins, our team focused on gene quantitative RNA expression [5,6]. Newborn blood is collected 24–48 hours after birth and it is of interest to know whether the gene expression findings in neonatal blood parallel or differ from those in umbilical cord blood from the same infant. We undertook this study to evaluate if gene expression, which can reflect many environmental exposures, is similar in umbilical cord blood and newborn blood when both are collected in the same way, spotted onto Guthrie filter papers and preserved in the same fashion.

METHODS

Participants

This study was approved by the Michigan State University and Sparrow Hospital Institutional Review Boards. A convenience sample of 14 pregnant women without recognized pregnancy complications was recruited from a Lansing, MI OB/GYN clinic. Study staff obtained written informed consent for the collection of umbilical cord blood and newborn blood by study physicians. Women were excluded if they delivered preterm or by cesarean. A total of 7 mother/infant pairs were available for analysis, after excluding 2 women who delivered by cesarean, one woman who delivered at a non-participating hospital, and 4 women in whom either the umbilical cord or newborn blood was not obtained.

Data Collection

After delivery of the infant and cutting of the umbilical cord, but prior to delivery of the placenta, study physicians filled 3–5 circles of 1.25 cm diameter on standard Guthrie cards (Whatman, Kent, UK) with blood from the umbilical cord. Michigan law mandates that hospitals obtain 5 circles of 1.25 cm diameter of neonatal blood on Guthrie cards via heel

stick blood draw between 24–72 hours after the newborn's birth. During this time, 3–5 additional neonatal blood spots were collected specifically for this study on a separate Guthrie card. All bloodspots were allowed to dry at room temperature. After the neonatal bloodspot was collected, the study physician recorded the infant's date of birth, gestational age, gender, race/ethnicity, maternal age, and delivery mode from the newborn's medical record. Prior to analysis, all blood spots were stored at ambient temperatures. The period from collection of specimen to mRNA extraction in the laboratory was 176.8 days on average.

RNA Isolation and Microarray Procedures

RNA isolation, cDNA synthesis, and microarray procedures on the bloodspot specimens were conducted in the Laboratory of Microarray Technology at the Van Andel Institute according to published protocols [6]. In brief, three 3-mm punches from each specimen were homogenized and RNA isolated using the illustra RNAspin Mini kit (GE Healthcare, Buckinghamshire, UK). All RNA is subjected to DNase treatment to remove DNA contamination. RNA quality and quantity were evaluated by the Agilent BioAnalyzer via a RNA Pico Lab Chip (Agilent Technologies, Santa Clara, CA). The WT-Ovation Pico RNA Amplification System (NuGEN Technologies, San Carlos, CA) was used to generate cDNA before labeling and hybridization onto the Agilent whole human genome gene expression 8×60K microarray. The microarrays were scanned with an Agilent G3 high-resolution scanner and microarray data were extracted using Feature Extraction software v.10.7.3.1. (Agilent).

Statistical Methods

Selection of gene sets for this study was based on our previous work, in which we investigated the expression of 7 gene sets (3 empirical; 4 canonical) representing four physiological pathways (inflammatory, hypoxic, thyroidal, and coagulative) hypothesized to contribute to the development of cerebral palsy. We found significant differences between children with CP and controls in gene regulation on the empirical inflammatory [7], hypoxic [8], and thyroidal [9] gene sets [10]. Inasmuch as these gene sets appear to be involved in important pathways relevant to long term outcomes, we assess expression of these gene sets, as well as total gene expression, in our comparison of umbilical cord and newborn blood.

To examine similarities between newborn blood and umbilical cord blood on overall expressed-gene and specific gene set expression, we calculated both pair-wise interclass correlation coefficients and the intraclass correlation coefficients. Means and standard deviations were calculated and results were displayed graphically. Additionally, a modified GAGE method was used to test the differentially expressed genes in the 7 pairs.

RESULTS

The total RNA yield was 9.87 ± 5.80 ng/ul and 8.38 ± 5.01 ng/ul for umbilical cord blood and newborn blood, respectively. The RNA integrity number was 2.07 ± 0.19 for umbilical cord blood and 2.03 ± 0.14 for newborn blood.

Microarray analysis of umbilical cord blood spots detected 9,749–19,101 expressed-genes, while in newborn bloodspot 10,859–17,539 mRNA species were detected. The mean number of genes expressed in the umbilical cord blood spot was 14,983; while the average number of genes expressed in the newborn blood spot was 13,339. Table 1 displays the correlation coefficients for each umbilical cord/newborn blood spot pair. The average Pearson correlation coefficient between paired umbilical cord and newborn bloodspots was 0.941(SD=0.018) for overall expressed-genes. The average correlation coefficient of genes expressed on the 3 specific genes sets investigated in this study was 0.949 (SD=0.242). Of 21,266 detected genes, 214 (1%) were differentially expressed (p-value<2.35e-6).

For comparison, we evaluated the correlation between all 21 possible pairs of cord blood specimens and newborn blood specimens. The obtained correlation coefficients also reached a high level, although not as high as those for paired sample. The average correlation coefficients for overall expressed genes were 0.808 (SD=0.102) and 0.836 (SD=0.110) among umbilical cord samples and among newborn blood samples, respectively (Supplemental Digital Content Figure S1). The average correlation coefficients on the 3 specific genes sets were 0.870 (SD=0.059) and 0.883 (SD=0.054) among umbilical cord samples and among newborn blood samples, respectively (Supplemental Digital Content Figure S2).

DISCUSSION

While several studies have established the reliability and reproducibility of gene expression in newborn blood spots, we believe this is the first study to assess the correlation between newborn blood and umbilical cord blood spotted on filter paper. We demonstrate that gene expression in newborn blood reflects umbilical cord blood very closely.

The principal findings of this study are that: 1) abundant gene expression was observed in examining both umbilical cord blood and newborn blood on Guthrie cards; 2) inflammatory, hypoxic, and thyroidal genes crucial for newborn adaptation were consistently expressed in both types of specimens; 3) overall gene expression and specific empirical gene set expression in umbilical cord blood and newborn blood are highly correlated for matched pairs (correlation coefficient > 0.94); and 4) gene expression does not change dramatically in healthy newborns between delivery and the first day or two of life since the between-pair correlations within the same individual are high.

It is therefore possible that newborn blood stored on Guthrie filter paper may be used as an alternative to umbilical cord blood in gene expression studies to investigate the physiological and environmental exposures at birth. We emphasize, however, that this study was done in healthy newborns. It is possible that newborns who become ill after birth may mobilize genes that were not expressed at the time of birth. Our findings support the value of neonatal blood spots for genome-wide gene expression profiling and pathway studies in perinatal research.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Table 1

Correlation coefficients for matched umbilical cord/newborn blood pairs for overall and specific gene set expression.

Cord/Newborn Blood Pair	Overall Gene Expression Correlation Coefficient	Specific Gene Sets Correlation Coefficient
1	0.959	0.974
2	0.936	0.958
3	0.942	0.947
4	0.913	0.901
5	0.963	0.970
6	0.946	0.959
7	0.927	0.949