Cytochrome P450 (*CYP2D6*) Genotype is Associated with Elevated Systolic Blood Pressure in Preterm Infants after Discharge from the Neonatal Intensive Care Unit

John M. Dagle, MD, PhD, Tyler J. Fisher, BA, Susan E. Haynes, MD, Susan K. Berends, ARNP, Patrick D. Brophy, MD, Frank H. Morriss, Jr., MD, MPH, and Jeffrey C. Murray, MD

Objective To determine genetic and clinical risk factors associated with elevated systolic blood pressure (ESBP) in preterm infants after discharge from the neonatal intensive care unit (NICU).

Study design A convenience cohort of infants born at <32 weeks gestational age was followed after NICU discharge. We retrospectively identified a subgroup of subjects with ESBP (systolic blood pressure [SBP] >90th percentile for term infants). Genetic testing identified alleles associated with ESBP. Multivariate logistic regression analysis was performed for the outcome ESBP, with clinical characteristics and genotype as independent variables.

Results Predictors of ESBP were cytochrome P450, family 2, subfamily D, polypeptide 6 (*CYP2D6*) (rs28360521) CC genotype (OR, 2.92; 95% CI, 1.48-5.79), adjusted for outpatient oxygen therapy (OR, 4.53; 95% CI, 2.23-8.81) and history of urinary tract infection (OR, 4.68; 95% CI, 1.47-14.86). Maximum SBP was modeled by multivariate linear regression analysis: maximum SBP = 84.8 mm Hg + 6.8 mm Hg if cytochrome P450, family 2, subfamily D, polypeptide 6 (*CYP2D6)* CC genotype + 6.8 mm Hg if discharged on supplemental oxygen + 4.4 mm Hg if received inpatient glucocorticoids (P = .0002).

Conclusions ESBP is common in preterm infants with residual lung disease after discharge from the NICU. This study defines clinical factors associated with ESBP, identifies a candidate gene for further testing, and supports the recommendation to monitor blood pressure before age 3 years, as is suggested for term infants. *(J Pediatr 2011;159:104-9)*.

Both term infants who are small for gestational age and preterm infants who have low birth weight by virtue of their prematurity are at an increased risk for hypertension as adults.[1-4] Proposed mechanisms to explain this phenomenon include increased sympathoadrenal activity,[5] increased arterial stiffness,[6] and altered kidney development.[7] Prematurity and low birth weight are among the few conditions in which blood pressure (BP) monitoring is recommended in children under age 3 years.[8] Because of the difficulty in accurately measuring BP in very young children and in defining a normal population of preterm infants, little data exist with respect to normal BP values in preterm-born infants during the first year of life. A multicenter study involving BP monitoring in 135 preterm infants during the first year of life found that mean BP rose as postnatal age increased, with a plateau of approximately 92 mm Hg at a postconceptional age of 40-44 weeks.[9] In centers that used both Doppler ultrasound and an oscillometric technique (currently the most commonly used method) for comparison, oscillometry tended to overestimate systolic BP (SBP) when measurements were in a low range. There is currently no universally recognized definition of hypertension in preterm infants at various postconceptional ages.

Several studies have reported an increased risk of elevated BP in preterm infants with bronchopulmonary dysplasia (BPD). Abman et al[10] reported elevated BP in 13 of 30 infants with BPD, and found that the diagnosis was often made after discharge from the neonatal intensive care unit (NICU). Anderson et al[11] reported elevated BP in 11 of 87 infants with BPD receiving home oxygen therapy. Finally, Alagappan and Mallory[12] reported elevated BP in 5 of 41 infants with BPD, with onset at a mean of 105 days after birth. All of these studies demonstrated an association between BPD and later-onset BP elevation, but the mechanisms linking these conditions remain unknown.

In the present study, we sought to identify genetic and clinical risk factors for elevated SBP (ESBP) in a cohort of high-risk preterm-born infants monitored in the neonatology follow-up clinic at the University of Iowa Children's Hospital.

BP	Blood pressure
BPD	Bronchopulmonary dysplasia
CYP2D6	Cytochrome P450, family 2, subfamily D, polypeptide 6
ESBP	Elevated systolic blood pressure
NICU	Neonatal intensive care unit
SBP	Systolic blood pressure
SNP	Single nucleotide polymorphism
TFAP2B	Transcription factor AP-2 beta
UTI	Urinary tract infection

From the Department of Pediatrics (J.D., S.H., S.B., P.B., F.M., J.M.) and Carver College of Medicine (T.F.), University of Iowa, Iowa City, IA

Supported by grants from the National Institutes of Health (R01 HD052953, R01 HD57192, P30 ES05605, and K08 DK064333) and the March of Dimes (FY2006-575 and FY2008-260). The authors declare no conflicts of interest.

 10.1016/j.jpeds.2011.01.002

Methods

The study cohort was assembled from an existing cohort of infants admitted to the NICU at University of Iowa Children's Hospital since 2000. Parents provided informed consent on behalf of themselves and their children for the collection of blood and/or buccal swabs for biobanking. This Institutional Review Board–approved program was established to provide biological material for subsequent use in investigations of genetic contributions to diseases in infants.[12,13] We studied a non-random convenience cohort of 305 preterm infants with the following characteristics: birth at <32 weeks gestation, evaluation at the neonatology follow-up clinic at University of Iowa Children's Hospital, and available DNA samples from our neonatal DNA repository. Approximately 90% of our study cohort was Caucasian, by parental report. Clinical data were abstracted retrospectively from the infants' medical records.

Phenotyping

Because there are no universally accepted criteria for defining normal BP values in preterm-born infants during the first year of life, we defined ESBP as a SBP >90th percentile using values for term infants.[13] We calculated the corrected age at which each BP measurement was obtained in a preterm subject for comparison with term infant BP values at various chronological ages using the following equation: corrected age (weeks) = chronological age (weeks) - (40 - gestational age at birth [weeks]). An advanced registered nurse practitioner measured SBP, diastolic BP, in the 305 study infants at all follow up clinic visits using a calibrated oscillometric device (Dinamap GE Pro 300 V2; GE Medical Systems Information Technologies, Milwaukee, Wisconsin) following standard clinic protocols. The BP measurements abstracted for this study were obtained with the infant calm, either held in a parent's lap or in a car seat, in the right upper extremity using an appropriate-sized cuff. Nearly 82% of the study infants were evaluated on more than one occasion, as indicated by their clinical condition during infancy. Maximum SBP was defined as the highest value obtained in a calm infant at any follow-up clinic visit. BP measurements were generally obtained between 9 a.m. and 4 p.m., and there was no segregation of sicker infants to any specific times of the day.

Gestational age was determined by the clinical team who initially admitted the infant to the NICU, generally based on last menstrual period, prenatal ultrasound, and/or physical examination, in decreasing order of priority.

DNA Processing and Genotyping

DNA was extracted from infant cord blood and from venous blood, buccal swabs, or saliva from parents. Allelic variation was determined using the TaqMan genotyping system (Applied Biosystems, Foster City, California), as described previously.[14] Allele scoring was done using Sequence Detection Systems version 2.2 (Applied Biosystems). The genotype data were uploaded into a Progeny database (Progeny Software, South Bend, Indiana) that also contained phenotype data for the infant cohort.

Analytic Strategy

The first phase of the study was a candidate gene survey using single nucleotide polymorphism (SNP) genotype information from a larger ongoing study of genetic risk factors for prematurity. The genes included in the initial analysis generally related to 1 of 4 biological pathways: xenobiotic metabolism, smooth muscle contraction, inflammation, or folic acid metabolism. Non-random allele inheritance was assessed by the transmission disequilibrium test as implemented in the Family-Based Association Test software (FBAT, Harvard School of Public Health, Boston, Massachusetts).[15-17] All SNPs associated with ESBP in the first year of life with a P value $\leq .001$ were identified for further study, recognizing the possibility of identifying false-positive signals with this liberal P value. Next, each SNP identified in this manner was included as the independent variable in a multivariate logistic regression analysis for the outcome of ESBP, adjusting for clinical covariates that were identified on univariate and multivariate logistic regression analyses as being associated with ESBP.

Statistical Analyses

Means and standard deviations of normally distributed continuous variables were compared using analysis of variance. Distribution frequencies were compared using the $\chi 2$ or Fisher exact test. Multivariate logistic regression analysis was performed to determine significant predictors of the event in preterm infants of observing a maximum SBP >90th percentile for term infants. Both stepwise selection and backward elimination algorithms were used. Multiple linear regression analysis was used to model significant genetic and clinical predictors of maximum SBP. In the models, all variables were expressed as categorical, except for the continuous variables maximum SBP and gestational age.

Results

The Figure shows the maximum SBP value for each of the 305 preterm infants in the study cohort at the corrected age when measured. Using the defined phenotype of ESBP, 221 of the 305 preterm infants (72%) were identified as having one or more measurements indicating ESBP. Table I lists characteristics of the infants with ESBP and those without (controls). There was no difference between the groups with respect to birth weight; however, the infants with ESBP were born at a slightly earlier gestational age (27.3 weeks vs 28.1 weeks; $P = .007$). The 2 groups were similar with respect to sex, weight for gestational age, maternal steroid administration, and rates of serious intraventricular hemorrhage (> grade I), patent ductus arteriosus, and use of umbilical catheters. Nine infants in the ESBP group had been diagnosed with ESBP during NICU hospitalization, one of whom had been discharged on antihypertensive