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Determination of Genetic Predisposition to Patent Ductus Arteriosus in Preterm Infants

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Abstract

Patent ductus arteriosus (PDA) is a common morbidity associated with preterm birth. The incidence of PDA increases with decreasing gestational age to about 70% in infants born at 25 weeks gestation. Although medical treatment with non-steroidal anti-inflammatory drugs (NSAIDs) is used to close the ductus arteriosus, approximately 30% of infants with a PDA do not respond to pharmacologic attempts at closure. We investigated whether single nucleotide polymorphisms (SNPs) in genes that regulate smooth muscle contraction, xenobiotic detoxification, inflammation and other processes are markers for persistent patency of the DA. Initially, 377 SNPs from 130 genes of interest were evaluated in DNA samples collected from 204 infants with a gestational age of less than 32 weeks. A family-based association test (FBAT) was performed on genotyping data to evaluate over-transmission of alleles. P-values of less than 0.01 were detected for genetic variations found in seven genes. The analysis was then replicated with an independent set of 162 infants, focusing on the seven markers with initial p-values less than 0.01, and one genetic variant in the angiotensin II type I receptor *(AGTR1)* previously shown to be related to PDA. Of the initial positive signals, SNPs in the transcription factor AP-2 beta (*TFAP2B*) and TNF receptor-associated factor 1 (*TRAF1*) genes remained significant, both with p-values of 0.005. An *AGTR1* polymorphism previously reported to be associated with PDA following prophylactic indomethacin administration was not associated with the presence of a PDA in our population (p = 0.48). Overall, our data support a role for a genetic contribution to the risk of PDAs in preterm infants.

Introduction

Patent ductus arteriosus (PDA) is a common complication occurring in preterm infants and has been associated with the development of chronic lung disease, necrotizing enterocolitis, and

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intraventricular hemorrhage. The ductus arteriosus (DA) is an important *in utero* vascular connection between the aorta and pulmonary artery. At birth, the pulmonary artery pressures are high and there is limited shunting from the aorta to the pulmonary arteries via the ductus arteriosus. As the pulmonary artery pressures begin to fall during the first hours to days of life, significant left to right shunting of blood from the systemic circulation to the pulmonary circulation occurs. This shunting causes alterations in systemic blood flow and decreases flow to skin, muscle, kidneys and the gastrointestinal tract (1). Additionally, pulmonary over-circulation caused by the left to right shunting across the PDA can lead to pulmonary edema and a worsening of the infant's already compromised respiratory status (2). Finally, the presence of a PDA has recently been shown to have a negative effect on cerebral perfusion, resulting in impaired oxygen delivery to an already vulnerable premature brain (3).

Postnatal closure of the DA occurs in two phases. First, after birth, increasing PaO2 and decreasing amount of circulating prostaglandins allow the smooth muscle of the ductus arteriosus to contract, functionally limiting luminal blood flow. After this physiologic occlusion has occurred, hypoxia of the medial layer of the DA occurs resulting in the elaboration of inflammatory mediators and growth factors. These compounds subsequently induce fibrosis, resulting in permanent anatomic closure of the DA, creating the ligamentum arteriosum (1).

Notably, not all preterm infants develop a PDA. The most recent data from the Vermont Oxford Network (2006) of nearly 40,000 preterm infants with a birth weight 501 to 1500 grams show the overall incidence of PDA to be 37.2% (4). The incidence of PDA in infants with gestational ages of 24, 25 and 26 weeks was 76.9%, 69.5% and 61.5%, respectively.

There have been recent efforts to delineate a genetic cause of PDA. However, these studies have focused primarily on PDAs associated with syndromes in small cohorts of patients, and have generally excluded preterm infants (5–7). In one such analysis, mutations in the *MYH11*, a gene encoding smooth muscle myosin heavy chain, were found to be causative for two kindreds that presented with thoracic aortic aneurysm and a PDA (8). Mutations associated with Char syndrome, a disorder characterized by facial dysmorphism, hand anomalies, and PDA, were found in *TFAP2B*, a gene encoding a transcription factor found in neural crest-derived cells (5,7).

One study has addressed PDA in preterm infants and their response to medical management; examining 159 infants born before 33 weeks gestation who received prophylactic indomethacin. The authors investigated whether a polymorphism in the *AGTR1* gene (rs5186) influenced PDA closure with indomethacin. Infants with the CC genotype of this locus were found to have a lower risk of PDA than those infants with AA or CA genotypes following early indomethacin administration (9).

In this study, we evaluated the hypothesis that common variants in candidate genes might play a role in the development of a PDA. The infants in this analysis are a defined cohort from a larger study population in which possible genetic contributions to preterm delivery were investigated. All genotypes available were analyzed using a hypothesis generating strategy, although our *a priori* hypothesis was that genes regulating pathways controlling smooth muscle contraction or those associated with syndromes including PDA would be most likely to be associated with PDA in preterm infants.

Methods

This study used a two-phased approach. The first analysis of 204 infants was a data-mining, candidate gene survey using genotype information from a larger study investigating genetic contributions to prematurity. Using a family based association test, we chose seven SNPs with

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p-values less than 0.01 for further study. A second phase of study was then performed on these genes of interest by adding an additional 162 infants to the study population and repeating the statistical analysis on the total sample of 366 infants.

Sample Population

Since 2000, blood or buccal swabs from infants (and their parents) admitted to the Neonatal Intensive Care Unit at the University of Iowa Children's Hospital have been collected and banked. This IRB-approved program is designed to generate samples for use in genetic studies evaluating diseases of the infant. A second IRB approval was obtained to access these samples and the associated clinical information necessary for this study. This population is a subgroup of one that has been described previously (as have some of the SNPs used in this study), but evaluated for preterm labor as an endpoint with respect to the progesterone receptor (10) and genes affecting cholesterol metabolism (11). Approximately 90% of the sample population is Caucasian.

A total of 366 infants less than 32 weeks gestation were included for analysis. The first analysis, in which 377 SNPs were screened for possible genes of interest, included 204 infants (collected between 2000 and 2005). The second analysis, which encompassed those markers with p-values of <0.01 from the initial screen, included an additional 162 infants (collected between 2005 and 2007). In addition, a few new genes and SNPs were investigated in the second analysis that were not under consideration during the first phase of the study (*e.g*., *EPAS1, and AGTR1*). The gestational ages for the study population are presented in Figure 1. Classification of disease state was performed by a chart review of each infant. The diagnosis of PDA was made by a pediatric cardiologist after the third day of life by standard echocardiography. No infant received prophylactic indomethacin (*i.e*., in the first two days of life). In the first phase of analysis, PDA was present in 69 (33.8%) of the study infants and absent in 135 (66.2%). Of the 69 infants diagnosed with a PDA, 16 underwent surgical ligation for closure of the PDA, all after failure of closure with indomethacin. Of the 162 infants added into the second phase of the study, 57 (35.2%) were diagnosed with a PDA and 24 underwent surgical ligation, again after failure of the PDA to close following indomethacin treatment. Table 1 lists the demographics of the infants in the study. On average, infants with PDAs were born two weeks earlier and were 300 grams lighter than infants without a PDA. The proportion of male infants in each group was similar. Consistent with their earlier gestational age, infants with a PDA were more likely to develop an intraventricular/periventricular hemorrhage than those without a PDA ($p=0.02$). The group of infants with a PDA included 91 singletons, 30 twins and 5 triplets. Those without PDA included 171 singletons, 58 twins, 7 triplets and 4 quadruplets.

DNA Processing and Genotyping

DNA was extracted from cord blood for the infants in the analysis and from venous blood, buccal swabs or saliva from parents. Allelic variation was determined using the TaqMan genotyping system (Applied Biosystems, Foster City, CA, USA), as previously described (10). Allele scoring was done using the Sequence Detection Systems software (version 2.2, Applied Biosystems, Foster City, CA, USA). The genotype data were uploaded into a Progeny database (Progeny Software, LLC, South Bend, IN, USA), also containing phenotypic data, for subsequent statistical analysis.

Candidate genes

Candidate genes were chosen based on a review of the current literature as well as hypotheses of biologic plausibility. Genes in the smooth muscle pathway, such as the prostaglandin synthases as well as those that had been associated with PDAs in syndromes, were specifically chosen for analysis of PDA in these preterm infants. As part of this study utilized a data mining approach, some of the genes evaluated were those that had been previously reported or

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hypothesized to be associated with preterm birth. SNPs within each gene were selected using data from the International HapMap project (www.hapmap.org), balancing the greatest coverage of the gene with the fewest number of tagging and/or known functional SNPs required. In general, a minor allele frequency of 0.1 was chosen as a lower cutoff for a SNP to ensure that an adequate number of individuals within the population would be carriers of the minor allele. Genes selected for analysis are listed in Table 2. A complete listing of the SNPs with their dbSNP identification ("rs") numbers can be found in the online supplemental material.

Statistical analysis

Genotyping data for each SNP were assessed using the program PedCheck for any departures from Mendelian inheritance patterns (12). Alleles at each marker were tested for association with PDA, using the Family Based Association Test (FBAT) (13–15). Additionally, haplotype FBAT (HBAT) was performed for sliding windows of 2, 3 and 4 SNPs across genes when appropriate.

Each infant was part of a trio of father, mother and child. If two infants in a family shared the same affection status (i.e., both with or both without PDA) then the nuclear family was the analysis unit. Otherwise, the infants with differing PDA status formed two separate trios with the same parents for the subgroup analyses. Given the multiple testing of these 377 SNPs in the first phase of the study, for statistical significance at an alpha level of 0.05, the most conservative correction (Bonferroni) would require p-values of less than 0.0001 to be considered formal evidence of association. In order to effectively explore the possible association between PDA and candidate genes while avoiding false negative results, we chose those SNPs with p-values < 0.01 as criteria for further consideration in subsequent replication studies in which all of the PDA cases were analyzed together.

Results

In the initial phase of the analysis, seven markers were found to be positively associated with the development of a PDA in our study population, with a p-value of < 0.01. One marker in each of the following genes was identified: TNF receptor-associated factor 1 (*TRAF1*), cholesteryl ester transfer protein, plasma (*CETP*), cytochrome P450, family 2, subfamily D, polypeptide 6 (*CYP2D6*), prostaglandin I2 (prostacyclin) synthase (*PTGIS*), corticotrophin releasing hormone receptor 1 (*CRHR1*), hepatic lipase (*LIPC*) and transcription factor AP-2 beta (*TFAP2B*). P-values and SNP identification can be found in Table 3. A plot of all genetic variants considered in the analysis and the negative log of their p-value is shown in Figure 2.

In the replication phase of our analysis we evaluated the seven SNPs which had p-values less than 0.01 in the initial candidate gene phase and found that the p-values for five of the seven markers increased, suggesting initial false positives. Notably, the p-values of two of the initial seven markers remained significant, suggesting that they are truly associated with the presence of a PDA in our population: *TFAP2B* (rs987237: G allele; p=0.005) and *TRAF1* (rs1056567: T allele; p=0.005). Interestingly, we also found that the A allele of the TFAP2B SNP rs987237 was positively associated with the absence of a PDA. As is the case with most candidate gene association studies, additional replication in a larger, distinct population is needed to further support our findings.

An attempt was also made to delineate markers that were associated with the failure to respond to medical management and the subsequent need for surgical ligation. Although the number of infants requiring surgical ligation (after failing indomethacin therapy) was rather small (n=40), we did find a borderline positive association with two of the studied polymorphisms: *TFAP2B*, rs987237 (p=0.04 with 10 informative families) and *EPAS1*, rs1867785 (p=0.03 with

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13 informative families). These results also need to be replicated studying a larger population of infants with persistent PDAs following NSAID therapy.

Haplotype analysis of the genotyped SNPs was performed to examine whether combinations of alleles might be positively associated with the presence of the PDA. In this analysis, one gene, (*TFAP2B*) showed an association with the presence of a PDA, while 2 genes (*TRAF1* and *PTGIS*) were associated with the absence of a PDA, as shown in Figure 3. With respect to TFAP2B, 14% of our population had a G allele at both rs6930924 and rs987237 and this combination was associated with the presence of a PDA (p=0.004). Conversely, the presence of a G allele at rs6930924 and an A allele at rs987237 was associated with the absence of a PDA (p=0.02). Haplotype analysis of both *TRAF1* and *PTGIS* genes demonstrate a negative association with PDA, suggesting a protective effect of specific allele combinations. As shown in the middle panel of Figure 3 (*TRAF1*), 24% of the study population had a C allele at both rs1056567 and rs10985070 and this combination was negatively associated with the presence of a PDA (p=0.003). Likewise in the *PTGIS* gene (lower panel, Figure 3), 47% of the study population had a G allele at rs49394 and an A allele at rs693649, and this combination was also negatively associated with the presence of a PDA (p=0.01). No other genes analyzed in this way had haplotype blocks that were associated with the presence or absence of PDA.

Because decreasing gestational age is a major risk factor for the development of a PDA, our data set was analyzed to determine whether the genetic polymorphisms that were positively associated with PDA were significant due to a positive association with preterm delivery at less than 32 weeks. None of the genes that we have identified as being associated with PDA had a positive association with preterm delivery (p>0.01, see Table 3), thus the genetic variations identified were not merely surrogates for the confounding variable of preterm delivery.

Finally, we attempted to replicate findings from the only prior report linking genetic sequence variations with PDA (9). We investigated six genetic variations covering the angiotensin II type 1 receptor gene (including the previously studied *AGTR1*-A1166C polymorphism, rs5186) for association with PDA. Using FBAT analysis, we were unable to detect a distortion in allele transmission at any loci in triads containing a preterm infant with PDA. Thus, no significant association was found between these polymorphisms, including rs5186 (p=0.48), and the presence of a PDA.

Discussion

Previous studies have begun to address the role of genetic factors influencing common neonatal morbidities. A retrospective, multicenter study of 450 twin pairs found a genetic contribution for several morbidities of the preterm infant such as bronchopulmonary dysplasia, necrotizing enterocolitis, and intraventricular hemorrhage (16). Retinopathy of prematurity has also been found to have a strong genetic predisposition (17). As mentioned earlier, only one paper to date has focused on genetic polymorphisms contributing to the PDA in preterm infants (9).

Our approach was to evaluate sequence polymorphisms within candidate genes for association with isolated (non-syndromic) PDA in high-risk preterm infants. We identified seven SNPs associated with the development of a PDA with a p-value of less than 0.01. Another sixteen markers were found to have a p-value between 0.01 and 0.05. We chose a p-value of 0.01 to provide a first level cutoff for markers that might be of interest and warranted replication of results in the second sample population. Replication with a smaller number of SNPs tested does not require the extreme rigor of the Bonferroni adjustment as the initial phase of this study, which used a large number of SNPs and a modest number of cases. How to appropriately analyze large numbers of samples and hypotheses from a statistical standpoint represents a

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significant challenge that is now arising in large genome-wide association studies where hundreds of thousands of markers are tested on thousands of cases (18).

Sequence variations in one SNP (rs987237) in *TFAP2B* (transcription factor AP-2B), the gene mutated in Char syndrome, was found to be associated with both PDA and failure of the PDA to close with indomethacin in our population of normal preterm infants. This gene is expressed in neural crest derivatives and is generally involved with development, cell-cycle control, and apoptosis (19,20). Embryologic studies using the chick model system have identified cardiac neural crest cells in the wall of the ductus arteriosus, further supporting a role for a gene controlling differentiation of neural crest derivatives in the persistent patency of the ductus arteriosus (21). Recently, *TFAP2B* and *EPAS1* were both found to be expressed in the smooth muscle of the mouse DA, playing key roles in ductal closure in this animal model by participating in a transcriptional network that regulates ductal smooth muscle development. (Ronald Clyman, personal communication). The specific *TFAP2B* marker of interest (rs987237) is located in a highly conserved region between exons three and four containing a number of putative transcription factor binding sites. The SNP rs987237 is present in a haplotype block that, in Caucasians, extends from the intronic region between exons one and two to beyond the end of the gene. This block encompasses exons two, four, and five, where several mutations reported to cause Char syndrome are located (7). The actual genetic variation (s) responsible for PDA in preterm infants could lie anywhere within this relatively large haplotype block. More detailed mapping of this region and confirmation of the finding in other preterm populations is necessary to further define the actual etiologic polymorphism.

A second SNP (rs1056567), in a haplotype block adjacent to the *TRAF1* gene (tumor necrosis factor receptor-associated factor 1), was also found to be associated with PDA in our population. This protein mediates the activity of NF-kappaB and plays a role in modulating both the inflammatory and apoptotic pathways (22,23). Both apoptosis and inflammation are known to play an important role in the second (more permanent) phase of ductal closure, fibrosis following muscular contraction and hypoxia. The SNP rs1056567 is also in a haplotype block with a second gene, *PHF19* (PHD finger protein 19). *PHF19*, which is a human homologue of the Drosophila polycomb-like gene, encodes a protein that binds to DNA and is postulated to play a role in transcriptional regulation (24).

Haplotype analysis determines whether combinations of neighboring polymorphisms, rather than individual sequence variations alone, are positively associated with a phenotype. In addition to a two-SNP window in the *TRAF1* gene, a second two-SNP combination was found to be associated with PDA in the *PTGIS* (prostaglandin I2 synthase) gene. This gene is responsible for the production of prostaglandin I2 (prostacyclin), a potent vasodilator which plays a critical role in fetal patency of the ductus arteriosus (reviewed in (25)). It is possible that alterations in the regulation of this gene could lead to abnormal postnatal closure of the DA in a preterm infant, since prostacyclin levels are elevated in preterm infants and the concentration of a prostacyclin metabolite (6-ketoprostaglandin F1-alpha) has been shown to correlate with ductal diameter (26).

None of the genetic polymorphisms that were positively associated with persistent patency of the DA were located in coding regions; therefore they would not be expected to alter protein structure. They could, however, alter gene expression by changing transcriptional regulatory regions or methylation patterns. Recent reports suggest that haplotype structure may be more important in determining the effect of a variant on function rather that the individual SNP (27). While none of the genetic variants that we identified is likely to be a causative factor for PDA, sequence variations located within the identified haplotype blocks they represent may be etiologic. Our approach has identified a region of interest for further fine mapping for potential genetic contributors to PDA.

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One limitation of a hypothesis-generating (*i.e*., data-mining), candidate gene approach such as ours is the large number of observations that are produced by the analysis. Because of these multiple observations, it becomes difficult to delineate what p-value represents an appropriate level of significance. We recognize the importance of this issue in the present study. Our use of p=0.01 as a first-phase criteria was a compromise to limit false positives, but may have also led to us to miss some significant genes where our power to detect an affect was limited by sample size. In fact, the second phase of our study demonstrated that five of the seven original positive signals were likely false positives rather than truly associated with PDA. Employing the traditional Bonferroni correction for multiple comparisons in the initial phase of the study would have led to a very stringent level for statistical significance (0.00013), eliminating all candidate genes in the study. This degree of stringency is likely not appropriate when investigating common complex human diseases where an individual gene, along with environmental forces, may play a variable, but important, role in the development of a disease.

PDAs are found with high frequency in preterm infants. It is possible then, that the significant allelic variation described in this report may be associated with preterm birth and are positive simply because PDA is a common morbidity of the preterm infant. In order to eliminate preterm birth as a possible confounding factor in the analysis for predisposition to PDA, we also performed an analysis for association with preterm delivery with the same data set of infants less than 32 weeks gestation. None of the polymorphisms that were positively associated with PDA were found to be associated with preterm birth overall.

Gene-environment interactions almost certainly play a role in PDA. It is very likely that extreme prematurity is the environmental condition upon which a genetic predisposition to PDA becomes manifest, a hypothesis supported by the very low incidence of PDA in term infants. The cardiovascular environment of a preterm neonate is very different from that of a term infant in a number of ways (*e.g*., lower PaO_2, lower systemic blood pressure, need for positive pressure ventilation, etc.) that may promote persistent patency of the DA. Additional conditions, such as birth via cesarean section and lower hematocrit at birth, have been associated with failure of the PDA to close with indomethacin treatment (28). It has also been demonstrated that preterm infants of mothers who received indomethacin tocolysis have an increased incidence of symptomatic PDA (29). Prematurity and the abnormal anatomy and physiology that goes along with it, coupled with a genetic predisposition, may lead to the development of a PDA. Infants born at term, even with a genetic risk, may not have a PDA because they are simply not exposed to the inciting environment.

There are practical benefits in delineating genetic factors associated with PDA. It is common practice to attempt closure with non-steroidal anti-inflammatory medications such as indomethacin or ibuprofen. The number of such attempts before surgical closure is undertaken varies by institution. Medical treatment of the PDA, however, is not without potential risks and side effects. Recent studies have shown that adverse effects from the early use of indomethacin may offset the benefits of early DA closure. While early closure of the PDA has been shown to reduce incidence of IVH, no reduction in BPD has been seen (30). If an infant’s genotype is associated with failure of medical closure and could reliably predict the need for surgical ligation, we may one day incorporate genotype data into whether to subject the infant to medical therapy before definitive closure of the PDA surgically. In this study we did see a borderline significant association of alleles in the *TFAP2B* and *EPAS1* genes with the subsequent need for surgical ligation that, if replicated, might afford an opportunity for such early predictions. Finally, identifying genes and pathways that play a role in PDA closure can advance our understanding of developmental cardiovascular physiology in humans.

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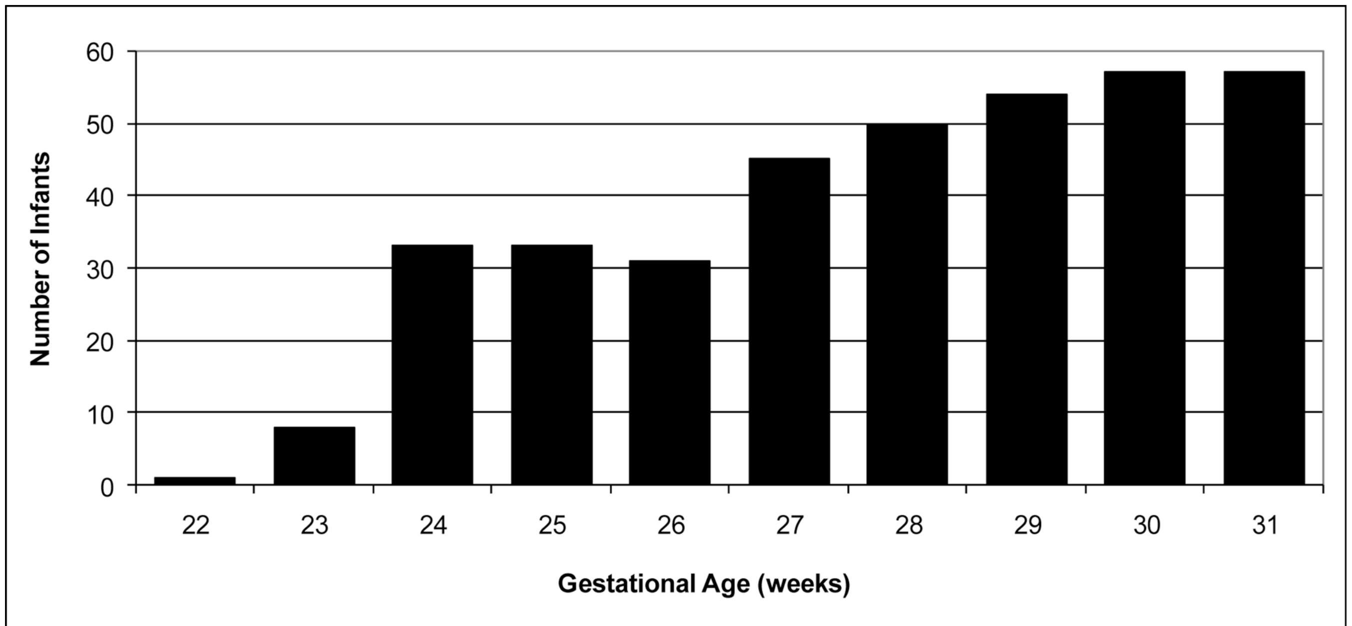

Figure 1.
Frequency distribution of the 366 study infants by gestational age.

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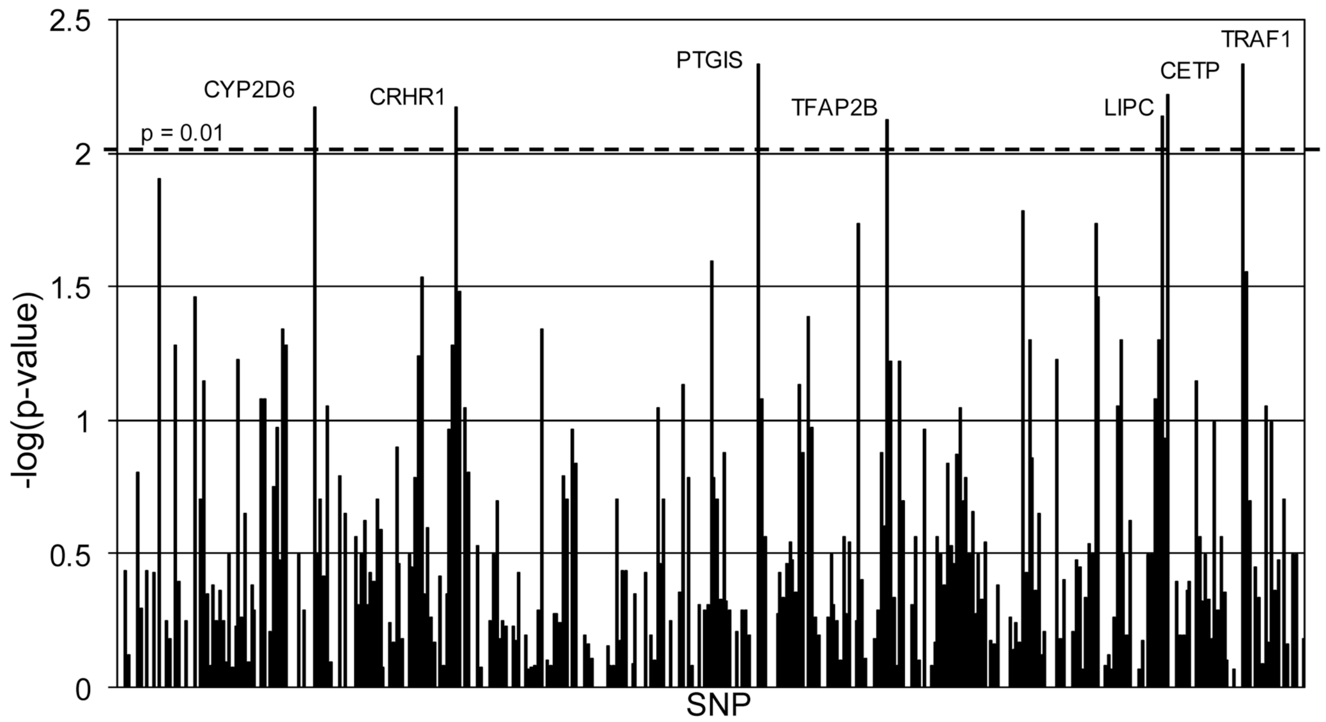

Figure 2.
Results of the family-based test (FBAT) for genetic association with PDA. The negative log of the p-value is plotted for each SNPs association with PDA. SNP signals reaching about the dotted line represent p-values less than 0.01.

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TFAP2B

2 SNP window			Global p	Hap p (assoc)	Hap freq
rs2272903	rs6930924	rs987237			
--			--	--	--
	G	G	0.01	0.004 (+)	14 %

TRAF1

2, 3 & 4 SNP windows				Global p	Hap p (assoc)	Hap freq
rs10985066	rs1056567	rs10985070	rs2239657			
G	T			0.07	0.07(+)	57%
G	C				0.02(-)	33%
	C	C		0.03	0.003(-)	24%
		--		0.30	--	--
G	C	C		0.04	0.005(-)	24%
	C	C	G	0.04	0.01(-)	18%
G	C	C	G	0.04	0.01(-)	18%

PTGIS

2 SNP window						Global p	Hap p (assoc)	Hap freq
rs1502245	rs6095545	rs6090996	rs522962	rs493694	rs693649			
--						0.38	--	--
	--					0.50	--	--
		--				0.56	--	--
			--			0.49	--	--
				G	A	0.07	0.01 (-)	47%

KEY: Global p = overall p-value for association between PDA and the indicated haplotype window.
Hap p (direc) = p-value for associate between PDA and a specific haplotype composed of the alleles indicated in the bars.
In parentheses is the direction of the association: “+” for positive association or “-” for negative association.
Hap freq = estimated haplotype frequency for the specific haplotype composed of the alleles indicated within the bars

Figure 3.

Haplotype analysis for association with PDA in the *TFAP2B*, *TRAF1*, and *PTGIS* genes. Haplotypes for three genes were analyzed using a 2-SNP window (*TFAP2B* and *PTGIS*) or a combination of 2-, 3- and 4-SNP windows (*TRAF1*). Allele combinations yielding an individual haplotype with a p-value less than 0.05 are shaded gray. The sign in parentheses after the p value indicates whether the allele combination is positively or negatively associated with PDA.

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Table 1

Study infant demographics

	PDA	No PDA
Number of infants	126	240
MeanBirth Weight (grams)	896	1220
Mean Gestational Age (weeks)	26.3	28.7
Percent male	57.1%	54.6%
IVH (Total number)	31	35
IVH Grade		
I	6	21
II	7	5
III	9	6
IV	9	3

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Table 2

Genes included in the PDA analysis

ABCA1	*CYP1A2*	*FMO3*	*IL1RN*	*NQO1*	*SFTPD*
ABCC11	*CYP1B1*	*FOLR1*	*IL4*	*NR3C1*	*SHMT1*
ADRB2	*CYP2C19*	*GART*	*IL5*	*OPRM1*	*SLC19A1*
AKR1C3	*CYP2C9*	*GP1BA*	*IL6*	*OXT*	*SLC24A5*
ALDH2	*CYP2D6*	*GP6*	*IL8*	*OXTR*	*TBX1*
APOA1	*CYP2E1*	*GPR51*	*ITGA2*	*PGR*	*TBX5*
APOA4	*DARC*	*GSTT1*	*ITGB3*	*PLA2G4A*	*TFAP2B*
APOA5	*DDC*	*HLA-B*	*KCNN3*	*PLUNC*	*TGFB1*
APOB	*DEFB1*	*HLA-G*	*LCAT*	*PTEN*	*TGFBR1*
APOC2	*DHCR24*	*HMGCR*	*LDLR*	*PTGER1*	*TGFBR2*
APOC3	*DHCR7*	*HPGD*	*LIPC*	*PTGER2*	*TLR4*
APOE	*DHFR*	*HSD11B1*	*LPL*	*PTGER4*	*TNFA*
BIRC2	*EDN1*	*HSD11B2*	*MBL2*	*PTGES*	*TNFAIP3*
BIRC3	*EDN2*	*HSD3B1*	*MMP1*	*PTGES2*	*TNFRSF1A*
CETP	*EP300*	*IFNG*	*MMP9*	*PTGFR*	*TNFRSF1B*
CFH	*EPHX1*	*IGF1*	*MTHFD1*	*PTGIS*	*TRADD*
CHD7	*ESR1*	*IGF2*	*MTHFR*	*PTGS1*	*TRAF1*
CHRNA4	*F13*	*IL10*	*MTR*	*PTGS2*	*TRAF2*
CRHR1	*F2*	*IL10RA*	*MTRR*	*PTPN11*	*VEGF*
CYP11A1	*F5*	*IL1A*	*NAT2*	*RPAIN*	*ZFHX1B*
CYP17A1	*FAS*	*IL1B*	*NNMT*	*SERPINA6*	
CYP1A1	*FMO1*	*IL1R2*	*NOS2A*	*SFTPB*	

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Table 3

SNPs associated with the presence of PDA in infants < 32 weeks gestation

		P-values			
Gene	**SNP**	**PDA-1#**	**PDA-2***	**No PDA**	**Prematurity**
PTGIS	rs493694	0.005	0.29	0.08	0.05
TRAF1	rs1056567	0.005	0.005	0.70	0.03
CETP	rs711752	0.006	0.12	0.73	0.23
CRHR1	rs173365	0.007	0.21	0.07	0.03
CYP2D6	rs28360521	0.007	0.02	0.34	0.02
LIPC	rs1973028	0.007	0.06	0.71	0.39
TFAP2B	rs987237	0.007	0.005	0.10	0.52

initial phase- 69 infants with PDA

* replication phase- a total of 126 infants with PDA, including the original 69 infants.