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Genetic Modifiers of Patent Ductus Arteriosus in Term Infants

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Abstract

Objective—To identify single nucleotide polymorphisms (SNPs) in specific candidate genes associated with patent ductus arteriosus in term infants.

Study design—We conducted an initial family-based, candidate gene study to analyze genotype data from DNA samples obtained from 171 term infants and their parents enrolled in the National Birth Defect Prevention Study (NBDPS). We performed transmission disequilibrium testing using a panel of 55 SNPs in 17 genes. Replication of SNPs with $p<0.1$ in the NBDPS trios was performed using a case-control strategy in an independent population.

Results—Transmission disequilibrium test (TDT) analysis of the NBDPS trios resulted in 6 SNPs reaching the predetermined cutoff ($p<0.1$) to be included in the replication study. These 6 SNPs were genotyped in the independent case-control population. A SNP in *TGFBR2* was found to be associated with term PDA in both populations after correcting for multiple comparisons. (rs934328, TDT $p=2\times10^{-4}$, case-control $p=6.6\times10^{-5}$).

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The authors declare no conflicts of interest.

Conclusions—These findings confirm the importance of the TGF-beta pathway in the closure of the term ductus arteriosus and may suggest new therapeutic targets.

Keywords

candidate gene; TGFBR2; TRAF1; CREBB

The ductus arteriosus, a vascular connection between the pulmonary artery and the ascending aorta, shunts blood away from the lungs during fetal development. Patency of the fetal ductus arteriosus relies upon the combination of low fetal systemic arterial oxygen tension, elevated circulating prostaglandins, and low levels of endogenous vasoconstrictors. The spontaneous functional closure of the ductus arteriosus generally occurs within the first 24-48 hours after birth in term infants (1). Failure of the ductus arteriosus to undergo closure after birth results in a patent ductus arteriosus (PDA), which increases the risk of congestive heart failure as pulmonary vascular resistance drops, and may ultimately result in irreversible pulmonary vascular disease (1).

PDA affects approximately 1 in 2,000 to 1 in 5,000 term infants each year, constituting 5% to 7% of all congenital heart disease in term infants (2). PDA occurs more frequently in preterm infants less than 32 weeks gestation and often requires medical treatment with inhibitors of prostaglandin biosynthesis (3). The reported increased prevalence (5%) of PDA among siblings of individuals with a PDA suggests a complex etiology for this condition, with both genetic and environmental factors playing a role (2, 4, 5).

A mouse model was utilized to examine the effects of PGE_2 receptor disruption in PDA and found disruption of this gene resulted in a PDA and subsequent death (6). Research on other congenital heart lesions in humans also supports the causal role of genetics in cardiovascular anomalies such as PDA, as previous work suggests that allelic variants in specific genes may contribute substantially to their etiology (7). Additionally, our prior work examining candidate genes for PDA in preterm infants suggests that genetic factors, such as *TFAP2B*, play a role in this condition (8, 9).

In humans, loci for dominant and recessive forms of syndromic PDA have been mapped, but no specific genes have been identified for isolated (non-syndromic) term PDA cases (2, 10). PDA is found at high rates in several human genetic syndromes, including those with defined chromosomal aberrations or single-gene mutations, such as Char syndrome (10, 11). Char syndrome is a rare autosomal dominant form of PDA with additional hand abnormalities and facial dysmorphisms caused by mutations in the *TFAP2B* gene (10, 11). This condition provides one example of a single gene defect in which less deleterious mutations/variants might result in isolated structural lesions of the cardiovascular system.

Identification of additional genes involved in the etiology of term PDA is vital to understanding the pathogenesis of cardiac development and to improving treatment for infants with this abnormality. Understanding the mechanisms responsible for PDA may also aid in developing targeted genetic therapies. To investigate genes that may be associated with development of PDA, we chose a study design that allowed us to take advantage of an available population-based sample of PDA cases, and literature that suggested biologically

plausible candidate genes. Our goal was to identify single nucleotide polymorphisms (SNPs) that are present in specific candidate genes associated with PDA in term infants.

Methods

The Institutional Review Board at the University of Iowa approved this study. The original study population consisted of 171 trios from the National Birth Defects Prevention Study (NBDPS). The NBDPS is a population-based, case-control study designed to examine environmental and genetic risk factors for over 30 major birth defects (12). A signed consent form was obtained from parents of case children. Eligible case children for the current study were term infants delivered between October 1, 1997, and December 31, 2005, diagnosed with one or more NBDPS defects (Table I; available at www.jpeds.com), including a PDA (12). Cases were ascertained in Iowa using multisource, active case finding and detailed medical record abstraction. A clinical geneticist confirmed birth defect diagnoses. Cases that had known chromosomal or other defined (single gene, teratogen) diagnoses or known additional congenital heart disease were excluded from the study.

The replication phase of the study included 573 cases and 1200 controls from New York State. The New York State samples were archived newborn blood spot cards from infants with birth weight >2500g (birth weight was used as a surrogate for gestational age as gestational age was unavailable) born between January 1, 1998 and December 31, 2005 in New York State. Cases were identified from the New York State Congenital Malformations Registry (CMR) and controls were a random sample of live births. The New York State CMR is statewide and includes all hospitals that serve children. PDA was reported by the hospital.

Data for cases from the NBDPS were obtained through part of the NBDPS protocol. Mothers of eligible case children were asked to complete a telephone interview that included items about pre- and/or post-conception reports of illness, medications, diet, substance use, and occupation. Following completion of the interview, mothers, fathers and case children were asked to provide buccal cell samples using cytobrushes. Genomic DNA was obtained from these cytobrushes according to standard NBDPS extraction and quality control protocols (13). Cases in the replication study were identified through the New York State Congenital Registry as infants with isolated PDA born to New York residents at 37 weeks or greater gestation. Cases were linked to the New York State Newborn Screening program to obtain blood spot cards for DNA extraction. Controls were randomly selected from the Newborn Screening program. A majority of cases and controls from the NBDPS and New York State Congenital Registry (90% and 100%, respectively) were non-Hispanic, Caucasian individuals.

Seventeen genes, either associated with syndromic forms of PDA or with regulation of normal PDA closure, were selected as candidates (Tables II and III). A particular focus was placed on genes representing prostaglandin, apoptosis, and inflammation pathways. After identifying high-priority candidate genes, single nucleotide polymorphisms (SNPs) in the candidate genes were identified using HapMap data to define the haplotype blocks (http://www.hapmap.org) (14). SNPs were selected based on the likelihood that the SNP altered the

function of the gene product, high heterozygosity to enhance power, and as tagging SNPs in haplotype blocks. To enhance power, only those polymorphisms with a minor allele frequency greater than 10% in the Caucasian population were considered for analysis. Ultimately, the SNPs with the highest minor allele frequency near or within a given haplotype block were selected. Association studies were performed on DNA samples using an initial starting panel of 55 SNPs (Table IV) to determine allelic variation in the 17 selected genes. Candidate gene SNPs that were found significant at $p < 0.1$ in the initial analysis were genotyped in the replication population (NY samples).

Genotyping was performed using TaqMan® Assay-On-Demand™ probes and TaqMan® Assay-On-Design™ probes by Applied Biosystems (Foster City, CA, USA). Following PCR amplification on a PE 9700 thermocycler, the results were read using the Applied Biosystems 7900 HT instrument with Sequence Detection System software (version 2.2, Applied Biosystems, Foster City, CA, USA).

Statistical Analyses

The genotype data were validated using PedCheck software (15), which affords secondary analyses to identify Mendelian incompatibilities that might represent cases of deletions or sample errors. Hardy-Weinberg equilibrium was tested in each population using a chi-square test. Any SNP with a chi-square *p* value less than .01 in the unaffected individuals was excluded from further analyses. All analyses were completed using PLINK v1.07 (16, 17). The NBDPS trio data were analyzed using Transmission Disequilibrium Testing (TDT; 18), a method that evaluates over-transmission of specific alleles (i.e., a nonrandom allele distribution) from parents to affected cases (19). A family-based analysis was not possible for the New York State case-control data, as no parental DNA was available. Therefore, the case-control data were analyzed using logistic regression assuming an additive genetic model.

Results

Statistical analysis for the initial 171 NBDPS trios revealed ten SNPs reaching the predetermined cutoff to be included in the replication study ($p < 0.1$). Four of the ten SNPs were excluded from further analyses, as the chi-square p-values for the test of Hardy-Weinberg equilibrium were less than .01 in unaffected individuals. The remaining six SNPs (Table IV) meeting the predetermined cutoff and with acceptable chi-square p-values for Hardy-Weinberg equilibrium were genotyped in the additional 1773 New York case-control samples.

The number of informative families for the TDT analysis, risk alleles, odds ratios (OR) with 95% confidence intervals, and p values for the six SNPs genotyped in both the NBDPS and New York case-control populations are presented in Table V. As TDT analysis only includes trios in which at least one parent is heterozygous for the specified SNP, the number of informative families in TDT analysis varied and was substantially smaller than the total sample (n=171 trios). For three of the SNPs (rs3761846, rs130021, and rs2238777), the allele conferring risk in the NBDPS trios is reversed in the New York State case-control sample (indicated by an odds ratio less than one in the New York case-control sample).

Three of the six SNPs achieved significance after correction for multiple comparisons ($p < 0.01$; Table V) in the NY case-control population. Additionally, rs934328 in *TGFBR2* was found to be significant after correction for multiple comparisons in both the NBDPS and NY case-control populations (Table V). Importantly, the risk allele was the same for this SNP in both the NBPDS trio data and the New York State case-control data.

Discussion

The initial analysis found several SNPs to be associated with the occurrence of PDA in term infants using a cutoff of p<0.1, and three of these SNPs (rs934328 in *TGFBR2*, rs3761846 in *TRAF1*, and rs130021 in *CREBBP)* were found to be significant after applying a Bonferroni correction in the replication analyses, although two of these SNPs (rs3761846 in *TRAF1* and rs130021 in *CREBBP*) had a reversal of the risk allele in the New York case-control sample. In theory and occasionally in practice, a reversal of the risk allele can occur if the populations are of different ancestry. This is possible in the current study, although it is more likely that these represent false positives, especially given that both samples were predominantly non-Hispanic Caucasian.

Odds ratios for the risk alleles were similar in magnitude to odds ratios in other studies of common pediatric disease. The SNP found to be significant in both samples, rs934328 in *TGFBR2*, occurs in an intronic region and thus may not have a functional role, but may be a surrogate for an etiologic variant in the gene that is in linkage disequilibrium with rs934328. *TGFBR2* was identified for the current study based on biological plausibility, as transforming growth factor-beta receptor (*TGFBR*) genes are potent developmental regulators of cell proliferation and differentiation. Variation within *TGFBR2* could potentially alter cell cycle progression leading to phenotypic malformations in target tissue types. Certain mutations in *TGFBR2* cause Loeys-Dietz syndrome, a syndrome characterized by a variety of clinical features, including thoracic aortic aneurysm and PDA (20).

Further investigation through additional genotyping or sequencing is required to ascertain whether other variants in linkage disequilibrium with the SNP are driving the association found in the current study. Additionally, the candidate gene analysis, a hypothesis-driven approach, is limited in that only genes were included with plausible biological function for contributing to PDA. However, a genome-wide approach, which is hypothesis-free, would allow for detection of variants throughout the genome, including genes for which function remains largely unknown.

Limitations of this study include the modest number studied in the family-based analysis, the high degree of heterogeneity likely in PDAs, and the lack of any connection to environmental covariates. An additional limitation is the variability of surveillance data due to the documentation or lack thereof of PDAs in the medical records and effects of non-participation on the representativeness of the sample. It is recognized that the identification of false-positives is always possible when performing multiple genetic testing, however a conservative method was used to correct for multiple comparisons. Such positive results need to be followed up with analysis of additional SNPs in and around the particular gene

based on haplotype structure. Additional cases of PDA in term newborns need to be tested as well, as large sample sizes are required to evaluate the implication of the effect of a genetic variant in a disease appropriately . Major strengths of the current study include the use of TDT analysis in the family cohort and an independent replication cohort to test positive signals from the initial analysis.

PDA is a common congenital heart defect that affects thousands of term and preterm newborns each year. Determining the genetic contributions to PDA may provide opportunities to identify the pathophysiologic mechanisms that are amenable to prevention or intervention and improve the care of newborns with PDA. Our analyses support a genetic pathogenesis for term PDA. These findings suggest strong associations between variations in several genes and the occurrence of PDA in term infants. Further genetic investigation in larger sample sets and utilizing more comprehensive gene coverage (i.e. sequencing or genome-wide approaches) could help to identify other modifier genes of PDA and could further elucidate the molecular mechanisms responsible for this complex trait.

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Abbreviations

NBDPS	National Birth Defects Prevention Study
PDA	patent ductus arteriosus
SNP	single nucleotide polymorphism
TDT	Transmission Disequilibrium Test

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Table 1

Number of other birth defects present in the NBDPS case children included in the current study.

Birth Defect	Number of Children Affected
Heterotaxy without CHD	1
Amniotic bands	1
Anorectal atresia	2
Bladder exstrophy	1
Cataract	1
Choanal atresia	1
Cloacal exstrophy	1
Cleft lip and/or palate	5
Diaphragmatic hernia	5
Anopthalmia/micropthalmia	1
Esophageal atresia	2
Holoprosencephaly	1
Hydrocephaly	2
Hypospadias	4
Limb reduction	1
Omphalocele	1
Any other heart defect	151

Table 2

Genes included in the current study that have been previously associated with syndromes showing increased prevalence of patent ductus arteriosus.

Syndrome	Gene (# of SNPS)	Chromosome Locus
Mowat-Wilson	*SMADIP1* (4)	2q22
Loeys-Dietz	*TGFBR2* (11), *TGFBR1* (5)	3p22, 9q22
Char	*TFAP2B* (2)	6p12
CHARGE	*SEMA3E* (2), *CHD7* (3)	7q21.11, 8q12.2
VACTERL	*PTEN* (2)	10q23.3
Noonan	*PTPN11* (2)	12q24
Holt Oram	*TBX5* (6)	12q24.1
Rubinstein-Taybi	*CREBBP* (3), *EP300* (2)	16p13.3, 22q11.21
DiGeorge	*TBX1* (2)	22q13.2
Visceral Heterotaxy	*ZIC3* (1)	3X26.2

Table 3

Genes included in the current study that have been previously associated with pathways involved in normal closure of patent ductus arteriosus

Pathway	Genes (# of SNPs)	Chromosome Locus
Inflammation	*TRAF1* (3), *BIRC2* (2)	9q33-q34, 11q22
Nitric oxide synthesis	*NOS2A* (2)	17q11.2-112
Prostaglandin metabolism	*PTGIS* (3)	20q13.13

Table 4

Initial panel of SNPs genotyped in the NBDPS sample

BIRC2	rs10895294, rs7934594
CHD7	rs1483208, rs4738813, rs4738816
CREBBP	**rs130021,** rs2239316, rs11076785
EP300	rs9611505, rs9611510
SEMA3E	rs2713170, rs3801525
SMAD1P1	rs11904051, rs12471396, rs1365779, rs7568133
NOS2A	rs7215373, rs944725
PTEN	rs1234212, rs2735343
PTPN11	rs7953150, rs7977332
PTGIS	**rs493694,** rs693649, rs6095545
TBX1	**rs2238777,** rs2301558
TBX5	rs10744823, rs1265501, rs1895585, rs1895602, rs2236018, rs883079
TFAP2B	rs2272903, rs6930924
TGFBR1	rs1013186, rs10283455, **rs10760671,** rs334348, rs6478974
TGFBR2	rs1036095, rs1036097, rs1864615, rs2116142, rs3087465, rs3773634, rs3773656, rs3773663, rs3863075, rs5020833, **rs934328**
TRAF1	**rs3761846,** rs4836834, rs876445
ZIC3	rs2746112

Note. SNPs meeting inclusion criteria ($p < 0.1$) for genotyping in the NY sample are in bold font

Table 5

Gene, SNP, associated allele, odds ratios (OR), 95% confidence intervals (CIs) and p values for the 6 SNPs genotyped in both the NBDPS and NY case-control populations

Gene	SNP	Allele	Informative N *	NBPDS OR (95% CI)	NBDPS p value	NY OR (95%CI)	NY p value
TGFBR2	rs934328	T	91	2.25 (1.44-3.51)	**2×10^{4}**	1.37 (1.17-1.59)	**6.6×10^{-5}**
TGFBR1	rs10760671	G	47	2.13 (1.56-3.94)	.01	1.15 (0.96-1.37)	0.12
TRAF1	rs3761846	C	77	1.48 (0.94-2.34)	.09	0.80 (0.70-0.93)	**2.8×10^{-3}**
CREBBP	rs130021	A	61	1.90 (1.12-3.23)	.01	0.73 (0.63-0.85)	**5.5×10^{-5}**
PTGIS	rs493694	A	69	1.88 (1.14-3.07)	.01	1.09 (0.94-1.26)	0.25
TBX1	rs2238777	G	66	1.64 (0.99-2.70)	.05	0.86 (0.75-0.99)	0.04

Note.

* Informative N refers to the number of families included in the TDT analysis of the NBDPS samples. This N is the number of families in which at least one parent is heterozygous for the SNP, a requirement for inclusion in TDT analysis; The NBDPS sample is comprised of 171 trios; the NY case-control sample is comprised of 573 cases and 1200 controls and data were analyzed using case-control association testing; SNPs remaining significant after applying a Bonferroni correction are indicated in bold font.