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Variations in *CRHR1* are Associated with Persistent Pulmonary Hypertension of the Newborn

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Abstract

Persistent pulmonary hypertension of the newborn (PPHN) is associated with substantial infant morbidity and mortality. Recently, genetic associations have been found in idiopathic pulmonary arterial hypertension. We performed a family-based candidate gene study to examine a genetic association to PPHN and sequenced the *BMPR2* gene in 72 individuals. We enrolled 110 families with infants diagnosed with PPHN based on inclusion criteria. After medical chart review, 22 subjects were excluded based on predefined criteria, DNA samples from 88 affected infants and at least one parent were collected and genotyped. Thirty-two single nucleotide polymorphisms (SNPs) in twelve genes involved in vasoconstriction/vasodilation, lung development, surfactant regulation, or vascular endothelial cell function were investigated using family-based association tests. PPHN was significantly ($p<0.05$) associated with genetic variants in corticotropin releasing hormone receptor 1, *CRHR1* (rs4458044, $p=9.9\times10^{-5}$; rs173365, p=0.02) and corticotropin releasing hormone-binding protein, *CRHBP* (rs10062367, p=0.009 and rs10055255, p=0.003).

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Association with *CRHR1* rs4458044 passed the Bonferroni threshold for significance. No mutations were found in the *BMPR2* gene. We describe previously unreported genetic associations between PPHN and *CRHR1* and *CRHBP*. These findings may have implications for further understanding the pathophysiology of PPHN and treatment.

INTRODUCTION

Persistent pulmonary hypertension of the newborn (PPHN) is a serious and often rapidly progressive disease. If untreated, PPHN can lead to severe hypoxemia, right ventricular failure and death. Combined with greater recognition, advances made in the treatment of neonatal pulmonary disease, such as mechanical ventilation, inhaled nitric oxide (iNO) and extracorporeal membrane oxygenation (ECMO) have greatly improved the survival and outcomes of infants with PPHN. Even so, PPHN remains a disease of substantial mortality and neonates that survive may suffer long-term consequences as a result of the associated hypoxemia and invasive therapies (1–4).

Idiopathic PPHN occurs in 1–2 per 1,000 live born infants (1, 2, 5), typically presenting as hypoxemic respiratory failure within the first day of life in term or late preterm infants without associated congenital anomalies or cyanotic cardiac lesions. In normal physiology, fetal lungs have high pulmonary vascular resistance (PVR) and receive only 5–10% of cardiac output. At birth, PVR normally decreases, pulmonary blood flow increases dramatically, amniotic fluid clears from the lungs and effective gas exchange begins. In PPHN, fetal lungs fail to undergo this normal physiologic transition. PVR remains high, restricting pulmonary blood flow, resulting in hypoxemia, which leads to vasoconstriction, decreased gas exchange, acidemia and hypercarbia. On pathological study, neonates with PPHN are found to have marked pulmonary vascular remodeling with torturous vessels and excessive muscularization from pulmonary arteriole smooth muscle cell proliferation (6).

Pulmonary hypertension is a complex and heterogeneous disease. Although many hypotheses have been proposed, the exact etiology leading to PPHN remains unknown (6). Recent studies of pulmonary hypertension in adults (pulmonary arterial hypertension) and neonates (PPHN) have increased our understanding of the genetic basis of these distinct diseases. Genetic susceptibility to PPHN has been inferred based on studies of environmental risk factors, such as non-steroidal anti-inflammatory drugs and selective serotonin reuptake inhibitors (SSRIs) taken during pregnancy and variation in the development and severity of disease (7–9). Genes involved in the transforming growth factor-beta (TGF-beta) super family (10, 11), nitric oxide pathway (12–14) stress and inflammatory response (15–17), surfactant regulation (18, 19) and glucocorticoids (20, 21) have all been implicated in adult and/or neonatal pulmonary disease. Nearly 300 disease-causing genetic variants have been described in bone morphogenic protein receptor, type II (*BMPR2*), associated with >70% of familial (non-neonatal) pulmonary hypertension cases (22, 23). Nitric oxide synthase 3 (*NOS3*) polymorphisms in T-786C and Glu298Asp have been shown to contribute to altered pulmonary vascular reactivity in adults (12). We therefore evaluated 32 single nucleotide polymorphisms (SNPs) in 12 genes with biologic plausibility of contributing to PPHN.

METHODS

Study Design & Population

This study was approved by the University of Iowa Institutional Review Board (IRB 200307031). Neonates diagnosed with and treated for PPHN at the University of Iowa Children's Hospital (UICH) between 1993 and 2009 (and their families) were considered for

enrollment into the study. Inclusion criteria for this family-based, candidate gene association study were: a neonate with hypoxemic respiratory failure with the clinical diagnosis of pulmonary hypertension. All participants provided informed consent. After medical chart review, patients were excluded from genetic analysis based on predetermined exclusion criteria: gestational age < 35 weeks, multiple major congenital anomalies, congenital diaphragmatic hernia, cyanotic heart disease and/or the inability to obtain a DNA sample from the neonate and at least one parent. Neonates with hypoxic respiratory failure were diagnosed with PPHN by the medical team using echocardiography, preductal/postductal oxygen saturation difference >10%, and/or a clinical response to iNO. Echocardiographic findings consistent with PPHN included elevated pulmonary artery pressure compared with systemic pressure, right-to-left or bidirectional patent ductus arteriosus shunting and right-to-left or bidirectional shunting through the patent foramen ovale. In total, 110 families were enrolled in the study. Following medical chart review, twenty-two subjects were excluded from genetic analysis.

Samples were obtained retrospectively from either a DNA repository at the University of Iowa (n=60) or recruited after medical record review (n=28) to capture families not represented in the DNA repository. A maternal interview was conducted for families not previously enrolled in the DNA repository database. Samples were obtained from venous blood, cord blood, buccal swabs or saliva samples. DNA was extracted using standard protocols (Qiagen Maxi Kit, Hilden, Germany).

Genotyping

Candidate genes were identified by a review of the literature and genes associated with adult or neonatal pulmonary disease and/or with biologic plausibility were selected. Thirty-two SNPs in 12 genes were selected and genotyped utilizing Applied Biosystems (Foster City, CA) TaqMan chemistry, as noted in Table 1. SNPs were selected according to haplotype blocks to minimize number of SNPs selected per gene, high minor allele frequency to increase informativity of each SNP and known functional impact. Allelic end-point fluorescence was measured and analyzed on a 7900HT PCR system (Applied Biosystems) and interpreted with Sequence Detection System software (SDS, version 2.3, Applied Biosystems). Greater than 98% of samples tested provided usable genotype information. Genotypes were entered into a laboratory database (Progeny, South Bend, IN) in order to generate datasets for analysis.

Statistical Analysis

Statistical analyses of the genotype data were performed with a transmission disequilibrium test (TDT), using family-based association test software (FBAT, Cambridge, MA) (24–26). Our primary outcome was neonatal hypoxic respiratory failure with clinical or echocardiographic findings consistent with PPHN. TDT measures over-transmission of an allele from heterozygous parents to offspring by comparing whether transmission proportions are compatible with Mendelian probabilities. This method maintains its robustness by evaluating genetic linkage only in the face of genetic association and has been shown to have sufficient power to detect even disease determinates of a relatively small effect (27). To correct for the multiple tests performed in this study, a conservative Bonferroni correction would place significance at $p<0.0016$ using a standard α of 0.05. However, given the exploratory nature of this initial study, less stringent values are also of interest.

While performing subject chart reviews, we observed a high number of abnormal congenital adrenal hyperplasia (CAH) newborn screen (NBS) results in our cohort. This test measures the level of 17-hydroxyprogesterone (17-OHP), a substrate in the steroid hormone pathway

upstream of both cortisol and testosterone. CAH is due to a deficient enzyme in this pathway (usually 21-hydroxylase) leading to the buildup of 17-OHP and corresponding insufficient steroid hormone. All CAH tests were processed by the University of Iowa Hygienic Laboratory. To test the hypothesis that abnormal steroid hormone metabolism is linked to PPHN, we obtained the 17-OHP values for our PPHN affected cases and 87 unaffected controls matched for gender, gestational age, birth weight and year of NBS test. As very preterm infants have higher rates of false-positive CAH tests attributed to an immature hypothalamic-pituitary-adrenal (HPA) axis, we only analyzed term cases and controls (≥37weeks gestation; 58 cases, 60 controls) to separate gestational age effect (28). Standard clinical procedure is to repeat abnormal (borderline or presumptive positive) NBS CAH tests. We used the first obtained 17-OHP result for our analysis. We looked at medical records and repeat 17-OHP tests to assess if any subjects were true-positive CAH. We had no true-positive CAH subjects in cases or controls and all repeat 17-OHP values normalized. Data was checked for normality and transformed using the Box-Cox statistical method (29). Analysis was performed using linear regression, adjusting for major lot changes in the assay. Categorical results were compared using Fischer's exact test.

Sequencing

Each of the 13 exons of *BMPR2* was sequenced to include at least 50 base pairs of flanking intronic sequencing using methods described previously (30). All affected cases with adequate DNA sample were sequenced (n=72); 68 (94%) provided adequate sequencing results.

RESULTS

We analyzed genotype data for 88 cases and their families. Of these, 72 cases were part of a complete triad (mother, father, affected infant) and 16 were part of a diad (one parent, affected infant). Our proband cohort was predominantly male (67%) and Caucasian (89%). Average gestational age was 38.3 (+/−1.8) weeks. Average birth weight was 3512g; 31 (35%) cases were large for gestational age (LGA). Sixty (68%) cases required iNO or ECMO. Thirty-four cases (39%) received hydrocortisone for blood pressure support in addition to vasopressors, which a total of 47 (53%) cases received. A description of the study cases and mothers is provided in Table 2.

Associations were identified in Corticotropin Releasing Hormone Receptor 1, *CRHR1* (rs4458044, $p=9.9\times10^{-5}$ and rs173365, p=0.02) and Corticotropin Releasing Hormone Binding Protein, *CRHBP* (rs10062367, p=0.009 and rs10055255, p=0.003) in infants with PPHN. The result for rs4458044 remains significant after using the Bonferroni correction (p<0.0016). The major allele was overtransmitted in *CRHR1* rs4458044 and *CRHBP* rs10062367 and rs10055255. The minor allele was overtransmitted in *CRHR1* rs173365.

For the newborn screen analysis, there was no significant difference in gestational age, birth weight or race between cases and controls. Mean 17-OHP was significantly higher in cases verses controls ($p=1.7\times10^{-4}$), as shown in Table 3. Our term PPHN affected cases had a 14% abnormal CAH test result rate verses 0% for unaffected controls (p=0.002). In 2000, Iowa's rate of false-positive CAH tests for all babies was 72 out of 38,141 tests or 0.19%, when using the more conservative estimate of assuming all babies lost to follow-up may have had false-positive results. (31).

No previously unreported consensus splice site, missense or nonsense mutations were found in sequenced *BMPR2* exons. A previously reported missense mutation (rs2228545) was heterozygous in five cases. According to HapMap samples, this SNP has a population

frequency of 5% (32). We did not find overtransmission of *BMPR2* rs13010656 (p=0.50), which is in linkage disequilibrium with the sequence variant.

DISCUSSION

We have identified genetic variants in *CRHR1* and *CRHBP* associated with PPHN. Our data show a robust and highly significant association with variations in the *CRHR1* gene, particularly rs4458044. Both the Corticotropin Releasing Hormone (CRH) receptor 1 (encoded by *CRHR1*) and binding protein (encoded by *CRHBP*) interact with CRH in the HPA axis and the myometrium, with cortisol as the ultimate end product. We found no evidence that mutations in *BMPR2*, previously associated with adult onset pulmonary arterial hypertension, make a significant contribution PPHN.

Corticotropin-releasing hormone binding protein binds CRH, decreasing its bioavailability and hence controlling the reactivity of CRH in pregnancy. *CRHBP* is located on chromosome 5; significant results, rs10062367 and rs10055255, are located between exons 6 and 7. In contrast, CRHR1 is a seven-transmembrane receptor with high affinity for CRH located in the anterior pituitary and myometrium, which plays an essential role in transmitting the ACTH signal for cortisol production. We investigated SNPs along *CRHR1*, located on chromosome 17; significant results were found in intronic regions between exons 4 and 5 (rs173365) and exons 1 and 2 (rs4458044). Downstream rs4458044, in the same intronic region, is a transcription factor binding site for peroxisome proliferator-activated receptor gamma (PPAR-γ). PPAR-γ is an essential regulator of pulmonary smooth muscle cell proliferation and vascular tone. Decreased expression of PPAR-γ has been associated with pulmonary hypertension and neonatal chronic lung disease (33). No other SNPs or genes yielded significant results.

Cortisol plays a vital role in fetal development. It is commonly accepted that maturation of the fetal HPA axis and fetal steroid production is necessary for *in utero* lung development and ex-utero pulmonary transition (29, 34, 35). Glucocorticoid supplementation for threatened preterm birth has been an extremely effective therapy in preventing neonatal respiratory distress syndrome. Yet, fetuses with conditions of deficient cortisol secretion, such as congenital adrenal hyperplasia, do not necessarily have lung immaturity, suggesting fetal cortisol is not requisite for fetal lung development. In mice, *CRHR1* knockouts are phenotypically normal when born to a *CRHR1+/−* mother, but *CRHR1−/−* pups born to knockout females die within 48 hours due to respiratory distress. On pathologic review, the pup lungs show significant dysplasia, characterized by hypercellularity, immaturity and intra-alveolar hemorrhage. Reports do not comment on pulmonary vascular changes. This lethal neonatal phenotype is completely rescued with maternal cortisol supplementation (34, 36, 37). Thus it appears that either maternal or fetal cortisol is sufficient for normal lung development, but that one source is absolutely required.

CRH, CRHR1 and CRHBP have different secretion patterns, expression and roles in pregnancy than in other physiologic states (38, 39). Given this, it is possible that the polymorphisms we describe only have a significant clinical effect on the pulmonary vasculature *in utero* and during the transition from fetus to neonate. In fact, while PPHN overall has a high burden of morbidity and mortality, follow-up of neonates who successfully responded to PPHN treatment found that the capacity of long-term normal development with no clinically significant pulmonary sequelae exists (40, 41). If the genetic polymorphisms we describe are associated with decreased CRH binding to CRHR1 or an excessive binding by CRHBP, the resulting decreased signal by CRH would diminish the activity of the HPA axis and affect either fetal lung functional development or the capacity to adequately transition to *ex utero* life.

In critical illnesses, the HPA axis is activated, resulting in increased cortisol secretion, a crucial effect for homeostasis (42). Transient adrenal insufficiency, marked by low cortisol despite increased physiologic demand due to stress, has been described in both preterm (43, 44) and term infants (45–47) and is associated with increased hemodynamic instability, poor outcomes and longer illnesses (48, 49). Studies have found that infants with severe lung disease requiring ventilation had lower rates of cortisol in the first seven days of life and that early treatment with corticosteroids can decrease pulmonary oxidative stress and increased pulmonary arterial response to vasodilators (50–52). When we probed deeper into the high number of abnormal CAH NBS results in our cohort (14%), we found that mean 17-OHP levels were significantly higher in term PPHN cases verses controls ($p=1.7\times10^{-4}$). As we only noticed the abnormal NBS results incidentally during retrospective chart review, a limitation of our study is that we were not able to measure cortisol levels to confirm whether an abnormally high 17-OHP was the result of a buildup of substrate, as the CAH screen is designed to measure, or an overall activation of the HPA axis. Clinically, many of our PPHN cases showed signs of transient adrenal insufficiency such as hypotension, hemodynamic instability, and inadequate stress response. Forty-seven (53%) cases received vasopressors, of which 34 (39%) also received postnatal glucocorticoids. Further investigation into the cortisol levels of neonates with PPHN may provide insight into the biologic consequences of genetic variation in *CRHR1* and *CRHBP*. If adrenal insufficiency is causally linked to PPHN and/or the hemodynamic instability that often accompanies it, glucocorticoids may be an important adjuvant treatment for both PPHN and hypotension, in addition to or instead of vasopressors.

In addition to not confirming 17-OHP results with cortisol measurements, an important limitation of our study is its retrospective nature over 16 years. While many clinical treatment advances occurred during this time, these do not affect the genetics of the affected individuals and therefore our study. Efforts were taken to minimize retrospective selection bias. All neonates diagnosed with PPHN at our center were thoroughly investigated for inclusion. Second, given that UICH is a major referral center in Iowa, our cohort was skewed toward PPHN requiring more invasive treatments, like iNO and ECMO. Due to power issues and our focus on genetic contributions rather than clinical outcome, we did not separate subjects for heterogeneity, such as treatment required or possible inciting factors like meconium aspiration. Third, not all cases of PPHN were confirmed with echocardiography. Finally, our case-control study was limited to a 1:1 study. However, given state-wide NBS population statistics, we do not anticipate our results would markedly differ with additional controls. Thirty-five percent of our cohort was LGA, which reflects epidemiology studies that report this risk factor for PPHN (53). Strengths of the study include that it was adequately powered for an uncommon neonatal disease, allowing us to draw statistically relevant conclusions. Second, we replicated all of our significant findings with a SNP in complete linkage disequilibrium to ensure our results were not technical artifacts. Third, all tests and methods were performed at single centers, minimizing differences in understanding of standard protocols, techniques and style.

In conclusion, while other studies looking at PPHN have focused primarily on vasodilators/vasoconstrictors, surfactant, vascular growth factors and more recently serotonin receptors, we introduce that the HPA axis and specifically corticotropin releasing hormone signaling and bioreactivity resulting in altered cortisol levels may play a vital role in pulmonary transition from fetal to neonatal life. To our knowledge, this is the first report identifying genetic variants in *CRHR1* and *CRHBP* associated with PPHN.

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ABBREVIATIONS

17-OHP	17-hydroxyprogesterone
BMPR2	Bone morphogenetic protein receptor type II
CAH	Congenital Adrenal Hyperplasia
CRH	Corticotropin Releasing Hormone
CRHBP	Corticotropin Releasing Hormone Binding Protein
CRHR1	Corticotropin Releasing Hormone Receptor
ECMO	Extracorporal Membrane Oxygenation
HPA	Hypothalamic-Pituitary-Adrenal
iNO	Inhaled Nitric Oxide
NBS	Newborn Screen
PPAR	Peroxisome Proliferator-Activated Receptor
PPHN	Persistent Pulmonary Hypertension of the Newborn
PVR	Pulmonary Vascular Resistance
SNP	Single Nucleotide Polymorphism

REFERENCES

1. Walsh-Sukys MC, Tyson JE, Wright LL, Bauer CR, Korones SB, Stevenson DK, Verter J, Stoll BJ, Lemons JA, Papile LA, Shankaran S, Donovan EF, Oh W, Ehrenkranz RA, Fanaroff AA. Persistent pulmonary hypertension of the newborn in the era before nitric oxide: practice variation and outcomes. Pediatrics. 2000; 105:14–20. [PubMed: 10617698]
2. Farrow KN, Fliman P, Steinhorn RH. The diseases treated with ECMO: focus on PPHN. Semin Perinatol. 2005; 29:8–14. [PubMed: 15921147]
3. John E, Roberts V, Burnard ED. Persistent pulmonary hypertension of the newborn treated with hyperventilation: clinical features and outcome. Aust Paediatr J. 1988; 24:357–361. [PubMed: 3149464]
4. Clark RH, Huckaby JL, Kueser TJ, Walker MW, Southgate WM, Perez JA, Roy BJ, Keszler M. Clinical Nitric Oxide Research Group (CINRGI). Low-dose nitric oxide therapy for persistent pulmonary hypertension: 1-year follow-up. J Perinatol. 2003; 23:300–303. [PubMed: 12774137]
5. Hageman JR, Adams MA, Gardner TH. Persistent pulmonary hypertension of the newborn. Trends in incidence, diagnosis, and management. Am J Dis Child. 1984; 138:592–595. [PubMed: 6720647]

6. Abman SH. New developments in the pathogenesis and treatment of neonatal pulmonary hypertension. Pediatr Pulmonol Suppl. 1999; 18:201–204. [PubMed: 10093143]
7. Chambers CD, Hernandez-Diaz S, Van Marter LJ, Werler MM, Louik C, Jones KL, Mitchell AA. Selective serotonin-reuptake inhibitors and risk of persistent pulmonary hypertension of the newborn. N Engl J Med. 2006; 354:579–587. [PubMed: 16467545]
8. Chambers CD, Johnson KA, Dick LM, Felix RJ, Jones KL. Birth outcomes in pregnant women taking fluoxetine. N Engl J Med. 1996; 335:1010–1015. [PubMed: 8793924]
9. Alano MA, Ngougmna E, Ostrea EM Jr, Konduri GG. Analysis of nonsteroidal antiinflammatory drugs in meconium and its relation to persistent pulmonary hypertension of the newborn. Pediatrics. 2001; 107:519–523. [PubMed: 11230592]
10. Harrison RE, Berger R, Haworth SG, Tulloh R, Mache CJ, Morrell NW, Aldred MA, Trembath RC. Transforming growth factor-beta receptor mutations and pulmonary arterial hypertension in childhood. Circulation. 2005; 111:435–441. [PubMed: 15687131]
11. Sztrymf B, Yaici A, Girerd B, Humbert M. Genes and pulmonary arterial hypertension. Respiration. 2007; 74:123–132. [PubMed: 17318011]
12. Casas JP, Cavalleri GL, Bautista LE, Smeeth L, Humphries SE, Hingorani AD. Endothelial nitric oxide synthase gene polymorphisms and cardiovascular disease: a HuGE review. Am J Epidemiol. 2006; 164:921–935. [PubMed: 17018701]
13. Pearson DL, Dawling S, Walsh WF, Haines JL, Christman BW, Bazyk A, Scott N, Summar ML. Neonatal pulmonary hypertension--urea-cycle intermediates, nitric oxide production, and carbamoyl-phosphate synthetase function. N Engl J Med. 2001; 344:1832–1838. [PubMed: 11407344]
14. Villanueva ME, Zaher FM, Svinarich DM, Konduri GG. Decreased gene expression of endothelial nitric oxide synthase in newborns with persistent pulmonary hypertension. Pediatr Res. 1998; 44:338–343. [PubMed: 9727710]
15. Lavoie PM, Pham C, Jang KL. Heritability of bronchopulmonary dysplasia, defined according to the consensus statement of the national institutes of health. Pediatrics. 2008; 122:479–485. [PubMed: 18762515]
16. Nickel N, Kempf T, Tapken H, Tongers J, Laenger F, Lehmann U, Golpon H, Olsson K, Wilkins MR, Gibbs JS, Hoeper MM, Wollert KC. Growth differentiation factor-15 in idiopathic pulmonary arterial hypertension. Am J Respir Crit Care Med. 2008; 178:534–541. [PubMed: 18565955]
17. Silverman ES, Breault DT, Vallone J, Subramanian S, Yilmaz AD, Mathew S, Subramaniam V, Tantisira K, Pacak K, Weiss ST, Majzoub JA. Corticotropin-releasing hormone deficiency increases allergen-induced airway inflammation in a mouse model of asthma. J Allergy Clin Immunol. 2004; 114:747–754. [PubMed: 15480311]
18. Pavlovic J, Papagaroufalis C, Xanthou M, Liu W, Fan R, Thomas NJ, Apostolidou I, Papathoma E, Megaloyianni E, DiAngelo S, Floros J. Genetic variants of surfactant proteins A, B, C, and D in bronchopulmonary dysplasia. Dis Markers. 2006; 22:277–291. [PubMed: 17264398]
19. Kunig AM, Parker TA, Nogee LM, Abman SH, Kinsella JP. ABCA3 deficiency presenting as persistent pulmonary hypertension of the newborn. J Pediatr. 2007; 151:322–324. [PubMed: 17719949]
20. Rogers AJ, Tantisira KG, Fuhlbrigge AL, Litonjua AA, Lasky-Su JA, Szefler SJ, Strunk RC, Zeiger RS, Weiss ST. Predictors of poor response during asthma therapy differ with definition of outcome. Pharmacogenomics. 2009; 10:1231–1242. [PubMed: 19663668]
21. Melnick M, Choy HA, Jaskoll T. Glucocorticoids, tumor necrosis factor-alpha, and epidermal growth factor regulation of pulmonary morphogenesis: a multivariate in vitro analysis of their related actions. Dev Dyn. 1996; 205:365–378. [PubMed: 8901048]
22. Sztrymf B, Coulet F, Girerd B, Yaici A, Jais X, Sitbon O, Montani D, Souza R, Simonneau G, Soubrier F, Humbert M. Clinical outcomes of pulmonary arterial hypertension in carriers of BMPR2 mutation. Am J Respir Crit Care Med. 2008; 177:1377–1383. [PubMed: 18356561]
23. Machado RD, Eickelberg O, Elliott CG, Geraci MW, Hanaoka M, Loyd JE, Newman JH, Phillips JA3rd, Soubrier F, Trembath RC, Chung WK. Genetics and genomics of pulmonary arterial hypertension. J Am Coll Cardiol. 2009; 54(Suppl 1):S32–S42. Review. [PubMed: 19555857]

24. Spielman RS, McGinnis RE, Ewens WJ. Transmission test for linkage disequilibrium: the insulin gene region and insulin-dependent diabetes mellitus (IDDM). Am J Hum Genet. 1993; 52:506–516. [PubMed: 8447318]
25. Horvath S, Xu X, Laird NM. The family based association test method: strategies for studying general genotype--phenotype associations. Eur J Hum Genet. 2001; 9:301–306. [PubMed: 11313775]
26. Laird NM, Horvath HS, Xu X. Implementing a unified approach to family-based tests of association. Genet Epidemiol. 2000; 19:S36–S42. [PubMed: 11055368]
27. Risch N, Teng J. The relative power of family-based and case-control designs for linkage disequilibrium studies of complex human diseases. I. DNA pooling. Genome Res. 1998; 8:1273–1288. [PubMed: 9872982]
28. Lee JE, Moon Y, Lee MH, Jun YH, Oh KI, Choi JW. Corrected 17-alpha-hydroxyprogesterone values adjusted by a scoring system for screening congenital adrenal hyperplasia in premature infants. Ann Clin Lab Sci. 2008; 38:235–240. [PubMed: 18715851]
29. Box GEP, Cox DR. An analysis of transformations. J Roy Statistical Society [Ser B]. 1964; 26:211–252.
30. Ehn NL, Cooper ME, Orr K, Shi M, Johnson MK, Caprau D, Dagle J, Steffen K, Johnson K, Marazita ML, Merrill D, Murray JC. Evaluation of fetal and maternal genetic variation in the progesterone receptor gene for contributions to preterm birth. Pediatr Res. 2007; 62:630–5. [PubMed: 17805208]
31. National Newborn Screening and Genetics Resource Center (NNSGRC). National Newborn Screening Report. Austin: University of Texas; 2003. Chapter 9: Congenital Adrenal Hyperplasia; p. 121-133.
32. International HapMap Consortium. The International HapMap Project. Nature. 2003; 426:789–796. [PubMed: 14685227]
33. Simon DM, Mariani TJ. Role of PPARs and retinoid X receptors in the regulation of lung maturation and development. PPAR Res. 2007; 2007:91240. [PubMed: 17710236]
34. Muglia L, Jacobson L, Dikkes P, Majzoub JA. Corticotropin-releasing hormone deficiency reveals major fetal but not adult glucocorticoid need. Nature. 1995; 373:427–432. [PubMed: 7830793]
35. Garbrecht MR, Klein JM, Schmidt TJ, Snyder JM. Glucocorticoid metabolism in the human fetal lung: implications for lung development and the pulmonary surfactant system. Biol Neonate. 2006; 89:109–119. [PubMed: 16195667]
36. Smith GW, Aubry JM, Dellu F, Contarino A, Bilezikjian LM, Gold LH, Chen R, Marchuk Y, Hauser C, Bentley CA, Sawchenko PE, Koob GF, Vale W, Lee KF. Corticotropin releasing factor receptor 1-deficient mice display decreased anxiety, impaired stress response, and aberrant neuroendocrine development. Neuron. 1998; 20:1093–1102. [PubMed: 9655498]
37. Muglia LJ, Bae DS, Brown TT, Vogt SK, Alvarez JG, Sunday ME, Majzoub JA. Proliferation and differentiation defects during lung development in corticotropin-releasing hormone deficient mice. Am J Respir Cell Mol Biol. 1999; 20:181–188. [PubMed: 9922208]
38. Brosnan PG. The hypothalamic pituitary axis in the fetus and newborn. Semin Perinatol. 2001; 25:371–384. [PubMed: 11778908]
39. Grammatopoulos D, Dai Y, Chen J, Karteris E, Papadopoulou N, Easton AJ, Hillhouse EW. Human corticotropin-releasing hormone receptor: differences in subtype expression between pregnant and nonpregnant myometria. J Clin Endocrinol Metab. 1998; 83:2539–2544. [PubMed: 9661640]
40. Hoskote AU, Castle RA, Hoo AF, Lum S, Ranganathan SC, Mok QQ, Stocks J. Airway function in infants treated with inhaled nitric oxide for persistent pulmonary hypertension. Pediatr Pulmonol. 2008; 43:224–235. [PubMed: 18203182]
41. Bernbaum JC, Russell P, Sheridan PH, Gewitz MH, Fox WW, Peckham GJ. Long-term follow-up of newborns with persistent pulmonary hypertension. Crit Care Med. 1984; 12:579–583. [PubMed: 6734225]
42. Lamberts SW, Bruining HA, de Jong FH. Corticosteroid therapy in severe illness. N Engl J Med. 1997; 337:1285–1292. [PubMed: 9345079]

43. Martins PG, Procianoy RS. Cortisol and 17-alpha-hydroxy-progesterone levels in infants with refractory hypotension born at 30 weeks of gestation or less. Braz J Med Biol Res. 2007; 40:577–582. [PubMed: 17401502]
44. Quintos JB, Boney CM. Transient adrenal insufficiency in the premature newborn. Curr Opin Endocrinol Diabetes Obes. 2010; 17:8–12. [PubMed: 19881342]
45. Tantivit P, Subramanian N, Garg M, Ramanathan R, deLemos RA. Low serum cortisol in term newborns with refractory hypotension. J Perinatol. 1999; 19:352–357. [PubMed: 10685256]
46. Fernandez EF, Watterberg KL. Relative adrenal insufficiency in the preterm and term infant. J Perinatol. 2009; 29(Suppl 2):S44–S49. [PubMed: 19399009]
47. Fernandez E, Schrader R, Watterberg K. Prevalence of low cortisol values in term and near-term infants with vasopressor-resistant hypotension. J Perinatol. 2005; 25:114–118. [PubMed: 15526013]
48. Kamath BD, Fashaw L, Kinsella JP. Adrenal insufficiency in newborns with congenital diaphragmatic hernia. J Pediatr. 2010; 156:495–497. [PubMed: 20056240]
49. Michalaki M, Margeli T, Tsekouras A, Gogos CH, Vagenakis AG, Kyriazopoulou V. Hypothalamic-pituitary-adrenal axis response to the severity of illness in non-critically ill patients: does relative corticosteroid insufficiency exist? Eur J Endocrinol. 2010; 162:341–347. [PubMed: 19903798]
50. Chandrasekar I, Eis A, Konduri GG. Betamethasone attenuates oxidant stress in endothelial cells from fetal lambs with persistent pulmonary hypertension. Pediatr Res. 2008; 63:67–72. [PubMed: 18043518]
51. Ng PC, Lam CW, Lee CH, Ma KC, Fok TF, Chan IH, Wong E. Reference ranges and factors affecting the human corticotropin-releasing hormone test in preterm, very low birth weight infants. J Clin Endocrinol Metab. 2002; 87:4621–4628. [PubMed: 12364445]
52. da Costa DE, Nair AK, Pai MG, Al Khusaiby SM. Steroids in full term infants with respiratory failure and pulmonary hypertension due to meconium aspiration syndrome. Eur J Pediatr. 2001; 160:150–153. [PubMed: 11277374]
53. Hernandez-Diaz S, Van Marter LJ, Werler MM, Louik C, Mitchell AA. Risk factors for persistent pulmonary hypertension of the newborn. Pediatrics. 2007; 120:e272–e282. [PubMed: 17671038]

TABLE 1

CANDIDATE GENES

Genes/SNPs were selected based on literature review and biologic plausibility.

Gene	Gene Name	Markers
BMPR-2	Bone Morphogenic Protein Receptor type II	rs6747299, rs6436149, rs13010656
SFTP-B	Surfactant Protein B	rs1030862
EPAS-1	Endothelial PAS Domain Protein	rs1867785
PDE-5A	Phosphodiesterase 5A	rs2389886, rs3822194, rs1480931
SOD-3	Superoxide Dismutase 3	rs2536512
NR3C1	Glucocorticoid Receptor	rs12656106, rs33383
CRHBP	Corticotropin Releasing Hormone Binding Protein	rs10062367, rs1700678, rs10055255, rs1875999
VEGFA	Vascular Endothelial Growth Factor	rs2010963
NOS-3	Nitric Oxide Synthase 3	rs1808593, rs2373962, rs10277237
SFTP-C	Surfactant Protein C	rs8192306, rs2070687, rs7592
SFTP-D	Surfactant Protein D	rs2243639, rs721917, rs2256703
CRHR1	Corticotropin Releasing Hormone Receptor I	rs173365, rs242944, rs4458044, rs7225082, rs11079718, rs1876831, rs8064870

TABLE 2

COHORT DESCRIPTORS

Characteristics of PPHN cases and mothers of cases.

NEONATAL CHARACTERISTICS	**n=88**
Male	59 (67%)
Birth weight	3512 +/−596 grams
Gestational Age	38.3 +/−1.8 weeks
Caucasian	78 (89%)
Postdate (>40weeks)	11 (13%)
Premature (<37 weeks)	18 (20%)
APGAR <7 at 5 minutes	16 (18%)
Pneumonia	19 (22%)
Pneumothorax	26 (30%)
Meconium Aspiration	21 (24%)
Large for Gestational Age	31 (35%)
Discharge on Oxygen	13 (15%)
MATERNAL CHARACTERISTICS	
Maternal Age at Delivery	28.9 +/−5.7 years
Cesearan Section Mode of Delivery	45 (51%)
Complications of Delivery	39 (44%)
Group B Strep Positive	10 (11%)
History of Depression	9 (10%)
SSRI Treatment during Pregnancy	4 (5%)
COHORT DESCRIPTORS	
Triads (proband + mother + father)	72 (82%)
Diads (proband + 1 parent)	16 (18%)
Treated with iNO &/or ECMO	60 (68%)
UICH Hospital of Birth	23 (26%)
Families with >1 case infant	3 (3%)

TABLE 3
NBS CASE-CONTROL 17-OHP ANALYSIS

Term PPHN cases and unaffected controls matched for birth weight, year of birth, gender, and gestational age.

	Cases	Control	p-values
Total Number	58	60	
Males	39 (67%)	44 (73%)	0.55
Females	19 (33%)	16 (27%)	0.55
Caucasian	52 (90%)	46 (77%)	0.34
Gestational age (Mean +/− SD)	39.0 weeks (+/−1.4)	39.1 weeks (+/−1.1)	0.46
Birth weight (Mean +/− SD)	3676 grams (+/−571)	3565 grams (+/−457)	0.25
Number of abnormal NBS CAH results	8 (14%)	0 (0%)	**0.002**
Borderline	6 (10%)	0 (0%)	
Presumptive Positive	2 (4%)	0 (0%)	
17-OHP (Mean +/− SD)	30 ng/mL (26)	19 ng/mL (9)	**0.00017**