Research Article ISSN 2689-1085

Journal of Pediatrics & Neonatology

Association of the *SOD2* Rs5746136 *C>T* Polymorphisms with The Risk of Persistent Pulmonary Hypertension of The Newborn

Lemus-Varela ML[1], García-Valdez LM[1], Ramírez-Patiño R[2], Zúñiga-González GM[3] and Gallegos-Arreola MP[4]*

[1]*Department de Neonatología, Hospital de Pediatría, UMAE, Centro Médico, Nacional de Occidente, Instituto Mexicano del Seguro Social (IMSS), Guadalajara, Jalisco, México.*

[2]*Department of Medical and Life Sciences. Ciénega University Center. University of Guadalajara. Ocotlán México.*

[3]*Laboratorio de Mutagénesis, División de Medicina Molecular, Centro de Investigación Biomédica de Occidente, Instituto Mexicano del Seguro Social, Guadalajara, Jalisco, México.*

[4]*División Genética, Centro de Investigación Biomédica de Occidente, Instituto Mexicano del Seguro Social, Guadalajara, Jalisco, México.*

***Correspondence:**
Dr. Martha Patricia Gallegos Arreola, Sierra Mojara 800, Col. Independencia, C.P. 44340, División de Genética, Centro de Investigación Biomédica de Occidente, Instituto Mexicano del Seguro Social, Tel: +52 33 36170060 (31936), Fax: +52 33 36181756.

Received: 02 May 2021; **Accepted:** 28 May 2021

Citation: Lemus-Varela ML, García-Valdez LM, Ramírez-Patiño R, et al. Association of the *SOD2* Rs5746136 *C>T* Polymorphisms with The Risk of Persistent Pulmonary Hypertension of The Newborn. J Pediatr Neonatal. 2021; 3(2): 1-5.

ABSTRACT

Background: *Persistent pulmonary hypertension of a newborn (PPHN) is a serious condition with a high morbidity and mortality rate, with a complex therapeutic approach. Recently, genetic associations have been found in patients with PPHN, the role of some polymorphisms that explain its pathogenesis has not been well defined.*

Objective: *To determine the frequency of association of SOD2 gene polymorphisms (rs5746136 C>T) in PPHN in the Mexican population.*

Methods: *We included 155 Mexican infants; 76 with PPHN, confirmed by echocardiography (study group), and 79 healthy newborns (control group) and by polymerase chain reaction (PCR) and RFLPs was identified the rs5746136 polymorphism in SOD2 gene.*

Results: *The group with PPHN had a lower gestational age (35.6 ± 2.81 weeks versus 38.1 ± 1.9; P=0.008) and a lower mean body weight (2,622 ± 626 g versus 2,992 ± 565 g, P=0.052) than the controls respectively. The rs5746136 C>T polymorphism was associated with PPHN susceptibility, when PPHN patients and the control group were compared for the TT genotype (OR 8.1, 95%CI 2.65-24.9; P=0.0001), the CT/TT genotype (OR 6.5, 95% CI 3.12-13.5; P=0.0001), and the T allele (OR 4.3, 95% CI 2.63-7.02; P=0.0001).*

Conclusion: *We found significant differences in the association of rs5746136 C>T polymorphism of SOD2 gene in PPHN of the Mexican population analyzed.*

Keywords

eNOS, Persistent pulmonary hypertension of the newborn, Polymorphisms SOD2, rs5746136.

Abbreviations

PPHN: persistent pulmonary hypertension of the newborn; SOD: Superoxide dismutase; eNOS: Endothelial nitric oxide synthase; NO: nitric oxide.

Introduction

Persistent pulmonary hypertension of the newborn (PPHN) results from failure to initiate or to sustain the transition from fetal to neonatal circulation, which normally occurs after birth [1]. This serious condition is characterized by sustained elevation of pulmonary vascular resistances, and in the setting of normal or low systemic vascular resistance; which leads to extra-pulmonary right to left shunting across the patent *ductus arteriosus* and/or foramen *ovale.* This can result in life threatening hypoxemia, right ventricular failure and even death [2].

PPHN occurs mostly in term or near-term neonates, in 2/1000 live births can result secondary to neonatal pulmonary disease such as meconium aspiration syndrome, pneumonia, sepsis, respiratory distress syndrome, congenital diaphragmatic hernia, pulmonary hypoplasia. PPHN carries high rate of morbidity and mortality, particularly in limited resource settings (low income and/or developing country), Echocardiography remains the gold standard for diagnosis of PPHN and faces a complex therapeutic approach such as: inhaled nitric oxide (iNO), high frequency oscillatory ventilation, extracorporeal membrane oxygenation, and other pulmonary vasodilators agents like sildenafil can reduce the mortality rate [3].

Recent studies have increased our understanding of the genetic basis of the PPHN, such as: genes involved in the potassium channel family, endothelin system and the nitric oxide (NO) pathway that regulate pulmonary vascular resistance by the activity of endothelial oxide synthase (eNOS) [4]. However, with in to our knowledge there are not reports related to *SOD2* polymorphisms.

The gradual accumulation of genetic and epigenetic events might influence the development of PPHN [5]. Studies have shown an association between superoxide dismutase (SOD), a key enzyme that plays a primary role in removing reactive oxygen species (ROS), and PPHN [6,7]. ROS are chemical compounds that contain one or more unpaired electrons and are derived from oxygen molecule. Among the most important ROS are hydrogen peroxide, superoxide, and the hydroxyl radical [5]. They have a short half-life, are highly reactive and ultraviolet rays, ionizing radiation, pharmaceutical drugs and the metabolism of oxygen produce them in aerobic systems. Under physiological conditions, ROS activate inflammatory cells [5,7,8], which in the absence of efficient detoxification mechanisms (antioxidants) to counteract these free radicals, can cause an imbalance and might produce adverse effects and enhance the development of different disease manifestations, including PPHN [5,8-10].

Regarding PPHN, ROS is likely to have counterproductive effectives, in addition to stimulating vascular smooth muscle cell proliferation and increasing vascular tone. ROS may directly regulate eNOS and NO [5,8-10]. There are some studies that have shown that the administration recombinant human superoxide (rhSOD), increased eNOS activity and expression, decrease oxidative stress, increased tetrahydrobiopterin, a cofactor critical to the function of eNOS, hence to the stimulation of NO production and ultimately pulmonary vasodilation [10]. In an experimental model, it has shown that rhSOD restored endogenous eNOS function [11].

The SOD2 SNP rs5746136 in the 3'UTR is located 65 base pair (bp) downstream of the poly A site and <1 kb upstream from SP1 and the NF-kB transcription element sequences. The polymorphism has been studied in some diseases as oral cancer, diabetes mellitus 1, heroin dependency, and lower gestational age and birth weight in in preterm infants [12-15]. The variation in the reported frequency also depends on the population analyzed, and the *T* allele showed a frequency in controls of 26-45% in China and Iran population [12,15].

The participation of *SOD2* as a modulator of the pulmonary hypertension and vascular muscularization in heterozygous *SOD2* −/+ mice model; suggest that polymorphisms in the *SOD2* gene could have an important role in the risk to the development of PPHN [16].

However, the association studies of rs5746136 polymorphism have not been examined with PPHN. The aim of this study was to evaluate the possible relationship between SOD2 gene polymorphism and the occurrence of PPHN in a Mexican population of infants born at term or near term.

Materials and Methods

DNA was extracted from peripheral blood lymphocytes using standard protocols [17]. Blood samples were collected from 155 Mexican infants; 76 with PPHN, confirmed by echocardiography (study group), and 79 healthy newborns (control group). The patient and control groups were not sex matched and no familial samples were included. All patients were residents of the metropolitan area of Guadalajara, Jalisco, México and were recruited from June 2018 to November 2019. All samples were obtained after the parents of patients and controls provided a written informed consent, as approved by the local committee for health research and the ethics committee of the pediatrics hospital of the western medical center of the Mexican Social Security Institute. (R 2013-1302-045). All procedures performed in studies involving human participants were in accordance with the 1964 Declaration of Helsinki.

Amplification of the SNP rs5746136 of *SOD2* gene was performed via PCR using the following primers: 5'-AAAAACCACTGGGT-GACATCTAC-3' and 5'-AAGGGAAACACTCGGCTTTCT-3', as described previously by Liu et al [12]. The PCR amplifications were performed in a total of 15 microliters containing 0.2 mM dNTPs (Invitrogen, Carlsbad, CA USA), 5 pmol of primers, 2.5 mM $MgCl_2$, 2.5 U of Taq polymerase (Invitrogen, Carlsbad, CA USA), and 50 ng of genomic DNA. The annealing temperature was 60°C. The PCR product was digested with Taq I restriction enzyme. In the previous electrophoretic procedure amplified products were separated on 8% polyacrylamide gels (19:1), followed by silver staining [18]. The fragments of 42 bp, and 52 bp were

identified as *CC* genotype, the fragments of 42bp, 52bp and 94bp as *CT* genotype and the fragments 94 as *TT* genotype.

For statistical analysis, we used Student *t* and allele frequencies of polymorphism rs5746136 was obtained by direct counting. The Hardy–Weinberg equilibrium was tested by a goodness-of-fit Chi-square test to compare the observed genotype frequencies with the expected frequencies among control subjects. Odds ratios (OR) and 95% confidence intervals (CI) were also calculated. A two-tailed P <0.05 was considered statistically significant. The association analysis by the Odds ratio and binary logistic regression analysis between the studied groups were performed using the PASW Statistic Base 18 software, 2009 (Chicago, IL, USA).

Results

The group with PPHN had a lower gestational age (35.6 ± 2.81 weeks *versus* 38.1 ± 1.9 P=0.008), therefore the average body weight was lower in this group: 2,622 ± 626 g, in contrast to the 2,992 ± 565 g in control group (P=0.05). About mortality in the PPHN group, 21 patients died (27.6%, Table 1).

Table 1. General characteristics of PPHN patients and controls

Variable	PPHN (n=76)	Control (n=79)	*P-value*
Gestational age (weeks)	35.6 ± 2.81	38.1 ± 1.9	**0.008**
Weight (g)	2,622 ± 626	2,992 ± 565	**0.052**
Female n(%)	10 (47.6)	11 (52.4)	0.750
Male n(%)	11 (52.4)	10 (47.6)	
Vaginal delivery n(%)	5 (23.8)	4 (19)	0.707
C-section n(%)	16 (76.2)	17 (81)	
Mortalidad n(%)	21 (27.6%)	0 (0)	**0.001**

General aspects of the included patients are shown.

Table 2. Genotype and allelic distribution of rs5746136 polymorphism of *SOD2* gene in PPHN patients and controls

polymorphism		PPHN*		Controls*		OR	95%(CI)	*P*- value
rs5746136	*Genotype*	(n=76)	%	(n=79)	%			
	CC	(14)	19	(47)	60	1		
	CT	(39)	51	(28)	35	1.8	(0.96-3.52)	0.088
	TT	(23)	30	(4)	5	**8.1**	**(2.65-24.9)**	**0.0001**
	CT/TT	(62)	81	(32)	40	**6.5**	**(3.12-13.5)**	**0.0001**
	Allele $^{(2n)}$	$^{(2n=152)}$		$^{(2n=158)}$				
	C	(67)	0.440 7	(122)	0.772 1	**0.23**	**(0.14-0.37)**	**0.0001**
	T	(85)	0.559 3	(36)	0.227 9	**4.3**	**(2.63-7.02)**	**0.0001**

OR, odds ratio; CI, confidence intervals; *P*-value significant < 0.05. *Hardy-Weinberg equilibrium in controls of rs5746136 (X^2 = 0.004, P=0.9483) and PPHN patients (X^2 =0.1272, P= 0.7213) of the *SOD2* gene polymorphism.

The rs5746136 polymorphism in the *SOD2* gene was significantly different between PPHN patients and controls. The genotypes *TT* (OR 8.1, 95%CI 2.65–24.9, P =0.0001) and *CT/TT* (OR 6.5, 95%CI 3.12–13.5, P =0.0001) and *T* allele (OR 4.3, 95%CI 2.63–7.02, P =0.0001) were observed as risk factors for developing PPHN (Table 2).

No significant differences were observed when comparing the rs5746136 polymorphism stratified by gestational age, weight, male, female, vaginal delivery, and C-section (p>0.05) between PPHN and control groups.

Discussion

The general treatment of patients with PPHN is geared toward maintenance of normothermia, normal serum electrolytes, normal intravascular volume, correction of acidosis, adequate sedation and analgesia, adequate ventilation and oxygenation with optimal lung recruitment; for which many times it is necessary to use high frequency ventilation, it is important avoid hyperoxia and hypocarbia. Inotropic and vasoactive agents are commonly initiated early to increase cardiac output, maintain adequate systemic blood pressure, and enhance oxygen delivery to the tissue [19,20].

The therapeutic gold standard is iNO, demonstrating improved oxygenation and reduced need for extracorporeal membrane oxygenation (ECMO); however, in about 30 to 40% of sick newborns, these improvements in oxygenation and hemodynamics are not sustained and affected infants often require rapid transfer to an ECMO center despite the initiation of iNO. Treatment with exogenous antioxidants has been reported to have a protective impact on NO signaling pathways in animal models associated with neonatal oxidative stress [21].

NO can combine with superoxide to produce peroxynitrete, a potent and highly reactive nitrogen species, at a rate that is much faster than the dismutation of superoxide by the superoxide dismutase (SOD) enzymes. Peroxynitrite can then go on to cause vascular dysfunction through a variety of mechanisms including direct oxidative damage, promotion of vasoconstriction, and uncoupling and downregulation of eNOS expression and function [22].

Animal studies have provided ample evidence of the impact of oxidative stress on the NO signaling pathways, including effects on phosphodiesterase type 5 (PDE5), NO, and guanylate cyclase soluble. In addition, studies utilizing extracellular SOD (ecSOD) knockout mice have shown that NO availability is affected by relative abundance of ecSOD, a major antioxidant enzyme in the pulmonary vasculature that increases available NO by reducing the amount of extracellular superoxide thereby decreasing NO and superoxide interactions [23].

On the other hand, among the antioxidant defense systems activated by ROS, these include some classic antioxidant defense enzymes, such as SOD, catalase and glutathione peroxidase, distributed in the mitochondria, peroxisomes and cytoplasm, as well as non-classical enzymes [heme oxygenase-1; Phase II detoxifiers (glutathione reductase and transferase) and non-enzymatic (vitamins E, C and catechins)] [24]. When the production of free radicals is excessive

in the body and the antioxidant defense capacity is inefficient, then oxidative stress is generated, which can become severe and even cause cell death [5,24].

Some polymorphisms have been described in the gene SOD2 and have been associated with different diseases [5,12-15]. It is known that the participation of SOD2 as a modulator of the pulmonary hypertension and vascular muscularization mice model; induces the generation of ROS and at the mitochondrial level is the initial trigger of a vicious cycle of oxidative stress in mice [17]. Chronic intermittent hypoxia (CIH) induced down-regulating of SOD2 increased pulmonary hypertension and vascular muscularization. It could be the mechanism of CIH leading to pulmonary hypertension [25].

There are no studies that described the relationship of rs5746136 polymorphism and PPHN, which was observed in this study. Although, it should be noted that the confidence intervals were high in the *TT* genotype due to the small sample size. Xu et al [26] observed the association between SOD2 rs4880 polymorphism and the pulmonary arterial hypertension susceptibility, and concluded that this polymorphism influenced the pulmonary arterial hypertension susceptibility by altering the expression of *SOD2*.

According to the above, it is possible to think that the regulation of the activity of the enzyme SOD2 on vascular and smooth muscle function, influence the expression of *SOD2*, which was associated with pulmonary arterial hypertension susceptibility [26].

We might suggest that in PPHN, carrying the *TT*, *CT/TT* genotypes and *T* allele rs5746136 polymorphism of *SOD2*, a decreased production of SOD2 enzyme, could be generated, and inefficient elimination of free radicals has a risk effect in PPHN patients.

According to our knowledge, this is the first study to report this association, however, we could elucidate that the progression of PPHN is associated with adverse clinical outcomes and it may modify the expression of different molecular factors including stress oxidative mechanisms, which could alter the regulation of cellular processes [27]. On the other hand, the progression of PPHN is not only related to the monogenic inheritance of a protein variant, but it also depends on the interaction of several genes that are involved in multiple metabolic pathways and epigenetic events [27].

In addition, is considerable interest in the identification of alterations in the signaling pathways that regulate pulmonary vasoconstriction; despite advances in neonatal respiratory care and increased understanding of the importance of aberrant NO signaling in the pathobiology of PPHN, clinicians continue to face the dilemma of how to best treat neonates with PPHN, remains the most extensively studied neonatal disease. These polymorphisms contribute at least in part, to the increase in pulmonary vascular resistance and vascular remodeling characteristic in PPHN and are associated with PPHN severity. We speculate that the presence of these polymorphisms may be related to the poor or absent response to inhaled NO, seen in some infants and could help improve therapeutic approaches.

Conclusion

In conclusion, our results showed that the rs5746136 polymorphism was associated with PPHN risk when comparing controls and PPHN patients for the genotypes *TT, CT/TT* and *T* allele, respectively.

References

1. Nakwan N, Jain S, Kumar K, et al. An Asian Multicenter retrospective study on persistent pulmonary hypertension of the newborn: incidence, etiology, diagnosis, treatment and outcome. J Matern Fetal Neonatal Med. 2020; 33: 2032-2037.
2. Kaluarachchi DC, Smith CJ, Klein JM, et al. Polymorphisms in urea cycle enzyme genes are associated with persistent pulmonary hypertension of the newborn. Pediatr Res. 2018; 83: 142-147.
3. Nakwan N. The practical challenges of diagnosis and treatment options in persistent pulmonary hypertension of the newborn: a developing country´s perspective. Am J Perinatol. 2018; 35: 1366-1375.
4. Byers HM, Dagle JM, Klein JM, et al. Variations in CRHR1 are associated with persistent pulmonary hypertension of the newborn. Pediatr Res. 2012; 71: 162-167.
5. Zelko IN, Mariani TJ, Folz RJ. Superoxide dismutase multigene family: a comparison of the CuZn-SOD (SOD1) Mn-SOD (SOD2) and EC-SOD (SOD3) gene structures, evolution, and expression. Free Radic Biol Med. 2002; 33: 337-349.
6. Wedgwood S, Lakshminrusimha S, Schumacker PT, et al. Hypoxia Inducible Factor Signaling and Experimental Persistent Pulmonary Hypertension of the Newborn. Front Pharmacol 2015; 6: 47.
7. Storme L, Aubry E, Rakza T, et al. Pathophysiology of Persistent Pulmonary Hypertension of the Newborn: Impact of the Perinatal Environment. Arch Cardiovasc Dis. 2013; 106: 169-177.
8. Wiener HW, Perry RT, Chen Z, et al. A polymorphism in SOD2 is associated with development of Alzheimer's disease. Genes Brain Behav. 2007; 6: 770-775.
9. Ying L, Lagabalyila Z, Bo L, et al. Correlation between superoxide dismutase 1 and 2 polymorphisms and susceptibility to oral squamous cell carcinoma. Exp Ther Med. 2014; 7: 171-178.
10. Firth AL, Yuan JXJ. Bringing down the ROS: a new therapeutic approach for PPHN. Am J Physiol Lung Cell Mol Physiol. 2008; 295: 976-978.
11. Farrow KN, Lakshminrusimha S, Reda WJ, et al. Superoxide dismutase restores eNOS expression and function in resistance pulmonary arteries from neonatal lams with persistent pulmonary hypertension. Am J Physiol Lung Cell Mol Physiol. 2008; 295: 979-987.

12. Liu Y, Zha L, Li B, et al. Correlation Between Superoxide Dismutase 1 and 2 Polymorphisms and Susceptibility to Oral Squamous Cell Carcinoma. Exp Ther Med. 2014; 7: 171-178.
13. Mohammedi K, Bellili-Muñoz N, Driss F, et al. Manganese Superoxide Dismutase (SOD2) Polymorphisms, Plasma Advanced Oxidation Protein Products (AOPP) Concentration and Risk of Kidney Complications in Subjects With Type 1 Diabetes. PLoS One. 2014; 9: 96916.
14. Poggi C, Giusti B, Vestri A, et al. Genetic polymorphisms of antioxidant enzymes in preterm infants. J Matern Fetal Neonatal Med. 2012; 25: 131-134.
15. Boroumand F, Mahmoudinasab H, Saadat M. Association of the SOD2 (rs2758339 and rs5746136) Polymorphisms with the Risk of Heroin Dependency and the SOD2 Expression Levels. Gene. 2018; 649: 27-31.
16. Fu C, Hao S, Liu Z, et al. SOD2 Ameliorates Pulmonary Hypertension in a Murine Model of Sleep Apnea via Suppressing Expression of NLRP3 in CD11b + Cells. Respir Res. 2020; 21: 9.
17. Gustincich S, Manfioletti G, Del Sal G, et al. A fast method for high-quality genomic DNA extraction from whole human blood. Biotechniques. 1991; 11: 298-300.
18. Sanguinetti CJ, Dias E, Simpson AJ. Rapid silver staining and recovery of PCR products separated on polyacrylamide gels. Biotechniques. 1994; 17: 914-921.
19. Qasim A, Jain SK. Milrinone use in persistent pulmonary hypertension of the newborn. Neoreviews. 2020; 21: 165-178.
20. Rafat N, Schaible T. Extracorporeal membrane oxygenation in congenital diaphragmatic hernia. Front Pediatr. 2019.
21. Gien J, Nuxoll C, Kinsella JP. Inhaled nitric oxide in emergency medical transport of the newborn. Neoreviews. 2020; 21: 157-164.
22. Bartesaghi S, Radi R. Fundamentals on the biochemistry of peroxynitrite and protein tyrosine nitration. Redox Biol. 2018; 14: 618-625.
23. Dillard J, Perez M, Chen B. Therapies that enhance pulmonary vascular NO-signaling in the neonate, Nitric Oxide. 2020.
24. Liu J, Wang Z. Increased Oxidative Stress as a Selective Anticancer Therapy. Oxid Med Cell Longev. 2015; 294303: 1-12.
25. Jernigan NL, Naik JS, Weise-Cross L, et al. Contribution of reactive oxygen species to the pathogenesis of pulmonary arterial hypertension. PLoS One. 2017; 12: 0180455.
26. Xu M, Han L, Yuan C, et al. Role for Functional SOD2 Polymorphism in Pulmonary Arterial Hypertension in a Chinese Population. Int J Environ Res Public Health. 2017; 14: 266.
27. Gallegos-Arreola MP, Ramírez-Hernández MA, Figuera LE, et al. The rs2234694 and 50 bp Insertion/Deletion polymorphisms of the SOD1 gene are associated with breast cancer risk in a Mexican population. Eur Rev Med Pharmacol Sci. 2020; 24: 8017-8027.