VASCULAR DYSFUNCTION BEYOND PATHOLOGICAL PREGNANCIES. AN INTERNATIONAL EFFORT ADDRESSED TO FILL THE GAPS IN LATIN AMERICA

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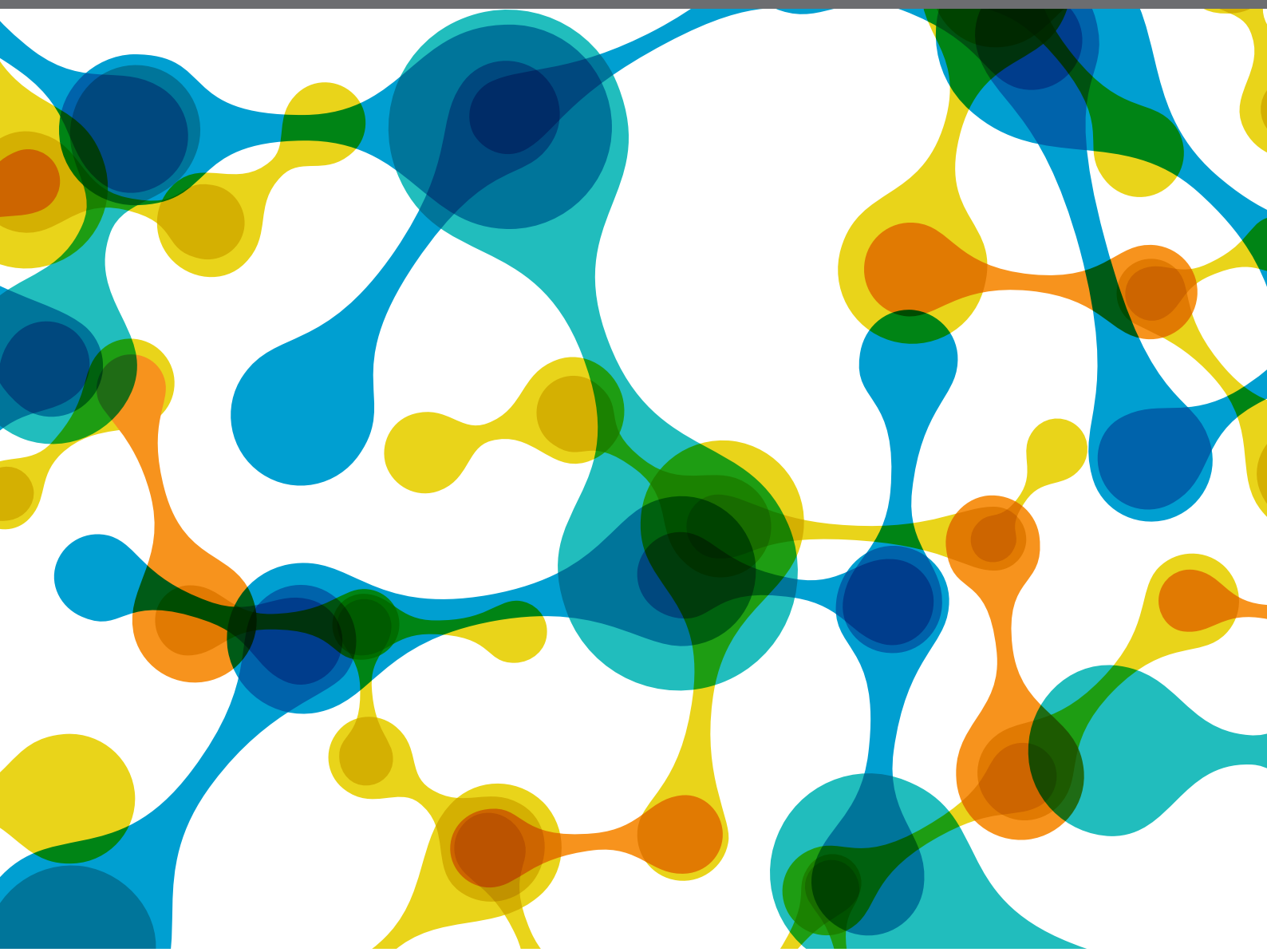

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VASCULAR DYSFUNCTION BEYOND PATHOLOGICAL PREGNANCIES. AN INTERNATIONAL EFFORT ADDRESSED TO FILL THE GAPS IN LATIN AMERICA

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Pregnancy is a physiologically stressful condition that generates a series of functional adaptations in the cardiovascular system. The impact of pregnancy on this system persists from conception beyond birth. Recent evidence suggests that vascular changes associated with pregnancy complications, such as preeclampsia; gestational diabetes; growth restriction; autoimmune diseases; among others, affect the function of the maternal and offspring vascular systems, after delivery and may be extended until adult life. Since the vascular system contributes to systemic homeostasis, defective development or function of blood vessels predisposes both mother and infant to future risk for chronic disease. In Latin American countries, like other low (LIC) and middle-income countries (MIC) worldwide, the rate of morbi-mortality due to both pregnancy complications and cardiovascular diseases have a higher incidence than in high-income countries (HIC). But, investigation in LIC and MIC, in particular in Latin America, still fall short of what would be expected considering the magnitude of those diseases. Although there are obvious deficiencies in terms of economies and scientific infrastructure between HIC and MIC or LIC, Latin American strength in terms of scientific productivity in this filed could be underestimated due to language limitation and publication in journals not indexed in major citation databases, resulting in low impact publications. As a result, we could speculate that potentially unique features of vascular disease associated to pregnancies complications can be unnoted in the global scientific community. Then, we would like to encourage researchers in vascular biology, in which, many groups in Latin America have contributed to both better understand vascular dysfunction associated to pregnancy diseases and show

the gaps in the literature, to overcome this hidden effect of our scientific production. This effort also will homogenize clinical concepts and knowledge that may strength the scientific effort in Latin America.

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EDITORIAL
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Editorial: Vascular Dysfunction Beyond Pathological Pregnancies. An International Effort Addressed to Fill the Gaps in Latin America

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Keywords: vascular, dysfuction, Latin America, pregnancy, pathological pregnancy

Editorial on the Research Topic

Vascular Dysfunction Beyond Pathological Pregnancies. An International Effort Addressed to Fill the Gaps in Latin America

Pregnancy is a physiologically stressful condition that generates a series of functional adaptations in the cardiovascular system. Recent evidence suggests that vascular changes associated with pregnancy complications may impair the function of the maternal and offspring vascular systems after delivery, being possibly extended until adult life.

In Latin American countries, like other low (LIC) and middle-income countries (MIC) worldwide, the rate of morbi-mortality due to both pregnancy complications and cardiovascular diseases have a higher incidence than in high-income countries. Paradoxically, research in this field is limited in Latin America (Giachini et al., 2017). Then, in addition to the scientific and public health implications of the maternal morbi-mortality in LIC and MIC, we also aimed to overcome geographic limitations. Therefore, our Research Topic titled "Vascular Dysfunction Beyond Pathological Pregnancies. An International Effort Addressed to Fill the Gaps in Latin America" intends to positively contribute in the scientific field, but also to visualize the challenging need for more investigation in our countries.

A highly diverse human population is observed within Latin America, and then many, risk factors for pregnancy complications are present in Latin American women. For example, the evaluation of genetic variants is critical to identify candidate genes that may contribute to the pathophysiology of pre-eclampsia in any specific population. Michita et al. have provided an integrative view of the genes evaluated by Latin American research groups, displaying a specific role on different aspects related to pre-eclampsia. They also discussed important topics related to pregnancy vascular disorders, which may be related to pre-eclampsia development, including epigenetics, transplantation biology, and non-coding RNAs.

Another interesting aspect on the pathophysiology of pre-eclampsia is that not only the mother, but also the father may be involved in the early onset of the disease, as revised by Galaviz-Hernandez et al. Indeed, the existence of a paternal antigen in the placenta has already been proposed in the specialized literature. For instance, evidences of paternal contribution include nulliparity, number

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of partners, among others, are remarked in the Galaviz-Hernandez et al. manuscript. Interestingly, not only maternal but also paternal obesity is a risk factor for pre-eclampsia.

Maternal nutritional condition is a key component in normal pregnancy development; and malnutrition by excess constitutes another increasing risk factor to pregnancy morbidity including pre-eclampsia. Despite that, as Lopez-Jaramillo et al. remark, not only obesity increases the risk of pre-eclampsia, but also some nutritional deficiencies of essential elements, predispose the mother to suffer pregnancy complications. On this regard, Lopez-Jaramillo et al. have discussed the alterations in the L-arginine/nitric oxide pathway that are commonly observed during obesity and may represent a key element in pre-eclampsia.

Another aspect related to nutritional deficiencies as well as obesity in pregnancies is the oxi-redox balance. In this regard, Alcala et al. review current evidences related with the negative impact of obesity (a well-characterized low-state chronic inflammation and high oxidative stress condition) to generate an unhealthy environment that predispose to development of adverse outcomes during gestation. Nevertheless, they also discuss the controversial results on antioxidant supplementation as a therapeutic tool during obese pregnancies.

Another risk factor to pathological pregnancies is the antiphospholipid syndrome (APS), a well-known condition linked with endothelial dysfunction. Velásquez et al. have compared the current understanding about the mechanisms of endothelial dysfunction induced by patient-derived anti-phospholipids auto-antibodies (aPL) under the two main clinical manifestations of APS: thrombosis and gestational complications, either alone or in combination. Analyzing current evidences in the field, Velásquez et al. challenge the current knowledge proposing that the mechanism of aPL-induced endothelial dysfunction depends on clinical manifestation of APS.

Placentation is clearly a key process for normal pregnancy development and many groups in Latin America have studied the placenta function. Indeed, the Latin American Society for Materno Fetal Interaction and Placenta (SLIMP) agglutin groups of researchers in this field. Teran et al. analyze the role of coenzyme Q10 (CoQ10) in placentation during pre-eclampsia. In their manuscript, extend the well-described role of CoQ10 as antioxidant and part of the mitochondria respiration chain into an effective intervention to reduce pre-eclampsia occurrence in Ecuadorian pregnant women.

We also highlight participation of other components such as aquaporins (AQPs) in the placentation process. On this regard, Szpilbarg et al. explain us how AQPs, a family of proteins that are known to work as water channel proteins, may display an additional role in the cellular homeostasis. This family of proteins also participates in cell signaling process including migration and apoptosis, which in turn are remarked component of normal placentation. Szpilbarg's manuscript details how a defective expression and activity of AQPs may result in the characteristic impaired placentation and systemic endothelial dysfunction underlying pre-eclampsia.

Continuing with analysis of placentation, Abán et al., provide current evidences about the interactions between endocannabinoids (ECS) and nitrergic signaling pathways during normal and pathological placentation process, observed both in intrauterine growth restriction and pre-eclampsia. In particular, ECS (a group of lipid-signaling molecules that include amides, esters and ethers of long-chain polyunsaturated fatty acids) can activate cannabinoid receptors, such as CB1 and CB2, leading to generation of nitric oxide (NO). Despite current knowledge about the relationship between ECS and NO synthesis, the underling molecular mechanisms, as well as its implications for abnormal placentation are still unclear.

In addition, Lima et al. present their work related with disturbances in the polysaccharide metabolism that may result in intracellular saccharide deposition, modulating cellular function. *O*-GlcNAcylation is a reversible post-translational modification that has been implicated as a modulator of protein function, both in physiological and pathological conditions including those in placental tissue. The interplay between *O*-GlcNAcylated placental proteins and the possible implications of this post-translational modification through placental development and pregnancy were also discussed.

Alterations in pregnancy due to pre-eclampsia, intrauterine growth restriction, or any other placental alterations are linked to systemic endothelial dysfunction, which then has profound implications in future cardiovascular health in both mother and her children. We started this analysis with the Galvis-Ramírez et al. manuscript, who reviewed the role of the structural domains of heparin sulfate (HS) in the process of selective permeability through the glomerular filtration barrier (GFB) and how these domains may be implicated in the glomerular inflammation processes observed in pre-eclamptic pregnancies.

Another key biological barrier forming by endothelial cells is the blood brain barrier (BBB), a tightly sealed monolayer of brain microvascular endothelial cells characterized by absence of fenestrations, low number of pinocytic vesicles and junctional complex formed by tight junctions and adherent junctions. In particular, it is known that the majority of maternal deaths resulting from pre-eclampsia are attributed to the coexistence of neurological complications. Torres-Vergara et al. help us to better understand this process by reviewing preclinical studies related to how the BBB is impaired in pre-eclampsia predisposing to cerebral edema and therefore brain complications in the mother not only during her pregnancy but even years after that.

Following brain complications and extending to offspring, Lara et al. propose a challenging hypothesis about how pre-eclampsia might impair brain angiogenesis in the offspring. In particular, they speculate how angiogenesis, as a key event for favoring the correct neurodevelopment and function could be disrupted in children born to pre-eclampsia. Proposed mechanism for impaired brain angiogenesis should consider imbalance of pro-angiogenic factors, including the vascular endothelial growth factor (VEGF) and placental growth factor (PlGF), with anti-angiogenic factors such as soluble VEGF receptor type 1 (or Flt-1).

In conclusion, although there are obvious deficiencies in terms of economies and scientific infrastructure between countries,

Latin American researchers have been able to generate milestone knowledge and contribute in the better understanding of vascular alterations present in pregnancy complications, indeed, it is the spirit of the Iberoamerican consortium called RIVA-TREM (Red Iberoamericana de alteraciones Vasculares Asociadas a TRastornos del EMbarazo).

We challenge ourselves to continuing our effort to visualize productivity and more important potential geographic and cultural particularities that are partially considered in the specialized literature. Then, we would like to encourage vascular biology researchers in Latin America that have continuing contributing to both better understand vascular dysfunction associated to pregnancy diseases and show the gaps in the literature, to overcome this hidden effect of our scientific production (Alperin, 2014; Van Noorden, 2014). This effort also will homogenize clinical concepts and knowledge that may strength the scientific effort in Latin America focused in reducing maternal and fetal morbi-mortality in our countries.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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REVIEW
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Genetic Variants in Preeclampsia: Lessons From Studies in Latin-American Populations

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Placental vascularization is a tightly regulated physiological process in which the maternal immune system plays a fundamental role. Vascularization of the maternal-placental interface involves a wide range of mechanisms primarily orchestrated by the fetal extravillous trophoblast and maternal immune cells. In a healthy pregnancy, an immune cross-talk between the mother and fetal cells results in the secretion of immunomodulatory mediators, apoptosis of specific cells, cellular differentiation/proliferation, angiogenesis, and vasculogenesis, altogether favoring a suitable microenvironment for the developing embryo. In the context of vasculopathy underlying common pregnancy disorders, it is believed that inefficient invasion of extravillous trophoblast cells in the endometrium leads to a poor placental blood supply, which, in turn, leads to decreased secretion of angiogenic factors, hypoxia, and inflammation commonly associated with preterm delivery, intrauterine growth restriction, and preeclampsia. In this review, we will focus on studies published by Latin American research groups, providing an extensive review of the role of genetic variants from candidate genes involved in a broad spectrum of biological processes underlying the pathophysiology of preeclampsia. In addition, we will discuss how these studies contribute to fill gaps in the current understanding of preeclampsia. Finally, we discuss some trending topics from important fields associated with pregnancy vascular disorders (e.g., epigenetics, transplantation biology, and non-coding RNAs) and underscore their possible implications in the pathophysiology of preeclampsia. As a result, these efforts are expected to give an overview of the extent of scientific research produced in Latin America and encourage multicentric collaborations by highlighted regional research groups involved in preeclampsia investigation.

Keywords: preeclampsia, vasculopathy, endothelial damage, inflammation, SNPs, Latin America, polymorphism

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INTRODUCTION

In all pregnancies that can potentially lead to living birth, a major concern is the high prevalence of disorders that can affect healthy pregnancies. Maternal mortality is a global health issue. One of the eight goals of the United Nations Millennium Development Goals (MDG) was to reduce maternal mortality by three quarters from 1990 to 2015. As of 2013, the worldwide maternal mortality ratio has dropped 45%, yet maternal deaths are still the primary cause of death. For the same period, an

estimated 289,000 maternal deaths due to pregnancy- or childbirth-related complications occurred, particularly in developing countries, since mortality rates vary according to geographical area and different social and ethnic characteristics. These estimates expose the alarming healthcare situation in developing countries where the maternal mortality ratio is ∼14 times higher than in developed countries. Actual numbers might be even higher because only 51% of the countries evaluated in the MDG had data on maternal causes of death (United Nations, 2015). In Latin America, pregnancy vascular disorders are the leading cause of maternal mortality and morbidity (Khan et al., 2006). These disorders cover a wide range of clinically characterized phenotypes with a common underlying dysfunction in the endothelial and vascular systems, including preeclampsia (PE), and will be appropriately discussed in this review.

Owing to a lack of robust experimental animal models and ethical issues related to early pregnancy tissue usage, elucidation of the underlying mechanisms involved in the pathophysiology of pregnancy disorders remains the "holy grail" of reproductive biology. Considering that fetal cells inherit half paternal genetic material, this "non-self" status (compared to the mother) represents a challenge to the maternal immunological system. In this sense, a question naturally arises: How does the fetus avoid rejection by the maternal immune system? Since rejection occurs at different levels, it is reasonable to consider that genetic disparity, or the genetic background of the parents may account for an increased risk of pregnancy disorders (Goldenberg et al., 2009; Gardosi et al., 2013; Lisonkova and Joseph, 2013). Human pregnancy is a phenomenon that relies on immunological adaptations (Aghaeepour et al., 2017). Since maternal immune tolerance is essential to the maintenance of pregnancy, breakage of such tolerance is an accepted hypothesis for the occurrence of pregnancy-related disorders, including PE (Christiansen, 2013; Redman et al., 2014), which is briefly reviewed in sections Placental Vasculogenesis and Angiogenesis: Immune System and Vascular Remodeling During Pregnancy and Preeclampsia.

Pregnancy is a highly coordinated process that requires the involvement of a well-regulated network of biological mechanisms. Briefly, pregnancy establishment initiates through blastocyst implantation and endometrial invasion. Blastocyst invasion requires the expression of a wide range of factors by both maternal and fetal cells, including adhesion molecules, pregnancy hormones, and inflammatory mediators (Norwitz et al., 2001). In this context, inefficient blastocyst implantation is related to impaired endometrial vascular remodeling and immunological tolerance, which are commonly observed in a broad spectrum of pregnancy disorders. The extent of maternal physiological responses driven by the foreign developing embryo involves both maternal/paternal and fetal aspects. The response for such stimuli varies between healthy and pathological pregnancies, or even among individuals of the same group. This implies that the genetic variability is a critical component and accounts in the susceptibility for (but not limited to) pregnancy vascular disorders by influencing both local and systemic responses. In Latin America, the genetic and molecular basis of PE is a rapidly developing field of investigation, and many studies approaching basic science or even extending to cutting-edge technologies have been published and will be reviewed in the sections Genetic Studies in Latin-American Populations, Genetic Variation in Histocompatibility-Related Genes in PE, Gene Variants Involved in Metabolic Processes, and Variants in Detoxification, DNA-Repair, and Apoptosis-Related Genes.

Latin America contains a highly diverse human population. This admixed population is also under the influence of environmental factors, such as climate, lifestyle, and pathogen exposure. As pregnancy disorders are affected by both genetic and environmental factors, it is difficult to extrapolate data obtained in specific human populations to other ones. Therefore, we provide an extensive review of studies developed in Latin America (**Figure 1**) as a contribution to the understanding of pregnancy disorders, mainly focusing on PE. Since Brazil and Mexico are at the forefront of PE investigation in Latin America, we call attention to the lack of investigative studies in countries not represented here. Also, we highlight the urgent need for collaborative studies and extensive efforts to fill gaps in the current scenario of hypertensive pregnancy disorder epidemiology in Latin America. Here, we will discuss current knowledge about the role of the maternal immune system in pregnancy vasculogenesis and PE. Also, we will review the literature concerning genetic studies evaluating the contribution of single nucleotide polymorphisms (SNPs) in candidate genes from distinct biological systems and discuss their involvement in PE pathogenesis by analyzing data from Latin America as well as from other human populations when appropriate. For the sake of clarity, reference SNP cluster (rs#) will be cited as it appears in the text and the SNP nomenclature will be maintained according to the original cited article.

PLACENTAL VASCULOGENESIS AND ANGIOGENESIS: IMMUNE SYSTEM AND VASCULAR REMODELING DURING PREGNANCY

Tissue remodeling and angiogenesis are the results of a tightly regulated interaction between the immune system and the vascular system (Ribatti and Crivellato, 2009). In pregnancy, an adequate placental vascularization depends on the proliferation and differentiation of the trophoblast cells in the placental villi (Herr et al., 2010). Adaptation and changes in maternal anatomy and physiology are fundamental for the establishment of an adequate blood supply for the developing fetus (Boeldt and Bird, 2017). After implantation, the invasion of the endometrium by the cytotrophoblast drives the first steps of human placentation. Initially, myometrial spiral arteries are remodeled in the second trimester, changing from a high-resistance state of coiled vessels to dilated low-resistance vessels (Boeldt and Bird, 2017). In low-resistance vessels, the exchange of gas and nutrients is highly facilitated, since there is a decrease in blood flow to the intervillous spaces of the placenta (Boeldt and Bird, 2017). According to the immunological aspects of pregnancy, it is accepted that a mild pro-inflammatory stimulus is essential for local tissue remodeling, neovascularization, and

FIGURE 1 | The contribution from Latin American countries to the understanding of genetic predisposition in pregnancy vascular disorders. In the map, areas in red represent the number of published articles covered by our review in Latin America. Areas in weak red represent low numbers of publications, and areas in strong red represent countries/populations with a production of 50 articles or more.

the establishment of successful embryo attachment enabling fetal development (Chaouat, 2002). Decidual immune cells, invading trophoblasts and endothelial cells interact and orchestrate placental vascularization. Leukocytes represent 15–30% of all cells in human early pregnant decidua (Mincheva-Nilsson et al., 1994). The organization of these immune cells is unique and includes lymphoid cell clusters, and randomly distributed immune cells, such as uterine natural killer (uNK) cells, αβ-T, and γδ-T cells, dendritic cells (DCs), and macrophages. B cells and regulatory B cells are less represented in number, and their emerging roles in pregnancy are discussed elsewhere (Muzzio et al., 2013; Fettke et al., 2014; Mor et al., 2017; Esteve-Solé et al., 2018). uNK cells represent ∼70% of leukocytes in the decidua (Moffett-King, 2002), and are essential to the angiogenesis and maintenance of vascular stability by secreting specific sets of cytokines: the vascular endothelial growth factor C (VEGFC), the placental growth factor (PIGF), and angiopoietin 2 (ANG2) (Li et al., 2001).

PREECLAMPSIA

Worldwide, PE affects 2–8% of pregnant women. In addition, it accounts for ∼40% of preterm births (<35 weeks of gestation) (Khan et al., 2006; Duley, 2009). PE incidence differs mainly between low- and high-income countries. In Latin American countries, ∼26% of maternal deaths are attributed to PE. However, the actual impact of PE in developing countries is underestimated due to differences in PE diagnostic criteria and the fact that reporting the maternal cause of death is not compulsory in several countries (Giachini et al., 2017).

PE usually manifests in the second trimester. Although new definitions for PE include organ dysfunction (Tranquilli et al., 2014) and no longer require proteinuria if other severe PE features are present (ACOG, 2013), traditionally PE is defined by onset of hypertension after 20 weeks of gestation (systolic ≥140 mmHg; diastolic ≥90 mmHg), proteinuria (≥300 mg/24 h or protein/creatinine ratio ≥0.5 in random sample) and edema. While untreated PE can be lethal, the clinical complications vary and include seizures, liver rupture, pulmonary edema, and renal insufficiency (Adu-Bonsaffoh et al., 2013). Despite advances in the clinical management of PE (symptomatic treatment), the only effective treatment remains clinical intervention and delivery, resulting in low birth weight and premature birth. In fact, ∼23% of low birth weight and ∼20% of preterm birth occurrences in Latin America are attributed to PE (Bilano et al., 2014). In clinical practice, therapies involving antiplatelet agents such as low aspirin doses (Duley et al., 2007; Roberge et al., 2013; Xu et al., 2015; ACOG, 2018) and calcium supplementation in women with low calcium diets (Hofmeyr et al., 2014) have proven to bring small to moderate benefit to women with high risk pregnancies. Symptomatic treatments include different strategies targeting gestational hypertension (antihypertensive therapy), eclamptic seizures (anticonvulsive therapy), and other symptoms as reviewed elsewhere (Ramos et al., 2017).

The impact of PE on both maternal and fetal health goes beyond pregnancy, and represents a significant burden on public health services, especially, in low-income countries where the incidence rates can reach up to 6% in Latin America, 2.3% in Africa, and 3.2% in Asia (Bilano et al., 2014). Preeclamptic women have an increased risk of post-partum depression, cardiovascular disorders, metabolic diseases and hypertension later in life (Ramsay et al., 2003; Hoedjes et al., 2011; Behrens et al., 2017; Neiger, 2017; Timpka et al., 2017; Zoet et al., 2018), while newborns are at higher risk to develop autistic spectrum disorders, cerebral palsy, and bronchopulmonary dysplasia due to low birth weight and preterm birth (Hansen et al., 2010; Mann et al., 2010; Strand et al., 2013).

Despite extensive efforts in the last two decades, the etiopathology of PE is still unclear, although some environmental and genetic risk factors have been reported (Fong et al., 2014; Ye et al., 2017). The variety of candidate genes evaluated by Latin American research groups and the critical events of each stage of PE development are summarized in **Figure 2** (for more details see Redman, 2014; Redman et al., 2014). Classically, PE development follows a two-stage model including a pre-clinical and a clinical period (Redman, 1991). This model was recently updated into a sequential four- and six-stage model to accommodate all immune aspects of PE: In the first stage of PE, environmental and genetic factors represent a critical component. The latter element involves several genes from different signaling pathways, revealing the polygenic nature of PE (for example, it is suggested that limited exposure to paternal antigens likely increases PE risk, being clinically relevant in primiparous women). In the next stage, inefficient trophoblast invasion in the decidua may result

in poor placentation and abnormal uteroplacental perfusion. In the third stage, placental ischemia and hypoxia result in local oxidative stress and inflammatory response. Secondary to placental damage, in the fourth stage, impaired secretion of placental and maternal factors lead to the manifestation of the clinical symptoms of PE. In the fifth stage, diagnosis of PE is clear. At this stage, the vascular damage is augmented in response to systemic inflammation (i.e., Th1/Th17 cytokines). The last stage characterizes a more severe form of the disorder (observed in up to 40% of placentas) and involves atherosis, a focal lesion in the spiral arterial wall associated with placental infarction and arterial thrombosis (Harsem et al., 2007).

Placental hypoxia and impaired perfusion lead to the release of reactive oxygen species (ROS) and endothelial damage. Thus, the release of fetal cell debris and syncytiotrophoblast microparticles into maternal circulation prompts an intense pro-inflammatory response by maternal immune cells (Redman and Sargent, 2000; Sibai et al., 2005). Also worth mentioning is the pregnancy stress test hypothesis, which postulates that pregnancy is a maternal stress test for the vascular, metabolic or immunological systems (Williams, 2003; Roberts and Hubel, 2010; Myatt and Roberts, 2015). Following this idea, women with pre-existing vascular dysfunction would present a lower threshold for the stress test, and a higher predisposition to develop PE and chronic disorders later in life.

PE might also be the manifestation of two extreme situations converging in a common phenotype. Sometimes, in maternal PE, normal placentation occurs in women with the pre-existing chronic disease. Conversely, in placental PE, abnormal placentation results in poor placental perfusion (Valenzuela et al., 2012). This concept highlights a not exclusive dependency of PE in placentation failure and explains the variability of clinical phenotypes and timing of PE development.

Familial history and hypertensive disorders increase the risk of PE, implying that the genetic components are also risk-modifying factors (Bezerra et al., 2010). PE is a polygenic disorder, and although no single genetic variant is believed to be responsible for all cases of PE, individual *loci*, environmental factors, and epistasis are components that should not be neglected (Staines-Urias et al., 2012; Williams, 2016). In this sense, the evaluation of genetic variants in PE risk could partially explain disorder susceptibility and would be of great importance to identify candidate targets for gene-gene interaction analyses, as well as to better follow-up/management of women at higher risk.

GENETIC STUDIES IN LATIN-AMERICAN POPULATIONS

Pro- and Anti-inflammatory Mediators in PE

In Latin America, several immune-related genes have been evaluated, and most of the studies are summarized in **Table 1.** For example, costimulatory molecules play a role in immune cell differentiation and activation, SNPs in the *CTLA4* (rs231775), *CD28* (rs3116496), and *ICOS* (rs4675378) were evaluated in Brazilian women with PE (Pendeloski et al., 2011). An association between the *ICOS* (−1564 T/C) SNP and PE was suggested based on a lower frequency of the *ICOS* "T" allele and the "TT" genotype in PE cases compared to controls. A systemic inflammatory response mediated by cytokines can cause endothelial damage, and thus it plays a central role in PE severity. In this scenario, six SNPs of pro-inflammatory genes were studied: *IL1R1* (rs2234650), *IL12* (rs3212227), *IL18* (rs187238), *IL18* (rs1946519), *TLR2* (rs5743708), and *TLR4* (rs4986790). However, no differences in genotypic and allelic frequencies between PE and controls were observed (Franchim et al., 2011). In a Northern Mexico population study, the association between PE risk and the *TGFB1* SNPs: −800G/A (rs1800468), −509C/T (rs1800469), and +869T/C (rs1800470) and their haplotypes were evaluated. No association between PE development and the SNPs or haplotypes was observed, although the +869TT genotype was suggested as a protective factor against severe PE (Aguilar-Duran et al., 2014).

Since different cytokine profiles have been associated with PE development (Saito and Sakai, 2003), de Lima et al. (2009) investigated SNPs of cytokine genes in eclampsia and PE in northwestern Brazilian individuals. They evaluated the SNPs *TNFA* (−308 G>A), *IL6* (−174 G>C), *IFNG* (+874 A>T), *IL10* (−1082 A>G, −819 C>T, −592 C>A), and *TGFB1* (+869 T>C, +915 G>C). No differences in genotypes and allelic frequencies were observed (de Lima et al., 2009). However, in a previous study by the same group, individuals were stratified according to ethnic origin in Caucasian and non-Caucasian, and the association of PE with *TNFA* (−308), *TGFB1* (+10;25), *IL10* (−1082), *IL6* (−174), and *IFNG* (+874) SNPs was evaluated. Intriguingly, the *IL10* −1082G/G SNP was associated with PE in Caucasian women, which is the most frequent allelic variant in people of African ancestry (Daher et al., 2006). A possible association between SNPs in *IL1B* was investigated in Brazilian women with severe PE. In this study, the "rs1143630 T" allele was associated with PE (Leme Galvão et al., 2016). In a Maya-Mestizo population sample, no association between *TNFA* (−308G/A, −850C/T) SNPs and PE was observed (Canto-Cetina et al., 2007). Another study reported no association of *IL10* (rs1800896), *IL6* (rs1800795), and *IL1RA* variable number of tandem repeats (VNTR) in intron 2 with PE susceptibility in Mexican-Mestizo women and Maya-Amerindian women from Mexico (Valencia Villalvazo et al., 2012).

The influence of *TNFA*, *IL6*, *IFNG,* and *IL10* gene SNPs and their relationship with cytokine plasma levels in severe PE, normotensive pregnancy, and in non-pregnant women from Brazil was investigated by Pinheiro et al. (2015). The SNPs evaluated in the study were *TNFA* (−308), *TGFB1* (+10;25), *IL10* (−1082), *IL6* (−174), and *IFNG* (+874). Interesting, they observed higher IL-10 levels in normotensive pregnant women compared to preeclamptic women. Conversely, higher plasma levels of IL-6 and IFN-γ were detected in PE in comparison to non-pregnant and normotensive pregnant women. Also, a positive correlation between IFN-γ plasma levels and the *IFNG* +874T allele was observed, and when the three groups were evaluated separately, a positive correlation between IL-6 levels

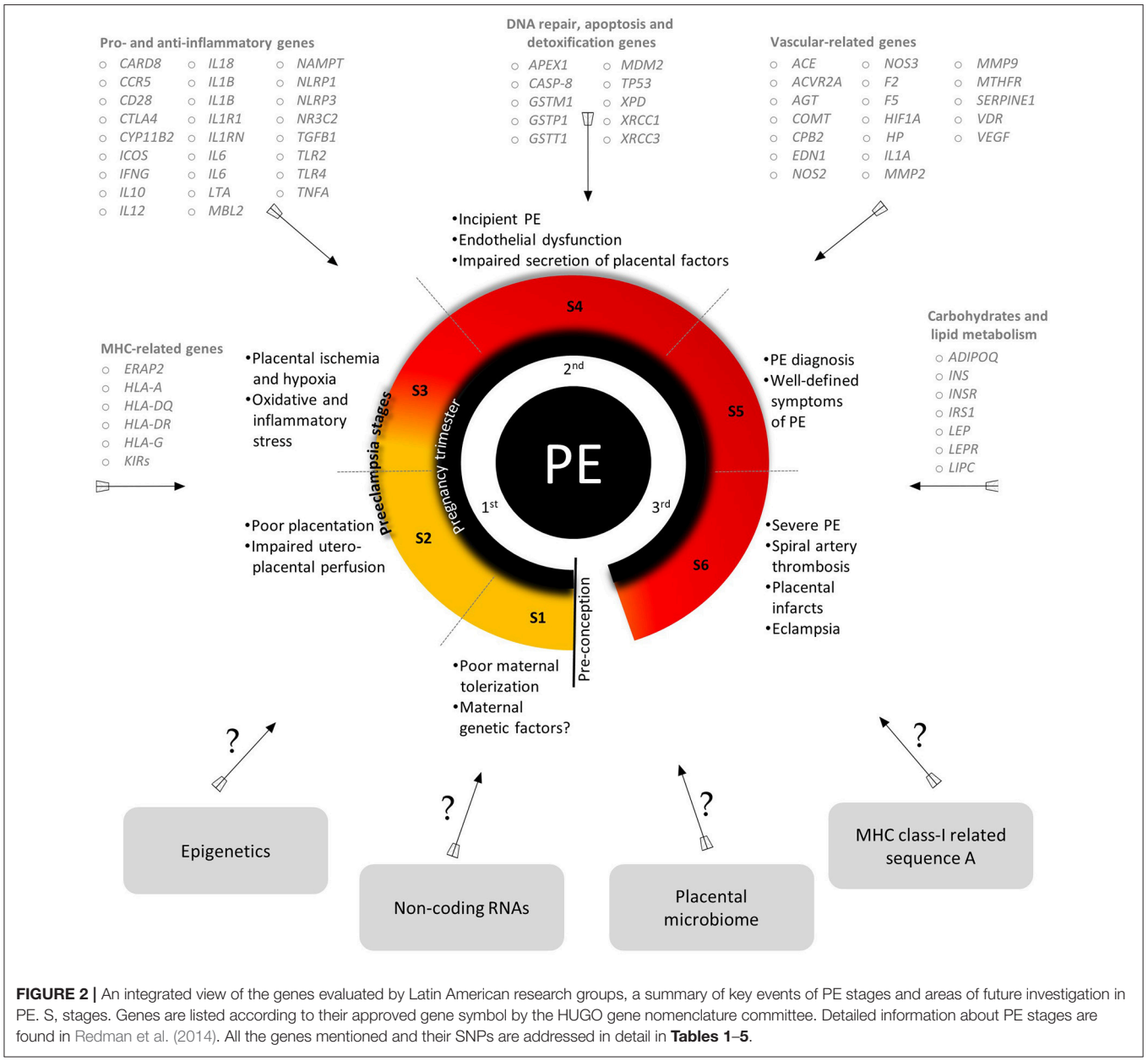

FIGURE 2 | An integrated view of the genes evaluated by Latin American research groups, a summary of key events of PE stages and areas of future investigation in PE. S, stages. Genes are listed according to their approved gene symbol by the HUGO gene nomenclature committee. Detailed information about PE stages are found in Redman et al. (2014). All the genes mentioned and their SNPs are addressed in detail in **Tables 1**–**5**.

and the presence of the IL6 −174C allele in normotensive pregnant women was observed (Pinheiro et al., 2015).

Mannose-binding lectin (MBL) is a pro-inflammatory protein that modulates inflammation and ultimately induces apoptosis (Turner, 2003). Polymorphisms in the *MBL2* gene located at exon 1: at codons 54 (allele B, rs1800450), 57 (allele C, rs1800451), and 52 (allele D, rs5030737) were evaluated in women with PE and in healthy pregnant controls from Brazil. The absence of all the variants characterize the wild-type allele "A." In this study, an association between genotypes coding for low MBL levels and a severe PE was evidenced. In the AD genotype, the C and D alleles were more frequent in PE compared to controls. Moreover, in relation to MBL levels, three groups of haplotypes were observed: group 1 (H/L)YA/(H/L)YA and (H/L)YA/LXA genotypes were related to high MBL serum levels; group 2 encompasses LXA/LXA and (H/L)YA/O genotypes, which were related to intermediate MBL serum levels; and group 3 was defined by low MBL serum levels, resulting in MBL deficiency, corresponding to LXA/O and O/O genotypes (Vianna et al., 2010). Cysteine-cysteine chemokine receptor type 5 (CCR5) is an essential receptor for inflammatory reactions expressed in leukocytes and other cell types (Barmania and Pepper, 2013). CCR5Δ32 is a 32-base pair deletion in the *CCR5* that, in homozygosis, results in a lack of expression of the functional CCR5 on the cell surface. Heterozygous carriers express lower levels of functional CCR5 compared to wild-type homozygous individuals (Venkatesan et al., 2002). Considering the intense inflammatory response in PE, and based on the high frequency

of the deletion allele in healthy pregnant women, Telini et al. (2014) suggested a protective role of the *CCR5Δ32* allele against PE development in a Brazilian study.

Adipocytokines are involved in trophoblast invasion and successful placentation. Visfatin is an adipocytokine also known as nicotinamide phosphoribosyltransferase (NAMPT), which has a potential role in the pathophysiology of metabolic disorders such as hypertension and obesity (Dahl et al., 2012). A study by Luizon et al. (2015) evaluated visfatin/NAMPT plasma levels in healthy pregnant women and in patients with gestational hypertension or PE, in the context of the *NAMPT* SNPs −423T<C (rs1319501) and rs3801266A<G in intron 1. No effects were observed concerning rs1319501. Nevertheless, gestational hypertensive patients carrying the rs3801266 "AG" and "GG" genotypes had higher visfatin/NAMPT levels compared to gestational hypertensive patients carrying the "AA" genotype (Luizon et al., 2015). Moreover, Luizon et al. (2017) evaluated whether *NAMPT* SNPs (rs1319501T>C, rs3801266A>G), haplotypes and gene-gene interactions in the NAMPT pathway could affect plasma visfatin/NAMPT levels, and the response to antihypertensive therapy in PE and hypertensive pregnant women. Low circulating visfatin/NAMPT levels were seen in non-responsive PE patients with the rs1319501 TC+CC genotypes. Conversely, high circulating visfatin/NAMPT levels were detected in non-responsive PE patients with the rs3801266 AG+GG genotypes. Haplotype analysis revealed an association of the 'C, A' haplotype with response to antihypertensive treatment and with low visfatin/NAMPT levels in PE (Luizon et al., 2017). Since lymphotoxin alpha (LTα) is an inflammatory mediator, this molecule was evaluated in the context of PE development, but no association of LTA +252 (rs909253) with PE risk was reported in a Brazilian study (Pissetti et al., 2015).

In order to evaluate the contribution of the inflammasome in PE development, SNPs in the genes coding for nod-like receptors with a pyrin domain 1 (*NLRP1*), *NLRP3*, caspase recruitment domain 8 (*CARD8*), and *IL1B* were studied in a Brazilian population. The *NLRP1* rs12150220A/T SNP was associated with PE. The minor "T" allele was more frequent in PE compared to healthy pregnancy controls, indicating that this allele might be relevant in PE susceptibility. A strong association with *NLRP1* (rs12150550) was also observed in this study, suggesting a role for this molecule in the pathogenesis of PE. Besides, *NLRP1* SNPs produce six main haplotypes, and the rs11651270/C-rs12150220/A-rs2670660/A combination was less frequent amongst PE women compared to controls, suggesting a protective effect against PE (Pontillo et al., 2015).

In the context of gestational hypertension, the relationship between aldosterone levels and SNPs of the aldosterone synthase (*CYP11B2*) gene (−344T/C) and the mineralocorticoid receptor gene (S810L) was investigated in a Mexican population. No differences in genotype distributions or in aldosterone levels were found (Ramírez-Salazar et al., 2011). Similar results were obtained for a Brazilian population (de Vasconcelos et al., 2009).

In summary, several studies covered in this review (**Table 1**), and other approaches have reinforced that PE is a polygenic disorder and manifests as complex phenotypes, resulting from both maternal and fetal genetic features (Triche et al., 2014). In Latin American populations, conflicting results regarding genetic variants and PE risk were observed, implying that genetic variability does account for this complex phenomenon. Therefore, the search for potential genetic components involved in PE, or its severity, is of paramount importance for a better understanding of the genetic basis of PE pathophysiology (**Figure 2**). Importantly, we observed a worrying lack of family-based studies evaluating the genetic components of both the fetus/placenta and its biological parents. Thus, such an approach would provide a more actual picture of the genetic risk factors involved in PE and possibly a more accurate disease monitoring and clinical management.

Vascular and Angiogenic Mediators

Studies also examined gene variants involved in vasculogenesis and angiogenesis, given the importance of establishing an adequate and efficient placental vascular system for a favorable gestational outcome (Herr et al., 2010). Studies from Latin America are summarized in **Table 2**. Nitric Oxide (NO) has a primary role in the circulatory system. Also, NO is a critical regulatory molecule in ovulation, embryo implantation, pregnancy maintenance, labor, and delivery. Imbalances in NO levels during gestation were suggested as a cause of the development of pregnancy-induced hypertension and PE (Maul et al., 2003). Several studies have evaluated SNPs in both endothelial and inducible nitric oxide synthase genes (*eNOS* and *iNOS*, respectively). In a multicenter study in Colombia, Serrano et al. (2004) evaluated the role of *eNOS* SNPs: Glu298Asp, −786T→ C, and VNTR b/a (27 bp-tandem repeat, where "a" and "b" refer to PCR product size, comprising 420 bp for "b" and 393 bp for "a" alleles) as potential risk factors for PE. Young Colombian women homozygous for the Asp298 allele were reported to have an increased risk for PE. The authors suggested that homozygous women for the Asp298 allele are more susceptible to endothelial dysfunction and at increased risk for PE development, since the homozygous state is likely to generate low NO levels. In addition, the presence of the "Asp298-786C-4b" haplotype was associated with an increased PE risk (Serrano et al., 2004). Similarly, Sandrim et al. (2008) compared the same *eNOS* SNPs in women with and without PE from Brazil. Interestingly, the study observed that two *eNOS* haplotypes ("T Glu a" and "C Glu a") were associated with PE and gestational hypertension. The same SNPs were also evaluated in a Maya-Mestizo Mexican population. The Asp298 allele was associated with PE in a recessive model. In addition, the "−786C-4b-Asp298" haplotype was more frequent in PE than in controls, whereas the "−786T-4b-Asp298" and "−786C-4b-Glu298" haplotypes had lower frequencies or were absent in patients (Díaz-Olguín et al., 2011). In another study, Alpoim et al. (2014) evaluated these same *eNOS* SNPs in early and late severe preeclamptic Brazilian women, and in a group of normotensive/healthy pregnant controls. The frequency of the 894T allele was higher in late severe PE compared to early severe PE. Also, the overall 894T frequency was higher in PE when compared to controls. Regarding the VNTR b/a SNP, higher "aa" genotype and "a"

TABLE 1 | Summary of studies developed in Latin America evaluating the role of genetic variation in pro- and anti-inflammatory mediators in PE.

Factors	Sample size[†]	Key findings	Country	References
ICOS (T-1564C) *CTLA4* (A49G) *CD28* (T17C)	130/260	Association with protection for PE: ICOS−1564T allele and−1564TT genotype.	Brazil	Pendeloski et al., 2011
TGFB1 (G800A, C509T, T869C)	175/253	Association with protection for severe PE: TGFB1 869TT genotype.	Mexico	Aguilar-Duran et al., 2014
IL1R1 (rs2234650) *IL12* (rs3212227) *IL18* (rs187238, rs1946519) *TLR2* (rs5743708) *TLR4* (rs4986790)	109/174	No association with PE.	Brazil	Franchim et al., 2011
TNFA (G308A) *IL6* (G174C) *IFNG* (A874T) *IL10* (A1082G, C819T, C592A) *TGFB1* (T869C, G915C)	165/101[a]	No association with PE.	Brazil	de Lima et al., 2009
TNFA (G308A) *TGFB1* (T10C, C25G) *IL10* (G1082A) *IL6* (G174C) *IFNG* (A874T)	151/189[b]	Association with PE risk: IL10−1082GG genotype in white women.	Brazil	Daher et al., 2006
IL1B (rs1143630)	169/287	Association with PE risk: IL1B rs1143630 'T' allele.	Brazil	Leme Galvão et al., 2016
TNFA (G308A,C850T)	105/200	No association with PE.	Mexico	Canto-Cetina et al., 2007
IL10 (G1082A) *IL6* (G174C) *IL1RA* (86bp-VNTR)	411/613	No association with PE.	Mexico	Valencia Villalvazo et al., 2012
TNFA (G308A) *IL6* (G-174C) *IFNG* (A874T) *IL10* (A1082G, C819T, C592A) *TGFB1* (T869C,G915C)	116/165[c]	Association with protection for PE: IL6−174C allele.	Brazil	Pinheiro et al., 2015
MBL2 allele B (rs1800450), allele C (rs1800451), allele D (rs5030737)	157/162	Association with PE severity: "AD" genotype, "C" and "D" alleles.	Brazil	Vianna et al., 2010
CCR5 (CCR5Δ32)	155/144	Association with protection for PE: CCR5Δ32 allele.	Brazil	Telini et al., 2014
NAMPT (rs3801266)	389/212[d]	Association with GH: rs3801266 "AG" and "GG" genotypes.	Brazil	Luizon et al., 2015
NAMPT (rs1319501; rs3801266)	379/207[e]	Association with PE risk: rs1319501 "TC+CC" and rs3801266 "AG+GG" genotypes.	Brazil	Luizon et al., 2017
LTA (+252A>G)	30/115	No association with PE.	Brazil	Pissetti et al., 2015
NLRP1 (rs11651270, rs12150550, rs2670660) *NLRP3* (rs35829419, rs10754558) *CARD8* (rs2043211, rs6509365) *IL1B* (rs1143634)	286/309	Association with risk for PE: rs12150220 (L155H) and the "rs11651270/C-rs12150220/A-rs2670660/A" haplotype.	Brazil	Pontillo et al., 2015
CYP11B2 (T344C) *MR* (S810L)	100/100	No association with PE.	Mexico	Ramírez-Salazar et al., 2011
CYP11B2 (T344C)	185/118[f]	No association with PE.	Brazil	de Vasconcelos et al., 2009

[†] *Pooled cases/controls.*

[a] *Cases were grouped according severity: PE (n = 92) and eclampsia (n = 73).*

[b] *Studied population was grouped according to skin color (white and non-white); white: PE (n = 56) and control (n = 92); non-white: PE (n = 95) and control (n = 97).*

[c] *Cases were compared to healthy pregnant (n =107) and non-pregnant women (n = 58).*

[d] *Cases correspond to PE (n = 208) and gestational hypertension (GH) cases (n=181).*

[e] *Cases were grouped according to disorder severity and response to anti-hypertensive therapy: PE responsive (n = 60) and non-responsive (n = 145); GH responsive (n = 120) and non-responsive (n= 54).*

[f] *Cases were grouped in PE (n = 70) and GH (n = 115).*

allele frequencies were observed in early severe PE compared to late severe PE and controls. Also, the "T-b-C" haplotype was more frequent in late severe PE compared to early severe PE and controls.

Although anti-hypertensive treatment has never been demonstrated to reverse PE outcome, its usage could prevent cardiovascular and cerebrovascular adverse consequences, due to severe and rapid elevations of the blood pressure, being a critical tool for clinical PE management. In this sense, it was proposed that anti-hypertensive therapy can enable maintenance of gestation and increase the gestational age of delivery, thus decreasing adverse fetal and maternal outcomes (Podymow and August, 2008). In this context, an elegant study compared the distribution of *eNOS* variants in gestational hypertensive and PE cases who were responsive to anti-hypertensive therapy versus cases who did not respond to treatment. Interestingly, a difference in the overall distribution of *eNOS* haplotypes was observed when PE responsive to treatment groups and PE nonresponsive to treatment groups were compared. The "C Glu a" haplotype was more frequent in the responsive PE group than in the nonresponsive PE group, and the "T Asp a" haplotype was less frequent in the active PE group than in the nonresponsive PE group. This was a pioneer study approaching the genetic background in the context of gestational hypertension treatment (Sandrim et al., 2010b).

The distribution of two *eNOS* Tag SNPs, rs743506 and rs7830, as well as the SNPs T-768C, Intron-4, and G894T, among healthy pregnant controls, gestational hypertensive subjects, and PE subjects was assessed by Muniz et al. (2012). No differences were detected among genotype frequencies in the three groups studied. However, the haplotype H5 "CbGGC" ("C" of rs2070744, "b" of intron 4, "G" of rs1799983, "G" of rs743506, and "C" of rs7830) was more frequent in the control group compared to gestational hypertensive and PE individuals, suggesting a potential protective effect against hypertensive disorders development in pregnancy.

Two *iNOS* SNPs, C-1026A (rs2779249) and G2087A (rs2297518), were evaluated in Brazilian healthy pregnant/control, gestational hypertension, and PE groups. The "GA" genotype and the "A" allele for the G2087A were more commonly found amongst PE subjects. No differences were observed concerning the other variants evaluated (Amaral et al., 2012).

Considering that increased levels of hemoglobin (Hb) and haptoglobin (Hp) complexes contribute to impaired NO bioavailability in PE (Sandrim et al., 2010a), the role of a haptoglobin SNP (duplication of exons 3 and 4 of *HP* gene) was evaluated in PE and non-pregnant women, in the context of NO bioavailability. Higher NO consumption was detected in association with increased cell-free Hb in plasma from PE patients carrying the allele *HP2* (duplicated exons 3 and 4 of the *HP1*), suggesting a functional association between *HP* SNPs and the hemodynamic imbalances observed in PE (Sertório et al., 2013).

Thrombin-activated fibrinolysis inhibitor (*TAFI*) gene has also attracted attention in the context of SNPs and their possible association with vascular disorders in pregnancy. In this scenario, a case-control study investigated the possible association between PE and *TAFI* SNPs (G505A, C1040T, and G-438A), together with TAFIa plasma levels in a Mexican-Mestizo population. No associations with increased PE risk were observed. However, due to higher plasma TAFIa levels and the presence of the G505A mutant genotype, together with wild-type forms of C1040C and G-438G, it was suggested that *TAFI* SNPs in the coding region or in nearby regulatory elements could contribute to variations in TAFIa plasma concentrations (Acosta-Tejeda et al., 2011).

The methylenetetrahydrofolate reductase (MTHFR) enzyme is critical for homocysteine (HCY) metabolism, where it catalyzes the NADPH-linked reduction of 5,10-MTHF to 5-MTHF, and subsequently the methylation of HCY to methionine in a vitamin B12-dependent manner (Barbosa et al., 2008). In a Tunisian study, low MTHFR activity levels were associated with mild to moderate increases in plasma HCY levels in placental vascular complications (Klai et al., 2011). In the same study, the *MTHFR* A1298C variant was associated with recurrent pregnancy loss, intrauterine growth restriction, and placental abruption. In the context of PE, a differential distribution of the *MTHFR* C677T alleles was associated with thrombosis markers and endothelial activation in a study with Mexican women (Rojas et al., 2010). Moreover, a possible association between C677T SNP of *MTHFR* gene and PE was investigated in pregnant women from the Yucatan Peninsula in southeastern Mexico, although no differences between cases and controls were observed (Pérez-Mutul et al., 2004).

In another study evaluating *MTHFR* (C677T) in Maya-Mestizo PE women, it was observed that *MTHFR* "T" allele and the "TT" genotype were more frequent in controls, suggesting a decreased risk of PE in women carrying this variant (Canto et al., 2008). Amongst a Mestizo-Ecuadorian population, the prevalence of C677T and A1298C *MTHFR* SNPs was also investigated in the context of PE, with the "CC" genotype of A1298C occurring in higher prevalence in PE women than controls (Chedraui et al., 2014). Nevertheless, contradictory results regarding PE, the placental genotype, and allele frequencies of the *MTHFR* C677T were observed (Chedraui et al., 2015). Interestingly, for the C677T SNP, the mutant "TT" genotype was threefold more frequent in preeclamptic placentas than controls. In a Chilean population, epistatic interactions between *MTHFR* and catechol-O-methyltransferase (COMT) gene were evaluated in maternal-fetus dyads. The increased PE risk was observed exclusively when the fetus harbored both the *COMT* "ATCA" haplotype (respectively composed by the SNPs rs6269, rs4633, rs4818, rs4680) and the *MTHFR* 677T allele (Hill et al., 2011a).

SNPs in the vascular endothelial growth factor (*VEGF*) gene are also largely studied in PE. Importantly, the low production of VEGF by peripheral blood mononuclear cells is associated with PE (Cardenas-Mondragon et al., 2014). The possible role of SNPs at the promoter region of *VEGF* was addressed by Sandrim et al. (2009). The study reported an association between PE development and the SNPs −2578C/A (rs699947), −1154G/A (rs1570360), and −634G/C (rs2010963). Importantly, inter-ethnic differences account for differential allelic and haplotype distributions, and this is particularly relevant for Latin American populations. In this context, the authors observed that *VEGF*

TABLE 2 | Summary of studies developed in Latin America evaluating the role of genetic variation in vascular- and angiogenesis-related genes in PE.

Factors	Sample size[†]	Key findings	Country	References
eNOS (−786T>C, intron-4 b/a, Glu298Asp)	322/522	Association with PE risk: 298Asp/Asp genotype and *eNOS* C-b-Asp haplotype.	Colombia	Serrano et al., 2004
eNOS (−786T>C, intron-4 b/a, Glu298Asp)	216/110[a]	Association with PE and GH risk: *eNOS* C-a-Glu haplotype. Association with protection for PE and GH: *eNOS* T-a-Glu haplotype	Brazil	Sandrim et al., 2008
e*NOS* (−786T>C, intron-4 b/a, Glu298Asp)	127/263	Association with PE risk: 298Asp/Asp genotype and *eNOS* C-b-Asp.	Mexico	Díaz-Olguín et al., 2011
eNOS (−786T>C, intron-4 b/a, Glu298Asp) MMP2 (C-1306T) MMP9 (C-1562T)	77/266	Association with PE risk and severity: −786CC genotype and −786C allele, respectively.	Brazil	Leonardo et al., 2015
eNOS (−786T>C, intron-4 b/a, Glu298Asp)	98/103[b]	Association with late-onset PE risk: 298Asp/Asp genotype and 298Asp allele; intron-4 aa genotype and a allele; *eNOS* C-b-Asp.	Brazil	Alpoim et al., 2014
eNOS (−786T>C), intron-4 b/a, Glu298Asp	152/152[c]	Association with anti-hypertensive therapy in PE, *eNOS* haplotypes: C-a-Glu responsive and T-a-Asp non-responsive.	Brazil	Sandrim et al., 2010a
eNOS (−786T>C, intron-4 b/a, Glu298Asp, rs743506, rs7830)	295/122[d]	Association with protection for PE and GH: eNOS C-b-Glu-G-C haplotype.	Brazil	Muniz et al., 2012
eNOS (C-1026A, G2087A)	353/212[e]	Association with PE risk: 2087GA genotype and the 2087A allele.	Brazil	Amaral et al., 2012
HP (Hp1-1, Hp2-1, Hp2-2)	92/105	No association with PE risk. Nitric Oxide byproducts in PE associated with Hp2-1 and Hp2-2 genotypes.	Brazil	Sertório et al., 2013
TAFI (G505A, C1040T, G-438A)	87/87	No association with PE.	Mexico	Acosta-Tejeda et al., 2011
MTHFR (C677T) *FV LEIDEN* (G1691A) PROTHROMBIN (G20210A)	28/41	No association with PE.	Mexico	Rojas et al., 2010
MTHFR (C677T)	148/490[f]	No association with PE	Mexico	Pérez-Mutul et al., 2004
MTHFR (C677T, A1298C)	150/150	Association with PE risk: 1298CC genotype.	Ecuador	Chedraui et al., 2014
MTHFR (C677T)	125/274	Association with protection for PE: 677TT genotype and 677T allele.	Mexico	Canto et al., 2008
VEGF (C-2578A, G-1154A, G-634C)	195/108[g]	Association with protection for PE: *VEGF*−2578C/-1154G/-634C haplotype. Low proportion of-2578AA and−634GG genotypes in white PE women.	Brazil	Sandrim et al., 2009
VEGF (C936T, C-2578A)	52/28	Association with protection for PE: *VEGF*−2578A allele.	Brazil	Cunha et al., 2011
VEGF (C2578A, G634C)	113[h]	No association with PE.	Brazil	Sandrim et al., 2015
VEGF (G634C) *IL1A* (rs3783550)	79/210	Association with PE risk: *IL1A* rs3783550 "A" allele.	Brazil	Silva et al., 2015
VEGF (A2578C, C1498T, A1154G, C634G,C936T)	31/31[i]	No association with PE.	Ecuador	Chedraui et al., 2013
eNOS (T786C, VNTR, G894T) *MTHFR* (C677T) *AGT* (C704T)	230/350	No association with PE.	Mexico	Coral-Vázquez et al., 2013
MMP9 (C1562T, (CA)n repeats)	300/176[j]	Association with risk for GH: *MMP9* C1562 T allele. No association with PE.	Brazil	Palei et al., 2010
eNOS (T786C, VNTR, G894T) *MMP9* (C1562T, (CA)n repeats) *VEGF* (C2578A, G634C)	229/102[k]	Association with protection for PE: combination of MMP9-1562CC with VEGF-634GG genotypes. Association with PE risk: combination of MMP9-1562CC with VEGF-634CC or MMP9-1562CT with VEGF-634CC or-634GG genotypes.	Brazil	Luizon et al., 2012
MMP2 (C1306T, C735T)	263/130[l]	No association with PE.	Brazil	Palei et al., 2012a
MMP9 (C1562T, (CA)n repeats)	399/214[m]	Association with GH: combination of the "T" allele for the C1562T and "H" allele of 90(CA)13–25.	Brazil	Palei et al., 2012b

(Continued)

TABLE 2 | Continued

Factors	Sample size[†]	Key findings	Country	References
MTHFR (C677T) *Factor II* (G20210A) *FV LEIDEN* (G1691A) *PAI1* (4G/5G I/D)	75/145	No association with PE.	Brazil	Dalmáz et al., 2006
MTHFR (C677T) *FV LEIDEN* (G1691A)	33/62	No association with PE.	Mexico	Dávalos et al., 2005
ACVR2A (rs1424954, rs1014064, rs1424941, rs2161983, rs3768687)	613/693[n]	Association with severe early-onset PE risk: SNPs rs1014064 "G," rs1424954 "A," and rs2161983 "A."	Brazil	Ferreira et al., 2015
ACE (287 bp I/D in intron 16)	51/71	No association with PE.	Brazil	Galão et al., 2004
FV LEIDEN (G1691A) *Factor II* (G20210A) *MTHFR* (C677T)	30/83	No association with PE.	Brazil	Dusse et al., 2007
ACE (287 bp I/D in intron 16)	66/37	Association with risk for PE: *ACE* "DD" genotype.	Mexico	González-Garrido et al., 2017
EDN1 (G5665T)	61/49[o]	Association with protection for PE: paternal *EDN1* "GG" and "GT" genotypes.	Mexico	Galaviz-Hernandez et al., 2016
MTHFR (C677T, A1298C)	50/50[p]	Association with risk for PE: *MTHFR* 677TT genotype.	Ecuador	Chedraui et al, 2015
ACE (287 bp I/D in intron 16)	665/1,046	No association with PE.	Colombia	Serrano et al., 2006
HIF1A (C1772T, G1790A)	150/105	No association with PE.	Mexico	Nava-Salazar et al., 2011
VDR (*FokI, ApaI, BsmI*)	316/213[q]	No association with PE.	Brazil	Rezende et al., 2012
COMT (rs6269, rs4633, rs4680, and rs4818), *MTHFR* (C677T)	528/575[r]	Association with PE risk: "ATCA" haplotype of *COMT* (SNPs rs6269, rs4633, rs4818, rs4680, and MTHFR 677T)	Chile	Hill et al., 2011a

[†] *Pooled cases/controls.*
[a] *Cases were stratified in PE (n = 113) and gestational hypertension (GH, n = 103).*
[b] *Cases were stratified in early severe PE (n = 53) and late severe PE (n = 45).*
[c] *Cases were stratified in PE (n = 152) and GH (n = 152).*
[d] *Cases were stratified in PE (n = 157) and GH (n = 138).*
[e] *Cases were stratified in PE (n = 187) and GH (n = 166).*
[f] *Sample size composed by PE cases (n = 148), health pregnant woman (N = 177), and health non-pregnant volunteers (313).*
[g] *Cases were stratified in PE (n = 94) and GH (n = 101).*
[h] *Sample size was composed by 113 PE white women who were responsive (n = 46) and non-responsive (n = 67) to anti-hypertensive treatment.*
[i] *Sample size was composed by 62 cord vessels of singleton gestations with severe PE (n = 31) and controls (n = 31).*
[j] *Cases were stratified in PE (n = 154) and GH (n = 146).*
[k] *Cases were stratified in PE (n = 122) and GH (n = 107).*
[l] *Cases were stratified in PE (n = 133) and GH (n = 130).*
[m] *Cases were stratified in PE (n = 214) and GH (n = 185).*
[n] *Cases were stratified in PE (n = 443), eclampsia (n = 138), and HELLP syndrome (n = 693).*
[o] *Sample size composed by PE cases (n = 61) and their partners (n = 61), and the control group was health pregnant woman (N = 49) and their partners (n = 49).*
[p] *Sample size composed by 100 placental tissues of PE cases (n = 50) and controls (n = 50).*
[q] *Cases were stratified in PE (n = 162) and GH (n = 154).*
[r] *Sample size was composed by maternal-fetus dyads from PE cases (n = 528) and controls (n = 575).*

−2578A and −1154A alleles were more frequent in European-descendants subjects compared to Afro-descendants, while no inter-ethnic differences were observed regarding the G-634C SNP. Ethnic origin is also correlated with differences in *VEGF* haplotypic frequencies (Muniz et al., 2009). Cunha et al. (2011) evaluated *VEGF* variants +936C/T and −2578C/A in PE cases and controls. The *VEGF* −2578A allele showed a higher frequency in the control group, suggesting a possible protective effect against PE, while no association of *VEGF* +936C/T was observed in PE or controls (Cunha et al., 2011).

In the context of antihypertensive therapy, *VEGF* SNPs (C-2578A and G-634C) were evaluated in European-derived Brazilian women with PE classified according to response to antihypertensive therapy. No associations were observed, suggesting that these *VEGF* SNPs does not influence the antihypertensive therapy responsiveness in PE (Sandrim et al., 2015). The *VEGF* G-634C and *IL1A* (rs3783550) SNPs were evaluated in Brazilian women with PE and in controls. An association between *IL1A* (rs3783550) SNP and PE development was observed in this population sample. However, no differences were observed regarding the *VEGF* G-634C variant (Silva et al., 2015).

An elegant study investigated *VEGF* SNPs (−2578 A/C, −1498 C/T, −1154 A/G, −634 C/G, and +936 C/T) in samples

from cord vessels of singleton gestations with severe PE. Additionally, they investigated NO plasmatic levels, asymmetric dimethyllarginine and VEGF levels in fetal circulation. The SNPs showed similar distributions in cases and controls. Significantly higher NO plasma levels in umbilical vessels were seen in PE. Arterial VEGF levels were significantly lower in PE cases, and a positive correlation was found between NO and asymmetric dimethylarginine levels amongst PE cases (Chedraui et al., 2013).

The influence of SNPs in *eNOS*, *MTHFR*, *GSTP1*, and angiotensinogen (*AGT*) genes on PE was evaluated by Coral-Vázquez et al. (2013). The *eNOS* variants covered in the study were: −786T→C (rs2070744), VNTR (27 bp) in intron 4, and G-894T→Glu298Asp (rs1799983). The C-704T→Met235Thr (rs699) was the variant studied in *MTHFR*, the C-704T→Met235Thr (rs699) in *AGT*, and the A-313G→Ile105Val (rs1695) in *GSTP1*. No differences in the distribution of the genotypes or haplotypes between controls and PE cases were observed.

Matrix metalloproteinases (MMPs) are enzymes responsible for the degradation of various extracellular matrix molecules. In pregnancy, a disturbance in MMP activity could indicate abnormal trophoblast invasion. Moreover, detection of MMP up-regulation could reflect an interaction between oxidative stress and inflammatory mediators, which could result in the delivery of cell debris in maternal circulation and accumulation in various maternal organs (Chen and Khalil, 2017). The involvement of MMPs in vascular disorders of pregnancy may worsen the response to antihypertensive therapy (Palei et al., 2012a). In this context, a study investigated two matrix metalloproteinase 9 (*MMP9)* SNPs, the g.−1562C>T (rs3918242) and microsatellite g.−90(CA)13-25 (rs3222264). They report an association of the *MMP9* SNP with gestational hypertension, but not with PE (Palei et al., 2010).

The association of PE and SNPs of nitric oxide synthase 3, *NOS3* (G894T, T-786C, and a VNTR with intron 4), *MMP2* (C-1306T), and *MMP9* (C-1562T) genes was investigated through a prospective case-control study in a southeastern Brazilian population. No association with PE development was found regarding *MMP2* and *MMP9* variants. Considering the *NOS3* gene, the SNP T-786C showed association with PE development (Leonardo et al., 2015). Luizon et al. (2012) evaluated whether epistatic interactions among seven clinically relevant SNPs of *eNOS* (T-786C, rs2070744, a VNTR in intron 4, and Glu298Asp, rs1799983), *MMP-9* [C-1562T, rs3918242 and −90(CA)13-25, rs2234681] and *VEGF* (C-2578A, rs699947, and G-634C, rs2010963) could be associated with PE or gestational hypertension. Significant interactions between the *MMP9* and *VEGF* genes were seen in PE samples (Luizon et al., 2012). The *MMP2* SNPs: g-1306 C>T (rs243865) and g-735 C>T (rs2285053) were analyzed in the context of both PE and gestational hypertension together with circulating MMP-2 and tissue inhibitor of metalloproteinase (TIMP)-2 levels. High MMP-2/TIMP-2 ratios were observed in gestational hypertensive patients, but no differences in the genotype and allelic frequencies were found (Palei et al., 2012a). The same above-mentioned approach was used regarding *MMP9* SNPs: g.−90(CA)13-25 (rs3222264) and g.−1562C>T (rs3918242) and circulating levels of MMP-9 and TIMP-1. Higher plasma concentrations of MMP-9 and TIMP-1 were detected in gestational hypertensive patients compared to controls. TIMP-1 levels were higher in PE cases, but MMP-9 and MMP-9/TIMP-1 ratios were similar between PE and gestational hypertensive subjects. Haplotype analyses suggested that the presence of the H4 haplotype increases susceptibility to gestational hypertension (Palei et al., 2012b).

Dalmáz et al. (2006) assessed the prevalence of four thrombophilic genes in women with mild or severe PE in Southern Brazil. Variants studied include the *MTHFR* C677T, prothrombin gene (*F II*) G20210A, *Factor V* (*FV Leiden*) G1691A, and insertion/deletion (4G/5G) in the plasminogen activator inhibitor type 1 (*PAI1*) gene promoter region. No association between PE and the SNPs was observed (Dalmáz et al., 2006). *MTHFR* C677T and *Factor V Leiden* SNPs were also investigated as potential genetic risk factors for eclampsia and PE in a group of women from western Mexico, without statistically significant results (Dávalos et al., 2005). Furthermore, *Factor V Leiden* (G1691A), *Factor II* (G20210A), and *MTHFR* (C677T) variants were investigated in the context of inherited thrombophilia in Brazilian PE women and controls. Again, no differences were observed (Dusse et al., 2007).

The gene *ACVR2A* encodes the activin A type II receptor (ActRIIA), an essential factor for pregnancy establishment during decidualization, trophoblast invasion, and placentation. Concerning the regulation of trophoblast invasion, abnormal decidual *ACVR2A* expression may affect placentation and lead to PE development (Yong et al., 2018a). In this context, five *ACVR2A* SNPs (rs1424954, rs1014064, rs1424941, rs2161983, and rs3768687) were investigated in a northwestern Brazilian population approaching PE cases and controls. These five SNPs showed no association with PE. Nevertheless, haplotype analysis revealed a strong association among SNPs rs1014064, rs1424954, and rs2161983 and severe early-onset PE (Ferreira et al., 2015).

Considering that the cardiovascular system of a pregnant woman adapts to allow and support increased blood flow toward the placenta, angiotensin-converting enzyme gene (*ACE*) SNPs were investigated in vascular disorders of pregnancy. A common 287-bp insertion/deletion SNP within *ACE* (*ACE*-I/D) was investigated as a possible risk factor for PE development in a south Brazilian population. The allele frequencies of this *ACE* variant were not associated with PE development (Galão et al., 2004). Subsequently, a case-control study and meta-analysis were also unable to show the association between the *ACE*-I/D variant and PE (Serrano et al., 2006). In a Mexican population, González-Garrido et al. (2017) evaluated the *ACE*-I/D SNP in relation to ACE activity and oxidative damage in PE. Higher ACE activity was found in PE cases compared to controls. Also, higher "DD" genotype and "D" allelic frequencies were found in PE compared to the control group. In summary, the results suggested that *ACE*-I/D SNP, high ACE activity, body mass index and oxidative damage are important factors in the pathogenesis of PE (González-Garrido et al., 2017).

The Endothelin 1 protein is a potent vasoconstrictor molecule, and its encoding gene, *END1*, is also a candidate gene for PE. A case-control study investigated women affected with PE and their partners in comparison to healthy pregnant women and their

partners regarding the *EDN1* rs5370 SNP. A negative association between the rs5370 SNP and PE in the male sub-group was observed, while no association was observed between cases and controls in the female sub-group (Galaviz-Hernandez et al., 2016). This study reminds us of the importance of including the paternal genetic background and the effect of the male genetic contribution in pregnancy outcomes.

Hypoxia-inducible factor (HIF) is a highly conserved transcription factor that coordinates an adaptive response in physiological and pathophysiological situations. Several cell types up-regulate *HIF* in response to low oxygen levels. In human pregnancy, HIF signaling in the gravid uterus is critical for fetal and placental development (Macklin et al., 2017). The Influence of *HIF1A* C1772T and G1790A SNPs was evaluated in PE patients and controls in a Mexican population, although no association of these variants with PE risk was observed (Nava-Salazar et al., 2011).

Finally, vitamin D is an essential molecule during pregnancy. The levels of its active form increase throughout healthy gestation and are critical for an adequate calcium supply for fetal growth (Urrutia and Thorp, 2012). Given its importance and relevance in gestational outcome, Vitamin D Receptor (*VDR*) SNPs have been studied in PE and gestational hypertension. Rezende et al. (2012) evaluated three *VDR* SNPs (*FokI, ApaI,* and *BsmI*) in a Brazilian population, and also investigated the potential association of hypertensive pregnancy disorders with *VDR* haplotypes. No differences in genotype, allele, or haplotype frequencies were observed between PE or pregnant hypertensive women and controls, these findings suggested that the investigated SNPs do not influence pregnancy outcome (Rezende et al., 2012).

Studies in vascular and angiogenic gene polymorphisms have shown conflicting results in Latin American populations (**Table 2**). Besides genetic variants alone, PE-associated haplotypes and the interaction among SNPs of distinct genes further support the importance of exploratory studies in this rapidly developing field. The conflicting results evidenced in this review are partially explained by the differences in the genetic background of distinct Latin American populations, which result from high admixture (Salzano and Sans, 2014). Besides, the different genotypic and allelic frequencies of the studied SNPs corroborate the PE classification as a complex disease. For a better understanding of the whole scenario involving this disease, robust studies and several exploratory studies still need to be put into practice. Also, the publication of negative results is important, mainly for the correct performance of meta-analyses encompassing preexisting data that would better reflect the actual frequency of genetic variants in Latin American populations.

GENETIC VARIATION IN HISTOCOMPATIBILITY-RELATED GENES IN PE

The major histocompatibility complex (MHC) is fundamental to the immunological system allowing the development of immune responses against foreign antigens or immunogenic epitopes through recognition of self- and non-self. Traditionally, the MHC complex is defined by two well-known genetic *loci*: MHC class-I and MHC class-II, although MHC class-III and -IV also exist and are relevant to complex diseases (Gruen and Weissman, 2001; Yau et al., 2016). MHC class-I members split in "classical" [human leukocyte antigen (*HLA*)-*A*, -*B,* and -*C*] and "non-classical" (*HLA-E*, -*F*, -*G* and -*H*: or MHC-Ib) molecules. Classical genes are ubiquitously expressed on virtually all nucleated cells (with a few exceptions), are highly polymorphic, and their primary function is as peptide presenting molecules. On the contrary, expression of non-classical molecules are restricted to some cellular types (for example, EVT), have a limited degree of polymorphism, and do not present peptides as a major function but rather act as signaling molecules to immune cells. Classical MHC class-II (*HLA-DR*, -*DQ,* and -*DP*) expression is restricted to antigen presenting cells, such as B cells, macrophages, and DCs. MHC class-III and IV are otherwise very distinct molecules comprising members of the complement system and induced-stress/inflammatory proteins, respectively (Gruen and Weissman, 2001).

The role of the MHC-Ib molecules in pregnancy has been a focus since the discovery of HLA-G expression in human trophoblast cells (Kovats et al., 1990). In the maternal-placental interface, an exciting aspect is the expression of HLA-G, -E, -F, and -C antigens on EVT cells (Hackmon et al., 2017).

Among the non-classical MHC-I molecules, HLA-G is a most enigmatic member. It interacts with several maternal immune cells, including those in the decidua (i.e., dNK, decidual macrophages, dCD4+, dCD8+), and has the potential to inhibit or activate their immunologic functions. Recently, it was reported that soluble HLA-G (sHLA-G) affinity for its cognate receptors [i.e., dimers binding to LILRB1 (leukocyte Ig-like receptor 1) with increased affinity] is likely impacted by sHLA-G heterodimerization in inflamed patients, which is likely to occur in PE and explains the variable findings reported so far (Veit et al., 2015). Also, LILRB1 receptors bind to β2-microglobulin(m)-associated HLA-G, whereas the LILRB2 receptors bind to non-β2m-associated HLA-G molecules. Alternative splicing of the gene results in seven isoforms: four membrane-bound HLA-G isoforms (HLA-G1 to -G4) and three soluble isoforms (HLA-G5 to G7). HLA-G1 undergoes proteolytic cleavage by metalloproteinase-2 (MMP-2) giving rise to sHLA-G1 (Rizzo et al., 2013).

In Latin America, several groups have evaluated the role of candidate genes belonging to the MHC loci and PE susceptibility (**Table 3**). *HLA-G* is the most studied *MHC* gene due to its immunotolerogenic properties, and several aspects of *HLA-G* have been explored. An SNP located in the 3′ untranslated region (UTR) of the gene, namely 14-bp insertion(ins)/deletion(del) (rs66554220), is well-known due to its influence on mRNA stability which affects the expression patterns of the gene (Rousseau et al., 2003; Porto et al., 2015). We have recently reported that specific haplotypes and variants in the 3′UTR increase the risk for recurrent pregnancy loss in Brazilian women (Michita et al., 2016). Also, we suggested that a maternal 14 bp del/del homozygous status might predispose primiparous women to PE (Vianna et al., 2007). An increased risk for

PE was also observed in neonates who preferentially inherit the maternal *HLA-G**0104 allele (Carreiras et al., 2002), which has been associated with the 14 bp del allele present in the UTR-3 haplotype (Castelli et al., 2014). In another study, a concomitantly low frequency of CD8+CD28- T cells (CD8+T memory cells), low monocyte (CD14+HLA-G+), and low T cell (CD3+HLA-G+) counts in PE women were associated with a pro-inflammatory status, which was confirmed by pro-inflammatory cytokine measurements (Vianna et al., 2016); however, no differences in 14 bp ins/del and +3142C/G (rs1063320) SNP frequencies between PE and non-PE women were observed. Similarly, in a Mexican PE cohort, although HLA-G expression was not evaluated, a reduced frequency of CD3+ T cells was observed in third trimester decidual tissue, and most importantly, dNK cells (CD3-CD56+CD16-CD9+) persisted throughout pregnancy and shared the same phenotype as the ones detected in early pregnancy (Sánchez-Rodríguez et al., 2011). This implies that long-term persistence of dNK cells could play important physiological roles in labor by the secretion of inflammatory mediators and fighting against infectious agents. Still considering HLA-G, Ferreira et al. (2017) reported that the 14 bp variant had no influence on PE predisposition, although the specific contribution of this SNP for PE in primiparous women was not evaluated. Hitherto, the role of the 14 bp variant in PE has been a matter of debate (Vianna et al., 2007; Pabalan et al., 2015; Ferreira et al., 2017). However, in a recent meta-analysis, the ethnicity (European-derived) and the 14 bp ins/ins genotype status in neonates were pointed as likely involved in PE risk in primiparous women (Pabalan et al., 2015).

PE development probably involves the interaction of maternal and fetal features. Also, a contribution of paternal origin has been suggested (Dahl et al., 2014; Saftlas et al., 2014). Functional variants within the endoplasmic reticulum aminopeptidase gene (*ERAP2*) have been associated with PE in non-Latin American populations (Johnson et al., 2009). In a study by Hill et al. (2011a), the *ERAP* variants rs2549782 and rs17408150 were evaluated in Chilean dyads (mother-neonate) and African American subjects (78% were dyads) with PE. In this study, no influence of *ERAP* SNPs in PE predisposition was reported. The lack of association with PE risk could be partially explained by differences in population structure and linkage disequilibrium patterns (Hill et al., 2011b). A study evaluating Venezuelan dyads reported an increased risk for PE in both mothers and neonates carrying the *HLA-DRB1**07 *DQA1**0201 *DQB1**0201 haplotype. In addition, mothers carrying the *HLA-DRB1**06/07 allele were more likely to be infected by the human cytomegalovirus (HCMV) (Carreiras et al., 2002). Since the recent Zika virus epidemics in Brazil (Schuler-Faccini et al., 2016), the relevance of viral infections during pregnancy is once more in the spotlight. Of note, recent evidence suggests that some viral infections modify the threshold of placental cell immunologic response to bacterial lipopolysaccharides (LPS) resulting in an exacerbated inflammatory response, and thus contributing to the development of pregnancy disorders including PE (Cross et al., 2017; Nourollahpour Shiadeh et al., 2017).

Other polymorphic loci immunologically relevant in PE comprise the *KIR* (Killer-cell immunoglobulin-like receptors) family. This gene family encompasses both activating (S) and inhibitory (L) receptors and can be functionally characterized in two additional groups: A (inhibitory) or B (activating) group. There is evidence of maternal *KIR* contribution in PE development. Indeed, it was suggested that the predominance of inhibitory receptors in PE women conferred an increased risk for PE in Mexican women (Sánchez-Rodríguez et al., 2011). Interestingly, a higher frequency of CMV-positivity was observed in third trimester Mexican women carrying the inhibitory *KIR* bB03|tA01 haplotype (*KIR* A) (Alvarado-Hernández et al., 2016), reinforcing the theory that imbalances between activating and inhibitory receptors expressed on cytotoxic cells influence viral infection predisposition and are possibly a risk-modifying factor for pregnancy disorder development.

GENE VARIANTS INVOLVED IN METABOLIC PROCESSES

Changes in maternal metabolism occur during gestation, allowing adaptation to the energetic and nutritional needs of the developing fetus and ensuring its healthy development. Some changes involve the metabolism of carbohydrates and lipids. Such metabolic changes occur in a spatial and temporal manner as pregnancy develops. Early in gestation, glucose and insulin levels are comparable to those of non-pregnant women, with a slight increase in insulin sensitivity (Butte, 2000). A decrease in insulin sensitivity occurs naturally, becoming evident in the second trimester, however, a noticeable loss of insulin sensitivity can lead to systemic resistance, hyperglycemia, and gestational diabetes mellitus (DM). The effects of hyperglycemia in pregnancy are associated with several adverse clinical outcomes for both mother and newborn, the latter associated with overweight and cardiometabolic risk later in life (Thaware et al., 2015; Zhu et al., 2016; Tam et al., 2017). It was suggested that gestational hyperglycemia or pre-pregnancy DM are risk factors for gestational disorders, including PE (Wendland et al., 2008). Interestingly, the expression of cytokines (i.e., IL-10 and TNF-alpha) relevant to the pathophysiology of PE (Daher et al., 2006; Pinheiro et al., 2015) is associated with maternal glycemia (Moreli et al., 2012), implying that maternal glycemia not only affects the metabolic status but also the immunological profile of pregnant women.

Some studies have evaluated the role of critical mediators in metabolic processes and their influence on PE development (**Table 4**). Adiponectin (ADIPOQ) is an adipokine, a term referring to adipose tissue-derived signaling molecules with broad biological functions (Ruan and Dong, 2016). ADIPOQ enhances cellular insulin sensitivity and thus is involved in adipose tissue expansion. Besides metabolic signaling, ADIPOQ has anti-inflammatory, anti-atherogenic and anti-proliferative functions, but paradoxically it is associated with coronary diseases (Sattar and Nelson, 2008). In addition, it enhances human EVT cell invasion *in vitro* by means of MMP-9 and -2 expression and TIMP-2 repression (Benaitreau et al., 2010). Expression of both MMPs in EVT cells may

TABLE 3 | Summary of studies in Latin America evaluating the role of genetic variants in histocompatibility-related genes in PE.

Factors	Sample size[†]	Key findings	Country	References
HLA-A, *-G*, *-DRB1*, *-DQA1*, *-DQB1* alleles	27/29[a]	Association with PE risk: *HLA-G**0104 allele, *DRB1**07 *DQA1**0201 *DQB1**0201 haplotype and *DRB1**07 and/or *DRB1**06 alleles in presence of HCMV detection.	Venezuela	Carreiras et al., 2002
HLA-G (14 bp ins/del)	157/162	No association with PE.	Brazil	Vianna et al., 2007
KIR inhibitory(*2DL1, 2DL2, 2DL3, 2DL4, 2DL5, 3DL1, 3DL2, 3DL3*); activating (*2DS1, 2DS2, 2DS3, 2DS4, 2DS5, 3DS1*); pseudogenes (*2PQ1, 3DP1*)	90/86	No association with PE.	Mexico	Sánchez-Rodríguez et al., 2011
HLA-G (14 bp ins/del, +3142C>G).	26/32[b]	No association with PE.	Brazil	Vianna et al., 2016
HLA-G (14 bp ins/del)	409/332[c]	No association with PE.	Brazil	Ferreira et al., 2017
ERAP2 (rs2549782, rs17408150)	528/575[d]	No association with PE.	Chile	Hill et al., 2011b

[†] *Pooled cases/controls.*
[a] *Samples were mother-neonate dyads.*
[b] *Controls were grouped in non-PE (n = 25) and healthy group (n = 7).*
[c] *Cases were grouped in PE (n = 246), eclampsia (n = 57), and HELLP (n = 106). PE, preeclampsia; HLA, human leukocyte antigen; HCMV, human cytomegalovirus; ins, insertion; del, deletion; KIR, killer cell immunoglobulin-like receptor; ERAP2, endoplasmic reticulum aminopeptidase-2*
[d] *Only Chilean mother-neonate dyads.*

increase membrane cleavage of the immunomodulatory molecules MIC-A and HLA-G (Sun et al., 2011; Rizzo et al., 2013). SNPs in *ADIPOQ* influence basal expression of the gene and predispose occurrences of metabolic disorders in French and Japanese populations (Hara et al., 2002; Fumeron et al., 2004). In a cohort of Brazilian PE women *ADIPOQ* variants −11391G/A (rs17300539), −11377C/G (rs266729), 45T/G (rs2241766), and 276G/T (rs1501299) were evaluated. The rs266729 GG genotype presented a higher frequency in PE (Machado et al., 2014). The −11377G allele is suggested to decrease the affinity of nuclear proteins in the *ADIPOQ* promoter and putatively the transcriptional activity (Bouatia-Naji et al., 2006; Wang et al., 2009; Zhang et al., 2009). Therefore, preeclamptic -11377GG genotype carriers are likely to express low levels of adiponectin, resulting in impaired control of glycemia. Also, −11377G allele carriers have been associated with chronic hypertension (Ong et al., 2010), recurrent pregnancy loss (Dendana et al., 2018), and gestational diabetes (Pawlik et al., 2017) in non-Latin American populations.

Lipid metabolism and plasmatic concentration are regulated by an enzyme encoded in lipase hepatic gene *LIPC*. *LIPC* −514C/T (rs1800588) is a promoter SNP which influences hepatic lipase levels. In fact, the −514TT genotype is associated with the lowest enzyme activity, although the variant effect is variable among non-Latin American populations (Tahvanainen et al., 1998; Ordovas et al., 2002; Isaacs et al., 2004). This variant was evaluated in a cohort of PE Peruvian women (Enquobahrie et al., 2005). Although no direct association with PE risk was observed, overweight status during pregnancy was a modifying risk factor for PE in *LIPC* −514TT genotype.

Changes in insulin responsiveness are essential in pregnancy and affect both mother and fetus. As pregnancy develops, maternal insulin resistance increases, which in turn facilitates glucose transport across the placenta and stimulates fetal insulin production, favoring normal fetal growth and development (Farrar, 2016). Hyperinsulinemia is harmful and resembles the endothelial dysfunction observed in PE pathophysiology (Muniyappa and Sowers, 2014). An interesting Mexican study evaluating the role of genetic variants of genes involved in insulin responsiveness in PE development focused on: insulin [(*INS*); *Pst*I (rs3842752) and *Mae*III (rs689)], insulin receptor [(*INSR*); *Nsi*I (rs2059806)], and insulin receptor substrate [(*IRS1*); Ala513Pro (rs1801276) and Gly972Arg (rs1801278)] (Machorro-Lazo et al., 2009). Although no statistical difference in SNPs frequencies was observed, a previous study evaluating different ethnic groups in Mexico observed differences in the *Mae*II, *Pst*I, and *Nsi*I genotype distribution when stratified by fasting insulin and serum triglyceride levels (Flores-Martínez et al., 2004; Sánchez-Corona et al., 2004). Also, the *IRS1* 972Arg allele was associated with gestational diabetes in a meta-analysis (Zhang et al., 2013) and the *INSR Nsi*I SNP (rs2059806AA genotype) was associated with PE in an Australian cohort and also in PE newborns small for the gestational age in a Sinhalese cohort (Andraweera et al., 2017). The lack of association with PE is possibly due to the stringent inclusion/exclusion criteria of the study since pregnant women with undiagnosed insulin resistance before pregnancy were excluded.

As insulin signaling involves an intricate network of molecules, it is unlikely that a single gene or SNP results in an insulin-resistant phenotype. Nevertheless, SNPs in leptin (*LEP*) and leptin receptor (*LEPR*) genes seem to have the potential to

TABLE 4 | Summary of studies in Latin America evaluating the role of genetic variants within genes involved in metabolic changes during pregnancy.

Factors	Sample size[†]	Key findings	Country	References
ADIPOQ (-11391G>A,−11377C>G, 45T>G, 276G>T)	240/161[a]	Association with PE risk: −11377GG genotype.	Brazil	Machado et al., 2014
INS (*Pst*I, *Mae*III) *INSR* (*Nsi*I) *IRS1* (Ala513Pro, Gly972Arg)	43/46	No association with PE.	Mexico	Machorro-Lazo et al., 2009
LEP (G2548A) LEPR (Gln223Arg, Lys109Arg)	146[b]	Association with GH clinical findings: LEP 2548AA genotype with BMI and 2548G allele with systemic BP; LEP 109 Lys/Lys genotype with BMI and Insulin resistance.	Brazil	Farias et al., 2017
LIPC (-514C>T)	157/180	Association with PE risk: *LIPC* −514TT genotype in overweight pregnant women.	Peru	Enquobahrie et al., 2005

[†] *Pooled cases/controls.*

[a] *Cases were grouped in PE (n = 127) and gestational hypertension (n = 113).*

[b] *Prospective cohort of pregnant women. PE, preeclampsia; ADIPOQ, adiponectin; INS, insulin; INSR, insulin receptor; IRS1, insulin receptor substrate-1; LEP, leptin; LEPR, leptin receptor; GH, gestational hypertension; BP, blood pressure; BMI, body mass index; LIPC, hepatic lipase.*

influence blood pressure during pregnancy as an indirect effect on insulin sensitivity and BMI, and therefore are relevant in PE pathophysiology (Fan and Say, 2014; Taylor et al., 2015). In a Brazilian study, *LEP* G2548A (rs7799039), *LEPR* Q223R (rs1137101), and K(Lys)109R(Arg) (rs1137100) variants were evaluated regarding their influence on maternal blood pressure during pregnancy and the postpartum period (Farias et al., 2017). Although no association with leptin levels and SNPs were observed, homozygous individuals for 2548AA genotype had lower BMI in early pregnancy, and the effect of BMI on blood pressure levels was higher in 2548AA homozygous carriers compared to G allele carriers (GA+GG). On the contrary, 2548GG+GA showed a positive increase in systemic blood pressure in early pregnancy. In a more recent study, the 2548A allele was associated with an increased risk for gestational weight gain (Martins et al., 2017). The influence of G2548A SNP in leptin levels during pregnancy is still not evident (Sugathadasa et al., 2010; Yang et al., 2016; Farias et al., 2017). Nevertheless, in non-pregnant Brazilian women, associations with obesity risk and increased leptin levels for 2548GG genotype and 2548G allele were reported (Hinuy et al., 2008). In PE, plasma levels of leptin are higher than in normotensive pregnant women (Sugathadasa et al., 2010). Also, women with impaired fasting glucose have higher levels of both insulin and leptin compared to euglycemic pregnant women (Yang et al., 2016). These observations are relevant since leptin-induced obesity is associated with hyperglycemia, hypertension, and endothelial damage.

VARIANTS IN DETOXIFICATION, DNA-REPAIR, AND APOPTOSIS-RELATED GENES

Vascular dysfunction is one hallmark of PE that is intensified by positive feedback involving altered maternal immune tolerance and placental hypoxia. In addition, endothelial damage observed in PE is the *prima facie* of impaired clearance of oxidative stress byproduct by endogenous detoxifying agents. Oxidative stress causes membrane lipid peroxidation, DNA damage and is possibly implicated in the pathogenesis of essential hypertension (González, 2014). Functional SNPs in candidate genes of the detoxification system, DNA repair, and apoptosis genes have been suggested to play roles in PE development (**Table 5**). Glutathione-S-transferase (GST) is an endogenous detoxifying enzyme superfamily that protects against oxidative stress and exogenous toxins or xenobiotics. The functional variant *GSTP1* 313A/G (rs1695) lies within the active site of the GSTP1 enzyme, and the 313G allele (valine) is associated with low catalytic activity (Ali-Osman et al., 1997). Studies evaluating this variant in different continental cohorts of PE have reported conflicting results (Zusterzeel et al., 2000; Gerhardt et al., 2004; Canto et al., 2008; Coral-Vázquez et al., 2013; Gao et al., 2016). On the one hand, it was observed that *GSTP1* 313G allele and 313GG/AG genotypes are protective factors for PE development in Maya-Mestizo women (Canto et al., 2008), a finding inconsistent with a Dutch study (Zusterzeel et al., 2000). This same variant had no influence on severe PE development in Mexican-Mestizo women (Coral-Vázquez et al., 2013), highlighting differences in results according to ethnic origin. Studies evaluating the role of *GSTP1* 313A/G in PE risk reported conflicting results, probably due to the high inter-variation and intra-variation (i.e., admixture) of the *GSTP1* 313G allele frequency (Zerbino et al., 2018). Another interesting *GST* variant is the complete deletion of *GSTM1* and *GSTT1* (Anvar et al., 2011). It is reported that Mexican-Mestizo women homozygous for *GSTT1* null genotype have a higher risk for PE, and those double homozygous for both *GSTM1* and *GSTT1* null genotypes have a 5-fold increased risk for PE (Sandoval-Carrillo et al., 2014a). These findings contribute to the conflicting body of evidence as pointed out by a meta-analysis (Anvar et al., 2011; Ge et al., 2015). Although the frequency of single deletions varies (Palma-Cano et al., 2017), we hypothesized that populations showing a high frequency of both *GSTs* deletions could have a high frequency of individuals carrying both deletion

TABLE 5 | Summary of the studies in Latin America evaluating the role of genetic variants in genes involved in detoxification, DNA repair and apoptosis in PE.

Factors	Sample size†	Key findings	Country	References
GSTP1 (313A>G)	125/274	Association with protection for PE: 313GG and AG genotypes.	Mexico	Canto et al., 2008
GSTP1 (313A>G)	230/352	No association with PE.	Mexico	Coral-Vázquez et al., 2013
GSTM1, *GSTT1*	112/233	Association with PE risk: *GSTT1* deletion, and combined *GSTM1/GSTT1* deletion (highest risk).	Mexico	Sandoval-Carrillo et al., 2014a
APEX1 (Asp148Glu) *XPD* (Lys751Gln) *XRCC* (Arg399Gln) *XRCC3* (Thr241Met)	202/350	Association with PE risk and disorder severity: *APEX1* 148Glu allele.	Mexico	Sandoval-Carrillo et al., 2014b
TP53 (Arg72Pro) *MDM2* (309T>G)	119/99	No association with PE.	Brazil	Busatto et al., 2015
CASP-8 (*rs13416436, rs2037815*)	55/162	No association with PE.	Brazil	Orlando et al., 2018

† *Pooled cases/controls. PE, preeclampsia; GSTP, glutathione s-transferase Pi-1; GTSM1, glutathione s-transferase Mu-1; GSTT1, glutathione s-transferase Theta-1; APEX1, Apex nuclease 1; XPD, Xeroderma pigmentosum complementation group D; XRCC, x-ray repair cross-complementing protein; XRCC3, x-ray repair cross-complementing protein 3; TP53, tumor protein p53; MDM2, mouse double minute-2 homolog; CASP8, caspase-8.*

alleles, implying an increased risk to oxidative stress-related disorders such as PE or vasculopathies.

Most DNA damage caused by endogenous ROS generated from oxidative stress is corrected by the DNA repairing machinery through diverse pathways (see Chatterjee and Walker, 2017). It is not clear whether DNA damage is an effect or cause of PE pathophysiology, although impaired DNA repair is observed in placental tissue from PE women (Tadesse et al., 2014). Also, accumulation of DNA errors results in cell death, and DNA repair efficiency is impacted by genetic variation in DNA repair genes. Hitherto, few studies have investigated such variants in PE development (Vural et al., 2009; Saadat et al., 2012; Sandoval-Carrillo et al., 2014b). In a study enrolling Mexican women with PE, SNPs in DNA repair genes from nucleotide and base excision pathways, homologous recombination and single-strand break repair mechanisms were evaluated. Among the variants evaluated, a possible role for the functional variant T1349G (Asp148Glu; rs1130409) in the apurinic/apyrimidinic (AP) endonuclease (*APEX1*) gene in PE development was observed. Although no difference in overall genotype distribution between PE and normotensive pregnant women was observed, consistent with a previous study (Vural et al., 2009), the 1349G (148Glu) allele frequency was higher in PE subjects compared to normotensive women. Also, the G allele frequency was higher in severe PE compared to mild-PE (Sandoval-Carrillo et al., 2014b). Although a functional study reported no difference in endonuclease activity between APEX1-148Glu and APEX1-148Asp molecules (Hadi et al., 2000), the role of this variant in PE is supported by impaired enzyme functionality (impaired DNA-binding and endonuclease activity) associated with the 1349G (148Glu) allele (Almutairi et al., 2015), and also by the fact that APEX-1 is essential for the base excision repair pathway, apoptosis, response to oxidative stress, and cell cycle control.

Essential for genomic stability and cell cycle control, the tumor suppressor protein p53 is also implicated in human reproduction (Kang and Rosenwaks, 2018). Our research group has investigated the role of the *TP53* Arg72Pro (rs1042522) and *MDM2* 309T/G (rs2279744) variants in PE development (Busatto et al., 2015). Despite a lack of association with PE risk in our study, it is reported that *MDM2* 309GG genotype confers an increased risk for PE in an Iranian population (Salimi et al., 2017). Interestingly, MDM2 309G allele frequency in normotensive and PE women was similar in both studies, although genotypic frequencies differed. These findings highlight that interaction among SNPs from the regulatory *TP53* network are likely to account for observed differences and should be addressed in further studies (Jacovas et al., 2015). Genetic variants in apoptosis-related genes, such as *CASPASE-8* (rs13416436T/A and rs2037815G/A) were evaluated in PE in a small cohort of Brazilian women, although no association with disorder risk was observed (Orlando et al., 2018).

FUTURE DIRECTIONS: CHALLENGES AND PERSPECTIVES

Over the past decade, our understanding of the molecular basis of many disorders has increased in an unprecedented manner. Despite improvements in understanding the contribution of paternal, maternal, and placental factors in PE pathophysiology, the identification of reliable predictive biomarkers for PE remains elusive. We do not wish to distract from the importance and biological implications of the many other advances in PE understanding, however, based on our knowledge we suggest future directions/studies and challenges in PE research by highlighting and discussing some emerging trends from distinct but related biological fields (**Figure 2**).

MHC Class-I Related Sequence A

It is well known that some biological aspects inherent to host immunologic tolerance to solid organ allograft transplantation (tx) could overlap to some extent with those directly related to human pregnancy (sometimes considered as a naturally

occurring grafting event). Relevant in human pregnancy, the MHC Class-Ib molecules are becoming a target of studies in human transplantation, since the rejection of allografts fully matched for HLA antigens still occur. In this context, the non-classical MHC class-I related sequence A (MIC-A or MICA), a stress-induced protein has attracted attention due to its immunomodulatory properties (Baranwal and Mehra, 2017; Risti and Bicalho, 2017). MICA has restricted tissue expression in normal physiological conditions (i.e., gastrointestinal tract and endothelial cells) (Baranwal and Mehra, 2017). *MICA mRNA* transcripts are detected in decidual, placental, and trophoblast cells from healthy pregnancies, although the MICA molecule is barely detected on placental tissues (Mincheva-Nilsson et al., 2006; Apps et al., 2008). It has been proposed that soluble MICA (sMICA) in pregnant women may participate in fetal immune escape (Mincheva-Nilsson et al., 2006; Huang et al., 2011), although high levels of sMICA were considered a predictive biomarker for *in vitro* fertilization failure (Porcu-Buisson et al., 2007). Indeed, in pathological situations, *MICA* expression patterns might change. A dimorphism known as MICA-129Val/Met (rs1051792), is reported to influence both sMICA levels and affinity to the NKG2D receptor expressed on cytotoxic cells, including uNK cells. It was observed that soluble NKG2D has a higher affinity to 129Met molecules (range 10- to 50-fold) compared to 129Val MICA (Steinle et al., 2001). Thus, this variant seems relevant in inflammatory disorders (Isernhagen et al., 2016), but its influence on pregnancy disorders is yet to be addressed. Besides, high sMICA levels are observed in PE and other vascular pregnancy disorders, often being absent in healthy pregnancies. Further, sMICA maternal plasma from PE women downregulates NKG2D expression on CD3-CD56+NK cells from healthy donors (Haumonte et al., 2014), suggesting that sMICA impairs vascular remodeling through downregulation of NK effector functions by means of interferon-gamma secretion and cytotoxicity (Haumonte et al., 2014; Zhou et al., 2014). Additionally, microvesicles derived from early placenta harbor MICA which has potential to downregulate NKG2D (Hedlund et al., 2009).

Non-coding RNAs and Epigenetics

In the era of genomics, next-generation DNA sequencing is becoming a technique accessible to most laboratories. The possibility of massively interrogating millions of DNA strands at the same time has fostered research in the search of causal genetic variation involved in PE pathophysiology (see Yong et al., 2018b). The profile of non-coding RNA (ncRNA) in distinct tissues, body fluids and disorders has revealed a universe of RNAs, which is currently under extensive investigation. Traditionally, ncRNAs are divided into two classes based on size: small ncRNA (<200 nt) and long ncRNA (>200 nt). The small ncRNA includes microRNA (miRNA), small interfering RNA, small nuclear RNA, small nucleolar RNA, ribosomal RNA, transfer RNA, and P-element-induced-wimpy testis (piwi)-interacting RNA. Their regulatory activity extends to different levels of transcription and post-transcriptional control (Anfossi et al., 2018). Although little is known about long non-coding RNA (lncRNA) functions, they participate in several biological processes such as epigenetic regulation, transcriptional and post-transcriptional control, regulation of miRNAs (by acting as sequence decoys) and acting as scaffolds for protein complex (Li et al., 2014), suggesting a more extensive biological versatility compared to small ncRNAs.

Currently, ncRNAs are considered promising diagnostic tools and disease progression biomarkers in the clinical setting, because their level of presence is expected to correlate with repressive activity (Bounds et al., 2017). In PE, an increasing number of studies have identified potential regulatory ncRNAs, most of them miRNAs such as miR-155 and miR-210 (Bounds et al., 2017; Wei et al., 2017; Winger et al., 2018; Yoffe et al., 2018). miRNAs are of particular interest due to their high stability in body fluids (Brase et al., 2010) and their potential to be released inside microvesicles (Salomon et al., 2017). An emerging role of genetic variants within miRNAs (even virally encoded miRNAs) highlights their influence in the susceptibility to viral infections (Ellwanger et al., 2018).

Despite the promising applications of ncRNAs as biomarkers in distinct pathologies, the increasing complexity for use due to ncRNA heterogeneity as well as the diversity of methodologies implemented for their isolation (Anfossi et al., 2018) highlight some challenges that should be addressed, including the need for sample collection, processing, and analysis standardization in order to increase the feasibility and replicability of studies.

It is clear that epigenetics is a mechanism involved in the development of a healthy pregnancy. Partially methylated domains (PMDs) are regions showing reduced average methylation levels which cover up to 40% of the genome. PMDs are observed in only a few cell types: cultured cells, malignant cells and placental cells (Hansen et al., 2011; Schroeder and LaSalle, 2013). Interestingly, PMD covers 37% of the placental genome, and most of the genes in PMD are repressed, suggesting that repression of specific genes within PMD during pregnancy is needed for healthy development (Schroeder et al., 2013). Following this reasoning, disruption in the epigenetic program could lead to placental dysfunction and associated disorders (Robinson and Price, 2015). Some studies have evaluated the methylation status, or methylome, of the placenta in pregnancy disorders such as preterm birth (Hong et al., 2017), intrauterine growth restriction (Hillman et al., 2015), and PE (Blair et al., 2013; Anton et al., 2014; Chu et al., 2014; Liu et al., 2014). Owing to the fact that different methodologies exist, the comparison between studies is not always possible. Nonetheless, two genes (*DAPK3* and *PAPPA2*) were observed to share methylation patterns in preeclamptic placenta (Blair et al., 2013; Chu et al., 2014; Bianco-Miotto et al., 2016). Apart from pregnancy disorders, the methylation patterns in placental PMD suggest a causal link to autism spectrum disorders because behavioral genes are overrepresented in placental PMD (Schroeder et al., 2016). There is also a suggestion of an interaction between environmental factors and DNA, altering epigenetic features and therefore susceptibility to many disorders including PE (Chelbi and Vaiman, 2008).

Analysis of DNA methylation in cord blood cells may improve our knowledge of epigenetic signatures in pregnancy (and PE) and improve understanding of their implications

for adult life. For example, hypomethylation of the 11β-hydroxysteroid dehydrogenase type-2 (*HSD11B2*) gene promoter is suggested to increase fetal glucocorticoid levels identified as risk factors for metabolic diseases (Hu et al., 2014). In a study evaluating cord blood in early preeclamptic women, different sets of genes from lipid metabolism, cellular proliferation and inflammation showed variable levels of methylation in their promoter regions, suggesting that early epigenetic signatures are detected in newborns and could be associated with predisposition to cardiovascular diseases in adulthood (Ching et al., 2015). Nevertheless, whether early risk epigenetic modifications remain constant and act as disease triggers or risk-modifying factors is still an open question.

Genomic imprinting is closely associated with parental origin, which highlights that epigenetic disruption can result in abnormal expression of imprinted genes in the placenta and contributes to PE development. The distal-less homeobox-5 (*DLX5*) gene is paternally imprinted (maternally expressed gene) in normal healthy placenta, but its status is upregulated in PE as a result of the loss of paternal imprinting. *DLX5* was upregulated in up to 70% of PE placentas correlating positively with classical PE markers (i.e., PlGF:sFLT). Of note, overexpression of DLX5 *in vitro* led to reduced proliferation and endoplasmic reticulum stress of trophoblast cells (Zadora et al., 2017). GATA-binding protein 3 (*GATA3*), a gene relevant to trophoblast invasion, was also identified as a candidate for future research concerning dysregulated imprint and pregnancy disorders (Chiu and Chen, 2016; Zadora et al., 2017).

Overall, the methylome opens new perspectives for comprehension of the phenomenon of inherited traits unrelated to classical nucleotide sequence changes in the genome (SNPs or mutations) and how they affect phenotype. The future is promising, but some important issues should be addressed. For example, PMD is overrepresented in the placental methylome, but most of the studies published so far have ignored them, raising the question of whether PMD occurs in specific trophoblast cell lineages or at specific stages of development (Schroeder et al., 2013; Bianco-Miotto et al., 2016). Interestingly, methylation patterns in early extraembryonic tissues resemble those commonly observed in cancer (Smith et al., 2017), implying that comprehension of the epigenomic landscape of these two phenomena would provide some clues to the inherent process of cellular invasion, proliferation, and vasculogenesis. Also, the paradox of high methylation of CpG islands in genes within placental PMD is yet to be addressed. Lastly, future studies should differentiate hypomethylation patterns occurring in PMD regions from those occurring in other genomic regions (Schroeder et al., 2013).

Placental Microbiome—Friend or Foe

The fact that microorganisms are detected in the placenta, the womb, and the fetus, once thought of as sterile entities, has attracted much attention. The detection of bacterial DNA in the placenta (Aagaard et al., 2014; Collado et al., 2016) and in the amniotic fluid (Collado et al., 2016) has brought the "placental microbiome" into the spotlight. This concept challenges the traditional belief that newborns acquire their first bacteria only

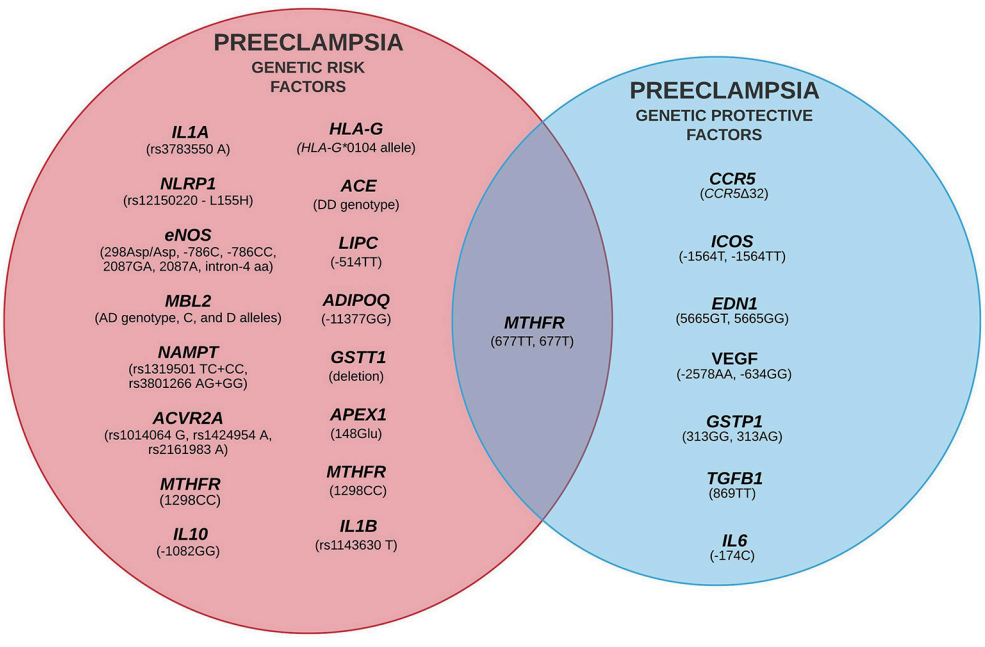

FIGURE 3 | Summary of preeclampsia-associated genetic variants addressed in Latin American studies. Only single locus polymorphisms are shown. Risk and protection associated variants are shown where the intersection represents variants found in both protective and risk factors in different studies. Additional information and haplotypes are detailed in **Tables 1**–**5**.

as they pass through the birth canal. The observation that *Enterococcus faecium* from human breast milk orally inoculated in pregnant mice can be detected in the amniotic fluid, and the pup's meconium (Jiménez et al., 2005, 2008; Aagaard et al., 2014) further supports the concept of the placental microbiome. In this same line, it seems that the newborn gut microbiome shares similarities to the maternal oral microbiome (Aagaard et al., 2014). The nature of symbiosis between extraembryonic tissues and the local community of microorganisms is still unknown. Although studies support the existence of fetal microbiomes, there is currently skepticism surround the concept, as discussed in other studies (Lauder et al., 2016; Perez-Muñoz et al., 2017).

The presence of placental microbiota in normal pregnancy (Aagaard et al., 2014; Parnell et al., 2017) intuitively implies that an altered microbiome would underlie pregnancy disorders such as chorioamnionitis and preterm birth (Antony et al., 2015; Prince et al., 2016). In this sense, a novel mechanism by which viruses may alter immunologic tolerance to intrauterine bacteria was suggested (Cross et al., 2017). It demonstrated that polymicrobial exposure of human fetal membranes (FM; amnion and chorion) explanted to bacterial LPS and virus [Herpes simplex virus type 2 (HSV2)] samples result in the aberrant expression of IL-1β, which is commonly observed in chorioamnionitis and preterm birth (Gomez-Lopez et al., 2017). The mechanism is not fully understood, however, it involves downregulation of the MER tyrosine kinase proto-oncogene (MERKT) receptor, allowing the activation of Nod-like receptor protein-3 (NLRP3) also known as the NLRP3 inflammasome through a synergistic signaling by LPS/TLR4 (TLR: toll-like receptor-4) and viral double strand dsRNA/TLR3 (dsRNA: double strand RNA) (Cross et al., 2017). It is worth mentioning that some viruses exploit TAM receptors for cell attachment and entry, but whether they are surrogates capable of suppressing TLR signaling is unclear (Best, 2013; Bhattacharyya et al., 2013). This observation is relevant since NLRP3 expression seems to be higher in the placental villi of preeclamptic women compared to normotensive women (Weel et al., 2017). However, if polymicrobial exposure underlies NLRP3 expression in preeclamptic placentas, and if different herpesviruses (i.e., congenital Cytomegalovirus infection) besides the ones evaluated are also able to reduce LPS threshold response are still open questions.

GENERAL CONCLUDING REMARKS

In Latin America, several studies approached the molecular basis of PE pathogenesis, documented by the increasing amount of scientific study and its impact on local and international scientific communities. In this endeavor, Brazil and Mexico are at the forefront of scientific production. However, we call attention to the need for studies in other Latin American countries, since these regions are characterized by a highly genetically diverse human population. Additionally, PE and gestational hypertensive disorders are a heavy burden in Latin America, strongly affecting maternal and fetal health.

Several genetic variants influencing PE predisposition were reported (**Figure 3**), some consistently associated with PE across different populations, despite disparities in the genetic/ethnic background inherent in Latin American populations. Genetic intra- and inter-variation have a great influence on genetic predisposition to PE. Although a comprehensive literature review was performed in this study, it may not be representative of the genetic variability present in Latin America since human population studies focus on small samples and therefore may not represent the genetic variability of entire local populations. Additionally, in developing countries, medical specialties (i.e., high-risk pregnancy care) are often centralized in the biggest cities. Therefore, replication of studies in different populations and multicentric collaborative studies are encouraged and would provide a better evaluation of the maternal genetic components of PE development in Latin America. Finally, PE and other hypertensive pregnancy disorders are the primary cause of maternal-fetal morbidity and mortality in low- and middle-income countries, representing a significant burden on public healthcare services. Therefore, it is imperative that public health policies assure prenatal care, perinatal monitoring, and health education in order to reduce the risk of pregnancy-related complications.

AUTHOR CONTRIBUTIONS

RM designed the review, planned the topics, wrote the review, reviewed the literature, and designed the figures and tables. VK wrote the review, reviewed the literature, designed the figures, and proofread the review. JC designed the review, contributed to writing the topics and critically reviewed the manuscript.

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Paternal Determinants in Preeclampsia

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Preeclampsia is a condition associated with high rates of maternal-fetal morbidity and mortality. It usually occurs in 3–10% of nulliparous women and 18% of previously affected women. Different lines of evidence have demonstrated the role of the father in the onset of preeclampsia. The placenta is the cornerstone of preeclampsia and poses important paternal genetic determinants; in fact, the existence of a "paternal antigen" has been proposed. Nulliparity is a well-known risk factor. Change of partner to a woman without history of preeclampsia increases the risk; however, this change decreases in women with history of the condition. High interval between pregnancies, short sexual intercourse before pregnancy, and conception by intracytoplasmic sperm injection suggest a limited exposure to the so-called paternal antigen. A man who was born from a mother with preeclampsia also increases the risk to his partner. Not only maternal but also paternal obesity is a risk factor for preeclampsia. Fetal HLA-G variants from the father increased the immune incompatibility with the mother and are also significantly associated with preeclampsia in multigravida pregnancies. An analysis of a group of Swedish pregnant women showed that the risk for preeclampsia is attributable to paternal factors in 13% of cases, which could be related to genetic interactions with maternal genetic factors. This review aimed to evaluate the evidences of the father's contribution to the onset of preeclampsia and determine the importance of including them in future studies.

Keywords: preeclampsia, paternal, primipaternity, placenta, immunology, genetics

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BACKGROUND

Preeclampsia is a condition in pregnant women associated with high rates of maternal and fetal morbidity and mortality worldwide. This disease is characterized as a systemic syndrome with *de novo* hypertension occurring after 20 weeks of gestation as well as proteinuria (300 mg in 24 h) (NHBPEP, 2000). In 2013, the Task Force on Hypertension in Pregnancy established new diagnostic criteria for hypertension in pregnancy.

Preeclampsia is related to deficient placental implantation, with multisystem consequences (generalized endotheliosis) in the mother (Roberts and Hubel, 2009). Its incidence in industrialized

countries ranges from 3 to 5% (Stone et al., 1995; Dahlstrom et al., 2006; Wallis et al., 2008), which reaches up to 16% in Nigeria (Osungbade and Ige, 2011). The frequency of maternal and perinatal deaths in Mexico is 34 and 33%, respectively (Peralta Pedrero et al., 2006).

Preeclampsia is classified as mild and severe and can complicate to hemolysis, elevated liver enzymes, and low platelet count (HELLP) syndrome and eclampsia (Geller et al., 2004).

The pathophysiology of preeclampsia is characterized by placental hypoxia and/or ischemia leading to overexpression of hypoxia-inducible factor 1 (HIF1), which in turn increases the expression of the soluble isoform of vascular endothelial growth factor (sFlt) (Nevo et al., 2006). Hypoxic placenta also releases the soluble endoglin, antagonizing the production of endothelial nitric oxide synthase (eNOS) through sequestering of transforming growth factor beta1 (TGFb1) (Sandrim et al., 2008). In addition, the proangiogenic factors such as the placental growth factor (PIGF) and vascular endothelial growth factor (VEGF) (Reuvekamp et al., 1999). Together, they can trigger the antiangiogenic state in the mother, which resulted in generalized endothelial dysfunction.

Risk factors for disease development include nulliparity, pregnancy with multiple products, previous history of preeclampsia, vascular and connective tissue diseases, maternal age of >35 years, Afro-American ethnicity, preexistent renal disease, arterial hypertension, type 2 diabetes, and obesity (Eiland et al., 2012).

Previous studies on preeclampsia have considered the mother's participation almost exclusively. However, the placenta is a transient biparental organ with maternal and paternal contributions. In this way, changes on the expression profile of genes involved in the metabolism and transport in the placenta depend on various maternal and paternal genetic profiles (Zusterzeel et al., 2002; Dekker et al., 2011).

Increasing evidence has demonstrated the father's role in the onset of preeclampsia. In Astin et al. (1981) reported a case of a man who fathered two consecutive women who developed severe preeclampsia and passed away, suggesting the existence of a "fatal father factor."

The risk of preeclampsia is genetically attributable to the mother (35%), fetus (20%), and couple (13%) (Cnattingius et al., 2004). Women and men who were born from a pregnancy with preeclampsia are at higher risk to have a baby, result of a preeclamptic pregnancy (Esplin et al., 2001). The change of partner increases the risk for preeclampsia in 1.6%, which increases up to 2.9% in a woman whose second pregnancy is the result of union with a man who has had a previous partner with preeclampsia (Lie et al., 1998). Change of partner plays a predisposing or protective role, depending of the presence or absence of the disease in the first pregnancy (Wikström et al., 2012). The presence of fetal variants of HLA-G from the father and those outside the mother generate a paternal-fetal susceptibility component for the development of preeclampsia (Tan et al., 2008).

Paternal genetic material plays an important role for the onset of preeclampsia. A triploid (69 XXX) partial mole, with paternal isodisomy in the placenta and a disomic fetus, increases preeclampsia-like symptoms at 19 weeks of gestation (Yoneda et al., 2013). Although preeclampsia is a human-specific disorder, animal models of the disease have been created to show the role of the paternal genetic factors. A female transgenic mouse for human angiotensinogen gene was mated with a male transgenic mouse for human renin gene, originating a preeclampsia-like syndrome (Takimoto et al., 1996).

In this review, we aimed to describe different lines of evidence regarding the paternal contribution on preeclampsia.

EPIDEMIOLOGICAL AND CLINICAL EVIDENCE

Preeclampsia has been traditionally considered as a disease during first pregnancies; however, an early study by Need (1975) showed that a woman, pregnant by different fathers, had a healthy twin pregnancy with the first men, but severe preeclampsia occurred in the second one. The analysis of 34,201 patients revealed the presence of 47 multigravid patients with severe preeclampsia. A new partner was demonstrated in 13 among them (19.1%), and significant difference was observed than those in the control group ($p < 0.01$) (Feeney and Scott, 1980). Ikedife (1980) showed that change of partners was observed in 34 (74%) out of 46 multiparous patients with eclampsia. A woman with three different husbands presented preeclampsia only in the first pregnancy of the second and third spouses. Therefore, preeclampsia is not just a primiparous woman disease but also a condition related to the first pregnancy with a particular partner (Chng, 1982). The Guadeloupe study revealed an increased change of partner in multiparous women who were affected with preeclampsia (Robillard et al., 1993). The same group demonstrated that the incidence of preeclampsia was 11.9% among primigravidae, 4% among multigravidae without a change of paternity, and 24% among multigravidae with a new partner; however, these numbers depend on the duration of sexual cohabitation before conception (Robillard et al., 1994).

When evaluating paternal vs. maternal half-sisters with preeclampsia, Lie et al. (1998) found an increased risk for the disease in the former. Likewise, they showed that 13% of primiparous mothers with preeclampsia had recurrence in the second pregnancy, decreasing the influence of partner's change (11.8%), which is opposite in mothers without preeclampsia in their first pregnancy (Lie et al., 1998).

The same results were obtained by Li and Wi (2000). Pipkin (2001) observed that partner's change increases the risk for preeclampsia occurrence in primiparous women.

A woman pregnant by a partner who previously fathered a woman with preeclampsia was highly at risk to develop the disease. In 2004, Cnatinggius et al. evaluated three different scenarios: (1) mothers without preeclampsia and no partner change (control group); (2) mothers with previous preeclampsia, current preeclampsia, and change of spouse who was not fathered by a previous couple with preeclampsia; and (3) mothers with preeclampsia and change of couple who fathered another woman with preeclampsia. The maternal effect was 45%, meanwhile the paternal one was 10% (Cnattingius et al., 2004).

Conversely, Chigbu et al. (2009) evaluated two groups of Nigerian women with and without change of partner in their second pregnancy and found no differences for the development of preeclampsia.

Marti and Herrmann (1977) found that preeclampsia is associated with shorter exposure to spermatozoa in younger women and more frequent use of barrier contraceptive methods. Therefore, the authors coined the term "immunogestosis" to denote the immune nature of preeclampsia. The use of barrier contraceptive methods was significantly higher in women with preeclampsia compared to healthy women [odds ratio (OR) = 2.48], and the number of sexual contacts was inversely related to the risk of preeclampsia (Klonoff-Cohen et al., 1989). Conversely, Mills et al. (1991) and Ness et al. (2004) found no differences in the use of barrier contraceptive methods between women with and without preeclampsia.

Shorter sexual intercourse increases the shorter antigenic seminal exposure (Beer, 1989). The risk of pregnancy-induced hypertension (PIH) was increased when conception is within 12 months of sexual cohabitation: 40% in 0–4 months, 23% in 5–8 months, 15% in 9–12 months, and 5% after 12 months (Robillard et al., 1994). The same author compared patients with simple PIH versus those with preeclampsia and eclampsia and observed that the sexual cohabitation times are shorter (9.5 months) compared to those of unaffected women (26.3 months) (Robillard and Hulsey, 1996). Koelman et al. (2000) found a lower frequency of oral sex in women with preeclampsia compared to healthy pregnant women ($p = 0.0003$), suggesting a protective role. The authors report lower amounts of soluble HLA A and HLA B in the seminal fluid, in partners of preeclamptic women (Koelman et al., 2000). In Robertson et al. (2003) proposed how repeated semen exposure protects preeclampsia, based on four lines of evidence: (1) the semen contains antigens shared by the conception; (2) after the seminal contact, the maternal mucosa can mount a regulated immune response to semen antigens; (3) semen contains among others, high amounts of TGFb that can inhibit type 1 immunity; and (4) TGFb-dependent changes in T-lymphocytes allow a hypo-responsiveness to paternal antigens. The exposure to seminal fluid through the vagina is inversely correlated with the risk of preeclampsia occurrence, whereas the oral exposure to seminal fluid has no effect on disease development (Saftlas et al., 2014).

A clear increase in the frequency of preeclampsia when the father (OR = 2.1) and mother (OR = 3.3) are products of preeclampsia-complicated pregnancies has been observed; however, this study did not consider the changes of paternity (Conde-Agudelo, 2001). In men who were born after a pregnancy complicated by preeclampsia, the risk in the first pregnancy was moderately increased compared with men who were born after a pregnancy without preeclampsia (OR = 1.5, 1.3–1.7).

This observed risk increases when severe or early preeclampsia is considered (OR = 1.9, 1.4–2.5) (Skjaerven et al., 2005). Lie (2007) found similar numbers: fathers who came from a preeclamptic pregnancy had a 1.5-fold risk [95% CI 1.3–1.7] of fathering a preeclamptic pregnancy.

Since, Need et al. (1983) reported a significantly higher frequency of preeclampsia in women with abortions (15.7%) than those with normal pregnancy (4.7%). They also found that azoospermia and oligospermia present a lower frequency of preeclampsia of 8.7 and 13.6%, respectively, which could be related to the minimal antigenic sperm exposure. The frequency of preeclampsia was higher in the donor insemination program than those in the father insemination program (OR = 1.20, 95%) (Smith et al., 1997). Hoy et al. (1999) revealed a higher frequency of preeclampsia in donor-inseminated women vs. natural-inseminated women (OR = 1.4, 95% CI). In Salha et al. (1999) compared 72 infertile women subjected to sperm, ovum, or embryo donation, with the same number of pregnant women through insemination with their own ovum or partner's spermatozoa (control group). Fourteen patients developed preeclampsia, 13 of whom belong to the group of donated gametes and 1 to the control group. Kyrou et al. (2010) found a marginally significant ($p = 0.05$) higher frequency of preeclampsia in women conceiving by a sperm donor compared to partners in spermatozoa insemination. The type of spermatozoa and number of previous insemination cycles were the variables that influenced the risk of preeclampsia ($p = 0.012$); in fact, the authors observed that the fewer the number of the insemination cycles, the higher the risk of preeclampsia (Kyrou et al., 2010). A recent meta-analysis of seven studies showed the association with preeclampsia in women conceiving with donor sperm (OR = 1.63) (González-Comadran et al., 2014). Another study evaluated the risk of preeclampsia in infertile women subjected only to sperm donation via intrauterine insemination (IUI) or *in vitro* fertilization (IVF), compared to those with primary sperm donation (IUI or IVF) followed by egg donation. A higher frequency of preeclampsia in the latter was observed; therefore, the authors conclude that double gamete donation is associated with increased risk for preeclampsia (Bartal et al., 2018).

The aforementioned studies support the theory of the immunological basis of preeclampsia.

IMMUNOLOGICAL EVIDENCE

Preeclampsia is a state in which alloantigen (placenta of paternal origin) must be recognized to avoid rejection (Saito et al., 2007). Trophoblast cells must express paternal alloantigens that must be recognized by the mother's immune system. Extravillous trophoblast express different HLA-C, E, F, and G (Hackmon et al., 2017). HLA-C is the ligand of immunoglobulin-like receptors (KIR) that are expressed in decidual natural killer (NK) cells (Sharkey et al., 2008). HLA-C and KIR are polymorphic; therefore, many maternal/paternal different combinations are possible. Two KIR haplotypes exist A and B, with the latter stimulating the expression of chemokines and angiogenic cytokines, promoting trophoblast invasiveness. Therefore, haplotype B could be protective for preeclampsia (Redman and Sargent, 2010). Seminal priming triggers a cascade of events for placental recognition or rejection. Seminal fluid contains high amounts of TGF-b that induces T-regulatory cells (Treg); these cells modulate immune responses in an antigen-specific way. Therefore, the effects of HLA-C/KIR interaction plus the seminal priming activity of TGF-b could in some

extent explain the immune nature of father's involvement in preeclampsia (Redman and Sargent, 2010).

The immune nature of preeclampsia was observed by Need (1975), who demonstrated histoincompatibility in a mother who developed preeclampsia with her second partner through the evaluation of HL-A typing. In Feeney et al. (1977) found a lower incidence of preeclampsia in previously blood-transfused women, compared with the same number of non-transfused primigravidas. A similar effect is observed in patients with kidney transplantation (Feeney et al., 1977). In the same year, Marti and Herrmann, (1977) found a correlation between the number of exposures to semen and lower frequency of preeclampsia; they coined the term immunogestosis to explain both, the immunologic tolerance and immunologic enhancement that abrogates immunoreaction against paternal and fetal histocompatibility antigens.

Birkeland and Kristofferson (1979) evaluated the immune response in mothers with and without preeclampsia and found no leukocyte antigens against the father in women with normal pregnancies, meanwhile these antigens were identified in one woman with severe preeclampsia. The evaluation of HLA A, B, and DR in women with severe and mild preeclampsia as well as their husbands and babies revealed a higher frequency of DR4 in all family members in severe preeclampsia (Kilpatrick et al., 1987). However, HLA A, B, and C are expressed in low amounts in the trophoblast, and HLA-G protein is exclusively expressed in trophoblast cells in high amounts. The evaluation of 1597del/C allele was not associated with preeclampsia (Aldrich et al., 2000). Three polymorphisms in HLA-G were evaluated in 68 primigravida trios, but were not associated with preeclampsia (Bermingham et al., 2000). The evaluation of 15 alleles in 4 exons of HLA-G in 155 family triads showed an overrepresentation of a homozygous HLA-G genotype in 40 pre-eclamptic offspring compared to 70 controls (p = 0.002) among primiparous women; further analyses suggested that the differences between pre-eclamptic cases and controls were primarily accomplished by a different transmission from the father of a 14 bp deletion/insertion polymorphism in the 3′UTR region (14 bp del/in) (p = 0.006) (Hylenius et al., 2004). In Tan et al. (2008) observed a significant association between paternally inherited HLA-G allele G*0106 in the fetus and an increased risk for preeclampsia, but only in multigravid pregnancies. The 14 bp del/in was evaluated in three different combinations: mother/offspring, father/offspring, and couples; heterozygosity in the mother plus double insertion in babies was significantly higher in severe early-onset preeclampsia (p = 0.023), and the frequency of double deletion in both the father and baby was lower in severe early-onset preeclampsia (p = 0.024). The analysis of couples did not reveal significant differences between cases and controls (Zhang et al., 2012). A recent meta-analysis of 1,625 cases and 2,145 controls in all members of the triads evaluated the influence of 14 bp del/in in the onset of preeclampsia; the results did not reveal the association with the disease in offspring, mothers, or fathers. A stratification showed the association of 14 bp del/in with preeclampsia in European Caucasian offspring, but not found in African descent population (Pabalan et al., 2015).

Semen is not sterile, and its microbes have also been considered as a potential cause of preeclampsia. Repeated exposure to semen seems to create a memory protecting women from preeclampsia and the same could be possible in the case of semen microbes. Therefore, common elements of preeclampsia and infections such as Galectin13, Toll-like receptors, and antiphospholipid syndrome are found (Kenny and Kell, 2018).

GENETIC EVIDENCE

The genetic nature of preeclampsia has also been evaluated through (1) familial cases, (2) twin studies, (3) consanguinity studies, (4) candidate gene evaluation, and (5) linkage analysis. Genes are involved in different pathophysiological mechanisms involved in preeclampsia. Genetic studies that included mother/father/children triads, which allow to determine the sole or joint contribution for preeclampsia, were limited.

The evaluation of thrombophilic genes methyle netetrahydrofolate reductase *(MTHFR)* and *FVL* in 92 mother/father/child triads revealed an increased risk only in mothers carrying two mutated copies of *MTHFR* and one mutated allele in *FVL*. Therefore, the risk of preeclampsia is not increased in the presence of fetal *MTHFR* or *FVL* mutations (Vefring et al., 2004). In van Dijk et al. (2010) observed a methylated paternal copy of *STOX1* gene and an unmethylated active maternal copy of the gene. Maternal transmission of this gene has been demonstrated in preeclampsia (van Dijk and Oudejans, 2011). The evaluation of *GSTP1, eNOS*, and *LPL* genes in 167 preeclamptic and control triads in a Greek cohort, which significantly demonstrated higher frequencies of Val105 allele (*GSTP1*) and Glu298Asp (*eNOS*) in control vs. preeclamptic groups in mothers, fathers, and child. The -93 polymorphism (*LDL*) was higher in preeclampsia only in mothers, but not in fathers or children. The transmission disequilibrium test revealed no differences in the rate of transmission of the studied common vs. mutated alleles (Pappa et al., 2011).

In Zhou et al. (2013) found a significant association of C4599A polymorphism in *AGTR2* gene with preeclampsia in mothers with body mass index (BMI) of $\geq$25 kg/m^2 in their partners and studied children. Galaviz-Hernandez et al. (2016) evaluated the polymorphism rs5370 of *EDN1* gene in mothers with preeclampsia and their partners, showing a significant negative association with the disease in case of fathers (OR = 0.42; CI 95%, 0.18–0.94, p = 0.034), which was strengthened after adjusting the paternal protective factors. The evaluation of two polymorphisms in *SOD1* (+35A/C) and *SOD2* (Ala16Val (C/T) genes in 698 mother/father/infant triads revealed a significant association with preeclampsia in fathers with Ala16Val (TT genotype) [OR = 2.77 (1.32–5.81), p = 0.007]. This study revealed essentially the same risk for preeclampsia in both combined TT genotypes in mothers and fathers [OR = 6.80 (2.32–19.95), $p < 0.001$] and mother/father/infant triads [OR = 6.46 (2.16–19.31), $p < 0.001$] (Luo et al., 2018). Polymorphisms in the thrombophilic genes factor V Leiden, prothrombin, and *MTHFR* were evaluated in women, fetus, and fathers as risk factors for pregnancy-associated complications, including preeclampsia. The authors

found significant differences between cases and controls in maternal Factor V and fetal *MTHFR*, but no differences on any of the polymorphisms analyzed in the father's group (Nevalainen et al., 2018).

CONCLUSION AND PERSPECTIVES

Epidemiological, clinical, immunological, and genetic evidences supported the contribution of fathers in the onset of preeclampsia. Despite this, few studies were intended to evaluate the spouses' role. In this way, the evaluation of paternal-derived immune and genetic materials was performed in order to identify the risk and prognostic markers of preeclampsia. Recent advances in placental epigenetics, along with the use of OMICS tools, ensure the identification of molecular markers associated with the role of fathers in the development of preeclampsia.

AUTHOR CONTRIBUTIONS

CG-H gathered and analyzed the data, and wrote the manuscript. MS-M gathered and organized the data and references. ET organized the data. MS-M, JG-O, and ET critically lectured the manuscript. BL-R gathered the data.

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Obesity and Preeclampsia: Common Pathophysiological Mechanisms

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Preeclampsia is a disorder specific of the human being that appears after 20 weeks of pregnancy, characterized by new onset of hypertension and proteinuria. Abnormal placentation and reduced placental perfusion associated to impaired trophoblast invasion and alteration in the compliance of uterine spiral arteries are the early pathological findings that are present before the clinical manifestations of preeclampsia. Later on, the endothelial and vascular dysfunction responsible of the characteristic vasoconstriction of preeclampsia appear. Different nutritional risk factors such as a maternal deficit in the intake of calcium, protein, vitamins and essential fatty acids, have been shown to play a role in the genesis of preeclampsia, but also an excess of weight gain during pregnancy or a pre-pregnancy state of obesity and overweight, which are associated to hyperinsulinism, insulin resistance and maternal systemic inflammation, are proposed as one of the mechanism that conduce to endothelial dysfunction, hypertension, proteinuria, thrombotic responses, multi-organ damage, and high maternal mortality and morbidity. Moreover, it has been demonstrated that pregnant women that suffer preeclampsia will have an increased risk of future cardiovascular disease and related mortality in their later life. In this article we will discuss the results of studies performed in different populations that have shown an interrelationship between obesity and overweight with the presence of preeclampsia. Moreover, we will review some of the common mechanisms that explain this interrelationship, particularly the alterations in the L-arginine/nitric oxide pathway as a crucial mechanism that is common to obesity, preeclampsia and cardiovascular diseases.

Keywords: preeclampsia, obesity, endothelial dysfunction, nitric oxide, cardiovascular risk

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INTRODUCTION

Obesity is considered a risk factor for preeclampsia and there are many common mechanisms that link obesity with a higher risk of developing preeclampsia (Spradley et al., 2015). Moreover, preeclampsia, similar to obesity, is associated with an increased risk of future cardiovascular diseases for the mother (Bellamy et al., 2007). Preeclampsia is a specific disease of the human being characterized by hypertension, edema of extremities, and proteinuria occurring after 20 weeks of gestation. It affects many organ systems and leads to high maternal mortality and morbidity worldwide (Lopez-Jaramillo et al., 2009).

Hypertensive disorders are amongst the most common disorders that affect pregnant women and are major contributors to maternal deaths. In a systematic review conducted by the World Health Organization (WHO), 16% of maternal deaths in developed countries were attributed to hypertensive disorders, 9% in the regions of Africa and Asia, and as high as 25% in Latin America and the Caribbean (Khan et al., 2006). The WHO-review named hypertensive disorders during pregnancy the leading cause of maternal deaths in industrialized countries, responsible for 16% of maternal deaths. Regionally, hypertensive disorders are responsible of 25,000 maternal deaths in Africa, 22,000 in Asia, 3,800 in Latin America and the Caribbean, and 150 maternal deaths in industrialized countries (Khan et al., 2006). The two disorders most associated with hypertension during pregnancy are eclampsia and preeclampsia. Preeclampsia can be characterized by hypertension, proteinuria, edema of extremities, persistent severe headaches, visual disturbances, sudden onset of swelling of hands and feet, and hyperreflexia, amongst many more. It affects coagulation, the renal, respiratory, and central nervous system and can have detrimental consequences on the placenta and the baby (Duley, 2009). Most maternal hypertensive deaths are attributed to eclampsia as opposed to preeclampsia. Eclampsia occurs when preeclampsia goes untreated and the hypertensive mothers experience seizures. It is estimated that hypertensive disorders complicate 5–10% of all pregnancies and preeclampsia arises in 2–8% of them, although it is difficult to gather accurate data on the prevalence of preeclampsia worldwide because of differences in the definitions and in the symptoms that are used as diagnostic criteria (Khan et al., 2006). However, there are important variatons in the prevalence of preeclampsia between lower middle-income countries and high-income countries. For instance, preeclampsia is diagnosed in 3% of all pregnancies in the United States (Wallis et al., 2008), and 3.3% in New Zealand (Stone et al., 1995) while in Colombia it is present in 9% and in Haiti in 17% (Lopez-Jaramillo et al., 2005, 2007). In the United States, from 1987 to 2004 the average annual incidence rate of preeclampsia and gestational hypertension were 2.7 and 2.1% (Wallis et al., 2008). In the same 18-year period, the rate of preeclampsia and gestational hypertension has increased significantly. From 1987–1988 to 2003–2004, the age-adjusted rate of preeclampsia rose by 24.6%. Meanwhile, the rates for gestational hypertension increased by almost threefold from 1.07 to 3.6%. The rate for eclampsia during the same time decreased by 22% from 1.04 in 1987–1995 to 0.82 in 1996–2004, although this change was not significant. The risk of developing preeclampsia is highest amongst women $<$20 years of age, but women $\geq$35 years of age also have an increased risk of developing preeclampsia (Wallis et al., 2008).

Although the terminology and methods for classifying and diagnosing the hypertensive disorders of pregnancy differ from country to country and region to region, some common risk factors have been identified. These risk factors include, maternal age, pre-pregnancy overweight and obesity, sedentarism, insulin resistance and diabetes, subclinical infections and inflammation, and nutritional deficiencies during pregnancy as low intake of calcium and essential fatty acids (Lopez-Jaramillo et al., 1989; Lopez-Jaramillo et al., 1990b, 1997, 2005, 2008b, 2011; Lopez-Jaramillo, 1996; Otto et al., 1997, 1999; Herrera et al., 2001, 2005, 2006, 2007; Teran et al., 2001; Catov et al., 2007; García et al., 2007; Sierra-Laguado et al., 2007; Wang et al., 2008; Ramírez-Vélez et al., 2011; World Health Organization [WHO], 2011; Reyes et al., 2012,a,b).

OBESITY, PREECLAMPSIA AND CARDIOVASCULAR DISEASES

The relationship between preeclampsia and obesity has been greatly studied. Similar to how the trend of preeclampsia has increased over the past 25 years, obesity prevalence has also been on the uprise. Over the past 30 years, the percentage of overweight or obese women in the US has increased by 60% (Wang et al., 2008). The WHO estimates that the female prevalence of overweight and obesity is 77% in the United States, 73% in Mexico, 69% in South Africa, 37% in France, 32% in China and 18% in India, with a wide variation within each continent (World Health Organization [WHO], 2011). Numerous studies have shown that obesity is associated with many complications during pregnancy, including fetal overgrowth, fetal malformations, spontaneous miscarriage, gestational diabetes, thromboembolic complications, stillbirth, preterm deliveries, cesarean section, and hypertensive complications (Yogev and Catalano, 2009). A strong direct correlation was found between an increasing body mass index (BMI) and the risk of developing preeclampsia and pregnancy induced hypertension (Fernández Alba et al., 2018). The adjusted risk of developing preeclampsia doubled for overweight mothers with a BMI of 26 kg/m^2, and almost tripled for obese mothers with a BMI of 30 kg/m^2 (Bodnar et al., 2005). The increased risk was found to affect not only Caucasian and African American mothers (Bodnar et al., 2007), but also mother from other ethnics around the world (Hauger et al., 2008). Not only were the early or mild forms of preeclampsia found to augment with a raise in the BMI, but also the late and severe forms, which are associated with greater perinatal morbidity and mortality (Catov et al., 2007). Further contributing to the relationship between preeclampsia and obesity, a study found that weight loss reduces the risk of developing preeclampsia (Magdaleno et al., 2012). Despite the fact that weight loss is not recommended during pregnancy, studies have found that excessive maternal weight gain is correlated with an increased risk of preeclampsia (Fortner et al., 2009), thus weight loss is recommended in women with obesity or overweight that are planning to be pregnant (Yogev and Catalano, 2009).

THE MECHANISMS LINKING OBESITY AND PREECLAMPSIA

Metabolic Factors: Hyperinsulinemia and Insulin Resistance in Preeclampsia

Many different mechanisms have been proposed as explanations of the physiopathology of preeclampsia, at a point that it is

called the "disease of the theories" (Widmer et al., 2007). **Table 1** shows some of the characteristics that are common to obesity and preeclampsia.

The initial phase in the development of preeclampsia is an altered invasion of the cytotrophoblast cells of fetal origin into the uterus and the spiral arterioles, situation that results in a decreased remodeling of these arterioles with a consequent lower blood flow to the placenta (Soma et al., 1982). The placenta in hypoxic conditions releases different substances into the maternal circulation, these include anti-angiogenic soluble fms-like tyrosine kinase 1 (sFlt-1) factors, and pro-inflammatory factors like tumor necrosis factor alpha (TNF-α) (Reyes et al., 2012), which are associated to maternal endothelial dysfunction (Roberts K.A. et al., 2011). As we have demonstrated, these factors are increased in the plasma of preeclamptic women (Teran et al., 2001; Reyes et al., 2012). This sequence of alterations is one of the proposed mechanisms linking obesity to the risk of preeclampsia (Kao et al., 2016), clinical and experimental evidence suggests that obesity may affect placental function and perfusion, through some of the metabolic alterations that are associated to obesity as hyperlipidemia, hyperinsulinemia, or hyperleptinemia; however, the exact mechanisms are not well-known (Hunkapiller et al., 2011). These metabolic markers are known to be elevated in plasma of obese pregnant women and even higher in women with preeclampsia. Moreover, it has been reported that the levels of total serum cholesterol in the first and second trimesters of gestation predict the onset of preeclampsia (Dey et al., 2013), and we have reported a lipid profile alterations consisting of increased levels of low-density lipoproteins (LDLs), low high-density lipoproteins levels (HDLs), and increased levels of triglycerides in women with preeclampsia (Lopez-Jaramillo et al., 1998; Reyes et al., 2012a,b). It has been reported that LDL reduces extravillous cytotrophoblast migration and promotes trophoblast apoptosis (Pavan et al., 2004). Also, high levels of triglycerides and free fatty acids, which are increased in obesity, increase the risk of preeclampsia and are elevated in preeclampsia (Hubel et al., 1996). These two conditions are known to stimulate the nuclear receptor peroxisome proliferator-activated receptor-γ (PPAR-γ). PPAR-γ expression is increased in placentas from preeclamptic pregnancies, and increased levels of this receptor inhibit the invasiveness of trophoblast cells (Fabbrini et al., 2009; Holdsworth-Carson et al., 2010).

One of the most important characteristics of obesity is insulin resistance and hyperinsulinemia, and we have shown that hyperinsulinemia and insulin resistance precede the clinic manifestation of preeclampsia (Sierra-Laguado et al., 2007). Experimental studies showed that hyperinsulinemia produces a shallower implantation site and an intrauterine growth restriction associated with an altered nitric oxide (NO) synthesis (Skarzinski et al., 2009). Moreover, the group of Granger and colleagues, reported that increasing insulin levels toward the end of pregnancy raises blood pressure in rats (Palei et al., 2013). Took together, these clinical and experimental data support the view of the crucial role played by insulin resistance as one of the common mechanisms linking obesity to preeclampsia (López-Jaramillo et al., 2006; Sierra-Laguado et al., 2007).

TABLE 1 | Common characteristics of obesity and preeclampsia.

Characteristic	Obesity	Preeclampsia
Hyperinsulinism	++	++
Insulin resistance	++	++
Leptin	++	++
Adiponectin	– –	(+–)?
TNF-α*	++	++
IL-6*	++	++
hs-CRP*	++	++
Lipid profile alterations	++	++
↓ Flow mediated vasodilatation	++	++

**TNF-α, tumor necrosis factor alpha; IL-6, interleukin 6; hs-CRP, high sensitivity C-reactive protein (CRP).*

Role of Adiponectin, Leptin, and Proinflammatory Cytokines in Preeclampsia

We have determinated the levels of cytokines that are produced in the adipose tissue in patients with diseases as metabolic syndrome and type 2 diabetes mellitus, showing that plasma adiponectin levels are decreased, while proinflammatory cytokines as TNF-α and interleukin-6 (IL-6) are elevated, developing a proinflammatory state characterized by insulin resistance and endothelial dysfunction (Teran et al., 2001; Gómez-Arbeláez et al., 2013; Lopez-Jaramillo et al., 2014; Lopez-Jaramillo, 2016). In patients with severe coronary artery disease, in who we quantify a number of dysmetabolic and inflammatory markers and test of endothelial dysfunction, it was observed that the only differences between patients with and without abdominal obesity was a decrease in plasma concentrations of adiponectin and an increase in leptin plasma levels present in patients with abdominal obesity. Moreover, when we evaluated the vascular reactivity *ex vivo* in rings of the internal mammary artery, we observed a low vascular response to acetylcholine and a high vasoconstriction reaction to angiotensin II (Rueda-Clausen et al., 2010). Leptin and adiponectin are both produced by adipocytes, however, while adiponectin has an anti-inflammatory activity by down-regulating the expression and release of proinflammatory cytokines, leptin has a pro-inflammatory activity. Moreover, adiponectin acts improving the sensibility to insulin but in contrast leptin increases the resistance to the action of insulin. These results suggest that in obese people the increased production of leptin and the decreased production of adiponectin are associated with the systemic low-degree inflammation and insulin resistance, observed in preeclampsia, type 2 diabetes mellitus and cardiovascular diseases, explaining the increased risk of developing these diseases present in people with obesity, particularly abdominal obesity.

In support of this proposal, it has been reported that pregnant obese women that develop preeclampsia have increased leptin levels in relation to healthy pregnant women (Hendler et al., 2005). Also, it has been described that leptin reduces cytotrophoblast proliferation (Liu et al., 2009), and as discussed

above, an early alteration observed in preeclampsia is a poor cytotrophoblast proliferation, migration, and invasiveness of these cells into the uterus, it has been suggested that hyperleptinemia may play a role in placental ischemia and the consequent development of preeclampsia (Spradley et al., 2015), as shown by Mendieta Zerón et al. (2012). It was reported that chronic plasmatic leptin elevations in pregnant rats, increased blood pressure and placental factors that have a role in preeclampsia (Palei et al., 2015). Elevations in circulating leptin are associated to increased circulating levels of TNF-α in obese pregnant rats (Palei et al., 2015), and we have shown that women with preeclampsia have increased levels of TNF-α, IL-6 and C-reactive protein (CRP) (Teran et al., 2001). Experimental and clinic reports have demonstrated that TNF-α is increased in response to placental ischemia and hypoxia (Benyo et al., 2001; Peltier et al., 2011). However, other sources must contribute to the increase of circulating levels of TNF-α during preeclampsia, as peripheral blood leukocytes (Chen et al., 1996). Moreover, TNF-α mRNA levels are greater in mononuclear cells from peripheral blood and placental macrophages withdrawn from obese pregnant women (Challier et al., 2008).

We have proposed that the systemic low-degree inflammation that is present in women with preeclampsia is associated to obesity and insulin resistance, and that this low-degree inflammation state is an important mechanism that alters the endothelial function, and that it is proposed as one of the basic mechanism that precede the clinical manifestation of the disease. This proposal is supported by the results of two nested case-control studies that included Colombian pregnant women with preclampsia, demonstrating increased levels of CRP and leukocytes, a state of insulin resistance established by the homeostatic model assessment (HOMA), and a decreased flow-mediated vasodilation, as early as in the first trimester of gestation (García et al., 2007; Sierra-Laguado et al., 2007).

The results of studies that have explored the levels of plasma adiponectin in preeclampsia has been inconsistent, showing increased, decreased or not difference in relation to the observed in healthy pregnancies (Ramsay et al., 2003; Suwaki et al., 2006). Adiponectin activates the endothelial nitric oxide synthase (eNOS); increasing the levels of the vasodilator NO (Zhu et al., 2008) and some cases of preeclampsia course with reduced levels of nitrite, the stable metabolite of NO (Lopez-Jaramillo et al., 2008a). In healthy pregnancies, a positive association between circulating adiponectin concentrations and nitrite levels has been reported, but in preeclampsia there are increased levels of adiponectin with reduced nitrite levels, suggesting that for some reason, not yet determinated, in preeclampsia adiponectin has no act in the eNOS (Eleuterio et al., 2013).

The Role of the Endothelial L-Arginine-Nitric Oxide Pathway in Obesity and Preeclampsia

Nitric oxide plays an important role in increasing blood flow by relaxing the smooth vascular muscle, it also reduces smooth muscle migration and growth, platelet aggregation and thrombosis, monocyte and macrophage adhesion, and inflammation (Caballero, 2003). Abdominal or central obesity leads to an imbalanced production of fat-derived metabolic products, hormones and adipokines that predispose to a state endothelial dysfunction (Accini et al., 2001). The different mechanisms by which obesity produces endothelial dysfunction have been studied and proven since some years ago by *in vivo* vascular function measurement in peripheral vessels of obese individuals (Toda and Okamura, 2013) showing that obesity reduces endothelium-dependent vasodilatation, eNOS protein expression and endothelial NO production, favoring a thicker media layer, enhancing vasoconstriction and drastically decreasing relaxation. Moreover, as discussed above, proinflammatory adipokines induces endothelial dysfunction. Recently, the Finnish Genetics of Pre-eclampsia Consortium (FINNPEC) cohort have confirmed the women that developed preeclampsia have increased pre-pregnancy BMI and altered inflammatory markers (Jääskeläinen et al., 2018). Leptin, another adipokine that is increased in obesity, induces activation of the NADPH oxidase, which impairs endothelium-dependent vasodilatation by increasing NO degradation (Fortuño et al., 2010). All this leads to a NO deficit which affects endothelial integrity and functioning, that by itself it is deleterious, but in association to pregnancy, where the endothelial function plays a fundamental role in the adequate remodeling of the uterine arteries, and in the hemodynamic adaptations, becomes determinant in the development of preeclampsia-eclampsia, diseases with a high rate of maternal and fetal morbidity and mortality (Witcher, 2018).

During a healthy pregnancy there is an increase of plasma volume, heart rate, cardiac output and on the activity of the renin-angiotensin system (Moutquin et al., 1985), however the blood pressure is lower or similar to the observed in a no pregnancy state, situation that is related with the characteristic increase in the peripheral vasodilation that is observed in the pregnant women (Lopez-Jaramillo, 1996). Nowadays there is evidence that demonstrate that this peripheral vasodilation is product of an increase in the production of NO and prostacyclin in vascular endothelial cells (Lopez-Jaramillo et al., 2008a; Félix et al., 1991). In addition, these substances suppress the leukocyte and platelet migration and adhesion to the vascular wall (Moncada et al., 1991). The NO synthases (NOSs) are a family of specialized enzymes that synthesize NO from the amino acid L-arginine. The endothelial NOS (eNOS) depends on NADPH and calcium (Moncada et al., 1991), and we have proven the critical role of concentrations of extracellular calcium in the production of endothelial NO and in the control of vascular tone via the activation of cGMP (Lopez-Jaramillo et al., 1990a) Importantly, eNOS is expressed in the human placental syncytiotrophoblasts and in the extravillous trophoblasts, suggesting that the production of NO in the placenta play an important role in the vascular adaptations that are necessary to guarantee a normal blood flow (Sladek et al., 1997). The reports showing that NO is inactivated by superoxide (O^{-2}) and that this free radical is increased in pregnancies complicated with subclinical infections or low degree inflammation, suggest that these risk factors for preeclampsia are associated to a lower activity of NO in response to an

increase oxidative stress (Lopez-Jaramillo et al., 2008a). In reality, it is now well-accepted than the production of NO in the vascular endothelium of all vascular beds play an important role in the hemodynamic adaptations that are necessary to carry on a healthy pregnancy. On the other hand, a lower production and/or an increased inactivation of NO explain the generalized peripheral vasoconstriction, one of the most important characteristics of preeclampsia (Lopez-Jaramillo et al., 2008a). However, one of the problems to determinate the role of NO in pregnancy has been the methodological approaches to quantify the production and activity of NO. We and others groups have used the measurements of NO_2/NO_3 levels in plasma and serum of a small group of women with preeclampsia and compared them with the levels observed in unpregnant women and/or in women with healthy. These studies have used different methods to quantify NO_2/NO_3, and they have report elevated, decreased, or unchanged levels of NO_2/NO_3 in preeclamptic women in relation to controls (Lopez-Jaramillo et al., 2008a). Others reports measuring the levels of cGMP in plasma, urine, or platelets, demonstrated consistently that women with preeclampsia have lower levels of this mediator of the action of NO (Teran et al., 2004; Baksu et al., 2005; Lopez-Jaramillo et al., 2008a). A global analysis of these results suggests that some patients with preeclampsia have a decreased production of NO demonstrated by low levels of NO_2/NO_3 and cGMP, but in other cases there is a normal or increased production of NO, as demonstrated by the presence of similar or higher levels of NO_2/NO_3 in relation to women with healthy pregnancies, but in these cases they also presented with a decreased bioactivity of NO, demonstrated by the low levels of cGMP (Lopez-Jaramillo et al., 1996). Importantly, to support the role of NO in pregnancy, there are the consistent results reporting that low mediated dilation in women with healthy pregnancies have a higher vasodilation response to hyperemia that the one observed in women with preeclampsia, even before clinical manifestations appeared (Lopez-Jaramillo et al., 2008a). The analysis of the different reports of the L-arginine-NO pathway led us to the conclusion that in face of the multicausality of this disease, there are different mechanisms that can explain the alterations in the activity of the NO (Lopez-Jaramillo, 2000; Lopez-Jaramillo et al., 2001b). For instance, we understand that in the cases of preeclampsia with low NO production (low NO_2/NO_3 levels) the alterations are related with a deficiency in the substrate L-arginine, a deficit in the cofactors that are needed for a normal activity of the eNOS, such as ionic calcium and BH_4, build-up of the endogenous inhibitor of eNOS, the asymmetric dimethylarginine (ADMA), or by the presence of polymorphic alterations of the eNOS that result in a lower enzymatic activity (Serrano et al., 2004; Lopez-Jaramillo et al., 2008a). In the cases of preeclampsia that courses with normal or high production of NO but with a decreased NO bioactivity, there exists an increased inactivation of NO by an increased oxidative stress (Lopez-Jaramillo et al., 2008a) associated with the presence of antibodies antireceptors AT1 of angiotensin II, early abnormal placentation with placental ischemia and hypoxia, insulin resistance, subclinical infections, and overweight and obesity (Herrera et al., 2001; Herrera et al., 2007; Lopez-Jaramillo et al., 2008a). In any case, both alterations of NO explain the endothelial dysfunction observed in preeclampsia.

The presence of overweight and obesity before pregnancy, and an excessive weight gain during gestation, are causes of endothelial dysfunction and preeclampsia as demonstrated by Pardo et al reported that women with a gestational weight gain superior to 0.42 kg per week, had markers of a reduced eNOS activity and vasorelaxation in the rings of isolated umbilical veins (Pardo et al., 2015).

Role of Endothelin in the Pathophysiology of Preeclampsia

Other studies have proposed a role for endothelin (ET-1) in preeclampsia. ET-1 is a 21 amino acid active oligopeptide derived from a bigger precursor known as preproendothelin. This active peptide is a powerful vasoconstrictor that acts binding to the endothelin type A (ET_A) receptor, located in vascular smooth muscle cells. Increased levels of ET-1 have been reported in women with preeclampsia, compared to the levels observed in a healthy pregnancy (Rust et al., 1997). Moreover, it looks like there is a correlation between the levels of ET-1 and the severity of the disease (Nova et al., 1991). It has been proposed that the reduced NO production that occurs in the placenta in conditions of hypoxia increases the production of ET-1. Some experimental evidences support this proposal, for instance it was reported that the administration of L-arginine reduces the levels of renal cortical preproET-1 mRNA expression in pregnant rats infused with sFlt-1 (Spradley et al., 2015). This result supports the view that NO regulates the synthesis of ET-1 in the kidney, and that when the production NO is decreased by an ischemic placenta, the synthesis of ET-1 increases with the consequent vasoconstrictor and hypertensive effects. Moreover, it has been reported that overweight and obese people with endothelial dysfunction have enhanced levels of ET-1 (Weil et al., 2011), and that an ET_A receptor antagonist attenuated the hypertension observed in a experimental model of rats with obesity induced by a high fat diet, that also had increased leptin levels (da Silva et al., 2004). However, in the review of Spradley et al. (2015) they found only indirect evidence that ET-1 is higher in obese preeclamptic women.

Genetic and Epigenetic Factors Associated to Obesity and Preeclampsia

Obesity and preeclampsia are diseases that result from multiple genetic and environmental factors (Spradley et al., 2015). These two conditions share many pathophysiological mechanisms, however, only 10% of obese women will develop preeclampsia (Roberts J.M. et al., 2011). As reviewed above, the women that develop preeclampsia have different metabolic alterations that precede the appearance of clinical symptoms and that are associated with lifestyles and socio-economic status (Lopez-Jaramillo et al., 2005). We have proposed that the participation of the well-known risk factors for preeclampsia is different depending of the ethnicity and the socio-economic conditions of a specific community. The socio-economic status of communities and of individuals is determining t nutritional habits and the

type and quality of the health system (World Health Organization [WHO], 1988; Lopez-Jaramillo et al., 2008a).

In the last decades, the population from low and medium incomes countries is experimenting important lifestyle changes as a result of a rapid and messy urbanization characterized by an increase in the intake of processed carbohydrates and a reduction of physical activity, conditions that have increased the prevalence of obesity and insulin resistance (Lopez-Jaramillo et al., 2001a). Moreover, ethnic differences have been reported in the prevalence of insulin resistance and metabolic syndrome (Ford et al., 2002), differences that could be associated to genetic factors or socio-economic and environmental conditions. Regardless of the cause, the relationship between insulin resistance and preeclampsia is stronger in low and medium income countries. To support this proposal the Prospective Urban and Rural Epidemiology (PURE) study have demonstrated a higher prevalence of diabetes mellitus type 2 in people with lower BMI coming from low and medium income countries compared to the prevalence found in people from high-income countries (Dagenais et al., 2016).

The existence of a role of genetic factors is demonstrated by the observation that despite of the improvement of the environmental and socio-economic factors, there is still a percentage of women that develop preeclampsia. Moreover, there is evidence showing an association between preeclampsia and polymorphisms or mutations of genes related to hypertension (Ward et al., 1993; Grandone et al., 1997; Sohda et al., 1997; Kupferminc et al., 1999; Ford et al., 2002). However, it looks like environmental and socio-economic factors have a protagonic role. For instance, various studies originated in Europe have demonstrated that pregnant women with preeclampsia have increased levels of ADMA, which are associated with endothelial dysfunction (Fickling et al., 1993; Holden et al., 1998;

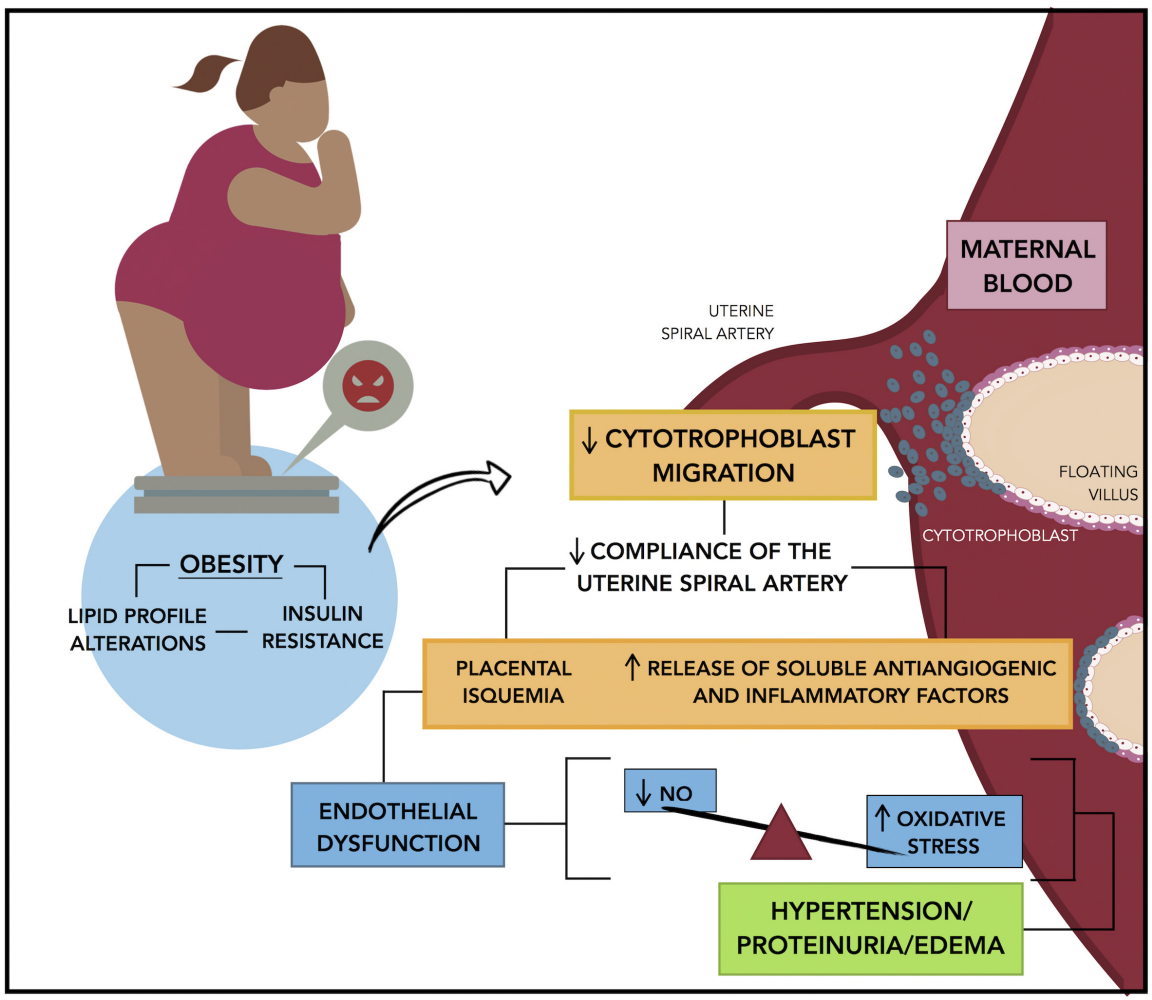

FIGURE 1 | Mechanisms linking obesity to preeclampsia. Insulin resistance that results of pre-pregnancy obesity or by an excessive weight gain during gestation is associated with a reduced cytotrophoblast migration and uterine spiral artery remodeling, which in turn conduce to placental hypoxia and ischemia. In this condition the placenta release of soluble anti-angiogenic factors and inflammatory factors into the maternal circulation promoting the endothelial dysfunction, which is characterized by a decrease in the endothelial production of nitric oxide and an increase in the oxidative stress, that results in the characteristic symptoms of preeclampsia: hypertension, proteinuria, and edema.

Ellis et al., 2001; Savvidou et al., 2003). Early in our research, we showed no difference in ADMA plasma levels between a small sample of Andean women: 22 unpregnant women, 22 women with a normal pregnancy and 22 pre-eclmaptic women (Lopez-Jaramillo et al., 1996). This result was later confirmed in a study (Maas et al., 2004) that included a bigger sample of Colombian women (67 women with preeclampsia and 93 healthy controls). We have proposed some possible explanations for this unexpected finding, to finally conclude that the probable reason is that the excess of the other risk factors present in our population is more important than ADMA plasma levels in the development of preeclampsia, situation that differs from that observed in the European population, where risk factors such as nutritional deficiencies do not exist and others as subclinical infections are detected and treated early in pregnancy. In this way of reasoning it is possible that endothelial dysfunction is mainly related to nutritional deficiencies, subclinical infections, and metabolic disorders in women with preeclampsia from developing countries, while genetic and immunological alterations seem to be the principal determinants for the development of preeclampsia in developed countries (López-Jaramillo et al., 2006).

The **Figure 1** resumes the proposed mechanisms that we believe are participating in the development of preeclampsia. The evidence reviewed in this article demonstrate that the process initiate with placental alterations. A decline in cytotrophoblast migration and remodeling of the uterine spiral artery conduce to placental ischemia. In this condition, soluble anti-angiogenic and inflammatory factors are released from the placenta into the maternal circulation, these factors affect the endothelial function, a critical event that conduce to the clinical manifestations of preeclampsia.

CONCLUSION

Since in medium and low-income countries the prevalence of obesity is increasing, and by the fact that preeclampsia is a major cause of maternal and perinatal morbidity and mortality in these countries, it is of crucial importance to understand how obesity impacts the pathogenesis of preeclampsia.

AUTHOR CONTRIBUTIONS

PL-J reviewed and approved the final version. All the authors have made a substantial contribution to this manuscript and participated in the literature review, design and redaction of the manuscript.

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Antioxidants and Oxidative Stress: Focus in Obese Pregnancies

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The prevalence of obesity in women of childbearing age around the globe has dramatically increased in the last decades. Obesity is characterized by a low-state chronic inflammation, metabolism impairment and oxidative stress, among other pathological changes. Getting pregnant in this situation involves that gestation will occur in an unhealthy environment, that can potentially jeopardize both maternal and fetal health. In this review, we analyze the role of maternal obesity-induced oxidative stress as a risk factor to develop adverse outcomes during gestation, including reduced fertility, spontaneous abortion, teratogenesis, preeclampsia, and intrauterine growth restriction. Evidences of macromolecule oxidation increase in reactive oxygen species generation and antioxidant defense alterations are commonly described in maternal and fetal tissues. Thus, antioxidant supplementation become an interesting prophylactic and therapeutic tool, that yields positive results in cellular, and animal models. However, the results from most meta-analysis studying the effect of these therapies in complicated gestations in humans are not really encouraging. It is still to be analyzed whether these therapies could work if applied to cohorts of patients at a high risk, such as those with low concentration of antioxidants or obese pregnant women.

Keywords: fertility, preeclampsia, miscarriage, latin-America, teratogenesis, intrauterine growth restriction, oxidative stress

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PREVALENCE OF OVERWEIGHT AND OBESITY AMONG WOMEN OF REPRODUCTIVE AGE

Prevalence of obesity in both developed and developing countries has increased among women over the last decades (Heslehurst et al., 2007; NCD Risk Factor Collaboration, 2016), including the prevalence in women of childbearing age, which has also raised dramatically worldwide (Nguyen et al., 2008; Menting et al., 2018; Schaefer-Graf et al., 2018), from 29.8% in 1980 to 38.0% in 2013 (Ng et al., 2014). Correa and Marcinkevage reported an average global prevalence of obesity in women of childbearing age ranging between 1% in Chad to 70.3% in Tonga (Correa and Marcinkevage, 2013).

Abbreviations: AGA, appropriate for gestational age; GSH, glutathione; GWG, gestational weight gain; IUGR, intrauterine growth restriction; LGA, large for gestational age; MDA, malondialdehyde; NAC, N-acetylcysteine; OR, odds ratio; OS, oxidative stress; PE, preeclampsia; ROS, reactive oxygen species; SGA, small for gestational age.

The available epidemiological data in Latin-American countries are dispersed, obtained from periodic national surveys in some cases, or from low-scale, punctual regional studies in others. As it is shown in the **Figure 1**, the highest prevalence of obesity in women of reproductive age was found in Mexico, while the lowest prevalence was found in Haiti.

The trend in the prevalence of obesity has become a matter of concern to preconception healthcare programs because pre-pregnancy obesity and excessive GWG is associated with an increased risk of adverse reproductive health outcomes (Dolin and Kominiarek, 2018; Most et al., 2018). Obesity reduces fertility and increases time taken to conceive (Poston et al., 2016). At the beginning of the gestation, obese pregnant women are more likely to have spontaneous and recurrent pregnancy loss (Chu et al., 2007). The rate of embryo malformations is also increased, showing mainly neural tube, and cardiac defects (Rasmussen et al., 2008). During mid-late gestation, obesity pregnancies have an increased risk for PE and gestational diabetes mellitus, both of which are associated with long-term morbidities post-partum (Milne et al., 2009; Poston et al., 2016). Obese women can also experience difficulties during labor and delivery. The chances of requiring a cesarean intervention in obese mothers double that of lean mothers, while the comorbidity (anesthetic complications, massive blood loss) reaches almost 34% of the gestations. For the newborn, there is a higher risk of LGA, macrosomia, shoulder dystocia and even obesity in childhood (Dennedy and Dunne, 2010; Nelson et al., 2010; Most et al., 2018).

Obesity is associated with a dysregulation in the metabolic balance comprising lipid metabolism, inflammatory or hormonal processes in addition to insulin resistance (Bozkurt et al., 2016). The pathogenesis of obesity is complex and includes metabolic and hormonal dysregulation, low-grade chronic inflammation and endoplasmic reticulum stress, among other processes that are closely interconnected. Several groups all over the world, including ourselves (Alcala et al., 2015), have focused our research in the role of oxidative stress as a central mechanism that may enhance the aforementioned conditions. In this context, we have shown how the use of antioxidants in long-term obesity reduces obesity-associated inflammation, insulin resistance and tissue fibrosis. In the following lines we will discuss the role of OS before, during and after gestation in the mother with pregestational obesity and in the offspring, reviewing the use of antioxidant therapies to ameliorate or prevent undesired negative outcomes.

OXIDATIVE STRESS

OS has been traditionally recognized as a key factor in the pathophysiology of numerous conditions, including cardiovascular and neurodegenerative diseases, cancer, diabetes, and obesity.

OS has been classically defined as an imbalance between ROS generation and its detoxification by antioxidant systems, in favor of the former. ROS are partially reduced, oxygen-containing metabolites (some of them are free radicals) that are generated because of normal cellular metabolism and environmental factors. They are extremely reactive and have the potential to oxidize lipids, proteins and DNA. On the other side, enzymatic (superoxide dismutase, catalase, glutathione peroxidase) and non-enzymatic (vitamin C and E, glutathione) antioxidants neutralize the effect of highly reactive ROS by transforming them into less reactive species and eliminating oxidation by-products, protecting cells from oxidative damage.

However, ROS, at a physiological concentration, behave as second messengers in several signaling pathways that are critical for the normal cellular function. Several studies in the last 10 years have described how ROS can modify the redox state of key residues of proteins (Jones et al., 2004) regulating enzyme activity. They have been reported to participate in different signaling pathways (NF-κB, PTP1b, ASK1, PTEN, REF1, p66hc, or IRP1) (Ray et al., 2012).

Adipose tissue has been proposed as the origin of obesity-induced OS, that is later transmitted to other tissues and may account for obesity-associated diseases such as hypertension, cardiovascular disease, or even cancer (Furukawa et al., 2004; Matsuda and Shimomura, 2013). Hyperglycemia, hyperleptinemia, endothelial dysfunction, hyperlipidemia and mitochondrial dysfunction are the main mechanisms that have been described to increase ROS-generation systems such as nitric oxide synthases (NOS) or NADPH oxidases (Savini et al., 2013). In the first stages of obesity, an upregulation of the antioxidant enzymes is observed to prevent oxidative damage, but as fat accumulates the antioxidant defense is overtaken, leading to OS (Alcala et al., 2015; Alcala et al., 2017).

In addition, oxidative stress has also been observed in healthy gestation. In the second trimester, there is a spike in oxygen supply and metabolic rate in placenta. If ROS levels are maintained under control, they regulate trophoblast proliferation, invasion and angiogenesis, required for a healthy pregnancy (Wu et al., 2015).

Thus, both obesity and gestation are characterized by an increased OS. When combined, OS is one of the proposed mechanisms involved in many reproductive and pregnancy disorders that may lead to adverse pregnancy outcomes (Malti et al., 2014).

OXIDATIVE STRESS, MATERNAL OBESITY AND PREGNANCY OUTCOMES

Fertility

Pre-existing obesity is an independent risk factor for anovulation, subfertility and infertility in women (Silvestris et al., 2018). Several studies show a positive correlation between maternal BMI and time-to-pregnancy (Gesink Law et al., 2007; Wise et al., 2010). It is estimated that for every unit of increase in the BMI, there is a 5% decrease in the probability of conception (van der Steeg et al., 2008). On the other hand, weight loss strategies may positively influence in fertility (Sim et al., 2014). A 10% of weight loss in overweight patients with infertility significantly improved conception and live birth rates (Kort et al., 2014).

The deleterious effect of obesity on reproduction is mainly driven by endocrine and metabolic alterations, which may

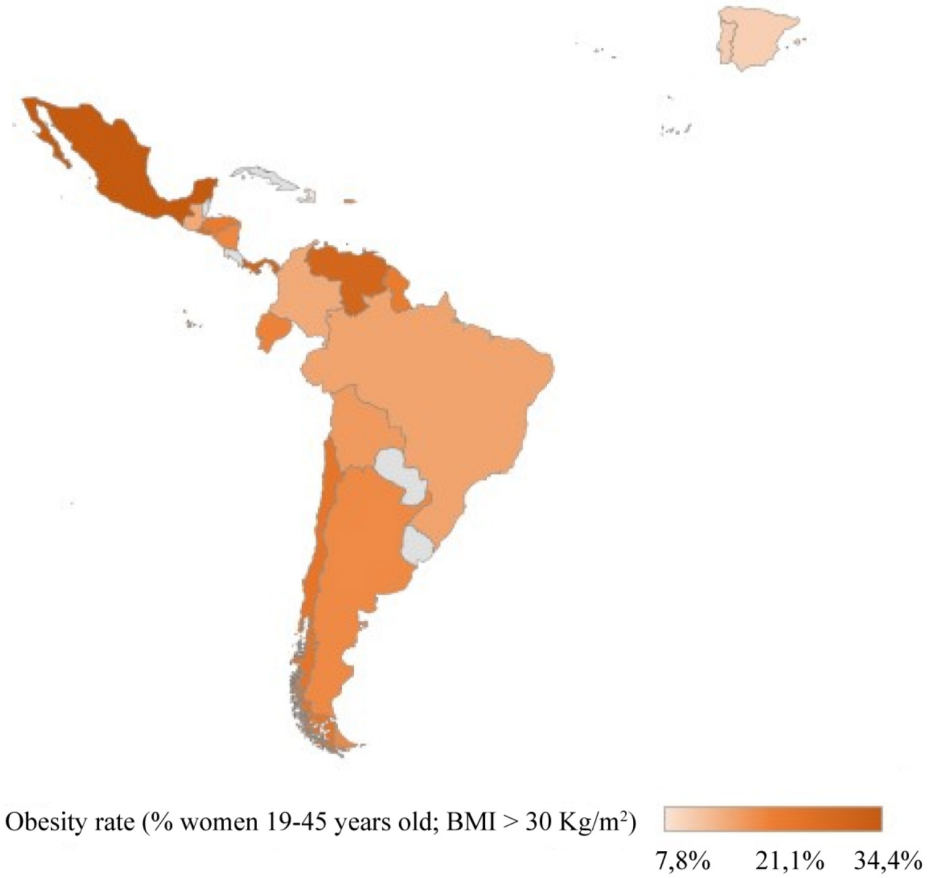

FIGURE 1 | Estimates of obesity in women of childbearing age in Latin-America. Percentage of obesity (BMI ≥ 30 Kg/m^2) in women between 18 and 45 years old. BOL, BRA, COL, ECU, ESV, GUA, HAI, HON, MEX, NIC, PER, and GUY obesity prevalence obtained from National Health Surveys and compilated in (Correa and Marcinkevage, 2013). VEN prevalence obtained from Instituto Nacional de Nutrición (2008–2010). CHI obesity prevalence obtained from Encuesta Nacional de Salud (ENS-2009) and reviewed in (Araya et al., 2014). PAN obesity prevalence obtained from cross-sectional studies (2010–2011) (Mc Donald et al., 2015). ARG obesity prevalence obtained from Encuesta Nacional de Nutrición y Salud (ENNyS-2007). PR obesity prevalence obtained from self-reported, prospective longitudinal cohort study (2011–2014) (Guilloty et al., 2015). SPA and POR obesity prevalence obtained from the European Health Interview Survey (EHIS 2014).

interfere with the neuroendocrine and ovarian function through a disruption in the hypothalamus-hypophysis-ovarian axis. The mechanisms underlying the defective endocrine program in obese women include metabolic alterations due to hyperinsulinemia, the effect of pro-inflammatory cytokines, endoplasmic reticulum stress, alterations in the mitochondria and OS (Silvestris et al., 2018).

In rodents, oocytes from dams fed on high fat diet showed abnormal mitochondrial morphology and increased activity, resulting in increased ROS production and GSH reduction (Igosheva et al., 2010) leading to meiosis failure (Han et al., 2017; Wang et al., 2018). Circulating markers of OS are also elevated in women with polycystic ovary syndrome, with a remarkable 50% decline in GSH concentration. In fact, GSH seems to be a critical player in both male and female fertility. The lack of the enzyme that catalyzes the rate-limiting step in GSH synthesis, the glutamate cysteine ligase, in female mice dramatically reduced preimplantation development (Nakamura et al., 2011; Lim et al., 2015).

The use of antioxidants to improve fertility in obese patients is still a matter of debate. A recent meta-analysis concludes that there is low-quality evidence of a beneficial effect of antioxidants to increase fertility (Showell et al., 2017). However, this report fails to independently analyze a cohort of women with pre-gestational obesity, where the oxidative insult may arise from the combination of two situations that are, independently, associated to oxidative stress. In this situation, the use of antioxidants such as resveratrol in preclinical studies using rodent models (Ghowsi et al., 2018; Jia et al., 2018), or in randomized control trials in humans using Mg and Zn (Afshar Ebrahimi et al., 2017) or NAC (Nasr, 2010; Maged et al., 2015) improved overall reproductive outcome.

Miscarriage and Obesity

Overweight and obese pregnant women present a higher risk of pregnancy loss and recurrent miscarriage compared to normal weight gestations (OR vary from 1.31 to 1.67) (Metwally et al., 2008; Boots and Stephenson, 2011). There is an even higher risk

for obese women of recurrent early miscarriage in spontaneous conception (OR: R: 3.51; 95% CI, 1.03–12.01) and miscarriage after ovulation induction (OR: 5.11; 95% CI, 1.76–14.83).

Recent results suggest a reduced regenerative capacity and plasticity of the endometrium in obese pregnant women (Murakami et al., 2013), factors that may predispose for pregnancy loss (Lucas et al., 2016).

Another key step for a successful pregnancy is a proper maternal-fetal exchange. From week 8 to 12 of gestation there is a peak in placental pO_2 when the maternal blood enters the placenta. This signal triggers the transcription of antioxidant genes (catalase, glutathione peroxidase, and superoxide dismutase) to overcome the prooxidant environment (Jauniaux et al., 2000). In obese pregnancies, together with a pre-established oxidative situation (Catalano and Shankar, 2017), there is a dysregulation of immune cells within the endometrium, characterized by a reduced presence of the anti-inflammatory Treg lymphocytes and an increase of natural killer lymphocytes (Quenby et al., 2009). This promotes early angiogenesis, increasing placental pO_2 prior to the establishment of a mature antioxidant system, depleting non-enzymatic antioxidants such as GSH and vitamin E (Hansen and Harris, 2013). Several authors have described an increase in OS markers in placenta from early and recurring pregnancy loss and suggested that increased ROS generation may be caused by premature establishment of maternal placental perfusion, which has been correlated with a higher risk of miscarriage (Miller et al., 2000; Burton and Jauniaux, 2004; Yiyenoglu et al., 2014).

However, current meta-analyses have shown no beneficial effect of the administration of vitamins (alone or in combination) prior to pregnancy or during the early stages of pregnancy (Balogun et al., 2016). Given the importance of GSH metabolism to fully develop a successful pregnancy, more studies should be carried out to evaluate the potential of NAC (a GSH precursor) to prevent obesity-related miscarriage. So far, the supplementation with NAC to women with recurrent unexplained pregnancy loss significantly increased the rate of living pregnancies beyond 20 weeks and the take-home baby rate (Amin et al., 2008).

Malformation

Congenital anomalies are the end-products of aberrant organogenesis in utero during the first trimester of gestation. A meta-analysis in 2009 revealed that newborns from obese mothers are at increased risk of severe congenital malformations, including neural tube defects and cardiovascular anomalies (Stothard et al., 2009). Results from a cohort including more than 1.2 million deliveries, showed that liveborn singletons from mothers with a BMI > 40 kg/m^2 almost double the risk of suffering major congenital malformations in the nervous system compared to the offspring of normal weight mothers (Persson et al., 2017).

The mechanisms involved in obesity-mediated teratogenesis are still unrevealed. Traditionally, the fuel-mediated teratogenesis hypothesis claims that exposing the embryo to an excessive amount of nutrients, mainly glucose and ketone bodies, may promote embryo malformations, inappropriate organ development and metabolic disturbances in the youth (Freinkel, 1980; Catalano, 2010; Plagemann and Harder, 2011).

OS, common feature in maternal obesity (Gallardo et al., 2015), has been suggested as a potential mechanism in the teratogenesis induced by diabetes (Viana et al., 1996) or chemical teratogens such alcohol, cocaine, valproate, or thalidomide (Hansen and Harris, 2013).

In addition to a direct effect on DNA damaging and repair (Wells et al., 2010), at a molecular level, OS has been shown to inhibit *Pax3* upregulation during early embryogenesis in a murine embryonic stem cell model (Wu et al., 2012). *Pax3* is a transcription factor required for neural tube development. As a result, cardiac neural crest and neuroepithelial cells undergo apoptosis by a process dependent on the p53 tumor suppressor protein (Wang et al., 2011). The supplementation with GSH and vitamin E has proven to be effective in the upregulation of Pax3 expression after an oxidative insult (Wu et al., 2012).

To the best of our knowledge, there is not any clinical trial, to test the potential protective effects of antioxidant supplementation in pre-gestational obese women. However, using retrospective, survey-based studies, a reduction in antioxidant consumption has been linked to increase odds of limb and neural tube defects in obese pregnant women (Chandler et al., 2012; Pace et al., 2018).

Preeclampsia and Cardiovascular Alterations

PE is a severe disease characterized by the presence of hypertension and proteinuria during the second and third trimester of gestation (Steegers et al., 2010). PE affects approximately 2–8% of all pregnancies (Ghosh et al., 2014) and is associated with substantially higher maternal and fetal morbidity and mortality worldwide, especially in Latin American countries where PE is one of the leading causes of maternal and fetal mortality (Giachini et al., 2017).

PE women exhibit at least a twofold increased risk of stroke, while risk of death due to ischemic heart disease is eight times higher when PE occurs before 34 weeks of gestation (Deanfield et al., 2005). Indeed, the American Heart Association has included PE as a risk factor for future cardiovascular disease (Bushnell et al., 2014).

Obesity has been listed as a major risk factor for PE (Marchi et al., 2015), together with higher waist circumference, blood pressure, insulin, proinsulin, glucose, C-reactive protein and triglycerides levels, and lower HDL cholesterol (Mongraw-Chaffin et al., 2010; Sliwa and Bohm, 2014).

The current well-accepted pathophysiology of PE involves a two-stage model: first, an incomplete remodeling of the spiral arteries communicating maternal and placental blood flow through a defective trophoblast invasion. This leads to the second stage, where the ischemia-reperfusion cycles triggers the release of harmful molecules including ROS, cytokines, antiangiogenic proteins, cell fragments, microparticles, and extracellular vesicles (Redman and Sargent, 2000, 2005; Tannetta et al., 2013; Hod et al., 2015). These elements may reach the maternal circulation and

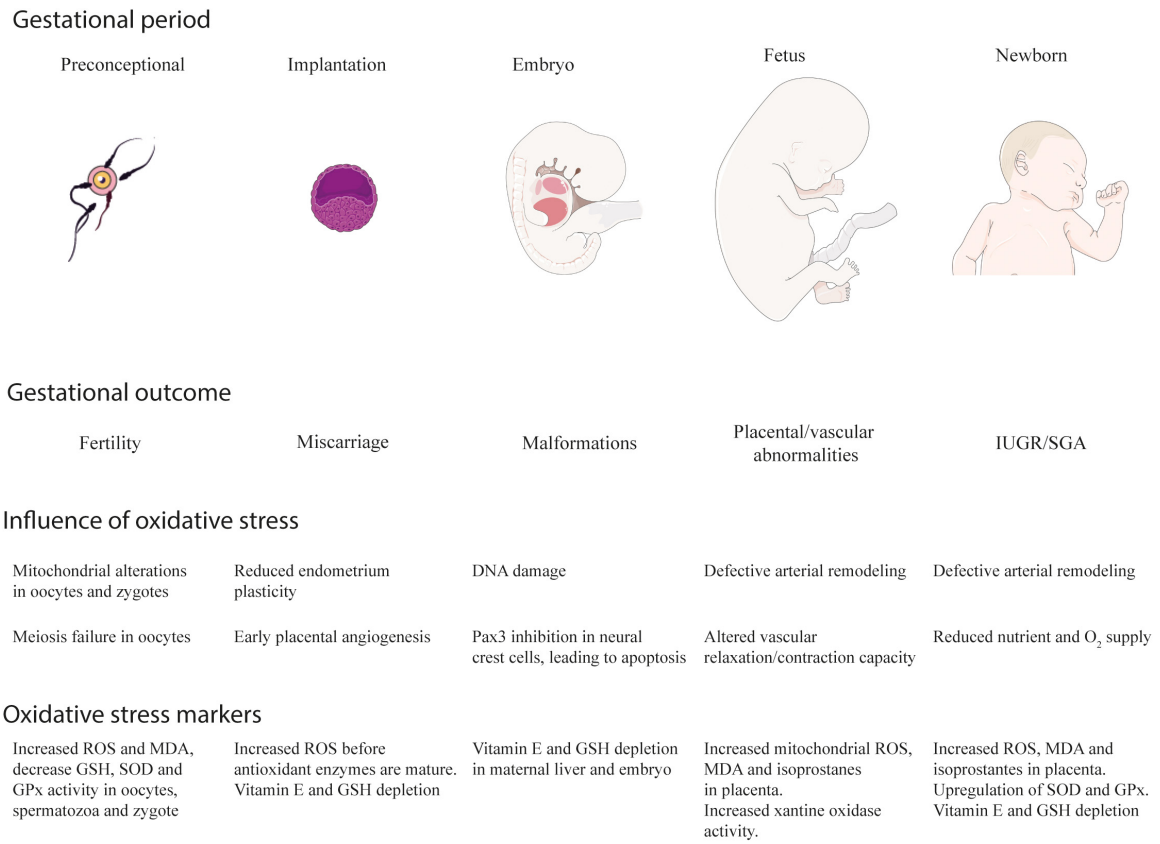

FIGURE 2 | Obesity, gestation and oxidative stress. Pregestational obesity increases the risk of suffering adverse outcomes prior (reduced fertility), during (spontaneous abortion, embryo malformations, preeclampsia, intrauterine growth retardation) and after gestation (small and large for gestational age deliveries) for both the mother and the fetus. Oxidative stress is a common feature in the etiology of obesity and can cause an accumulative effect with the oxidative stress that appears during gestation. The effects and the evidences of oxidative stress in obesity-complicated pregnancies are here summarized. IUGR, intrauterine growth retardation; SGA, small for gestational age; ROS, reactive oxygen species; MDA, malondialdehyde. Artwork was obtained from Servier Medical Art, licensed under a Creative Common Attribution 3.0 Generic License. http://smart.servier.com/.

are thought to be the causative factors of endothelial dysfunction (Roberts and Escudero, 2012).

OS, common in obesity, appears to be the central component of both placental and endothelial dysfunction (Aouache et al., 2018). Pregestational obesity modifies the arterial architecture in placenta (Avagliano et al., 2012) and its contraction/relaxation capacity, being more susceptible to maternal OS (Saker et al., 2008). In addition, obese placenta presents an increased generation of mitochondrial ROS caused by a defective respiratory chain (Hastie and Lappas, 2014), that has been linked to placental angiodysplasia (Ishii et al., 2014). The following episodes of hypoxia/reoxygenation induce the activity of the xanthine oxidase, an important source of superoxide (Hung et al., 2002).

Oxidative damage in the placenta leads to inflammation, apoptosis and the release of cellular debris into maternal circulation, along with several anti-angiogenic factors, cytokines and oxidants (MDA, isoprostanes) concomitant with a reduced antioxidant capacity (Atamer et al., 2005). These placental-derived factors act on the maternal vascular endothelium, inducing more OS and stimulating the production and secretion of pro-inflammatory cytokines, as well as vasoactive compounds. This results in a massive systemic endothelial dysfunction characterized by vascular inflammation and constriction (Goulopoulou and Davidge, 2015).

Preclinical experiments in cellular and animal models reported beneficial effects of antioxidants reducing maternal blood pressure and improving endothelial function (Chang et al., 2005; Roes et al., 2006; McCarthy and Kenny, 2016). However, meta-analysis from clinical trials in humans did not support the use of antioxidant therapy to reduce the risk of PE (Rumbold et al., 2008, 2015; Roberts et al., 2010). It is important to notice that none of these reviews stratify the population according to the BMI, so the effect of antioxidants on PE women with a preexisting oxidative situation has not been studied yet.

Intrauterine Growth Restriction and Obesity

An adequate transport of O_2 and nutrients in the mother-placenta-fetus circuit is mandatory for the normal development of gestation. An excess in the nutrient supply from obese mothers

(Rosario et al., 2015) has been strongly associated with alterations in fetal growth (Yu et al., 2013), increasing the risk of delivering LGA (Bove et al., 2014). Strikingly, epidemiological studies have also noticed that SGA deliveries are also more frequent among overweight and obese women (Radulescu et al., 2013). It is still to be confirmed whether pregestational BMI or GWG have more impact on fetal growth (Crane et al., 2009). In any case, both pregnancy outcomes, SGA and LGA, have an increased risk of suffering perinatal complications, including stillbirth (Yao et al., 2017) and complications later in life.

LGA newborns are the result of an increased flux of oxygen and nutrients together with placental overgrowth (Gaccioli et al., 2013), with a feature gene expression and more OS than those AGA (Saker et al., 2008). Even several years after delivery, in a prepubertal age, LGA children present more OS and insulin resistance than AGA (Chiavaroli et al., 2009).

On the other hand, IUGR in obesity is partly caused by a defective oxygen and nutrient supply to the placenta, which resembles the pathological basis of PE (Srinivas et al., 2009). However, not every case of IUGR can be explained by preexisting PE, so the presence of divergent molecular mechanisms has been proposed by some authors (Villar et al., 2006).

Nonetheless, OS is present in both conditions and may have a critical impact on the development of the disease. In fact, an increase in oxidative markers (MDA, isoprostanes, protein carbonyls) has been found in placenta, maternal and chord plasma (Longini et al., 2005; Biri et al., 2007; Zadrozna et al., 2009; Mert et al., 2012; Negro et al., 2017) of IUGR-complicated pregnancies, with and without PE. Enzymatic antioxidant defenses are also upregulated, as an increase in superoxide dismutase or glutathione peroxidase activities have been previously described. However, non-enzymatic antioxidants, such as GSH, vitamin E and C contents are depleted (Biri et al., 2007; Rajasingam et al., 2009; Zadrozna et al., 2009; Mert et al., 2012), reflecting an OS situation.

To our knowledge, there is not any published clinical trial in humans to determine the potential effect of antioxidants (vitamins or GSH-precursors) on the intrauterine growth from obese mothers. Retrospective, food intake survey-based studies, showed conflicting results. For example, in Spain no relation was found between the consumption of antioxidant vitamins and SGA frequency (Salcedo-Bellido et al., 2017) and a meta-analysis about the use of vitamin E during pregnancy showed no beneficial effect to prevent poor fetal growth in healthy pregnancies (Cohen et al., 2015; Rumbold et al., 2015).

CONCLUSION

Pregestational obesity affects approximately to 1 out of 3 women of childbearing age in the world. The excessive fat mass accumulation correlates with OS, caused by an overactivation of the ROS-generating systems (mainly NOS and NADPH oxidases) and a depleted antioxidant defense. Obesity-induced OS arises as a central factor that increases the risk of adverse outcomes in gestation, as it has been summarized in the **Figure 2**. Prior to gestation, obesity-related OS can cause decreased fertility due to a defective meiosis and mitochondrial alterations in the oocytes. On early gestation, OS increases the risk of miscarriage by reducing the plasticity of the endometrium and promoting early angiogenesis, that increases oxygen supply prior to the maturation of the antioxidant systems. OS can directly cause DNA damage and the inhibition of key genes for neural tube development and closure, responsible for some of the most common malformations observed in embryos from obese mothers. During the second trimester, placenta becomes another physiological source of ROS, with a physiological role on materno-fetal connection. However, the combination of both sources of OS in obese pregnancies can cause an overproduction of ROS, that may account for a defective vascularization of the placenta, leading to both hyperoxia and hypoxia. These two situations exacerbate the placental oxidative state and participate in the pathology of vascular conditions such as PE and IUGR.

Despite the multiple evidence of the oxidative disbalance along normal pregnancies, the use of antioxidants to prevent these outcomes is conflicting. While they have proven to be effective in preclinical studies in cellular and animal models, the reports of its application in large-scale clinical trials is often discouraging. However, the analyzed clinical trials in this mini-review do not specifically analyze a cohort of women with pregestational obesity. The design of specific clinical trials for this population, with a basal situation of increased OS, could potentially generate more promising results. Besides clinical recommendations to obese women, such as weight loss before conception and controlling GWG, specific studies on antioxidant therapies focused on this population could help clarifying the adequacy of targeting OS to prevent complications.

AUTHOR CONTRIBUTIONS

MA and MV participated in the conception of the work and in the preparation of the manuscript. SG-V, EC, EG-G, and MR-Á participated in the preparation of the manuscript and critically reviewed the final draft.

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Mechanisms of Endothelial Dysfunction in Antiphospholipid Syndrome: Association With Clinical Manifestations

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The endothelium is a monolayer of cells that covers the inner surface of blood vessels and its integrity is essential for the maintenance of vascular health. Endothelial dysfunction is a key pathological component of antiphospholipid syndrome (APS). Its systemic complications include thrombotic endocarditis, valvular dysfunction, cerebrovascular occlusions, proliferative nephritis, deep vein thrombosis, and pulmonary embolism. In women, APS is also associated with pregnancy complications (obstetric APS). The conventional treatment regimens for APS are ineffective when the clinical symptoms are severe. Therefore, a better understanding of alterations in the endothelium caused by antiphospholipid antibodies (aPL) may lead to more effective therapies in patients with elevated aPL titers and severe clinical symptoms. Currently, while *in vivo* analyses of endothelial dysfunction in patients with APS have been reported, most research has been performed using *in vitro* models with endothelial cells exposed to either patient serum/plasma, monoclonal aPL, or IgGs isolated from patients with APS. These studies have described a reduction in endothelial cell nitric oxide synthesis, the induction of inflammatory and procoagulant phenotypes, an increase in endothelial proliferation, and impairments in vascular remodeling and angiogenesis. Despite these lines of evidence, further research is required to better understand the pathophysiology of endothelial dysfunction in patients with APS. In this review, we have compared the current understanding about the mechanisms of endothelial dysfunction induced by patient-derived aPL under the two main clinical manifestations of APS: thrombosis and gestational complications, either alone or in combination. We also discuss gaps in our current knowledge regarding aPL-induced endothelial dysfunction.

Keywords: antiphospholipid syndrome, endothelial dysfunction, antiphospholipid antibodies, inflammation, thrombosis

INTRODUCTION

Antiphospholipid syndrome (APS) is an autoimmune disease characterized by a persistence (≥12 weeks) of moderate to high titers of immunoglobulin isotype G (IgG) and IgM antiphospholipid antibodies (aPL) reactive against either cardiolipin (aCL) or β2 glycoprotein I (aβ2GPI); or positive tests for lupus anticoagulant (LA). Clinically, APS is defined as either primary APS, when it occurs in the absence of any other related disease, or as secondary APS, when it is associated with other autoimmune pathologies, such as systemic lupus erythematosus (SLE) (Mackworth-Young et al., 1989). Another variant termed catastrophic APS is characterized by rapid episodes of thrombosis in small vessels of multiple organs causing a systemic dysfunction (Asherson et al., 2003). The clinical manifestations of APS include thrombosis and/or pregnancy complications (Miyakis et al., 2006). Systemic complications of APS include thrombotic endocarditis, valvular dysfunction, cerebrovascular occlusions, proliferative nephritis, deep vein thrombosis, and pulmonary embolism. Pregnancy complications associated with APS, also termed obstetric APS, are characterized by recurrent pregnancy loss, placental insufficiency, preeclampsia, and fetal growth restriction. Some female patients may present with obstetric APS in the absence of systemic thrombosis or purely obstetric APS (Bouvier et al., 2014). In contrast, there are female APS patients with prominent systemic thrombosis in whom it is not usual to find obstetric events. However, APS can present with both pregnancy complications and systemic thrombosis simultaneously. In patients with obstetric APS, pregnancy complications can be triggered by lower titers of aPL than those in patients with thrombotic APS (Meroni et al., 2018). Considering the above, there are several types of patients in which aPL induce different pathological events and thus, distinct mechanisms may be involved.

Patients with APS exhibit endothelial dysfunction. Mechanistically, vascular alterations in patients with APS, including arterial/venous hyperplasia with occlusion and stenosis in several organs (Amigo and García-Torres, 2000; Martínez-Sales et al., 2011), begins with the binding of aPL to the endothelium. This binding involves the participation of membrane receptors that, in some cases, may require the presence of serum β2GPI (Mineo, 2013; Padjas et al., 2016). Indeed, β2GPI is considered to be one of the major pathological autoantigens in APS (Ioannou et al., 2011). However, our understanding of endothelial dysfunction in APS is still limited. While patients with APS are not usually classified by their clinical manifestations, some clinical events indicate a greater association with thrombosis, pregnancy complications, or both. For instance, our group reported that serum from patients with APS exhibiting thrombosis and pregnancy loss simultaneously induced stronger deleterious effects on endothelial function, such as vascular remodeling and angiogenesis, than serum from patients with pregnancy complications alone (Velásquez et al., 2016). Thus, developing a better understanding of the pathophysiology and clinical manifestations of endothelial dysfunction in APS would facilitate the improvement of current treatment regimens, which are ineffective for some groups of patients (Espinosa et al., 2011; Scoble et al., 2011; Mekinian et al., 2015).

In this review, we have compared the current understanding about the mechanisms of endothelial dysfunction induced by patient-derived aPL under the two main clinical manifestations of APS: thrombosis and gestational complications, either alone, or in combination (summarized in **Table 1**). We also discuss gaps in our current knowledge regarding aPL-induced endothelial dysfunction.

Abbreviations: aPL, antiphospholipid antibodies; APS, antiphospholipid syndrome; ApoER2, apolipoprotein E receptor 2; obstetric APS, APS in pregnancy complications; aCL, antibodies anti- cardiolipin; C3a, C3b, and C5a, complement cascade active components; eNOS, endothelial nitric oxide synthase; IgG, immunoglobulin isotype G; IgM, immunoglobulin isotype M; IL-6, interleukin-6; IL-8, interleukin-8; LA, lupus anticoagulant; MMP-2, matrix metalloproteinases 2; MMP-9, matrix metalloproteinases 9; MAPK, mitogen-activated protein kinase; MCP-1, monocyte chemoattractant protein 1; mTOR, mammalian target of rapamycin complex; NF-κB, nuclear factor κB; NO, nitric oxide; PP2A, protein phosphatase 2A; RAPTOR, regulatory-associated protein of mTOR; S6, RICTOR, ribosomal protein; SLE, systemic lupus erythematosus; TF, tissue factor; TLR2, toll-like receptor 2; TLR4, toll-like receptor 4; VCAM-1, vascular cell adhesion molecule-1; aβ2GPI, antibodies β2 glycoprotein I.

IMPAIRED SYNTHESIS OF ANTITHROMBOTIC FACTORS IN APS

Impaired synthesis of the vasodilatory factor, nitric oxide (NO), has been described in patients with APS. Patients with APS displaying thrombosis exhibited low plasma levels of nitrites and nitrates, which are the stable metabolites of NO breakdown. Clinically, these low levels were associated with vascular occlusions, suggesting an enhanced risk of thrombotic, and inflammatory events (Ames et al., 2010). Additionally, aPL can act as endogenous antagonists of endothelial nitric oxide synthase (eNOS) through β2GPI, and this interaction may impair NO synthesis. In particular, attenuation of eNOS activation by aPL was mediated by reduced phosphorylation of eNOS serine 1179. This inhibition of eNOS phosphorylation was shown to be dependent upon protein phosphatase 2A (PP2A), β2GPI, and apolipoprotein E receptor 2 (ApoER2) (**Figure 1A**) (Ramesh et al., 2011; Sacharidou et al., 2018a). Furthermore, aPL inhibition of eNOS activity contributes to thrombus formation, increased leukocyte adhesion, and alterations in vascular tone. A pro-thrombotic phenotype of platelets and monocytes also appears to be triggered by aPL through ApoER2 (Sacharidou et al., 2018b). It remains unclear whether such apoER2-PP2A-eNOS activation/deactivation occurs in all clinical manifestations of APS or only in the more severe conditions associated with thrombosis. Additionally, it has not yet been clarified whether this pathway is activated in patients with obstetric APS. Therefore, there is a need for further studies that include patients with obstetric APS alone as a control.

The regulation of vascular tone mediated by other molecules in APS patients is an area worthy of research since we were unable to find any evidence in the literature of the assessment of endothelial control of vascular tone in APS patients. Therefore, this, and the participation of other vasoactive

TABLE 1 | Summary of the mechanisms of endothelial dysfunction in antiphospholipid syndrome and its association with clinical manifestations.

Clinical classification	aPL	Receptor	Target protein	Pathway	Model	Patients (gender) and clinical manifestation	Effect	References
Obstetric APS	NE-unknown aß2GPI?	NE-unknown	↑ C3/C9	NE-unknown	*In vivo: BALB/c female* *Ex vivo: uterine* endothelial cells	NA	↑ Fetal loss	(Agostinis et al., 2011)
	NE-unknown	NE-unknown	↑ C3a	NE-unknown	*In vivo: BALB/c female*	NS VT and PC	↑Fetal resorption	(Girardi et al., 2004)
Thrombotic APS	aß2GPI	apoER2	↓eNOS	↑PP2A	*In vivo:* C57BL/6 *in vitro:* endothelial cells *and* monocytes	2 men 2 women VT and 1 patient with PC	↑Monocyte adhesion ↑ Thrombus formation	(Ramesh et al., 2011)
	aß2GPI? alone or aCL dependent on ß2GPI?	apoER2	↓eNOS	↑PP2A Dab2/SHC1 recruitment ↓ p-Akt	*In vivo:* C57BL/6 (apoER2- $^{fl/fl}$ and apoER2- Δ) *in vitro:* HUVEC and HAEC cells	3 men 1 woman AT with CAPS, VT with CAPS and pulmonary hemorrhage, DVT and renal microthrombotic	↑ Thrombus size ↓ Time to occlusion	(Sacharidou et al., 2018a)
	Monoclonal aCL dependent on ß2GPI (CL15 and IS4) IS4 bind to ß2GPI alone	NE-unknown	↑ MCP-1	NF-κB?	*In vitro*: HUVEC cells	1 man (CL15) 1 woman (IS4) 1 patient with DVT 1 patient with DVT and SLE	↑Monocyte chemotaxis	(Cho et al., 2002)
	aßGPI (human) aß2GPI (rabbit)	Annexin A2	NE-unknown	NE-unknown	*In vitro*: HUVEC and Mono Mac 6 monocytes	NS LA+ and VT	↑Monocyte adhesion	(Zhang and McCrae, 2005)
	NE-unknown	NE-unknown	↑ C5/C3	NE-unknown	*In vivo: C57BL/6 C3$^{-/-}$ C5$^{-/-}$, CD1 male*	NS	↑ Thrombus size ↑Adhesion of leukocytes to endothelium	(Pierangeli et al., 2005)
	NE-unknown	NE-unknown	NE-unknown	NE-unknown (p38 MAPK?)	*In vitro*: HUVEC cells	28 women VT with or without PC	↑Endothelial MP E-selectin+	(Pericleous et al., 2013)
	LA	NE-unknown	NE-unknown	NE-unknown (p38 MAPK?)	*In vitro*: HUVEC cells and endothelial MP of human plasma	30 patients (no gender especified) VT and PC, NS	↑MP (E-selectin +, ICAM-1+ and CD31+)	(Combes et al., 1999)
Thrombotic and obstetric APS	NE-unknown	NE-unknown	↑ C3 convertase	NE-unknown	*In vivo: (BALB/c female, C57BL/6 C3$^{-/-}$ and CD1 male)*	1 patient NS LA+ and aCL+, multiple cerebrovascular accidents, livedo reticularis	↑ Thrombus size ↑Fetal resorption ↓Fetal weight	(Holers et al., 2002)

(Continued)

TABLE 1 | Continued

Clinical classification	aPL	Receptor	Target protein	Pathway	Model	Patients (gender) and clinical manifestation	Effect	References
	NE-unknown	TLRs?	NE- unknown	↑ROS ↑p-ATF-2 ↑p-p38 MAPK	*In vitro*: HUVEC cells and THP-1 monocytes	2 men 10 women DVT, SVT, AT, DVT with or without PC or SLE	↑ROS ↑ VCAM-1	(Simoncini et al., 2005)
	NE-unknown	TLRs?	NE- unknown	↑p-p38 MAPK NF-κB	*In vitro*: HUVEC cells	4 men 4 women DVT and PC	↑TF ↑ IL-8/IL-6 ↑iNOS	(Vega-Ostertag et al., 2005)
	aß2GPI	TLR2 and TLR4	NE-unknown	MyD88 ↑ NF-κB	*In vitro*: HUVEC cells and HMEC-1	NS 1 patient with APS	↑ E-selectin	(Alard et al., 2010; Raschi et al., 2014)
	aß2GPI?	NE- unknown	↑mTORC1 ↑mTORC2	RAPTOR (↑p-S6) RICTOR (↑p-Akt)	*In vitro*: HMEC-1 cells *ex vivo*: renal biopsies	49 men 57 women APS associated to nephropathy with anticoagulant medication	Nephropathies endothelial hyperplasia	(Canaud et al., 2014)
	NE-unknown	NE-unknown	↓MMP-2/9 ↓VEGF	↓ NF-κB	*In vitro*: HEEC cell *In vivo:* CD1 mice	6 women VT, AT with or without PC	↓Angiogenesis	(Di Simone et al., 2010)
	NE-unknown	NE-unknown	VEGF not altered	NE- unknown	*In vitro*: HUVEC and trophoblast cells	10 women VT with PC	↓Angiogenesis ↓Vascular remodeling	(Velásquez et al., 2016)
	NE-unknown	NE-unknown	NE-unknown	NE- unknown	Feto-placental vasculature and *ex vivo*: maternal serum	12 women VT with PC and SLE	Partial villous infarction, thrombosis ↑ ICAM-1 ↑ VCAM-1	(Lakasing et al., 2000)

NS, not specified; NE, not evaluated; NA, not applicable; aB2GPI, anti- β2 glycoprotein I; apoER2, apolipoprotein E receptor 2; eNOS, endothelial nitric oxide synthase; PP2A, protein phosphatase 2A; VT, vein thrombosis; PC, previous history of pregnancy complications; aCL, anti-cardiolipin, Dab2, disabled 2; SHC1, Scr homology 2 domain containing; p-Akt, protein kinase B; apoER2$^{fl/fl}$, apolipoprotein E receptor 2 floxed; apoER2Δ, apolipoprotein E receptor 2 modified; HUVEC, human umbilical cord vein endothelial cells; HAEC, human aortic endothelial cells; AT, arterial thrombosis; CAPS, catastrophic APS; DVT, deep vein thrombosis; MCP-1, monocyte chemoattractant protein-1; NF-κB, nuclear factor κB; SLE, systemic lupus erythematosus; p38-MAPK, p38 mitogen-activated protein kinases; MP microparticles; LA, lupus anticoagulant; TLRs, Toll-like receptors; ATF, activating transcription factor; ROS, reactive oxygen species; VCAM, vascular cell adhesion protein 1; SVT, superficial venous thrombosis; TF, tissue factor; IL-8/IL-6, interleukins 8 and 6; iNOS, inducible nitric oxide synthase; MyD88, myeloid differentiation primary response 88; HMEC-1, human dermal microvascular endothelial cells; mTORC1, mammalian target of rapamycin complex 1; mTORC2, mammalian target of rapamycin complex 2; RAPTOR, regulatory-associated protein of mTOR; S6, ribosomal protein; RICTOR, rapamycin-insensitive companion of mTOR; MMP-2/9, metalloproteinases 2/9; VEGF, vascular endothelial growth factor; HEEC, endometrial endothelial cells; ICAM, intercellular adhesion molecule 1. Question marks indicate current gaps in our knowledge.

substances, including prostacyclin or other arachidonic acid metabolites, endothelin-1, or purinergic metabolites might be the focus of future research. aPL can induce an imbalance in the production of thromboxane and prostacyclin that favor platelet activation, but the mechanism by which aPL alters the endothelial production of prostacyclin is unclear, since the reported results are controversial (Lellouche et al., 1991; Lindsey et al., 1992).

Another potential contributor to thrombosis formation in APS involves heparan sulfate, which is required for the adequate anticoagulant activity of anti-thrombin (Moon et al., 2005). β2GPI binds to heparan sulfate via an anionic-cationic interaction, thus offering epitopes for circulating aPL (Meroni et al., 1998). The intracellular consequences of the interaction between heparan sulfate-bound β2GPI and aPL have yet to be determined. However, in other autoimmune conditions, such as SLE and in patients with recurrent thrombosis, the functional activity of anti-thrombin is lost in the presence of antibodies against heparan sulfate (Cosgriff and Martin, 1981; Fillit et al., 1993). It is unclear how those changes are releted with endothelial dysfunction.

INCREASED INTERACTIONS BETWEEN THE ENDOTHELIUM AND MONOCYTES

In APS, increased interactions between monocytes and endothelial cells are associated with thrombosis (Clemens et al., 2009). aPL can directly activate monocytes, which in turn interact with the endothelium, resulting in pro-thrombotic events, such as the production of tissue factor (TF) (Kinev and Roubey, 2008; Shantsila and Lip, 2009). Conversely, the exposure of endothelial cells to aPL upregulates endothelial expression of monocyte chemoattractant protein 1 (MCP-1), which in turn promotes TF synthesis by monocytes (**Figure 1A**) (Cho et al., 2002), leading to thrombotic complications in patients with primary APS (Cuadrado et al., 1997).

In the presence of β2GPI, aCL directly induce activation of the mitogen-activated protein kinase (MAPK) pathway in monocytes. These aPL stimulated phosphorylation of p38 MAPK, nuclear factor κB (NF-κB) translocation to the nucleus, and the upregulation of TF. The aPL-induction of monocyte TF could be prevented by the p38 MAPK inhibitor SB203580 (Yasuda et al., 2005). Interestingly, IgG from patients with APS induced monocyte TF expression and adherence to endothelial cells *in vitro*, and in an *in vivo* mouse model induced thrombosis and leukocyte adherence; all via p38 MAPK activation (Vega-Ostertag et al., 2007). aβ2GPI exhibit high-affinity binding to other molecules, such as annexin A2 that is expressed on the surface of endothelial cells. Annexin A2 serves as an anchor for the binding of phospholipids and aPL, especially aβ2GPI, as described in a cohort of LA-positive patients with a history of venous thrombosis. Although the participating intracellular pathways remain unknown, endothelial activation evidenced by increased monocyte adhesion was observed after aβ2GPI/annexin A2 binding (**Figure 1A**) (Zhang and McCrae, 2005).

In summary, the adhesion of monocytes to the endothelium is pivotal in mediating aPL-induced TF production and formation of thrombotic clots. Additionally, the adhesion of monocytes to the endothelium would be expected to potentiate inflammation. However, there has been no clear association between this event and the clinical characteristics of patients so far, mainly because the reports have not included a control group of patients with obstetric APS.

PROTHROMBOTIC ENDOTHELIAL MICROPARTICLES RELEASED IN APS

Endothelial dysfunction is associated with the synthesis of endothelial extracellular particles, such as microvesicles (Morel et al., 2006; Pericleous et al., 2009). *In vitro* studies demonstrated a significant increase in the release of microparticles from endothelial cells cultured in the presence of IgG from patients with APS and clinical manifestations of thrombosis with or without pregnancy complications, when compared to control IgG. In contrast, aPL from patients with pregnancy complications, but without thrombosis, did not significantly alter endothelial microparticle release (Pericleous et al., 2013). Another study showed an increase in the production of microparticles expressing pro-adhesive and pro-coagulant proteins in endothelial cells stimulated with plasma from patients positive for LA, some of whom also had APS (Combes et al., 1999).

In APS, the molecular pathways involved in the aPL-induction of endothelial microparticle release have not been determined. However, generation of these particles seems to be dependent on p38 MAPK activation (**Figure 1A**) (Curtis et al., 2009), and thus based on our previous discussion could favor thrombotic episodes. Since the study of circulating microparticles allows both the detection of biomarkers for disease risk factors and the identification of intercellular communication mechanisms that contribute to pathological states, future studies focusing on the role of these microparticles in patients with APS are of high priority.

COMPLEMENT-DEPENDENT ENDOTHELIAL ACTIVATION IN APS

aPL trigger activation of the complement cascade generating the active components, C3a, C3b, and C5a. C5a is a potent anaphylatoxin that can induce thrombosis through chemotactic actions (Erkan and Salmon, 2016). Endothelial cells express complement receptors, such as C5aR that interacts with C5a. The binding of this receptor to its ligand induces TF production (Wojta et al., 2002). Using C3, C5, and C5aR deficient mice, as well as blocking anti-C5 monoclonal antibodies, aPL-induced endothelial activation and thrombosis was shown to be complement dependent (**Figure 1A**) (Pierangeli et al., 2005; Vega-Ostertag et al., 2007). Similarly, when the complement component C3 convertase was inhibited in an experimental model, the induction of thrombosis, fetal resorption, and low fetal weight by aPL was reduced. This

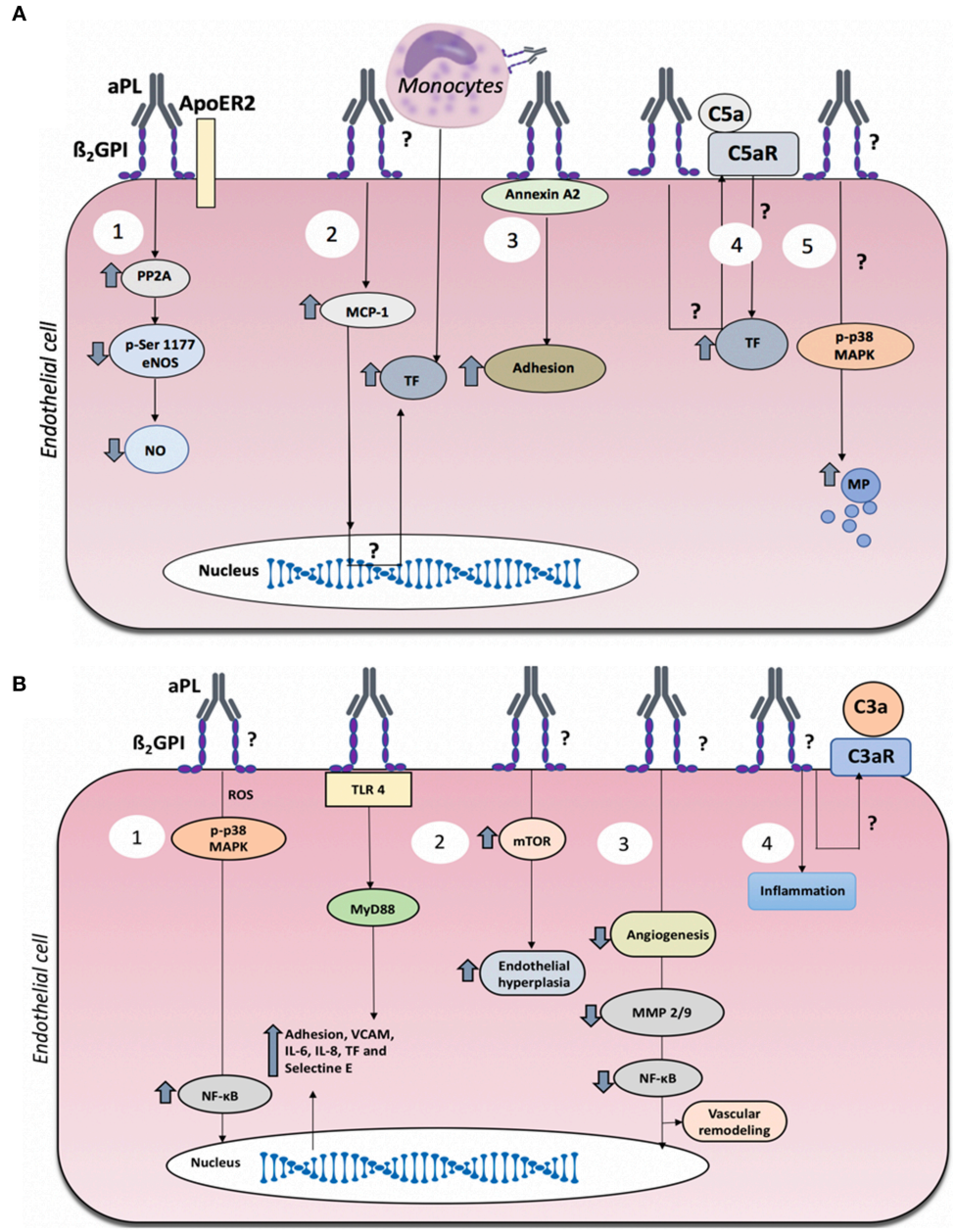

FIGURE 1 | Mechanisms of endothelial dysfunction in antiphospholipid syndrome (APS). **(A)** Mechanisms of endothelial dysfunction associated with thrombosis in APS. Thrombotic events in APS may be associated with several events triggered by aPL: (1) Reduced nitric oxide generation via ApoER2. (2) Elevated endothelial cell production of MCP-1, which favors the adhesion of monocytes to the endothelium, resulting in increased TF. (3) Binding of anti-β2GPI antibodies to annexin A2/β2GPI complexes on the plasma membrane induces elevated expression of adhesion molecules. (4) Complement C5a generation which in turn induces TF expression. (5) Increased production of endothelial microparticles (MP). **(B)** Mechanisms of endothelial dysfunction associated with obstetric APS with or without thrombosis. Obstetric APS may be associated with several events triggered by aPL: (1) Induction of inflammation via the TLR, MyD88, MAPK, and NF-κB pathways. (2) mTOR-mediated endothelial proliferation. (3) reduced maternal vascular remodeling. (4) inflammation and placental damage associated with complement activation.

indicates the relevance of complement in the pathophysiology of APS featuring both thrombosis and pregnancy complications (**Figure 1B**) (Holers et al., 2002). However, despite the strong experimental association between aPL-mediated complement activation, endothelial dysfunction, and thrombus formation, there remains a lack of strong clinical data to support these mechanistic relationships. In contrast, a clinical study has shown that complement activation early in pregnancy is predictive of adverse pregnancy outcomes in women with aPL (Kim et al., 2018).

PRODUCTION OF PROINFLAMMATORY FACTORS IN PATIENTS WITH APS

Endothelial cells treated with IgG-aPL from men and women with different clinical manifestations of APS showed increased reactive oxygen species (ROS) generation, which resulted in increased endothelial expression of vascular cell adhesion molecule-1 (VCAM-1) via p38 MAPK activation (Simoncini et al., 2005). In another study that examined aPL from men and women with clinically active APS, IgG-aPL treatment of endothelial cells activated the cells to express increased TF, interleukin-6 (IL-6), and IL-8. These prothrombotic and proinflammatory responses were mediated by activation of the p38 MAPK and NF-κB pathways (**Figure 1B**) (Vega-Ostertag et al., 2005). Unfortunately, in these studies, the patients were not classified by gender or clinical manifestation, which hinders the precise understanding of the mechanisms described in association with the pathophysiology of APS.

One mechanism by which aPL may mediate endothelial cell inflammation is through activation of the innate immune receptors, Toll-like receptor 2, and 4 (TLR2 and TLR4). Dimeric β2GPI can bind TLR2 and TLR4, which favor aPL binding (Alard et al., 2010; Raschi et al., 2014). aPL with β2GPI reactivity from patients with thrombosis and pregnancy complications induced endothelial cell activation through activation of MyD88, a key component of the TLR2, and TLR4 signaling pathways (**Figure 1B**). Notably, β2GPI shares molecular mimicry with certain pathogenic microorganisms that can activate TLR4 (Raschi et al., 2003). However, further studies are needed whereby patients are grouped according to their clinical manifestations and gender to obtain a more detailed understanding of the pathological effects of aPL on the endothelium.

ENDOTHELIAL DYSFUNCTION IN WOMEN WITH OBSTETRIC APS

In cases of obstetric APS there is endothelium involvement, despite the often lack of thrombotic events (Viall and Chamley, 2015). aPL specific for β2GPI are the most pathologic obstetrically by targeting the uterine endothelium, as well as the placental trophoblast and endometrial/decidual stroma, where high basal levels of β2GPI are normally expressed (Agostinis et al., 2011; Meroni et al., 2018). Unlike systemic APS, which is a thrombotic disease, obstetric APS is associated with inflammation at the maternal-fetal interface, and poor placentation associated with reduced trophoblast invasion and limited uterine spiral artery remodeling (Viall and Chamley, 2015). Thus, patients with obstetric APS may present a fundamentally different disease in contrast to patients with thrombotic APS; and the same aPL might induce both clinical conditions through these distinct mechanisms (Meroni et al., 2018).

In some women with obstetric APS who do not experience thrombosis, placental damage has been associated with inflammatory events (Asherson et al., 2006). Placental injury in obstetric APS has also been associated with complement activation (**Figure 1B**) (Alijotas-Reig, 2010). Moreover, in a murine model of obstetric APS it was demonstrated that heparin prevented gestational complications through the inhibition of complement activation (Girardi et al., 2004).

While there has been an abundance of studies showing that aPL deleteriously affect placental function (Stone et al., 2006; Tong et al., 2015), how aPL affect uterine endothelial function is a significantly understudied area. In particular, studies using aPL from patients with pregnancy morbidities in the absence of thrombosis are warranted.

VASCULAR REMODELING AND ANGIOGENESIS IN APS: IMPLICATIONS FOR PLACENTATION

Endothelial cells are key components of the angiogenesis process. For instance, a study using uterine endothelial cells showed that aPL from women with APS reduced *in vitro* angiogenesis when compared to serum from normal subjects (D'Ippolito et al., 2012). The authors did not detail the clinical characteristics of the patients, making it unclear whether the alterations in angiogenesis caused by aPL may be associated with thrombosis, pregnancy complications or both. Angiogenesis requires the degradation of the vascular basement membrane and remodeling of the extracellular matrix through the production of matrix metalloproteinases (MMP) (Han et al., 2001). *In vivo* angiogenesis assays were used by Di Simone et al. (2010) to document impaired angiogenesis of aPL following the subcutaneous implantation of angioreactors in the dorsa of CD1 mice. The expression of the MMP-2/-9 was decreased in presence of aPL and this was associated with a reduced activation of NF-κB (**Figure 1B**) (Di Simone et al., 2010).

On the other hand, impaired angiogenesis in women with APS may have profound implications in pregnancy, since placentation requires new vessel formation. Currently, it is unclear whether APS-associated defects in angiogenesis during placental development is more severe in patients with thrombosis who experience pregnancy complications.

Vascular remodeling of the uterine spiral arteries by the invading extravillous trophoblast is another important process of normal placentation. In obstetric APS, endothelial-trophoblast interactions are impaired, leading to reduced uterine spiral artery remodeling (Viall and Chamley, 2015; Silva and Serakides, 2016). In our previous studies we used an *in vitro* model of uterine spiral artery remodeling in which we could measure vessel-like tube structures after the co-culture of first trimester extravillous trophoblast cells with either human endometrial endothelial cells or human umbilical cord vein endothelial cells (Alvarez et al., 2015; Velásquez et al., 2016). In these studies we found that serum from women with obstetric APS either with or without thrombosis decreased the trophoblast-endothelial interactions and thus, the formation of stable vessel-like tube structures. In the feto-placental vasculature of women with APS and SLE, findings of partial villous infarction, intravascular thrombosis, and fibrin deposits associated with increased levels of intercellular adhesion

molecule-1 and vascular cell adhesion molecule-1 have also been reported (Lakasing et al., 2000).

Future studies, however, are still needed to better understand how aPL impair placental angiogenesis and to elucidate the clinical consequences. Additionally, *in vitro* analyses using serum or IgG-aPL, must consider the clinical parameters and gender of the included patients.

PROLIFERATION MARKERS ASSOCIATED WITH ENDOTHELIAL HYPERPLASIA

In renal biopsies from patients with APS, the endothelium exhibits activation of mTOR, a kinase involved in the regulation of cell proliferation. To detect the activation of mTOR (mTORC1 or mTORC2), the phosphorylation of ribosomal protein S6 and serine 473 of Akt in endothelial cells of the renal vasculature was evaluated. Among the several interesting findings from this study, we highlight the following. First, aPL isolated from patients with APS induced mTOR activation in endothelial cells *in vitro*. Second, treatment with rapamycin inhibited mTOR activation via RAPTOR and RICTOR. Third, in patients with catastrophic APS, the same mTOR activation was observed in the renal endothelium and was accompanied by severe vascular constriction. Finally, in patients with APS and renal transplantation who were treated with rapamycin, mTOR activation was not detected and the patients showed reduced renal lesions and an absence of endothelial hyperplasia between 3 and 12 months after transplantation. The authors concluded that the mTOR signaling pathway is involved in the development of the endothelial dysfunction that result in the clinical manifestations of APS (**Figure 1B**) (Canaud et al., 2014). However, these authors did not specify the clinical characteristics of their patients with APS and, therefore, it is unclear whether this pathological mechanism of aPL is associated with thrombosis, pregnancy complications or both. These results reinforce necessity of better clinical characterization of subjets to better pathophysiology understanding.

CONCLUSION

Patients with APS are classified into different groups depending on the severity of their clinical manifestations and on their aPL titers. Endothelial dysfunction is a key component of APS. However, our understanding of the precise mechanisms by which aPL induce endothelial dysfunction remains limited, in part, because patients are not always classified according to their clinical manifestations. Nonetheless, some mechanistic events do indicate a greater association with thrombosis, pregnancy complications, or both. In **Table 1**, we summarize the gaps in knowledge to highlight research topics that warrants further attention. Future studies involving patients with APS should consider clinical characteristics and gender to better understand the pathophysiology of endothelial dysfunction in this disease.

AUTHOR CONTRIBUTIONS

MV wrote the draft of the manuscript. MR, VA, CE, and ÁC critically revised the manuscript. ÁC generated the original idea and proposed topics for revision.

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Mitochondria and Coenzyme Q10 in the Pathogenesis of Preeclampsia

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Hypertensive disorders during pregnancy constitute one of the main causes of maternal and perinatal morbidity and mortality across the world and particularly in developing countries such as Ecuador. However, despite its impact on public health, the primary pathophysiological processes involved are yet to be elucidated. It has been proposed, among other theories, that an abnormal placentation may induce an endothelial dysfunction, which is ultimately responsible for the final clinical manifestations. Mitochondria, particularly from trophoblastic cells, are responsible for the production of energy, which is extremely important for normal placentation. The malfunction in this supply of energy may produce higher levels of free radicals. In both production of energy and free radicals, coenzyme Q10 (CoQ10) plays a crucial role in electron transport. As such, the role of CoQ10 in the genesis and prevention of preeclampsia has become the focus of a number of research groups, including that of the authors. Developing an in-depth understanding of these mechanisms might allow us to design new and feasible strategies with which we can reduce preeclampsia, particularly in the Latin-American countries.

Keywords: preeclampsia, placenta, mitochondria, coenzyme Q10, pregnancy

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INTRODUCTION

Maternal mortality remains a significant public health problem in Ecuador (The Maternal Health Study [MNPI], 1999; Centro de Estudios de Población y Desarrollo Social [CEPAR], 2004; Instituto Nacional Ecuatoriano de Estadísticas y Censos [INEC], 2017). The main cause for maternal mortality is preeclampsia – a disease characterized by hypertension, increased vascular resistance, endothelial dysfunction, proteinuria, and coagulopathy – that usually manifests in the second trimester of pregnancy (National High Blood Pressure Education Program Working Group Report on High Blood Pressure in Pregnancy, 1990). Preeclampsia is defined as an increase in blood pressure that is equal to or greater than 140/90 mmHg and 24 h of proteinuria equal to or greater than 300 mg/dl (Solomon and Seely, 2001). The presence of preeclampsia during pregnancy also causes complications to the newborn and is associated with low birth weight, preterm delivery, and neonatal mortality (López-Jaramillo, 1993). However, despite several years of basic research, the genesis and the pathophysiology of preeclampsia still remain unknown (Armaly et al., 2018).

For several years, we have dedicated our research efforts toward acquiring a better understanding of preeclampsia and designing new strategies, concepts, and hypotheses that might allow us to unlock the enigma of the etiology of preeclampsia (Calle and Teran, 2018). In this paper, we describe the evidence that highlights the putative role of abnormalities in placental implantation and, more

specifically, in terms of mitochondrial function at the placental level on the genesis of preeclampsia. The effects of these alterations upon the maternal endothelium are discussed elsewhere (Roberts and Redman, 1993; Evora, 2000; Granger et al., 2001; Saito and Nakashima, 2014).

DEVELOPMENT OF THE PLACENTA DURING NORMAL PREGNANCY AND IN PREECLAMPSIA

The placenta is a temporary organ that forms a physical and functional connection between the mother and the embryo/fetus during development. However, in contrast to other organs, the environment in which the placenta develops during the first 8–10 weeks is poor in oxygen; it has been demonstrated that this is essential for blastocyst implantation and further embryo and placental development (Genbacev et al., 1997; Goldman-Wohl and Yagel, 2002).

The trophoblast cells differentiate into the embryo and are derived from the trophectoderm and develop into the blastocyst during development; these cells are essential in maintaining the further development of a normal pregnancy (Genbacev et al., 1997). The trophoblast adheres to the uterus and begins to penetrate into the endometrial stroma. Previous research has shown that proteolytic enzymes such as metalloproteinase act as facilitators in the penetration process by degrading the extracellular matrix (Goldman-Wohl and Yagel, 2002).

The implantation of the blastocyst and primitive placental formation are associated with active penetration of the trophoblast into the endometrial epithelium and adjacent stroma in a low-pressure oxygen environment, which facilitates this process (Genbacev et al., 1997). Subsequently, when the trophoblast is in an environment of high oxygen pressure (Genbacev et al., 1997), it begins the process of differentiation from an early proliferative to an invasive phenotype that will continue to penetrate up to the first third of the myometrium (Zhou et al., 1997b). Interestingly, these cells can also invade the maternal spiraled arteries, which helps to refurbish and replace endothelial and muscular cells (Zhou et al., 1997b). This procedure, known as the conversion, is the replacement of the fetal by maternal endothelium and a loss of elasticity in the arterial vessels. Arteries, then by losing their elasticity, become more compliant, like veins, to increase its capability, according to the demand during pregnancy. In this way, blood flow increases in order to supply nutrients and oxygen to the embryo/fetus during development.

The syncytiotrophoblast is responsible for nutrient exchange, and it is derived from the fusion of the trophoblast cells, which creates an impermeable cellular wallpaper (Goldman-Wohl and Yagel, 2002). In addition to the maintenance of the placental nutrients, the syncytiotrophoblast is also responsible for gaseous exchange as well as the production of hormones and growth factors.

Dependence on oxygen is necessary for normal placental development (De Marco and Caniggia, 2002). The processes underlying the differentiation and the invasion of the trophoblast into the endometrium consume significant amounts of energy (Widschwendter et al., 1998). Based on this requirement, placental development requires an organ acting as an oxygen sensor to modulate energy production. Experimental studies have demonstrated that mitochondria and, more specifically, respiratory chain complexes appear to be responsible for such regulation (De Marco and Caniggia, 2002).

To determine the mechanisms responsible for how and why the placenta and trophoblastic cells can detect oxygen levels may have significant clinical relevance because preeclampsia or the retardation of intrauterine growth involves changes in placentation.

ABNORMAL PLACENTATION DURING PREECLAMPSIA

Several studies have shown that an alteration in placental function is linked to the development of preeclampsia. This pathology can occur in pregnancies without a fetus being present, for example, in the condition referred to as mole hydatidiform (Page, 1939) or via poor placentation (Goldman-Wohl and Yagel, 2002) and thus cause an increase in the placental mass, which is the characteristic of gestational diabetes (Solomon and Seely, 2001) or multiple pregnancies (Thornton and Macdonald, 1991). Notably, preeclampsia resolves after delivery (Redman, 1991). Furthermore, histopathological studies have demonstrated morphological changes in vessels from preeclamptic placentas in a process that has come to be known as "endotheliosis" (Gerretsen et al., 1981). A number of experimental studies have analyzed these observations (Gerretsen et al., 1981; Redman, 1991; Colburn et al., 1994; Graham and McCrae, 1996; Lim et al., 1997; Zhou et al., 1997a) and have noted that during preeclampsia, there are two functional abnormalities: first, invasion of the trophoblast to the uterine parenchyma is not deeper and invasion to the vasculature does not reach up to the decidua portion and into the spiral arteries. As a consequence, maternal vessels do not complete the normal physiological changes known as conversion. Consequently, the diameter of the myometrial vessels in preeclampsia is less than half of those in normal pregnancy (Brosens et al., 1972); furthermore, these vessels maintain their responsiveness to vasoconstrictors such as angiotensin or epinephrine (van der Graaf et al., 2013). Second, the number of vessels showing evidence of trophoblastic invasion is reduced in comparison with normal pregnancy (Khong et al., 1986).

However, there has been significant debate over the potential causes of such alterations. An immunological hypothesis targets a potential maternal sensitization process against fetal tissue. It has been shown that longer sexual cohabitation (Dekker and Baha, 2001) and sexual practices that expose the maternal environment to the partner's semen, for example, oral sex or intercourse without a condom (Koelman et al., 2000), can reduce a women's risk for the further development of preeclampsia (Saftlas et al., 2014).

In the maternal decidua, there is a significant concentration of leucocytes; of these, the most important are the natural killers

TABLE 1 | Studies involving the activity of the mitochondria during preeclampsia.

Study	Relevant findings
Torbergsen et al., 1989	• Features of preeclampsia as disturbed ion transport and prostaglandin synthesis, vasoconstriction, platelet aggregation, and hyperuricemia may be explain by mitochondrial dysfunction.
Folgerø et al., 1996	• Mutations in mitochondrial transfer ribonucleic acid genes in two families are associated with a high occurrence of preeclampsia and eclampsia.
Matsubara et al., 1997	• Number of mitochondria positive for COX staining markedly decreased in the placentae of the women with preeclampsia.
	• Dysfunction of trophoblast's mitochondria may be present in placenta of patients with preeclampsia.
Wang and Walsh, 1998	• Increase of the amount of placental mitochondria in preeclampsia.
	• Mitochondrial lipid peroxidation increased in preeclampsia.
	• Mitochondrial generation of superoxide an important source of oxidative stress in preeclampsia.
Staff et al., 1999	• The content of free 8-iso-prostaglandin F2a in decidual tissue from preeclampsia was significantly elevated than in control tissue.
Sikkema et al., 2001	• Oxidative stress in placenta of preeclamptic women is increased.
Zamudio, 2003	• Placenta from women living at high altitude has increased villous vascularization and thinning of the villous membranes, that together increases oxygen diffusion capacity.

(NK cells). During preeclampsia, the trophoblast experiences an increased level of cellular lysis by maternal NK cells (Colburn et al., 1994), which is responsible for an abnormal sensitization process. Therefore, during preeclampsia, there is a reduction in the expression of human leucocyte antigen G (HLA-G) in the invasive trophoblast (Colburn et al., 1994), making these cells more susceptible to attack by NK cells present in the maternal decidua. This process seems to be maintained throughout pregnancy, meaning that trophoblastic cells do not have the resources to support appropriate implantation. In these cases, the production of lysis enzymes in the extracellular matrix from the maternal decidua is abnormal (Lim et al., 1997) and the enzymes produced have structural problems and, therefore, are not able to function appropriately (Graham and McCrae, 1996). Consequently, the trophoblast cannot break down the maternal extracellular matrix and, hence, invasion is not possible.

In addition, during the process of placentation, several factors, such as growth factors and intercellular adhesion molecules, control trophoblast differentiation and also the process of conversion. During preeclampsia, these factors and their functions are often abnormal; thus, trophoblast invasion and conversion are also abnormal (Zhou et al., 1997a; Goldman-Wohl and Yagel, 2002).

Finally, for all these cellular processes, it is known that the trophoblast requires oxygen to produce energy at the mitochondrial level (Genbacev et al., 1997; De Marco and Caniggia, 2002). Several reports have been now published showing an abnormal mitochondrial function in the trophoblast cells during preeclampsia (Furui et al., 1994; Vuorinen et al., 1998; Wang and Walsh, 1998; Roberts and Lían, 2002).

MITOCHONDRIA AND PREECLAMPSIA

Mitochondria are intracellular organelles responsible for energy production through the respiratory chain in a process that uses oxygen to form adenosine triphosphate (ATP), which is known as oxidative phosphorylation (Davidson, 1994). This process is characterized by the transport of electrons from NADH and $FADH_2$ to the five separate enzymatic complexes in the mitochondrial membrane. Oxygen is the final receptor of electrons at the end of the respiratory chain, where it is ultimately reduced to water. However, during this process, normally 2–3% of oxygen is not completely reduced and leads to the formation of reactive oxygen species (ROS), particularly, superoxide (O_2^-, 16). Superoxide is mainly generated by coenzyme Q10 (CoQ10) partially reduced – and by complex III in the respiratory chain (Lenaz, 2001). Consequently, superoxide can be converted into hydrogen peroxide by superoxide dismutase in the mitochondria (Wang and Walsh, 1996).

Considering the demand for oxygen and energy required for normal placental development, it is logical to assume that any alterations at this level might compromise the overall process of placentation. Indeed, observational studies have demonstrated a higher incidence of preeclampsia in a family with mitochondrial dysfunction (Torbergsen et al., 1989). In addition, in women with preeclampsia (Folgerø et al., 1996) and their first-degree relatives, there is an abnormality in the expression of mitochondrial genes responsible for energy production, such as cytochrome C oxidase (Matsubara et al., 1997), or electron exchange processes preferentially favor oxidation (Wang and Walsh, 1998). On the contrary, it is well known that hypoxic conditions, for example, living at an altitude, lead to major susceptibility to the development of preeclampsia (Zamudio, 2003). In this sense, there is significant evidence showing that increased generation of ROS plays a key role in the development of preeclampsia (Davidge et al., 1992; Branch et al., 1994; Mikhail et al., 1994; López-Jaramillo et al., 1998; Little and Gladen, 1999; Staff et al., 1999; Sikkema et al., 2001). Reports have also confirmed that the origin of ROS is most likely from the placenta and, in particular, mitochondria (Wang and Walsh, 1998). A previous study reported that the number of mitochondria in placental tissue is far greater in women with preeclampsia and that these mitochondria have greater susceptibility to lipid peroxidation (Wang and Walsh, 1998); however, these observations were made in only a small number of patients (**Table 1**).

TABLE 2 | Studies involving Coenzyme Q10 (CoQ10) during normal pregnancy and preeclampsia.

Study	Relevant findings
Noia et al., 1996	• Low CoQ10 levels in cases of spontaneous abortion. • Increase in the plasma CoQ10 levels in relation to the contractile activity of the uterine muscle.
Noia et al., 1998	• CoQ10 levels were higher in fetuses with hypoxia and non-immune hydrops.
Teran et al., 2003	• Plasma CoQ10 levels were significantly higher in normal pregnant women in comparison to non-pregnant women. • During preeclampsia there is a significant decrease in plasma levels of CoQ10 compared to normal pregnant women.
Palan et al., 2004	• In pre-eclampsia there is decreased levels of CoQ10 and alpha-tocopherol, reducing the ability of antioxidant defense leading to the endothelial cell damage observed in preeclampsia.
Teran et al., 2005	• CoQ10 in placenta and umbilical cord blood from women with preeclampsia was significantly higher compare to normal pregnancy.
Teran et al., 2008	• Plasma and placental CoQ10 levels in normal pregnant women at sea level were significantly lower than in those living at high altitude. • Preeclamptic women displayed higher placental CoQ10 content, which was only significant among those living at sea level; while CoQ10 plasma levels were significantly lower only in preeclamptic women living at high altitude.
Teran et al., 2009	• Double blind, placebo controlled clinical trial with CoQ10 supplementation (200 mg/daily) from week 20 of pregnancy. • Thirty women (25.6%) in the placebo group compared with 17 women (14.4%) in the CoQ10 group developed preeclampsia (p = 0.035).
Hernandez et al., 2017	• At week 20 of pregnancy, plasma CoQ10 levels showed no difference between the control and supplemented groups. • In the CoQ10 group women who developed preeclampsia showed significantly higher placental levels than normal pregnant women did. • In mitochondria from preeclamptic women, levels of CoQ10 were no different among those in the placebo and CoQ10 groups.

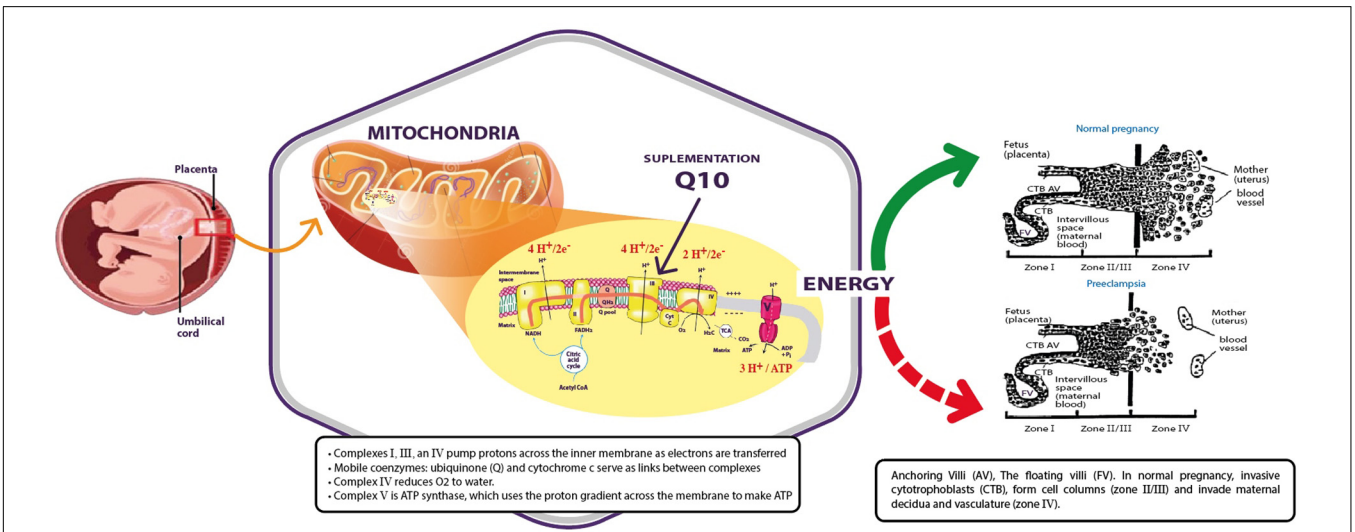

FIGURE 1 | Mitochondria produce energy for placental development during normal pregnancy (green arrow) that is abnormal in preeclamptic women (red dashed arrow). Supplementation of coenzyme Q10 might restore the activity of the mitochondria.

In this sense, CoQ10, the only non-polar electron transporter into the mitochondrial respiratory chain, plays a key role in both production of energy and formation of ROS (Davidson, 1994; Lenaz, 2001). Circulating CoQ10 is known to act as a potent antioxidant, either directly (Kaikkonen et al., 1999) or via the regeneration of vitamin E (Hodson and Watts, 2003). Therefore, it seems logical to suggest that CoQ10 could be involved in the pathogenesis of preeclampsia.

COENZYME Q10 DURING NORMAL PREGNANCY AND PREECLAMPSIA

The CoQ10 or ubiquinone, is a fat-soluble molecule synthesized endogenously from phenylalanine (benzoquinone ring) and mevalonic acid (responsible for isoprenoid units) and with a small contribution derived from the diet (Davidson, 1994). The CoQ10 participates in energy generation and plays a key role in mitochondrial respiration, as it is responsible for electron transport from complex I and II to complex III (Davidson, 1994). Thus, any alteration in the mitochondrial CoQ10 might result in reduced formation of energy and an increase in generation of ROS (Lenaz, 2001). On the contrary, CoQ10 acts as an antioxidant for lipoproteins, both membrane-bound and circulating (Hodson and Watts, 2003). In the later environment, the function of CoQ10 is related to its capability to increase the bioavailability of vasoactive substances such as nitric oxide (NO; Hodson and Watts, 2003; Teran, 2003).

Only a few studies have investigated CoQ10 during human pregnancy (Noia et al., 1996, 1998). These studies showed that

the levels of CoQ10 increase progressively, starting in the first trimester and continue to rise until delivery (**Table 2**). In 2003, the authors' group reported that Ecuadorian normal pregnant women, although with recognized nutritional deficiencies, had CoQ10 levels within the normal range as reported for other populations, but higher than non-pregnant women (Teran et al., 2003). However, the authors reported for the first time that women with preeclampsia show a significant reduction in CoQ10 levels (Teran et al., 2003), a result that was later confirmed by other authors (Palan et al., 2004). At that time, our working hypothesis was that during preeclampsia, there may be a "mechanism" that consumes CoQ10 (not related to diet), which might arise because of increased production of ROS. To investigate further this possibility, the author's group investigated CoQ10 levels in the placenta and the umbilical cord and interestingly found that the levels were significantly higher in women with preeclampsia (Teran et al., 2005), suggesting a compensatory accumulation. However, all those studies were done in Quito, a high-altitude city (2800 m above the sea level). So, in a subsequent study, plasma CoQ10 was measured in Ecuadorian women living at sea level; results showed that normal pregnant women had significantly lower levels of CoQ10, but in those with preeclampsia, the difference was not as evident as in those patients living at high altitude; these observations may be related to the small sample size of this study (Teran et al., 2008). Interestingly, placental content of CoQ10 was also significantly higher in preeclamptic women at sea level (Teran et al., 2008). It was also consistent with a later study showing that CoQ10 levels in mitochondria from placentas of women with preeclampsia were significantly higher compared with mitochondria from normal placentas (Teran et al., 2007; **Table 2**).

According to our present data, it is likely that women with preeclampsia have already-established alterations in their levels of CoQ10 compared with normal pregnant women; these changes occur both in the plasma and the placenta. However, they occur to a greater extent in the mitochondria. However, all of these previous studies were conducted in women, who had already developed the disease; consequently, it was not possible to determine if a reduction in CoQ10 was a cause or a consequence. With this information, we decided to set up a randomized, double-blinded, and placebo-controlled clinical trial providing 200 mg of CoQ10 daily to 235 pregnant women starting from week 20 up to delivery; we then determined the rate of preeclampsia in this cohort of patients. At the end of the study, there were more patients in the preeclampsia placebo group (25.6% vs. 14.4%), demonstrating, also for the first time, that supplementation of CoQ10 represents an effective intervention to reduce the risk of developing preeclampsia (Teran et al., 2009).

Post hoc analysis showed that before supplementation, at week 20 of pregnancy, plasma CoQ10 levels showed no difference between controls and the supplemented groups. Interestingly, at delivery, placental tissue showed no differences in the placebo group when compared between women with normal pregnancy and those with preeclampsia, while in the CoQ10 group, women with preeclampsia showed significantly higher placental levels compared with normal pregnant women. However, mitochondrial levels of CoQ10 in the placenta from pregnant women with preeclampsia receiving placebo did not show significant differences when compared with those receiving supplementation of CoQ10. These results suggest that in women with preeclampsia, although CoQ10 reduced preeclampsia and was present in high levels in placental tissue, the mitochondrial levels of CoQ10 did not change significantly (Hernandez et al., 2017).

CONCLUSION

In conclusion, preeclampsia is associated with abnormal placental development as a result of several factors; of these, the dysfunction of the mitochondria appears to be the most important. In that case, the lack of energy and the associated increase of free radicals might be due to the deficiency or the consumption of CoQ10, as supplementation of CoQ10 was shown to be an effective intervention for the reduction of the rate of preeclampsia (**Figure 1**).

AUTHOR CONTRIBUTIONS

ET, IH, and AC contributed to the conception and design of the studies. LT and ST organized the databases. ET, ST, and AC performed the statistical analysis. ET, IH, and ST wrote the first draft of the manuscript. C-GH, MS-M, and GM wrote sections of the manuscript. All authors contributed to manuscript revision, read, and approved of the submitted version.

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New Insights Into the Role of Placental Aquaporins and the Pathogenesis of Preeclampsia

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Accumulated evidence suggests that an abnormal placentation and an altered expression of a variety of trophoblast transporters are associated to preeclampsia. In this regard, an abnormal expression of AQP3 and AQP9 was reported in these placentas. Recent data suggests that placental AQPs are not only water channel proteins and that may participate in relevant processes required for a normal placental development, such as cell migration and apoptosis. Recently we reported that a normal expression of AQP3 is required for the migration of extravillous trophoblast (EVT) cells. Thus, alterations in this protein might lead to an insufficient transformation of the maternal spiral arteries resulting in fluctuations of oxygen tension, a potent stimulus for oxidative damage and trophoblast apoptosis. In this context, the increase of oxygen and nitrogen reactive species could nitrate AQP9, producing the accumulation of a non-functional protein affecting the survival of the villous trophoblast (VT). This may trigger the exacerbated release of apoptotic VT fragments into maternal circulation producing the systemic endothelial dysfunction underlying the maternal syndrome. Therefore, our hypothesis is that the alteration in the expression of placental AQPs observed at the end of gestation may take place during the trophoblast stem cell differentiation, disturbing both EVT and VT cells development, or during the VT differentiation and turnover. In both situations, VT is affected and at last the maternal vascular system is activated leading to the clinical manifestations of preeclampsia.

Keywords: extravillous trophoblast, villous trophoblast, AQP3, AQP9, human placenta, preeclampsia

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INTRODUCTION

Preeclampsia is a pregnancy complication characterized by high blood pressure and proteinuria which usually begins abruptly after 20 weeks of gestation. This condition is exclusively for human gestation and affects 7–10% of pregnancies worldwide (Giachini et al., 2017). In Latin-American countries, it is estimated that 26% of maternal deaths are related to preeclampsia (Khan et al., 2006; Abalos et al., 2013).

The consequences of preeclampsia are not limited to the pregnancy and may conduce to maternal permanent vascular and metabolic damage and future heart diseases. Moreover, emerging

evidence suggests that the adaptation to an adverse intrauterine environment may also affect the adult life of the newborn (Giachini et al., 2017).

Despite the importance of preeclampsia and several decades of extensive research, its etiopathogenesis remains unclear. It is well accepted that defects in placentation are the main predisposing factors for preeclampsia (Myatt, 2002; Huppertz, 2008; Hawfield and Freedman, 2009). However, the molecular basis of the abnormal placental development has not been sufficiently clarified yet. Several theories have been proposed to elucidate the origins of preeclampsia but none of them can explain the variability of cases. In fact, preeclampsia is a very heterogeneous syndrome and is classified by the severity of the disease as mild, moderate, and severe and by the time of delivery as early and late onset preeclampsia, with or without fetal growth restriction (Huppertz, 2008).

The most cited hypothesis on the etiology of preeclampsia considers this syndrome as a two-stage disorder (Hawfield and Freedman, 2009). The first stage is characterized by an insufficient transformation of the maternal spiral arteries resulting in a decrease in blood supply to the fetoplacental unit. Consequently, fluctuation in oxygen levels may increase the damage of the villous trophoblast (VT) concluding in the maternal endothelial dysfunction and the clinical manifestations of preeclampsia. Nevertheless, the link between preeclampsia and a failure of trophoblast invasion has recently raised serious doubts because the abnormal transformation of the uterine spiral arteries gives rise to the maternal symptoms only in some women (Huppertz, 2008, 2018). That is why, the current view regarding the etiology of preeclampsia has been recently updated toward the dysregulation of VT turnover.

In this regard, it was proposed that failures in the differentiation of the villous syncytiotrophoblast could accelerate the shedding of apoptotic syncytial aggregates (Norah et al., 2013). The detachment from the apical syncytiotrophoblast membrane of these structures into the maternal circulation, induce an antiangiogenic environment and enhance the maternal systemic inflammatory response producing the endothelial dysfunction. (Huppertz, 2008, 2018; Norah et al., 2013). Even more, a failure in the undifferentiated trophoblast stem cells, may affect VT and EVT lineage resulting also in an inadequate transformation of the uterine spiral arteries. In this condition, preeclampsia is associated with growth restriction (Huppertz, 2008, 2018).

Villous syncytiotrophoblast is involved in the fetal-maternal oxygen and nutrient exchange, and many placental transporters and channels are altered in preeclamptic placentas (Damiano et al., 2006; del Monaco et al., 2006; Castro-Parodi et al., 2009; Dietrich et al., 2013; Martínez et al., 2016; Szpilbarg and Damiano, 2017).

WATER TRANSPORT AND PLACENTAL AQUAPORINS

Fetal water requirements increase throughout gestation accompanying fetal growth. Emerging evidence show that water can pass across the placenta by transcellular and paracellular pathways, but the molecular mechanisms of these processes are not clarified yet. Previous research using isolated membrane vesicles showed that water moves through the syncytiotrophoblasts by lipid diffusion (Edwards et al., 1993; Jansson and Illsley, 1993; Jansson et al., 1999) discarding the transcellular route and the participation of water-channel cell membrane proteins known as aquaporins (AQPs).

However, in 2001 we described that AQP3 and AQP9 are present in the apical membrane of the human syncytiotrophoblast (Damiano et al., 2001). Both proteins are members of the aquaglyceroporin subfamily and allow the transport of water, urea, and glycerol. Exceptionally, AQP9 also facilitates the flux of neutral solutes such as monocarboxylates, purines, and pyrimidines (Ishibashi et al., 1998; Tsukaguchi et al., 1998). Uptake experiments showed that these AQPs may mediate the transcellular water, urea, and glycerol transport in human placenta (Damiano et al., 2006; **Table 1**).

However, in preeclamptic placentas we found that the expression of AQP9 increased and the cellular distribution of this protein changed, being localized not only in the apical and basal membranes but also in the cytosol of the syncytiotrophoblasts (Damiano et al., 2006). In contrast, we have recently reported that AQP3 expression considerably decreased in preeclamptic placentas compared to normal ones (Szpilbarg and Damiano, 2017).

Regarding AQPs functionality, water, and mannitol uptakes also decreased in preeclamptic placentas, compared to normal

TABLE 1 | Expression and functionality of AQP3 and AQP9 in normotensive and preeclamptic placentas.

	Normotensive Placentas	Preeclamptic Placentas
AQP3 EXPRESSION	Yes (Damiano et al., 2001; Zhu et al., 2009)	Yes, decreased (Szpilbarg and Damiano, 2017)
AQP3 LOCALIZATION	Apical membrane of syncytiotrophoblast cells (Damiano et al., 2001; Zhu et al., 2010)	Apical membrane of syncytiotrophoblast cells (Szpilbarg and Damiano, 2017)
AQP9 expression	Yes (Damiano et al., 2001)	Yes, increased (Damiano et al., 2006)
AQP9 localization	Apical membrane of syncytiotrophoblast cells (Damiano et al., 2001; Zhu et al., 2010)	Apical and basal membranes, and cytoplasm of syncytiotrophoblast cells (Damiano et al., 2006)
WATER UPTAKE, $pmol.\ g^{-1}.min^{-1}$		
Control	76 ± 6	$47 \pm 10^{*}$
+ $HgCl_2$ 0.3mM	$48 \pm 7^{\#}$ (Damiano et al., 2006)	36 ± 12 (Damiano et al., 2006)
Mannitol Uptake, $pmol.\ g^{-1}.min^{-1}$		
Control	14.6 ± 0.3	$5.5 \pm 0.6^{*}$
+ $HgCl_2$ 0.3mM	$7.8 \pm 0.8^{\#}$ (Damiano et al., 2006)	6.0 ± 2.3 (Damiano et al., 2006)

*$P < 0.05$ normotensive vs. preeclamptic placentas. #$P < 0.05$ in the absence vs. in the presence of $HgCl_2$, a general blocker of AQPs.

ones and were not sensitive to $HgCl_2$ (Damiano et al., 2006; **Table 1**).

Although the reduced uptake of water may be associated to AQP3 expression, the lack of sensitivity to $HgCl_2$ and the reduced uptake of mannitol, which can only permeate through AQP9, may suggest that both proteins are not functional in preeclamptic placentas.

Given that there is no evidence that the water fetal-maternal flux is altered in preeclampsia, the classical role of these placental AQPs exclusively in facilitation of trans-epithelial fluid transport has been called into question (Damiano, 2011; Martínez and Damiano, 2017). Recently, unexpected cellular roles of AQPs were reported, including organelle physiology, proliferation, apoptosis, and cell migration (Verkman, 2011; Kitchen et al., 2015). All of them are related to temporary cell volume changes.

It is important to note that human placenta is hemomonochorial, in which the syncytiotrophoblast, a single layer of polarized epithelium separates the maternal and fetal circulation (Stulc, 1997). Because of the similarities in the fetal-maternal transfer barrier, the guinea pig is the only animal model established to study placental transport. Despite of this, several studies were carried out in mice, rats, and sheep reporting changes in the expression of AQPs during pregnancy that could be associated with changes in placental water transfer and amniotic fluid homeostasis (Liu et al., 2004; Beall et al., 2007; Belkacemi et al., 2011). In addition, AQP3-knockout mice were viable and developed normally, but no studies ruled out placental or pregnancies alterations in these mice (Verkman, 2006; Lei et al., 2017).

On the other hand, preeclampsia is a disorder unique to human pregnancy, and no animal model could reproduce the complete syndrome. Taken into account these difficulties, the role of AQPs in human preeclamptic placenta was studied by *in vitro* models (Martínez and Damiano, 2017).

OXYGEN REGULATION OF PLACENTAL AQUAPORINS

It is well known that trophoblast differentiation is regulated by oxygen (Genbacev et al., 1997; James et al., 2006). During normal pregnancies, placentation occurs in a relatively hypoxic environment. Hypoxia inducible factor-1α (HIF-1α) may contribute to the adaptation of the placenta to fluctuations in the oxygen tensions (Patel et al., 2010).

In pathological conditions, intermittent hypoxia because of the decreased maternal blood flow into the intervillous space may produce an ischemia/reperfusion insult in the placenta (Hung and Burton, 2006).

Since hypoxia controls the expression of many genes involved in cell adaptation to stress, the regulation of placental AQPs by oxygen was studied. In normal placental explants exposed to oxygen deprivation, HIF-1α was detected and AQP9 protein drastically decreased (Castro-Parodi et al., 2013). However, in explants cultured in hypoxia/reoxygenation, HIF-1α was undetectable and AQP9 showed a significant increased. In this condition, we also observed that AQP9 was localized in the apical and basal membranes and in the cytosol of the syncytiotrophoblast as previously reported in preeclamptic placentas (Damiano et al., 2006; Castro-Parodi et al., 2013).

Regarding AQP3, hypoxia decreased the protein expression which was abnormally localized in the cytosol. After reoxygenation, AQP3 returned to the apical membrane of the syncytiotrophoblast, but its expression was not restored to control levels (Szpilbarg et al., 2016).

PLACENTAL AQUAPORINS AND APOPTOSIS

Trophoblast apoptosis increases progressively during gestation. This physiological process is required for normal turnover of VT and comprises the fusion of the mononuclear cytotrophoblast cells into the multinucleate syncytium (Smith and Baker, 1999; Huppertz et al., 2006; Sharp et al., 2010). These events lead to the release into maternal circulation of syncytial aggregates, which progressively increase during normal pregnancy and do not harm the mother (Burton and Jones, 2009).

In pregnancies complicated by preeclampsia, VT apoptosis is exacerbated compared to normotensive pregnancies (Sharp et al., 2010). It was proposed that an altered balance between proliferation and apoptosis could increase the formation of syncytial aggregates shedding into the maternal circulation. This favors the immunological and inflammatory processes of the mother and promotes the systemic endothelial dysfunction (Huppertz, 2008; Norah et al., 2013; Roland et al., 2016).

Concerning the role of AQPs in the programmed cell death, Jablonski et al. (2004) demonstrated that AQPs may mediate the loss water and subsequent cell shrinkage in the apoptotic cells. The proposed mechanism is that the K^+ efflux and the intracellular K^+ depletion generate an osmotic gradient that drives water out of the cell, a process known as apoptotic volume decrease (AVD). Inactivation of AQPs after AVD and the continuous efflux of ions K^+ decrease the ionic strength of the cytoplasm and activate the apoptotic caspases. (Chen et al., 2008).

Given this background, we studied the role of AQPs in the VT apoptosis. We observed that only the inhibition of AQP3 abrogates the apoptotic response of these cells (Szpilbarg et al., 2016). Thus, we provided evidence that AQP3 may be important in the regulation of VT apoptosis and consequently an abnormal expression of this protein could alter this tightly regulated process.

Along with this idea, we expected that an altered AQP3 may be one of the key factors in the development of preeclampsia. In this context, we assumed that the increase in trophoblast apoptosis observed in preeclampsia might correlate with an increase in AQP3, but we found a reduced expression of this protein in these placentas (Szpilbarg and Damiano, 2017). Consequently, the role of AQP3 in the apoptosis of the VT in preeclampsia remains unclear.

One possibility is that AQP3 decreases as an adaptive response of the trophoblast to reduce the apoptotic events observed in preeclampsia.

Another possible explanation is that the damage to the syncytiotrophoblast membranes produced by the intermittent hypoxia may create an unfavorable environment for AQP3 insertion into the plasma membrane, increasing its degradation. In fact, the apical membranes of syncytiotrophoblast from preeclamptic placentas are more rigid than normal ones, due to an increase in sphingomyelin that reduces the number of caveolae and the expression of Caveolin-1 (Levi et al., 2016). Moreover, analysis of the primary structure of AQPs revealed a putative caveolin-1-binding site which is required for AQPs functionality (Jablonski and Hughes, 2006). Consequently, an altered lipid composition may disrupt the ability of sphingomyelin and cholesterol to assemble into caveolae in the apical leaflet of the bilayer, affecting protein expression and cell signaling (Levi et al., 2016).

Regarding AQP9, we found that its blocking did not prevent the apoptotic response of the trophoblast (Szpilbarg et al., 2016), discarding a direct role of the AQP9 in this process. Furthermore, despite the increase in AQP9 protein expression, we observed a lack of its functionality for water and monocarboxylates in preeclamptic placentas (Damiano et al., 2006).

The placenta is a main source of reactive oxygen (ROS) and nitrogen (RNS) species which can nitrate tyrosine sites of proteins by enhancing the production of peroxynitrite (Webster et al., 2008; Myatt, 2010). Nitration of proteins can cause a loss of function. In recent studies (unpublished), we observed an increase of 3-nitrotyrosine AQP9 in preeclamptic placentas. In this context, we propose that this nitrated protein may impair the transfer of lactate, an end-product of anaerobic glycolysis.

Evidence suggests that AQP9 could play a role both in energy metabolism and in the clearance of free radicals. Recently, it was reported in preeclamptic placentas, a significant decrease in the protein expression and the function of GLUT-1 (Lüscher et al., 2017). Therefore, faced with the reduction of the glucose passage, the trophoblast might be forced to use another source of energy like lactate (Lüscher et al., 2017).

Lactate is an energy substrate and can also be involved in scavenging ROS as a source of NADH (Schurr and Gozal, 2011). Although the expression of monocarboxylate symporters MCT1 and MCT4 has been found in syncytiotrophoblast membranes (Settle et al., 2004), no data was found about their expression in preeclamptic placentas. For that reason, we hypothesize that the lack of functionality of AQP9 may increase ROS accumulation and adversely affect the survival of the trophoblast cells, like in other tissues (Miki et al., 2013; Akashi et al., 2015). Therefore, AQP9 may contribute to exacerbate the trophoblast apoptosis in an indirect manner.

In addition, supraphysiological concentrations of peroxynitrite can induce a decrease of protein degradation rates by the proteasome (Grune et al., 1998). Subsequently, we surmise that the changes in the cellular distribution of AQP9, observed in preeclamptic placentas (Damiano et al., 2006), may be due to the increase of this non-functional nitrated protein that cannot be degraded in the proteasome and accumulates in the cytosol of the syncytiotrophoblasts.

Given that preeclampsia is a syndrome of early placentation, we cannot discard that the abnormal expression of AQP3 and AQP9 observed at the end of gestation may be a consequence of a failure in the differentiation of the trophoblast stem cell. In this regard, we investigated the role of these proteins at early stages of placenta development.

PLACENTAL AQUAPORINS AND MIGRATION

To date, many AQPs were described in the blastocyst and in the early and term placenta (Escobar et al., 2012; Xiong et al., 2013). AQP3, AQP1, and AQP9 are the most abundant AQPs expressed in chorionic villi from first trimester suggesting that they may have a key role in the normal fetal growth and homeostasis.

A highly synchronized trophoblast differentiation, proliferation, and invasion are necessary to achieve a successful pregnancy. Nowadays, the molecular mechanisms that lead these complex processes remain unknown. Trophoblast cells differentiate to invasive extravillous trophoblast (EVT) or fuse to form the syncytium (Velicky et al., 2016). Thereby, EVT cells change their epithelial phenotype to an invasive mesenchymal phenotype. These events resemble the general epithelial-mesenchymal transition process and allow EVT to invade the endometrium. (DaSilva-Arnold et al., 2015; Davies et al., 2016). In these processes, mechanisms of migration and invasion displayed by trophoblast and malignant cells are similar. However, unlike tumors, the trophoblast behavior is tightly controlled (Soundararajan and Jagannadha Rao, 2004; Piechowski, 2016).

Moreover, increasing evidence demonstrated that AQPs may be involved in tumor cell migration. (Hu and Verkman, 2006; Nico and Ribatti, 2011; Cao et al., 2013; Ribatti et al., 2014; Marlar et al., 2017).

Considering that preeclampsia is related to an abnormal placentation and an altered placental expression of AQP3 and AQP9 was found after the onset of the maternal syndrome, we assumed that these proteins may participate in the early stages of placental development.

Along with this idea, we explored the contribution of AQPs to the EVT cell migration.

Our findings strongly show that only the blocking of AQP3 or the silencing of its expression reduce trophoblast cells migration. AQP9 is not involved in this process (Reca et al., 2018).

We believe that in preeclampsia complicated by IUGR, the aberrant expression of AQP3 may be present already in the undifferentiated trophoblast stem cell affecting both VT and EVT pathways. However, up to now, it is not possible to determine the expression or functionality of AQPs in early placentas that late in gestation will develop preeclampsia.

PUTATIVE PARTICIPATION OF AQP3 AND AQP9 IN THE PHYSIOPATHOLOGY OF PREECLAMPSIA

Emerging data suggests that placental AQPs may participate in relevant processes required for a normal placental development, such as cell migration and apoptosis (Szpilbarg et al., 2016; Reca et al., 2018).

The alteration in the expression of placental AQPs observed at the end of gestation may take place at two different steps during the trophoblast differentiation: (i) during the trophoblast stem cell differentiation disturbing both EVT and VT cells development or (ii) during the VT differentiation and turnover.

In both situations, the expression of AQPs in VT is affected and ultimately this may contribute with the development of the clinical symptoms of preeclampsia.

Figure 1 is a theoretical representation of our hypothesis of the putative contribution of AQPs to the multiple alterations that lead to the development of preeclampsia. In the first case (i), if the aberrant expression of AQP3 occurs during the first differentiation step of the trophoblast cell lineage or slightly afterward, it may affect EVT as well as VT development. Since AQP3 is required for the appropriated migration of EVT cells (Reca et al., 2018), the abnormal expression of this protein, might lead to a shallow trophoblast invasion and insufficient transformation of the maternal spiral arteries. In this situation, intermittent hypoxia affects placentation increasing ROS and RNS, a potent stimulus for VT apoptosis (Myatt and Cui, 2004; Hung and Burton, 2006). Our hypothesis is that the increase of ROS and RNS may lead to the nitration of AQP9, resulting in a non-functional protein. In this scenario, the lack of functionality of AQP9, may impair the transfer of lactate and promote more accumulation of ROS and enhance the VT cell death. This may trigger the exacerbated release of apoptotic syncytial aggregates into maternal circulation producing the systemic endothelial dysfunction underlying the maternal syndrome. The consequences of this set of alterations may lead to preeclampsia associated to growth restriction.

On the other hand (case ii), we propose that if the abnormal expression of AQP3 takes place only in the villous pathway, it may contribute, together with AQP9, to exacerbate the oxidative damage and apoptotic response of the trophoblast, increasing

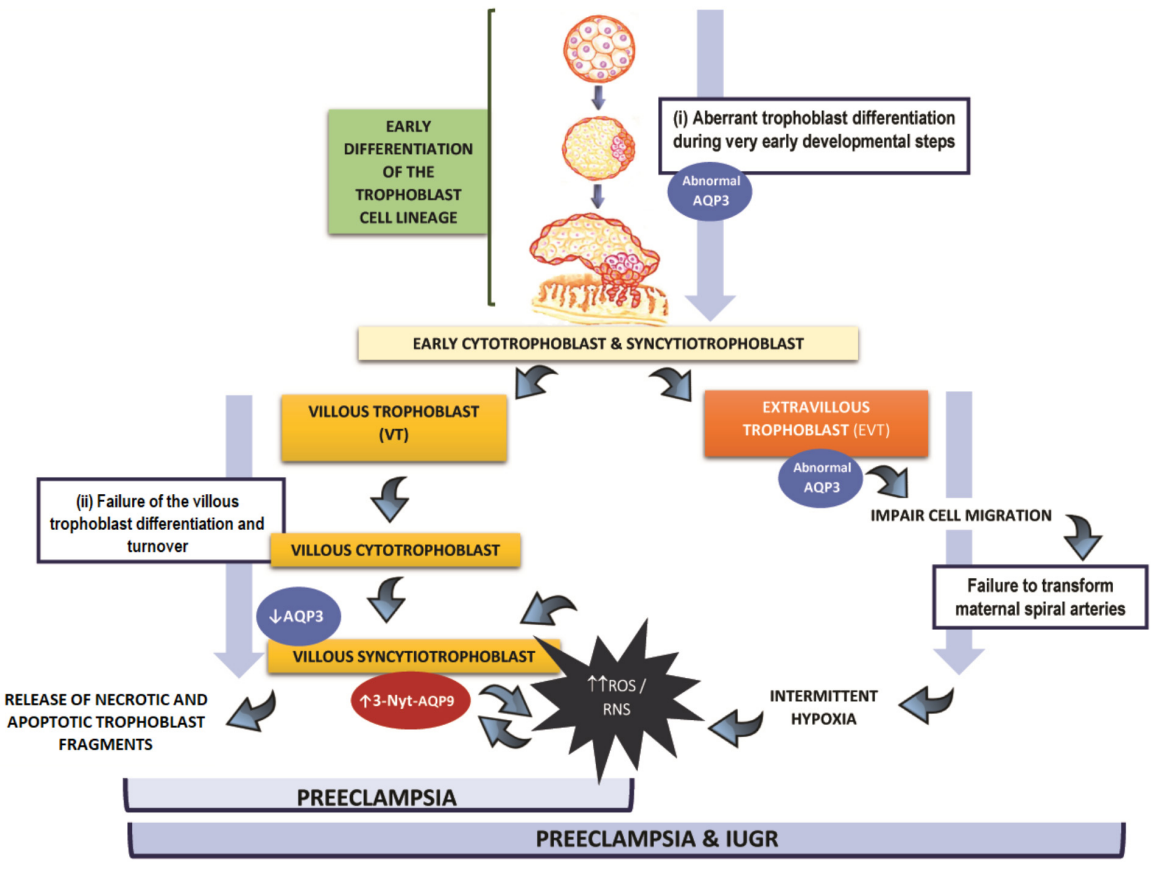

FIGURE 1 | Hypothesis of the participation of AQP3 and AQP9 in the physiopathology of preeclampsia: (i) Alterations in AQPs expression may occur during the trophoblast stem cell differentiation disturbing both extravillous and villous trophoblast cells development resulting in preeclampsia with fetal growth restriction. (ii) Alterations in AQPs expression occur during villous trophoblast differentiation resulting in preeclampsia without fetal growth restriction. In both scenarios, villous trophoblast cells are affected.

the shedding of trophoblast material into maternal circulation. Finally, this may collaborate to induce the injury of endothelial cells, resulting in preeclampsia without growth restriction.

In conclusion, abnormal expression of placental AQPs may appear at different points of trophoblast differentiation affecting VT alone or VT and EVT cells. Although preeclampsia is a complex and multisystemic disorder resulting from multiple simultaneous mechanisms, AQPs may be contributing as part of this network of alterations that give rise to the diverse clinical manifestations of preeclampsia.

AUTHOR CONTRIBUTIONS

AD defined the research topic. AS, YM, JR, MDP, and MC prepared the draft of the manuscript. NS and AD edited the text. NS, NM, AD co-wrote the manuscript. All authors approved the last version of the manuscript.

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Crosstalk Between Nitric Oxide and Endocannabinoid Signaling Pathways in Normal and Pathological Placentation

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Endocannabinoids are a group of endogenous lipid mediators that act as ligands of cannabinoid and vanilloid receptors, activating multiple signal transduction pathways. Together with enzymes responsible for their synthesis and degradation, these compounds constitute the endocannabinoid system (ECS), which is involved in different physiological processes in reproduction. The placenta, which is essential for the success of gestation and optimal fetal growth, undergoes constant tissue remodeling. ECS members are expressed in trophoblast cells, and current evidence suggests that this system is involved in placental development, apoptosis, and syncytialization. Impairment of endocannabinoid signaling has been associated with several pathological conditions such as intrauterine growth restriction and preeclampsia. Both clinical entities are characterized by dysregulation on vascular perfusion where nitrergic system performs a pivotal role. Nitric oxide (NO) is a potent local vasodepressor that exerts a critical role in the regulation of hemodynamic flow, contributing to the maintenance of low vascular resistance in the feto-placental circulation. NO production could be affected by different factors and growing evidence suggests that the endocannabinoid mediators may regulate nitrergic signaling. Herein, we review emerging knowledge supporting ECS-mediated regulation of NO production in normal placentation. Finally, we discuss how alterations in these systems could affect homoeostasis and contribute to the occurrence of placental-mediated pregnancy complications. Given the impact on women and perinatal heath, we will focus on current knowledge regarding the effects of ECS on nitrergic system in normal and pathological placentation.

Keywords: placenta, endocannabinoids, nitric oxide, preeclampsia, endothelial disfunction, anandamide

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INTRODUCTION

The placenta is a specialized transient organ essential for embryo growth and survival. In order to supply the metabolic demands of the developing fetus, this tissue performs numerous physiological functions such as gas exchange and efficient nutrient transfer. These events are crucial for the correct development of the feto-placental unit.

The placenta is an organ devoid of nerves; hence communication between mother and fetus takes place through blood-borne as well as locally produced substances. The syncytiotrophoblast (STB) is the main structural and functional epithelial layer that produces a variety of hormones such as human chorionic gonadotropin (hCG), placental lactogen, estrogen, progesterone, aldosterone, cortisol, placental growth hormone, among others. It can also release a large number of growth factors, cytokines, chemokines, and vasoactive compounds that synchronize placental blood flow, which is of outmost importance during gestation for fetal development (Gude et al., 2004).

Successful pregnancy is coordinated by a complex interplay of maternal, placental, and fetal endocrine signals. Inadequate migration of trophoblast cells and deficient remodeling of uterine spiral arterial walls lead to a reduction of placental blood flow and cause placental ischemia/hypoxia. In this context, vasoactive factors such as inflammatory cytokines, reactive oxygen species, hypoxia-inducible factors (HIFs), and anti-angiogenic factors are the major modulators of the systemic vascular endotheliosis. Both abnormalities in placental formation and function are often associated with human pregnancy complications such as intrauterine growth restriction (IUGR) and preeclampsia (PE).

PE is one of the leading causes of maternal and perinatal morbidity and mortality. In fact, it is the first direct cause of maternal death in Latin America (Giachini et al., 2017). This condition is characterized by hypertension ($\geq$140/90 mmHg) associated to proteinuria ($\geq$0.3 g/24 h) or thrombocytopenia (platelet count $<$ 100.00/μL), liver dysfunction, new onset renal failure (Serum creatinine $>$ 1.1 mg/dL), neurologic symptoms, or pulmonary edema (Brennan et al., 2014).

Endothelial dysfunction is one of the earliest manifestations of PE. To date, the pathogenesis of PE is complex and not well-understood, but it is accepted that an inappropriate remodeling of spiral uterine arteries leads to restricted supply of oxygen and nutrients to the placenta (Li et al., 2015). Vascular endotheliosis associated to PE can lead to a deregulation in the levels of vasodilator factors such as nitric oxide (NO). This altered environment causes placental ischemia and subsequent secretion of placental pro-inflammatory and anti-angiogenic factors into the maternal circulation such as soluble fms-like tyrosine kinase-1 (sFlt-1) and soluble endoglin (sEng), among others (Karumanchi, 2016). Furthermore, evidence of negative correlation between of circulating sFlt-1 and sEng on NO production has been reported in human samples (Sandrim et al., 2008) as well as in animal models of PE (Zhu et al., 2016). However, there are controversies among different studies that measure both circulating levels and urinary excretion of NO in normal and pathological conditions like PE (Ranta et al., 1999; Choi et al., 2002; López-Jaramillo et al., 2008). The discrepancy in the results could be due to different dietary intake of nitrites and nitrates or pharmacological treatments that are given to patients. In this regard, it should be noted that nifedipine, an antagonist of calcium channel widely used for hypertension treatment in preeclamptic patients, may alter NO levels (Berkels et al., 1994; Boccardo et al., 1996).

Furthermore, a number of reports also showed differences in the expression and activity of endothelial NO Synthase (eNOS) between normal and unhealthy pregnancies. (Myatt et al., 1997; Kim et al., 2006; Smith-Jackson et al., 2015; Motta-Mejia et al., 2017).

Distribution and activity of eNOS are regulated by different mechanisms. Trafficking between caveolar and non-caveolar compartments, protein–protein interaction, and phosphorylation are involved in the modulation and/or release of NO (Liaudet et al., 2000; Powe et al., 2011). Therefore, there is an extending interest in determining the specific cellular pathways that modulate the nitrergic signaling. Growing evidence indicates that the endocannabinoid system (ECS) is able to regulate the formation and/or release of NO (Lipina and Hundal, 2017).

The ECS is expressed in human placenta (Park et al., 2003; Aban et al., 2013; Costa et al., 2013) and previous results demonstrate that endogenous cannabinoids (ECs) could modulate NO production acting on different molecular targets (Poblete et al., 2005; Carney et al., 2009; Oddi et al., 2012; Krishnan and Chatterjee, 2015).

Herein, we discuss evidence that supports the role of these endogenous bioactive lipids in the regulation of NO signaling in healthy and pathological pregnancies.

ROLE OF NITRIC OXIDE IN THE PLACENTA

Throughout gestation significant circulatory adaptations occur that includes an increase in maternal blood volume and vasodilatation to maintain the fetal demands of oxygen and nutrients. Maternal uterine vascular remodeling is essential for normal fetal growth and NO plays a crucial role in this process (Myatt, 1992; Possomato-Vieira and Khalil, 2016).

Over the course of gestation the action of NO seems to support a low vascular resistance in the feto-placental circulation (Amit et al., 1998), maintain a vasodilator state of placental vessels, and attenuate the effects of vasoconstrictors (Myatt et al., 1992) being the main contributor to the regulation of physiological hemodynamic flow.

Nitric oxide is a potent gaseous mediator produced in different organs, including placenta (Farina et al., 2001; Shaamash et al., 2001; Cella et al., 2008; Aban et al., 2013).

During the third trimester, the growing fetus significantly enhances the metabolic demands on the placenta. Changes in vascular resistance allow the placenta to support fetal development and wellbeing. In this remodeling of placental blood-flow, both maternal and conceptus eNOS increase uterine arterial blood flow in normal pregnancy (Kulandavelu et al., 2012), and attenuation in its action may reduce placental perfusion and lead to an altered feto-placental signaling.

Nitric Oxide acts in multiple pathways. It diffuses into vascular smooth muscle cells, attaches to the receptor soluble guanylyl cyclase (sGC), and catalyzes the formation of cyclic guanosinemonophosphate (cGMP), resulting in vasodilation. Simultaneously, NO prevents the production and action of both endothelium-derived contracting factors and endothelin-1, thus

reducing the vasoconstrictor effect. Additionally, NO inhibits platelet aggregation and adherence to endothelial surfaces (Ignarro, 1990).

Nitric oxide and L-citrulline are produced from L-arginine through a reaction catalyzed by a family of calcium-calmodulin-dependent enzymes called NO synthases (NOS): Three major NOS isoforms have been identified: neuronal (nNOS or NOS1), inducible, (iNOS or NOS2), and endothelial (eNOS or NOS3). The nNOS and eNOS isoforms are frequently expressed constitutively and their activities are regulated by calcium availability. On the other hand, iNOS is independent of the intracellular calcium concentration and generates a high flow of NO. The tree isoforms of NOS employ flavin adenine dinucleotide (FAD), flavin mononucleotide (FMN), and (6R)-5,6,7,8-tetrahydro-L-biopterin (BH_4) as cofactors of the isozymes (Förstermann and Sessa, 2011) (**Figure 1**).

These enzymes are present in many cell types and tissues such as endothelium, nerves, immune cells, and placenta. In a normal pregnancy, eNOS is the most relevant member of this family and is the key enzyme when considering the production of NO (Moncada and Higgs, 2006).

In the human placenta, the eNOS isoform is expressed in the STBs and vascular endothelium (Kakui et al., 2003; Schiessl et al., 2005). Interestingly, extravillous trophoblast also produces NO while invading the maternal uterine spiral arteries but significantly higher NOS activity was found in the villous trophoblast. Ca^{2+}-dependent NOS activity was also identified in human term placentas, but it is substantially lower respect to samples from early placentas (Al-Hijji et al., 2003). On the other hand, iNOS is expressed in Hofbauer cells of the villous stroma (Myatt et al., 1997).

The placenta lacks of innervation, thus its vascular tone is modulated principally by local factors. In this context, the production of NO is essential for the development of normal placental endothelium, and promotes endovascular invasion by the cytotrophoblast (Zhou et al., 1997). These cells produce NO which acts on arterial walls to create a low-resistance, high-caliber uteroplacental unit (Noris et al., 2005).

The NO production is regulated by many molecules such as vascular endothelial growth factor (VEGF) and placental growth factor (PlGF). Both induce arterial vasodilation by increasing the endothelial calcium signaling, resulting in the release of endothelial NO. Additionally, endothelial shear stress produced by flowing blood stimulates endothelial NO release through a number of pathways, which involve opening of cation channels like TRPV1, TRPV4, among others (Vanhoutte et al., 2016). Protein–protein interactions represent another important mechanism for eNOS regulation. In this context, eNOS can interact with a variety of proteins such as calmodulin or caveolin resulting in an increase or a decrease in eNOS activity (Su, 2014).

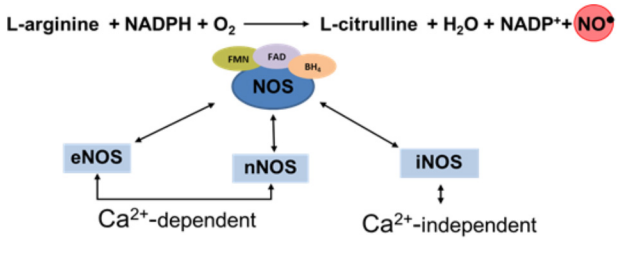

FIGURE 1 | Schematic diagram illustration the synthesis of NO.

THE ENDOCANNABINOID SYSTEM

Endocannabinoids are an emerging group of lipid-signaling molecules that include amides, esters and ethers of long-chain polyunsaturated fatty acids.

Endocannabinoids are produced on demand by cleavage of membrane phospholipids mainly through two-step reaction catalyzed by *N*-acyltransferase (NAT) and *N*-acylphosphatidylethanolamine-phospholipase D (NAPE-PLD) in the pathway of Anandamide (*N*-arachidonoylethanolamine; AEA) synthesis; and phospholipase C (PLC) and diacylglycerol lipases (DAGL) in the case of 2-arachidonoylglycerol (2-AG).

Both lipid mediators (AEA and 2-AG) are the main endogenous ligands of the cannabinoid receptors (CB1 and CB2) (Howlett et al., 2002). These receptors belong to the family of G-proteins coupled receptors (GPCRs) and activate multiple signaling pathways (Pertwee, 2006). In addition, these bioactive lipids can stimulate other membrane proteins such as the orphan G protein-coupled receptor 55 (GPR55) (Sharir et al., 2012; Gasperi et al., 2013), or the intracellular receptor peroxisome proliferator-activated receptors (PPAR). Additionally, other ECs such as oleoylethanolamide and palmitoylethanolamide can also bind to the peroxisome proliferator-activated receptor gamma (PPAR-γ) regulating food intake, lipid metabolism, and inflammatory processes (O'sullivan, 2007; Pistis and Melis, 2010). Moreover, the endocannabinoid AEA can bind to a non-selective cation channel, the transient receptor potential vanilloid 1 (TRPV-1), acting as an endovanilloid (Cella et al., 2008; Marzo and Petrocellis, 2010).

The action of AEA and 2-AG cease by enzymatic hydrolysis mediated by fatty acid amide hydrolase (FAAH) (McKinney and Cravatt, 2005; Fezza et al., 2008) and monoacylglycerol lipase (MAGL), respectively (Dinh et al., 2002).

In addition, there are other enzymes that constitute alternative biosynthetic and degradative pathways for this lipid mediators (Kozak et al., 2002; Pacher and Kunos, 2013).

Altogether, these enzymes and proteins involved in the production and signaling of endocannabinoids, along with these lipid ligands, constitute a complex system called ECS.

Endocannabinoid System in the Placenta

In the last years, enzymes that participate in AEA and 2-AG synthesis and release have been identified in human placenta (Aban et al., 2013; Costa et al., 2013), but until now only AEA levels were measured in this tissue (Marczylo et al., 2010).

The identification of the different components of the ECS in the placenta promoted the study of ECs in relevant physiological

TABLE 1 | Processes modulated by AEA and 2-AG in the human trophoblast.

Proliferation			
AEA	↓ mainly through CB2	BeWo	Habayeb et al., 2008a; Costa et al., 2014a, 2015b
2-AG	↓ mainly through CB2	BeWo	
Cell death			
AEA	↑ through CB1	hST	Aban et al., 2013
	↑ through TRPV-1	hCT	Costa et al., 2015b
	↑ mainly through CB2	BeWo	Habayeb et al., 2008a; Costa et al., 2014a
2-AG	↑ mainly through CB2	BeWo	
Syncytialization			
AEA	? morphological differentiation	hCT	Costa et al., 2014b
	- biochemical differentiation	hCT	
2-AG	↓ morphological differentiation through CB1 and CB2	hCT	Costa et al., 2015c
	↓ biochemical differentiation through CB1 and CB2	hCT	
Migration and invasion			
CB1−/−	↓ invasion	TSC	Sun et al., 2010
Protein biosynthesis			
AEA	↓ ecto-pALP activity, hCG secretion and aromatase expression through CB receptors	hST	Costa et al., 2015b
	- PAPP-A mRNA levels	hST	Costa et al., 2016
2-AG	↑ 3β-HSD mRNA levels through CB receptors	hST	
	- PAPP-A mRNA levels	hST	
Transport			
AEA	↓ K+ channel 1 (TASK-1)	hST	Bai et al., 2006; Wareing et al., 2006
	↓ folic acid transportation, acute treatment. Not mediated by CB receptors	BeWo	Araujo et al., 2009
	↑ folic acid transportation, chronic exposure. Not mediated by CB receptors	BeWo	

Increase (↑), decrease (↓), no effect (–); AEA, anandamide; 2-AG, 2-arachidonoylglycerol; CB1, cannabinoid receptor 1; CB2, cannabinoid receptor 2; TRPV1, transient receptor potential vanilloid 1; hCT, human cytotrophoblast; hST, human syncytiotrophoblast; TSC, trophoblast stem cells; PAPP-A, Pregnancy-associated plasma protein A; 3β-HSD, 3β-hydroxysteroid dehydrogenase; ecto-pALP, placental alkaline phosphatase; hCG, human chorionic gonadotropin.

processes such as proliferation, differentiation, apoptosis, and proteins biosynthesis, as well as in the transport of nutrients, oxygen, electrolytes, and other substances to the fetus. The results observed in these studies were extensively reviewed by Costa (2016) and the relevance of the ECS in trophoblast biology is summarized in **Table 1**.

In addition to the effects of ECs, phytocannabinoids such as delta-9-tetrahydrocannabinol (THC), the main psychoactive compound of marijuana, may affect the dynamics of placental development (Ortigosa et al., 2012; Costa et al., 2015a; Metz and Stickrath, 2015). In fact, it has been shown that THC can promote beneficial or detrimental effects on trophoblast cell viability and also impair morphological differentiation (Costa et al., 2015a). Additionally, chronic exposure to THC may affect the maternal–fetal transference of micronutrient (Araujo et al., 2009). For all the above mentioned, cannabis consumption during pregnancy may have serious alterations in human placentation causing negative pregnancy outcomes such as preterm birth (Dekker et al., 2012) and fetal growth restriction (El Marroun et al., 2009).

A similar mechanism seems to occur when high levels of endocannabinoids are detected during pregnancy. According to this, reports have shown that high plasma levels of AEA seriously interfere in the progression of pregnancy (Habayeb et al., 2008b; Taylor et al., 2011). In agreement with this observation, previous results from our laboratory demonstrated that NAPE-PLD and FAAH expression were impaired in PE placentas. Both proteins were mainly located in the apical membrane of STB in normal placentas although weak staining for FAAH was detected in some villi from PE tissues. Furthermore, high levels of FAAH activity were measured in normal tissues, but a lower activity of this metabolizing enzyme was detected in preeclamptic tissues (Aban et al., 2013). These findings suggest that pathological conditions may expose the fetus to unhealthy levels of the endocannabinoid, disturbing fetal development, and leading to neurophysiological abnormalities (Grant et al., 2017). However, the precise mechanisms by which the principal enzymes involved in the synthesis and degradation of AEA are deregulated in preeclamptic placentas are still unknown.

Other works have described alterations of several components of the ECS in normal and pathological human placentas. Acone et al. (2009) compared samples obtained from women undergoing elective cesarean section (non-laboring group) and women having a normal spontaneous delivery (laboring group) at term (Acone et al., 2009). Interestingly, CB1 expression was detected but FAAH protein was absent in the analyzed samples. On the other hand, Fügedi et al. (2014) observed higher levels of CB1 protein in the STB layer, as well as in the endothelial cells from preeclamptic placental tissue, although they did not find significant differences in CB2 and FAAH expression between preeclamptic and normal placental tissues (Fügedi et al., 2014).

It is worth to note the discrepancy in the results observed by different research groups on the altered expression of the ECS components, even when the same type of samples was analyzed. Such differences could be attributed to ethnicity, severity of the disease and/or differences in methodological procedures (e.g., sample processing, antibodies utilized). These disagreements must be analyzed and requires further elucidation.

CROSSTALK BETWEEN ECS AND NO IN REPRODUCTIVE TISSUES

Our understanding on the interaction between the ECS and nitrergic system has been enriched by several studies that demonstrated a strong influence of ECS on NO production. This regulation is mediated by endocannabinoids like AEA or 2-AG which exert stimulatory or inhibitory effects depending on tissue context, cell type, and/or activation of specific receptors (cannabinoid receptors or alternative molecular targets). Also, previous reports have provided evidence that a bidirectional modulation exists between the ECS and NO, and this crosstalk is extremely important since alterations in one or both systems would impact on cellular homeostasis or could trigger a pathological condition. A comprehensive review of these interactions is well described in Lipina and Hundal (2017).

Regulation of NO production by the ECS was demonstrated in different biological systems such as neurohypophysis (Luce et al., 2014), retina (Krishnan and Chatterjee, 2015), platelets (Signorello et al., 2011), heart (González et al., 2011), nephron (Mukhopadhyay et al., 2010a,b), and in energy metabolism (Tedesco et al., 2008). Nevertheless, little is known about the ECS-associated interaction with NO during pregnancy. The crosstalk between ECS and NO is relevant in reproductive tissues like bovine epithelial oviduct and spermatozoa (Osycka-Salut et al., 2012), as well as in murine and rat uterus and decidua (Vercelli et al., 2009b; Sordelli et al., 2011). NO is involved in various reproductive events including implantation, regulation of placental blood flow, and myometrial relaxation. However, there are limited reports that explain the mechanisms involved in regulation of ECS on NO production. In murine uterus incubated with lipopolysaccharide (LPS), AEA mediates LPS-induced NO production through activation of both cannabinoid receptors, CB1 and CB2. This lipid mediator increases iNOS expression and pharmacological blockade of CB1 and CB2 inhibit this effect suggesting the participation of both receptors. Moreover, LPS modulates the expression of the enzymes involved in AEA metabolism, producing alterations in AEA levels which results in different types of responses that affect NO production (Vercelli et al., 2009a). A similar mechanism was described in murine decidua, where AEA mediates LPS-induced NO synthesis through activation of both cannabinoid receptors. In this tissue, LPS has a deleterious effect on the implantation sites via CB1 receptor and it is believed that this could be associated to

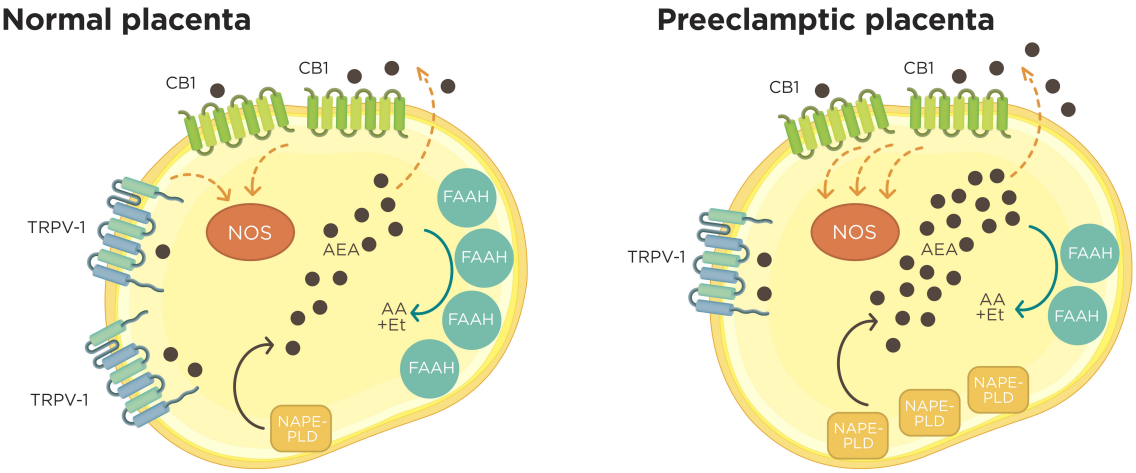

FIGURE 2 | Croostalk between ECS and nitrergic system in normal and preeclamptic placentas. In normal placenta, synthesized AEA from membrane lipids by NAPE-PLD leave to the trophoblast by simple diffusion or by carrier proteins and interact with CB1 receptor. The activation of CB1 by AEA results in the activation of NOS activity. Higher FAAH expression and activity contributes to the maintenance of a low AEA "tone" degrading this lipid to arachidonic acid (AA) and ethanolamine (Et). Inside the trophoblast cells, AEA also could regulate NOS activity through TRPV1 receptor. On the other hand, in preeclamptic placentas, an increased NAPE-PLD expression associated with lower FAAH expression and activity encourage a raise in AEA "tone" that contributes to a higher NO production at least in part through CB1 receptor.

septic abortion (Vercelli et al., 2009b). Furthermore, during the implantation process in rat uterus, AEA modulates NOS activity and NO production on implantation and inter-implantation sites in a specific manner, activating CB1 and/or CB2 depending on the presence or absence of the blastocyst (Sordelli et al., 2011).

The ECS regulates the homeostasis through a wide variety of mechanisms. It facilitates the intracellular communication between different cell types and contributes to maintaining the balance in the body. The placental abnormal expression of the ECS has been associated which serious pregnancy complication such as spontaneous miscarriage (Trabucco et al., 2009) and preterm birth (Sun et al., 2016).

Additionally, it was demonstrated that uterine deregulation of the ECS increases the levels of prostaglandins contributing to the mechanism by which infection causes preterm birth (Bariani et al., 2015). In this animal model, resveratrol administration prevented the changes in the uterine endocannabinoid profiling altered by LPS and diminished iNOS expression and NOS activity evidencing tocolytic effects (Bariani et al., 2017). Additionally, the loss of CB1 receptor has been linked to this pathology (Wang et al., 2008) while others demonstrated that THC has a preventive effect on preterm delivery in a LPS-induced murine model, suggesting the contribution of NO coupling through the CB1 receptor (Asghari-Roodsari et al., 2010).

Endocannabinoids have also been implicated in blood pressure regulation (Pacher et al., 2005). These lipid mediators can cause vasodilation through CB1, TRPV1, and NO-mediated or NO-independent mechanisms (Pacher and Steffens, 2009). Anandamide exerts its vasorelaxant effect on endothelium by upregulating the expression and activity of the inducible NO synthase (NO-mediated pathway) (Randall et al., 2002; Cella et al., 2008). Although there is no direct correlation between AEA serum levels and blood pressure, given these results it is possible to speculate that the decrease in AEA levels observed in preeclamptic pregnant woman (Molvarec et al., 2015) could contribute to their increase in blood pressure, which is a crucial factor characteristic of PE.

In rat placenta, a report from our laboratory demonstrates that AEA exerts a dual effect on NO production depending on which receptor is activated. While activation of TRPV-1 receptor stimulates NO production, the action of AEA on CBs decreases NOS activity, suggesting that AEA acts as a differential fine-tuning regulator of NO during pregnancy (Cella et al., 2008).

In fact, although AEA activates TRPV-1, the concentration required is higher than that needed for CB1 activation (Ross, 2003). On the other hand, an opposite effect is observed in human tissues. Interestingly, in human placenta at term both endogenous and exogenous AEA increase NOS activity through CB1 receptor (Aban et al., 2013). It is important to highlight that the activation of different receptors induces opposite responses, and this effect could be associated to changes in ECS which cause an appropriate AEA "tone", contributing to trigger one or other type of response. We speculate that the differences observed between rat and human placentas concerning to the effect of AEA on NOS activity may be due to the different gestational times analyzed, activation of different signaling pathways of CBs, and also to the expression of TRPV-1 that changes at the end of pregnancy.

In pathological conditions like PE, a higher basal NOS activity was observed in comparison to healthy normal samples. This observation, together with the altered expression pattern of the ECS metabolic enzymes, could result in higher AEA levels, which positively stimulate NOS activity and NO production (Aban et al., 2013) (**Figure 2**). Additionally, preliminary results obtained in our laboratory suggest that changes in the expression of some components of the ECS in human laboring placentas at term also modify NOS activity during labor (unpublished data).

Altogether, the results discussed in this review indicate that either the activation or the inhibition of the ECS can alter the production of NO, leading to beneficial or prejudicial biological responses depending on the cell type. Because the ECS and NO signaling are involved in the modulation of relevant aspects of placental physiology such as vasodilatation and placental blood flow, it is crucial for the tissues to keep their levels acutely regulated. Thus, it is expected that a crosstalk between these systems may contribute to the maintenance of the tissue homeostasis.

Given the relevance of the nitrergic signaling and the ECS in the development of placenta, this review may contribute to identify novel targets for the treatment of placental diseases such as PE.

SUMMARY

In order to understand the functionality of the placenta, we must take into account the complexity of the events that occur in this organ. In this review we have focused and discussed about the importance of ECS and NO in the physiological behavior of normal and pathological placentas. The ECS acts as a regulator of nitrergic system, modulating NO levels. Since NO is the main vasodilator in human placenta implicated in modulation of blood flow, alterations in this mediator may modify placental functions and can be associated to pathological conditions of pregnancy like PE. Herein we summarize recent experimental findings that support the importance of a crosstalk between AEA and NO and the contribution of CB1 signaling in placental development in normal and pathological conditions of pregnancy. Altogether this evidence proposes the ECS as a part of a relevant mechanism of the placenta and may serve as a possible pharmacological target given the relevance of this system in the regulation of NO and, consequently, in placental vascular dysfunction.

AUTHOR CONTRIBUTIONS

CA and MF have proposed the topic of this revision and designed the figures. All authors have contributed to information recrutment and write the present version.

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O-GlcNAc Modification During Pregnancy: Focus on Placental Environment

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Successful placentation is a key event for fetal development, which commences following embryo implantation into the uterine wall, eliciting decidualization, placentation, and remodeling of blood vessels to provide physiological exchange between embryo-fetus and mother. Several signaling pathways are recruited to modulate such important processes and specific proteins that regulate placental function are a target for the glycosylation with *O*-linked β-N-acetylglucosamine (*O*-GlcNAc), or *O*-GlcNAcylation. This is a reversible post-translational modification on nuclear and cytoplasmic proteins, mainly controlled by *O*-GlcNAc transferase (OGT) and *O*-GlcNAcase (OGA). *O*-GlcNAcylation has been implicated as a modulator of proteins, both in physiological and pathological conditions and, more recently, *O*-GlcNAc has also been shown to be an important modulator in placental tissue. In this mini-review, the interplay between *O*-GlcNAcylation of proteins and placental function will be addressed, discussing the possible implications of this post-translational modification through placental development and pregnancy.

Keywords: placental dysfunction, *O*-GlcNAc, post-translational modification, pregnancy, placentation

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INTRODUCTION

The hemochorial placenta allows blood coming from the maternal circulation to directly contact the fetal chorion, favoring nutrient exchange to the embryo and fetus (Croy et al., 2009). In addition to providing a complete environment for embryo-fetal development, this non-innervated organ also has important implications in endocrine and physiological control during pregnancy. These features display a tightly regulated placentation process requiring precise mechanisms to modulate embryo implantation, decidualization of the endometrium and uterine blood vessel remodeling to generate a functional placenta (Maltepe et al., 2010).

The placenta displays crucial functions during pregnancy, and its performance is associated with morphological integrity, providing the desirable environment for fetal development. Several maternal factors may impact placental function, including co-existence of diabetes, hypertension, and other conditions. Metabolic homeostasis, together with the development of gestational immune tolerance (Elliot and Crespi, 2006; Croy et al., 2009), favor an environment without stressors, and are requirements for successful placental development.

O-GlcNAcylation is a reversible and dynamic post-translational modification with *O*-linked β-N-acetylglucosamine (*O*-GlcNAc), targeting cytoplasmic and nuclear proteins at serine, threonine and tyrosine (Ser-Thr-Tyr) residues. This process occurs in several proteins in eukaryotic cells, and is analogous to protein phosphorylation (Lima et al., 2012; van der Laarse et al., 2018). Unlike other post-translational modifications, *O*-GlcNAc is regulated exclusively by two enzymes: *O*-GlcNAc transferase (OGT), which catalyzes the β -attachment of *O*-GlcNAc to the hydroxy groups of Ser-Thr-Tyr residues; and β-N-acetylglucosaminidase (OGA, or *O*-GlcNAcase), which catalyzes the hydrolytic cleavage of *O*-GlcNAc from proteins (Laczy et al., 2009; Lima et al., 2011). Interestingly, OGA and OGT are extensively expressed in placentas (Lubas et al., 1997; Gao et al., 2001) and several proteins that play important roles in placental function are targets for *O*-GlcNAcylation.

The most important source for *O*-GlcNAc formation is the hexosamine biosynthetic pathway (HBP). After glucose uptake, glucose can either be used in the glycolytic pathway or the HBP. The HBP uses fructose 6-phosphate to form glucosamine 6-phosphate, with glutamine serving as the donor of the amino group, whereas this reaction is catalyzed via glutamine: fructose 6-phosphate aminotransferase (GFAT), which is rapidly acetylated through the action of acetyl-CoA:d-glucosamine-6-phosphate N-acetyltransferase (GAT) and isomerized to *N*-acetylglucosamine-1-phosphate (GlcNAc-1-P). UDP-GlcNAc pyrophosphorylase then converts GlcNAc-1-P to UDP-GlcNAc, which serves as the donor for *O*-GlcNAc when OGT is activated. Glucosamine can also enter the cell through glucose transporters and is rapidly phosphorylated by hexokinase yielding glucosamine 6-phosphate, thereby passing the rate-limiting first step of the HBP (**Figure 1**; Lima et al., 2012).

In this review, we will present evidence of how this post-translational modification interacts with proteins that are important for placental function, discussing possible implications in pregnancy.

O-GLCNAC DURING PREGNANCY

O-GlcNAc Role During Pre-implantation, Implantation, and Embryo Development

Several stages of pregnancy may be affected by *O*-GlcNAc (**Figure 1**). Fast trophoblast proliferation, along with the development of the chorionic sac and chorionic villi marks the earliest stage of placental formation (Rossant and Cross, 2001). Maternal hyperglycemia may negatively impact pre-implantation by reducing the embryo's ability for glucose uptake, favoring miscarriage and congenital anomalies (Jungheim and Moley, 2008; Damasceno et al., 2017). In hyperglycemia and glucosamine incubation increased *O*-GlcNAcylation occurs, resulting in reduced cell proliferation and, therefore, impaired blastocyst formation, as demonstrated in an experimental model of mouse zygote cultures. Inhibition of OGT was able to partially restore cell proliferation and blastocyst formation under hyperglycemic conditions, suggesting that dysregulation of both HBP and *O*-GlcNAcylation may favor embryotoxic effects during pre-implantation development (Pantaleon et al., 2010). *O*-GlcNAc displays an important role not only in trophoblast proliferation, but also in embryonic cell differentiation. Naïve mouse embryonic stem cells, derived from pre-implantation embryos, maintain an undifferentiated state via augmented *O*-GlcNAc expression (Shi et al., 2013). The differentiation process from naïve to a primed state, as observed in cells from post-implantation embryos, requires OGA expression, whereas OGT expression is observed in maintenance of cell survival (Jang et al., 2012; Shi et al., 2013; Speakman et al., 2014). Interestingly, OGT also contributes to cell survival of primed embryo cells (O'Donnell et al., 2004) and expression of both OGT and OGA is required to revert primed to naïve cells (Miura and Nishihara, 2016). Later, it was demonstrated that the histone variant H2A is a target for *O*-GlcNAc at Ser^{40}, and this post-translational modification is required for H2A to contribute to the trophoblast stem cell differentiation process, being correlated with the evolution of placental tissue (Hirosawa et al., 2016). Indeed, H2A is required during mouse embryonic stem cell differentiation, allowing these cells to change gene expression. H2A is also highly expressed in early placental development (Kafer et al., 2015).

Post-translational modifications of histones represent an important mechanism of DNA damage repair. An example is the phosphorylation of the histone H2AX, of itself an effective repair mechanism. This process is, however, restrained to small compartments, and must occur within a limited time frame. *O*-GlcNAcylation also occurs in H2AX, limiting the expansion of DNA damage-induced phosphorylation of chromatin (Chen and Yu, 2016). Embryos from diabetic mothers display exacerbated DNA damage, evidenced by the co-localization of H2AX. *O*-GlcNAcylation was observed in diabetic blastocyst-stage embryos, favoring impairment of pre-implantation embryo development (Brown et al., 2018). That precursors coming from the metabolic flux have the ability to modulate nuclear function has been denominated metaboloepigenetics (Donohoe and Bultman, 2012); this mechanism provides evidence for an epigenetic contribution to the vertical transmission of diabetes.

OGA expression is also related to embryo/fetal viability since *Oga* gene disruption results in augmented levels of global *O*-GlcNAcylation. These genetically modified animals display high perinatal mortality, associated with low circulating glucose levels and smaller stores of glycogen in the liver. In this experimental model, other metabolic alterations were identified in heterozygous mice, including fat accumulation, reduced insulin sensitivity, glucose intolerance and hyperleptinemia. *Oga* disruption generated defective metabolic homeostasis, contributing to obesity and insulin resistance (Keembiyehetty et al., 2015).

Successful placentation involves decidual cells that encapsulate the implanting embryo, providing nutrition and favoring an immunological environment that allows trophoblast invasion and, later, placentation. It is mandatory for decidual cells to develop mechanisms to block stressor signals, favoring the integrity of this initial stage of the fetal-maternal interface

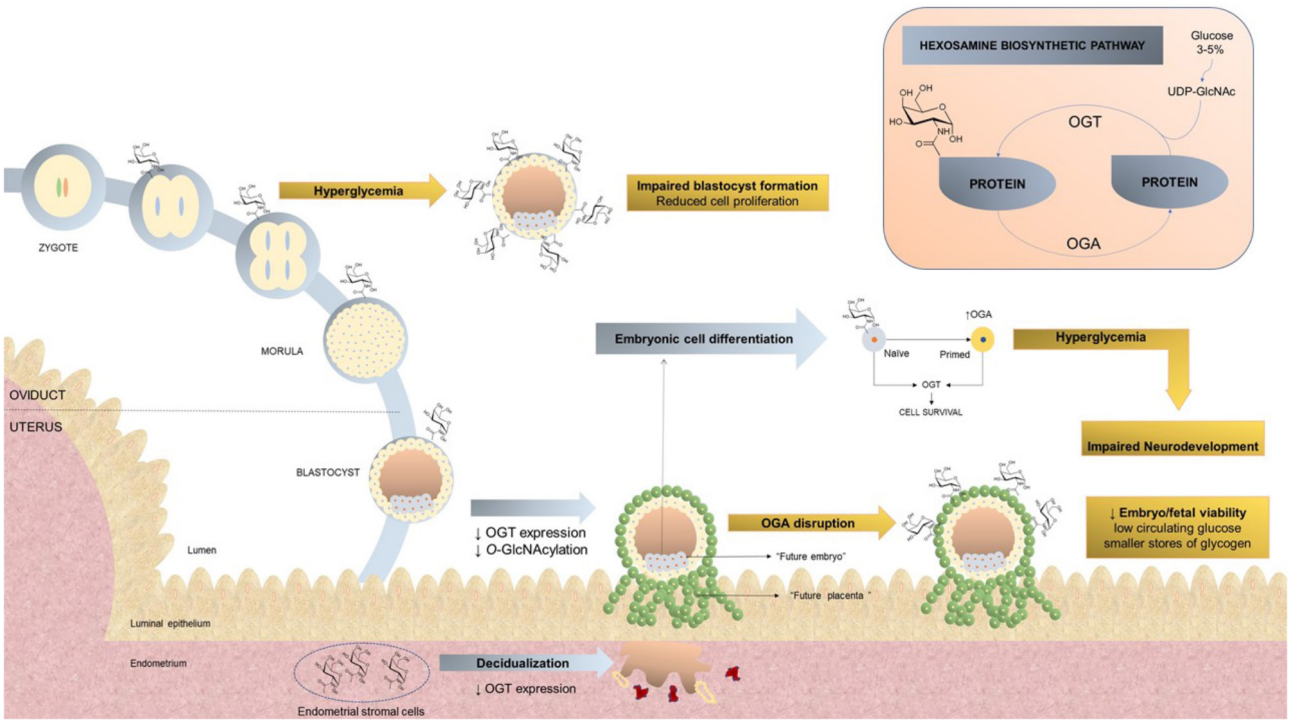

FIGURE 1 | Hexosamine biosynthetic pathway and *O*-glycosylation with *O*-linked β-N-acetylglucosamine during pregnancy. (Pink rectangle) Around 3–5% of intracellular glucose is converted through the HBP to substrate for enzymes involved in the synthesis of UDP-GlcNAc, consequently leading to protein modification through *O*-GlcNAc. OGT catalyzes the addition of *O*-GlcNAc to proteins, whereas OGA removes *O*-GlcNAc from proteins. (Bigger figure) Under physiological conditions, *O*-GlcNAc modifications to intracellular proteins are essential for embryogenesis, promoting the function and viability of various cell types. This process may be disrupted under specific conditions, including hyperglycemia or OGA disruption, where an increase in *O*-GlcNAc is observed, with a consequent reduction in cell proliferation, impaired blastocyst formation and decreased embryo viability. Abbreviations: HBP, Hexosamine biosynthetic pathway; *O*-GlcNAc, glycosylation with *O*-linked β-N-acetylglucosamine; OGA, β-N-acetylglucosaminidase; OGT, *O*-GlcNAc transferase.

(Weimar et al., 2012; Erlebacher, 2013). Decidualization of primary endometrial stromal cells results in reduced global *O*-GlcNAcylation, mediated by decreased OGT expression, without changes in OGA expression. Cell differentiation occurs simultaneously with enhanced expression of epidermal growth factor domain–specific *O*-linked GlcNAc transferase (EOGT), involving mechanisms of energy homeostasis and glucose and fatty acid metabolism, which explains, at least in part, adverse pregnancy outcomes observed in metabolic disorders (Muter et al., 2018).

O-GlcNAcylation impacts embryogenesis and alterations in the maternal glucose metabolism may disrupt neurodevelopment. Neural stem cells submitted to an *in vitro* hyperglycemic environment resulted in augmented global *O*-GlcNAcylation via OGT enhanced activity, and displayed neural tube defects, suggesting that inhibition of altered OGT might be beneficial in preventing birth defects in hyperglycemic pregnancies (Kim et al., 2017). Given the importance of this pathway on neurodevelopment, human embryonic stem cells were used to demonstrate that augmented global *O*-GlcNAcylation is associated with reduced neural progenitor proliferation and premature differentiation of cortical neurons. Therefore, *O*-GlcNAc regulation may represent an important mechanism observed in metabolically compromised pregnancies, contributing to neuronal impairment in the offspring (Parween et al., 2017).

O-GlcNAc and Immune System During Pregnancy

The embryo is recognized as non-self by the maternal immune system, and therefore, several adaptations are required to prevent rejection during implantation (Robertson and Moldenhauer, 2014). One important role of natural killer (NK) cells is to destroy cells that fail to express major histocompatibility complex (MHC) class I molecules; during pregnancy, in contrast to their primary function, placental NK cells tolerate cells from fetal tissue, which do not express maternal MHC I molecules (Ljunggren and Karre, 1990; King and Loke, 1991). A possible explanation for the tissue-specific behavior of placental NK cells is that non-classical MHC I molecules, including human leukocyte antigen-G (HLA-G), are expressed in fetal extra villous trophoblasts and may inhibit NK cell cytotoxicity during pregnancy (Carosella et al., 1999; Kumpel and Manoussaka, 2012). Trophoblast cells present in the maternofetal interface secrete a soluble HLA-G1 (sHLA-G1) isoform in the amniotic fluid, and are released into the maternal circulation, favoring systemic immunoinhibitory activity (McMaster et al., 1998). Indeed, sHLA-G1 secreted by

syncytiotrophoblast specifically induces the apoptosis of CD8+ T cells (Solier et al., 2002). Interestingly, NK cell cytotoxicity occurs simultaneously with reduced *O*-GlcNAcylation and seems to be inhibited by the sHLA-G1 α chain via an *O*-GlcNAc dependent-mechanism. When NK92 cells, an NK cell line, were submitted to a cytotoxicity assay using K562 cells as target cells, *O*-GlcNAc levels decreased inversely to cytotoxic activity (Yao et al., 2004). Preincubation with GST-HLA-Glα chain prevented the decrease of *O*-GlcNAc levels, reverting NK92 cytotoxicity.

Cytokine production is also modulated by *O*-GlcNAcylation. Augmented *O*-GlcNAc levels in the placenta during hyperglycemia coincided with augmented placental levels of interleukin (IL)-6 and tumor necrosis factor alpha (TNF-α). Interestingly, both cytokines positively correlated with placental weight, and negatively correlated with fetal weight and placental efficiency in hyperglycemic conditions (Dela Justina et al., 2017). Reduced embryo implantation and impaired blastocyst development was observed in an experimental mouse model of diabetes. These findings were related to an inflammatory imbalance, elicited by increased expression of pro-inflammatory cytokines in the uterus, including IL-1α, TNF-α, and interferon gamma (IFNγ), and a pronounced reduction of anti-inflammatory regulatory T-cells within the uterus-draining lymph nodes (Brown et al., 2018).

O-GlcNAC Regulation of Transcriptional Factors in Placental Tissue

Post-translational modifications are important key regulators of transcription factors. Considering the high occurrence of *O*-GlcNAcylation of nuclear proteins, *O*-GlcNAc has been implicated to be a major modulator of transcriptional activity (Ozcan et al., 2010). The first transcription factor described to be a target for *O*-GlcNAc modulation was specific protein 1 (Sp-1), a member of the Sp factors; removal of *O*-GlcNAc favored protein association, as demonstrated in HeLa cells (Roos et al., 1997).

Later, it was demonstrated that transcription factors from the placenta are also targets of *O*-GlcNAc modulation. The oncofetal protein gene (Pem) is expressed in a stage-specific manner during murine embryogenesis in placental, but not adult, tissues (Wilkinson et al., 1990). Murine placenta and embryonic expression of Pem is highly regulated, involving E74 like Ets transcription factor 1 (Elf-1) and Sp-1 transcription factors (Rao et al., 2002). Efl-1, which belongs to the Ets family, and Sp-1 are, similarly, modulated by *O*-GlcNAc (Jackson and Tjian, 1988; Juang et al., 2002; Chu and Ferro, 2005). Sp-1 and Elf-1 activate Pem promoter elements, favoring its transcription (Rao et al., 2002). *In vitro*, DNA binding and competition assays have demonstrated that *O*-GlcNAcylation of Sp-1 does not affect DNA binding itself, but reduces the ability of Sp-1 to interact with Elf-1. As a result, the activation of the Pem promoter element is reduced, and, thus, Pem expression (Lim and Chang, 2009). This was the first work to provide evidence that *O*-GlcNAc modulates transcription factors that regulate genes specifically expressed during embryogenesis, displaying a placental distribution.

The hypoxia-inducible factor-1alpha (HIF-1α), is a subunit of a heterodimeric transcription factor, hypoxia-inducible factor 1 (HIF-1), which is stabilized at the protein level in response to hypoxia. This transcription factor plays an essential role during vascular development of the placenta and OGA favors HIF-1α stabilization. OGA deficient mice display elevated *O*-GlcNAcylation along with defective placental vasculogenesis, characterized by reduced vasculature in the labyrinth region, directly contributing to fetal growth restriction. Interestingly, in this model OGA deletion reduced OGT activity, which resulted in HIF-1α suppression, reducing transcriptional activation of target genes (Yang et al., 2015). This evidences an important role of *O*-GlcNAcylation in cellular stress conditions. During proliferation, OGT deletion, which reduces *O*-GlcNAc levels, modulates HIF-1α favoring ER stress-mediated apoptosis, as observed in cancer cells (Ferrer et al., 2014).

Under hyperglycemic conditions, *O*-GlcNAc modifications also occur in nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), found in rat placental tissue (Dela Justina et al., 2017). This represents non-canonical activation of NF-κB, also described in other cells (Yang et al., 2008), in which augmented levels of *O*-GlcNAc bind to NF-κB, favoring its translocation to the nucleus, augmenting production of pro-inflammatory cytokines (Dela Justina et al., 2017).

O-GlcNAc and Maternal Stress

In several cell types, *O*-GlcNAcylation provides an important tool for cell survival in conditions of elevated stressors, and several forms of cellular injury result in dynamic changes to *O*-GlcNAcylation (Martinez et al., 2017). During pregnancy, exposure to stress contributes to maternal and fetal metabolic alterations and reduces placental growth. Both maternal stress and growth restriction have been consistently associated with metabolic dysfunction observed in offspring (**Figure 2**). High glucose levels, observed in mothers submitted to experimental models of stress, impact the gene expression profile of offspring. OGT is one of the genes affected and, in conditions of maternal stress, offspring display reduced OGT expression in the labyrinth region (Briffa et al., 2017).

A genome-wide array approach identified OGT as a cellular stress marker during pregnancy. Both OGT and *O*-GlcNAcylation were significantly lower when mothers were submitted to prenatal stress. In addition to being a biomarker of stress, OGT was also shown to be crucial for neurodevelopment, and its expression was affected to a greater extent in male, compared to female offspring (Howerton et al., 2013). Physiologically, *O*-GlcNAcylation is greatly decreased during early development and the shortest isoform of OGT (sOGT) is essentially undetectable during early development, but increases consistently after birth (Liu et al., 2012). To address the direct impact of placental OGT in programming the developing brain, an elegant study was conducted in a transgenic mouse model with targeted placental disruption of OGT (PI-OGT). Offspring from this model were compared to those of a mouse model of early prenatal stress (Howerton and Bale, 2014). These experiments confirmed that OGT is an important placental biomarker of maternal stress, resulting in a long-term harmful effect on offspring, which is suitable for metabolic and neurodevelopmental programing under these conditions.

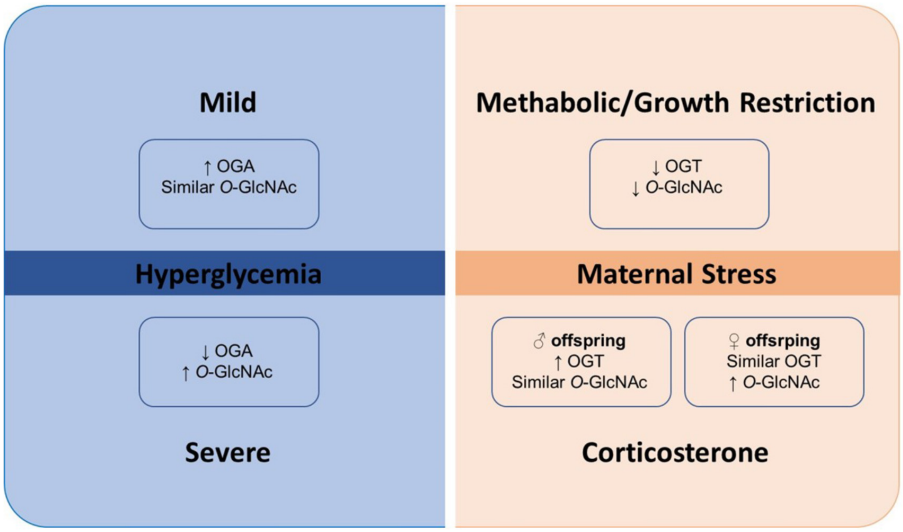

FIGURE 2 | Metabolic maternal stress and *O*-GlcNAc. *O*-GlcNAcylation appears to act as a stress sensor since it exerts its fundamental effects in response to stress. OGT has also been identified as a placental biomarker of cellular stress. During metabolic maternal stress and growth restriction, both OGT and *O*-GlcNAcylation were significantly lower. Placentas of female mice offspring had higher basal OGT expression compared to placentas of male offspring. However, following exposure to corticosterone, OGT expression raised in male placentas and remained the same in female placentas, simultaneously with increased global *O*-GlcNAcylation in female placentas, which was unmodified in male placentas. Abbreviations: *O*-GlcNAc, glycosylation with *O*-linked β-N-acetylglucosamine; OGA, β-N-acetylglucosaminidase; OGT, *O*-GlcNAc transferase.

It appears that OGT expression may change according to the kind and intensity of stress experienced by the mother, and affect offspring in a sexually dimorphic manner. Physiologically, the placenta of female mice offspring had higher basal OGT expression, compared to placentas of males. Following corticosterone exposure, OGT expression increased in placentas of males, but global *O*-GlcNAcylation was not modified, whereas, in placentas of females, OGT expression remained the same, and increased global *O*-GlcNAcylation was observed. These findings show that placentas from female offspring have a greater capacity to rapidly respond to maternal stress and suggests that offspring are affected by maternal stress in a sexually dimorphic pattern. This may impact future life, where males may be more suitable for diseases that are influenced by intra-uterus environment (Pantaleon et al., 2017). A deleterious consequence of maternal stress on offspring includes depression. Adult female rats displayed sex-specific depressive-like behavior when submitted to an intrauterine stress environment as offspring, while males did not display these symptoms (Liu et al., 2018). Interestingly, when these animals were subjected to a swimming exercise, depression symptoms were ameliorated, and this improvement was associated with OGT-related mitochondrial motility.

Metabolic maternal stress, as observed in placentas during severe hyperglycemia, resulted in increased *O*-GlcNAc levels compared to placentas from control and mildly hyperglycemic rats. OGA expression was reduced in placentas from the severely hyperglycemic rats, whereas augmented OGA was found in placentas from the mild hyperglycemic group, compared to control. No changes in OGT were observed during severe or mild hyperglycemia. *O*-GlcNAc overexpression in hyperglycemic conditions co-exist with placental dysfunction, which was characterized by morphometric alterations along with reduced placental index (Dela Justina et al., 2018).

PERSPECTIVES

The evidence relating *O*-GlcNAc to placental function is at present still limited, mainly due to the limited number of studies conducted so far. OGA and OGT expression and *O*-GlcNAc modification represent important modulator mechanisms involved in placental development. Several pathological conditions result in augmented *O*-GlcNAc levels and may impact these mechanisms, leading to impaired placental development and adversely affect fetal growth. Most of the work conducted has been performed in cell culture or in experimental models of hyperglycemia; future work would be served by evaluating *O*-GlcNAcylation in human placentas. In addition to hyperglycemia, high *O*-GlcNAc levels co-exist in other conditions, including hypertension (Zachara, 2012), kidney injury (Hu et al., 2017), high-fat diet (Lima et al., 2016), obesity (da Costa et al., 2018), cancer (Tiainen et al., 2016), among others. Therefore, a next step would be to verify how the altered *O*-GlcNAc levels observed in various pathological conditions might impact placental development.

The current knowledge on this topic also reveals a potential area for exploration in sexual dimorphism during placentation. This is particularly important at the time of the placental collection in animal studies, with respect to correct sex identification. Hence, it will be exciting to describe whether *O*-GlcNAcylation occurs in a similar pattern at different stages of placentation for male and female offspring.

Several conventional pharmacological and non-pharmacological treatments, including medicinal plants, exercise, among others, are used to improve pregnancy outcomes and fetal growth. It will be interesting to evaluate whether these strategies impact *O*-GlcNAcylation of placental proteins or improve gestational success in pathological conditions.

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AUTHOR CONTRIBUTIONS

FG and VL have proposed the topic of this revision and designed the figures. All the authors have contributed to information recruitment, revision design, to write and revise the present version. FG has proposed the topic of this revision and designed the figures. All the authors have contributed to design, write and revise the present version.

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REVIEW
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Novel Insights Into the Role of Glycans in the Pathophysiology of Glomerular Endotheliosis in Preeclampsia

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The polysaccharide heparan sulfate is ubiquitously expressed as a proteoglycan in extracellular matrices and on cell surfaces. In the glomerular filtration barrier, the action of the heparan sulfate is directly related to the function of glomerular filtration, mostly attributed to the sulfated domains that occur along the polysaccharide chain, as evidenced by fact that release of fragments of heparan sulfate by heparanase significantly increases the permeability of albumin passage through the glomerular endothelium, event that originates proteinuria. This review aims to show the importance of the structural domains of heparan sulfate in the process of selective permeability and to demonstrate how these domains may be altered during the glomerular inflammation processes that occur in preeclampsia.

Keywords: glomerular endothelia dysfunction, preeclampsia, heparan sulfate (HS), systemic inflammatory response, renal damage

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INTRODUCTION

Preeclampsia (PE) is characterized as a progressive multisystemic disorder diagnosed by hypertension and proteinuria after 20 weeks of pregnancy. In some clinical cases, proteinuria is absent, and the disease is diagnosed by another clinical findings such as thrombocytopenia, elevated liver enzymes, among others (Visintin et al., 2010; ACOG, 2013). Preeclampsia is accompanied by a mild to severe microangiopathy of target organs, such as the placenta, kidney, liver, and brain (Gilstrap and Ramin, 2002). The placental tissue is a determinant in the establishment of the disease, which is always "cured" after the delivery of the placenta. Evidence related to the role of the placenta in this disease has led to the proposal that failed trophoblast invasion is the central pathogenic mechanism (Kang et al., 2011). Thus, some authors have suggested that preeclampsia is a disease of two stages: in the first stage, poor placentation and the subsequent hypoxic and oxidative damage of trophoblast occur; whereas in the second stage, an endothelial dysfunction and hypertensive signs are evidenced (Goldman-Wohl and Yagel, 2009; Kang et al., 2011).

Preeclampsia exhibits diverse clinical manifestations such as mild or severe, early onset (<34 weeks) or late onset (>34 weeks), or the presence or absence of intrauterine growth retardation (Gilstrap and Ramin, 2002; Giachini et al., 2017). Accumulating evidence has shown that pathological features of preeclampsia involve a shallow trophoblast invasion and poor spiral artery remodeling, resulting in placental hypoperfusion. These events occur in the first trimester of pregnancy and initially compromise maternal–fetal interface one, but they result in increased production of antiangiogenic and inflammatory factors (Schiessl, 2007; Palei et al., 2013). Additionally, hypertensive disorders of pregnancy are associated with endothelial damage of target organs including the kidneys.

Although the clinical manifestations of PE are present after 20 weeks of pregnancy, some evidences indicate that the pathophysiological changes leading to the disease occur in the placental bed during the placentation process. Reported findings suggest that these events promote exacerbated systemic inflammatory responses and the subsequent production of soluble factors, including proinflammatory cytokines, as well as changes in the ratio of angiogenic/proangiogenic factors, which are the determinant variables of changes in the endothelium, the target organ of PE (Levine et al., 2004; Goswami et al., 2006).

Pregnancy has been described as a controlled state of mild inflammation, whereas a state of exacerbated inflammation apparently occurs in PE (Sargent et al., 2007). Our research group has shown that the activation of natural killer (NK) cells is increased compared with other cell populations, which is evidenced by the higher production of both pro- and anti-inflammatory cytokines in patients with early-onset severe PE (Bueno-Sanchez et al., 2013). However, other authors have found that the intracellular production of pro-inflammatory cytokines [interleukin-1 beta (IL-1β), tumor necrosis factor-alpha (TNF-α), IL-6, and IL-8] by circulating maternal monocytes is increased in patients with PE (Luppi and Deloia, 2006). The increased production of proinflammatory cytokines from different cellular sources (NK cells according to our data and monocytes or neutrophils according to other studies) initially triggers inflammatory changes in the vascular endothelium and then compromises the endothelium of other organs (Stella and Sibai, 2006). Accordingly, Rops et al. (2008) have shown that IL-1β and TNF-α may alter the expression of glycocalyx structures, including the specific domains of heparan sulfate (HS), a glycosaminoglycan present in the renal glomerular endothelium.

Histopathological changes in the glomerular endothelium, which partly explain the proteinuria observed in women with PE, are among the aforementioned changes (Karumanchi et al., 2005). Studies have shown that the structural integrity of the glomerular filtration barrier (GFB) consists of two cellular components, the glomerular endothelium, and podocytes, as well as an extracellular matrix structure with a high content of glycoconjugates, including the glomerular basement membrane (Karumanchi et al., 2005). Reports have suggested that the structural integrity of the GFB is crucial for proper functioning during the process of renal filtration. Thus, a structural damage of the glomerular filtration barrier can be explained by a competitive blocking of vascular endothelial growth factor (VEGF) in the murine model after administration of a synthetic VEGF antagonist. These mice presented nephrotic-range proteinuria and hypertension compared with controls (Eremina et al., 2008). These findings raise the possibility of assessing the biochemical aspects of the glomerular endothelium, including its function in filtration, as a means of clarifying the mechanisms of renal damage that occur in women with PE.

We have reported evidence of changes in the membrane distribution of podocin and CD2AP in podocytes, which are cellular components of the GFB, when stimulated with sera from women with PE (Henao et al., 2008). However, few studies have investigated the role of the glomerular endothelium in PE. In turn, Singh et al. (2007) have proposed a key role for the glycocalyx of glomerular endothelial cells in the GFB-mediated restriction of albumin permeability, suggesting elements that can be used to evaluate renal damage in PE. Among these carbohydrates, glycosaminoglycans, particularly HS glycosaminoglycans, are components of the glomerular endothelium glycocalyx that are associated with structures referred to as proteoglycans. HS activity is directly involved in glomerular filtration, and its involvement is mostly attributed to the sulfated domains throughout the polysaccharide chain. This is evidenced by the heparanase-mediated release of HS fragments, which significantly increase albumin permeability through the glomerular endothelium, albeit without affecting the transendothelial electrical resistance (Singh et al., 2007).

Based on the above evidence, this review aims to show the possible role of the structural domains of HS in the process of selective permeability through the GFB and how these domains may be altered during the glomerular inflammation processes that occur in PE.

THE SULFATED DOMAINS OF HS MEDIATE ITS INTERACTIONS WITH DIFFERENT PROTEINS

Heparan sulfate proteoglycans (HSPGs) are biomolecules with structural and regulatory functions that are capable of establishing ionic interactions with several proteins through structural regions in the polysaccharide chains. HSPGs consist of a protein core covalently bound to the side chains of HS (Coombe and Kett, 2005). This polysaccharide consists of alternating units of glucuronic acid (GlcA) and *N*-acetylglucosamine (GlcNAc), which undergo various enzymatic modifications including *N*-deacetylation and *N*-sulfation of the GlcNAc units (Carbon 6 and Carbon 3), epimerization in the C5 of GlcA to iduronic acid (IdoA) and *O*-sulfation in Carbon 2 (Kreuger et al., 2006). The various modifications do not occur randomly along the polysaccharide chain. Instead, they have the distribution of a typical domain because of limitations imposed by substrate specificity (and factors that are still unknown). Disaccharide units alternating between GlcNS and IdoA with 2-*O*-sulfate substitutions (NS domains) provide highly sulfated regions that may be interspersed with *N*-acetylated GlcNAc and GlcA regions lacking substitutions by sulfate group residues (NA domains). Similarly, forms with alternating NS/NA domains may also be found, albeit without *O*-sulfate groups (Maccarana et al., 1996; Sasisekharan and Venkataraman, 2000). The study of HS structures, which have been characterized in several mouse tissues, shows that the disaccharide composition, total degree of *N*- and *O*-sulfations and domain organization are specific to each tissue (Ledin et al., 2004). Furthermore, immunohistochemistry analyses have revealed the selective expression of different HS glycotopes in rat kidney tissues (van den Born et al., 1995; van Kuppevelt et al., 1998). The structural complexity of HS domains is even greater when considering that the HS chains may be modified after biosynthesis by endo-6-*O*-sulfatases, which generate HS fragments that have been functionally implicated in various signaling pathways (Ai et al., 2003).

Furthermore, HS domains may undergo endoglycosidic cleavage by heparanases, thereby releasing extracellular HS fragments (Vlodavsky et al., 1999). In summary, these observations suggest that HS biosynthesis is highly regulated at different points of enzymatic processing in events that apparently vary with the tissue conditions or cellular environment.

This structural diversity could largely explain the interactions between HS and various proteins. Although the types of chemical interactions between HS and proteins have been difficult to elucidate, some proteins are known to require HS sequences with a well-defined length and structure, especially proteins whose net charge is positive, thereby suggesting that such interactions are not as nonspecific as initially thought.

The best characterized interaction between HS and a protein involves the binding of HS to antithrombin III, which is involved in hemostasis. Evidence first reported by Lindahl et al. (1980) shows that the pentasaccharide GlcNAc6S-GlcAGlcNS3S-IdoA-GlcNS is essential for the high-affinity interaction with antithrombin to occur and, therefore, for anticoagulant activity. However, these results most notably demonstrate the importance of specific HS structural domains, including the presence of a 3-sulfate group in the terminal glucosamine residue, which is crucial for such activity (Lindahl et al., 1980).

Another example of an interaction between HS and a protein involves a specific 3-*O*-sulfated domain of the GlcN3S unit that apparently mediates the specific binding of the herpes simplex virus (HSV) protein gD to the cell surface during viral infection (Shukla et al., 1999). According to some authors, in addition to this domain, the most common sulfate substituents in biosynthesis (3-*O* sulfate and 2-*N* sulfate) could also be specifically arranged in a sequence for selective binding to a protein (Salmivirta et al., 1996). Interaction studies involving the fibroblast growth factor protein family have shown that a particular type of sulfated domain (6-*O*-sulfate) could contribute more to the interaction than other domains (Salmivirta et al., 1996). These findings and the strict regulation of HS biosynthesis suggest that HS–protein interactions are mostly mediated by specific saccharide-binding domains with restricted specificity.

THE GLOMERULAR ENDOTHELIAL GLYCOCALYX IS A BARRIER THAT IS SELECTIVELY PERMEABLE TO ALBUMIN

Evidence shows that the NS domains of HS mediate its interactions with some proteins, and these interactions may be associated with its biological action. This suggests that the negative charges of the HS domains in the GFB could play a role in its selective permeability to positively charged proteins, including albumin, which are retained in the plasma. Conversely, small peptides, electrolytes, and even immunoglobulins are filtered into the urine during the glomerular filtration process (Singh et al., 2007).

The GFB is described as a set of three tissue layers: glomerular endothelium, glomerular basement membrane, and podocytes (**Figure 1**). Special large epithelial cells with cytoplasmic extensions (pedicels or foot processes) called podocytes are found toward the outer surface of the glomerular basement membrane and the fenestrated glomerular endothelium toward the innermost location, which is in contact with the plasma (Satchell, 2013). The GFB is highly selective and permeable to water and small molecules. However, for some proteins including albumin, only 0.008% of the plasma fraction is filtered into the urinary space compared with 0.2% through other systemic capillaries (Norden et al., 2001). Some authors have highlighted proteins expressed in the filtration diaphragm between pedicels because proteinuria is usually accompanied by changes in the distribution of these proteins, thereby suggesting a causal relationship between the expression of diaphragm proteins and albumin excretion. However, the size of the podocyte clefts is inconsistent with the size of albumin, which is much smaller, thereby raising questions of whether this could explain urinary albumin excretion as previously proposed (Haraldsson and Sorensson, 2004; Henao et al., 2008). Accordingly, some examples indicate that proteinuria may be found in diseases such as nephrotic syndrome even when podocyte integrity is maintained. Animal experiments show that the down-regulation of circulating VEGF or neutralization by either antibodies or Soluble fms-like tyrosine kinase-1 (sFIt-1) may play a key role in inducing proteinuria without changes in podocyte proteins in various renal diseases or PE, wherein a high level of sFIt-1 has been associated with the endothelial dysfunction observed in these women (Maynard et al., 2003; Sugimoto et al., 2003; Stillman and Karumanchi, 2007). This condition has also been examined in humans. A previous study involving 27 cases of nephrotic syndrome with minimal changes at different stages that assessed podocyte morphology using electron microscopy showed greater changes in podocyte pedicels in the more chronic forms of the disease. However, no significant differences in proteinuria were found between the study groups formed according to the time of evolution, thereby conclusively indicating that changes in podocytes are unrelated to protein loss (van den Berg et al., 2004).

By the other hand, it is known that HSPGs are abundant in the glomerular basal membrane and it has been thought to play a major role in the charge-selective glomerular filtration barrier. However, knockout of Ext3 (an enzyme that adds GlcNAc to the fixed tetrasaccharides on the core protein in proteoglycans) in podocytes and glomerular basal membrane did not lead to overt proteinuria (Aoki et al., 2018). Aoki et al. (2018) found a bumpy glomerular basal membrane and podocyte effacement by electron microscopy using a Extl3KO mice model compared with Diabetes Mellitus and high sodium intake mice groups, however they had some limitations in their study because the follow-up period was too short for remarkable renal impairments and establishment of hypertension. Additionally, renal function tests and HS urine measurements were not assessed in all groups of mice, which would help to support the possible role of HSPGs in the physiology of the GFB.

Thus, the summarized evidence shows that changes in podocytes or glomerular basal membrane fail to explain completely the onset of proteinuria, suggesting that the glomerular endothelium and its glycocalyx structures could

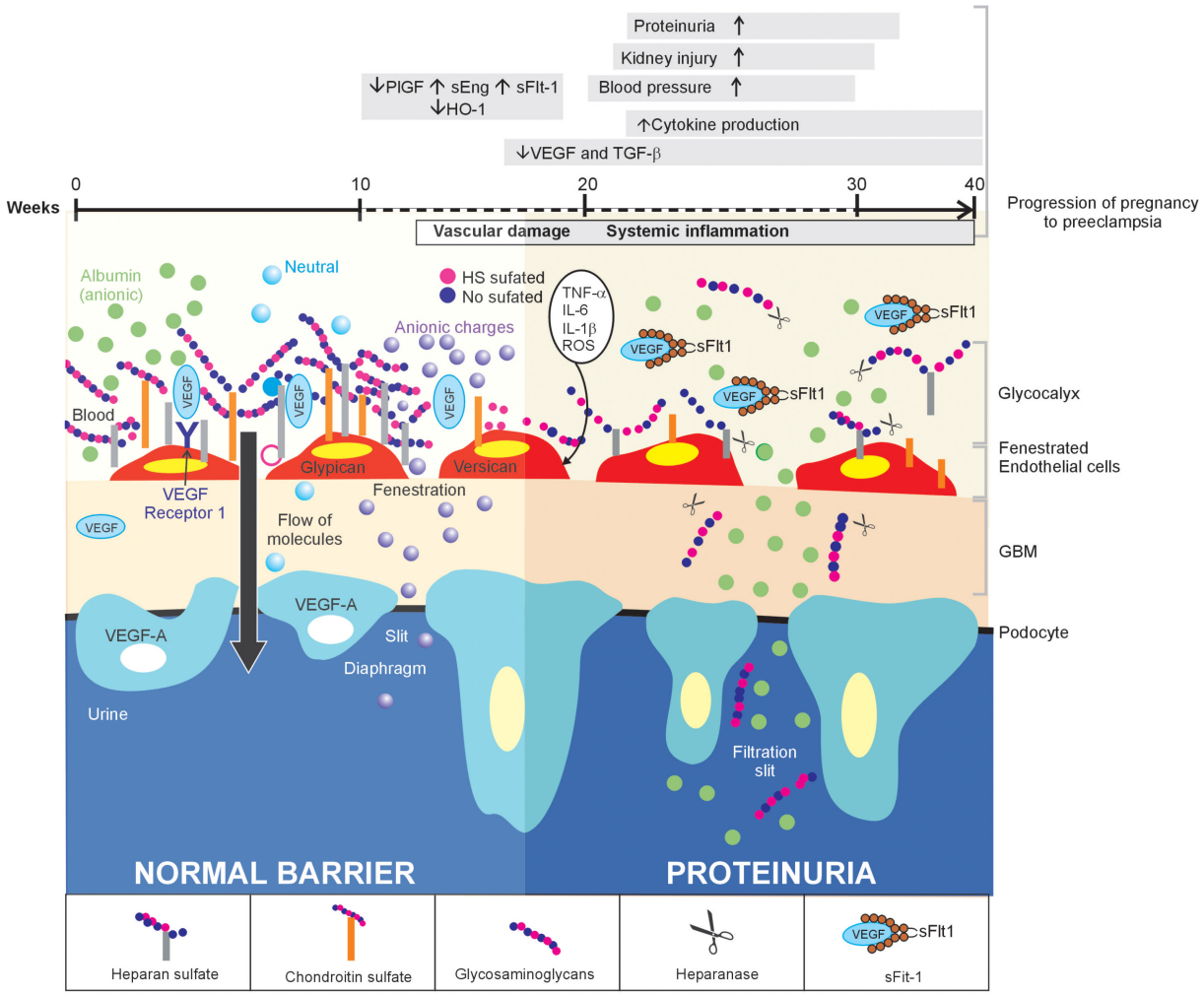

FIGURE 1 | Inflammation of the glomerular endothelium causes the loss of HS, thereby triggering proteinuria in women with PE: a proposed model. In normal conditions (left side), the GFB performs its function of filtration, which is characterized by selective permeability to albumin due to HS in the glomerular endothelial glycocalyx, through its three layers. When a glomerular endothelial inflammation is established (right side), as is the case of PE, the increase in pro-inflammatory cytokines promotes the heparanase-mediated endoglucosidic activity of HS, which leads to the loss of anionic sites and, consequently, proteinuria.

be involved in selective permeability to albumin because this the first layer in contact with plasma and, therefore, the first structure to provide a barrier to albumin. Reported evidence on glomerular endothelium damage in diseases including PE, which is characterized by the presence of hypertension with the onset of proteinuria in most diagnosed cases, suggests that the endothelium may play a key role in the onset of proteinuria in this disease (Maynard et al., 2003). Other studies support a selective permeability layer (glycocalyx) at the endothelial level (Sorensson et al., 1998; Ohlson et al., 2000; Ciarimboli et al., 2003; Jeansson and Haraldsson, 2006).

The glomerular endothelium has two key structural characteristics. The first is a set of fenestrations or pores that are sufficiently large enough (36 Å or 3.6 nm) for water and other molecules, including albumin, to move through the endothelial cell layer (Levick and Smaje, 1987). This characteristic alone contradicts the aforementioned evidence implying the additional presence of a barrier within the fenestrations. The second component is the glycocalyx, which covers the fenestrations and has molecular and charge characteristics that could restrict albumin movement through the glomerular endothelium. Its complex structure consists of proteoglycans, including syndecan, glypican, or biglycan – with HS, *N*-, and *O* – glycoproteins containing sialic acid that is expressed on the endothelium luminal surface (Reitsma et al., 2007; Weinbaum et al., 2007). Jeansson was one of the first researchers to study in detail the selective permeability of the GFB in mice and proposed that hyaluronic acid, chondroitin sulfate, sialic acid, and HS are important for selective permeability because of their electrical charges (Jeansson and Haraldsson, 2006). A key observation is the increased ratio of HS and hyaluronic acid over sialic acid (Avasthi and Koshy, 1988). This observation in particular suggests that HS may make a greater contribution to the electrical charge of the GFB because hyaluronic acid lacks

TABLE 1 | Involvement of HS and heparanase expression in proteinuric diseases.

Disease/Animal Model	Species	Glomerular Heparanase Expression	HS Expression	Proteinuria	Reference
Diabetic nephropathy	Human	Increased	Reduced	(+)	Makino et al., 1992; van den Born et al., 1993; Tamsma et al., 1994; Katz et al., 2002; Maxhimer et al., 2005; van den Hoven et al., 2006; Wijnhoven et al., 2008; Garsen et al., 2016
Systemic lupus erythematosus	Human/mouse	Increased	Reduced	(+)	Seyger et al., 1998; Rops et al., 2007b; Kim et al., 2017; Szymczak et al., 2017
Membranous glomerulonephritis	Human/mouse	Increased	Reduced	(+)	van den Born et al., 1993; Garsen et al., 2016; Szymczak et al., 2017
Dense deposit disease	Human/Rat	Increased	Reduced	(+)	Smith et al., 2007; Zaferani et al., 2012
Ig A nephropathy	Human	Increased	Reduced	(+)	Sakagami et al., 2004; Celie et al., 2008; Szymczak et al., 2017
Minimal change disease	Human	Not analyzed	Reduced	(+)	Dong et al., 2009

significant negative charges when compared with the sulfated domains of HS. This hypothesis may be supported by studies conducted by Singh et al. (2007), who assessed the glycocalyx structure in a glomerular endothelial cell line to examine its relevance to endothelial-selective permeability. They found that the removal of HS after treatment with human heparanase (the only endogenous enzyme described in mammals that degrades HS) was associated with increased albumin movement through the fenestrations without changing the transendothelial electrical resistance. These results suggest a possible role for the glycocalyx in restricting protein movement through the GFB and raise the possibility that heparanase levels in humans are related to proteinuria in kidney damage (Jin and Zhou, 2017). Additionally, the HS fragments induced by heparanase from GFB could contribute to release local proinflammatory cytokines or chemokines in the extracellular space, modifying the inflammatory response, and the endothelial glycocalix (Digre et al., 2017; Martin et al., 2017; O'Callaghan et al., 2018) (**Table 1**).

Immortalized human glomerular endothelial cells have a glycocalyx of approximately 200 nm in culture (Singh et al., 2007). The enzymatic removal of glycans, including HS and sialic acid, increases the rate of albumin passage through the monolayers of the glomerular endothelial cells. This has been demonstrated in studies by Singh et al. (2007) and in another by Wijnhoven et al. (2007), who assessed the loss of sialic acid from the glomerular endothelial glycocalyx with neuraminidase, which was directly correlated with the increase in urinary albumin excretion. Glycan expression in the glomerular endothelium may be modulated at high glucose concentrations, which reduce the quantity of HS glycoaminoglycans and increase the passage of FITC-labeled albumin without affecting the interendothelial junctions (Singh et al., 2011). Reactive oxygen species may also cause changes to the glycocalyx of glomerular endothelial cells by depolarizing glycosaminoglycans, thereby inducing an increase in transendothelial albumin movement (Singh et al., 2013). The importance of HS in the GFB to the passage of albumin has been confirmed by assays in which heparanase knockout mice failed to develop albuminuria, whereas the albuminuria exhibited by the wild-type diabetic mice was reduced with a heparanase inhibitor (Gil et al., 2012).

RENAL DAMAGE IN WOMEN WITH PE INVOLVES A LOSS OF ANIONIC SITES IN THE GLOMERULAR ENDOTHELIUM

Normal pregnant women have higher levels of glomerular protein filtration than do non-pregnant women. Conversely, proteinuria above 300 mg has been detected in 24-h urine samples from women with PE (ACOG, 2013), which indicates a loss of the histological integrity of the GFB. Methods, including dextran sieving, have been described for assessing the restriction of albumin permeability. This polysaccharide is not reabsorbed or secreted by renal tubules. Therefore, it reflects the average permeability of the entire barrier. With sieving and mathematical models, researchers have identified a loss of the restriction of permeability to albumin, particularly at the end of pregnancy in women with PE, allowing for greater excretion than at the beginning of pregnancy or compared with the non-pregnant women who served as controls (Moran et al., 2003). Could the loss of anionic charges of HS from the glomerular endothelium glycocalyx explain the loss of albumin in women with PE?

This question may be partly answered with the following evidence: Histological analyses of this hypertensive disorder show that the lesions on the filtration barrier are mainly limited to endothelial cells, with no significant changes in podocytes (Lafayette et al., 1998; Strevens et al., 2003). Thus, if the damage occurs in the glomerular endothelium, some of its cellular structures must be altered (including the glycocalyx). A study has revealed a decrease in the electrical charge of the glomerular endothelium by morphometrically

assessing the glomerular anionic charge and examining the pathological changes in the renal histology of African women with early onset PE (Naicker et al., 1997). Furthermore, the results showed a strong correlation between the number of anionic sites and the severity of proteinuria (Naicker et al., 1997).

Evidence supporting this earlier hypothesis and providing a more accurate approximation can be found in a more recent study. Khedun et al. (2002) evaluated 84 patients, including normotensive and proteinuric hypertensive pregnant women, as well as women with PE. This result showed a correlation between the reduction in glomerular charges and proteinuria severity. They also assessed HS levels using spectrophotometric methods with dimethylmethylene blue (DMB) and observed an increase in urinary glycosaminoglycan excretion.

The results discussed thus far indicate that HS may play a role in selective permeability as a function of the electrical charge when present in the glomerular endothelial glycocalyx. Thus, anionic charges may generate repulsion to proteins, including albumin, particularly the sulfated domains found throughout the HS chain. However, the specific chemical structures of these domains have not yet been identified.

In this scenario, it is not very clear how damage to the glomerular endothelium and the loss of HS in the glycocalyx occur. Inadequate placentation could lead to an increase in oxidative stress, thereby explaining the increased release of soluble factors including sFIt-1 (Levine et al., 2004; Goswami et al., 2006). This VEGF antagonist prevents proper communication between the podocytes that produce this antagonist and the glomerular endothelium that expresses the VEFGR receptors, thereby causing a loss of endothelial fenestrations (Sugimoto et al., 2003). Moreover, increased production of IL-1, IL-8, and TNF-α resulting from systemic inflammatory responses in women with PE could trigger glycocalyx loss due to increased heparanase activity, as will be discussed shortly (Luppi and Deloia, 2006; Bueno-Sanchez et al., 2013) (Graph 1).

SYSTEMIC INFLAMMATORY RESPONSE IN PE COULD HELP TO EXPLAIN THE PROTEINURIA?

HS involvement has been described in several steps of the inflammatory process: initial binding and leukocyte rolling into the inflamed endothelium, stable adhesion of activated leukocytes to the endothelium, glycocalyx degradation, and, finally, leukocyte migration (Parish, 2006; Taylor and Gallo, 2006; Rops et al., 2007a).

Some studies have shown that HS is capable of interacting with L-selectin and *P*-selectin, thereby participating in the leukocyte rolling process of the adhesion step. Norgard-Sumnicht showed that there is an interaction between Sulfur-35-(an analog of HS with more substitutions by sulfate groups)-labeled heparin and L-selectin through a calcium-dependent process in endothelial cell cultures (Norgard-Sumnicht et al., 1993). Supporting this hypothesis, the ability of HS to bind L-selectin and *P*-selectin was assessed in *in vitro* models of endothelial cells, and a direct correlation was observed. The same study assessed the ability of heparin to interact with these adhesion molecules, and the results showed that the binding was dependent on highly sulfated NS domains, which had a higher binding affinity for adhesion molecules in heparin than HS (Norgard-Sumnicht et al., 1993).

These and other findings suggest that the HS-analog heparin could be useful as a powerful anti-inflammatory agent by inhibiting the function of L-selectin and *P*-selectin (Koenig et al., 1998). It has reported strong evidence that HS is the ligand for L-selectin in the endothelium during inflammatory processes. In this study, mice in which the enzyme *N*-deacetylase-*N*-sulphotransferase-1 (NDST-1), which is required to add sulfate groups to HS chains, was knocked out exhibited normal development, although neutrophil infiltration was observed in several tissues. These effects resulted from changes in HS that were specific to endothelial cells. In contrast, the results showed that leukocytes migrated to inflammatory sites when partially sulfated HS was expressed (Wang et al., 2005).

In addition to interacting with L-selectin and *P*-selectin, HS also interacts with cytokines to allow for leukocyte extravasation. Indeed, approximately 45 chemokines have been implicated in the recruitment of leukocytes during endothelial inflammation according to bioinformatic analyses. Furthermore, it is noteworthy that all of these chemokines have the ability to interact with HS in four possible ways at the binding sites (Lortat-Jacob et al., 2002). Reported evidence that chemokines are unable to perform their full range of functions at sites of inflammation unless they can bind to HS highlights the importance of this interaction. Different approaches have shown that HS may control the function of chemokines. First, HS may sequester chemokines by increasing the local concentration, thereby facilitating binding to its receptors that are expressed in leukocytes. Studies using IL-8 have shown that HS may induce oligomerization, and this form is more active. Similarly, the removal of glycosaminoglycan from CHO cells expressing chemokine receptors resulted in decreased binding to RANTES (regulated on activation, normal T cell expressed and secreted), MCP-1 (Monocyte Chemoattractant Protein-1), and IL-8 (Hoogewerf et al., 1997). Second, without chemokines, leukocytes are unable to form selectin-mediated stable interactions with the endothelium or migrate directionally through the endothelium. A study reported such evidence by showing that endothelium-bound chemokines induce an association between lymphocytes and their antigen receptor (lymphocyte function-associated antigen 1 – LFA1), whereas nonsoluble chemokines failed to exhibit this behavior (Shamri et al., 2005). Lastly, chemokine transport through the endothelial cell layer, known as the transcytosis process, apparently depends on HS expression (Wang et al., 2005). Concerning data from Talsma et al. (2018) indicate a pivotal role of endothelial HS in the development of renal inflammation and fibrosis in diabetic nephropathy in mice (Talsma et al., 2018). The results showed that a decrease in sulfations of endothelial HS induced an increased glomerular macrophage infiltration, mannose binding lectin complement deposition, and glomerulosclerosis (Talsma et al., 2018).

Leukocytes that bind to endothelial cells through different selectins (a process in which different cytokines are involved as mentioned above) are apparently able to cross the subendothelial space and, therefore, the basement membrane, which consists of a dense glycocalyx forming a sort of barrier. Leukocytes use several glycosidase-like enzymes that degrade glycocalyx structures to overcome the barrier. Although several enzymes have been reported, heparanase, an endo-β-glucoronidase, is the most studied in the inflammatory process. HS cleavage resulting from heparanase activity not only allows for the passage of leukocytes through the endothelium but also contributes to the increase in vascular permeability observed in inflammation (Edovitsky et al., 2006). Several studies have reported heparanase expression during the inflammatory response. Different cells, including platelets, leukocytes, neutrophils, and endothelial cells, are able to produce heparanase and degrade the extracellular matrix after stimulation with cytokines, such as IL-1, IL-8, and TNF-α (Bartlett et al., 1995; Vlodavsky et al., 1999).

Normal pregnancy is reportedly characterized by a controlled local inflammatory response, which is evident during the luteal phase of the menstrual cycle before implantation and develops as pregnancy progresses. In contrast, an exacerbated systemic inflammatory response, which may alter the endothelium and, therefore, different organs including the kidney, has been observed in PE (Sargent et al., 2007). Accumulated evidence suggests that PE might be an immune system-mediated disease, mainly an exacerbated pro-inflammatory state of pregnancy in which an injured endothelium promotes changes in leukocyte recruitment, release of pro-inflammatory cytokines, dysregulation of angiogenic pathways, and disturbances of the glomerular filtration barrier (Germain et al., 2007; Kanasaki and Kalluri, 2009). This systemic inflammation could be explained by the increase in placental factors, including soluble factors and proinflammatory cytokines, or by placental oxidative stress. The soluble receptor for VEGF, known as sFIt-1, is a possible inducer of this inflammation. sFIt-1 is able to neutralize the angiogenic functions of VEGF, and systemic inhibition would lead to generalized endothelial dysfunction because sFIt-1 is a key factor related to angiogenesis (Maynard et al., 2003). HS release from inflamed endothelium can modulate and promote some pathophysiological aspects evidenced in PE, such as the augment of neutrophil trafficking or availability of pro-inflammatory cytokines among others (Rashid et al., 2007; Goodall et al., 2014). These HS actions has not been explored in the context of inflammatory response in PE but some indirect evidences support a possible relationship between renal damage and the systemic pro-inflammatory state observed in PE.

Thus, previous reports described the histopathological finding of glomerular endotheliosis as pathognomonic of PE (Strevens et al., 2003). However, this pathological finding has been reported in women with various glomerular lesions absent from PE and in some pregnant women without complications (Strevens et al., 2003). Furthermore, an increase in proinflammatory cytokines, including TNF-α, is typical of endothelial dysfunction in pregnant women with PE (Luppi and Deloia, 2006; Rops et al., 2008; Bueno-Sanchez et al., 2013). In our laboratory, we have evaluated the effects of TNF-α, IL-1β, VEGF, and sFlt-1 in the release of heparan sulfate on a cell line of human glomerular endothelial Genc. Our fluorescence results by confocal microscopy show a decrease in the expression of 50% heparan sulfate ($P < 0.05$) after treatment with TNF-α (1, 5, and 25 ng/mL) for 24 h, largely explained by the increase in heparanase expression observed by Western blotting in a concentration dependent effect. These results were confirmed by the increase in glycosaminoglycans (800 mg/L ± 78) in culture supernatant compared to basal (400 mg/L ± 94) (Galvis-Ramirez, 2017). The enzymatic elimination of heparan sulfate induced by TNF-α contributes to the deterioration of the glycocalyx of the glomerular endothelium, which could partially explain the proteinuria observed in preeclampsia.

This inflammatory response scenario leads us to propose a hypothesis according to which PE occurs. The increase in pro-inflammatory cytokines leads to leukocyte activation and deposition in the glomerular endothelium. This triggers heparanase activity, thereby increasing glycocalyx excision, especially of HS, which would explain the loss of anionic sites in the GFB and, therefore, the associated proteinuria. Although mechanisms underlying the relation between renal disease and systemic endothelial cell dysfunction remain incompletely understood, a structural defect in the endothelial surface layer has been proposed as a mechanistic link between vascular dysfunction and albuminuric kidney disease. This approach could be common to all inflammatory diseases, as it is shown in **Table 1**, in which HS and heparanase are differentially expressed. Thus, inflammatory diseases whose etiology are different may promote an endothelial glycocalix dysfunction by several associated pathways and initiate albuminuria. More evidence needs to be provided in order to verify this hypothesis.

In the case of preeclampsia, this approach is based on the findings of Rops et al. (2007a), who assessed the glomerular endothelial expression of different HS domains in a mouse model of lupus nephritis and in biopsies from patients with this same condition. They observed a decrease in the *N*- and 6-*O*-sulfate domains in biopsies from patients and associated this decrease with albuminuria. A second study from the same research group assessed the adhesion of a cell line consisting of 32Dd3 granulocytes and monocytes to immortalized glomerular endothelial cells that were stimulated with two cytokines, TNF-α and IL-8. Their results showed that TNF-α activates the rolling of granulocytes and monocytes into endothelial cells, and this adhesion is mediated by HS and not by other proteoglycans. This was based on the evidence showing that the use of synthetic heparanase reduced the amount of adhesion under basal conditions, and this degraded HS was also found in the cell culture supernatant. A key observation was the increase in the relative expression of heparanase under stimuli with cytokines. However, the evaluation of the specific HS domains that may play a role in the adhesion process stands out the most among these results. Inhibition assays using antibodies directed against specific HS sequences revealed that the *N*- and 6-*O*-sulfate domains are crucial for leukocyte adhesion to the glomerular endothelium under dynamic flow conditions (Rops et al., 2008). These results and evidence reported in this review

support our hypothesis, and these phenomena identified under other conditions may be occurring in the inflammatory processes that are triggered in PE.

CONCLUSION

HS may play a key role in PE by interacting with different proteins through their sulfated domains. Furthermore, HS contributes to the GFB in a process known as selective permeability to the passage of albumin through the effects of the negatively charged glomerular endothelial glycocalyx. The HS-sulfated domains may contribute to this effect and, therefore, to the selective permeability of the GFB to albumin. Proinflammatory cytokines levels are increased in women with PE and systemic inflammation, which ultimately activates heparanase activity. Therefore, HS could be cleaved from the glycocalyx, which would partially explain the proteinuria observed in PE.

AUTHOR CONTRIBUTIONS

JQ-C, MG-R, and JB-S contributed to the conception, interpretation of data, and design of the manuscript. Additionally, all of them revised it critically for important intellectual content and provided approval for publication of the content. MG-R is a master student who looked for and selected the bibliography. JQ-C and JB-S designed the paper and proposed the hypothesis.

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Drug Transport at the Brain and Endothelial Dysfunction in Preeclampsia: Implications and Perspectives

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Transport of drugs across biological barriers has been a subject of study for decades. The discovery and characterization of proteins that confer the barrier properties of endothelia and epithelia, including tight junction proteins and membrane transporters belonging to the ATP-binding cassette (ABC) and Solute Carrier (SLC) families, represented a significant step forward into understanding the mechanisms that govern drug disposition. Subsequently, numerous studies, including both pre-clinical approaches and clinical investigations, have been carried out to determine the influence of physiological and pathological states on drug disposition. Importantly, there has been increasing interest in gaining a better understanding of drug disposition during pregnancy, since epidemiological and clinical studies have demonstrated that the use of medications by pregnant women is significant and this condition embodies a series of significant anatomical and physiological modifications, particularly at excretory organs and barrier sites (e.g., placenta, breast) expressing transporter proteins which influence pharmacokinetics. Currently, most of the research in this field has focused on the expression profiling of transporter proteins in trophoblasts and endothelial cells of the placenta, regulation of drug-resistance mechanisms in disease states and pharmacokinetic studies. However, little attention has been placed on the influence that the cerebrovascular dysfunction present in pregnancy-related disorders, such as preeclampsia, might exert on drug disposition in the mother's brain. This issue is particularly important since recent findings have demonstrated that preeclamptic women suffer from long-term alterations in the integrity of the blood-brain barrier (BBB). In this review we aim to analyze the available evidence regarding the influence of pregnancy on the expression of transporters and TJ proteins in brain endothelial cells, as well the mechanisms that govern the pathophysiological alterations in the BBB of women who experience preeclampsia. Future research efforts should be focused not

only on achieving a better understanding of the influence of preeclampsia-associated endothelial dysfunction on drug disposition, but also in optimizing the pharmacological treatments of women suffering pregnancy-related disorders, its comorbidities and to develop new therapies aiming to restore the integrity of the BBB.

Keywords: blood-brain barrier, ABC transporters, SLC transporters, tight junction proteins, endothelial dysfunction, preeclampsia, eclampsia, brain alterations

INTRODUCTION

Worldwide, there has been an increase in the number of prescribed and over-the-counter medications taken by pregnant women (Mitchell et al., 2011; Beyene and Beza, 2018; Navaro et al., 2018). In Latin America, the true extent of the use of therapeutic drugs among pregnant women is not well characterized, but in countries such as Uruguay, 96% of pregnant women take medications and 78% use two or more (Viroga et al., 2013). A prospective cohort study conducted in Brazilian, Argentinian and Peruvian populations showed that immunodeficiency virus (HIV)-infected pregnant women exhibit a better adherence to anti-HIV therapy when compared to post-partum (Kreitchmann et al., 2012). The above-mentioned statistics are significant since pregnancy is a physiological condition associated with anatomical and physiological modifications capable of influencing the disposition of drugs, including increased blood volume, enhanced basal metabolism, and modified hormone levels, among others.

Treatment of chronic diseases within pregnancy carries a risk for both the mother and fetus, as the administered drug could cross the placenta and reach the fetus circulation, with deleterious consequences (Jentink et al., 2010; Tomson et al., 2018). Pharmacokinetic studies carried out in animal models and human suggest that exposure to drugs is reduced in pregnancy since there is an increase in both the renal glomerular filtration rate (thereby increasing renal elimination) and hepatic metabolism mediated by isoforms of cytochrome P450 enzymes and uridine 5′-phosphate glucuronosyltransferases (Pariente et al., 2016; Koren and Pariente, 2018). Furthermore, the expression and activity of transporters involved in drug disposition appears to be modified in excretory organs (Hebert et al., 2008) in a similar fashion to that observed with metabolizing enzymes.

Pregnant women suffering from psychiatric disorders and other central nervous system (CNS) diseases often require pharmacotherapy to stabilize their symptoms, which are likely to continue after labor due to their chronic nature. Clinical studies of pregnant women receiving antiepileptic and antidepressive pharmacotherapy demonstrated that the reduced drug exposure, due to increased clearance, is associated with an increase in the seizure rate (Reisinger et al., 2013) and decreased plasma levels of serotonin reuptake inhibitors, respectively (Westin et al., 2017). These outcomes clearly demonstrate that pregnancy could have a negative impact on the clinical effect of these drugs.

Furthermore, women under pharmacological treatment for epilepsy (Borthen, 2015) or depression (Palmsten et al., 2012) have a higher risk of suffering complications derived from pathophysiological alterations associated with pregnancy, such as preeclampsia. This pathological condition, that is present in 2–8% of all pregnancies (Duley, 2009), is a disorder characterized by hypertension and proteinuria after the twentieth gestational week, which may evolve to vasogenic edema, eclampsia (seizures) and cerebrovascular stroke if not properly controlled (American College of Obstetricians and Gynecologists, Task Force on Hypertension in Pregnancy, 2013; Cipolla, 2013; Hammer and Cipolla, 2015). It is also reported that 75% of maternal deaths due to preeclampsia are related to cerebrovascular complications including eclampsia, intracranial hemorrhage, and edema (Zeeman, 2009).

Preeclampsia is associated with impaired systemic endothelial function (Roberts et al., 1989) and, in the brain of preeclamptic women, this endothelial dysfunction presents in the form of impaired integrity of the BBB (Bergman et al., 2018), which is apparently maintained even post-partum (Bergman et al., 2016). The influence of endothelial dysfunction on drug disposition in the brain has been studied in disease states, including stroke (Huang et al., 2017) however there is a lack of studies investigating the effect of endothelial dysfunction on brain drug disposition in pregnancy-related disorders. The latter issue is extremely important and needs to be addressed since preeclamptic women may need to receive medications to control symptoms within pregnancy, and/or at some point post-partum, particularly for treatment of chronic conditions, e.g., epilepsy, depression and HIV-infection. Furthermore, it is likely that women with brain endothelial dysfunction could experience increased exposure to the effects of endogenous factors and potentially harmful xenobiotics.

While the function of the BBB and transport of molecules across brain endothelial cells (BECs) has been extensively studied in non-pregnant populations, much less is known about BBB physiology during pregnancy and pregnancy-related disorders. Therefore, this review will summarize the findings related to the effect of pregnancy and preeclampsia on the expression and activity of proteins involved in the transport of drugs at the BBB.

OVERVIEW OF THE BLOOD-BRAIN BARRIER

The BBB (**Figure 1A**) is a highly restrictive and specialized neurovascular network comprised of BECs, a basal membrane composed on collagen, fibronectin and laminin, pericytes, neurons and glial cells (Abbott, 2013; Daneman and Prat, 2015). In essence, the BBB isolates the brain parenchyma from the systemic circulation, regulating the supply of nutrients,

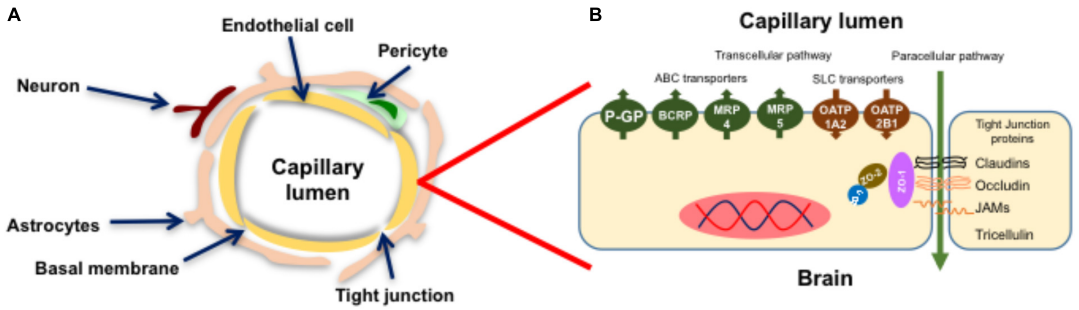

FIGURE 1 | Structure of the blood-brain barrier. The BBB is a neurovascular unit comprised of endothelial cells, a basal membrane, pericytes, astrocytes and neurons **(A)**. Brain endothelial cells (BECs) express ABC/SLC transporters and high levels of Tight Junction (TJ) proteins that confer the barrier properties of the BBB **(B)**. Figure adapted from Abbott et al. (2010) and O'Brien et al. (2012) with permission from their publishers.

controlling the bidirectional transport of endogenous mediators and protecting the CNS from exposure to harmful compounds, i.e., xenobiotics and metabolites.

Unlike other vascular beds, the endothelial cells of the BBB express a unique phenotype (**Figure 1B**) with higher levels of expression of tight junction (TJ) proteins, membrane transporters belonging to the ATP-binding cassette (ABC) and Solute Carrier families (SLC), and metabolizing enzymes (Decleves et al., 2011; Shawahna et al., 2011; Daneman and Prat, 2015; Liao et al., 2017).

Findings of *in vitro* and animal models have consistently demonstrated that the transport of molecules across the BBB is strongly regulated by membrane transporters and TJ proteins (Cecchelli et al., 1999; Cantrill et al., 2012; Helms et al., 2016). More recently, the use of imaging techniques and probes have allowed the *in vivo* analysis of transporter functionality, e.g., P-glycoprotein (P-GP) activity at the BBB in both healthy (Bauer et al., 2015) and disease states (Shin et al., 2016).

OVERVIEW OF TIGHT JUNCTION PROTEINS IN BRAIN ENDOTHELIAL CELLS

The paracellular transport of molecules at the BBB is highly selective, and this feature is associated with the expression of high levels of TJ proteins. These proteins act as a biological adhesive, anchoring together adjacent BECs via transmembrane proteins attached to intracellular scaffolding proteins (Haseloff et al., 2015).

The transmembrane proteins occludin, claudins and Junctional Adhesion Molecules (JAMs) form complex strands that interact between cells, reducing paracellular diffusion (Haseloff et al., 2015; Keaney and Campbell, 2015). However, in order to maintain this restrictiveness, TJs are linked to the cytoplasmic zonula occludens (ZO) proteins that provide a structural bridge to the actin cytoskeleton. In zones where there is contact between three BECs, the TJ protein tricellulin (MARVELD2) plays a pivotal role in modulating paracellular permeability by reducing the passage of large molecules (Reinhold and Rittner, 2017).

Since the discovery that the permeability of the BBB could be regulated through reversible disruption of TJs, this principle has served as an approach for the delivery of therapeutics that would not cross this barrier by conventional means, e.g., passive diffusion, carrier-mediated transport (Dithmer et al., 2017; Sol et al., 2017).

OVERVIEW OF TRANSPORTERS INVOLVED IN BRAIN DRUG DISPOSITION

Data from *in vitro* and animal models have helped establish which membrane transporters impact brain drug disposition. Furthermore, the International Transporter Consortium et al. (2010) has published and updated recommendations (Hillgren et al., 2013) for decision-making processes related to drug-transporter interactions that could be translated to clinical settings. In this regard, the ABC transporters P-glycoprotein (P-GP; Cordon-Cardo et al., 1989), Multidrug Resistance-associated Proteins (MRPs) MRP4 and MRP5 (Huai-Yun et al., 1998; Seetharaman et al., 1998), Breast Cancer Resistance Protein (BCRP; Eisenblatter and Galla, 2002; Eisenblatter et al., 2003), and the Organic Anion Transporting Polypeptides (OATPs) OATP1A2 and OATP2B1 SLC transporters (Roth et al., 2012), are considered the most clinically important transporters within the BBB. Although there is evidence (from pre-clinical models) that other SLC transporters expressed in the BBB, including members of the Monocarboxylate Transporter (MCT; Lee and Kang, 2016), Organic Anion Transporter (OAT) subfamilies (Hosoya and Tachikawa, 2011) are involved in the uptake of drugs, this review will exclusively focus on the transporter proteins expressed in human BECs. The characteristics of these protein families in human BECs are briefly summarized in **Table 1** and the following section.

ATP-Binding Cassette Transporters

P-glycoprotein is a 170 kDa efflux transporter encoded by the *ABCB1* gene in human and the *abcb1a/abcb1b* genes in rodent. This protein is located at the luminal side of BECs and is described as a phenotypical marker (Sugawara et al., 1990;

TABLE 1 | Human BEC ABC and SLC transporters involved in drug disposition.

Transporter	Gene	Molecular weight (kDa)	Detected at protein level	Localization and function	References
ABC					
P-GP	ABCB1	170	Yes	Luminal/Efflux	Dauchy et al., 2009; Shawahna et al., 2011; Uchida et al., 2011
BCRP	ABCG2	70 (Monomer)	Yes	Luminal/Efflux	Eisenblatter et al., 2003; Dauchy et al., 2009; Shawahna et al., 2011; Uchida et al., 2011
MRPs	ABCC4	170	Yes	Luminal/Efflux	Nies et al., 2004; Shawahna et al., 2011
	ABCC5	160	Yes		
SLC					
OATP1A2	SLC21A3	≈70	Yes	Luminal/Uptake	Lee et al., 2005; Roth et al., 2012
OATP2B1	SLC21A9	≈77	Yes	Luminal/Uptake	Bronger et al., 2005; Roth et al., 2012

Dauchy et al., 2008; Cantrill et al., 2012). P-GP exhibits a broad substrate specificity that includes anticancer drugs (Mealey and Fidel, 2015), antidepressant drugs (O'Brien et al., 2012), antiepileptic drugs (Stepien et al., 2012), cardiotonic drugs (Ledwitch et al., 2016), HIV protease inhibitors (Liu et al., 2017) and immunosuppressants (Picchianti-Diamanti et al., 2014) among others. This characteristic implies the transporter has a predominant role in regulation the disposition of xenobiotics, including therapeutic drugs, thereby acting as a mechanism of detoxification and drug resistance. Some studies have also proposed a role for P-GP in the transport of endogenous mediators including steroids, bilirubin (Cascorbi, 2011) and amyloid-β, the peptide responsible of the formation of amyloid plaques in Alzheimer's disease (Zhong et al., 2016).

The BCRP is an ABC transporter encoded by the *ABCG2* gene in human and *abcg2* gene in rodent, and is expressed at the luminal domain of BBB endothelial cells (Eisenblatter et al., 2003). BCRP is a monomeric protein (70 kDa) that requires the formation of at least a homodimer (and can even form homotetramers) to be functionally active (Ni et al., 2010). There is significant overlap in the substrate specificity of P-GP and BCRP and, like P-GP, BCRP can significantly influence drug transport in the body (Poguntke et al., 2010). In human BECs, BCRP is expressed at higher levels than P-GP (Shawahna et al., 2011; Uchida et al., 2011), but its overall contribution to the transport of substrates is less well understood than P-GP. As well as transporting drug substrates, BCRP also participates in transport of hormones (and conjugated metabolites) (Grube et al., 2018) and urate, a product of purine metabolism whose accumulation causes gout (Woodward et al., 2009; Fujita and Ichida, 2018).

The MRP transporters are encoded by the *ABCC* class of genes in human and *abcc* genes in rodents. MRPs mediate the transport of a diverse array of drugs and endogenous molecules including hormones, prostaglandins, leukotrienes and their conjugates (glucuronides, sulfates, and glutathione) (Zhou et al., 2008; Zhang et al., 2015; Bloise et al., 2016). Members of the MRP family are not as highly expressed in BECs as P-GP and BCRP in human BECs, but the findings of proteomic (Shawahna et al., 2011; Uchida et al., 2011) and transcriptomic (Warren et al., 2009) analyses have demonstrated that MRP4, an isoform located at the luminal side, is expressed at detectable levels in human BECs. Luminal expression of MRP5 in human BECs has also been confirmed by means of fluorescent immunohistochemistry (Nies et al., 2004).

Solute Carrier Transporters

The OATPs, a group of SLC transporters belonging to the SLCO subfamily, are ubiquitously expressed throughout the body, and at the human BBB, luminal expression of OATP1A2 (Lee et al., 2005) and OATP2B1 (Bronger et al., 2005), has been reported. OATPs mediate the uptake and efflux of endogenous and exogenous molecules, which tend to possess amphiphilic characteristics (Roth et al., 2012) and substrates include prostaglandins, steroid and thyroid hormone conjugates (Grube et al., 2018), bile acids and therapeutic drugs (Kalliokoski and Niemi, 2009; Roth et al., 2012).

EXPRESSION AND FUNCTIONALITY OF BLOOD-BRAIN BARRIER TIGHT JUNCTIONS PROTEINS AND DRUG TRANSPORTERS IN PREGNANCY

During normal pregnancy there is an increase in blood levels of several endogenous mediators including hormones and their metabolites, pro-inflammatory cytokines, chemokines, matrix metalloproteinases and growth factors (Chavan et al., 2017; Chen and Khalil, 2017). For example, clinical studies report that pro-inflammatory cytokines including interleukin-6 (IL-6) and markers of cyclooxygenase-2 activity were increased in healthy women, suggesting that pregnancy is characterized

by a mild, sub-clinical systemic inflammatory state (Palm et al., 2013; Danielsen et al., 2014). Under this physiological pro-inflammatory condition, expression and functionality of BBB TJ proteins and ABC/SLC transporters involved in drug disposition could be modified as is observed in other conditions (Keaney and Campbell, 2015; Qosa et al., 2015). Although little is known of the influence of pregnancy on the expression of BBB transporters and TJ proteins, recent studies are addressing this issue.

Tight Junction Proteins and Pregnancy

The influence of pregnancy on the integrity of TJs has primarily been studied in endothelial cells of the placenta (Marzioni et al., 2001; Ahn et al., 2015), and to date, we are not aware of any studies reporting the effects of a healthy pregnancy on the expression of TJ proteins in the maternal BBB. During normal pregnancy, high plasma levels of vascular endothelial growth factor (VEGF), an angiogenic mediator that increases BBB permeability through changes in the expression of TJ proteins (Lafuente et al., 2006) including claudin-5 and occludin (Argaw et al., 2012), have been reported (Evans et al., 1998). However, despite elevated plasma levels of VEGF, the permeability of the BBB remains unaltered in normal pregnancy (Cipolla, 2013). Indeed, studies have shown that the serum collected during late pregnancy attenuates the effects of VEGF on permeability of cerebral veins isolated from non-pregnant rats and rats in late-pregnancy (Schreurs et al., 2012). The authors attributed this outcome to the fact that, in late pregnancy, high plasma levels of soluble Fms-like tyrosine kinase 1 (sFlt1), a splice variant of the VEGF receptor (VEGFR) lacking activity, counteracts the effects of VEGF (Cipolla, 2013).

Membrane Transporters

Current evidence suggests that expression levels of ABC transporters within the maternal BBB vary throughout pregnancy, although this outcome has only been demonstrated in animal models. Studies report the expression of rodent P-gp and mrp1 in the BBB of pregnant mice is higher at mid-gestation and decreases in late-gestation (Coles et al., 2009), whilst positron emission tomography (PET) studies conducted on macaques (Chung et al., 2010) report that P-GP activity increased from mid-gestation to late-gestation, as evidenced by reduced accumulation of the radiolabelled P-GP substrate ^{11}C-verapamil. However, the latter study did not confirm if this effect was a result of increased P-GP expression. No studies to date have reported the influence of pregnancy on the expression of SLC transporters in the maternal BBB, although it has been demonstrated expression of rodent Oatp1a4 (a rodent isoform that shows a high homology with human OATP1A2) in the BBB of the newborn increases with maturation (Harati et al., 2013).

ATP-binding cassette and SLC transporters govern movement of a whole array of endogenous and exogenous molecules through endothelial cells of the BBB. Consequently, this transcellular passage of substances may be significantly affected by pregnancy-dependent changes in transporter expression. However, despite the potential implications of modification of the barrier properties of the maternal BBB, to date, the precise mechanisms by which pregnancy could influence the expression of the above transporters are unclear and are only relatively recently being investigated.

A recent study reported that acute exposure of isolated hippocampal rat brain capillaries to serum obtained from pregnant rats reduced P-GP activity (Johnson et al., 2018). The authors hypothesized that this inhibition of P-GP, mediated by high levels of circulating serum factors, was associated with the increased incidence of seizures in normal pregnant rats. However, this study did not identify the molecules responsible for reduced P-GP activity. Furthermore, the findings are in contrast to those reported in studies investigating the relationship between seizures and P-GP activity, which suggest glutamate-mediated induction of cyclooxygenase-2 activity is responsible for the up-regulation of P-GP expression and activity in BECs in animal models of epilepsy (Zibell et al., 2009; van Vliet et al., 2010) and in capillaries isolated from human brains (Avemary et al., 2013). These findings are particularly important since studies suggest that in normal pregnancies and in preeclampsia, the cerebral levels of glutamate are reduced when compared to non-pregnant women (Nelander et al., 2018).

The specific effects of endogenous factors on the expression and activity of ABC transporters during pregnancy have been studied more in-depth on the developing fetal BBB than in the maternal BBB. Studies have reported that primary cultures of guinea pig BECs, obtained from late-gestational fetuses and postnatal pups, are highly responsive to the effects of glucocorticoids and pro-inflammatory cytokines, with hydrocortisone and dexamethasone (Iqbal et al., 2011) increasing P-gp activity and IL-1β, IL-6 and TNFα (Iqbal et al., 2012) decreasing P-gp activity. However, despite the opposing effects exerted by glucocorticoids and cytokines on P-gp expression and activity, a later report demonstrated that co-treatment with the synthetic glucocorticoid dexamethasone, apart from increasing the expression of the transporter, enhanced the inhibitory actions of IL-1β, IL-6, and TNFα on P-GP activity (Iqbal et al., 2016). The authors suggested that this enhancement of cytokine inhibitory actions is the result of a dexamethasone-mediated increase in the expression of pro-inflammatory cytokines receptors.

Transforming Growth Factor β (TGFβ), a protein found at high levels in plasma during pregnancy (Forbes and Westwood, 2010), has also been reported to regulate the expression of P-GP in BECs, and Baello et al. (2016), have reported TGFβ-mediated up-regulation of P-GP expression and activity, through activation of the ALK1 and ALK5 signaling pathways, in BECs isolated from male fetuses and postnatal guinea pig pups.

ENDOTHELIAL DYSFUNCTION AT THE BRAIN IN PREGNANCY-RELATED DISORDERS

Pregnancy alone can be considered as an inflammatory (but not pathological) state. One hallmark of pregnancy-related disorders, including preeclampsia, is the manifestation of endothelial dysfunction promoted by high levels of factors released from the placenta (Escudero et al., 2009; Myatt and Roberts, 2015).

The brain vasogenic edema present in later stages of preeclampsia is apparently the result of impaired autoregulation of cerebral blood flow and increased BBB permeability (Cipolla and Kraig, 2011; Cipolla, 2013; Hammer and Cipolla, 2015), but the pathophysiological mechanisms involved are still unclear. *In vitro* studies have demonstrated that when rat cerebral vasculature was exposed to plasma from normal and preeclamptic human pregnancies, there was an increase in BBB permeability. Interestingly, this effect was more marked following treatment with preeclamptic plasma (Amburgey et al., 2010). Furthermore, this study demonstrated that inhibition of VEGF receptor tyrosine kinase activity reversed the effect elicited by the treatment with preeclamptic plasma, suggesting that VEGF could be involved in modulating vascular permeability.

The pro-inflammatory cytokine TNFα is also believed to contribute to the increased BBB permeability in preeclampsia. TNFα infusion in healthy pregnant rats at gestational day 19 increased the water content in the anterior cerebrum without increasing the BBB permeability (Warrington et al., 2015). However, when pregnant rats were subjected to a reduction of

TABLE 2 | Drugs employed for treatment of chronic diseases in pregnancy and preeclampsia as substrates/inhibitors of ABC/SLC transporters expressed in human brain endothelial cells.

Drug	ABC Transporter		SLC Transporter		Reference
	Substrate	Inhibitor	Substrate	Inhibitor	
Antidepressants					
Citalopram		P-GP			Weiss et al., 2003a; O'Brien et al., 2012
Fluoxetine		P-GP			Weiss et al., 2003a; O'Brien et al., 2013
Paroxetine	P-GP	P-GP			Weiss et al., 2003a; O'Brien et al., 2012
Antiepileptics					
Carbamazepine	P-GP	P-GP			Weiss et al., 2003b; Zhang et al., 2012
Lamotrigine	P-GP, BCRP	P-GP			Weiss et al., 2003b; Luna-Tortos et al., 2008; Romermann et al., 2015
Oxcarbazepine	P-GP				Weiss et al., 2003b; Zhang et al., 2011; Antunes Nde et al., 2016
Phenobarbital	P-GP				Luna-Tortos et al., 2008
Antihypertensives					
Labetalol	P-GP				Incecayir et al., 2013
Nicardipine	P-GP				Kadono et al., 2010
Nifedipine	P-GP				Choi et al., 2013
Antirretroviral drugs					
Abacavir	P-GP, BCRP	P-GP, BCRP			Pan et al., 2007; Storch et al., 2007
Atazanavir	P-GP	P-GP, BCRP		OATP2B1	Perloff et al., 2005; Storch et al., 2007; Weiss et al., 2007; Fujimoto et al., 2009
Darunavir	P-GP	P-GP	OATP1A2	OATP2B1	Annaert et al., 2010; Hartkoorn et al., 2010; Kis et al., 2010
Efavirenz	BCRP	P-GP, BCRP		OATP2B1	Storch et al., 2007; Weiss et al., 2007; Kis et al., 2010
Indinavir	P-GP			OATP1A2	Lee et al., 1998; Van Der Sandt et al., 2001; Campbell et al., 2015
Lopinavir	P-GP	P-GP, BCRP	OATP1A2		Lee et al., 1998; Janneh et al., 2007; Weiss et al., 2007; Hartkoorn et al., 2010
Nelfinavir	P-GP	P-GP, BCRP		OATP2B1	Kim et al., 1998; Gupta et al., 2004; Storch et al., 2007; Weiss et al., 2007; Kis et al., 2010
Nevirapine		P-GP, BCRP			Storch et al., 2007; Weiss et al., 2007
Raltregavir	P-GP, BCRP				Hashiguchi et al., 2013
Ritonavir	P-GP	P-GP, BCRP		OATP1A2, OATP2B1	Van Der Sandt et al., 2001; Gupta et al., 2004; Weiss et al., 2007; Hartkoorn et al., 2010
Saquinavir	P-GP	P-GP, BCRP	OATP1A2	OATP1A2, OATP2B1	Kim et al., 1998; Lee et al., 1998; Gupta et al., 2004; Janneh et al., 2005; Weiss et al., 2007; Hartkoorn et al., 2010; Kis et al., 2010
Zidovudine	P-GP, BCRP, MRP4	BCRP			Schuetz et al., 1999; Wang et al., 2003; Pan et al., 2007

uterine perfusion pressure (RUPP), a model of placental ischemia that emulates preeclampsia and impairs the maternal cerebral blood flow, they exhibited an increase in both the water content at the anterior cerebrum and BBB permeability, which was counteracted by treatment with the TNFα inhibitor etanercept.

Findings from other studies conducted in the RUPP model have helped to elucidate how BECs respond to the circulating factors present in preeclampsia. When RUPP was performed in pregnant rats at gestational day 14, edema and increased maternal BBB permeability in the anterior cerebrum were observed, with increased expression of the protein aquaporin 4 and no changes in the expression of TJ proteins (Warrington et al., 2014). However, it has been reported the same procedure led to post-partum edema and increased maternal BBB permeability in the posterior cortex, probably due to reduced expression of the TJ protein occludin (Clayton et al., 2018).

The increased BBB permeability reported by both Warrington et al. (2014) and Clayton et al. (2018), is partly supported by clinical studies which demonstrated that in women developing preeclampsia, blood levels of S100B, neuronal specific enolase (NSE) and neurofilament light chain (NfL), three markers of cerebral injury, were higher than those observed in women with normal pregnancies (Bergman et al., 2018). Indeed, another report showed that in preeclamptic women, the levels of S100B and NSE were still high 1-year post-partum, suggesting that the alterations in the integrity of the BBB are manifest for a substantial period of time following delivery (Bergman et al., 2016).

PHARMACOLOGICAL MANAGEMENT OF PREECLAMPSIA

Hypertension is one of the pathological features of preeclampsia and is routinely managed with the use of antihypertensive drugs including nifedipine, nicardipine, labetalol, hydralazine and methyldopa (Odigboegwu et al., 2018). Although it remains unclear whether the BBB permeability of these drugs is affected during preeclampsia, it is noteworthy that several antihypertensives, namely labetalol, nicardipine and nifedipine, are substrates of efflux transporters including P-GP (Thiel-Demby et al., 2009; Choi et al., 2013; Incecayir et al., 2013). The therapeutic management of neurological complications associated with preeclampsia, including seizures, primarily relies on the intravenous or intramuscular administration of magnesium sulfate, a drug with demonstrated ability to prevent the development of eclampsia and reduce maternal mortality (Altman et al., 2002).

The mechanism of action by which magnesium sulfate exerts its effects is unknown, but despite this limitation, it is widely considered a neuroprotective agent capable of reducing BBB permeability in animal models of brain injury (Li et al., 2017). Furthermore, a recent study demonstrated that magnesium sulfate reduced the water content in the anterior cerebrum, as well protein, cytokine, chemokine and VEGF levels in cerebrospinal fluid of rats subjected to the RUPP procedure (Zhang and Warrington, 2016). The above findings are important since the transport of drugs and endogenous mediators across the choroid plexus, which constitutes the blood-cerebrospinal fluid barrier, is a subject that is receiving increasing interest. A better characterization of the mechanisms that govern transport of molecules across this barrier will certainly help to understand brain drug disposition in both healthy and disease states such as preeclampsia.

To date, as the specific effects of magnesium sulfate on the expression/functionality of BBB TJ proteins and drug transporters are unknown, future studies addressing this subject will prove crucial in identifying potential therapeutic targets and in developing treatment strategies.

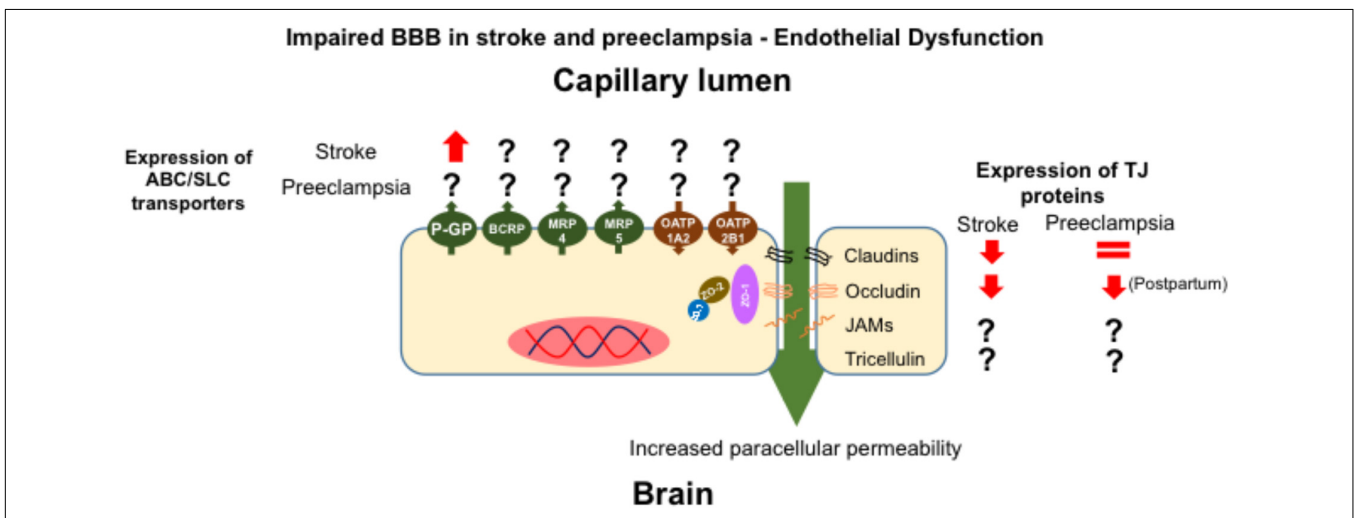

FIGURE 2 | Effect of the endothelial dysfunction elicited by preeclampsia and stroke on the expression of transporters and TJ proteins. In both stroke and preeclampsia there is a decrease in the expression of TJ proteins, but in preeclampsia, this change appears to be evident post-partum. In stroke, endothelial dysfunction increases the expression of P-glycoprotein.

HOW BLOOD-BRAIN BARRIER ENDOTHELIAL DYSFUNCTION COULD ALTER BRAIN DRUG DISPOSITION IN PREECLAMPSIA

The effect of endothelial dysfunction elicited by preeclampsia on brain drug disposition is unknown. A preeclampsia-mediated increase in BBB permeability could potentially result in increased permeation of endogenous, blood-borne substances, including placental derived sFlt-1, and hormones, into the brain. Furthermore, a less restrictive BBB could allow more extensive penetration of therapeutic drugs into the CNS, resulting in increased side effects. In preeclamptic women, in addition to hypertension, which is treated with antihypertensive P-GP drug substrates, comorbidities, including epilepsy, depression and HIV infection, are often reported (Pariente et al., 2016). Since there is evidence that drugs belonging to these pharmacological groups are substrates and/or of ABC/SLC transporters (O'Brien et al., 2012; Stepien et al., 2012; Alam et al., 2016; Han et al., 2017), there is an obvious need to better understand the effect of preeclampsia on BBB transporter physiology and the effects of transporter modifications on brain drug disposition. A list of drugs used for treatment of chronic diseases in pregnancy and preeclampsia is presented in **Table 2**.

Although the precise mechanisms, and effects, of alterations in BBB permeability associated with preeclampsia have not yet been elucidated, it is possible to gain an insight into the potential consequences of BBB modification from clinical situations in which the expression of BBB drug transporters and BBB integrity are altered. In this regard, ischemic and hemorrhagic stroke are life-threatening conditions whose outcomes include severe BBB disruption (Knowland et al., 2014; Keep et al., 2018). In rodent models of ischemic stroke, based on middle cerebral artery occlusion (MCAO), an increase in brain water content and a decrease in the expression of TJ proteins, including occludin and claudin-5, have been observed (Huang et al., 2017). In functional terms, the cited reports demonstrated an increase in BBB permeability, i.e., increased brain levels of drugs transported through the paracellular pathway. Interestingly, MCAO also resulted in a time dependent up-regulation of P-GP expression (Cen et al., 2013; DeMars et al., 2017), which may serve as a compensatory protective mechanism, especially for drugs that are substrates of this transporter.

Pathological similarities are observed in both ischemic stroke and preeclampsia, including neuroinflammation, vasogenic edema and increased BBB permeability (**Figure 2**). To date, there is a lack of studies addressing the effect of preeclampsia on BBB physiology and, in particular, the effects of this disorder on TJ complexes, which govern paracellular permeability, and on ABC/SLC transporters, which regulate transcellular permeability. However, there is potential to monitor ABC transporter functionality, particularly P-GP activity, in women who have a history of preeclamptic pregnancies using non-invasive PET studies employing ^{11}C-verapamil as tracer (Shin et al., 2016). Future studies could employ this technique in women who had suffered preeclampsia or eclampsia, in order to measure P-GP activity and investigate whether there is a correlation between transporter activity and propensity of seizures.

CONCLUDING REMARKS

The BBB is a highly restrictive but dynamic system that regulates the transport of ions and molecules into and out of the brain. Consequently, alterations in its function could result in an increased CNS exposure to potentially toxic xenobiotics, including therapeutic drugs, and endogenous factors. Given the findings that preeclampsia is associated with increased BBB permeability, there is an urgent and fundamental need to characterize the functionality of BBB ABC and SLC transporter proteins involved in CNS drug disposition through the use of appropriate pre-clinical models and execution of clinical studies.

A better understanding of preeclampsia-associated changes in BBB physiology would not only allow characterization of the processes responsible for pathophysiological changes, but could help improve the therapeutic management of women experiencing, or those who had experienced, preeclamptic pregnancies. Indeed, this knowledge will help to reduce the risk of acute and chronic complications caused by alterations in BBB function elicited by preeclampsia.

AUTHOR CONTRIBUTIONS

PT-V designed and wrote the manuscript. CE and JP contributed to the writing of the manuscript and provided a critical revision of its contents.

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Are the Cognitive Alterations Present in Children Born From Preeclamptic Pregnancies the Result of Impaired Angiogenesis? Focus on the Potential Role of the VEGF Family

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Lara E, Acurio J, Leon J, Penny J, Torres-Vergara P and Escudero C (2018) Are the Cognitive Alterations Present in Children Born From Preeclamptic Pregnancies the Result of Impaired Angiogenesis? Focus on the Potential Role of the VEGF Family. Front. Physiol. 9:1591. doi: 10.3389/fphys.2018.01591

Evidence from clinical studies has proposed that children born from preeclamptic women have a higher risk of suffering neurological, psychological, or behavioral alterations. However, to date, the mechanisms behind these outcomes are poorly understood. Here, we speculate that the neurodevelopmental alterations in the children of preeclamptic pregnancies result from impaired angiogenesis. The pro-angiogenic factors vascular endothelial growth factor (VEGF) and placental growth factor (PlGF) are key regulators of both vascular and neurological development, and it has been widely demonstrated that umbilical blood of preeclamptic pregnancies contains high levels of soluble VEGF receptor type 1 (sFlt-1), a decoy receptor of VEGF. As a consequence, this anti-angiogenic state could lead to long-lasting neurological outcomes. In this non-systematic review, we propose that alterations in the circulating concentrations of VEGF, PlGF, and sFlt-1 in preeclamptic pregnancies will affect both fetal cerebrovascular function and neurodevelopment, which in turn may cause cognitive alterations in post-natal life.

Keywords: preeclampsia, angiogenesis, neurovascular, neurocognitive, vascular endothelial growth factor, placental growth factor, sFlt-1

INTRODUCTION

Preeclampsia is a multisystemic syndrome of unknown etiology that affects pregnant women after 20 weeks of gestation. The clinical presentation of preeclampsia is characterized by hypertension with co-morbidities including proteinuria or alterations in coagulation and liver function, thrombocytopenia, pulmonary edema, and brain or visual impairments (ACOG, 2013). The global incidence of preeclampsia ranges from 2 to 8% of all pregnancies, with the condition being the leading cause of maternal morbidity and mortality, premature birth and perinatal death in both developed and developing countries (Duley, 2009). Furthermore, 30% of newborns to

preeclamptic mothers experience some form of adverse perinatal outcome, including prematurity and intrauterine growth retardation among others (Villar et al., 2006). Although the remaining percentage (∼70%) of newborns may be considered healthy, evidence suggests that children born to preeclampsia have a higher risk of suffering cardiovascular-related diseases compared to children born to normal pregnancies (Davis et al., 2012a).

The etiology of preeclampsia is not well known, but the most accepted hypothesis targets the placenta as a primary source of endogenous factors that, upon release to the systemic circulation, impair the functionality of endothelial cells. Previous reports have proposed that anti-angiogenic factors, including the soluble fms-1-like tyrosine kinase-1, (sFlt-1 or sVEGFR-1) and soluble endoglin (sENG) are responsible for the endothelial dysfunction present in preeclampsia (see details in Roberts and Escudero, 2012).

Furthermore, multiple studies have shown alterations in the balance of pro-angiogenic and anti-angiogenic factors in the placenta at term, in the fetal-placental circulation, and in the circulation of preeclamptic women and their children years after the preeclamptic incident (Maynard and Karumanchi, 2011; Rana et al., 2014; Karumanchi, 2016).

In addition to imbalances in pro and anti-angiogenic processes, endothelial dysfunction is a well-recognized feature in the offspring born to preeclampsia, the consequences of which include cardiovascular disease in adulthood (Davis et al., 2012a,b). In this manuscript, we extend this concept to propose that impaired angiogenesis affects the cognitive development of children born from preeclamptic women.

EVIDENCE OF COGNITIVE ALTERATIONS IN THE OFFSPRING BORN TO PREECLAMPTIC PREGNANCIES

The pathophysiology of pregnancy-related disorders such as preeclampsia is associated with impaired placental function that compromises the interaction between mother and fetus (Barker, 1995; Krause et al., 2009; Escudero et al., 2014b; Thornburg, 2015). These alterations in the mother-fetus interaction apparently generate adaptive changes in the functionality of the endocrine, metabolic, vascular and immune systems of the fetus, with consequences in post-natal life. In this regard, several reports have proposed that the offspring of preeclamptic pregnancies have a higher risk of developing metabolic, neurological and cardiovascular disorders in adulthood (Tenhola et al., 2003; Wu et al., 2008, 2009, 2011; Robinson et al., 2009; Jayet et al., 2010; Davis et al., 2012a; Tuovinen et al., 2012; Escudero et al., 2014b; Alsnes et al., 2016; Pinheiro et al., 2016; Ratsep et al., 2016a,b; Figueiro-Filho et al., 2017a).

At a cognitive level, several studies have described that children born to preeclamptic women exhibit an increased risk of developing cerebral palsy (Szymonowicz and Yu, 1987), cerebral stroke (Kajantie et al., 2009), impaired neurological development (Spinillo et al., 1994), developmental delays at the age of 5 years (Warshafsky et al., 2016), poor cognitive development (Cheng et al., 2004), intellectual disability (Griffith et al., 2011), anxiety (Tuovinen et al., 2012), depressive symptoms (Tuovinen et al., 2010), attention deficit disorder and hyperactivity (Getahun et al., 2013), and other mental disorders (Tuovinen et al., 2014) in comparison with children born to normotensive women. In agreement with this body of evidence, a recent systematic review concluded that preeclampsia is associated with neurocognitive alterations in children (Figueiro-Filho et al., 2017b).

BRAIN ALTERATIONS IN OFFSPRING BORN TO PREECLAMPSIA

Pre-clinical studies have attempted to establish an explanation for the variety of pathoneurological consequences associated with preeclampsia. Studies report that the brain weight at birth of the offspring born to hypertensive pregnant rats was significantly lower compared to the offspring born to their normotensive counterparts (Liu et al., 2016). Furthermore, reduced brain weight was associated with reduced expression of markers of neurogenesis, markers of neuroproliferation in the cortex and with severe impairments in spatial learning and memory.

In the clinical setting, Dr. Ana Croy's group have been conducting pioneering studies which attempt to correlate cognitive parameters with brain anatomy (Ratsep et al., 2016a,c) or neuronal networking (Mak et al., 2018). The findings of these magnetic resonance imaging (MRI) studies, in 10 children born to preeclampsia and 10 children born to normotensive pregnancies, demonstrated that at least five brain regions, including the cerebellum, temporal lobe, brainstem, and right and left amygdala, were larger in children born to preeclampsia compared with controls (Ratsep et al., 2016c). The study also demonstrated cerebral blood flow in both parietal and occipital lobes in children born to preeclamptic women was lower than in controls. The authors proposed that changes in brain vasculature may have preceded the structural alterations. Consistent with these findings, the MRI studies of Figueiro-Filho et al. (2017a) reported increased volumes of the tract for the superior longitudinal fasciculus and the caudate nucleus, amongst other alterations, in the brains of children born to preeclamptic pregnancies.

More recently, a study suggested that children born from preeclamptic pregnancies demonstrated higher levels of connectivity between the left amygdala and bilateral frontal pole; the right amygdala and the left frontal pole, and between the medial prefrontal cortex and precuneus, compared to matched-control children (Mak et al., 2018). Furthermore, this study also found that children born from preeclamptic pregnancies exhibit decreased connectivity between the medial prefrontal cortex and the left occipital fusiform gyrus.

The body of findings available therefore provides strong, albeit preliminary, evidence for the hypothesis that the neurological and cognitive impairments present in children delivered from preeclamptic pregnancies are likely the result of neurodevelopmental alterations. Furthermore, complementary to this hypothesis is the fact that impaired vascular development

in the fetal brain could be a contributing factor to preeclampsia-associated impairments.

Crucially, a more in-depth understanding of (i) the potential confounding factors which impact fetal development, such as altered placental functionality (hypoxia), (ii) subject-specific data, including the weight of the newborn, gestational age at birth and the socioeconomic and nutritional context in which the newborn develops, and (iii) the physiological and morphological modifications in the cerebral vasculature of children from preeclamptic women, will be key to strengthening this hypothesis.

EVIDENCE OF ALTERATIONS IN THE VASCULATURE OF CHILDREN BORN TO PREECLAMPTIC WOMEN

Angiogenesis, defined as the formation of new vessels from an existing vessel, is widespread during fetal and neonatal development, although it is rarely observed in adults under normal conditions (Carmeliet and Ruiz de Almodovar, 2013; Watson et al., 2016, 2017). Although several processes drive vascular development, angiogenesis is one of the most studied in the context of preeclampsia. Conversely, vascular remodeling or "vascular pruning," the process by which pro-angiogenic signaling is suppressed, resulting in accelerated apoptosis of endothelial cells (Watson et al., 2017) and organized loss of blood vessels, is also observed in the fetus. Consequently, angiogenesis and vascular remodeling are complementary processes, the relative extents of which can influence vascular development not only in the fetuses of normotensive and preeclamptic pregnancies, but can impact vascular architecture in neonates, and in individuals in later life.

Although studies have investigated the characteristics of accessible vascular networks in individuals born to preeclamptic pregnancies, no studies report on how preeclampsia influences the anatomy and physiology of the blood brain barrier in neonates, children, and adults.

Our research group (Escudero et al., 2013, 2014a; Acurio et al., 2014, 2016) and others (Staff et al., 2005; Tsao et al., 2005; Stark et al., 2009; Antonios et al., 2012; Touwslager et al., 2012; Yu et al., 2016) have studied how angiogenesis may be impaired in the children born to preeclamptic pregnancies. Interestingly, it has been shown that full-term babies of hypertensive pregnancies (including preeclampsia) exhibited a reduction in the maximum capillary density per square millimeter on the plantar surface of the big toe compared with control infants (Antonios et al., 2012).

The above results are in agreement with previous findings (Stark et al., 2009; Touwslager et al., 2012) that demonstrated structural and functional alterations in the microvasculature of newborns and increased vascular remodeling in the skin of 3-month old babies born to hypertensive pregnancies (Yu et al., 2016). Also, it is reported (Yu et al., 2012) that children born to preeclampsia exhibited a 45% reduction in the risk of prematurity-associated retinopathy, a condition characterized by increased retinal angiogenesis. Furthermore, studies report the retinal arteriolar caliber, but not the retinal venular caliber, was narrower in school-age children from hypertensive pregnancies, including preeclamptic pregnancies, than in children born from normotensive pregnancies (Yesil et al., 2016). Since prematurity-associated retinopathy is a condition characterized by an increase in the formation of blood vessels in the retina, differences in retinal arteriolar caliber are suggestive of differences in vascular remodeling in the two cohorts.

However, as far as we are aware, although Ratsep et al. (2016c) demonstrated alterations in blood flow to the parietal and occipital lobes in the offspring of preeclamptic pregnancies, the effects of preeclampsia on blood brain barrier anatomy and physiology in neonates, children, and adults are yet to be established. In this manuscript we propose that cerebral angiogenesis is altered in children born to mothers with preeclampsia, thereby initially influencing the early neonatal stage and subsequently throughout life (**Figure 1**).

Since preeclampsia-associated alterations in vascular networks (Stark et al., 2009; Antonios et al., 2012; Touwslager et al., 2012; Yesil et al., 2016; Yu et al., 2016), neurocognition (Wu et al., 2009; Dang et al., 2016; Ratsep et al., 2016a) and neuroanatomy (Ratsep et al., 2016a,b,c; Figueiro-Filho et al., 2017a; Mak et al., 2018) have been reported, there is an obvious need for an improved understanding of the molecular mechanisms underpinning such physiological changes. Vascular endothelial growth factor (VEGF), placental growth factor (PlGF) and soluble fms-like tyrosine kinase (sFlt-1) play key roles in angiogenesis and neurogenesis (Ruiz de Almodovar et al., 2009; Carmeliet and Ruiz de Almodovar, 2013) and have therefore been extensively studied in preeclampsia. These important biomarkers are discussed in more detail in the next section.

VEGF AND ANGIOGENESIS AND NEUROGENESIS

The VEGF family of proteins is one of the key regulators of both angiogenesis and neurogenesis (Carmeliet and Ruiz de Almodovar, 2013). This family is comprised of five members including VEGF-A, VEGF-B, VEGF-C, VEGF-D, and PlGF, which activate vascular endothelial growth factor receptors (VEGFRs), namely VEGFR-1, VEGFR-2, and VEGFR-3, a group of membrane receptors possessing tyrosine kinase activity. Once activated these receptors mediate several processes including cell migration, proliferation and survival (Shibuya, 2013). In addition, VEGFRs are involved in development of the morphological features of blood vessels and regulate vascular permeability (Olsson et al., 2006). The activity of VEGF and PlGF is regulated by sFlt-1, a circulating variant of VEGRF-1 that prevents their binding to their membrane receptors, thus decreasing their activity (Ahmad et al., 2011). Deletion of VEGF (Carmeliet et al., 1996), VEGFR1 (Fong et al., 1995) or VEGFR2 (Shalaby et al., 1995), or even tyrosine residues (Y1175) responsible for VEGFR2 activation (Sakurai et al., 2005) causes severe failure in angiogenesis, and are embryonic lethal in mice. Furthermore, mice deficient in VEGFR1 die at the embryonic stage due to hypertrophy and disorganization of blood vessels (Fong et al., 1995), rather than a lack of vessel formation.

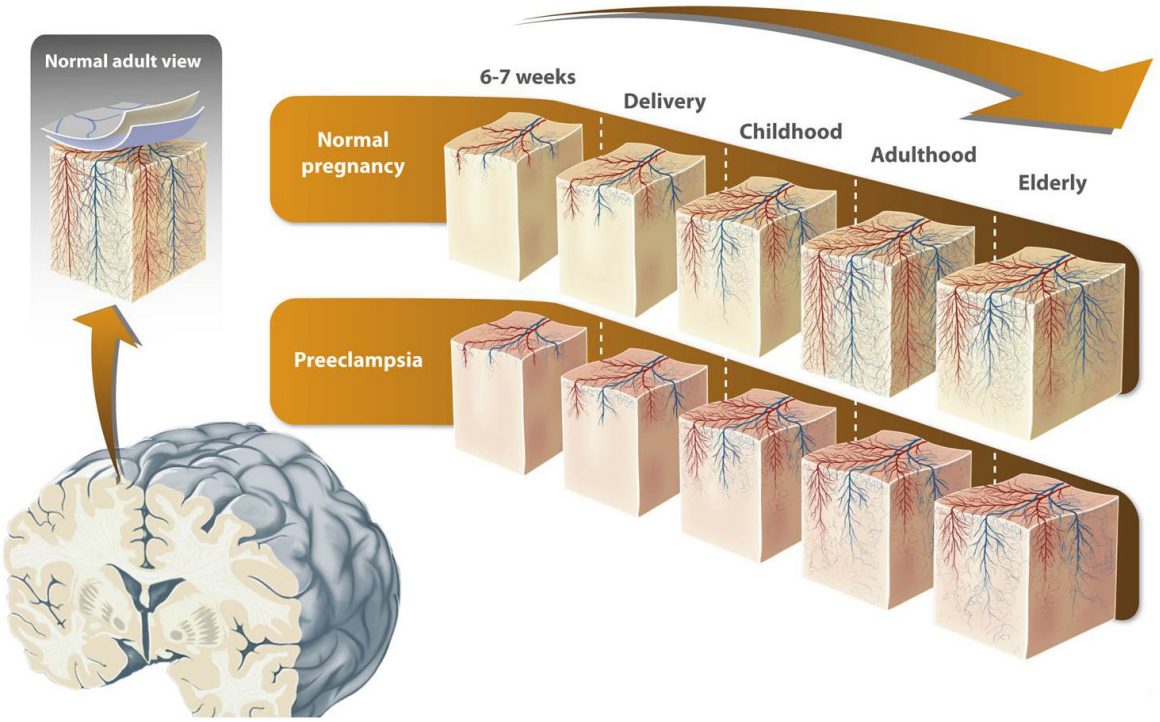

FIGURE 1 | Proposed model of alterations in brain vascular development in offspring born to preeclampsia. Vascular system components in the cerebral cortex emerge directly or indirectly from the pial capillary anastomotic plexus located in the pial lamella (the inner meningeal compartment). The microvascular compartment in the brain cortex is a key component not only for the blood brain barrier but also for cortex development itself. The process of microvasculature formation in the human brain cortex, in which both new vessel formation (angiogenesis) and vascular remodeling takes place, is highly dynamic and not totally understood. Vessel formation in the brain cortex starts during fetal development and continues in the post-natal stage. It is proposed that in the elderly, cortical vessel formation decreases and/or vascular remodeling increases. Since preeclampsia is characterized by an imbalance in pro and anti-angiogenic markers, it has been speculated that cerebral angiogenesis is altered in children born to mothers with preeclampsia. This alteration might be present throughout life, and may explain the greater risk of cognitive alterations in these individuals. The figure is reproduced with permission from the copyright holder.

Several reports have demonstrated that in preeclampsia there is an increase in circulating levels of placental derived sFlt-1 (and other anti-angiogenic molecules such as sENG), thereby generating a state of systemic vascular dysfunction in the mother (see details in Karumanchi, 2018).

VEGF-mediated activation of VEGFR2 leads to cell migration, proliferation and angiogenesis (Koch and Claesson-Welsh, 2012; **Figure 2A**), and activation is mediated by phosphorylation of the tyrosine 951 (Y951) and tyrosine 1175 (Y1175) residues. Consequently, alterations in angiogenesis may well be a multi-factorial process, influenced not only by circulating levels of ligand and levels of receptor expression, but by actual kinase-mediated phosphorylation of the receptor itself. Few publications have described activation of VEGFR2 (i.e., tyrosine phosphorylation) in preeclampsia, the findings of which are conflicting (Ahmad and Ahmed, 2004; Escudero et al., 2014a).

Activation of VEGFRs has also been linked with neural development (Ruiz de Almodovar et al., 2009; Carmeliet and Ruiz de Almodovar, 2013) and *in vitro* and animal studies (see details in Carmeliet and Ruiz de Almodovar, 2013) report VEGF and PlGF stimulate multiple processes, including neurogenesis, enhanced neuronal survival, axonal growth, and migration and proliferation of glial cells.

Therefore, VEGFRs are excellent candidates to study to further understand the mechanism(s) of angiogenesis impairment and neural development in children born to preeclamptic pregnancies.

VEGF FAMILY AND PREECLAMPSIA

Preeclamptic women exhibit high plasma levels of sFlt-1 (Levine et al., 2004), and circulating levels of anti-angiogenic sFlt-1 in the fetoplacental circulation and children born to preeclamptic mothers are summarized in **Table 1**. Since the discovery of elevated sFlt-1 plasma levels in preeclamptic women, several studies have attempted to characterize the role of sFlt-1, PlGF, and VEGF in the pathophysiology of the condition (see details in Karumanchi, 2016, 2018), and the roles of these factors in preeclampsia are discussed below.

VEGF, sFlt-1 and Angiogenesis in the Offspring of Preeclamptic Pregnancies

Elevated levels of sFlt-1 have been reported in blood collected from umbilical cord (Staff et al., 2005; Tsao et al., 2005) and in adults (between 20 and 30 years old) originally born

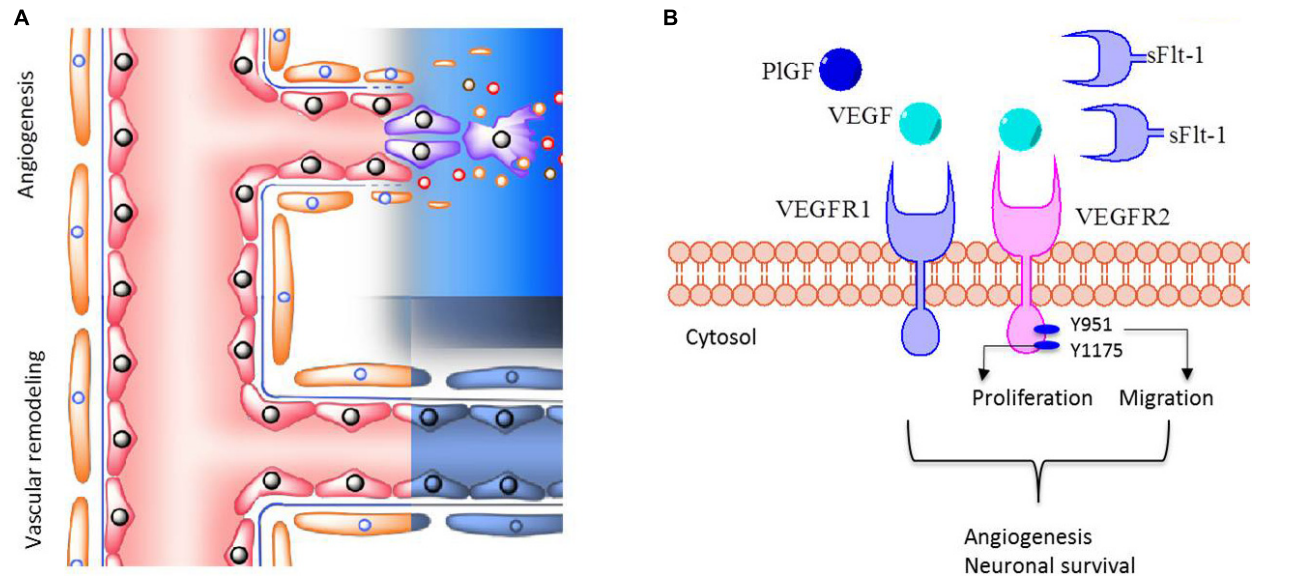

FIGURE 2 | VEGFR1 and VEGFR2 in brain vessel formation. **(A)** The extent of perfusion of brain tissue depends on both angiogenesis and vascular remodeling processes, which are mediated by pro- and anti-angiogenic factors. **(B)** VEGF and PlGF activate VEGFR1 and VEGFR2. Phosphorylation of tyrosine 951 (Y951) or Y1175 is associated with activation of VEGFR2, leading preferentially to cell migration or cell proliferation, respectively. These regulatory mechanisms are involved in angiogenesis and neuronal survival. In contrast, sFlt-1 prevents the binding of VEGF and PlGF to VEGFRs, thus decreasing their pro-angiogenic and neuronal survival activity. In this manuscript it is postulated that children born to preeclampsia exhibit a reduced number of blood vessels in the brain cortex, through impaired angiogenesis and/or increased vascular remodeling. We also propose that the underling mechanism might involve dysregulation in VEGFR2 activation due to high sFlt-1 circulating levels in preeclampsia.

TABLE 1 | Summary of findings of angiogenic factors in children born to preeclampsia in comparison with matched normotensive controls.

Age	Type of study	Sample size	Type of sample	Findings in preeclampsia	Reference
23–36 weeks of gestation	Cohort	123	Umbilical cord blood	VEGF was positively correlated, and sFlt-1 was negatively correlated, with birth weight and percentiles of weight for gestational age. Higher cord blood VEGF levels were associated with reduced risk of postnatal growth failure. The above biomarker associations were attenuated after adjustment for maternal preeclampsia.	(Voller et al., 2014)
30 weeks of gestation	Prospective	4108	Umbilical cord blood	High sFlt-1 (and reduced PlGF) were associated with reduced growth from the gestational stage to 6 years of age.	(Bergen et al., 2015)
Newborns at birth	Case-control	70	Umbilical cord blood	Elevated sFlt-1 in children of mothers with preeclampsia.	(Staff et al., 2005)
Newborns at birth	Cross-sectional	39	Umbilical cord blood	Lower VEGF levels and higher sFlt-1 levels.	(Olmos et al., 2013)
0–30 years	Prospective	204	Antecubital vein blood	Elevated levels of sFlt-1.	(Lewandowski et al., 2015)
5–8 years		43	Antecubital vein blood	No difference in sFlt-1 levels.	(Kvehaugen et al., 2011)

sFLT-1: soluble receptor tyrosine kinase similar to fms-1; VEGF: Vascular endothelial growth factor; PlGF: Placental growth factor.

to preeclamptic pregnancies (Lewandowski et al., 2015). Also, studies have shown that neonates born from preeclamptic pregnancies exhibit lower levels of VEGF and higher levels of sFlt-1 when compared to children born to normotensive women (Olmos et al., 2013). In addition, it has been suggested that high levels of sFlt-1 in the umbilical cord are associated with impaired function of both endothelial cell progenitors (Xia et al., 2007) and human umbilical vein endothelial cells (Yu et al., 2016). Furthermore the impact of sFlt-1 on tissue function has been reported, and in the thyroid gland, a highly vascularized tissue, there is a negative association between thyroid hormone production and umbilical cord blood levels of sFlt-1 (Korevaar et al., 2014). Moreover, in the broader context of the systemic effects of sFlt-1 on infant growth and development, it has been reported that high umbilical levels of sFlt-1 are negatively associated with

growth in infants in uncomplicated pregnancies (Bergen et al., 2015), and sFlt-1 levels are also negatively correlated with body weight percentile in preterm babies (Voller et al., 2014).

At the central nervous system level, the precise role of sFlt-1 in modifying neuroanatomical and vascular architecture, and circulatory characteristics in the brain is unknown. Interestingly, high circulating levels of sFlt-1 are associated with adult psychiatric and neurological alterations (Kim et al., 2007; Fulzele and Pillai, 2009; Emanuele et al., 2010; Lee et al., 2010; Lizano et al., 2016, 2017; Pillai et al., 2016) and studies also reveal a reduction in total frontal lobe volume in patients with schizophrenia/schizoaffective disorder (Pillai et al., 2016). Whether this association is the result of alterations in microvascular development in those patients is unknown, however, gene transfer of sFlt-1 in rats leads to a reduction in brain edema and in blood brain barrier permeability, suggesting a direct effect on brain endothelium (Kumai et al., 2007).

A limited number of studies have investigated the effect of sFlt-1 on brain development, including those of Carver et al. (2014) which report that, in a mouse model of preeclampsia, adenoviral transfer of sFlt-1 to the mother was associated with sex-dependent neuroanatomical alterations in the offspring at 6 months of age, which were partly counteracted by treating mothers with pravastatin (Carver et al., 2014).

If high levels of sFlt-1 in the mother or in the fetus effectively impair the proper development of brain blood vessels, and potentially brain development, in the offspring born from preeclamptic pregnancies (**Figure 2B**), sFlt-1 would represent a reliable prognostic biomarker that would help to predict adverse outcomes in children born from preeclamptic pregnancies.

PlGF and Cerebral Circulation in Offspring of Preeclampsia

PlGF is highly expressed at all brain development stages (Luna et al., 2016) and is considered the key cerebral angiogenic factor (Liu et al., 2006; Gaal et al., 2013). Mechanistically, PlGF stimulates growth of neurons (Dewerchin and Carmeliet, 2012; Carmeliet and Ruiz de Almodovar, 2013) and formation of new blood vessels in the brain (Gaal et al., 2013) although the influence of PlGF on brain vasculature in preeclampsia has not been investigated.

However, studies report low levels of PlGF in maternal and fetal blood (Schlembach et al., 2007) and low levels of PlGF mRNA in the placenta (Andraweera et al., 2012) of preeclamptic pregnancies compared to normotensive pregnancies. Furthermore, low maternal levels of PlGF in the second trimester of gestation were associated with a narrower retinal arteriolar caliber (but not with a narrower retinal venular caliber) in childhood, compared to children who had experienced a normotensive pregnancy (Gishti et al., 2015). These findings suggest a highly selective role for PlGF in retinal vascular development.

A more direct relationship between PlGF levels during pregnancy and brain angiogenesis in offspring has been documented by Lecuyer et al. (2017), who demonstrated low placental levels of PlGF were associated with a reduction in radial distribution of fetal cerebral cortical microvasculature. Studies in normotensive mice virally transfected with PlGF (Gaal et al., 2013) and in PlGF ($Plgf^{-/-}$) deficient mice (Freitas-Andrade et al., 2012; Luna et al., 2016) highlight the key role PlGF in the development of brain vasculature. Furthermore, seminal studies from Dr. Ana Croy's group found that $Plgf^{-/-}$ mice showed anatomical alterations in the cerebral macrocirculation at the level of anterior communicating cerebral arteries and anterior collateral vessels forming Willis' polygon. These structural changes in cerebral circulation observed in $Plgf^{-/-}$ mice were associated with impaired cognitive function (Luna et al., 2016), however, the latter study did not characterize the brain microcirculation, nor the profile of pro or anti-angiogenic factors in the $Plgf^{-/-}$ mice. More recently, the same group has reported that $PlGF^{-/-}$ mice also exhibited alterations in the development of neurons (Chaballe et al., 2011) and the retina (Kay et al., 2017).

IS IT ALL ABOUT BRAIN ANGIOGENESIS?

Mechanistically, impaired brain angiogenesis is unlikely to be the unique alteration that fully explains the complexities of the adverse cognitive outcomes present in children born from preeclamptic pregnancies. Considering the hypothesis of fetal programming, it is feasible to speculate that other factors including oxidative stress, inflammation, neuronal structural modifications and epigenetic modifications driven by intrauterine hypoxia, among others, are involved. Furthermore, the contribution of many other post-natal factors, including socioeconomic environment, breastfeeding and social interactions to increase brain stimulation, to cognitive function in children born from preeclamptic pregnancies need to be established. Several excellent reports, including (Van den Bergh, 2011; Babenko et al., 2015) address the concept of fetal programming and alterations at brain level.

CONCLUDING REMARKS

The syndrome of preeclampsia is a frequent complication of pregnancy, affecting both the mother and the fetus. This disorder is characterized by an imbalance between pro and anti-angiogenic factors released from the placenta and may lead to a series of adverse consequences in individuals throughout their post-natal life.

The evidence discussed in this review suggests that children of mothers with preeclampsia have a higher risk of developing cognitive disorders. However, despite increased research in this field, it is still unclear whether other confounding factors, such as intrauterine growth restriction, prematurity or asphyxia, contribute to the cognitive disorders in children born to preeclamptic pregnancies, and further studies are required to establish the underlying mechanisms responsible for such cognitive disorders.

Herein, we present supporting evidence for alterations in the characteristics of brain microvasculature in the offspring born to preeclamptic women, although some studies include a small sample size that indirectly suggests structural alterations. While we highlight a potential key role of impaired brain vessel formation as an underling mechanism for cognitive alterations in children born to preeclampsia, it is also unlikely that this would be a unique mechanism for explaining the complex alterations in brain development and cognitive deficiencies observed. Furthermore, both inflammation and nutrient access (both over and undernutrition) represent potential contributory factors involved in brain alterations of offspring born to preeclamptic pregnancies.

We acknowledge that focusing on impaired VEGFR-mediated brain angiogenesis is an oversimplification of the complexity of the underling mechanisms of adverse cognitive outcomes in offspring born to preeclampsia. However, as indicated in this review, literature in this field is extremely limited and there is a substantial need for more focused research to establish the precise contribution of preeclampsia, independent of other confounding factors, on cognitive alterations in children. In particular, a better understanding of the roles of VEGF, PlGF, and sFLT-1 in impaired brain angiogenesis may aid in early diagnosis and management, and also in development of new treatment interventions.

AUTHOR CONTRIBUTIONS

EL wrote the draft of the manuscript. JA, JL, JP, and PT-V critically revised the manuscript. CE generated the original hypothesis and proposed the development of the review article.

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