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Review

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Mechanisms linking exposure to preeclampsia *in utero* and the risk for cardiovascular disease

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Abstract

Preeclampsia (PE) is now recognised as a cardiovascular risk factor for women. Emerging evidence suggests that children exposed to PE *in utero* may also be at increased risk of cardiovascular disease (CVD) in later life. Individuals exposed to PE *in utero* have higher systolic and diastolic blood pressure and higher body mass index (BMI) compared to those not exposed to PE *in utero*. The aim of this review is to discuss the potential mechanisms driving the relationship between PE and offspring CVD. Exposure to an adverse intrauterine environment as a consequence of the pathophysiological changes that occur during a pregnancy complicated by PE is proposed as one mechanism that programs the fetus for future CVD risk. Consistent with this hypothesis, animal models of PE where progeny have been studied demonstrate causality for programming of offspring cardiovascular health by the preeclamptic environment. Shared alleles between mother and offspring, and shared lifestyle factors between mother and offspring provide alternate pathways explaining associations between PE and offspring CVD risk. In addition, adverse lifestyle habits can also act as second hits for those programmed for increased CVD risk. PE and CVD are both multifactorial diseases and, hence, identifying the relative contribution of PE to offspring risk for CVD is a very complex task. However, considering the emerging strong association between PE and CVD, those exposed to PE *in utero* may benefit from targeted primary CVD preventive strategies.

Introduction

Preeclampsia (PE) is a pregnancy-specific disorder that complicates 2%–5% of all pregnancies and is a leading cause of maternal morbidity and mortality worldwide.[1] PE was traditionally diagnosed when a pregnant woman presented with increased blood pressure and proteinuria. According to the current International Society for the Study of Hypertension in Pregnancy guidelines, PE is defined as an onset of hypertension (systolic blood pressure higher than 140 mmHg or diastolic blood pressure higher than 90 mmHg on two occasions that are 4–6 h apart) after 20 weeks gestation and the coexistence of one or more of the following new-onset conditions: (1) proteinuria (spot urine protein/creatinine >30 mg/mmol [0.3 mg/mg] or >300 mg/day or at least 1 g/l ['2 + '] on dipstick testing), (2) organ dysfunction (such as renal insufficiency [creatinine >90 umol/l; 1.02 mg/dl], liver involvement (elevated transaminases – at least twice upper limit of normal ± right upper quadrant or epigastric abdominal pain), neurological complications (examples include eclampsia, altered mental status, blindness, stroke or more commonly hyperreflexia when accompanied by clonus, severe headaches when accompanied by hyperreflexia, persistent visual scotomata), haematological complications (thrombocytopenia – platelet count below 150,000/dl, DIC, haemolysis) and (3) uteroplacental dysfunction evidenced by fetal growth restriction.[2] Although the hypertension and proteinuria resolve after pregnancy, women who develop PE are at increased risk of vascular diseases in later life. Previous systematic reviews and meta-analyses have shown that women who develop PE are at more than two-fold increased risk of developing coronary artery disease (CAD) compared to women who have non-preeclamptic pregnancies.[3-5] Early onset PE (<34 weeks gestation) is associated with nearly eight-fold increased risk of subsequent CAD for the woman compared to late onset disease and severe PE (blood pressure >160/110 mmHg plus proteinuria >0.3 g/24 h or diastolic blood pressure >110 mmHg plus proteinuria >5 g/24 h) is associated with a higher risk of CAD compared to mild PE.[5,6] Women who develop PE are at increased risk of other vascular diseases including hypertension, thromboembolic disorders, cerebrovascular events and diabetes compared to women who do not experience PE.[3-5] The evidence linking PE with

cardiovascular disease (CVD) has resulted in PE being recognised by the American Heart Association as a female-specific, cardiovascular risk factor.[7]

In addition to the maternal risk, emerging evidence suggests that children born to women who experience PE may also be at increased risk of CVD in adulthood. A recent population-based cohort study of 231,298 deliveries demonstrated a significant linear association between PE (no PE, mild PE, severe PE and eclampsia) and rates of CVD in the offspring (0.24% vs. 0.33% vs. 0.51% vs. 2.73%, respectively, $p < 0.001$).[8] Furthermore, compared to children of normotensive pregnancies, those exposed to PE *in utero* (mild, severe and eclampsia) had significantly higher rates of hypertension (0.06% vs. 0.11% vs. 0.14% vs. 1.37%, respectively, $p < 0.001$), arrhythmias (0.13% vs. 0.18% vs. 0.18% vs. 1.37%, respectively, $p = 0.016$) and heart failure (0.04% vs. 0.03% vs. 0.18% vs. 0.00%, respectively, $p = 0.004$)[8]. Over the last decade, many studies have also reported a greater prevalence of conventional CVD risk factors in children exposed to PE *in utero* compared to children not exposed to PE *in utero*. We recently conducted a systematic review and meta-analysis of prospective and retrospective studies that had compared CVD risk factors among those exposed to PE *in utero* compared to those not exposed to PE *in utero*.[9] This pooled evidence from 36 studies and over 53,000 participants found that systolic blood pressure, diastolic blood pressure and body mass index (BMI) were significantly higher among those exposed to PE *in utero* compared to those not exposed to PE *in utero*.[9] The link between maternal PE and offspring CVD is likely to be due to abnormalities in vascular function. This is supported by the finding of impaired vascular responses in some offspring animal models that mimic PE[10] as well as the majority of human studies of those exposed to PE *in utero*.[11] Endothelial dysfunction, a hallmark of PE and an early biological marker of CVD, is evident in children and adolescents exposed to PE *in utero*.[12,13] Endothelium-dependent differences in microvascular function have also been reported in children born to women who experienced PE as early as in the neonatal period.[14] Furthermore, carotid intima-media thickness is increased among young adults in their 20s exposed to PE *in utero*.[15] A study that compared aortic intima-media thickness (aIMT) and cord blood lipid profiles of neonates born to women with PE with those of neonates born of healthy pregnancies showed significantly increased aIMT and cord blood triglyceride and decreased cord blood high density lipoprotein cholesterol levels in neonates of preeclamptic pregnancies compared to the control group.[16] These findings suggest the possibility that an early atherogenic phenotype independent of blood pressure may contribute to the link between exposure to PE *in utero* and later life CVD.

The above evidence demonstrates a clear association between PE and later life CVD for both women and their children. CVD is a major global health burden that results in the greatest number of deaths worldwide. The World Health Organization estimates that 17.6 million people died from CVD in 2012 accounting for 31.43% of global mortality.[17] In the context of an increasing global obesity epidemic, this calls for an urgent need to develop potential primary preventive strategies for at-risk populations. The association between maternal PE and offspring CVD is well known. However, the mechanisms underlying this link are poorly understood. This review outlines the evidence for developmental programming, epigenetics and shared genetics as potential mechanisms driving the relationship between PE and offspring CVD.

Developmental programming of CVD risk by in utero exposure to PE

Developmental programming of chronic diseases is now an established paradigm. The link between early life environmental factors and later life disease susceptibility was initially proposed by David Barker who in early 1990s showed that restricted growth in fetal life was associated with mortality from CVD.[18,19] This concept that exposure to unfavourable conditions *in utero* at times of critical organ development may lead to later life disease risk was originally called the Barker Hypothesis/fetal origins of disease hypothesis and now the Developmental Origins of Health and Disease hypothesis.[20] While most of the initial work on this topic linked intrauterine undernutrition with later life CVD and metabolic disease risk, subsequent epidemiological studies have shown that numerous intrauterine exposures including major pregnancy complications (PE, intrauterine growth restriction [IUGR], spontaneous preterm birth and gestational diabetes mellitus), maternal obesity and smoking during pregnancy and exposure to environmental chemicals can each trigger propensity for a myriad of cardiovascular, metabolic, immunological, reproductive and neurodevelopmental disorders in the offspring.[21]

Depending on the severity of the insult during critical periods of rapid growth and development, permanent tissue adjustments can occur which lead to long-term functional changes in vital organs. Although PE is a multifactorial disorder, several pathophysiological mechanisms including angiogenic imbalance, excessive inflammation, ischaemia/perfusion and imbalances in the renin angiotensin system are all implicated in its pathogenesis.[22,23] During a pregnancy complicated by PE, the placenta releases circulating factors, including sFlt and sEng,[24,25] into the maternal circulation as a result of syncytiotrophoblast stress due to a variety of insults including placental ischemia/hypoxia, contributing to excessive inflammation and generation of reactive oxygen species.[26-29] Consistent with a causal role for these placental factors in programming of progeny CVD, overexpression of sFlt in a mouse model of PE mimicking the placenta of a pregnancy affected by PE results in elevated systolic and diastolic blood pressure in male offspring.[30] Higher than normal levels of these placental circulating factors lead to placental oxidative stress, inflammation and lipid peroxidation[31,32] which can have permanent effects on the susceptibility of that fetus to CVD later in life. Increased oxidative stress and placental oxidative DNA damage are associated with fetal growth restriction in offspring from pregnancies complicated by PE,[33] and there is evidence of oxidative stress and DNA damage in cord blood from offspring of PE-affected pregnancies.[34] Exposure to hypoxia in a rat model of PE leads to impaired placental function, and offspring exhibit inflammation and atherosclerosis.[35-37]

The impact of perinatal exposure to inflammation also has various impacts on the fetus, including changes to the fetal immune system[38,39] (e.g. lower abundance of regulatory T [Treg] cells) which persist into early childhood.[39] Exposure to intrauterine inflammation as a result of placental dysfunction may lead to increased inflammation and reduced capacity to dampen inflammation due to a deficiency in Treg cells in later life. Such immune programming may contribute to progeny CVD, since inflammation is central in the pathogenesis of atherosclerosis and CVD.[39,40]

Animal studies provide mechanistic evidence that each of these exposures programs offspring blood pressure. The strength of most of the animal models is that they avoid the confounding factors of shared genetics and shared lifestyles and focus specifically on

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in utero exposure. The models of PE in which programming of progeny cardiometabolic outcomes has been most extensively characterised are in rodents. These include the surgically induced reduced uterine perfusion pressure (RUPP) rat, and PE induced by maternal treatment during pregnancy with hypoxia, the nitric oxide synthase (NOS) inhibitor L-NAME or angiotensin II auto-antibodies. Postnatal outcomes have also been well characterised in progeny of mice where elevated sFlt1 was achieved by adenoviral transfection of dams during pregnancy. In each of these models, progeny are lighter at birth but catch up in body weight to that of controls during or before adulthood.[37,41-51] Development of obesity is variable, with obesity reported in adult female, but not male, RUPP rats with ageing,[52,53] but normal body and central fat abundance in adult progeny of *sFlt1*-transduced mice of both sexes.[54] Likewise, effects of *in utero* exposure to a preeclamptic phenotype on postnatal glucose tolerance are sex- and age-dependent in the RUPP rat,[53,55] L-NAME-treated rat[56] and *sFlt1*-transduced mice[51] models of PE. Progeny cardiac dysfunction is common to all of these models, although with some variation in phenotype and different sets of outcomes studied in each model. Hypertension at baseline and/or in response to stress or adrenaline has been reported in progeny of the RUPP rat,[10,41-45,52,57] hypoxic rat[47,48,58,59] and in males but not females in the *sFlt1*-transduced mice model of PE.[30,54] Interestingly, progeny of the L-NAME-treated rat are normotensive as young and mature adults.[50,60] Greater arterial vasoconstriction in response to stimuli, greater renal sympathetic nerve activity, contributions from circulating sex steroids and impaired vasodilation are all implicated as mechanisms for hypertension in progeny of the RUPP rat.[10,42,43,52,57] Similarly, in progeny of hypoxic rat dams, phenylephrine-induced vasoconstriction is greater, and both endothelium-dependent and endothelium-independent vasodilatation are reduced compared to control progeny.[36,47,48,61,62]

Progeny heart structure and function are also impaired in several of these models. For example, cardiac and cardiomyocyte size are normal at 3 months of age in young male adults whose mothers were exposed to intermittent hypoxia from early pregnancy (10% O_2 for 3 h/day from GD7 to 21)[59]. At 5 months of age, hypertensive adult male progeny from these dams exhibit cardiac hypertrophy, with normal cardiomyocyte size but greater left ventricular collagen expression of collagen I and III.[59] Furthermore, lesions suggestive of developing atherosclerosis are present in the aortas of adult male progeny at 5 months of age of dams housed continuously in 10.5% O_2 from GD5-delivery,[37] and in aged adult male and female progeny at 16 months of age who were exposed to hypoxia *in utero* 10% O_2 for 3 h/day from GD7 to 21.[35] Although their relative heart weights are normal, male progeny of dams treated with angiotensin-II-like auto-antibodies (which bind and activate the angiotensin II-type I receptor) have enlarged cardiomyocytes, with areas of disorganisation, necrotis and apoptosis evident in their left ventricles as young adults at 5 months postnatal age.[49] The function of isolated hearts from these progeny in a Langendorff apparatus is normal under basal conditions, but they have impaired recovery and larger infarcts following ischaemia-reperfusion.[49] Cardiac hypertrophy is also evident throughout the first month of postnatal life in progeny of rats treated with L-NAME before and throughout pregnancy.[63]

The fact that hypertension and impaired metabolism occur in progeny in these models of induced PE provides direct evidence of causality for the exposure. Nevertheless, these models lack the initiating processes that lead to spontaneous PE in humans. Interpretation of the programming mechanisms in progeny exposed to PE *in utero* and evaluation of potential maternal or postnatal interventions also need to consider the stage of cardiovascular development when *in utero* exposure occurs. For example, the switch from cardiomyocyte proliferation to hypertrophy occurs postnatally in rodents but before birth in other species including humans.[64] A recent report that progeny of preeclamptic baboons exhibit elevated systolic blood pressure during a dietary salt loading challenge[65] provides direct evidence of cardiovascular programming by *in utero* exposure to PE in species that are more mature at birth, although studies with greater animal numbers are needed to assess any sex differences.

When combined with epidemiological evidence from human studies, these suggest several plausible mechanisms for the hypertensive phenotype seen in offspring of preeclamptic pregnancies.[11] Several studies have shown reduced numbers of nephrons and cardiomyocytes in offspring born with low birthweight or born preterm, two pregnancy complications that often co-exist with PE.[66] A reduction in the number of nephrons contributes to a reduction in the rate of renal ultrafiltration that affects the circulating blood volume leading to increased blood pressure.[67] Altered nephron number is associated with glomerular hypertrophy and reduced renal vascular dilation contributing to risk of hypertension.[68,69] Alterations in the renin–angiotensin–aldosterone system (RAAS) including upregulation or downregulation of specific receptors within the kidney, blood vessels and the placenta have also been implicated in the development of renal dysfunction.[70] Emerging evidence suggests that epigenetic processes which are strongly influenced by the environment may play a key role in developmental programming of adult CVD.

The role of epigenetics in the relationship between PE and increased risk of CVD in offspring

Epigenetics refers to modifications that result in gene expression changes, often through chromatin remodelling, without altering the underlying DNA sequence[71] These modifications include DNA methylation, histone modifications, non-coding RNA (ncRNA) and other modifications that can result in altered gene expression.[71] Epigenetic changes have been widely studied in developmental processes, especially their role in determining cell lineages, and also in disease states as they can mediate aberrant gene expression.[71] In addition, epigenetic modifications are routinely used for diagnostic and prognostic biomarkers of adverse outcomes. Epigenetic modifications are also altered in response to environmental exposures and can potentially indicate whether an adverse exposure early in life is associated with future chronic disease risk.[71]

The most widely studied epigenetic alteration is DNA methylation which is the addition of a methyl group to a cytosine catalysed by enzymes known as DNA methyltransferases. DNA methylation is involved in development, imprinting and X chromosome inactivation, as well as numerous other processes.[71] Hypermethylation at gene regulatory regions or promoter associated CpG islands is often associated with gene repression. Histone modifications refer to changes to the histone tails including acetylation, methylation or phosphorylation and together with DNA methylation impact chromatin packing and hence accessibility of transcription factors to the regulatory DNA sequences controlling gene expression. ncRNAs consist of short ncRNAs like microRNAs, piwiRNAs, snoRNAs or long non-coding RNAs (lncRNA) and can inhibit transcription and translation or alter protein trafficking and folding to name just a few of their actions.[72]

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Epigenetic changes are considered likely mechanisms that link maternal PE with offspring CVD with the possibility of passing on the risk to subsequent generations, by germ-line inheritance of epigenetic marks.[73,74] Animal models, which allow for tight regulation and control of the adverse exposure, have repeatedly shown that adverse events *in utero* are associated with increased risk of chronic disease in offspring.[73,75,76] Similar studies in humans are difficult, not only due to the lack of control of 'other' life-time environmental exposures but also due to genetic predispositions and additionally access to the appropriate tissue for assessment. However, epidemiological studies do show evidence for changes in DNA methylation as potential mediators or biomarkers of *in utero* exposure to PE when assessed in the placenta[77,78] and cord blood.[79] Whether these epigenetic changes are associated with an increased risk of CVD in the offspring later in life is unknown as no studies have yet reported epigenetic alterations in the adult offspring born to women who experience PE. This is not surprising due to the length of follow-up required for these studies and also access to the right tissue sample for the epigenetic analyses. However, there are a few studies that demonstrate an association between PE exposure and epigenetic changes in offspring. In a 2015 study by Julian et al., DNA methylation in blood from young adult men born following hypertensive pregnancies ($n = 5$) compared to controls ($n = 19$) showed differential methylation in the genes *SMOC2*, *ARID1B* and *CTRHC1*.[80] Murray et al. reported that DNA methylation in cord blood leukocytes of *ANRIL* predicted pulse wave velocity and heart rate in the child at 9 years of age.[81] Therefore, with the appropriate longitudinal cohorts, in future, we may be able to determine whether epigenetic change induced by *in utero* exposure to PE underlies increased risk of future CVD risk in the offspring.

The placenta may mediate this future CVD risk in the offspring as several studies have shown altered DNA methylation in placentas from women who experience PE compared to healthy pregnancies (reviewed in ref.[77]). The genes shown to have aberrant DNA methylation include those with a role in trophoblast proliferation, differentiation and invasion, as well as genes involved in vascular function and immunological processes.[77] Epigenetic alterations in these critical genes may impact the function of the placenta during pregnancy resulting in an adverse environment for the fetus.[82] The fetus adapts to this adverse environment, resulting in epigenetic alterations that mediate future CVD risk. More work is required to define how PE impacts the intrauterine environment, what epigenetic alterations this induces in the fetus and if these epigenetic alterations are still evident in adult offspring. Dissecting out the epigenetic mechanism is made difficult in humans by the need for longitudinal cohorts as well as determining which tissue to assess.

The role of shared genetics in the relationship between PE and increased risk of CVD in offspring

The link between PE and CVD in offspring could also be explained by shared genetic factors that predispose individuals to vascular diseases that manifest at different time points during the life course.[83] The incidence of PE is higher among women who were born of a pregnancy complicated by PE,[84] while the risk of fathering a pregnancy complicated by PE is also higher among men who previously fathered a pregnancy complicated by PE with a different partner[84] and among men who were themselves the product of a pregnancy complicated by PE,[85] all suggesting that PE is at least in part heritable. Further evidence for a genetic mechanism is provided by studies that show that genetic variants that are known risk factors for CVD are more prevalent among women who experience PE, men who father pregnancies complicated by PE and among infants born of pregnancies complicated by PE compared to unaffected populations.[86-89] Genetics, therefore, may act as a confounding factor in the association between maternal PE and offspring CVD. The underlying mechanism for some offspring who experience CVD in adult life after exposure to PE *in utero* may not be the effect of PE but genetic susceptibility to CVD, and these individuals may experience CVD even if they had not been exposed to PE *in utero*. This theory is supported by the findings of genetic variants that are common in both PE and CVD from a study on genetic dissection of the 2q22 PE susceptibility locus.[90] This study identified four independent single nucleotide polymorphisms (SNPs) within four genes that were associated with PE in an Australian family cohort: lactase (*LCT*, rs2322659), low-density lipoprotein receptor-related protein 1B (*LRP1B*, rs35821928), rho family GTPase 3 (*RND3*, rs115015150) and grancalcin (*GCA*, rs17783344).[91] These four SNPs were also associated with cardiovascular risk factors in an independent cohort of Mexican American families.[91] An attempt to confirm the above findings in an independent Australian population-based cohort of mothers and their adolescents showed nominal associations with cardiovascular risk factors (weight, blood glucose and triglycerides) for all four SNPs.[92] These findings suggest the possibility that certain genetic variants may predispose individuals to different vascular diseases, with some women developing PE during pregnancy, some men fathering pregnancies complicated by PE and some developing CVD.

The role of shared environment and lifestyle in the relationship between PE and increased risk of CVD in offspring

Similar to shared alleles, shared lifestyle factors between mother and offspring may also confound the association between maternal PE and offspring CVD. It is well known that suboptimal environmental and lifestyle conditions including unhealthy diets, lack of exercise, low socioeconomic status and low educational levels are all associated with both PE and CVD. Since these factors are very likely to be shared between mothers and their children, they may underlie the association between maternal PE and CVD in offspring in a manner similar to the potential role of similar genes. These unfavourable lifestyle factors are also proposed to act as 'second hits' for some individuals. Some who are 'programmed' for increased disease risk only develop disease when exposed to a 'second hit'[93] at a later stage in life. These 'second hits' are often adverse lifestyle factors including smoking, unhealthy diets and sedentary lifestyles,[93] so shared adverse environment may contribute to CVD risk both directly and by providing a 'second hit' exposure to individuals who are susceptible as a consequence of their intrauterine exposures.

Maternal PE and offspring CVD: developmental programming, shared genes or shared lifestyle?

If the association between maternal PE and offspring CVD was solely due to developmental programming, adverse cardiovascular profiles would only be seen among those exposed to PE *in utero* and siblings born of normotensive pregnancies will be unaffected. On the other hand, if the association between maternal PE and offspring CVD is due to shared genetics and/or lifestyles, then offspring of the same parents from pregnancies unaffected by PE

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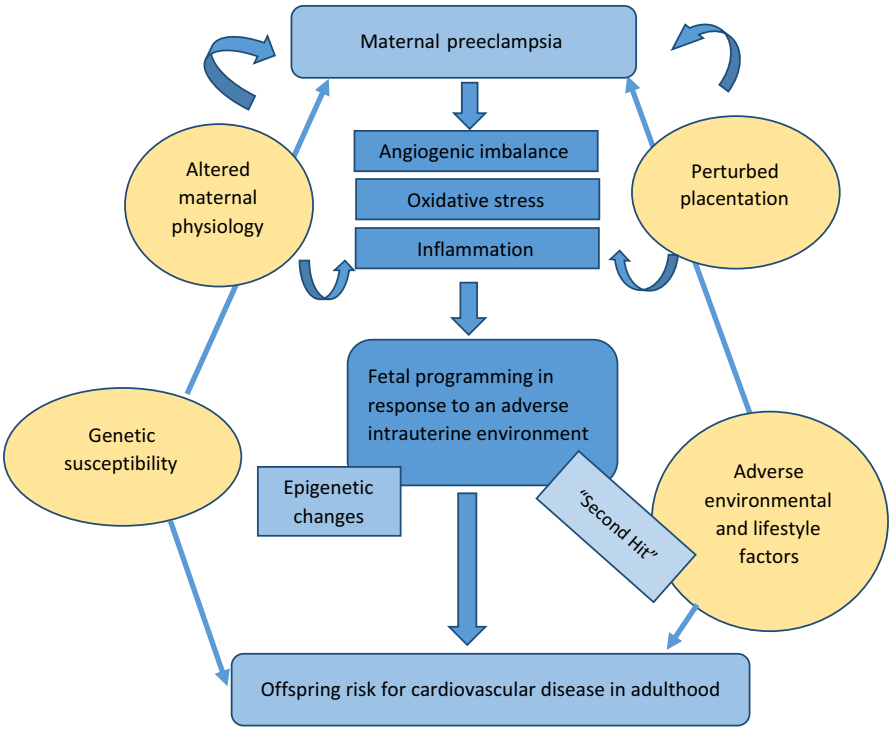

Fig. 1. Potential mechanistic link between exposure to PE *in utero* and offspring risk for CVD.

would demonstrate similar CVD risk to that of offspring of pregnancies complicated by PE. The HUNT study comprising three large population-based surveys (HUNT 1, $n = 77{,}212$; HUNT2, $n = 65{,}215$; HUNT 3, $n = 50{,}807$) conducted in Norway explored these questions by comparing CVD risk factors among siblings discordant for the exposure to hypertensive disease in pregnancy ($n = 472$ participants within 210 sibships).[94] In this large study, offspring exposed *in utero* to maternal hypertension including PE and their siblings born after a normotensive pregnancy had similar adverse CVD risk profiles, suggesting that shared genes or lifestyle may account for the association, rather than an intrauterine effect. However, other studies suggest that the increased CVD risk in the offspring could be a long-term consequence of fetal exposure to PE. In support of this theory, offspring who were born after a pregnancy complicated by PE display marked vascular dysfunction (higher pulmonary artery pressure and lower flow-mediated dilation), but their siblings born after a normotensive pregnancy display normal vascular function.[13] In this study, the birthweight of offspring exposed to PE *in utero* was approximately 400 g lower than the control group. This suggests the possibility of an alternative explanation. Birthweight correlates linearly with nephron number in adults and children, with nephron number increasing by 257,426 per kg increase in birthweight[95] and low nephron number being associated with higher blood pressure (reviewed in ref.[66]). Effects of PE exposure *in utero* on postnatal blood pressure may therefore be in part mediated by effects of fetal growth restriction on renal function. Interpretation of associations between exposure to PE *in utero* and later life CVD becomes more complicated by the fact that offspring of pregnancies complicated by PE can also be born with low birthweight and prematurity. Low birthweight and prematurity are both associated with a congenital reduction in nephron number, raised blood pressure, proteinuria and chronic kidney disease in adulthood.[66] Therefore, developmental programming as a consequence of these conditions may confound the associations between PE and offspring CVD risk. Low birthweight and prematurity both suggest exposure to a more severe placental disease and are mostly seen in early onset PE. Due to the interactions between genes, environment and potentially programming mechanisms, understanding the relative contribution of each of these mechanisms to the link between PE and CVD is complex and a combination of all three mechanisms appears the most plausible explanation.

Conclusion

Considering the current evidence, PE appears to have a long-term impact on the cardiovascular health of the offspring. Epidemiological evidence from long-term follow-up studies on humans shows that those exposed to PE *in utero* have higher blood pressure and higher BMI compared to those not exposed to PE *in utero*. The possible link between exposure to PE *in utero* and offspring risk for CVD is shown in Fig. 1. Three main mechanisms could explain the association between maternal PE and offspring CVD, and these are not mutually exclusive. Firstly, shared non-genetic (environmental and lifestyle exposures) risk factors may account for the association. Secondly, contributions from shared genetic variants to both PE and CVD risk are a plausible explanation. Thirdly, developmental programming due to exposure to the preeclamptic intrauterine environment can result in long-lasting effects on the cardiovascular health of the offspring. Animal models provide important insights into possible mechanistic pathways as they avoid confounding factors of shared lifestyle factors and can address specific *in utero* exposures. The different phenotypes of PE including early vs. late, severe vs. non-severe also make the interpretation of findings from different studies harder as each phenotype has different confounding factors.

In conclusion, there is strong evidence to demonstrate an association between maternal PE and offspring CVD. However, the mechanistic pathways leading to the risk for CVD after intrauterine exposure to PE are not yet clear. It is very likely that there is an

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overlap among all three mechanisms explored in this review and that dissecting the relative contribution of each pathway is impossible. Considering the risk for CVD among offspring exposed to PE *in utero*, primary preventive strategies are warranted in this target population.

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