A perspective on pre-eclampsia and neurodevelopmental outcomes in the offspring: does maternal inflammation play a role?

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Abbreviations:

Attention deficit hyperactivity disorder (ADHD); Autism spectrum disorder (ASD); Diffusion tensor MR imaging (DTI); Hypertensive disorders of pregnancy (HDP); Intellectual disability (ID); Interleukin (IL); International Society for the study of Hypertension in Pregnancy (ISSHP); Magnetic resonance (MR); Pervasive Developmental Disorder - Not Otherwise Specified (PDD-NOS); soluble fms-like tyrosine kinase-1 (sFlt-1); Tumor necrosis factor (TNF);

Abstract:

Pre-eclampsia is a leading cause of maternal death and maternal and perinatal morbidity. Whilst the clinical manifestations of pre-eclampsia often occur in late pregnancy, the molecular events leading into the onset of this disease are thought to originate in early pregnancy and result in insufficient placentation. Although the causative molecular basis of pre-eclampsia remains poorly understood, maternal inflammation is recognised as a core clinical feature. While the adverse effects of pre-eclampsia on maternal and fetal health in pregnancy is well-recognised, the long-term impact of pre-eclampsia exposure on the risk of autism spectrum disorder (ASD) in exposed offspring is a topic of on-going debate. In particular, a recent systematic review has reported an association between exposure to pre-eclampsia and increased risk of ASD, however the molecular basis of this association is unknown. Here we review recent evidence for; 1) maternal inflammation in pre-eclampsia; 2) epidemiological evidence for alterations in neurodevelopmental outcomes in offspring exposed to pre-eclampsia; 3) long-term changes in the brains of offspring exposed to pre-eclampsia; and 4) how maternal inflammation may lead to altered neurodevelopmental outcomes in pre-eclampsia exposed offspring. Finally, we discuss the implications of this for the development of future studies in this field.

Key words: Pre-eclampsia; autism; behaviour; maternal; inflammation; placenta; cytokine.

1. Introduction:

Autism spectrum disorder (ASD) is a complex neurodevelopmental disorder, characterised by impairments in social and communication skills, as well as restricted and repetitive patterns of behaviour (Lord et al., 2018; Thapar et al., 2017; Xiao et al., 2014). ASD is among the most common neurodevelopmental conditions with a prevalence of approximately 1% globally, and 1.5% in developed countries (Lord et al., 2018; Lyall et al., 2017).

While there is a general consensus that genetics play the major role in the aetiology of ASD (Sandin et al., 2014), the environmental contribution is estimated to be between 17-50%. (Sandin et al., 2017; Sandin et al., 2014). Therefore, it is important to investigate factors potentially contributing to the likelihood of development of ASD. Several environmental risk factors, including prenatal and perinatal factors have been examined in an attempt to explain the aetiology of ASD (Lord et al., 2018). In particular, a recent systematic review examining the association between hypertensive disorders of pregnancy (HDP) and neurodevelopmental disorders reported an association between pre-eclampsia and ASD in exposed offspring (Maher et al., 2018). However, while the molecular basis of this association is not known, it may involve maternal inflammation given its link to ASD (Brown et al., 2014), and given that maternal inflammation is a core feature of pre-eclampsia (Lau et al., 2013) (Figure 1). Therefore, the objectives of this paper are to review and provide a perspective on the:

1. Evidence for maternal inflammation in pre-eclampsia;
2. Epidemiological evidence for alterations in neurodevelopmental outcomes in offspring exposed to pre-eclampsia;
3. Evidence for long-term changes in the brains of offspring exposed to pre-eclampsia;
4. Evidence for how maternal inflammation may lead to altered neurodevelopmental outcomes in pre-eclampsia exposed offspring.

2. Hypertensive disorders of pregnancy:

Hypertensive disorders of pregnancy (HDP) may be chronic (pre-dating pregnancy or diagnosed before 20 weeks' gestation) or arise *de novo* (either pre-eclampsia or gestational hypertension). HDP are one of the most common gestational complications affecting 3-10% of all pregnancies and are made up of a collection of hypertensive conditions including pre-existing hypertension (chronic hypertension), gestational hypertension, white coat hypertension and pre-eclampsia (Brown et al., 2018a). Of these, pre-eclampsia is one of the leading causes of maternal mortality and morbidity and has recently been redefined by the International Society for the study of Hypertension in Pregnancy (ISSHP) as gestational hypertension

(systolic BP $\geq$ 140 and/or diastolic BP $\geq$90 mmHg) accompanied by one or more of the following new-onset conditions at or after 20 weeks' gestation (Brown et al., 2018b):

1. Proteinuria;
2. Other maternal organ dysfunction, including:
    - Acute kidney injury (creatinine > 90umol/L; 1mg/dL)
    - Liver involvement (elevated transaminases e.g. ALT or AST >40 IU/L) with or without right upper quadrant or epigastric abdominal pain)
    - Neurological complications (examples include eclampsia, altered mental status, blindness, stroke, clonus, severe headaches, persistent visual scotomata)
    - Haematological complications (thrombocytopenia – platelet count below 150,000/uL, DIC, hemolysis)
3. Uteroplacental dysfunction (such as fetal growth restriction, abnormal umbilical artery Doppler wave form analysis, or stillbirth).

Previously thought to be simply due to impaired trophoblast invasion followed by the development of the clinical manifestations of the disease, it is now appreciated that the underlying aetiology of pre-eclampsia is far more complex. Beginning with genetic susceptibility, followed by an abnormal immune adaptation to pregnancy, this in turn leads to impaired placentation and the subsequent perfusion of the intervillous space by oxygenised arterial blood resulting in excessive or deficient placental derived factors in the maternal circulation (Chaiworapongsa et al., 2014a; Chaiworapongsa et al., 2014b). The endothelial dysfunction, resulting from placental ischemia and release of placental products which occurs in pre-eclampsia appears to occur as a result of oxidative stress and is mediated by high levels of free radicals and low levels of antioxidants (Roberts and Cooper, 2001; Roberts and Gammill, 2005; Roberts and Hubel, 2004; Roberts and Lain, 2002; Roberts and Speer, 2004; Roberts et al., 1989). Vasoactive factors released include soluble fms-like tyrosine kinase-1 (sFlt-1), cytokines, angiotensin II and type 1 receptor autoantibodies (Conrad and Benyo, 1997; Maynard et al., 2003; Rinehart et al., 1999; Roberts et al., 1991; Wallukat et al., 1999). These factors target the maternal vascular endothelium giving rise to the maternal syndrome of hypertension, proteinuria, organ and uteroplacental dysfunction which may be followed by acute atherosis in the spiral arteries predisposing to spiral artery thrombosis and placental infarcts (Lindheimer and Katz, 1981; Redman, 2014). In addition, there is an increasing awareness that pre-eclampsia leads to a state of exaggerated maternal inflammation as a direct result of the underlying pathophysiology, and perhaps also indirectly by risk factors such as

maternal obesity which is known to lead to chronic low-grade inflammation (Chaiworapongsa et al., 2014b; Jaaskelainen et al., 2018; Nelson et al., 2010; Segovia et al., 2014; Spradley et al., 2015). Therefore pre-eclampsia may be a common cause of maternal inflammation during pregnancy, which is a recognised risk factor for adverse neurodevelopmental outcomes (Knuesel et al., 2014). Consequently, there has been a growing interest in studying maternal inflammation and subsequently neurodevelopmental outcomes in offspring exposed to pre-eclampsia.

3. Maternal inflammation in pre-eclampsia: a potential role for Interleukin-6 and tumor necrosis factor (TNF)-α?

In uncomplicated pregnancies there is a normal systemic inflammatory response in which cytokines promote the infiltration of the spiral arteries by invading trophoblast cells (Redman et al., 1999). This is an important feature of normal placentation and occurs early in pregnancy. However this normal inflammatory response becomes exaggerated in pre-eclampsia resulting in disruptive activation of monocytes, granulocytes and the endothelium resulting in a state of maternal inflammation (Redman and Sargent, 2003). It has been proposed that there may be a genetic susceptibility to inflammation in pre-eclampsia yet many studies are conflicting (for recent review see (Thakoordeen et al., 2018). A meta-analysis of maternal polymorphisms in interleukin (IL)-6 (174G/C) (n = 396 pre-eclampsia and n = 507 normotensive) and tumor necrosis factor (TNF)-α (308G/A) (n = 1888 pre-eclampsia and n = 2497 normotensive) found that these were not associated with pre-eclampsia, despite maternal IL-6 and TNF-α levels being significantly higher in patients with pre-eclampsia (Xie et al., 2011). This suggests that elevations in cytokines may be as a result of the primary pathophysiology. However some subsequent studies have reported opposing findings (Sowmya et al., 2015) and this remains a topic of on-going investigation (Thakoordeen et al., 2018). However what is clear is that many clinical studies have now reported that women with pre-eclampsia have increased levels of inflammatory cytokines including IL-6, TNF-α, IL-12 and IL-16, which cause structural and functional changes in endothelial cells, promote the formation of endothelin and reduce acetylcholine induced vasodilatation (Benyo et al., 2001; Conrad and Benyo, 1997; Greer et al., 1994; Gu et al., 2008; Kupferminc et al., 1994; Lefer and Ma, 1993; Marsden and Brenner, 1992; Meekins et al., 1994; Pober and Cotran, 1990; Vince et al., 1995; Visser et al., 1994). A systematic review and meta-analysis published in 2013 tested the association between pre-eclampsia and maternal circulating levels of IL-6 (n = 425 pre-eclampsia and n = 363

normotensive), IL-10 (*n* = 180 pre-eclampsia and *n* = 175 normotensive) and TNF-α (*n* = 1015 pre-eclampsia and *n* = 925 normotensive) (Lau et al., 2013). Third trimester maternal circulating levels of IL-6, IL-10 and TNF-α were significantly higher in women with pre-eclampsia compared to normotensive controls (Lau et al., 2013). Subsequently a number of studies have extended and corroborated these findings.

A study by Mihu et al examined maternal cytokine concentrations between 28 and 41 weeks' gestation in an uncomplicated pregnancy group (*n* = 78), a pre-eclampsia group (*n* = 80), and a non-pregnant control group (*n* = 72) and reported elevations in IL-6 and TNF-α in the pre-eclampsia group (Mihu et al., 2015). In support of this, a study by Valencia-Ortega et al also examined IL-6 levels in age-matched pregnant women with (*n* = 50) and without pre-eclampsia (*n* = 50). They reported that maternal serum concentrations of IL-6 were significantly higher in late-onset pre-eclampsia, compared to early-onset pre-eclampsia or uncomplicated pregnancy (Valencia-Ortega et al., 2018). Moreover while mid-gestation circulating IL-6 levels were associated with pre-eclampsia, IL-6 was only significantly associated with term pre-eclampsia, suggesting that elevations in IL-6 may be a late stage feature of pre-eclampsia (Taylor et al., 2016b). This is consistent with a study of women with pre-eclampsia (*n* = 208) and normotensive controls (*n* = 411) which showed that first and second trimester levels of IL-6 were not associated with pre-term pre-eclampsia (Taylor et al., 2016a). Interestingly given that we and others have reported that the stage of pregnancy in which offspring are exposed to maternal inflammation is a key determinant of neurodevelopmental outcomes in exposed offspring (Aguilar-Valles and Luheshi, 2011; Boksa, 2010; Fortier et al., 2007; Meyer et al., 2006; Straley et al., 2014; Straley et al., 2017), it is possible that the effects of pre-eclampsia on offspring neurodevelopmental outcomes may vary depending on the severity and clinical course of the disease.

These findings are also supported by animal modelling of the pathophysiological mechanisms that underlie the development of pre-eclampsia. Specifically, the reduced uterine perfusion pressure (RUPP) rat model of placental ischemia mimics many of the clinical characteristics of pre-eclampsia. Placental ischaemia generated by reductions in uterine perfusion pressure in pregnant rats increases blood pressure, reduces glomerular filtration rate (GFR), increases sFlt-1 concentrations, elevates production of pro-inflammatory cytokines and reactive oxygen species (ROS) and leads to intrauterine growth restriction (IUGR) (Granger et al., 2006). Recent studies in the RUPP model have described an immune imbalance characterised by increased pro-inflammatory $CD4^+$ T cells and pro-inflammatory cytokines in

addition to a reduction in regulatory T cells and anti-inflammatory cytokines (Cornelius, 2018). Specifically there is substantial evidence of increased serum levels of pro-inflammatory cytokines IL-6 (Gadonski et al., 2006) and TNF-α (LaMarca et al., 2008) in response to placental ischemia in the RUPP model compared to sham controls. Initial work infusing TNF-α at day 14 of gestation in pregnant rats reported a significant increase in mean arterial blood pressure and a reduction in renal iNOS production coincident with two-fold increase in plasma TNF-α levels (Alexander et al. 2002). This work was extended to examine the role of endothelin in mediating the effect of TNF-α-induced hypertension in pregnant rats (LaMarca et al. 2005). The TNF-α-induced hypertension was associated with an increase in preproendothelin expression in placenta, aorta and kidneys. Additionally, pre-treating these pregnant rats with an endothelin receptor A antagonist prior to TNF-α infusion abolished the increase in mean arterial pressure. Interestingly, chronic infusion of TNF-α had no effect on mean arterial blood pressure in virgin rats (LaMarca et al. 2005). Furthermore, inhibition of TNF-α using the soluble receptor etanercept significantly reduced mean arterial pressure and rescued fetal growth restriction in RUPP rats (LaMarca et al. 2008). A study by Gadonski et al examined the role of IL-6 in generating pre-eclampsia-like characteristics by infusing pregnant rats with IL-6 for 5 days resulting in a 2-3 fold increase in serum IL-6 levels. As a result of the increase in circulating IL-6 levels these rats had elevated mean arterial pressure, reduced renal plasma flow and reduced glomerular filtration rates (Gadonski et al., 2006). Interestingly, these pre-eclampsia-like characteristics were not evident in virgin rats infused with IL-6 (Gadonski et al., 2006). These data indicate that elevations in maternal IL-6 may be part of the maternal inflammatory pathophysiology of pre-eclampsia.

4. The epidemiological evidence for alterations in neurodevelopmental outcomes in offspring exposed to pre-eclampsia:

We recently conducted a systematic review synthesising published, epidemiological evidence examining the association between HDP and neurodevelopmental disorders in the offspring (Maher et al., 2018). The primary outcomes included in the review were ASD and attention deficit hyperactivity disorder (ADHD). Secondary outcomes included behavioural outcomes such as Asperger's Syndrome, Pervasive Developmental Disorder - Not Otherwise Specified (PDD-NOS), behavioural difficulties using standardised checklists, as well as cognitive functioning, developmental delay and intellectual disability. In total, 61 papers were included in the review: 20 for ASD (six cohort studies and 14 case-control studies), 10 for ADHD (five

cohort studies and five case-control studies) and 31 secondary outcome papers (25 cohort studies and six case-control studies).

Pooled results from this study showed that exposure to HDP (including pre-eclampsia, gestational hypertension and chronic hypertension) was associated with a 35% increase in the odds of ASD when compared to those unexposed to HDP (OR=1.35; 95% CI: 1.11-1.64) (Maher et al., 2018). Subgroup analysis examining pre-eclampsia alone and ASD increased the odds ratio to 1.50 (95% CI: 1.26-1.78), whereas all other HDP (which may include pre-eclampsia) were associated with a non-significant increase in the odds of ASD (OR: 1.25, 95% CI: 0.90-1.73) (Maher et al., 2018) (see Table 1). However, it is important to note that the epidemiological evidence in this area is largely inconsistent. For example, some studies suggested that exposure to pre-eclampsia may be associated with a statistically significant increase in the likelihood of ASD, when compared to unexposed offspring (Buchmayer et al., 2009; Curran et al., 2018; Dodds et al., 2011; Lyall et al., 2012; Mann et al., 2010; Nath et al., 2012; Polo-Kantola et al., 2014; Walker et al., 2015), while others proposed a positive other HDP-ASD relationship (Curran et al., 2018; Dodds et al., 2011; Nath et al., 2012; Polo-Kantola et al., 2014). Similarly, there are studies that alluded to a positive pre-eclampsia-ASD relationship, (Bilder et al., 2009; Buchmayer et al., 2009; Burstyn et al., 2010; Hultman et al., 2002; Krakowiak et al., 2012; Larsson et al., 2005; Mrozek-Budzyn et al., 2013) and others a HDP-ASD relationship (Bilder et al., 2009; Buchmayer et al., 2009; Hultman et al., 2002; Krakowiak et al., 2012) but failed to meet statistical significance. Conversely, some older studies are suggestive of a protective association between pre-eclampsia and ASD (Deykin and MacMahon, 1980; Glasson et al., 2004; Langridge et al., 2013; Lyall et al., 2012; Mason-Brothers et al., 1990; Matsuishi et al., 1999), and other HDP-ASD (Lyall et al., 2012; Langridge et al., 2013), but only two of these found a statistically significant relationship (Langridge et al., 2013; Mason-Brothers et al., 1990).

Given the non-significant pooled estimate seen with other HDP and ASD, it is difficult to hypothesise whether the type of HDP is an important factor in determining the impact on ASD risk in exposed offspring. The subgroup analysis by Maher et al (Maher et al., 2018) reported a highly statistically significant association between pre-eclampsia and ASD but a non-significant risk of ASD with other HDP (including pre-eclampsia). This may suggest that the association observed occurs not as a result of exposure to hypertension but as a result of exposure to a mediator of the complex syndrome of pre-eclampsia such as inflammation. More research is needed on the association between the type of HDP and ASD in order to examine whether pre-eclampsia only, or all HDP display a significant association with ASD.

Although the findings show an apparent HDP-ASD relationship, results may need to be interpreted with caution as several limitations were identified among ASD studies. Firstly, misclassification bias could have resulted from a lack of validated questionnaires and maternal reporting of exposure and ASD status when determining exposure and outcome status of subjects (Curran et al., 2018; Krakowiak et al., 2012; Lyall et al., 2012; Walker et al., 2015). Secondly, confounding is of particular concern in observational studies due to the lack of randomisation process, potentially leading to spurious findings. The vast majority of studies identified in the systematic review failed to control for a combination of key variables, calling into question the validity of findings. For example, only one study controlled for a combination of key variables such as maternal age, socio-economic status, ethnic origin and maternal depression (Curran et al., 2018). Finally, several studies contained small sample sizes, evident in 5 of 20 studies which had fewer than 10 cases of ASD exposed to HDP (Bilder et al., 2009; Deykin and MacMahon, 1980; Matsuishi et al., 1999; Mrozek-Budzyn et al., 2013; Nath et al., 2012). However, results of larger studies (> 10 exposed cases) that controlled for at least one potential confounder in the analysis phase of the study ranged from an OR of 1.36 to 2.36 for pre-eclampsia and 0.96 to 2.83 for other HDP (which may have included pre-eclampsia) (Maher et al., 2018).

In addition, while the results of the systematic review also suggest an association between pre-eclampsia and ASD, this association may not be specific to ASD (Maher et al., 2018). For example, adjusted pooled results in Maher et al also proposed that offspring exposed to HDP were 30% more likely to have ADHD compared to those unexposed. Subgroup analyses investigating a pre-eclampsia-ADHD relationship in isolation did not change this estimate, while the odds of ADHD was associated with a 70% increase in relation to other HDP (Maher et al., 2018). Moreover, while the evidence remains inconsistent among secondary outcome studies included in the review, there were some patterns of association between HDP and intellectual disability (ID) despite methodological differences between studies (Eaton et al., 2001; Griffith et al., 2011; Langridge et al., 2013; Salonen and Heinonen, 1984). For example, results from Griffith et al 2011 suggested that pre-eclampsia/eclampsia was associated with a 38% increase in the odds of ID (95% CI: 1.16, 1.64) (Griffith et al., 2011). Similarly, the relative risk for an eclampsia-'mental retardation' relationship classified according to ICD coding was 3.03 in Danish offspring less than 15 years old (Eaton et al., 2001). Langridge et al measured ID using the American Association on Mental Retardation classification system and suggested an association between HDP and moderate ID in Western Australia (OR: 1.39, 95% CI: 1.25, 1.54) (Langridge et al., 2013). Lastly, Salonen and

Heinonen used a standardised set of tests for mental performance and suggested that HDP was associated with an increased likelihood of 'mental retardation' in children aged 9-10 years in Eastern Finland (RR: 6.1, 95% CI: 1.3, 28.9) (Salonen and Heinonen, 1984).

Collectively, the epidemiological evidence points to an apparent relationship between pre-eclampsia exposure in particular, and ASD risk in exposed offspring. However, the specificity of the effects of pre-eclampsia on ASD risk, could in fact be associated with poor neurodevelopmental outcome in general as opposed to being specific to ASD (Bodnar et al., 2018). Given the available evidence that pre-eclampsia and other HDPs may impact neurodevelopmental outcomes (Maher et al., 2018), there has been an increasing focus on identifying any neuroanatomical alterations in the brain of offspring exposed to pre-eclampsia.

5. Evidence for long-term changes in the brains of offspring exposed to pre-eclampsia:

An increasing body of work has now shown that the brains of women with pre-eclampsia can undergo structural and functional changes as a result of pre-eclampsia with the suggestion that this may predispose to developing neurological deficits later in life (for a comprehensive review see (Ijomone et al., 2018). However, aside from the maternal neurological changes, there is increasing interest in the long-term changes in brains of exposed offspring. In rodent models, we have recently shown that a mild prenatal hypoxia-ischemia insult which mirrors a specific aspect of the pre-eclamptic pathology just prior to delivery, did not affect brain or birth weight, but led to social behavioural deficits in exposed offspring at postnatal day 30 (Driscoll et al., 2018). The offspring also had elevations in circulating adrenocorticotropic hormone and corticosterone indicating an exaggerated stress response, coupled with elevations in IL-6 and *IL-1β* but not *TNF-α* mRNA and protein in the brain and blood samples (Driscoll et al., 2018), which have been shown in a recent systematic review and meta-analysis to be elevated in ASD (Masi et al., 2015). These cytokines may also contribute to the evolution behavioural abnormalities in the post-natal period given that postnatal administration of IL-1Ra protected against prenatal-LPS-induced changes in brain development and associated functional deficits (Girard et al., 2012). These data suggest there may be long-term changes in the brains of pre-eclampsia exposed offspring, and characterising these changes has been the focus of recent investigations in human populations.

A recent imaging study has examined regional brain volumes and cerebral vasculature of children aged 7 to 10 years after exposure to pre-eclampsia (Ratsep et al., 2016). Specifically children that had been exposed to pre-eclampsia (mean age = 9.79 ± 0.89 years; n = 10; 5 male, 5 female) were matched based on age and sex to those born from an uncomplicated pregnancy

(mean age = 9.66 ± 1.07 years; *n* = 10; 5 male, 5 female). This cohort then underwent magnetic resonance (MR) imaging to identify any brain structural and vascular anatomic differences. While there were no significant differences in total intracranial brain volume between the control group and children from mothers exposed to pre-eclampsia, the pre-eclampsia group had significant larger regional brain volumes in five of twenty-one regions analysed that included the cerebellum, temporal lobe, left amygdala, right amygdala and the brainstem (Ratsep et al., 2016). It is important to note however that there were no significant differences in gestational age (controls = 39.47 ± 1.38 weeks vs. pre-eclampsia = 37.16 ± 3.34 weeks), there was a significant difference in birth weight (controls = 3.42 ± 0.36 kg vs. pre-eclampsia = 2.67 ± 0.79 kg) in this study which may have confounded these results (Ratsep et al., 2016).

Interestingly, however, these alterations in regional brain volumes have also been reported in children with ASD (Ha et al., 2015; Lainhart, 2015). In particular, the increases in amygdala volume has been reported in a number of studies (Nordahl et al., 2012). In addition, a recent follow up study in this same cohort as Ratsep et al, employed diffusion tensor MR imaging (DTI) to examine myelination patterns and white matter connectivity and six brain regions of interest were identified for analysis by tractography (middle occipital gyrus, caudate nucleus and precuneus, cerebellum, superior longitudinal fasciculus, and cingulate gyrus) (Figueiro-Filho et al., 2017). They reported increased tract volumes in a number of these brain regions including the superior longitudinal fasciculus, which is strongly related to language and communication pathways (Kamali et al., 2014). Interestingly, while the molecular mechanisms that underlying these neuroanatomical changes are unknown, a recent study have shown that exposure of fetal cortical neurons to serum of women with established pre-eclampsia lead to increases in axonal growth and branching (Curran et al., 2018). This suggests that pre-eclampsia exposure may alter neurodevelopmental trajectories, but to our knowledge this causative basis of altered brain volumes in offspring exposed to pre-eclampsia are currently unknown and in addition these studies require confirmation in larger patient cohorts.

6. How might maternal inflammation in pre-eclampsia alter neurodevelopmental outcome?

Given the epidemiological evidence for an association between pre-eclampsia and neurodevelopmental outcome, then a key question is what are the mechanisms that mediate this association? Given that maternal inflammation is a core feature of the maternal pathophysiology of pre-eclampsia (Lau et al., 2013) and systematic evidence has reported

maternal inflammation as a risk factor for ASD (Jiang et al., 2016), it is possible that pre-eclampsia-induced maternal inflammation is a determinant of fetal neurodevelopmental outcome.

Arguably IL-6 is one of the best characterised mediator of the impacts of maternal inflammation on fetal neurodevelopmental outcome. Animal models of maternal inflammation have shown that maternal administration of the viral mimetic, poly(I:C), lead to elevations in maternal and fetal IL-6 levels, and alter neurobehavioural outcomes in the offspring (Meyer et al., 2006). Blocking IL-6 signalling through maternal co-administration of anti-IL-6 antibodies with poly(I:C), prevented the poly(I:C)-induced social deficits and transcriptional changes in the brains of exposed offspring (Smith et al., 2007). Interestingly there is also increased IL-6 expression and signalling in the placenta in the poly(I:C) model suggesting that conditions that increase maternal-placental IL-6 signalling may lead to detrimental effects in the fetal brain (Hsiao and Patterson, 2011). This has recently been addressed in an elegant study by (Wu et al., 2017) who addressed the role of maternal IL-6. The authors crossed *il-6*$^{+/+}$ males with *il-6*$^{-/-}$ females (resulting in a pregnant dam who cannot mount an IL-6 response), and in parallel crossed *il-6*$^{-/-}$ males with *il-6*$^{+/+}$ females (resulting in a pregnant dam who can mount an IL-6 response). Poly(I:C) administration to these pregnant dams led to increases in fetal brain IL-6 levels only in offspring from *il-6*$^{+/+}$ females (Wu et al., 2017). Moreover, conditional deletion of the IL-6 receptor in the placental trophoblast prevented the maternal poly(I:C)-induced fetal brain inflammatory response, neuroanatomical changes and anti-social and repetitive/anxiety-like behaviour in exposed offspring (Wu et al., 2017).

While the majority of these studies have been carried out in rodent models, a recent study in humans reported the association between maternal IL-6 in pregnancy and the structural connectivity of frontolimbic circuitry, which is critical for socioemotional and cognitive development, in thirty infants (Rasmussen et al., 2018). Specifically, diffusion tensor imaging revealed that maternal IL-6 levels averaged across pregnancy were inversely associated with fractional anisotropy (a measure of brain connectivity) and offspring cognition at 12 months of age (Rasmussen et al., 2018). Other studies have also show that third trimester maternal IL-6 levels, are associated with neonatal functional connectivity and with both fetal heart rate variability and toddler cognitive development (Spann et al., 2018). This is in agreement with the report that higher average maternal IL-6 was prospectively associated with larger right amygdala volume and selected stronger bilateral amygdala connectivity (Graham et al., 2018). Interestingly, larger newborn right amygdala volume and stronger left amygdala connectivity

mediated the association between higher maternal interleukin-6 concentrations and lower impulse control at 24 months of age (Graham et al., 2018). Moreover, mothers of children with ASD with intellectual disability had significantly elevated mid-gestational levels IL-6 (Jones et al., 2016). However while these correlations do not prove causation, recent work has shown that maternal depressive symptoms are associated with higher maternal inflammation, including IL-6, which mediates the effect on maternal report of infant negative affect (Gustafsson et al., 2018). This study provides proof-of-principle that pre-eclampsia-induced elevations in maternal IL-6 may act as a mediator of the pre-eclampsia-ASD association. Collectively these data support the premise that fetal exposure to pre-eclampsia-induced alterations in maternal IL-6 and maternal-placental IL-6 signaling may increase the risk of adverse neurodevelopmental outcomes, and perhaps even increase the risk of neurodevelopmental disorders in genetically predisposed offspring.

7. Conclusions and future perspectives:

Future epidemiological research examining the association between pre-eclampsia and ASD in particular and neurodevelopmental disorders in general, should address the limitations and gaps in the current literature we have recently discussed (Maher et al., 2018). In particular, large population-based cohort studies with valid methods to identify women with HDP and children with ASD are needed. It is important that such studies be able to adjust for key potential confounders such as maternal and paternal age, maternal body mass index, socio-economic status factors, behavioural factors, family history of mental disorders, ethnic origin and maternal morbidity such as diabetes. In addition, such studies should attempt to assess whether observed associations between HDP and ASD is HDP type specific, whether the association is specific to ASD, or ASD and other neurodevelopmental and psychiatric disorders. Whether other pregnancy complications and early life events have effect modification or mediation role in the HDP-ASD association is worth investigating as such analyses may improve our understanding of the association and the potential mechanisms. Moreover an important gap in the literature is the potential impact of antihypertensive medications on any observed association. In other words, is the observed association between pre-eclampsia and ASD related to the HDP or pharmacological treatments used during pregnancy? This is an important question for future research.

In future work it will also be important to examine neuroanatomical and neurobehavioural outcomes across the life span using multiple pre-clinical models of pre-

eclampsia, and in clinical cohorts. While the longitudinal nature of these studies are challenging in humans, imaging and developmental assessments of adequately powered cohorts of offspring exposed to pre-eclampsia and appropriate matched controls will be important given recent studies showing changes in the brains of pre-eclampsia-exposed offspring (Figueiro-Filho et al., 2017; Ratsep et al., 2016). Combining this with animal modelling will allow the role of maternal inflammation and in particular IL-6 as mediator of the association to be determined, using elegant approaches reported by Wu et al (Wu et al., 2017). It will also be important to not limit studies of potential mediators to IL-6 and explore a range of other potential inflammatory mediators including but not limited to TNF-α and IL-17 (Choi et al., 2016; Jones et al., 2016). Moreover, given a recent study showing that most significant genetic variants associated with schizophrenia converge on a developmental trajectory sensitive to events that affect the placental response to *in utero* stressors, including pre-eclampsia (Ursini et al., 2018), understanding the placental response in pre-eclampsia and how this may predict or be associated with neurodevelopmental outcomes in pre-eclampsia-exposed offspring (O'Keeffe and Kenny, 2014), will be important questions for future research.

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Figures and Figure Legend

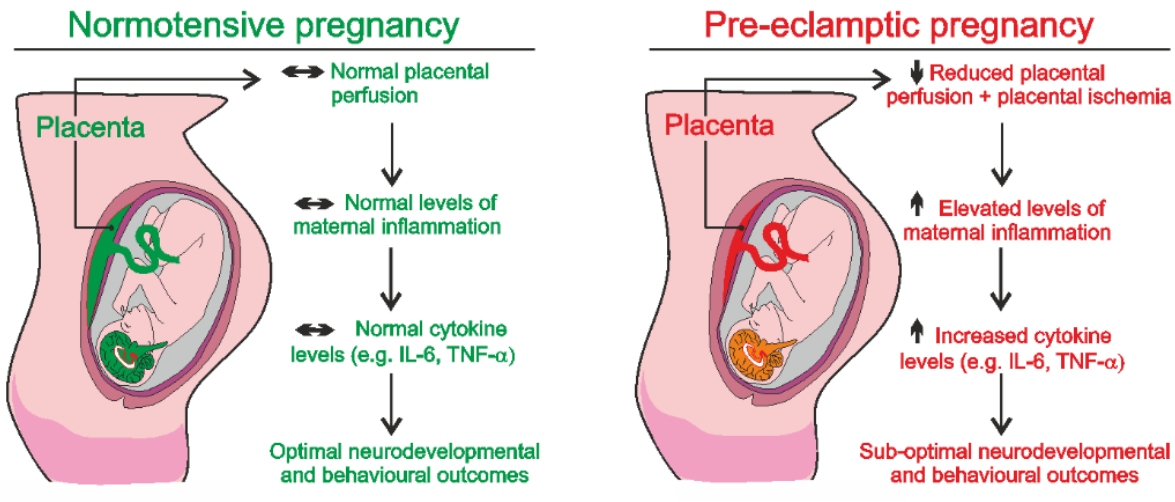

Figure 1. Schema showing an overview of how pre-eclampsia may impact neurodevelopmental outcomes in exposed offspring. While physiological levels of maternal inflammation plays a role in an uncomplicated pregnancy, the decrease in placental perfusion in pre-eclampsia leads to the increased production of pro-inflammatory cytokines including IL-6 and TNF-α. These cytokines may disrupt placenta signalling and/or cross to the fetal circulation to alter fetal neurodevelopmental trajectories, which may increase the risk of sub-optimal neurodevelopmental outcomes in offspring exposed to pre-eclampsia.

Table 1: Summary of studies examining pre-eclampsia and ASD

Author	Design	N	Prenatal Stressor	Outcomes	Results	cOR/RR (95% CI)	aOR/RR (95% CI)
Walker et al., 2015	Case-control	867	PE from medical records or maternal self-reporting in telephone interview	ADOS ADI-R	↑ odds ASD*	2.58 (1.31, 5.11)	2.36 (1.18, 4.72)
Mrozek-Budzyn., 2013	Case-control	288	PE from medical records or self-reporting	ICD-10	↑ odds ASD	2.05 (0.58, 7.28)	-
Lyall et al., 2012	Cohort	66445	Toxemia self-reported in questionnaire	Maternal-reported	↑ odds ASD*	1.24 (0.99, 1.55)	1.36 (1.04, 1.78)
Burtsyn et al., 2010	Cohort	216342	PE from APHP delivery records	ICD-9	↑ odds ASD	1.91 (1.30, 2.81)	1.49 (1.00, 2.22)
Mann et al., 2010	Cohort	87677	PE/eclampsia from billing records for Medicaid-eligible women (ICD-9)	ICD-9 from Medicaid billing records or DDSN	↑ odds ASD*	1.85 (1.38, 2.48)	1.69 (1.26, 2.27)
Buchmayer et al., 2009	Case-control	7296	PE from MBR (ICD-9, ICD-10)	ICD-9, ICD-10	↑ odds ASD*	1.41 (0.98, 2.03)	1.64 (1.08, 2.49)
Larsson et al., 2005	Case-control	18148	PE from MBR	ICD-8 and ICD-10 from PCR	↑ odds ASD	1.54 (0.83, 2.86)	-
Glasson et al., 2004	Case-control	1627	PE (ICD-9)	DSM	↓ odds ASD	0.90 (0.50, 1.62)	-
Eaton et al., 2001	Case-control	103021	Eclampsia from MBR	ICD from PCR	↓ odds ASD	0.82 (NR)	-
Matsuishi et al., 1999	Case-control	232	Toxemia	DSM-III-R	↓ odds ASD	0.82 (0.18, 3.79)	-
Mason Brothers et al., 1990	Case-control	285	Toxemia from medical records	DSM-III	↓ odds ASD	0.36 (0.16, 0.83)	-
Deykin et al., 1980	Case-control	364	Toxemia from medical records and interview data	≥1 symptoms of impaired relatedness to the environment, stereopathy and impaired language development	↓ odds ASD	0.83 (0.25, 2.70)	0.90 (0.50, 1.62)

*Adjusted result was statistically significant.

95% confidence interval (95% CI); Pre-eclampsia (PE); Autism Diagnostic Observation Schedule (ADOS); Autism Diagnostic Interview, Revised (ADI-R); Alberta Perinatal Health Program (APHP); Department of Disabilities and Special Needs, South Carolina (DDSN); Medical Birth Register (MBR); Psychiatric Central Register (PCR); Diagnostic and Statistical Manual of Mental Disorders (DSM); not reported (NR); Diagnostic and Statistical Manual of Mental Disorders–3rd Edition Revised (DSM-III-R