Commentary

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The Impact of Maternal Inflammatory Conditions During Pregnancy on the Risk of Autism: Methodological Challenges

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In the current issue of *Biological Psychiatry: Global Open Science*, Croen *et al.* (1) report the results of a case-control study that examined the association between inflammatory conditions during pregnancy and the risk of autism spectrum disorder (ASD) and other neurodevelopmental disorders. They proposed that maternal inflammation during pregnancy stemming from immune or metabolic dysregulation would adversely impact ASD risk, and that individual and combinations of maternal conditions may lead to different outcomes in offspring. They found that maternal asthma (odds ratio [OR] = 1.62 [95% CI, 1.15–2.29]) and obesity (OR = 1.51 [95% CI, 1.07–2.13]) are associated with a higher risk of ASD in offspring. Strikingly, the combination of asthma and extreme obesity had the greatest odds of ASD (OR = 16.9 [95% CI, 5.13–55.71]), among female children only. This suggests that while individual maternal conditions can impact a child's risk of ASD, the combination of certain maternal conditions may have a significantly greater impact on this risk than either condition alone.

The research on associations between pregnancy risk factors and the risk of child morbidity, including neurodevelopmental disorders, is not without complex methodological issues. These associations can be confounded by familial genetic and environmental factors and confounding by indication. For example, studies on pregnancy risk factors and the risk of ASD should adjust for maternal—and possibly family—history of mental health. In the last 2 decades, tens of studies and meta-analyses have reported statistically significant associations between various pregnancy risk factors, such as Caesarean section, preeclampsia, maternal age, maternal obesity, and child morbidity, such as autism, asthma, and autoimmune disease, among others [see Keag *et al.* (2)].

These associations are not examined using randomized controlled trials, as this is not feasible and is often impossible, considering that the pregnancy risk factors being examined, lead to potential residual confounding. To give an example of confounding: in the last 2 decades, there has been a large body of evidence suggesting that children born by Caesarean section are at increased risk of ASD, asthma, and type 1 diabetes, among others, compared with children born by vaginal birth (2). The proposed associations were thought to be explained by the hygiene hypothesis where children born by Caesarean section, especially elective Caesarean section, are not exposed to vaginal microflora; subsequently, their immune systems are compromised, leading to an increased risk of morbidity (3). We, and others, examined these associations using data from Scandinavian national health data registers and were able to conduct sibling-control studies. The sibling-control analyses, in simple terms, indirectly control for shared familial factors such as shared genes, undiagnosed maternal morbidity including mental health, and lifestyle factors that remain constant across pregnancies. These factors are difficult to control in observational studies. When conducting traditional cohort analyses, the previously reported findings were confirmed; however, when we did the sibling-control analyses, the associations almost disappeared, especially in relation to elective Caesarean section compared with vaginal delivery (4). The conclusion was that although children born by elective Caesarean section were at increased risk of ASD, the association was not causal, and can be explained by familial genetic and lifestyle factors or due to confounding by indication, i.e., indications of Caesarean section. Similar findings were found regarding asthma, attention-deficit/hyperactivity disorder, type 1 diabetes, and adult psychosis. The association between Caesarean section and type 1 diabetes was suggested to be not causal using the sibling-control study design with Swedish data but also by controlling for maternal type 1 diabetes with Danish data. The sibling-control approach was also helpful in showing that although association between preeclampsia and ASD was attenuated from a hazard ratio (HR) of 1.25 (95% CI, 1.19–1.30) to 1.17 (95% CI, 1.06–1.28), the association remained statistically significant and that children who were exposed to preeclampsia and born small for gestational age were at increased risk of ASD even when using sibling-control analyses (5). It should be noted, however, that the sibling-control analysis is not without limitations and could be biased toward the null in some studies.

Croen *et al.* (1) proposed that maternal inflammation during pregnancy would adversely impact ASD. A potential role for maternal inflammation in mediating the association between preeclampsia and ASD is supported by preclinical studies showing that offspring from the L-NAME preeclampsia mouse model exhibited ASD-like phenotypes and elevated nuclear factor-κB signaling in the fetal cortex (6). Tumor necrosis factor α (TNF-α) neutralization during gestation in this model ameliorated ASD-like phenotypes and normalized nuclear factor-κB levels in the exposed offspring (6). In terms of the impact of fetal sex, it has been shown that lipopolysaccharide-stimulated peripheral blood mononuclear cell cytokine production, including that of TNF-α in early pregnancy, was greater in women carrying females versus males, despite no changes in serum cytokine levels in pregnancy in relation to

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fetal sex (7). It is also worth noting that a mouse model of maternal asthma-allergy showed elevated levels of inflammatory cytokines, including TNF-α, in both male and female maternal asthma-allergy offspring brains compared with control animals (8). This is important when considered in the context of a recent study that measured the levels of 60 cytokines and growth factors in maternal midgestational and cord blood samples from individuals with ASD (n = 457) and control individuals (n = 497) from the Norwegian Autism Birth Cohort (9). They reported a significant association between several maternal midgestational cytokines, including TNF-α, and ASD in males (adjusted OR [aOR] = 2.63 [95% CI, 2.18–3.18]) and to a great extent in females (aOR = 8.42 [95% CI, 4.03–17.59]) (9). TNF-α was one of the few cytokines in cord blood to also show this association in both males (aOR = 1.71 [95% CI, 1.44–2.03]) and females (aOR = 3.24 [95% CI, 1.95–5.36]) (9).

Croen *et al.* (1) addressed the familial confounding issue by conducting two additional analyses: polygenic risk scores and Mendelian randomization. These analyses are important as they suggested that the observed associations between maternal asthma, obesity, and the combination of both maternal asthma and obesity were not driven by the conditions themselves nor by shared genetic risk. Instead, they are likely to be driven by other shared risk factors, such as air pollution, as suggested by the authors. These results and the examples used above regarding Caesarean section and preeclampsia demonstrate the complexity of these associations and show that using traditional cohort methods alone does not answer the research questions adequately and may lead to conclusions that are not robust. This integration of different study designs and different analytical approaches that address different sources of bias, within studies or programs of research, is key for moving this research field forward and improving causal inference in fetal origins of disease studies.

Other issues in such studies are that maternal and family history factors are often based on maternal reporting, which could be problematic depending on the question asked. In addition, there could be misclassification bias when the condition, such as mental health, is not diagnosed. In some studies, maternal mental health is ignored completely, leading to reporting results that are confounded. Studies that report results that are confounded not only lead to wrong conclusions—they also cause distress to mothers who may feel guilty that they caused harm to their children, especially when the exposure is something like obesity or elective Caesarean section. Another issue is that studies rarely consider the full reproductive life of the mother to examine whether exposures such as preeclampsia and obesity (in instances where the mother's obesity status changes between pregnancies) have an impact on subsequent pregnancies or just the pregnancy that was affected by that factor. In a recent study (10), we examined the intergenerational effect of preeclampsia on ASD and found that when the mother and grandmother had preeclampsia, the HR for ASD was even higher (HR = 1.58 [95% CI, 1.02–2.46]) than when only the mother of the child had preeclampsia (HR = 1.31 [95% CI, 1.19–1.43]).

Considering the importance of these associations in our understanding of the etiology of the condition, such as ASD, and its implications on future research and the development of interventions, it is important that observational studies are done with the required rigor to produce results that are as unbiased and unconfounded as possible and subsequently improve causal inference. What is needed at this stage is joint efforts where well-designed epidemiology studies with integration of evidence from different study designs and analytical approaches and adequate statistical power are conducted in parallel with animal and/or genetic studies. Such initiatives have a much better chance of identifying causal associations, leading to opportunities to develop appropriate interventions targeting specific factors that lead to reduced risk of specific poor child outcomes.

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Article Information

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