Intersection of epigenetic and immune alterations: Implications for Fetal Alcohol Spectrum Disorder and mental health

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Abstract

Prenatal alcohol exposure can impact a wide range of physiological systems, resulting in a host of structural, neurocognitive, and behavioral abnormalities. Included in the adverse impacts associated with prenatal alcohol exposure are alterations in immune function, such an increased incidence of infections, and alterations in immune/neuroimmune parameters throughout the life-course. Epigenetic patterns have also been shown to be highly sensitive to alcohol exposure, with widespread alcohol-related alterations to epigenetic profiles resulting from prenatal alcohol exposure, including changes in DNA methylation, histone modifications, and miRNA expression. Importantly, epigenetic programs are crucial for immune system development, impacting key processes such as immune cell fate, differentiation, and activation. In addition to their role in development, epigenetic mechanisms are emerging as attractive candidates for the biological embedding of environmental factors on immune function and as mediators between early-life exposures and long-term health. Here, following an overview of the impact of prenatal alcohol exposure on immune function and epigenetic patterns, we discuss the potential role for epigenetic mechanisms in the reprogramming of immune function and the consequences for health and development. We highlight a range of both clinical and animal studies to provide insights into the array of immune genes impacted by alcohol exposure by way of epigenetic reprogramming. Finally, we discuss potential consequences of alcohol-related reprogramming of immune/neuroimmune functions and their effects on the increased susceptibility to mental health disorders.

Reprogramming of physiological systems by prenatal alcohol exposure

Alcohol (ethanol) exposure *in utero* can have numerous adverse effects on a developing fetus. In humans, prenatal alcohol exposure (PAE) can lead to Fetal Alcohol Spectrum Disorder (FASD), which refers to the broad spectrum of structural, neurocognitive, and behavioral abnormalities or deficits that can occur following PAE[1]. The magnitude of these effects is variable and depends on factors such as timing and level of maternal alcohol use, physiological and genetic background, and overall maternal health and nutrition[2]. Despite the innate variability of these moderating factors, children across the entire spectrum of FASD display long-term cognitive and neurobehavioral alterations, including neurocognitive impairment (i.e., cognition, learning, memory, executive function), impaired self-regulation (i.e., attention, impulsivity, behavioral regulation, stress responsiveness, mood/affect, sleep), and deficits in adaptive functioning (i.e., communication, social behavior, activities of daily living)[3-8]. Taken together, these findings highlight the complex reprogramming effects of PAE on neurobehavioral and neurobiological systems. However, the mechanisms underlying the pervasive and multisystem impacts of alcohol are not yet fully understood.

The Developmental Origins of Health and Disease (DOHaD) hypothesis provides an important framework to interpret both the transient and long-term effects of PAE[9]. This hypothesis developed through mounting evidence indicating that early-life exposures or events can have a long-lasting impact on adult health outcomes[10-13]. Importantly, the DOHaD hypothesis suggests that early environments can exert their influence on long-term health through the mechanism of fetal programming, by which early life experiences shape development of neurobiological and physiological systems, altering their function over the lifespan. However, the mechanisms that drive the reprogramming of physiological systems are not yet fully understood.

Disruption of immune development has been recognized as a “pathway to pathology”, impacting risk for both childhood and adult diseases[14]. Specifically, the field of developmental immunotoxicity has identified a wide range of agents capable of immune disruption or programming of

93 the immune system with long-term health consequences, and these include diet, environmental
94 chemical, physical factors such as UV radiation, and notably alcohol/drug exposure, as well as
95 psychological factors[14]. While the molecular mechanisms underlying these effects have yet to be firmly
96 established, epigenetic mechanisms are emerging as attractive candidates for the biological embedding
97 of environmental factors on immune function, as they may link external stimuli and physiological
98 systems to influence health and behavior into later life[15-18]. In the present review, we address the impact
99 of PAE on immune function and epigenetic patterns, highlighting a potential role for epigenetic
100 mechanisms in the reprogramming of immune function and risk for mental disorders.
101
102 **Impact of prenatal alcohol exposure on immune and neuroimmune function**
103 Clinical data examining alcohol-induced alterations in immune competence in children and
104 adults with FASD remain somewhat limited [reviewed in[19, 20]]. However, one of the earliest and most
105 consistently clinically demonstrated effects of PAE is increased infection rates. Specifically, early
106 investigations identified that children with FAS have a higher incidence of a range of major and minor
107 infections, including recurrent otitis media, upper respiratory tract infections, urinary tract infections,
108 sepsis, pneumonia, and acute gastroenteritis[21-23]. In addition, decreased eosinophil and neutrophil cell
109 counts in alcohol-exposed compared to unexposed children, and decreased leukocyte response to
110 mitogens ([21]; reviewed in[24]) were observed. More recently, Gauthier and colleagues reported that very
111 low birth weight newborns exposed to alcohol *in utero* have a 15-fold higher incidence of early-onset
112 sepsis compared to matched controls[25]. Furthermore, high levels of maternal drinking (binge drinking),
113 specifically during the second trimester, has also been shown to increase the risk of infection by
114 approximately 4-fold, compared to that of unexposed newborns[26]. And similarly, Libster and
115 colleagues have also reported increased rates of adverse infection outcomes in young children (< 2
116 years of age) with PAE who were hospitalized for acute respiratory infections[27].

Several studies have also explored the link between PAE and atopic disorders (mainly dermatitis, eczema and asthma). Specifically, PAE is associated with an increased risk of dermatitis[28, 29]; however, the risk of eczema following PAE is less clear, as there are conflicting reports of PAE increasing risk of eczema[30] and having no associations with eczema[31, 32]. By contrast, there are consistent reports of a lack of association between PAE and asthma, with studies including a range of developmental time points and alcohol exposure levels[30, 31, 33-35].

Although the mechanisms underlying the immunoteratogenic effects of PAE remain unclear, alcohol-induced immune system alterations can have negative developmental outcomes. For example, alcohol consumption increases circulating cytokine levels[36, 37], with chronic alcohol consumption during pregnancy increasing levels of key cytokines in both the fetus and mother[38]. Importantly, as maternal cytokine induction can have a considerable impact on fetal development[39], alcohol-induced maternal immune system activation may drive some of the adverse developmental outcomes that occur following PAE. Indeed, our work has shown that alcohol consumption during pregnancy alters the maternal cytokine milieu, through activation and/or inhibition of key cytokine networks. Importantly, these distinct maternal immune profiles could be used to predict neurodevelopmental status (i.e., typical development vs. neurodevelopmental delay), identifying children with high risk or resilience to alcohol-induced neurodevelopmental delay[40]. We have also shown that child cytokine networks are themselves disrupted following PAE and that network activity patterns again differ based on neurodevelopmental status of the child (i.e., typical development vs. neurodevelopmental delay)[41], further linking cytokine disruptions to altered developmental outcomes. Finally, while studies investigating immune outcomes in older individuals is extremely limited, there is evidence for increased rates of atopic conditions and elevated lymphocyte counts in adolescents with PAE[33]. Overall, despite clear evidence of immune alterations associated with PAE, and associated developmental alterations, the mechanism(s) underlying the impact of alcohol exposure on immune function, remain to be determined. Importantly, *in utero* alcohol exposure appears to induce long-

lasting changes in immune function; however, most immune cells themselves are not long-lived, and as such, mechanistic investigations will be required to fill this gap.

Animal model experiments have allowed for more in depth explorations of the immune disturbances associated with PAE, and in particular, have allowed for investigations into the impact of PAE on the neuroimmune system. Work from a range of animal models has shown that alcohol exposure generally increases cytokine production within the brain, a marker of neuroimmune activation (reviewed in **Table 1**). In third trimester equivalent exposure models, alcohol increased cytokine levels in the cerebellum, cortex, and hippocampus in alcohol exposed animals[42, 43]. Our laboratory has also identified alterations in cytokine levels in the brain following alcohol exposure throughout gestation (i.e., first and second trimester equivalent), finding increased cytokine levels in the hippocampus, and prefrontal cortex but decreased cytokine levels in the hypothalamus[44]. Despite inherent differences between these models, such as method and timing of alcohol administration, species, and cytokine detection method, the overall concordance of these findings highlights that neuroinflammation may be a cross-cutting feature in both FASD and animal models of PAE.

In addition to alterations in cytokine levels, alcohol exposure can alter microglial levels and/or activational status[42, 43, 45-50] [reviewed in[51-53]]. Importantly, during early development, microglia, resident macrophages of the CNS, exist in an activated state. In this state, microglia produce cytokines[54, 55] and contribute to brain development through their important roles in neurobiological processes, including phagocytosis of newborn neurons[56], synaptic pruning and maturation[57], remodelling of synaptic circuits[58], and synaptic plasticity[59]. As a result, alterations in microglial populations and activational status may be an important mechanism through which alcohol exposure impacts early brain development. By weaning, microglia transition to a quiescent state and remain relatively inactive throughout adulthood, unless activated by injury or immune challenge [54]. However, alcohol exposure may impair/delay the transition of microglia to a quiescent state[43] and as such, may result in heightened responses to challenges such as infections, with potential consequences for

167 behavior and cognition [reviewed in[60]. Thus, microglia are uniquely poised to retain an immunological
168 memory of early-life insults, such as exposure to alcohol, due the long-lived nature of these cells amid
169 a more ephemeral immune cell background. Nevertheless, the mechanisms underlying the impact of
170 alcohol exposure on cytokine levels and microglial activation remain unknown. Based on evidence of
171 fetal programming by PAE and from other models where epigenetic alterations of microglia are
172 associated with neuroinflammation[61, 62], we propose that epigenetic influences may be a critical link,
173 tying together alcohol exposure and long-term impacts on immune function and subsequent health
174 outcomes.
175
176 **Epigenetic mechanisms bridge early-life environments and long-term health**
177 Epigenetics refers to modifications of DNA and/or its regulatory factors that mediate the
178 accessibility of DNA, which can, in turn, modulate gene expression and cellular functions without
179 changes to underlying genomic sequences[63]. These regulatory factors include histone modifications,
180 non-coding RNA (ncRNA), and direct DNA modifications, such as methylation and
181 hydroxymethylation. In general, epigenetic patterns are closely associated with cellular specification
182 and differentiation, highlighting their role in the regulation of cellular functions[64]. As each cellular
183 subtype is closely associated with a characteristic epigenomic landscape that provides long-term
184 stability to its identity, cell type is the main driver of stable epigenetic patterns. However,
185 environmental stimuli can also influence epigenetic patterns throughout the genome, although with
186 subtler effects than ontogenic profiles. These mechanisms rely on an apparent paradox between the
187 stability of cell-specific profiles and plasticity in response to external cues to modulate both short- and
188 long-term epigenetic regulation[65, 66]. Overall, epigenetic patterns act in concert to fine-tune the cellular
189 response to external stimuli and regulate cellular functions [reviewed in[67]]. Importantly, emerging
190 evidence suggests that epigenetic patterns, such as DNA methylation, may mediate the relationship

between environmental insults and chronic disease, highlighting a potentially crucial role in our understanding of the biological embedding of early-life exposures[18].

Prenatal alcohol exposure alters epigenetic programs

The initial evidence that epigenetic mechanisms might be involved in programing of physiological function by PAE originated from studies of gene expression. Genome-wide alterations to gene expression patterns occur in the brains of fetal, neonatal, and adult animals following PAE, highlighting that the effects of alcohol may shift early developmental trajectories and lead to persistent alterations in adulthood[68-70], with a recent study identifying large-scale alterations to neuroimmune gene networks of the olfactory system[71] . Our work has also shown that PAE animals show changes in the brain's transcriptome both under basal conditions and in response to an immune challenge, suggesting reprogramming of neuroendocrine and neuroimmune function by alcohol[72]. Studies investigating possible epigenetic mechanisms of developmental alcohol exposure followed closely behind the initial gene expression studies, identifying widespread alterations to epigenetic profiles in both central nervous system and peripheral tissues, including DNA methylation, histone modifications, and miRNA expression [reviewed in[73]]. These findings have highlighted a potential role for epigenetic factors in the reprogramming of neurobiological functions by PAE in both animal models and clinical cohorts of FASD. Although relatively few epigenome-wide studies have been performed to date, several have identified alcohol-induced alterations to genes involved in immune function[74-85] (**Table 2**). These findings provide further evidence that the immune deficits observed following PAE may be linked to changes in epigenetic patterns.

Epigenetic programs play a key role in immune system development and function

Epigenetic programs are crucial to the broader development and function of the immune system, playing important roles in the regulation of immune cell development and identity and

neuroinflammatory processes[86]. Given the vital role of epigenetic mechanisms in the regulation of cell fate, it is perhaps not surprising that epigenetic mechanisms play an important part in the developmental cascades associated with immune cell differentiation[87]. In particular, epigenetic processes regulate stem-cell properties of progenitor cells and become increasingly specialized as immune cells progress through lineage commitment. The vital importance of these patterns is exemplified by the deficits in immune development and function in animals lacking components of the epigenetic machinery, including DNA methyltransferase (DNMTs) and histone deacetylases (HDAC)[87-90]. miRNAs also play a key role in immune system development, displaying unique expression signatures in different cellular subtypes, including microglia, granulocytes, and monocytes, which likely help modulate their specific functions and developmental trajectories[91-94]. Moreover, the activation state of immune effectors relies on epigenetic mechanisms, particularly histone modifications, to induce the phenotypic alterations necessary for their rapid response to pathogens[87, 95-98]. Of particular relevance to the current review, the epigenetic profiles of microglia are closely linked to neuroinflammatory processes, reflective of their role in the immune response of neural tissues[61, 62, 99]. Importantly, the epigenetic responsivity of microglia to both external and internal signals may play a crucial role in modulating the inflammatory status of the brain, which has important ramifications for neurobiological functions[100]. Overall, immune system development occurs in parallel with epigenetic changes in immune cells, which are responsive to both environmental and biological cues.

Several lines of evidence also suggest that developmental exposures can influence epigenetic patterns within the developing organism to potentially alter immune function and susceptibility to neurobiological deficits later in life[101]. For instance, increased maternal care can alter IL-10 expression and DNA methylation in microglial cells to diminish morphine-induced addictive behavior [102, 103]. These findings highlight the role of early life experiences in shaping developmental trajectories within the immune system and suggest that epigenetic mechanisms could play an integral role in the reprogramming of immune functions by PAE.

Epigenetic mechanisms may influence the immune alterations associated with PAE

Studies investigating epigenetic mechanisms involved in PAE effects have also identified alterations to cytokines, chemokines, and signalling pathways involved in the cellular response to immune molecules. **Table 2** outlines the findings from genome-wide studies of PAE, highlighting epigenetic alterations to genes involved in immune response and regulation (immune gene annotations obtained from immport.org, May 2021)[104]. Of particular note, results from work on a cohort of children with FASD showed that DNA methylation levels in buccal epithelial cells showed alterations in HLA-DPB1, a component of the major histocompatibility complex previously associated with rheumatoid arthritis[79, 81, 105, 106]. Importantly, both evidence from animal models[107] and reports from a recent informal health survey in adults with FASD[108] indicate that the incidence of rheumatoid arthritis is higher following PAE. While the findings of altered HLA-DPB1 were identified in a peripheral tissue not involved in immune modulation, they may provide insight into changes in global epigenetic patterns associated with altered immune profiles in children with FASD. Members of the complement system, a key immune pathway that promotes inflammatory responses to combat infection[109], also appear across multiple studies, from animal models to clinical cohorts of FASD. For instance, CFP displays alterations in both mouse and human embryonic cells exposed to alcohol, while C1R and C1S show alterations in the peripheral tissue of individuals with FASD[75, 79].

Through the use of animal models, which have the advantage of being able to assess the brain directly, several candidate genes involved in the reprogramming of neuroimmune functions have been identified as being altered following PAE, particularly within genes within the TNF receptor CXC/CC chemokine families[75, 77, 79]. Furthermore, almost every study of epigenetic patterns and PAE identified alterations to genes involved in cytokine/chemokine/interleukin expression and signaling, showing clear parallels and links to changes in circulating levels described earlier. Importantly, several immune system genes were common across both model organisms and human populations, including CFP, CRH, CSF1, FGFR2, ITGAL, PGFRA, PTHLH, and VIPR2, which may point to potentially pathways

necessitating mechanistic follow-up studies to assess whether they may be suitable targets for intervention.

Beyond the genes directly involved in immune system functioning, several immune-related transcription factors also show differential epigenetic profiles following PAE and could play a role in the altered genomic response to immune signals. Of note, PPARG , a transcription factor that promotes anti-inflammatory processes[110], shows differential DNA methylation and expression in the brain following PAE and has been previously implicated in the prevention alcohol-induced cell death[50, 75]. Finally, the polycomb group proteins are also altered by PAE and have been implicated in the deficits caused by PAE, as they play a key role in modulating the stem-cell properties of neural stem cells and immune cell progenitors[111, 112]. Taken together, epigenetic alterations to immune genes provides a potential mechanistic link between PAE and long-lasting immune system dysfunction.

Finally, and in addition to DNA and protein-based epigenetic alterations, several differentially expressed miRNAs known to be critical regulators of neuroimmune function have also been identified in PAE models, such as miR-9, -21, -153, -155, and -335[62, 113-117]. For example, alcohol exposure increases miR-155 expression, which typically promotes the secretion of pro-inflammatory cytokines by microglial cells following Toll-like receptor activation[117, 118]. By contrast, alcohol decreases miR-21 expression, a neuroprotectant that suppresses FasL levels, which may lead to greater vulnerability to microglial-induced cell death[113, 114, 119]. These data suggest that developmental alcohol exposure may shift the balance of different neuroimmune cell types in the brain, as well as the cytokines they produce, setting the stage for more robust neuroinflammatory responses. This possibility represents an important consideration for epigenetic studies, as cell type proportions are the major drivers of epigenetic patterns and must be taken into consideration when analyzing these types of data. To this point, a recent single-cell RNA-sequencing study of GD14.5 mice exposed to binge-levels of alcohol showed that PAE altered the cell cycle status of microglia in the ventricular zone, suggesting that alcohol may shift the developmental trajectories of neuroimmune pathways and mechanisms[120]. These

results also highlight the importance of timing in the study of PAE, as identifying the developmental trajectories of key neurobiological pathways may provide profound insight into the mechanisms that drive the effects of PAE on neurodevelopmental and physiological outcomes. Taken together, these findings suggest a complex interplay between the immune system and epigenomic profiles, which may partially influence the neurobiological and neuroinflammatory profiles observed following PAE, and, in the future, may enable us to describe unique immune and neuroinflammatory signatures in FASD.

Epigenetic dysregulation of immune function – the missing link between PAE and mental health disorders?

Individuals with FASD experience higher rates of mental health problems. In the general population, approximately 20% of individuals experience a mental health disorder[121], whereas 90% of individuals with FASD have a mental health disorder, with anxiety and depression among the most common[122-124]. Although the molecular mechanisms underlying this increased vulnerability in alcohol-exposed individuals remains unclear, alterations in the epigenetic regulation of immune genes resulting in abnormal immune/neuroimmune functioning has been implicated in the pathophysiology of a number of mental health disorders[125]. For instance, individuals diagnosed with major depressive disorder (MDD) show increases in circulating leukocytes and proinflammatory cytokine production [reviewed in[126]], with higher childhood levels of IL-6 and C-reactive protein (CRP) potentially predating the onset of depression[127]. Importantly, these differences are linked to epigenetic alterations, as blood cells from individuals with a lifetime history of depression show alterations to DNA methylation in IL-6 and CRP[128]. Beyond these gene-specific epigenetic alterations, recent evidence from human studies shows that epigenetic risk scores for higher inflammatory status, measured through CRP levels, are associated with increased internalizing and externalizing behaviors in children[129].

Taken together, these findings suggest a correlation between alterations in immune function and increased risk of mental health disorders, which may be mediated, at least in part, through epigenetic alterations. While this connection has yet to be specifically evaluated following PAE, the high prevalence of mental disorders in individuals with FASD and lasting alterations to immune function and epigenetic programs highlight a need for future mechanistic studies that explore this complex bidirectional relationship.

Conclusions and future directions:

As a whole, it is becoming increasingly apparent that a multisystem approach is needed to gain a better understand of mechanisms underlying the teratogenic effects of alcohol. To that end, we propose that immune disturbances arising as a result of *in utero* alcohol exposure may have long-term consequences extending beyond immune function (protection from pathogens) to include an impact on mental health and that this may be occurring through the mechanism of epigenetics. However, the findings from epigenetic studies must be interpreted with caution, as the vast majority are correlative in nature, rather than causative. As such, they do not provide a direct link between molecular mechanisms and disease and must be further assessed prior to making inferences as to causality. Nevertheless, the findings from epigenetic studies have provided important insights into potential regulatory mechanisms of immune reprogramming and may represent future targets to investigate the molecular underpinnings of alcohol-induced deficits.

Furthermore, interactions between the gut microbiome, immune system, and brain are now emerging as potential moderators of neural function and potentially disease, although their connection to neuroepigenetics and neuroinflammation remain mostly unknown[130-135]. Moving forward, and with this multisystem approach in mind, it will be important that future research also consider the impact of PAE on the gut-brain-immune axis[136], as to date, there is no research in this area. It is, however, known that chronic alcohol consumption results in compromised gut-barrier function and increased rates of

dysbiosis[137, 138] and as a result, *in utero* alcohol exposure would be expected to have an impact on the immature, developing gut. Moreover, dysbiosis during early life is linked to a proinflammatory state and an increased incidence of inflammatory-related diseases in adulthood[139-141]. Alterations in the microbiome may also confer increased risk of disease by altering immune system development and potentially inducing long-term epigenetic changes in immune regulators[125, 139]. Importantly, the establishment of the gut microbiome appears to rely partially on epigenetic mechanisms to establish microbe-T-cell mutualism, suggesting a complex interplay between physiological systems to dynamically regulate interactions between the microbiome and immune system [87]. Thus, in the context of the present overview, future work to investigate the impact of *in utero* alcohol exposure on the gut microbiome and the gut-brain-immune axis will complement the growing body of work on immune and epigenetic alterations in preclinical PAE models and clinical studies of individuals with FASD.

Finally, a better understanding of mechanisms underlying the teratogenic effects of PAE will also pave the way for the development of more informed, targeted intervention strategies for individuals with FASD. Unlike other neurodevelopmental disorders where the underlying cause(s) are still under investigation, such as autism spectrum disorder[142] or schizophrenia[143], alcohol is a known teratogen and intervention is the key to better long-term outcomes. Due to the pervasiveness of immune disturbances across PAE models[43, 144], and the link between immune function and overall physical and mental health[145], the immune system may be an ideal pharmacological target for individuals with FASD. Moreover, immune activation/cytokines play a key role in brain development, and increasing evidence demonstrates that altered immune activation may underlie altered cognition, attention, behavior, self-regulation, and adaptive functioning. Thus research on immune-based interventions will have broad implications for improving overall function of individuals with FASD [reviewed in[146]]. As such, future investigations examining the safety and utility of anti-inflammatory agents applied during early postnatal life will be important. This is particularly urgent in that currently, with the exception of ongoing work to evaluate the therapeutic potential of choline supplementation [147, 148] and evaluation of

pioglitazone in animal models[43] there are relatively few available drugs specifically shown to significantly improve the outcomes of PAE.

As a whole, the collective findings from animal models and clinical studies of FASD point to a compelling relationship between the immune system and epigenetic pathways, which may have important causal links to the long-term and multisystem effects of PAE. Ultimately, additional research in this area will not only provide deeper insight into the molecular mechanisms that influence mental health processes, but also help identify novel interventions and therapeutic strategies that may alleviate the health consequences arising from alcohol exposure.

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Conflicts of interest

The authors declare no conflicts of interest.

References:

1. Astley SJ, Clarren SK. Diagnosing the full spectrum of fetal alcohol-exposed individuals: introducing the 4-digit diagnostic code. *Alcohol Alcohol*. Jul-Aug 2000;35(4):400-10.
2. Pollard I. Neuropharmacology of drugs and alcohol in mother and fetus. *Semin Fetal Neonatal Med*. Apr 2007;12(2):106-13. doi:S1744-165X(06)00111-9 [pii] 10.1016/j.siny.2006.12.001
3. Carter RC, Jacobson JL, Molteno CD, Dodge NC, Meintjes EM, Jacobson SW. Fetal Alcohol Growth Restriction and Cognitive Impairment. *Pediatrics*. Aug 2016;138(2)doi:10.1542/peds.2016-0775
4. Lynch ME, Kable JA, Coles CD. Prenatal alcohol exposure, adaptive function, and entry into adult roles in a prospective study of young adults. *Neurotoxicol Teratol*. Sep-Oct 2015;51:52-60. doi:10.1016/j.ntt.2015.07.008
5. Panczakiewicz AL, Glass L, Coles CD, et al. Neurobehavioral Deficits Consistent Across Age and Sex in Youth with Prenatal Alcohol Exposure. *Alcohol Clin Exp Res*. Sep 2016;40(9):1971-81. doi:10.1111/acer.13153
6. Doyle LR, Mattson SN. Neurobehavioral Disorder Associated with Prenatal Alcohol Exposure (ND-PAE): Review of Evidence and Guidelines for Assessment. *Curr Dev Disord Rep*. Sep 2015;2(3):175-186. doi:10.1007/s40474-015-0054-6
7. Astley SJ, Olson HC, Kerns K, et al. Neuropyschological and behavioral outcomes from a comprehensive magnetic resonance study of children with fetal alcohol spectrum disorders. *Can J Clin Pharmacol*. Winter 2009;16(1):e178-201.
8. Streissguth AP, O'Malley K. Neuropsychiatric implications and long-term consequences of fetal alcohol spectrum disorders. *Semin Clin Neuropsychiatry*. Jul 2000;5(3):177-90.
9. Hellemans KG, Sliwowska JH, Verma P, Weinberg J. Prenatal alcohol exposure: fetal programming and later life vulnerability to stress, depression and anxiety disorders. *Neurosci Biobehav Rev*. May 2010;34(6):791-807. doi:10.1016/j.neubiorev.2009.06.004
10. Wadhwa PD, Buss C, Entringer S, Swanson JM. Developmental origins of health and disease: brief history of the approach and current focus on epigenetic mechanisms. *Semin Reprod Med*. Sep 2009;27(5):358-68. doi:10.1055/s-0029-1237424
11. Barker DJ, Osmond C. Infant mortality, childhood nutrition, and ischaemic heart disease in England and Wales. *Lancet*. May 10 1986;1(8489):1077-81.
12. Barker DJ, Winter PD, Osmond C, Margetts B, Simmonds SJ. Weight in infancy and death from ischaemic heart disease. *Lancet*. Sep 9 1989;2(8663):577-80.
13. Barker DJ, Gluckman PD, Godfrey KM, Harding JE, Owens JA, Robinson JS. Fetal nutrition and cardiovascular disease in adult life. *Lancet*. Apr 10 1993;341(8850):938-41.
14. Dietert R. Macrophages as targets of developmental immunotoxicity. *OA Immunology*. 2014;18(2(1))
15. Shulha HP, Cheung I, Guo Y, Akbarian S, Weng Z. Coordinated cell type-specific epigenetic remodeling in prefrontal cortex begins before birth and continues into early adulthood. *PLoS Genet*. Apr 2013;9(4):e1003433. doi:10.1371/journal.pgen.1003433
16. Kobor MS, Weinberg J. Focus on: epigenetics and fetal alcohol spectrum disorders. *Alcohol Res Health*. 2011;34(1):29-37.
17. Yuen RK, Neumann SM, Fok AK, et al. Extensive epigenetic reprogramming in human somatic tissues between fetus and adult. *Epigenetics Chromatin*. May 05 2011;4:7. doi:10.1186/1756-8935-4-7
18. Fujii R, Sato S, Tsuboi Y, Cardenas A, Suzuki K. DNA methylation as a mediator of associations between the environment and chronic diseases: A scoping review on application of

mediation analysis. *Epigenetics*. 2021;ahead-of-print:1-27. doi:https://doi.org/10.1080/15592294.2021.1959736
19. Bodnar T, Weinberg, J. Prenatal alcohol exposure: impact on neuroendocrine-neuroimmune networks. . In: Cui C, Grandison L, Noronha A, ed. *Neural-Immune Interactions in Brain Function and Alcohol Related Disorders*. Springer; 2013:312-362.
20. Reid N, Moritz KM, Akison LK. Adverse health outcomes associated with fetal alcohol exposure: A systematic review focused on immune-related outcomes. *Pediatr Allergy Immunol*. Nov 2019;30(7):698-707. doi:10.1111/pai.13099
21. Johnson S, Knight R, Marmer DJ, Steele RW. Immune deficiency in fetal alcohol syndrome. *Pediatr Res*. Jun 1981;15(6):908-11. doi:10.1203/00006450-198106000-00005
22. Ammann AJ, Wara DW, Cowan MJ, Barrett DJ, Stiehm ER. The DiGeorge syndrome and the fetal alcohol syndrome. *Am J Dis Child*. Oct 1982;136(10):906-8.
23. Church MW, Gerkin KP. Hearing disorders in children with fetal alcohol syndrome: findings from case reports. *Pediatrics*. Aug 1988;82(2):147-54.
24. Gottesfeld Z, Abel EL. Maternal and paternal alcohol use: effects on the immune system of the offspring. *Life Sci*. 1991;48(1):1-8.
25. Gauthier TW, Manar MH, Brown LA. Is maternal alcohol use a risk factor for early-onset sepsis in premature newborns? *Alcohol*. Jun 2004;33(2):139-45. doi:10.1016/j.alcohol.2004.06.003
26. Gauthier TW, Drews-Botsch C, Falek A, Coles C, Brown LA. Maternal alcohol abuse and neonatal infection. *Alcohol Clin Exp Res*. Jun 2005;29(6):1035-43.
27. Libster R, Ferolla FM, Hijano DR, et al. Alcohol during pregnancy worsens acute respiratory infections in children. *Acta Paediatr*. Nov 2015;104(11):e494-9. doi:10.1111/apa.13148
28. Carson CG, Halkjaer LB, Jensen SM, Bisgaard H. Alcohol intake in pregnancy increases the child's risk of atopic dermatitis. the COPSAC prospective birth cohort study of a high risk population. *PLoS One*. 2012;7(8):e42710. doi:10.1371/journal.pone.0042710
29. Linneberg A, Petersen J, Gronbaek M, Benn CS. Alcohol during pregnancy and atopic dermatitis in the offspring. *Clin Exp Allergy*. Nov 2004;34(11):1678-83. doi:10.1111/j.1365-2222.2004.02101.x
30. Wada K, Konishi K, Tamura T, Shiraki M, Iwasa S, Nagata C. Alcohol Intake During Pregnancy and Offspring's Atopic Eczema Risk. *Alcohol Clin Exp Res*. May 2016;40(5):1037-43. doi:10.1111/acer.13048
31. Shaheen SO, Rutterford C, Zuccolo L, et al. Prenatal alcohol exposure and childhood atopic disease: a Mendelian randomization approach. *J Allergy Clin Immunol*. Jan 2014;133(1):225-32 e1-5. doi:10.1016/j.jaci.2013.04.051
32. Apfelbacher CJ, Diepgen TL, Schmitt J. Determinants of eczema: population-based cross-sectional study in Germany. *Allergy*. Feb 2011;66(2):206-13. doi:10.1111/j.1398-9995.2010.02464.x
33. Oleson DR, Magee RM, Donahoe RM, Falek A, Coles CD. Immunity and prenatal alcohol exposure. A pilot study in human adolescents. *Adv Exp Med Biol*. 1998;437:255-64.
34. Yuan W, Sorensen HT, Basso O, Olsen J. Prenatal maternal alcohol consumption and hospitalization with asthma in childhood: a population-based follow-up study. *Alcohol Clin Exp Res*. May 2004;28(5):765-8. doi:10.1097/01.alc.0000125348.23133.88
35. Magnus MC, DeRoo LA, Haberg SE, et al. Prospective study of maternal alcohol intake during pregnancy or lactation and risk of childhood asthma: the Norwegian Mother and Child Cohort Study. *Alcohol Clin Exp Res*. Apr 2014;38(4):1002-11. doi:10.1111/acer.12348

36. Crews FT, Bechara R, Brown LA, et al. Cytokines and alcohol. *Alcohol Clin Exp Res*. Apr 2006;30(4):720-30. doi:10.1111/j.1530-0277.2006.00084.x
37. He J, Crews FT. Increased MCP-1 and microglia in various regions of the human alcoholic brain. *Exp Neurol*. Apr 2008;210(2):349-58. doi:10.1016/j.expneurol.2007.11.017
38. Ahluwalia B, Wesley B, Adeyiga O, Smith DM, Da-Silva A, Rajguru S. Alcohol modulates cytokine secretion and synthesis in human fetus: an in vivo and in vitro study. *Alcohol*. Jul 2000;21(3):207-13.
39. Deverman BE, Patterson PH. Cytokines and CNS development. *Neuron*. Oct 15 2009;64(1):61-78. doi:10.1016/j.neuron.2009.09.002
40. Bodnar TS, Raineki C, Wertelecki W, et al. Altered maternal immune networks are associated with adverse child neurodevelopment: Impact of alcohol consumption during pregnancy. *Brain Behav Immun*. Oct 2018;73:205-215. doi:10.1016/j.bbi.2018.05.004
41. Bodnar TS, Raineki C, Wertelecki W, et al. Immune network dysregulation associated with child neurodevelopmental delay: modulatory role of prenatal alcohol exposure. *J Neuroinflammation*. Jan 28 2020;17(1):39. doi:10.1186/s12974-020-1717-8
42. Topper LA, Baculis BC, Valenzuela CF. Exposure of neonatal rats to alcohol has differential effects on neuroinflammation and neuronal survival in the cerebellum and hippocampus. *J Neuroinflammation*. Sep 4 2015;12:160. doi:10.1186/s12974-015-0382-9
43. Drew PD, Johnson JW, Douglas JC, Phelan KD, Kane CJ. Pioglitazone blocks ethanol induction of microglial activation and immune responses in the hippocampus, cerebellum, and cerebral cortex in a mouse model of fetal alcohol spectrum disorders. *Alcohol Clin Exp Res*. Mar 2015;39(3):445-54. doi:10.1111/acer.12639
44. Bodnar TS, Hill LA, Weinberg J. Evidence for an immune signature of prenatal alcohol exposure in female rats. *Brain Behav Immun*. Nov 2016;58:130-141. doi:10.1016/j.bbi.2016.05.022
45. Fernandez-Lizarbe S, Pascual M, Guerri C. Critical role of TLR4 response in the activation of microglia induced by ethanol. *J Immunol*. Oct 1 2009;183(7):4733-44. doi:10.4049/jimmunol.0803590
46. Ruggiero MJ, Boschen KE, Roth TL, Klintsova AY. Sex Differences in Early Postnatal Microglial Colonization of the Developing Rat Hippocampus Following a Single-Day Alcohol Exposure. *J Neuroimmune Pharmacol*. Jun 2018;13(2):189-203. doi:10.1007/s11481-017-9774-1
47. Gursky ZH, Johansson JR, Klintsova AY. Postnatal alcohol exposure and adolescent exercise have opposite effects on cerebellar microglia in rat. *Int J Dev Neurosci*. Oct 2020;80(6):558-571. doi:10.1002/jdn.10051
48. Chastain LG, Franklin T, Gangisetty O, et al. Early life alcohol exposure primes hypothalamic microglia to later-life hypersensitivity to immune stress: possible epigenetic mechanism. *Neuropsychopharmacology*. Aug 2019;44(9):1579-1588. doi:10.1038/s41386-019-0326-7
49. Boschen KE, Ruggiero MJ, Klintsova AY. Neonatal binge alcohol exposure increases microglial activation in the developing rat hippocampus. *Neuroscience*. Jun 02 2016;324:355-66. doi:10.1016/j.neuroscience.2016.03.033
50. Kane CJ, Phelan KD, Han L, et al. Protection of neurons and microglia against ethanol in a mouse model of fetal alcohol spectrum disorders by peroxisome proliferator-activated receptor-gamma agonists. *Brain Behav Immun*. Jun 2011;25 Suppl 1:S137-45. doi:10.1016/j.bbi.2011.02.016
51. Kane CJM, Drew PD. Neuroinflammatory contribution of microglia and astrocytes in fetal alcohol spectrum disorders. *J Neurosci Res*. Aug 2021;99(8):1973-1985. doi:10.1002/jnr.24735

52. Wilhelm CJ, Guizzetti M. Fetal Alcohol Spectrum Disorders: An Overview from the Glia Perspective. *Front Integr Neurosci*. 2015;9:65. doi:10.3389/fnint.2015.00065
53. Mahnke AH, Adams AM, Wang AZ, Miranda RC. Toxicant and teratogenic effects of prenatal alcohol. *Curr Opin Toxicol*. Apr 2019;14:29-34. doi:10.1016/j.cotox.2019.08.002
54. Ling EA, Wong WC. The origin and nature of ramified and amoeboid microglia: a historical review and current concepts. *Glia*. Jan 1993;7(1):9-18. doi:10.1002/glia.440070105
55. Fujita S, Tsuchihashi Y, Kitamura T. Origin, morphology and function of the microglia. *Prog Clin Biol Res*. 1981;59A:141-69.
56. Marin-Teva JL, Dusart I, Colin C, Gervais A, van Rooijen N, Mallat M. Microglia promote the death of developing Purkinje cells. *Neuron*. Feb 19 2004;41(4):535-47.
57. Paolicelli RC, Bolasco G, Pagani F, et al. Synaptic pruning by microglia is necessary for normal brain development. *Science*. Sep 9 2011;333(6048):1456-8. doi:10.1126/science.1202529
58. Tremblay ME, Stevens B, Sierra A, Wake H, Bessis A, Nimmerjahn A. The role of microglia in the healthy brain. *J Neurosci*. Nov 9 2011;31(45):16064-9. doi:10.1523/JNEUROSCI.4158-11.2011
59. Xavier AL, Menezes JR, Goldman SA, Nedergaard M. Fine-tuning the central nervous system: microglial modelling of cells and synapses. *Philos Trans R Soc Lond B Biol Sci*. Oct 19 2014;369(1654):20130593. doi:10.1098/rstb.2013.0593
60. Bilbo SD, Schwarz JM. The immune system and developmental programming of brain and behavior. *Front Neuroendocrinol*. Aug 2012;33(3):267-86. doi:10.1016/j.yfrne.2012.08.006
61. Chauhan A, Quenum FZ, Abbas A, et al. Epigenetic Modulation of Microglial Inflammatory Gene Loci in Helminth-Induced Immune Suppression: Implications for Immune Regulation in Neurocysticercosis. *ASN Neuro*. Jul-Aug 2015;7(4)doi:10.1177/1759091415592126
62. Jovicic A, Roshan R, Moisoi N, et al. Comprehensive expression analyses of neural cell-type-specific miRNAs identify new determinants of the specification and maintenance of neuronal phenotypes. *J Neurosci*. Mar 20 2013;33(12):5127-37. doi:10.1523/JNEUROSCI.0600-12.2013
63. Bird A. Perceptions of epigenetics. *Nature*. May 24 2007;447(7143):396-8. doi:10.1038/nature05913
64. Ziller MJ, Gu H, Muller F, et al. Charting a dynamic DNA methylation landscape of the human genome. *Nature*. Aug 22 2013;500(7463):477-81. doi:10.1038/nature12433
65. Boyce WT, Kobor MS. Development and the epigenome: the 'synapse' of gene-environment interplay. *Dev Sci*. Jan 2015;18(1):1-23. doi:10.1111/desc.12282
66. Aristizabal MJ, Anreiter I, Halldorsdottir T, et al. Biological embedding of experience: A primer on epigenetics. *Proceedings of the National Academy of Sciences*. 2020;117(38):23261. doi:10.1073/pnas.1820838116
67. Allis CD, Jenuwein T. The molecular hallmarks of epigenetic control. *Nat Rev Genet*. Aug 2016;17(8):487-500. doi:10.1038/nrg.2016.59
68. Zhou FC, Zhao Q, Liu Y, et al. Alteration of gene expression by alcohol exposure at early neurulation. *BMC Genomics*. Feb 21 2011;12:124. doi:10.1186/1471-2164-12-124
69. Green ML, Singh AV, Zhang Y, Nemeth KA, Sulik KK, Knudsen TB. Reprogramming of genetic networks during initiation of the Fetal Alcohol Syndrome. *Dev Dyn*. Feb 2007;236(2):613-31. doi:10.1002/dvdy.21048
70. Hard ML, Abdolell M, Robinson BH, Koren G. Gene-expression analysis after alcohol exposure in the developing mouse. *J Lab Clin Med*. Jan 2005;145(1):47-54. doi:10.1016/j.lab.2004.11.011

71. Gano A, Prestia L, Middleton FA, Youngentob SL, Ignacio C, Deak T. Gene expression profiling reveals a lingering effect of prenatal alcohol exposure on inflammatory-related genes during adolescence and adulthood. *Cytokine*. 2020/09/01/ 2020;133:155126. doi:https://doi.org/10.1016/j.cyto.2020.155126
72. Lussier AA, Stepien KA, Neumann SM, Pavlidis P, Kobor MS, Weinberg J. Prenatal alcohol exposure alters steady-state and activated gene expression in the adult rat brain. *Alcohol Clin Exp Res*. Feb 2015;39(2):251-61. doi:10.1111/acer.12622
73. Lussier AA, Weinberg J, Kobor MS. Epigenetics studies of fetal alcohol spectrum disorder: where are we now? *Epigenomics*. Mar 2017;9(3):291-311.
74. Chater-Diehl EJ, Laufer BI, Castellani CA, Alberry BL, Singh SM. Alteration of Gene Expression, DNA Methylation, and Histone Methylation in Free Radical Scavenging Networks in Adult Mouse Hippocampus following Fetal Alcohol Exposure. *PLoS One*. 2016;11(5):e0154836. doi:10.1371/journal.pone.0154836
75. Khalid O, Kim JJ, Kim HS, et al. Gene expression signatures affected by alcohol-induced DNA methylomic deregulation in human embryonic stem cells. *Stem Cell Res*. May 2014;12(3):791-806. doi:10.1016/j.scr.2014.03.009
76. Laufer BI, Kapalanga J, Castellani CA, Diehl EJ, Yan L, Singh SM. Associative DNA methylation changes in children with prenatal alcohol exposure. *Epigenomics*. 2015;7(8):1259-74. doi:10.2217/epi.15.60
77. Liu Y, Balaraman Y, Wang G, Nephew KP, Zhou FC. Alcohol exposure alters DNA methylation profiles in mouse embryos at early neurulation. *Epigenetics*. Oct 01 2009;4(7):500-11.
78. Marjonen H, Sierra A, Nyman A, et al. Early maternal alcohol consumption alters hippocampal DNA methylation, gene expression and volume in a mouse model. *PLoS One*. 2015;10(5):e0124931. doi:10.1371/journal.pone.0124931
79. Portales-Casamar E, Lussier AA, Jones MJ, et al. DNA methylation signature of human fetal alcohol spectrum disorder. *Epigenetics Chromatin*. 2016;9:25. doi:10.1186/s13072-016-0074-4
80. Zhou FC, Balaraman Y, Teng M, Liu Y, Singh RP, Nephew KP. Alcohol alters DNA methylation patterns and inhibits neural stem cell differentiation. *Alcohol Clin Exp Res*. Apr 2011;35(4):735-46. doi:10.1111/j.1530-0277.2010.01391.x
81. Lussier AA, Morin AM, MacIsaac JL, et al. DNA methylation as a predictor of fetal alcohol spectrum disorder. journal article. *Clin Epigenetics*. January 12 2018;10(1):5. doi:10.1186/s13148-018-0439-6
82. Lussier AA, Bodnar TS, Mingay M, et al. Prenatal Alcohol Exposure: Profiling Developmental DNA Methylation Patterns in Central and Peripheral Tissues. *Front Genet*. 2018;9:610-610. doi:10.3389/fgene.2018.00610
83. Sharp GC, Arathimos R, Reese SE, et al. Maternal alcohol consumption and offspring DNA methylation: findings from six general population-based birth cohorts. *Epigenomics*. 2018;10(1):27-42. doi:10.2217/epi-2017-0095
84. Frey S, Eichler A, Stonawski V, et al. Prenatal Alcohol Exposure Is Associated With Adverse Cognitive Effects and Distinct Whole-Genome DNA Methylation Patterns in Primary School Children %U https://www.frontiersin.org/article/10.3389/fnbeh.2018.00125. *Front Behav Neurosci*. 2018;12(125 %M):%7 %8 2018-June-26 %9 Original Research %# %! Prenatal alcohol exposure affects DNA methylation and cognitive development %* %<. doi:10.3389/fnbeh.2018.00125 %W %L
85. Cobben JM, Krzyzewska IM, Venema A, et al. DNA methylation abundantly associates with fetal alcohol spectrum disorder and its subphenotypes. *Epigenomics*. May 2019;11(7):767-785. doi:10.2217/epi-2018-0221

86. Garden GA. Epigenetics and the modulation of neuroinflammation. *Neurotherapeutics : the journal of the American Society for Experimental NeuroTherapeutics*. 2013;10(4):782-788. doi:10.1007/s13311-013-0207-4
87. Obata Y, Furusawa Y, Hase K. Epigenetic modifications of the immune system in health and disease. *Immunol Cell Biol*. Mar 2015;93(3):226-32. doi:10.1038/icb.2014.114
88. Akimova T, Beier UH, Liu Y, Wang L, Hancock WW. Histone/protein deacetylases and T-cell immune responses. *Blood*. Mar 15 2012;119(11):2443-51. doi:10.1182/blood-2011-10-292003
89. Dovey OM, Foster CT, Conte N, et al. Histone deacetylase 1 and 2 are essential for normal T-cell development and genomic stability in mice. *Blood*. Feb 21 2013;121(8):1335-44. doi:10.1182/blood-2012-07-441949
90. Lee PP, Fitzpatrick DR, Beard C, et al. A critical role for Dnmt1 and DNA methylation in T cell development, function, and survival. *Immunity*. Nov 2001;15(5):763-74.
91. Hashimi ST, Fulcher JA, Chang MH, Gov L, Wang S, Lee B. MicroRNA profiling identifies miR-34a and miR-21 and their target genes JAG1 and WNT1 in the coordinate regulation of dendritic cell differentiation. *Blood*. Jul 09 2009;114(2):404-14. doi:10.1182/blood-2008-09-179150
92. Johnnidis JB, Harris MH, Wheeler RT, et al. Regulation of progenitor cell proliferation and granulocyte function by microRNA-223. *Nature*. Feb 28 2008;451(7182):1125-9. doi:10.1038/nature06607
93. Taganov KD, Boldin MP, Chang KJ, Baltimore D. NF-kappaB-dependent induction of microRNA miR-146, an inhibitor targeted to signaling proteins of innate immune responses. *Proc Natl Acad Sci U S A*. Aug 15 2006;103(33):12481-6. doi:10.1073/pnas.0605298103
94. Schmidl C, Delacher M, Huehn J, Feuerer M. Epigenetic mechanisms regulating T-cell responses. *J Allergy Clin Immunol*. Sep 2018;142(3):728-743. doi:10.1016/j.jaci.2018.07.014
95. Aung HT, Schroder K, Himes SR, et al. LPS regulates proinflammatory gene expression in macrophages by altering histone deacetylase expression. *FASEB J*. Jul 2006;20(9):1315-27. doi:10.1096/fj.05-5360com
96. Nicodeme E, Jeffrey KL, Schaefer U, et al. Suppression of inflammation by a synthetic histone mimic. *Nature*. Dec 23 2010;468(7327):1119-23. doi:10.1038/nature09589
97. Satoh T, Takeuchi O, Vandenbon A, et al. The Jmjd3-Irf4 axis regulates M2 macrophage polarization and host responses against helminth infection. *Nat Immunol*. Oct 2010;11(10):936-44. doi:10.1038/ni.1920
98. Zhang Q, Cao X. Epigenetic regulation of the innate immune response to infection. *Nat Rev Immunol*. Jul 2019;19(7):417-432. doi:10.1038/s41577-019-0151-6
99. Kaminska B, Mota M, Pizzi M. Signal transduction and epigenetic mechanisms in the control of microglia activation during neuroinflammation. *Biochim Biophys Acta*. Mar 2016;1862(3):339-51. doi:10.1016/j.bbadis.2015.10.026
100. Cheray M, Joseph B. Epigenetics Control Microglia Plasticity. *Front Cell Neurosci*. 2018;12:243. doi:10.3389/fncel.2018.00243
101. Hinz D, Bauer M, Roder S, et al. Cord blood Tregs with stable FOXP3 expression are influenced by prenatal environment and associated with atopic dermatitis at the age of one year. *Allergy*. Mar 2012;67(3):380-9. doi:10.1111/j.1398-9995.2011.02767.x
102. Schwarz JM, Hutchinson MR, Bilbo SD. Early-life experience decreases drug-induced reinstatement of morphine CPP in adulthood via microglial-specific epigenetic programming of anti-inflammatory IL-10 expression. *J Neurosci*. Dec 07 2011;31(49):17835-47. doi:10.1523/JNEUROSCI.3297-11.2011

668 103. Wang J, Hodes GE, Zhang H, et al. Epigenetic modulation of inflammation and synaptic
669 plasticity promotes resilience against stress in mice. *Nat Commun*. Feb 2 2018;9(1):477.
670 doi:10.1038/s41467-017-02794-5
671 104. Bhattacharya S, Dunn P, Thomas CG, et al. ImmPort, toward repurposing of open access
672 immunological assay data for translational and clinical research. *Sci Data*. 2018;5:180015-
673 180015. doi:10.1038/sdata.2018.15
674 105. Liu Y, Aryee MJ, Padyukov L, et al. Epigenome-wide association data implicate DNA
675 methylation as an intermediary of genetic risk in rheumatoid arthritis. *Nat Biotechnol*. Feb
676 2013;31(2):142-7. doi:10.1038/nbt.2487
677 106. Raychaudhuri S, Sandor C, Stahl EA, et al. Five amino acids in three HLA proteins explain
678 most of the association between MHC and seropositive rheumatoid arthritis. *Nat Genet*. Jan 29
679 2012;44(3):291-6. doi:10.1038/ng.1076
680 107. Zhang X, Lan N, Bach P, et al. Prenatal alcohol exposure alters the course and severity of
681 adjuvant-induced arthritis in female rats. *Brain Behav Immun*. Mar 2012;26(3):439-50.
682 doi:10.1016/j.bbi.2011.11.005
683 108. Himmelreich M, Lutke, C, Travis E. The Lay of the Land: Fetal Alcohol Spectrum Disorder
684 (FASD) as a Whole-Body Diagnosis. In: Begun A, Murray, MM, ed. *The Routledge Handbook of*
685 *Social Work and Addictive Behaviors*. Routledge; 2020:191 - 215:chap 12.
686 109. Sarma JV, Ward PA. The complement system. *Cell Tissue Res*. 2011/01/01
687 2011;343(1):227-235. doi:10.1007/s00441-010-1034-0
688 110. Le Menn G, Neels JG. Regulation of Immune Cell Function by PPARs and the Connection
689 with Metabolic and Neurodegenerative Diseases. *Int J Mol Sci*. 2018;19(6):1575.
690 doi:10.3390/ijms19061575
691 111. Veazey KJ, Muller D, Golding MC. Prenatal alcohol exposure and cellular differentiation: a
692 role for Polycomb and Trithorax group proteins in FAS phenotypes? *Alcohol Res*. 2013;35(1):77-
693 85.
694 112. Aloia L, Di Stefano B, Di Croce L. Polycomb complexes in stem cells and embryonic
695 development. *Development*. Jun 2013;140(12):2525-34. doi:10.1242/dev.091553
696 113. Balaraman S, Winzer-Serhan UH, Miranda RC. Opposing actions of ethanol and nicotine on
697 microRNAs are mediated by nicotinic acetylcholine receptors in fetal cerebral cortical-derived
698 neural progenitor cells. *Alcohol Clin Exp Res*. Oct 2012;36(10):1669-77. doi:10.1111/j.1530-
699 0277.2012.01793.x
700 114. Sathyan P, Golden HB, Miranda RC. Competing interactions between micro-RNAs
701 determine neural progenitor survival and proliferation after ethanol exposure: evidence from an
702 ex vivo model of the fetal cerebral cortical neuroepithelium. *J Neurosci*. Aug 08
703 2007;27(32):8546-57. doi:10.1523/JNEUROSCI.1269-07.2007
704 115. Wang LL, Zhang Z, Li Q, et al. Ethanol exposure induces differential microRNA and target
705 gene expression and teratogenic effects which can be suppressed by folic acid supplementation.
706 *Hum Reprod*. Mar 2009;24(3):562-79. doi:10.1093/humrep/den439
707 116. Qi Y, Zhang M, Li H, et al. MicroRNA-29b regulates ethanol-induced neuronal apoptosis in
708 the developing cerebellum through SP1/RAX/PKR cascade. *J Biol Chem*. Apr 04
709 2014;289(14):10201-10. doi:10.1074/jbc.M113.535195
710 117. Ignacio C, Mooney SM, Middleton FA. Effects of Acute Prenatal Exposure to Ethanol on
711 microRNA Expression are Ameliorated by Social Enrichment. *Front Pediatr*. 2014;2:103.
712 doi:10.3389/fped.2014.00103
713 118. Cardoso AL, Guedes JR, Pereira de Almeida L, Pedroso de Lima MC. miR-155 modulates
714 microglia-mediated immune response by down-regulating SOCS-1 and promoting cytokine and

715 nitric oxide production. *Immunology*. Jan 2012;135(1):73-88. doi:10.1111/j.1365-
716 2567.2011.03514.x
717 119. Zhang L, Dong LY, Li YJ, Hong Z, Wei WS. miR-21 represses FasL in microglia and protects
718 against microglia-mediated neuronal cell death following hypoxia/ischemia. *Glia*. Dec
719 2012;60(12):1888-95. doi:10.1002/glia.22404
720 120. Salem NA, Mahnke AH, Konganti K, Hillhouse AE, Miranda RC. Cell-type and fetal-sex-
721 specific targets of prenatal alcohol exposure in developing mouse cerebral cortex. *iScience*.
722 2021/05/21/ 2021;24(5):102439. doi:https://doi.org/10.1016/j.isci.2021.102439
723 121. Nestler EJ, Barrot M, DiLeone RJ, Eisch AJ, Gold SJ, Monteggia LM. Neurobiology of
724 depression. *Neuron*. Mar 28 2002;34(1):13-25.
725 122. Pei J, Denys K, Hughes J, Rasmussen C. Mental health issues in fetal alcohol spectrum
726 disorder. *J Ment Health*. Oct 2011;20(5):438-48. doi:10.3109/09638237.2011.577113
727 123. O'Connor MJ, Shah B, Whaley S, Cronin P, Gunderson B, Graham J. Psychiatric illness in a
728 clinical sample of children with prenatal alcohol exposure. *Am J Drug Alcohol Abuse*. Nov
729 2002;28(4):743-54.
730 124. Famy C, Streissguth AP, Unis AS. Mental illness in adults with fetal alcohol syndrome or
731 fetal alcohol effects. *Am J Psychiatry*. Apr 1998;155(4):552-4. doi:10.1176/ajp.155.4.552
732 125. Alam R, Abdolmaleky HM, Zhou JR. Microbiome, inflammation, epigenetic alterations, and
733 mental diseases. *Am J Med Genet B Neuropsychiatr Genet*. Sep 2017;174(6):651-660.
734 doi:10.1002/ajmg.b.32567
735 126. Hodes GE, Kana V, Menard C, Merad M, Russo SJ. Neuroimmune mechanisms of
736 depression. *Nat Neurosci*. Oct 2015;18(10):1386-93. doi:10.1038/nn.4113
737 127. Khandaker GM, Pearson RM, Zammit S, Lewis G, Jones PB. Association of serum
738 interleukin 6 and C-reactive protein in childhood with depression and psychosis in young adult
739 life: a population-based longitudinal study. *JAMA Psychiatry*. Oct 2014;71(10):1121-8.
740 doi:10.1001/jamapsychiatry.2014.1332
741 128. Uddin M, Koenen KC, Aiello AE, Wildman DE, de los Santos R, Galea S. Epigenetic and
742 inflammatory marker profiles associated with depression in a community-based epidemiologic
743 sample. *Psychol Med*. May 2011;41(5):997-1007. doi:10.1017/S0033291710001674
744 129. Barker ED, Cecil CAM, Walton E, et al. Inflammation-related epigenetic risk and child and
745 adolescent mental health: A prospective study from pregnancy to middle adolescence. *Dev*
746 *Psychopathol*. Aug 2018;30(3):1145-1156. doi:10.1017/S0954579418000330
747 130. Alenghat T, Osborne LC, Saenz SA, et al. Histone deacetylase 3 coordinates commensal-
748 bacteria-dependent intestinal homeostasis. *Nature*. Dec 05 2013;504(7478):153-7.
749 doi:10.1038/nature12687
750 131. Stilling RM, Dinan TG, Cryan JF. Microbial genes, brain & behaviour - epigenetic regulation
751 of the gut-brain axis. *Genes Brain Behav*. Jan 2014;13(1):69-86. doi:10.1111/gbb.12109
752 132. Mohajeri MH, La Fata G, Steinert RE, Weber P. Relationship between the gut microbiome
753 and brain function. *Nutr Rev*. Jul 1 2018;76(7):481-496. doi:10.1093/nutrit/nuy009
754 133. Carabotti M, Scirocco A, Maselli MA, Severi C. The gut-brain axis: interactions between
755 enteric microbiota, central and enteric nervous systems. *Ann Gastroenterol*. Apr-Jun
756 2015;28(2):203-209.
757 134. Petra AI, Panagiotidou S, Hatziagelaki E, Stewart JM, Conti P, Theoharides TC. Gut-
758 Microbiota-Brain Axis and Its Effect on Neuropsychiatric Disorders With Suspected Immune
759 Dysregulation. *Clin Ther*. 2015/05/01/ 2015;37(5):984-995.
760 doi:https://doi.org/10.1016/j.clinthera.2015.04.002

135. Morais LH, Schreiber HL, Mazmanian SK. The gut microbiota–brain axis in behaviour and brain disorders. *Nature Reviews Microbiology*. 2021/04/01 2021;19(4):241-255. doi:10.1038/s41579-020-00460-0
136. Louwies T, Johnson AC, Orock A, Yuan T, Greenwood-Van Meerveld B. The microbiota-gut-brain axis: An emerging role for the epigenome. *Exp Biol Med*. 2020/01/01 2019;245(2):138-145. doi:10.1177/1535370219891690
137. Bull-Otterson L, Feng W, Kirpich I, et al. Metagenomic analyses of alcohol induced pathogenic alterations in the intestinal microbiome and the effect of Lactobacillus rhamnosus GG treatment. *PLoS One*. 2013;8(1):e53028. doi:10.1371/journal.pone.0053028
138. Keshavarzian A, Choudhary S, Holmes EW, et al. Preventing gut leakiness by oats supplementation ameliorates alcohol-induced liver damage in rats. *J Pharmacol Exp Ther*. Nov 2001;299(2):442-8.
139. Tamburini S, Shen N, Wu HC, Clemente JC. The microbiome in early life: implications for health outcomes. *Nat Med*. Jul 7 2016;22(7):713-22. doi:10.1038/nm.4142
140. Hooper LV, Littman DR, Macpherson AJ. Interactions between the microbiota and the immune system. *Science*. Jun 8 2012;336(6086):1268-73. doi:10.1126/science.1223490
141. Yan AW, Fouts DE, Brandl J, et al. Enteric dysbiosis associated with a mouse model of alcoholic liver disease. *Hepatology*. Jan 2011;53(1):96-105. doi:10.1002/hep.24018
142. Won H, Mah W, Kim E. Autism spectrum disorder causes, mechanisms, and treatments: focus on neuronal synapses. *Front Mol Neurosci*. 2013;6:19. doi:10.3389/fnmol.2013.00019
143. Fatemi SH, Folsom TD. The neurodevelopmental hypothesis of schizophrenia, revisited. *Schizophr Bull*. May 2009;35(3):528-48. doi:10.1093/schbul/sbn187
144. Topper LA, Baculis BC, Valenzuela CF. Exposure of neonatal rats to alcohol has differential effects on neuroinflammation and neuronal survival in the cerebellum and hippocampus. *J Neuroinflammation*. 2015;12(1):160. doi:10.1186/s12974-015-0382-9
145. Raison CL, Capuron L, Miller AH. Cytokines sing the blues: inflammation and the pathogenesis of depression. *Trends Immunol*. Jan 2006;27(1):24-31. doi:10.1016/j.it.2005.11.006
146. Drew PD, Kane CJ. Fetal alcohol spectrum disorders and neuroimmune changes. *Int Rev Neurobiol*. 2014;118:41-80. doi:10.1016/B978-0-12-801284-0.00003-8
147. Wozniak JR, Fuglestad AJ, Eckerle JK, et al. Choline supplementation in children with fetal alcohol spectrum disorders: a randomized, double-blind, placebo-controlled trial. *Am J Clin Nutr*. Nov 2015;102(5):1113-25. doi:10.3945/ajcn.114.099168
148. Nguyen TT, Risbud RD, Mattson SN, Chambers CD, Thomas JD. Randomized, double-blind, placebo-controlled clinical trial of choline supplementation in school-aged children with fetal alcohol spectrum disorders. *Am J Clin Nutr*. Nov 02 2016;doi:10.3945/ajcn.116.142075
149. Hicks SD, Middleton FA, Miller MW. Ethanol-induced methylation of cell cycle genes in neural stem cells. *J Neurochem*. Sep 2010;114(6):1767-80. doi:10.1111/j.1471-4159.2010.06886.x
150. Krishnamoorthy M, Gerwe BA, Scharer CD, et al. Ethanol alters proliferation and differentiation of normal and chromosomally abnormal human embryonic stem cell-derived neurospheres. *Birth Defects Res B Dev Reprod Toxicol*. Jun 2013;98(3):283-95. doi:10.1002/bdrb.21063
151. Laufer BI, Mantha K, Kleiber ML, Diehl EJ, Addison SM, Singh SM. Long-lasting alterations to DNA methylation and ncRNAs could underlie the effects of fetal alcohol exposure in mice. *Dis Model Mech*. Jul 2013;6(4):977-92. doi:10.1242/dmm.010975

Figure 1:

Summary figure: (1) Prenatal alcohol exposure (PAE) is known to result in (2) maternal immune system activation, altering the fine cytokine balance during pregnancy, which in turn impacts the developing immune system of the fetus. (3) Epigenetic modifications, including methylation changes and alterations in miRNAs, also occur as a result of PAE and are likely important mechanistic drivers of (4) life-long impairments in offspring immune function and (5) neuroimmune system activation, including microglial activation and central cytokine changes. Together, offspring central and peripheral immune system activation, by way of epigenetic changes, are hypothesized as driving, at least in part, the increased risk of mental health conditions, such as depression and anxiety, in alcohol-exposed offspring.

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Table 1. Studies showing the impact of prenatal/early postnatal (third trimester equivalent) alcohol exposure on central cytokine levels

Table 2. Differential effects of PAE on the epigenetic profiles of immune genes