Prenatal diet and childhood ADHD: exploring the potential role of *IGF2* methylation

“Evidence for diet-induced epigenetic effects may also highlight nutrition as an important modifiable intervention target.”

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Unhealthy diet during pregnancy is a risk factor for a wide range of negative health and psychiatric outcomes [1]. For example, high-sugar and fat diets associate not only with increased risk for noncommunicable diseases [2], such as diabetes and obesity, but also for neurodevelopmental disorders, such as attention-deficit hyperactivity disorder (ADHD) and conduct problems (CP) [3]. In the nutritional field, epigenetics is an important area of investigation, as nutrients and bioactive compounds can alter the expression of genes at the transcriptional level [4]. Because epigenetic modifications, such as DNA methylation, can be passed on during cell division and result in long-term phenotypic changes [5], they also provide a framework for understanding the biological mechanisms through which pre- and post-natal environmental exposures may influence disease vulnerability [6,7].

In a recent paper [8], we examined how exposure to unhealthy fats (e.g., vegetable oils in fast foods) and sugars (e.g., sweets) might associate with ADHD symptoms in children who follow an early onset (age ≤ 10 years; $n = 83$) versus low ($n = 81$) trajectory of CP, via DNA methylation of the *IGF2* gene. We focused on *IGF2* due to its relevance in metabolic function [9], placental and fetal growth [10] and the development of brain regions implicated in ADHD [11–13]. Data were drawn from the Accessible Resource for Integrated Epigenomics Studies [14], a sub-

sample of the Avon Longitudinal Study of Parents and Children, which includes: firstly, maternal reports of diet (32-week gestation, age 3 and 7 years); secondly, peripheral measures of DNA methylation (Illumina 450 k) at birth and age 7 years ($n = 671$, 49% male) and thirdly, repeated assessments of psychiatric symptomatology, including CP (ages 4–13 years) and ADHD (ages 7–13 years). Below, we summarize our key findings before discussing limitations and future directions.

Key findings

Prenatal unhealthy diet & neonatal *IGF2* methylation

Firstly, we found that a maternal unhealthy diet during pregnancy prospectively associated with higher *IGF2* methylation at birth across all (i.e., both CP and typically developing) children, even after adjusting for a range of inter-related risk factors (e.g., maternal smoking, psychosocial and contextual risks). This finding is consistent with previous research reporting an association between variation in offspring *IGF2* methylation and prenatal dietary exposures, including periconceptional dietary supplementation (e.g., folic acid [15,16]; docosahexaenoic acid [17]), severe caloric restriction resulting from prenatal famine exposure [18], maternal obesity and BMI [17,19], as well as animal research on high-fat diet exposure [20]. The use of a prospective design additionally enabled us to examine longitudinal inter-

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relationships between unhealthy diet and *IGF2* methylation, spanning gestation to mid-childhood. We found that, firstly, the association between diet and *IGF2* methylation was specific to pregnancy (i.e., not observed postnatally) and secondly, whereas unhealthy diet showed high stability over time (i.e., what mothers ate in pregnancy related to what their children ate in childhood), *IGF2* methylation did not. While these findings do not permit causal inference (see limitations), they do point to gestation as a potentially critical developmental window for diet-induced changes in *IGF2* methylation, and suggest that *IGF2* methylation levels may be temporally dynamic.

IGF2 methylation at birth & ADHD symptoms in childhood

Secondly, we found that *IGF2* methylation at birth prospectively associated with higher ADHD symptoms in childhood (age 7–13 years), but that this association was specific to CP children. Interestingly, this specificity was not due to differences in dietary exposure, as both groups showed comparable levels of prenatal unhealthy diet as well as *IGF2* methylation at birth. Why would such a developmental risk pathway be specific to CP children? We know from a large body of literature that CP and ADHD are not only highly co-morbid, but that this co-occurrence is underpinned by greater genetic heritability and more severe environmental risk exposure, compared with either CP or ADHD alone [21,22]. Consequently, it is possible that, for CP children, the association between *IGF2* methylation and ADHD may be compounded by unmeasured genetic influences (e.g., variability in *IGF2*, related imprinted loci, such as *H19*, and/or broader polygenic effects) as well as environmental factors beyond those controlled for in the analyses (e.g., air pollutants [23]).

IGF2 methylation as a potential mediating mechanism

Thirdly, we found that among CP children, *IGF2* methylation at birth mediated the effect of prenatal diet on ADHD symptomatology. This finding supports previous data from animal studies documenting an indirect pathway linking prenatal diet and postnatal outcomes, via *IGF2* methylation. For example, Claycombe *et al.* [20] reported that in male rats, combined exposure to intra-uterine undernourishment and post-weaning high-fat diet associated with variation in *IGF2* methylation and expression, which in turn related to lower insulin sensitivity and higher adipose tissue growth. Here, we extend findings by implicating *IGF2* methylation as a potential mechanism not only mediating vulnerability to physical/metabolic outcomes but also for ADHD risk, a neurodevelopmental psychiatric

disorder. Of note, we found that mediation was specific to ADHD symptoms and did not extend to other co-occurring symptoms (e.g., anxiety, depression) among CP children. Together, these findings are in line with the developmental origins of health and disease [7] and latent vulnerability [24] hypotheses, whereby early-risk exposure may alter biological systems in a way that may increase long-term vulnerability to physical and psychiatric disease.

Limitations & future perspective

Conclusions drawn from our study are limited in a few important ways. Firstly, ADHD and CP are complex, multi-determined psychiatric phenotypes. As such, other factors, such as genetic and environmental influences (beyond diet), are likely to be important. Moreover, the *IGF2* gene is located in a highly dynamic region that includes multiple transcripts from alternative promoters, relates to different biological functions and is differentially expressed across tissues and developmental periods, so that a more comprehensive epigenetic investigation in this gene will be required (e.g., contribution of histone modifications and miRNAs).

Secondly, we examined ADHD as a global construct. However, ADHD comprises of subdimensions of inattention, hyperactivity and impulsivity, and it will be of interest in future to establish whether effects may be general or domain-specific [25]. Thirdly, although the use of peripheral tissue samples hold potential for the identification of exposure/risk biomarkers, the extent to which findings may reflect methylation changes in the brain is unclear. In fact, a recent study [26] found that most DNA methylation markers in peripheral blood do not reliably predict brain DNA methylation status, making inferences on brain-relevant processes difficult. As such, it will be important to establish to what extent peripheral levels of *IGF2* methylation relate to *in vivo* structural/functional neural markers of ADHD.

Fourthly, more work will be needed to trace the specific biological pathways through which the observed effects manifest. The present findings allow only limited conclusions as to, firstly, how exactly diet affects *IGF2* methylation; secondly, why this effect is only observed prenatally and thirdly, why associations with ADHD are observed only in children with co-occurring CP. Addressing these questions will require the integration of intermediary variables, such as metabolites of nutrition, fetal growth trajectories (e.g., via sonar doppler; [27]), transcriptomic data, early indicators of neurodevelopment and *in vivo* brain-imaging measures. The use of more comprehensive, repeated assessments of prenatal diet will also enable us to trace

dietary effects on *IGF2* methylation from early-to-late gestation, as well as examining the role of other important nutrients (e.g., folic acid). Furthermore, the inclusion of genetic and environmental moderators may help to shed light on the specificity of ADHD risk to CP children (e.g., via molecular designs to study single-gene effects; or behavioral genetic designs to investigate diet interaction with genetic heritability as a whole).

Finally, because our study was based on correlational data, we were not able to establish causality. This will require the use of a multimethod approach for strengthening causal inference, including the implementation of (two-step) mendelian randomization [28], negative control (e.g., using paternal prenatal diet effects as a negative control to maternal effects) and cross-species designs.

Implications & translational potential

Data from our study and others suggest that *IGF2* methylation may be sensitive to diet-related exposures – particularly during pregnancy – and mediate vulnerability for negative developmental outcomes, such as ADHD risk. Although promising, this evidence is currently preliminary and in need of replication. Consequently, findings should be interpreted with caution and considered more as well-grounded hypotheses for further investigation. Bearing this in mind, there are

a number of ways in which findings may be used in future to inform policy and practice. In the first place, findings may refine existing models of how risk exposures, such as an unhealthy diet, become biologically embedded. Longitudinal modeling of environmental and epigenetic data may also be used to pinpoint specific windows of biological vulnerability (e.g., prenatal period) that may benefit most from preventive action. Evidence for diet-induced epigenetic effects may also highlight nutrition as an important modifiable intervention target. On the longer term, developments in knowledge, methodology and research designs will offer exciting opportunities for delineating the role of *IGF2* methylation, the neurodevelopment, as well as testing its potential clinical utility as an exposure indicator, disease biomarker and therapeutic target.

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