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LETTERS TO THE EDITOR

Brain weight in males is correlated with DNA methylation at *IGF2*

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The insulin-like growth factor 2 (*IGF2*) gene, located in a cluster of imprinted genes on chromosome 11p15.5, encodes a hormone involved in prenatal growth and development. Impaired function of *IGF2* is linked to intrauterine growth retardation,[1] with aberrant imprinting of this region being associated with congenital disorders characterized by growth abnormalities such as Beckwith–Wiedermann Syndrome and Silver–Russell Syndrome.[2] Epigenetic heterogeneity at *IGF2* is also a known feature of normal populations,[3] with variable imprinting reported in the brain[4] and considerable loss of imprinting detected in fetal cerebellum samples.[5] Several studies report that inter-individual differences in *IGF2* methylation may be environmentally mediated, particularly by prenatal exposure to famine. Individuals conceived during the Dutch Hunger Winter Famine of 1944–1945 were found to be hypomethylated across a differentially methylated region (DMR) in *IGF2*[6] and at the insulin-*IGF* (INS_IGF) DNA transcript, which aligns with the 3′ end of the *IGF2* gene.[7] Of note, the authors report a significant interaction between DNA methylation and sex, with the association between *IGF2* hypomethylation and famine exposure being restricted to males.[7] It thus appears that variable *IGF2* methylation may result from exposure to specific environmental factors during sensitive periods of development, and may be mediated by the sex of the exposed individual.

We hypothesized that variation in *IGF2* methylation affecting gene dosage could impact upon the development of the brain, which continues to grow and mature after birth. The cerebellum, in particular, has a very long period of development; it begins differentiating at a very early stage, yet is one of the last brain structures to reach maturity,[8] ultimately accounting for 10% of the total brain volume.[9] In the present study, we assessed inter-individual differences in *IGF2* methylation in the cerebellum using a set of post-mortem brain samples carefully characterized for brain weight, to determine whether a relationship exists between the degree of *IGF2* methylation and total brain weight at death.

Postmortem cerebellum samples, matched for age, gender, postmortem interval and pH, were provided by the Stanley Foundation Neuropathology Consortium (The Stanley Medical Research Institute, Bethesda, MD, USA). Brains were extracted from the skull in a standardized manner by a trained neuropathologist, and immediately after extraction the whole brain, including cerebellum and brain stem, was weighed. DNA was extracted using the Qiagen AllPrep Mini Kit (Qiagen, Valencia, CA, USA) and treated with sodium bisulfite using the EZ 96-DNA methylation kit (Zymo Research, Orange, CA, USA) following the manufacturer's standard protocol, with fully methylated and unmethylated samples included as assay controls. Bisulfite-treated DNA was PCR amplified using primers designed to amplify a known DMR of the *IGF2* gene (NCBI 36.1 Chr11:2154089-2154542). Quantitative DNA methylation analysis was conducted following bisulfite-PCR amplification using the Sequenom EpiTYPER system (Sequenom, San Diego, CA, USA). Cases with $<60\%$ complete DNA methylation data and CpG sites with $<80\%$ complete DNA methylation data were excluded from the analysis. In total, DNA methylation data spanning 17 CpG sites for 53 cerebellum samples (24 female and 33 male) were included in our analysis.

Spearman's rank correlations were performed using SPSS (version 15) to assess the correlation between brain weight and the density of 5-methylcytosine across the *IGF2* DMR. Although analyses in the entire sample revealed no significant correlation with brain weight ($r=0.209$, $P=0.120$), analyses split by sex revealed a strong positive correlation between average DNA methylation and brain weight in males. Several individual CpG units were significantly correlated with brain weight, with the mean level of DNA methylation across all CpG sites being strongly correlated ($r=0.415$, $P=0.016$) (Figure 1).

Although these data are preliminary, the finding that DNA methylation at *IGF2* is significantly correlated with brain weight is plausible given the known role of *IGF2* in mediating prenatal growth and development.[10] Several studies have previously highlighted a link between brain weight and psychiatric disorders, including schizophrenia.[11] This is interesting given that prenatal exposure to famine is associated with both adult schizophrenia[12] and loss of imprinting at *IGF2*.[6] Because the epigenome is known to be sensitive to environmental stressors during key developmental periods,[13] it is plausible that prenatal risk factors for psychiatric illness, such as exposure to famine, could act by epigenetically altering the

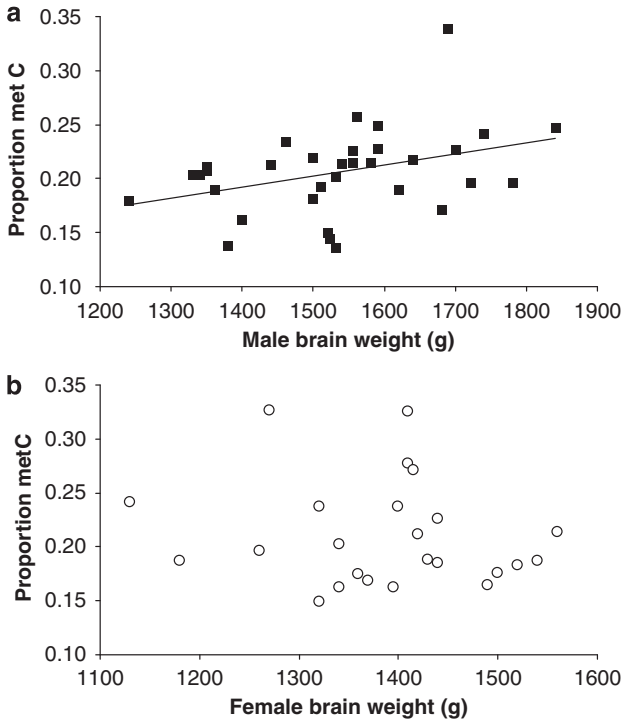

Figure 1 Correlation between density of 5-methylcytosine (metC) at *IGF2* in relation to brain weight in males (**a**) and females (**b**), showing significant correlation between DNA methylation and brain weight in males ($r = 0.415$, $P = 0.016$).

expression of genes involved in brain growth. Such alterations could manifest in abnormal brain development and ultimately result in psychopathology. The sex-specific nature of our correlation concurs with a previous study showing that hypomethylation at *IGF2* after prenatal exposure to famine occurs specifically in males.[7] Evidence suggests that *IGF2* expression in the brain is responsive to sex hormones,[14] which are known to alter DNA methylation at specific loci in the genome,[15] controlling gene expression in a sex-specific manner.

Future studies will aim to correlate *IGF2* DNA methylation with brain weight in additional samples and extend these analyses to other brain regions. Studies will also consider DNA methylation across other DMRs in *IGF2* and at other imprinted genes. Finally, although our current study was underpowered to detect disease-associated epigenetic changes, it would be interesting to examine the link between *IGF2* methylation and psychiatric illness given the evidence for an association between small brain size and disorders such as schizophrenia.[11]

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The reaction norm in gene × environment interaction

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In a recent commentary on gene × environment (GxE) interactions in *Molecular Psychiatry*, Belsky *et al.*[1] argue that many GxE findings are misinterpreted through adherence to a dominant model of disease vulnerability that assumes an ordinal interaction between specific genetic variation and the presence/absence of environmental adversity (diathesis stress). They cite numerous studies of people with putative 'risk' alleles of certain monoamine-regulating polymorphisms who experience psychiatric outcomes (for example, depression) more frequently in adverse circumstances, yet *less* frequently in salutary environments, compared with those with other genotypes. Belsky *et al.* suggest that this cross-over (or disordinal) interaction reflects 'differential susceptibility' to environmental influences (heightened plasticity) among individuals possessing these alleles ('differential' denoting the potential for both worse *and* better outcomes), rather than genetic vulnerability to outcomes that are specifically negative and expressed only in adversity. Differential susceptibility has distinct parallels in the 'reaction norm' (RN), a concept introduced by Woltereck in 1909 and a staple of experimental research in biology and evolutionary genetics. Here, I suggest that framing differential susceptibility within an RN perspective clarifies the role of phenotypic plasticity in GxE interaction.