RESEARCH SUMMARY

Differential DNA methylation in peripheral blood mononuclear cells in adolescents exposed to significant early but not later childhood adversity
Esposito, E.A., Jones. M.J., Doom. J.R., MacIsaac. J.L., Gunnar. M.R. and Kobor, M.S.
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It is now well known that adverse experiences in early childhood can have a variety of physical and psychological long-term effects, and research studies have shown similar patterns arising in other mammals, from rodents to monkeys. In humans, adults who experienced early childhood stress have been shown to perform less well at school and to be at greater risk of inflammatory diseases, mental health difficulties and alcoholism. Nevertheless, some children are more resilient than others and not all individuals in this position will be affected. Scientists are still trying to understand the physiological processes that link early adversity with these adult difficulties.

In this context, children who spend the first months or years of their life in orphanages and are then adopted into stable families can be worthwhile to study. These young people may have endured a variety of adverse experiences between conception and, perhaps, toddler-hood, including abuse, neglect, poverty and disease. Their experiences in later childhood are very different: people who choose to adopt institutionalised children are likely to be highly educated and at least reasonably affluent and to prove sensitive and responsive parents. We now know that institutionalised children who are adopted in early childhood in circumstances like these can catch up with their non-adopted peers with remarkable speed, although deficits often remain in some areas, including emotional regulation. Adopted children who have reached adolescence – by which time they will have spent the majority of their lives in supportive, well-resourced environments – are an important study group for investigating the long-term effects of early childhood adversity.

In non-human mammals, adverse experiences in early life have been associated with certain chemical changes to parts of the genetic material, DNA. These changes – and in particular the addition of small chemical groups known as methyl groups to the DNA bases, or DNA methylation – affect the amounts of proteins that are synthesised using that DNA as a template. Specific differences in DNA methylation patterns have been observed between maltreated children and their more fortunate peers, and even between children reared in orphanages and compatriots reared in poverty in their birth families.

Elisa Esposito of the University of Minnesota, Minneapolis, USA, Meaghan Jones of the University of British Columbia, Vancouver, Canada and their colleagues have carried out a study to compare patterns of DNA methylation in healthy adolescents who were born in Russia and Eastern Europe and adopted into families in the USA during early childhood with those in comparable US-born youngsters. All the subjects were Caucasian in origin; both groups of adolescents (50 adopted and 33 non-

adopted) contained approximately equal numbers of boys and girls, and the average age in both groups was 15. The adopted youth had entered their American families at on average 22 months; they had thus spent about 80% of their lives in similar advantaged environments to their non-adopted peers at the time of the study.

All young participants attended a single testing session during which a blood sample was taken and they and their parents completed questionnaires covering basic demographics and family history; health and behaviour; and stressful events experienced during the previous year. DNA was extracted from the blood samples and a list of methylated DNA positions was obtained for each one using standard techniques. Methylation sites that were common to all samples were removed from further analysis.

The questionnaires revealed some differences in medical history between the adopted and non-adopted youth. Adopted adolescents were significantly more likely to have been diagnosed with attention deficit hyperactivity disorder (ADHD); there were no such differences in other psychiatric diagnoses, including depression and anxiety. Adopted youths were also slightly more likely to report a physical health problem in the month before the test, although this was not statistically significant. There was no difference between the groups in the number and severity of general negative life events.

The DNA methylation profile obtained from a blood sample is known to depend strongly on the different types of white blood cell present in that sample. The researchers therefore used the data they had obtained to predict a distribution of cell types from each participant's DNA. Interestingly, this data showed a striking difference in cell type distribution between the adopted and non-adopted adolescents. Blood samples from adopted youth were predicted to contain fewer of one type of T-cell, known as $CD4^+$ T-cells; more of another T-cell type, $CD8^+$ T-cells; and fewer of the B-cells that secrete antibodies. The ratio between $CD4^+$ and $CD8^+$ numbers may affect the efficiency of the immune system in general, and this ratio was more skewed towards $CD8^+$ cells in those youths who had been diagnosed with ADHD. In order to understand more about this unexpected finding, Esposito, Jones and their co-workers performed the same analysis on a set of DNA methylation data obtained to compare Russian children raised in institutions with another group from non-adoptive, but poverty-stricken, families in the same country. Blood from both these groups of children was predicted to have $CD4^+$:$CD8^+$ cell ratios that are similar to those of the adolescents in this study who were born in Russia and eastern Europe and adopted into American families.

Many of the observed differences in DNA methylation patterns between the groups can be explained completely by this difference in white blood cell types. The researchers therefore removed this component of difference from their data before seeking further, more specific, differences between the groups. This analysis revealed a list of 30 DNA positions in 19 genes at which there was a very significant difference in methylation between the adopted and non-adopted youth. Methylation at four of these positions had been analysed in the Russian study but no differences had been

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observed at these positions between the institutionalised and simply poverty-stricken children.

One of the genes that Esposito and Jones found to be methylated more in the adopted youth was one for which methylation had previously been associated with exposure to cigarette smoke. This made sense because children in Russian and eastern European orphanages are known to be frequently exposed to smoke; the researchers therefore examined their data for further evidence of smoke-related DNA methylation patterns, and discovered several other genes with methylation patterns that indicated smoke exposure. The youngsters had not been asked about their current smoking patterns, so this alternative explanation could not be wholly ruled out. A wider analysis of all genes showing differences in methylation patterns – not just the most significant 19 – showed that genes associated with the nervous system and those associated with development were most likely to show methylation differences.

In summing up their findings, the researchers suggested that the differences in white blood cell composition between the adopted and non-adopted youth were consistent with the adopted youngsters having less efficient immune systems. Perhaps surprisingly, this did not seem to be associated with any significant differences in overall physical or mental health and, as similar ratios were calculated in blood samples from children reared in poor families in Russia, it was clearly not only an effect of institutional care. Furthermore, as the early life experiences of the two groups differed in many ways, including exposure to cigarette smoke as well as poverty and emotional neglect, further analysis involving larger groups will be necessary to fully understand DNA methylation as a mechanism through which adverse early-life experiences can influence an individual's physical and mental wellbeing many years later.

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