RESEARCH SUMMARY

Differentially Methylated Genes in Saliva are linked to Childhood Stress

Papale, L.A., Seltzer, L.J., Madrid, A., Pollak, S.D. and Alisch. R.S. (2018)
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It is well known that experiencing stress in childhood – particularly if this is chronic or severe – will put an individual at a higher risk of developing health problems later in life. Childhood stress has been associated with many conditions, but the clearest links are with depression and other mood disorders, anxiety, and difficulties in regulating emotions that may lead to aggressive behaviour. The prevalence of these disorders is a major public health concern: however, we still know little of the mechanisms through which childhood stress influences future health and behaviour.

We do know that an individual's environment and life experiences will influence the way in which his or her genes are expressed: that is, which genes are 'turned on' or 'turned off' in each cell at any time, switching on or off the synthesis of the proteins that carry out all biochemical functions. Changes in protein levels in cells lead to biochemical cascades that have profound influences on our health and behaviour. Therefore, studying how stress influences gene expression can help us understand the mechanisms linking childhood stress with disease.

One of the most important mechanisms through which life experiences affect gene expression is through small chemical changes to the DNA that makes up those genes. Our chromosomes are made up of long pieces of DNA, which itself consists of a sequence of chemical bases – cytosine, guanine, adenine and thymine – joined by a backbone. In all chromosomes, a tiny chemical group known as a methyl group is added to some of the cytosine bases. This trivial-seeming chemical change is DNA methylation; it often occurs on DNA regions known as promoters that lie close to the points where genes start. Methylation of gene promoters will reduce, or repress, the expression of those genes. Studies in young monkeys and human children have suggested that changes in the methylation patterns of some specific gene promoters are associated with both severe stress in early childhood and the risk of developing anxiety and mood disorders later in life.

So far, most of this work has concentrated on the expression of single genes that are already known to be associated with, for example, emotional regulation or stress. Human behaviour is so complex, however, that this pattern is likely to be repeated in many more genes. A group of researchers led by Seth Pollak and Reid Alisch at the University of Wisconsin, Madison, USA, have now analysed the link between childhood stress, DNA methylation and gene expression across the whole 23 chromosomes of the human genome.

The researchers collected saliva from 22 pre-teenage girls who had previously been involved in a study of stress and hormone regulation. Half the participants had experienced high or very high levels of stress as young children; the others, the control group, had only experienced the stresses and strains of normal childhood. There was no significant difference in the racial characteristics or age profile of the groups; about half the girls in each group identified themselves as Caucasian. They interviewed all participants and their parents separately using a semi-structured Youth Life Stress Interview to determine the types and levels of stress that they had experienced during childhood, and the behaviour of each girl was assessed by trained staff on a 5-point scale. High levels of stress were strongly associated with anxiety and poor behaviour; the girls who had experienced the most stress were more likely to have problems in their social lives, to react badly to upsetting thoughts, and to engage less in extracurricular activities.

DNA was extracted from the saliva, and each DNA sample was analysed to determine the extent of methylation and the positions on each chromosome where it occurred. Positions along the chromosomes at which the amount of methylation, varied significantly between stressed and unstressed girls were recorded. Gene expression in the saliva samples was analysed by measuring the level of RNA (ribose nucleic acid) associated with each gene; this nucleic acid is manufactured using the DNA sequence of genes as a template and is a precursor to the production of protein. This analysis produced two lists of genes, one in which methylation levels differed between the groups and the other in which expression levels varied These were compared to each other and then to a third list of genes that are already known to be associated with the stress response.

Pollak, Alisch and their co-workers discovered a total of 122 genes with stable differences in DNA methylation between the stressed and unstressed girls; both increases and decreases in methylation with stress were common, and 12 genes had both increases and decreases at different positions along their DNA sequence. Thirty-one of these genes had proven associations with the stress response reported in the scientific literature, and many others had links to potentially relevant biochemical pathways.

Turning to the link between stress and gene expression, the researchers found a total of 1,405 genes (very roughly 7% of the genes in the human genome) with significant differences in expression levels between the stressed and unstressed groups. About a quarter of these (349) have known links to the stress response.

The two genes that occur on all three lists – with a known role in the stress response and significant differences in DNA methylation and gene expression between the groups – are known by the acronyms *FHL3* and *NPC2*. The exact function of the first of these is unknown, although it binds to the main receptor protein that is activated by testosterone. Mutations in *NPC2* can cause cholesterol to accumulate in cells, which will affect the function of the mitochondria (which are, in effect, cells' 'power generators') This suggests that defects in cellular energy production and use might be a factor in the development of stress-related disorders.

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The researchers admit one limitation to their study: that it sought to link observations made in saliva with disorders that are largely psychological and neurological. However, they add that others have recently shown DNA taken from brain cells to have similar patterns of methylation to that from other tissues, including saliva, in the same individual. The links between DNA methylation, gene expression, childhood stress and health problems – particularly those involving emotion and mood – have now been clarified. Focusing in on some of the genes identified might, eventually, lead to the identification of drug targets for psychiatric disorders: but it would be even better to prevent the need for such drugs, by targeting the causes of early childhood stress.

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