RESEARCH SUMMARY

Involvement of circulating factors in the transmission of paternal experiences through the germline

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[Note: This summary frequently refers to the 'germ cells' and the 'germline'. This has nothing to do with infectious disease! In this context, the germ cells are those cells that pass on their genetic material to the offspring. They include the egg and sperm cells and the predecessor cells that form eggs and sperm after cell divisions. These cells together form the germline.]

Adverse life events, and the stress that they cause, can have long-term effects on the physical, as well as the mental health of the individuals involved. It is less well known that these effects can also be passed on to children born long after the initial stress has passed. This transmission of such signals from one generation to another is a type of heredity, but one that clearly cannot involve changes to gene sequences, which are fixed at conception. Instead, it is thought that stressful events in an individual can cause small chemical changes to DNA in germ cells that are the predecessors of sperm and eggs. These changes can be passed on to their offspring, and fathers as well as mothers are involved. Exactly how, and when, signals produced by stressful life events are transmitted into germ cells and give rise to these so-called epigenetic changes to their DNA is still unclear.

Many different chemicals circulate around the body and carry signals that communicate information between tissues and cells. These include molecules that are regulated by changes in physiology and are involved in epigenetic changes to genes in other tissues. This process has been observed in humans and in other mammals.

A large group of scientists from Switzerland and the UK, led by Isabelle Mansuy of the University of Zurich, Switzerland, set out to discover how these circulating factors are involved in the transmission of the stress of adverse experiences from fathers to their offspring and, perhaps, further generations. Most of their experiments involved a mouse model of early life stress, known as MSUS (for maternal separation plus unpredictable maternal stress). This involved separating mother mice from their pups for 3 hours at unpredictable times of day, every day for the first two weeks after birth. During the separation periods, the dams were stressed randomly for short periods, either by plunging them into cold water or by restraining them in a tube. When the pups reared in this way (MSUS mice) become adults, they have characteristic problems with their metabolism and behaviour that are transmitted to their own offspring.

Mansuy and her colleagues analysed the chemicals, or metabolites, that are found in the plasma of male MSUS mice and compared them with those in the plasma of similar mice raised normally (control mice). They discovered significant differences between the two groups: the MSUS mouse plasma had more fatty acids and other molecules involved in inflammation and fewer molecules involved in steroid synthesis, than that of the control mice.

The group collaborated with an SOS Children's Village in Lahore, Pakistan to find out whether the metabolic changes they observed in mice were replicated in human children. They identified a group of children between 6 and 12 years old who had lost their fathers and who had recently been moved into the orphanage because their mothers could no longer care for them. These children – named PLMS for paternal loss, maternal separation – are probably as nearly equivalent to the MSUS mice as it is possible for human children to be. They were matched with a group of children of similar ages with no known severe stresses, who lived with both parents and attended the same school. Analysis of serum obtained from both groups showed metabolic differences between the PLMS and control children that were similar to those observed between the MSUS and control mice.

The researchers considered that, out of all the significantly different circulating molecules, fatty acids and related compounds were the most likely to affect the germline. These acids bind to a group of proteins known as peroxisome proliferator-activated receptors, or PPARs, and, once they are bound, the receptors regulate the synthesis of proteins involved in metabolism, inflammation and, interestingly, cognition. Bile acids and steroid metabolites, which are also altered by MSUS, bind to receptors in the same family.

Different members of the PPAR family are found in different cells and tissues, and several of these were found to be more abundant, or more active, in the MSUS mice. The principal variant found in gametes is PPARg; the activity of this receptor in pre-sperm cells called spermatogonial cells was significantly higher when they were exposed to plasma from MSUS mice than that from normal mice. These cells were chosen because they are found in the developing mouse testis during the first two weeks of life: that is, at the time when the MSUS mice were exposed to separation and trauma.

It is possible to mimic the activation of a receptor directly by injecting a chemical, known as an agonist, that can bind to it causing it to respond in the same way as its natural ligand does. Mansuy and her colleagues injected a compound called tesaglitazar, which activates both the PPARa and PPARg receptors, into the body cavity of normal male mice. Forty-six days later, and so after spermatogonial cells affected by the injection had differentiated into sperm, these mice were mated with control females, and the resulting pups of both sexes were mated when adult with further control mice to produce 'grand-offspring'. The first and second-generation offspring of the original mice had low body weight and increased sensitivity to insulin, both traits that are observed in MSUS mice.

The group then tested compounds that might be assumed to have the opposite effect. PPARg antagonists bind to the receptors, preventing other molecules from binding but eliciting no response. One such antagonist, a compound with the unmemorable name of T0070907, was injected into adult control male mice, and these too were bred with control females to produce litters. The resulting pups were, when fully grown, indistinguishable from normal mice, suggesting that PPAR inhibition had no effect on their metabolism.

Next, Mansuy and her co-workers investigated whether injection with tesaglitazar affected the expression of RNA – the step before protein expression – in mouse sperm. The pattern of RNA expression in tesaglitazar-injected normal mice strongly suggested that those mice would produce higher concentrations of proteins involved in processes linked to PPAR activity. Furthermore, spermatogonial cells exposed to serum from either MSUS or tesaglitazar-injected male mice had higher levels of PPAR activity. These results strongly suggested a link between early stress and PPAR expression and activity.

They also found that the offspring of normal male mice that had been injected with serum from MSUS males had the same low body weight and increased insulin sensitivity as the original MSUS males, but that this pattern was not seen in the offspring of mice injected with tesaglitazar. This result, unsurprisingly, indicates that the complex, inheritable response to early stress cannot be wholly mimicked by the activity of a single receptor.

Taken together, all these results show that chemicals circulating in the blood and plasma are responsible for epigenetic changes to the DNA in the germ cells of male mice and for passing the effects of early-life stress down at least two generations from the father. PPAR receptor activation has been shown to be a factor in triggering these changes, although it is by no means the only one. Stress has been known for many years to activate the sympathetic nervous system and the hypothalamic–pituitary–adrenal (HPA) axis, but these results suggest that it also affects lipid (fat) metabolism. This might have clinical implications, since there is a known association between early stress and obesity. The observed similarities between children who have experienced seriously disrupted parenting and MSUS mice suggests that epigenetic modifications to the germline may also play a role in transmitting adverse experiences from a human father to his children.

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