REPRODUCTIVE ENDOCRINOLOGY

# Dad's diet – smRNA methylation signatures in sperm pass on disease risk

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Metabolic disease risk is thought to arise at the interface of genetics and the environment. A new study identifies an enzyme that modifies small non-coding RNA and is required for passing on the effects of high-fat diet from father to offspring.

*Refers to* Zhang, Y. et al. Dnmt2 mediates intergenerational transmission of paternally acquired metabolic disorders through sperm small non-coding RNAs. *Nat. Cell Biol.* **20**, 535–540 (2018).

The rapid rise in the incidence of obesity and associated metabolic disorders is a major public health challenge. Moreover, the frequency of disease risk has increased by an order of magnitude within a few decades[1]. Despite our awareness that metabolic disease arises at the interface between genetic susceptibility and environmental factors (such as overnutrition and inactivity), we have not been able to curb the incidence of these disorders. With the advent of cost-effective whole-genome sequencing technologies has come the search for the genetic underpinnings of metabolic disorders; however, to date, only a small percentage of metabolic disease risk has been associated with genetic variants[2]. This weak association with genetic variants has raised the intriguing possibility that metabolic disease risk could be transmitted from generation to generation via potentially modifiable non-genetic signals present in parental germ cells.

The idea that metabolic disease risk could be transmitted from one generation to another remains controversial and a subject of much debate, but paternal transmission of environmentally induced phenotypes across generations has been reported to occur following a number of qualitatively different environmental exposures (for example, toxins, postnatal stress and diet). The observation of these effects in response to paternal experience is strongly suggestive of signals transmitted through sperm, as male contributions to breeding are predominantly limited to sperm. While epigenetic marks such as DNA methylation and histone modifications have been speculated to directly pass on environmental 'memories', evidence for this has been minimal[3]. However, there is a growing body of work suggesting that small non-coding RNAs (smRNAs) transmitted via sperm can recapitulate parental phenotypes in their offspring. Indeed, it seems that stress and diet can induce changes in expression and composition of sperm smRNA content (primarily comprised of microRNAs (miRNAs), Piwi-interacting RNAs (piRNAs) and tRNA-derived small RNAs (tsRNAs)). Several groups have now shown that injection of sperm RNA (collected from mice that have experienced stress or dietary manipulations) can recapitulate at least some of the behavioural and metabolic phenotypes that are observed in offspring sired through natural breeding conditions[4–6].

In a 2016 study, Qi Chen and colleagues showed that, in addition to expression changes, sperm smRNAs carry methylation modifications that could be critical for conveying the phenotypic memories of previous generations[4]. The authors reported that sperm from male mice exposed to a high-fat diet (HFD) showed changes in the expression of tsRNAs, which also carried methylation marks at the $m^5C$ (the 5th carbon of cytosine bases) and $m^2G$ (the 2nd carbon of guanine bases) positions. Through a series of RNA injection experiments into naive zygotes, they further showed that it is the fraction of smRNAs containing the tsRNAs and not the miRNAs or longer non-coding fragments that seem capable of transmitting the metabolic phenotypic to offspring. Interestingly, Chen and colleagues found that mice that were injected with synthetic tsRNAs lacking RNA modifications were unable to recapitulate the phenotype, suggesting that these RNA modifications were necessary for non-genetic transmission[4].

In a new paper, Qi Chen, Yunfang Zhang and colleagues have taken their work a step further, using a genetic model to show that DNA methyltransferase enzyme 2 (DNMT2), a methyltransferase enzyme previously shown to modify mammalian tRNA, has a critical role in the non-genetic transmission of metabolic phenotypes[7] (FIG. 1). They first noted that HFD in mice increases DNMT2 expression in the caput epididymis, where sperm are thought to acquire their smRNA content during maturation. They then tested the functional relevance of this enzyme, by assessing the effects of HFD in a mouse model lacking DNMT2. Interestingly, sperm collected from HFD-exposed males lacking DNMT2 failed to recapitulate metabolic phenotypes in offspring, confirming that DNMT2-mediated methylation was a functionally necessary component of tsRNA-mediated paternal transmission. An extensive analysis of the possible modifications of these tsRNA fragments revealed that HFD induces increases in $m^5C$ and $m^2G$ methylation, which the authors suggest comprises a 'sperm RNA coding signature' that is essential for transmitting the effects of paternal HFD to offspring.

> "Zhang et al. provide an experimental framework for determining the mechanisms linking environmental exposures"

An intriguing question raised by these results is why might increases in $m^5C$ and $m^2G$ methylation be important for the non-genetic transmission of phenotypes across generations? Zhang and colleagues show that synthetic tsRNAs that lack a single $m^5C$ at position 38 had a markedly different secondary structure and were resistant to degradation by RNases, compared with

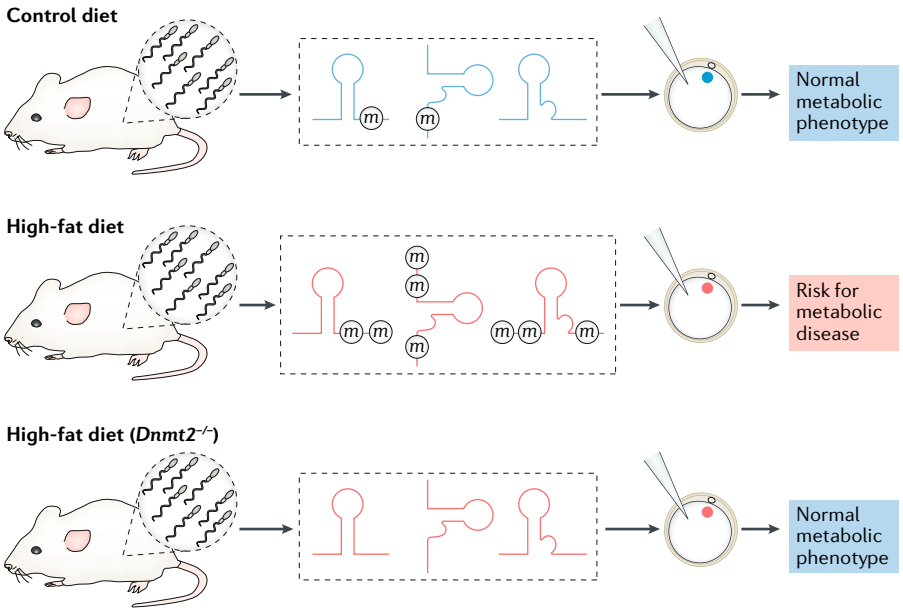


Fig. 1 | **Changes in tRNA-derived small RNAs following a high-fat diet.** Yunfang Zhang and colleagues report that a high-fat diet in mice results in changes in tRNA-derived small RNA (tsRNA) expression profiles as well as increased methyl modifications at these tsRNAs, which when injected into zygotes result in phenotypes associated with metabolic disease risk. This transmission is prevented in mice lacking a DNA methyltransferase enzyme (DNMT2; *Dmnt2*$^{-/-}$), suggesting that methyl modifications for tsRNAs are required for the transmission of the environment memories of a high-fat diet.

synthetic tsRNAs that had the same modification or completely lacked any modifications. Moreover, the more stable synthetic tsRNAs were associated with unique transcriptional responses when transfected into cells.

The synthetic tsRNAs that Zhang and colleagues examined represent sperm tsRNA modifications that are present in DNMT2-positive mice compared with DNMT2-null mice fed a normal diet. Given that there is no difference in phenotypic outcomes in offspring between these conditions in the injection experiments, it still remains unclear what the functional role(s) of the HFD-induced changes in tsRNA methylation might be, especially as the HFD diet increases methylation of tsRNAs more broadly across the different positions of the RNA molecule.

> “This work raises a broader issue about the relative importance of non-genetic contributions of sperm”

An interesting point to note is that the effect of HFD-induced changes in sperm RNAs on offspring metabolic phenotype is lost under natural mating conditions. Moreover, under these conditions offspring of DNMT2-positive mice seem to show increased signs of metabolic disorder compared with offspring of DNMT2-null mice (effects that are not present in the injection experiments). These effects could be due to the differential methylation that the authors report at position 38 of tsRNA-Gly, or, more likely, reflect the complexity of reproduction in a natural context. Much of the work linking sperm RNA to phenotypic outcomes has involved injecting RNA from a very large number of sperm cells (non-physiological levels), which could overestimate or mask the impact of specific sperm tsRNAs and their associated modifications on offspring development. Furthermore, we must recognize that mothers are not passive recipients of sperm and that reproduction involves a complex interplay between paternal contributions from sperm and maternal factors (oocyte content, intrauterine environment and postnatal care), all of which could further shape offspring metabolic phenotypes[8]. Therefore, it is not surprising that there are differences in the magnitude and direction of non-genetic transmission of phenotypes to offspring when comparing natural mating and in vitro reproduction[9].

This work raises a broader issue about the relative importance of non-genetic contributions of sperm on offspring development. While these effects have led many to speculate that they might represent an adaptive, more flexible mode of ‘inheritance’, many of these studies have been performed in isogenic rodent models, making it unclear how important these effects are in human populations where genetic variation might override or have complex interactions with non-genetic effects. The recent discovery that sperm RNA profiles are different between obese and healthy men is a relevant move towards closing this gap[10]. Critical next steps include deciphering mechanisms relating these sperm RNAs to phenotypic outcomes, and determining whether or not the effects of the sperm RNAs are simply an epiphenomenon.

Studies such as this by Zhang et al. provide an experimental framework for determining the mechanisms linking environmental exposures with genetic and non-genetic signals and metabolic risk to identify potentially relevant biomarkers of environmental compromise. This might have implications for the development of reversal strategies (pharmacological and/or behavioural), which might ultimately reduce the growing incidence of metabolic disease.

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**Competing interests**
The authors declare no competing interests.