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RESEARCH HIGHLIGHT

Paternal transgenerational glucose intolerance with epigenetic alterations in second generation offspring of GDM

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Gestational diabetes mellitus (GDM) has been shown to be associated with high risk of diabetes in offspring. In addition to intergenerational transmission (F1 offspring), intrauterine hyperglycemia also has effects on the second generation (F2 offspring). However, the mechanisms involved and the possibilities of transgenerational transmission are still unclear. In a recent study published in *Diabetes*, we have utilized GDM mouse model to identify hyperglycemic intrauterine environment causing a high risk of diabetes in offspring by altering epigenetic modification. Furthermore, the results indicate that the abnormality of phenotype and imprinted genes expression are more obvious in male offspring than that of female. The changes of epigenetics in sperm may contribute to transgenerational transmission.

A growing body of research suggests that exposure to the abnormal environment in uterus can lead to chronic health problems later in life.[1] Intrauterine hyperglycemia is a major characteristic of GDM and has been suggested as an important determinative factor for the risk of diabetes in adulthood, in addition to the effects of genetic factors.[2] The mechanism involved in the association between intrauterine hyperglycemia and a high risk of diabetes in offspring remains unclear.[3] In mammals, epigenetic reprogramming is involved in germ cells and early embryonic development.[4,5] Because erasure and establishment of the genomic imprints for some imprinted genes begin when migratory primordial germ cells enter the embryonic genital ridge through gametogenesis, epigenetic abnormalities that occur during

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this phase may be involved in transgenerational transmission.[6]

In the study, Ding et al.[7] established a GDM mouse model of intrauterine hyperglycemia. The female (♀) and male (♂) F1 adults of control and GDM mice were intercrossed to obtain F2 offspring of four groups: (i) C♂–C♀; (ii) C♂–GDM♀; (iii) GDM♂–C♀; and (iv) GDM♂–GDM♀. We found that intrauterine hyperglycemia induced impaired glucose tolerance (IGT) and abnormal insulin levels in F1 offspring, which are partly due to the deficient islet ultrastructure. IGT of male GDM offspring was more obvious than that of female offspring, suggesting that male fetus might be more sensitive to the intrauterine environment than female. Furthermore, the intrauterine hyperglycemia induced transgenerational transmission of glucose intolerance and abnormal insulin levels. In all F2-GDM offspring groups, IGT of male was obvious than that of female, suggesting the effect of intrauterine hyperglycemia on F2 males was more obvious than F2 females. Intrauterine environment with moderate hyperglycemia did not affect birth weight of F1-GDM, but increased birth weight of F2 offspring born from F1-GDM with IGT. After born from F1-GDM with IGT, the birth weight in the GDM♂–C♀ and GDM♂–GDM♀ groups significantly increased when compared with C♂–C♀, which showed sex-specific characteristics. In previous study, a number of diabetes-related animal models also show gender differences, with males having a more profound phenotype.[8] In the Nagoya–Shibata–Yasuda mice, there is a marked gender difference with almost all males developing hyperglycemia, but less than a third of females being affected.[9] Many genetically induced forms of insulin resistance have a more severe phenotype in males compared with females.[10]

The gender differences in transmission of phenotypes indicate that epigenetic mechanisms may be involved in transgenerational effect.

To define further the potential secretory defects that could contribute to glucose intolerance in offspring exposed to intrauterine hyperglycemia, we assessed glucose-stimulated insulin secretion of islets isolated from 8-week-old male mice. We found *in vitro* glucose-stimulated insulin secretion was impaired in both F1-GDM and F2-GDM offspring. Reduced glucose-stimulated insulin secretion may contribute to impaired glucose tolerance in both F1 and F2 offspring from intrauterine hyperglycemia. Many factors are related to β-cell dysfunction. In our study, we focused on the imprinted genes involved in islet development and the pathogenesis of diabetes, *Igf2*, *H19* and *Plagl1*. Down-regulated expression of *Igf2* and *H19* exhibited not only in F1-GDM but also in F2-GDM offspring through both paternal and maternal lines, confirming that dysregulation of *Igf2* leads to inappropriate insulin production and secretion and induces diabetes.[11,12] Moreover, in F2-GDM offspring, decreased level of *Igf2* and *H19* expression was associated with parental characteristics. *Igf2* reduced maximum through the maternal line, while *H19* was reduced maximum through the paternal line. In accordance with the tendency of phenotype changes, the change of imprinted genes expression was also more obvious in male offspring than that of female.

As imprinted genes, *Igf2* and *H19* allelic expression in mice is regulated by allele-specific methylation at four DMRs.[13] We collected islets from 8-week-old male mice of the control, F1-GDM and GDM♂–GDM♀ groups. We analyzed the methylation levels of 12 CpGs of the *Igf2* DMR0, 4 CpGs of the *Igf2* DMR1 and 13 CpGs of the *Igf2* DMR2

by bisulfite genomic sequencing PCR. Furthermore, we analyzed the methylation levels of the 12 CpGs located in the CCCTC-binding factor binding sites of the *H19* DMR. Although there were no differences in methylation of *Igf2* DMR0 and *Igf2* DMR1, compared with control, in islet of F1-GDM and GDM♂–GDM♀, the methylation levels were significantly higher in *Igf2* DMR2. The methylation levels of *H19* DMR were also significantly much higher than control. These results indicated that intrauterine hyperglycemia could cause hypermethylation at *Igf2* DMR2 and *H19* DMR in both F1 and F2 offspring, suggesting that altered expression of *Igf2* and *H19* in islets are associated with the altered modification of *Igf2* DMR2 and *H19* DMR.

By *in vitro* culture, we found that the *Igf2* and *H19* mRNA levels were significantly lower in fetal mouse islets exposed to a high glucose concentration (25 mmol l^{-1}) than to a physiological concentration (5 mmol l^{-1}). The effect of high glucose on *Igf2* and *H19* expression in fetal islets provided a probable explanation for intrauterine hyperglycemia directly influenced imprinted genes and induced β-cell dysfunction. However, during pregnancy period of adult female F1-GDM, the random glucose level was normal, which means fetus of C♂–GDM♀ and GDM♂–GDM♀ groups both developed in relatively normoglycemic intrauterine environment.

Especially in GDM♂–C♀ group, there was completely normal maternal metabolic environment during development *in utero*. The paternal factor of F1 offspring played more important role in growth and glycometabolism of F2 offspring, suggesting that epigenetic changes occurred in the germ line and inherited through meiosis,[6,14] may be an explanation for transgenerational transmission. Thus, we further investigated the expression of *Igf2* and *H19* in sperm of adult F1-GDM male mice. It is interesting that, no matter whether F1-GDM male mice with or without IGT, *Igf2* and *H19* were both downregulated in their sperm. Moreover, the change of *H19* was more obvious than *Igf2*.

Although relevant epigenetic modification needed further exploration, the study indicates the paternal influences on transgenerational transmission. Intrauterine hyperglycemia could alter imprinted genes expression of sperm to transmit the risk of disease to next generation.

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