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Research Paper

Prevalence of Prediabetes Risk in Offspring Born to Mothers with Hyperandrogenism

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ABSTRACT

Background: Excessive androgen exposure during pregnancy has been suggested to induce diabetic phenotypes in offspring in animal models. The aim of this study was to investigate whether pregestational maternal hyperandrogenism in human influenced the glucose metabolism in offspring via epigenetic memory from mother's oocyte to child's somatic cells.

Methods: Of 1782 reproductive-aged women detected pregestational serum androgen, 1406 were pregnant between 2005 and 2010. Of 1198 women who delivered, 1116 eligible mothers (147 with hyperandrogenism and 969 normal) were recruited. 1216 children (156 children born to mothers with hyperandrogenism and 1060 born to normal mother) were followed up their glycometabolism in mean age of 5 years. Imprinting genes of oocyte from mothers and lymphocytes from children were examined. A pregestational hyperandrogenism rat model was also established.

Findings: Children born to women with hyperandrogenism showed increased serum fasting glucose and insulin levels, and were more prone to prediabetes (adjusted RR: 3.98 (95%CI 1.16–13.58)). Oocytes from women with hyperandrogenism showed increased insulin-like growth factor 2 (IGF2) expression. Lymphocytes from their children also showed increased IGF2 expression and decreased *IGF2* methylation. Treatment of human oocytes with dihydrotestosterone upregulated IGF2 and downregulated DNMT3a levels. In rat, pregestational hyperandrogenism induced diabetic phenotypes and impaired insulin secretion in offspring. In consistent with the findings in human, hyperandrogenism also increased Igf2 expression and decreased DNMT3a in rat oocytes. Importantly, the same altered methylation signatures of *Igf2* were identified in the offspring pancreatic islets.

Interpretation: Pregestational hyperandrogenism may predispose offspring to glucose metabolism disorder via epigenetic oocyte inheritance.

Clinical trial registry no.: ChiCTR-OCC-14004537; www.chictr.org.

1. Introduction

Hyperandrogenism is a common endocrine disorder among reproductive aged women (Qiao and Feng, 2011). Excessive concentration of androgen in ovarian follicles may alter oocyte developmental competence that consequently decreases the fertilization rate and embryonic

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development during in vitro fertilization (IVF) (Sen et al., 2014; Murray et al., 2008; Teissier et al., 2000). Animal studies showed that intrauterine testosterone exposure induced metabolic alterations, including reduced glucose stimulated insulin secretion and impaired glucose tolerance, in offspring not only at early age but also in adulthood (Amalfi et al., 2012; Nohara et al., 2013). However, the long term health implications of children conceived by women with hyperandrogenism remain largely unknown.

Human epidemiological studies have indicated the inheritance of environment-induced phenotype (Huang and Sheng, 2014). Offspring of in utero malnutrition are associated with increased prevalence of type 2 diabetes risk (Petry and Hales, 2000). Famine experiences in grandfathers increased the risk of obesity and cardiovascular diseases in grandchildren (Painter et al., 2008). Although the exact mechanism is unclear, these studies indicate that the environmental factors are associated with the inheritance of disease risk in next generations.

Recently, a series of animal studies have revealed that adverse paternal factors increase the susceptibility to adult metabolic diseases in offspring through gametic epigenetic alterations (Carone et al., 2010; Radford et al., 2014; Wei et al., 2015). Paternal high-fat-diet exposure may program β-cell dysfunction in first-generation (F1) female offspring in rat (Ng et al., 2010). Intrauterine undernutrition impairs lipid metabolism in second-generation (F2) offspring by transmission of DNA methylation alteration via paternal linage (Martinez et al., 2014). Likewise, paternal prediabetes can be transgenerationally inherited through the male germ line depending on methylation changes in mice (Wei et al., 2014). A recent study in rodent, using IVF and embryo transfer (IVF-ET) and foster mother, demonstrated that a parental high-fat diet renders offspring more susceptible to developing obesity and diabetes in a sex- and parent of origin-specific mode (Huypens et al., 2016). Two studies showed that maternal diabetes and obesity resulted in altered oocyte methylation patterns of specific genes (Ge et al., 2013, 2014). Hence, it is very important to investigate whether oocytes, especially human oocytes, from mother with endocrine disorders are associated with increased risks of metabolic disorders in their children, and what mechanisms are involved.

The aim of this study was to investigate whether mothers with pregestational hyperandrogenism could predispose offspring to glucose metabolism disorder through epigenetic oocyte inheritance. Our results demonstrated that children born to mothers with pregestational hyperandrogenism manifested increased serum fasting glucose and insulin levels, and were more prone to prediabetes. High androgen levels significantly upregulated *IGF2*, an imprinting gene, expression in human oocytes. In parallel, study in rats clearly showed that pregestational hyperandrogenism induced diabetic phenotypes and impaired insulin secretion in offspring. Exposure of rat oocytes with high androgen concentration increased the expression level of *Igf2* not only in mothers' oocytes but also in β-cells of F1 pancreatic islets.

2. Materials & Methods

2.1. Participants and Prospective Cohort Study Design

The study was approved by the ethical committee of School of Medicine, Zhejiang University, Hangzhou, China. Total of 1782 women, who registered in Reproductive Center, Women's Hospital, School of Medicine, Zhejiang University for IVF between 2005 and 2010, voluntarily participated in this study. All participants provided informed written consents. Their basal hormones including testosterone (TTE), dehydroepiandrosterone (DHEAS) levels were examined at menstrual cycle day-3. Women were excluded if they were not pregnant till December 2010 (n = 355) or withdrew consents (n = 21). Among the remaining 1406 pregnant women, 38 were with ectopic pregnancy, 95 went through miscarriage and 75 women were lost to follow up before delivery. After delivery, 55 women declined child testing. The inclusion criteria for hyperandrogenism group were basal TTE level higher than 2.4 nmol/L and/or basal DHEAS level higher than 8.8 μmol/L. During the follow up period, 27 women without hyperandrogenism were lost to follow up. Finally, 156 children born to women with hyperandrogenism and 1060 children born to women without hyperandrogenism attended our face to face follow up. Twins were handled as 2 separate children. None of the children had significant medical conditions which might affect their development. Children were followed up for 2–7 years after they were born (mean ages about 5 years).

2.2. Anthropometrics and Glycometabolism of Children

Children were required to attend the hospital at 8 am after an overnight fasting. Blood pressure measurements were performed after 20 min of quiet rest. Prediabetes was diagnosed if fasting glucose was 5.6–6.9 mmol/L. Oral glucose tolerance test (OGTT) was administrated in 132 singleton children born to women without hyperandrogenism and 80 singleton children born to women with hyperandrogenism after taking glucose at a dose of 1.75 g/kg body weight.

2.3. Human Oocytes and Lymphocytes Collection

Human MII oocytes were donated by the patients undergoing IVF/intra-cytoplasmic sperm injection (ICSI) procedure. All patients signed consent forms. Oocytes (n = 10) from women with hyperandrogenism and oocytes (n = 12) from control women were directly used for immunofluorescence analysis. Additional 20 oocytes from control women were cultured with 0 M (n = 10) and 10^{-9} M (n = 10) DHT for 30 h at 37 °C in a humidified atmosphere of 5% CO_2 in air. Lymphocytes from children were separated using density gradient and stored at −80 °C for further experiments.

2.4. Pregestational Hyperandrogenism Rat Model

Rat experiments were approved by the Animal Care and Use Committee, Zhejiang University. Hyperandrogenism female rat model was established using testosterone propionate injection at age of 9 days as previously described (Fan et al., 2012). At 6 weeks old, serum testosterone/sex hormone-binding globulin (SHBG) levels were examined using ELISA kit (R&D Systems, Minneapolis, MN, USA). Ovarian CYP17a was examined by immunohistochemical analysis (anti-CYP17a antibody, 1:200, Bioss, MA, USA; and CWBIO DAB kit, Guangzhou, China).

Mature oocytes were collected from 6-week-old rats. Meanwhile, hyperandrogenism female rats were mated with normal male rats, and pregnancy was determined by the presence of copulation plug.

2.5. Rat Offspring Glycometabolism and Islet Isolation

Glucose tolerance tests (GTT) of offspring were performed at 3-week-old and 8-week-old rats (male and female numbers were even in each groups). Offspring pancreatic islets were isolated at embryonic day 20 (E20), 3 weeks and 8 weeks old, respectively, as previously reported (Ding et al., 2012).

2.6. Quantitative Real Time-PCR and Bisulfite Genomic Sequencing PCR (BSP)

Total RNA was extracted from human peripheral lymphocytes and rat islets using RNAiso™ Reagent (TAKARA, Dalian, China), and cDNA was reverse-transcribed using the PrimeScript™ RT Reagent Kit (TAKARA). The methylation status of human *IGF2*, *GRB10* genes and rat *Igf2* gene were analyzed by sequencing of bisulfite-treated DNA using the EpiTect bisulfite kit (Qiagen). The schematic diagrams of CpG sites in human IGF2 and rat Igf2 were shown in Supplementary Fig. 1.

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