Maternal inheritance of severe hypertriglyceridemia impairs glucose metabolism in offspring

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Abstract

Maternally inherited familial hypercholesterolemia (FH) impairs glucose metabolism and increases cardiovascular risks in the offspring to a greater degree than paternal inherited FH. However, it remains unknown whether hypertriglyceridemia affects glucose metabolism via inheritance. In this study, we sought to compare the impact of maternally and paternally inherited hypertriglyceridemia on glucose and lipid metabolism in mice. ApoCIII transgenic mice with severe hypertriglyceridemia were mated with non-transgenic control mice to obtain 4 types of offspring: maternal non-transgenic control and maternal transgenic offspring, and paternal control and paternal transgenic offspring. Plasma triglycerides (TG), total cholesterol (TC), fasting plasma glucose (FPG) and fasting insulin (FINS) were measured. ApoCIII overexpression caused severe hypertriglyceridemia, but the transgenic female mice had unaltered fertility with normal pregnancy and birth of pups. The 4 groups of offspring had similar birth weight and growth rate. The plasma TG of maternal and paternal transgenic offspring were nearly 40-fold higher than maternal and paternal control mice, but there was no difference in plasma TG between maternal and paternal transgenic offspring. Plasma triglycerides (TG), total cholesterol (TC), fasting plasma glucose (FPG) and fasting insulin (FINS) were measured. ApoCIII overexpression caused severe hypertriglyceridemia, but the transgenic female mice had unaltered fertility with normal pregnancy and birth of pups. The 4 groups of offspring had similar birth weight and growth rate. The plasma TG of maternal and paternal transgenic offspring were nearly 40-fold higher than maternal and paternal control mice, but there was no difference in plasma TG between maternal and paternal transgenic offspring. Although the FPG of the 4 groups of animals had no difference, the maternal transgenic mice showed impaired glucose tolerance, increased FINS levels and higher homeostasis model assessment insulin resistance index (HOMA-IR) than the other 3 groups. In conclusion, maternally inherited hypertriglyceridemia in ApoCIII transgenic mice displayed impaired glucose tolerance, hyperinsulinemia and increased HOMA-R, while paternally inherited hypertriglyceridemia did not have such impacts.

Keywords: Apolipoprotein CIII, Transgenic mice, Hypertriglyceidemia, Insulin resistance.

Introduction

Large epidemiological studies have shown that hypertriglyceridemia (HTG) is an independent atherosclerosis risk factor¹¹, HTG is also associated with multiple organ damage, such as pancreatitis¹², renal dysfunction¹³, impaired nervous system function¹⁴ and impaired glucose regulation¹⁵. The major causes

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for the development of HTG are related to impaired metabolism of triglyceride-rich lipoproteins (TRLs) and is one of main components of metabolic syndrome. In general, environmental factors, such as high-fat, high-carbohydrate diet and lack of physical activities, play important roles in HTG occurrence. Genetic factors like gene variants of the lipoprotein lipase (LPL) which hydrolyze triglycerides, and apolipoproteins (Apos) that modulate LPL activity and hepatic secretion of very low-density lipoprotein (VLDL), can all be operational through the interaction with environmental factors influencing the occurrence and extent of HTG.

It has been noticed that HTG is also often accompanied with pregnancy because the metabolism of glucose and lipid will change significantly in the course of pregnancy, with increasing concentrations of estrogen and insulin resistance. In the midterm of pregnancy, fat storage starts to increase with concomitant reduction of lipolysis, and adipose tissue will normally increase 1.5 to 2 fold in the third trimester in comparison to non-pregnant women. Plasma triglycerides (TG) will also rise 2–3 times, up to 200–300 mg/dL. It then gradually decreases to pre-pregnant levels 6 weeks after term⁶⁶. If women have HTG before pregnancy, they tend to have severe HTG during pregnancy, which may induce acute pancreatitis with higher maternal and perinatal mortality, along with fetal malformation, miscarriage, premature birth and retarded growth rate. Other adverse pregnancy outcomes are also significantly higher⁶⁸.⁶⁹

As an inhibitor of LPL⁶¹, high plasma levels of ApoCIII are closely related to severity of human HTG⁶⁰, while low plasma levels of ApoCIII are associated with low plasma TG and even with decreased incidence of coronary artery disease (CAD) as defined by angiography⁷⁰. In addition, intracellular ApoCIII stimulates hepatic secretion of TG-rich lipoprotein via enhanced packaging of larger TG-rich lipoprotein, VLDL1. Such stimulatory effect has been shown to be through the 2 charged amino acid residues (Lys 58 and Lys 60 in α-helix 5) and potentially resulted in more TG-rich lipoproteins into plasma pool, hence aggravating HTG⁷¹.

Therefore, the ApoCIII transgenic mice with plasma TG levels 5–10 times higher than non-transgenic littermates would serve as an ideal model to study HTG and its related abnormalities⁷² because most other animals, when fed high fat diet, would only have plasma TG levels at 50%–2 times higher than that of chow diet controls⁷³.⁷⁴

Although both genetic and environmental factors play important roles in the development of HTG, transgenic ApoCIII mice were often used as genetic HTG model. Owing to the inherited nature of transgenesis, one should bear in mind that the severity of phenotypes in the offspring may not be identical. In human studies, it was found that maternally inherited familial hypercholesterolemia (FH) offspring would have higher cholesterol levels and cardiovascular risk than that of paternally inherited offspring⁷⁵. The same results were also found in the hypercholesterolemic ApoE-deficient mice⁷⁶. However, it is unknown whether maternal or paternal inheritance would have different effects on the severity of HTG, which is closely related to hypercholesterolemia. Therefore, in the present study, severe HTG ApoCIII transgenic male and female mice were mated with the normal male and female mice, respectively, to observe whether the female HTG mice would have normal pregnancy and parturition. If either normal or ApoCIII transgenic females would be able to give birth, we will have some normal and some HTG transgenic offspring, which would allow us to study the impact of maternal and paternal inheritance on severity of HTG and the glucose metabolism which is closely related to TG metabolism.

**Materials and methods**

**Animals**

*ApoCIII* transgenic mice in C57B6 background were from Jackson Laboratories, United States. They were bred with ICR mice to 5 generations to obtain *ApoCIII* transgenic mice with ICR background for larger litter size and easy reproduction. Genotyping of the parental mouse was performed by PCR using genomic DNA obtained from the clipped tail. Primers used for the *ApoCIII* gene were 5'-AGC TGG CAT AGC AGA GGT GT-3' (forward), and 5'-GCA GCC TCT CAT TGG GAA AG-3' (reverse). Using a mixture of these primers, PCR was done with 35 cycles of 30 s of denaturation at 94°C, 30 seconds of annealing at 65°C and 30 seconds of elongation at 72°C. The PCR product was 173 bp in size.

Totally 8 female HTG *ApoCIII* transgenic mice were mated with 8 normal ICR male mice, and 6 *ApoCIII* transgenic males with 6 normal ICR females to obtain 150 pups in 14 litters. Two litters from respective transgenic parents were taken randomly for assay of blood lipids, glucose and insulin. There were 13, 10, 13 and 11 pups in each litter, respectively, and they were divided into 4 groups: maternal non-transgenic normal controls (MC) and transgenics (MT); paternal normal controls (PC) and transgenics (PT). Plasma TG and total cholesterol (TC) levels were measured after 2 hour fasting at 14 days after birth.
Measurement of plasma parameters

At 28 days after birth the blood from the above 4 groups of pups were collected by retroorbital bleeding after 4 hours fasting. TC, TG, glucose and insulin in plasma were measured with commercially available kits (Sigma, St. Louis, MO, USA for TC, TG and Glu; Linco Research Inc, MO, USA for insulin). In order to eliminate the influence of turbidity caused by severe HTG, plasma samples from PT and MT pups were ultracentrifugated at 20,000 rpm for 30 minutes at 4°C in an OptimaTM TLX Ultracentrifuge (Beckman Coulter, Inc., Brea, CA, USA) before glucose and insulin measurement.

Oral glucose tolerance tests (OGTT)

OGTT was performed by giving a glucose bolus (3 g/kg) by gavage after 16 hours fasting. Blood was collected by retroorbital bleeding at 0, 30, 60, 90 and 120 minutes. Plasma glucose levels were measured as described above.

Western blot of human ApoCIII.

Plasma (1 μL) was electrophoresed on 12% Bis-Tris PAGE gels. The size-separated proteins were transferred to nitrocellulose membranes (Millipore) and probed by a primary antibody of goat anti-human ApoCIII (#K74130G, Biodesign) at 1:1000 dilution. A rabbit anti-goat HRP conjugated secondary antibody (#7074, Cell Signaling Technology) at 1:5000 dilution was chosen for color development and visualization.

Homeostasis model assessment insulin resistance index (HOMA-IR)

HOMA-IR was calculated as follows: HOMA-IR = (fasting insulin × fasting glucose)/22.5

Statistical analysis

The data showed a normal distribution and were expressed as means ± standard deviations (SD). Significant differences in parameters among groups were assessed by One-Way Analysis of Variance (One-Way ANOVA) and multiple comparison for post-hoc pairwise test. Significant differences in comparison of plasma triglycerides among 4 groups of mice 14 days were assessed by Kruskal-Wallis test. The statistical analysis was performed in SPSS 11.5 (SPSS Inc, Chicago, IL, USA). Statistical significance was defined as P<0.05.

Results

Normal fertility in both male and female ApoCIII transgenic mice with severe HTG.

Presence of human ApoCIII in parental transgenic mice were validated by both PCR genotyping of genomic DNA, Western blot analysis of plasma and plasma TG levels, as shown in Fig. 1 A, B and C. ApoCIII overexpression caused severe HTG but the transgenic male mice had normal fertility and the transgenic female mice had normal fertility with normal pregnancy, birth of pups and lactation as well. Totally 8 female HTG

Fig. 1 Validation of parental ApoCIII transgenic mice and comparison of litter size. A. Genotyping of ApoCIII transgenic mice. M. DNA marker; 1,3,4 and 5, transgenic mice; 2,6,7,8 and 9, none transgenic animals. B. Western blot of plasma probed by anti-human ApoCIII polyclonal antibody. 1 and 2, none transgenic; 3 and 4, transgenic. Arrows indicate molecular weight. C. Plasma TG levels in ApoCIII transgenic and non-transgenic control female and male mice. * P<0.01. D. Litter size of pups from female and male CIII transgenic parental mice.
ApoCIII transgenic mice were mated with 8 normal male mice to obtain 85 pups. At 14 days after birth the blood of 85 pups were collected after 2 hours of fasting. TG were measured, 47 pups TG levels > 1500 mg/dL, which were defined as the MT group; 38 exhibited TG levels > 1500 mg/dL, which were defined as the MC group. Totally 6 male HTG ApoCIII transgenic mice were mated with 6 normal female mice to obtain 65 pups. Thirty-five pups had TG levels > 300 mg/dL, which were defined as the PC group; 30 pups exhibited TG levels > 1500 mg/dL, which were defined as the PT group. There were no statistical differences in the litter size (Fig. 1D), offspring birth weight and growth rate (Fig. 2) between female ApoCIII transgenic and male ApoCIII transgenic mice.

Unaltered plasma TG levels in ApoCIII transgenic offspring from maternal and paternal inheritance.

At 14 days after birth plasma TG of MT and PT were significantly increased than their respective MC, PC groups, 3676.3 ± 1921.3 vs 231.2 ± 55.2 mg/dL (P<0.01) and 3601.9 ± 1298.4 vs 195.4 ± 56.3 mg/dL (P<0.01), which were milky appearance in both MT and PT groups (Fig. 3A and B).

At 28 days after birth, the 4 groups of offspring had similar weight, TG of MT and PT were nearly 40-fold higher than their respective MC, PC groups, 3309.1 ± 676.54 vs 79.8 ± 14.9 mg/dL (P<0.01) and 3024.6 ± 970.0 vs 78.6 ± 25.9 mg/dL (P<0.01), but there was no difference in plasma TG between MT and PT groups. There were about 3 times increase in plasma TC levels between MT/PT groups and MC/PC groups (Table 1).

Impaired glucose tolerance in maternal transgenic offspring

Although the FBG of the 4 groups of animals had no difference, but the MT group animals’ BG of 15 minutes after oral glucose was significantly higher

Table 1. Comparison of body weight and blood lipids among 4 groups of mice 28 days after birth (mean ± SD)

| Characteristic          | MC     | MT     | PC     | PT     |
|-------------------------|--------|--------|--------|--------|
| Number                  | 12     | 11     | 13     | 11     |
| Body weight (g)         | 18.0 ± 1.4 | 18.4 ± 2.3 | 18.6 ± 1.3 | 19.2 ± 1.9 |
| Triglycerides (mg/dL)   | 79.8 ± 14.9 | 3309.1 ± 676.0* | 78.6 ± 25.9 | 3024.6 ± 970.0* |
| Cholesterol (mg/dL)     | 124.1 ± 24.4 | 457.6 ± 125.5* | 152.8 ± 20.1 | 533.6 ± 102.2* |

MC: maternal control; MT: maternal transgenic; PC: paternal control; PT: paternal transgenic. * indicates the significant difference between MT vs MC and PT vs PT groups (P<0.01).

Fig. 2 The growth curve of offspring from female and male ApoCIII transgenic parental mice. MC, maternal non-transgenic normal controls; MT, transgenics; PC, paternal normal controls; PT transgenics.

Fig. 3 Comparison of plasma triglycerides among 4 groups of mice 14 days after birth. A. Milky appearance of plasma from MT and PT ApoCIII transgenic offspring; B. Levels of plasma triglycerides. * P<0.01. MC, maternal non-transgenic normal controls; MT, transgenics; PC, paternal normal controls; PT transgenics.
that MC, PC, PT group (*P*, 0.01), 4 groups of animals’ BG after oral glucose 30 minutes, 60 minutes 120 minutes BG was no significant difference. The MT mice showed mild impaired glucose tolerance, than the other three groups. The PT mice showed normal glucose tolerance (Fig. 4).

Increased fasting insulin and HOMA-IR in maternal transgenic offspring

The MT mice showed significantly increased FINS levels and higher HOMA-IR than the other three groups. At 28 days after birth, the MT group had insulin resistance (Fig. 5).

Discussion

In this study, we utilized male and female *ApoCIII* transgenic mice with severe HTG to mate respective non-transgenic mice, and obtained maternal and paternal inherited HTG offspring and their respective controls. *ApoCIII* transgenic male mice were fertile and the transgenic females also had normal pregnancy, birth of pups and lactation. There were no statistical differences in litter size, birth weight and growth rate of transgenic and non-transgenic pups from either maternal or paternal inheritance, indicating that parental severe HTG from *ApoCIII* transgene did not affect the development and growth rate of the offspring with different genotypes. Severe HTG accompanied with *ApoCIII* overexpression was inherited following Mendelian law of inheritance with half of the offspring being normal and half being transgenic either paternally or maternally. The plasma levels of triglycerides in the PT and MT group were both over 10 times higher than their non-transgenic littermate controls (PC and MC) during lactation and after weaning, but there was no difference between the MT and PT group, implying no influence of paternal or maternal inheritance of *ApoCIII* transgenic on severity of HTG. This is in contrast to inheritance of hypercholesterolemia in *ApoE* deficient mice which showed stronger maternal influence on the development of hypercholesterolemia in the offspring than paternal inheritance.

However, the present study found that mice from maternally inherited HTG displayed impaired glucose tolerance, in comparison to paternally inherited HTG mice. The fasting insulin levels of MT were significantly higher than MC, PC, and PT though the fasting plasma glucose levels of MT were not affected. The value of HOMA-RI derived from fasting plasma insulin and glucose levels was then the highest in MT mice among all 4 offspring groups. The results suggest that maternally inherited HTG due to *ApoCIII* overexpression might affect glucose metabolism.

It is well known that diabetic patients often have HTG accompanied with low HDL, because insulin resistance was shown to be a cause for enhanced synthesis and secretion of hepatic VLDL. Recently, Caron et al. showed that glucose might have a direct effect by up-regulating *ApoCIII* gene at the transcriptional level, resulting in HTG in hyperglycemic patients. However, the impact of primary HTG on
insulin and glucose metabolism has not been well studied. Lin et al. reported the severe HTG patients with non-diabetic microalbuminuria have glucose intolerance and hyperinsulinemia, demonstrating the potential impact of severe HTG on glucose metabolism. In another clinical study, it is found that in a HTG cohort with LPL gene mutation (LPL291), HTG was positively correlated with type 2 diabetes, also implying abnormal glucose metabolism resulting from HTG. Our previous study showed that LPL-deficient mice with severe HTG had a significant reduction in the first-phase insulin secretion with late stage occurrence of insulin resistance and proliferation of pancreatic β cells.

In an early study using similar ApoCIII transgenic mice, Chen et al. did not find significant differences in glucose metabolism from non-transgenic controls either in normal or in streptozotocin-induced diabetes, although ApoCIII deficient mice displayed increased adipose tissue and insulin resistance. In the present study, we found that only the severe HTG mice from maternal inheritance had higher insulin levels and hence increased HOMA-RI after weaning. Since the transgenic offsprings were usually obtained from paternal inheritance (routine breeding of transgenic males with non-transgenic females), or a mixture of paternal and maternal inheritance, it is hence possible to draw such conclusion that severe HTG resulting from ApoCIII transgenic had no effect on glucose metabolism. In addition, the age of the animals may also influence the results of the study.

Although we did not explore the mechanisms of increased insulin resistance in maternally inherited HTG in the current study, it should be noted that the fetus in utero environment of the HTG dams may be an important contributor to later development of insulin resistance. As Goharkhay et al. have shown hypercholesterolemia in ApoE-deficient female mice induced methylation and demethylation in certain regions of the genome, causing up- and down-regulation of genes involved in cholesterol homeostasis of embryos, thereby increasing cholesterol levels and hence the susceptibility to AS in maternally inherited offspring. The exact mechanisms of the finding that maternal inheritance of HTG through ApoCIII transgenic expression can affect glucose metabolism in offspring in the current study certainly warrant further investigation.

Our finding that maternal inheritance of HTG can affect the glucose metabolism in offspring would be of great interest if such finding can be validated through the clinical studies that maternally inherited severe HTG could indeed affect glucose metabolism. More attention on potential impairment of glucose metabolism then will be placed to those who have mothers experiencing severe HTG during pregnancy and lactation period.

In conclusion, maternally inherited HTG in ApoCIII transgenic mice displayed impaired glucose tolerance, hyperinsulinemia and increased HOMA-R, while paternally inherited HTG did not have significant impact on the glucose metabolism.

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