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Loss of inherited genomic imprints in mice leads to severe disruption in placental lipid metabolism

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ABSTRACT

Introduction: Monoallelic expression of imprinted genes is necessary for placental development and normal fetal growth. Differentially methylated domains (DMDs) largely determine the parental-specific monoallelic expression of imprinted genes. Maternally derived DNA (cytosine-5-) -methyltransferase 1o (DNMT1o) maintains DMDs during the eight-cell stage of development. DNMT1o-deficient mouse placentas have a generalized disruption of genomic imprints. Previous studies have demonstrated that DNMT1o deficiency alters placental morphology and broadens the embryonic weight distribution in late gestation. Lipids are critical for fetal growth. Thus, we assessed the impact of disrupted imprinting on placental lipids.

Methods: Lipids were quantified from DNMT1o-deficient mouse placentas and embryos at E17.5 using a modified Folch method. Expression of select genes critical for lipid metabolism was quantified with RT-qPCR. Mitochondrial morphology was assessed by TEM and mitochondrial aconitase and cytoplasmic citrate concentrations quantified. DMD methylation was determined by EpiTYPER.

Results: We found that DNMT1o deficiency is associated with increased placental triacylglycerol levels. Neither fetal triacylglycerol concentrations nor expression of select genes that mediate placental lipid transport were different from wild type. Placental triacylglycerol accumulation was associated with impaired beta-oxidation and abnormal citrate metabolism with decreased mitochondrial aconitase activity and increased cytoplasmic citrate concentrations. Loss of methylation at the MEST DMD was strongly associated with placental triacylglycerol accumulation.

Discussion: A generalized disruption of genomic imprints leads to triacylglycerol accumulation and abnormal mitochondrial function. This could stem directly from a loss of methylation at a given DMD, such as MEST, or represent a consequence of abnormal placental development.

1. Introduction

Genomic imprinting is a molecular process that distinguishes parental alleles such that one allele is transcriptionally active. The majority of imprinted genes are organized in clusters containing a variable number of imprinted genes. The imprinted expression of genes is determined by differentially methylated domains (DMDs) within imprinting control regions [1].

Proper inheritance of methylation imprints is essential for development, with isoforms of the DNA (cytosine-5-) methyltransrease1 (DNMT1) protein playing a central role. The somatic isoform, DNMT1s, maintains methylation at most preimplantation stages [2,3]. The enzyme DNMT1o maintains DMD methylation in the 8-cell embryo. DNMT1o is synthesized in the maternal oocyte [4–6]. Post-implantation embryos and placentas derived from DNMT1o-deficient oocytes lose methylation on ~50% of the normally methylated alleles of their DMDs [2]. Maintenance of maternal DNA methylation is unaffected by DNMT1o deficiency.

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Imprinted genes are highly expressed in the placenta and regulate fetal growth [7]. Previous investigations have demonstrated that imprinted genes modulate nutrient delivery to the fetus [8–10]. While these and other data provide a connection between imprinted genes and placental carbohydrate storage and dissemination, little is known about the relationship between imprinting and placental lipids.

Transplacental supply of lipids is critical for fetal growth, particularly late in gestation [11,12]. Free fatty acids and cholesterol are the predominant lipids transferred from the maternal circulation to the fetus [13,14]. The placenta is also capable of *de novo* lipid synthesis. The mechanisms that determine delivery of lipids to the fetus remain incompletely understood. Given the important role imprinted genes play in regulating fetal growth and nutrient delivery, we hypothesized that genomic imprinting is a plausible regulator of placental lipids. The loss of DNMT1o provides an opportunity to analyze the collective contribution of genomic imprinting to these processes by stochastic elimination of DMD methylation at many sites.

We have previously reported that DNMT1o deficiency results in abnormal placental morphology and altered embryonic growth. Pathway analysis of microarray gene expression data from E17.5 DNMT1o-deficient placentas identified genes regulating the accumulation of lipids as dysregulated. Here we extended our research to investigate whether DNMT1o deficiency altered placental lipid accumulation at E17.5 [15].

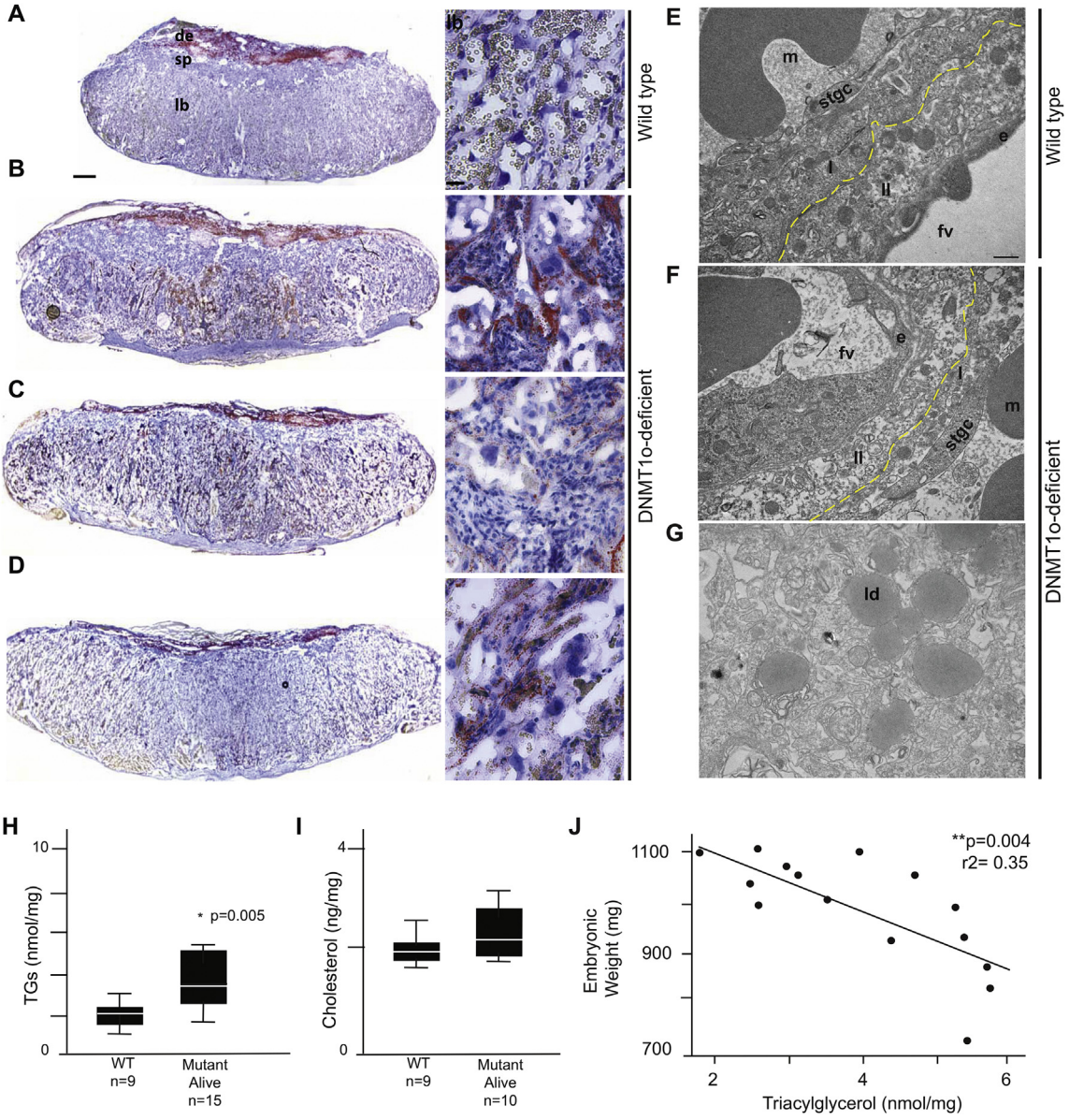

Fig. 1. A generalized disruption in genomic imprinting leads to accumulation of triacylglycerols in the placenta at E17.5 (A–D) Staining with oil red O in representative wild type (A) and DNMT1o-deficient placentas (B–D). Maternal decidua serves as a positive control. (E–G) Electron micrographs of labyrinth of E17.5 wild type (E) and DNMT1o-deficient placentas (F,G). Yellow dotted line indicates the junction between trophoblast layers I and II. (H,I) Box plots showing median values, upper and lower quartiles, and range of triacylglycerol and cholesterol among wild type and DNMT1o-deficient placentas. (J) Relationship between embryonic weight and placental triacylglycerols among mutants. TGs = Triacylglycerols. WT = Wild type. de = Decidua. sp = Spongiotrophoblast. lb = Labyrinth. fv = fetal vessel, e = endothelium. stgc = sinusoidal trophoblast giant cells. m = maternal. ld = lipid droplet. Scale bar (A–D) 400 μm (left panel) and 100 μm (right panel). Scale bar (E–G) 2 μm (left panel) and 500 nm (right panel).* Denotes significant difference compared to wild type by Kruskal–Wallis. **Analysis by linear regression.