Published in final edited form as:

Reprod Sci. 2021 February ; 28(2): 462–469. doi:10.1007/s43032-020-00348-7.

Catechol-O-methyltransferase and Pregnancy Outcome: an Appraisal in Rat

Khursheed Iqbal[1,2], **Pramod Dhakal**[1,2,3], **Stephen H. Pierce**[1,2], **Michael J. Soares**[1,2,4,5,6]

[1]Institute for Reproduction and Perinatal Research, University of Kansas Medical Center, Kansas, USA

[2]Department of Pathology and Laboratory Medicine, University of Kansas Medical Center, Kansas, USA

[3]Present address: Department of Animal Sciences, University of Missouri, Columbia, MO, USA

[4]Department of Pediatrics, University of Kansas Medical Center, Kansas, USA

[5]Department of Obstetrics and Gynecology, University of Kansas Medical Center, Kansas, USA

[6]Center for Perinatal Research, Children's Mercy Research Institute, Children's Mercy, Kansas, MO, USA

Abstract

Catechol-O-methyltransferase (COMT) has been shown to be a key regulator of pregnancy outcomes in mouse, and its deficiency is causative in the development of a preeclampsia-like disease process. Preeclampsia is a human pregnancy disorder associated with failure of intrauterine trophoblast cell invasion and trophoblast-guided uterine spiral artery remodeling, which are not well-developed in mouse. The purpose of this study was to investigate COMT in rat, a species with deep intrauterine trophoblast invasion. To accomplish this task, we used clustered regularly interspaced short palindromic repeats/Cas9-mediated genome editing of the rat *Comt* gene. A *Comt* null rat model was established and its fertility characterized. *Comt* null male and female rats were viable and fertile. COMT deficiency did not significantly impact pregnancy outcomes, including litter size, placental and fetal weights, Mendelian and sex ratios, or pregnancy-dependent adaptations to hypoxia. Collectively, our findings indicate that pregnancy-associated phenotypic outcomes of COMT deficiency are not equivalent in mouse and rat. In rat, COMT is not required for a successful pregnancy outcome.

Keywords

COMT; Rat genome editing; Placenta; Pregnancy

✉Khursheed Iqbal, kiqbal@kumc.edu.

Authors' Contributions KI and MJS designed the research; KI, PD, SHP, and MJS performed the research; KI and MJS analyzed data and wrote the paper.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s43032-020-00348-7) contains supplementary material, which is available to authorized users.

All animal procedures were approved by the KUMC Institutional Animal Care and Use Committee.

Competing Interests The authors declare that they have no competing interests.

Introduction

Catechol-O-methyltransferase (COMT) is an enzyme involved in the catabolism of catecholamines and other molecules possessing a catechol structure, including estrogens. COMT catalyzes the transfer of a methyl group from S-adenosyl-L-methionine (SAM) to a hydroxyl group of catechol-like compounds [1–5]. The COMT gene is highly conserved across species. COMT modifies 2-hydroxyestradiol to 2-methoxyestradiol (2ME) [4, 5], a compound with biological functions implicated in regulatory processes associated with pregnancy and placentation [6]. COMT and 2ME have been demonstrated to contribute to the regulation of angiogenesis, trophoblast development, and adaptations to hypoxia [7–12].

Deficits in COMT and 2ME have been implicated in the etiology of preeclampsia [6, 11, 13, 14]. Specific COMT polymorphisms have also been linked to preeclampsia [15–20]. Furthermore, a key report implicating COMT in the etiology of preeclampsia was published over a decade ago [8]. Using a COMT mutant mouse model [21], Kalluri and colleagues showed that deficits in COMT resulted in pregnancy complications characterized by a preeclampsia-like phenotype linked to the absence of 2ME [8]. Subsequently, COMT-deficient mouse models have been routinely used to interrogate molecular mechanisms underlying preeclampsia and fetal growth restriction [9, 22–27]. Unfortunately, more recent investigations exploring the connection between COMT in human pregnancy and its dysregulation in preeclampsia have not supported earlier observations [28–32]. Thus, uncertainty exists regarding the relative importance of COMT in pregnancy and in the etiology of diseases such as preeclampsia, and most notably, the merits of the COMT mutant mouse as a model for preeclampsia research.

Preeclampsia is a pregnancy disease best characterized in the human, where it is associated with failed deep trophoblast invasion [33, 34]. Mouse exhibits shallow trophoblast invasion and is not an optimal model for investigating placentation events linked to preeclampsia [35–37]. In contrast, rat shows deep placentation and robust intrauterine trophoblast invasion and is an excellent model for examining regulatory events at the uterine–placental interface [36, 38–40]. Implementation of appropriate animal models for research on human health–related issues, such as pregnancy and preeclampsia, is essential. Although mouse mutagenesis via gene targeting in embryonic stem cells has been a technique available for 30 years [41], routine manipulation of the rat genome has only been available for about a decade [42].

In this report, we sought to evaluate the involvement of COMT on pregnancy outcome in rat, a species characterized by extensive intrauterine trophoblast cell invasion. Clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 genome editing was used to generate a COMT-deficient rat model. Fertility and reproductive phenotypes were analyzed and compared between wild type and COMT null rats, including assessment of fetal and placental growth. We conclude that there are prominent species differences in the involvement of COMT in determining pregnancy outcomes.

Materials and Methods

Animals and Tissue Collection

Holtzman Sprague–Dawley rats were purchased from Envigo (Indianapolis, IN) and maintained under specific pathogen-free conditions in an Association for Assessment and Accreditation of Laboratory Animal Care–accredited animal facility at the University of Kansas Medical Center (KUMC). Rats were fed standard rat chow and water ad libitum and maintained in a 14-h light:10-h dark photoperiod (lights on at 0600 h). Time-mated pregnancies were established by co-housing adult female rats (8–12 weeks of age) with adult male rats (> 10 weeks of age). Detection of sperm or a seminal plug in the vagina was designated gestation day (gd) 0.5. Pseudopregnant females were generated by co-housing adult female rats (8–12 weeks of age) with adult vasectomized male rats (> 10 weeks of age). At the time of euthanasia, litter sizes and the viability of conceptuses were recorded, and tissues used for histological analysis were frozen in dry ice–cooled heptane and stored at − 80 °C until processed, whereas tissues used for biochemical analyses were frozen in liquid nitrogen and stored at − 80 °C until processed [43]. All animal procedures were approved by the KUMC Institutional Animal Care and Use Committee.

Transcript Analysis

Total RNA was extracted from tissues using TRIzol reagent (Cat. No. AM9738, Thermo Fisher, Waltham, MA). cDNAs were synthesized from total RNA (1 μg) for each sample using SuperScript 2 reverse transcriptase (Cat. No. 18064014, Thermo Fisher), diluted ten times with water, and subjected to reverse transcriptase quantitative PCR (RT-qPCR) to estimate mRNA levels. RT-qPCR primer sequences are presented in Supplemental Table 1. Real-time PCR amplification of cDNAs was carried out in a reaction mixture (20 μL) containing SYBR GREEN PCR Master Mix (Cat. No. 4309155, Applied Biosystems, Foster City, CA) and primers (250 nM each). Amplification and fluorescence detection were carried out using the ABI QuantStudio PCR system (Applied Biosystems). The delta–delta Ct method was used for relative quantification of the amount of mRNA for each sample normalized to 18S RNA.

Generation of a *Comt* Mutant Rat Model

Targeted mutations were generated using genome editing as previously described by our laboratory [44–46] with some modifications. In brief, 4- to 5-week-old donor rats were intraperitoneally injected with 30 units of equine chorionic gonadotropin (Cat. No. G4877, Sigma-Aldrich, St. Louis, MO), followed by an intraperitoneal injection of 30 units of human chorionic gonadotropin (Cat. No. C1063, Sigma-Aldrich) ~ 46 h later, and immediately mated with adult male rats. Zygotes were flushed from oviducts the next morning (gd 0.5) and maintained in M2 medium (Cat. No. MR-015-D, EMD Millipore, Burlington, MA) supplemented with bovine serum albumin (Cat. No. A9647, Sigma-Aldrich) at 37 °C in 5% CO_2 for 2 h. CRISPR RNAs (crRNAs) were designed to target Exon 2 (CATTCTGCTGCACGTAGCGCAGG) and Exon 4 (GAAGGTAGCGGTCTTTCCAGTGG) of the rat *Comt* gene (NM_012531.2). crRNAs were annealed with tracrRNA in equimolar concentrations to generate crRNA:tracrRNA duplexes (guide RNA). Guide RNAs were incubated with Cas9 nuclease to form

ribonucleoprotein (RNP) complexes. RNP complexes were microinjected into 0.5-day zygotes at a concentration of 25 ng/μL in Tris-EDTA buffer (pH 7.4). Genome editing reagents were obtained from Integrated DNA Technologies (Coralville, IA). Microinjections were performed using a Leica inverted microscope (Buffalo Grove, IL) and an Eppendorf Microinjection system (Hauppauge, NY). Manipulated zygotes were transferred to oviducts of pseudopregnant rats (20–30 zygotes per rat). Offspring were screened for deletions within the *Comt* gene. Deletion boundaries were verified by Sanger DNA sequencing. PCR primers used for genotyping of the genetically altered rats are listed in Supplemental Table 2. Germline transmission of the mutated gene was determined in F_1 rats by backcrossing F_0 founder rats with wild type rats. Detection of a mutation in F_1 rats identical to the mutation present in the parent F_0 rat was considered successful germline transmission. The *Comt* mutant rat model is available at the Rat Resource & Research Center (RRRC No. 882; University of Missouri, Columbia, MO; www.rrrc.us).

Western Blotting

Liver tissue was collected in radioimmunoprecipitation assay lysis buffer (Cat. No. sc-24948A, Santa Cruz Biotechnology, Santa Cruz, CA) containing a protease inhibitor cocktail (Cat. No. 11697498001, Sigma-Aldrich, St Louis, MO). Protein (20 μg) was separated on SDS-PAGE gels and transferred to Immun-Blot polyvinylidene difluoride membranes (Cat. No. 10600023, GE Healthcare, Milwaukee, WI) for 1 h at room temperature. Membranes were blocked in 5% non-fat milk in Tris buffered saline (pH 7.4)–0.1% Tween 20 (TBST) for 1 h at room temperature. Antibodies against COMT (1:4000 dilution, Cat. No. 611970, Clone 4/COMT/RUO, BD Biosciences, Franklin Lakes, NJ; 1:5000 dilution, Cat. No. A6200 and glyceraldehyde 3-phosphate dehydrogenase (GAPDH, 1:10,000 dilution, Cat. No. AM4300, Thermo Fisher)) were diluted in 5% non-fat milk in TBST and incubated at 4 °C overnight. Secondary antibodies were horseradish peroxidase–conjugated goat anti-rabbit IgG (1:8000 dilution, Cat. No. 7074S, Cell Signaling Technologies, Danvers, MA) and horseradish peroxidase–conjugated rabbit anti-mouse IgG (1:10,000 dilution; Cat. No. 7076S, Cell Signaling). Detection of protein expression was catalyzed by Luminata™ Crescendo Western HRP substrate (EMD Millipore).

Histological and Immunohistochemical Analyses

Immunohistochemical analyses were performed on 10-μm frozen tissue sections using indirect immunofluorescence. Primary antibodies to vimentin (Cat. No. V6630, Sigma-Aldrich) and pan-cytokeratin (1:1000 dilution, Cat. No. F3418, Sigma-Aldrich) were used in the analyses. Goat anti-mouse IgG conjugated with Alexa 488 (1:1000 dilution, Cat. No. A11029, Thermo Fisher) and goat anti-mouse IgG conjugated with Alexa 568 (1:400 dilution, Cat. No. A11031, Thermo Fisher) were used to detect primary antibodies. Fluoromount-G™, with 4′6-diamidino-2-phenylindole (DAPI, Cat. No. 00-4959-52, Thermo Fisher), was used to visualize nuclei and as mounting medium. 3-amino-9-ethylcarbazole (AEC, Cat. No. SK-4200, Vector Laboratories, Burlingame, CA) was used for chromogenic staining. Processed tissue sections were examined, and images were captured with a Nikon Eclipse 80i upright microscope equipped with a charge-coupled device camera (Nikon, Melville, NY).

Hypoxia Exposure

Female *Comt* null and wild type rats, aged 8 to 12 weeks, were mated in the evening with males of the corresponding genotype. Pregnant rats were randomized to prenatal hypoxia (10.5% O_2) or ambient oxygen conditions (21% O_2) from gd 6.5 to 18.5. Rats assigned to the prenatal hypoxia group were placed inside a gas-regulated chamber (Biospherix, Lacona, NY) that maintained an internal concentration of oxygen at 10.5 ± 0.3% [44]. Pregnant rats exposed to ambient conditions served as controls.

Statistical Analysis

All of the normally distributed data are expressed as mean ± standard error of the mean and were compared using the Student *t* test or analysis of variance that included genotype and treatment as sources of variation followed by post hoc tests. A P value < 0.05 was considered statistically significant.

Results

Expression of *Comt* Transcripts in Rat Placentation Sites

Comt transcripts were abundantly expressed in the rat placenta and the highest during the later stages of gestation (Fig. 1). *Comt* transcripts were expressed in both the junctional and labyrinth zones of the placenta. As gestation progressed, *Comt* transcript levels were more prominent in the labyrinth zone than in the junctional zone (Fig. 1).

Generation and Characterization of a *Comt* Null Rat Model

CRISPR/Cas9-mediated genome editing was used to generate *Comt*-targeted mutations. Genome editing resulted in a 1047-bp deletion, which included a partial deletion of Exon 2, complete deletion of Exon 3, and partial deletion of Exon 4 (Fig. 2a). The mutation led to complete removal of nucleotide sequence encoding the SAM-dependent methyltransferase domain and SAM-binding domain (Fig. 2b). These domains are critical for functional activity of the COMT enzyme [2]. Furthermore, the deletion resulted in a frameshift, premature appearance of a stop codon, and absence of detectable COMT protein in the liver of *Comt* mutant homozygotes (Fig. 2c). The *Comt* mutation was successfully transmitted through the germline and a colony established and maintained through *Comt* heterozygote breeding. Experimentation was performed on wild type and *Comt* homozygote littermates.

Effect of *Comt* Disruption on Fertility and Pregnancy

We next proceeded to evaluate the importance of COMT in fertility and pregnancy outcomes. *Comt* heterozygote intercrosses generated litters of expected size and Mendelian ratio (Table 1). At birth, rats of all *Comt* genotypes exhibited similar size and appearance. Consequently, phenotypic comparisons proceeded on wild type intercrosses versus homozygous *Comt* null intercrosses. Phenotypic assessments were made at gd 13.5 and gd 18.5. Litter size did not differ between *Comt* null pregnancies and wild type pregnancies (Fig. 3a). Additionally, the organization of gd 13.5 placentation sites was not significantly affected by COMT deficiency (Fig. 3b). Junctional zone and labyrinth zone compartments were well-defined in both genotypes, as was the extent of intrauterine trophoblast invasion at

gd 18.5. Postnatal litter size and developmental outcomes were similar among wild type intercross breeding and COMT-deficient intercross breeding. Thus, COMT deficiency does not adversely affect fertility or pregnancy outcomes.

Effect of Hypoxia on Pregnancy in COMT-Deficient Animals

In mouse, there is evidence that COMT deficiency adversely affects pregnancy-dependent adaptations to hypoxia [26]. Consequently, we compared prenatal exposure (gd 6.5 to 18.5) to ambient oxygen (~ 21.0% O_2) or hypoxia (10.5% O_2) in wild type versus COMT-deficient pregnancies (Fig. 4a). Placental and fetal weights did not differ based on genotype in either ambient or hypoxic environments. However, placental and fetal growth were similarly affected by hypoxia in wild type and COMT-deficient pregnancies. Placental weights were significantly greater and fetal weights significantly smaller following hypoxia exposure (Fig. 4b). Thus, in rat, COMT does not significantly affect placental or fetal growth and is not essential for pregnancy-dependent adaptations to hypoxia.

Discussion

COMT is a multi-faceted enzyme in cell signaling. Methylation of catechol substrates can result in the activation or inactivation of ligands with important roles in the regulation of numerous physiological processes [2–5]. In this report, we successfully generated a COMT-deficient rat model using CRISPR/Cas9 genome editing. The primary driver for our efforts was to further explore a role for COMT in pregnancy, as previously observed in mouse [8, 9, 23–27, 47]. In contrast to mouse, COMT deficiency in rat does not adversely affect pregnancy outcomes (present study). Litter size, placental and fetal development, and adaptations to a physiological stressor were similar in wild type and COMT-deficient pregnancies.

Kalluri and coworkers first reported that disrupting COMT in mouse results in pregnancies exhibiting a preeclampsia-like phenotype [8]. Pregnant COMT-deficient mice show decreased decidua/placenta weights, increased fetal lethality, elevated blood pressure, proteinuria, and kidney pathology. These pregnancy complications could be reversed by treating pregnant COMT null pregnant mice with 2ME [8]. The relationship of COMT deficiency with fetal growth restriction, proteinuria, and an assortment of vascular pathologies has similarly been reported by others [9, 23–25, 27, 47]. Mechanistic findings in mouse support correlative data linking COMT and 2ME deficits with human pregnancy complications [6]. Thus, the implication from this body of research was that the involvement of COMT in pregnancy-associated events was conserved and required for a healthy pregnancy.

Foundational support for viewing COMT as a regulator of human pregnancy is buttressed on mechanistic observations in mouse. However, the critical nature of COMT involvement in pregnancy does not extend to a closely related species, the rat, presenting a conundrum. There are few possible explanations. Rat is unique when compared with mouse and human and can exhibit adaptations that compensate for COMT deficiency, resulting in normal pregnancy outcomes, as observed in this report. These adaptations may include the existence of other methyltransferases that can substitute for COMT or, alternatively, regulatory

pathways affecting pregnancy that bypass the need for 2ME. A second possibility is that mouse may be the outlier and demonstrate heightened sensitivity to deficits in COMT during pregnancy. This assessment is consistent with our observations with rat (present study) and a subset of reports failing to establish a linkage between COMT and human pregnancy dysfunction [28–32]. A third potential explanation may be more complicated. The genetic backgrounds of the mouse and rat models used in the analyses may be relevant in interpreting the COMT mutant phenotypes [48, 49]. Mouse research on COMT and pregnancy utilized mice possessing a mixed C57BL/6/SV129 genetic background [8, 9, 24] or a C57BL/6 genetic background [23, 25–27, 47], whereas analysis of COMT in rat used the Holtzman Sprague–Dawley outbred rat (present study). Consequently, the existence of strain-specific co-morbidities may be central to a potential role for COMT in pregnancy outcome. Hypoxia exposure was used as a physiological challenge in the present report but was ineffective in eliciting a role for COMT in pregnancy-dependent adaptations. Nevertheless, our findings in rat indicate that a measure of caution should be the rule in extrapolating roles for human genes based on results from mutant animals that may not best model the human developmental or disease process under investigation.

Finally, the focus of our experimentation was notably biased by previous research implicating COMT in regulatory events transpiring at the uterine–placental interface [6, 8, 9]. It is evident that COMT is not an essential contributor to pregnancy outcome in rat. However, based on the expression profile of COMT during pregnancy, a role for COMT at the placental–fetal interface may reveal insights into its contribution to pregnancy. COMT expression was prominently upregulated within the labyrinth zone as gestation advanced. The labyrinth zone has a prominent barrier function [50–52]. Thus, COMT may be serving a protective role in preventing excessive catecholamine exposure during critical periods of fetal development, which may impact postnatal physiology or pathophysiological adaptations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank Stacy Oxley and Brandi Miller for administrative assistance.

Funding

This work was supported by NIH Grants ES028957, ES029280, and HD020676 and the Sosland Foundation.

References

1. Axelrod J Methylation reactions in the formation and metabolism of catecholamines and other biogenic amines. Pharmacol Rev. 1966;18:95–113. [PubMed: 5323771]
2. Guldberg HC, Marsden CA. Catechol-O-methyl transferase: pharmacological aspects and physiological role. Pharmacol Rev. 1975;27:135–206. [PubMed: 1103160]
3. Mannisto PT, Kaakkola S. Catechol-O-methyltransferase (COMT): biochemistry, molecular biology, pharmacology, and clinical efficacy of the new selective COMT inhibitors. Pharmacol Rev. 1999;51:593–628. [PubMed: 10581325]

4. Thomas MP, Potter BV. The structural biology of oestrogen metabolism. J Steroid Biochem Mol Biol. 2013;137:27–49. [PubMed: 23291110]
5. Kumar BS, Raghuvanshi DS, Hasanain M, Alam S, Sarkar J, Mitra K, et al. Recent advances in chemistry and pharmacology of 2-methoxyestradiol: an anticancer investigational drug. Steroids. 2016;110:9–34. [PubMed: 27020471]
6. Shenoy V, Kanasaki K, Kalluri R. Pre-eclampsia: connecting an-giogenic and metabolic pathways. Trends Endocrinol Metab. 2010;21:529–36. [PubMed: 20646932]
7. Mabjeesh NJ, Escuin D, LaVallee TM, et al. 2ME2 inhibits tumor growth and angiogenesis by disrupting microtubules and dysregulating HIF. Cancer Cell. 2003;3:363–75. [PubMed: 12726862]
8. Kanasaki K, Palmsten K, Sugimoto H, Ahmad S, Hamano Y, Xie L, et al. Deficiency in catechol-O-methyltransferase and 2-methoxyoestradiol is associated with pre-eclampsia. Nature. 2008;453:1117–21. [PubMed: 18469803]
9. Lee SB, Wong AP, Kanasaki K, Xu Y, Shenoy VK, McElrath TF, et al. Preeclampsia: 2-methoxyestradiol induces cytotrophoblast invasion and vascular development specifically under hypoxic conditions. Am J Pathol. 2010;176:710–20. [PubMed: 20075204]
10. Hernandez M, Hernandez I, Rodriguez F, Pertegal M, Bonacasa B, Salom MG, et al. Endothelial dysfunction in gestational hypertension induced by catechol-O-methyltransferase inhibition. Exp Physiol. 2013;98:856–66. [PubMed: 23104937]
11. Perez-Sepulveda A, Espana-Perrot PP, Norwitz ER, et al. Metabolic pathways involved in 2-methoxyestradiol synthesis and their role in preeclampsia. Reprod Sci. 2013;20:1020–9. [PubMed: 23456663]
12. Pinto MP, Medina RA, Owen GI. 2-methoxyestradiol and disorders of female reproductive tissues. Horm Cancer. 2014;5:274–83. [PubMed: 24764201]
13. Shen Z, Wu Y, Chen X, Chang X, Zhou Q, Zhou J, et al. Decreased maternal serum 2-methoxyestradiol levels are associated with the development of preeclampsia. Cell Physiol Biochem. 2014;34: 2189–99. [PubMed: 25562165]
14. Wantania J, Attamimi A, Siswishanto R. A comparison of 2-methoxyestradiol value in women with severe preeclampsia versus normotensive pregnancy. J Clin Diagn Res. 2017;11:QC35–8. [PubMed: 28511459]
15. Hill LD, York TP, Kusanovic JP, Gomez R, Eaves LJ, Romero R, et al. Epistasis between COMT and MTHFR in maternal-fetal dyads increases risk for preeclampsia. PLoS One. 2011;6:e16681. [PubMed: 21304959]
16. Krnjeta T, Mirkovic L, Ignjatovic S, et al. Protective role of maternal P.VAL158MET catechol-O-methyltransferase polymorphism against early-onset preeclampsia and its complications. J Med Biochem. 2016;35:312–8. [PubMed: 28356882]
17. Liang S, Liu X, Fan P, Liu R, Zhang J, He G, et al. Association between Val158Met functional polymorphism in the COMT gene and risk of preeclampsia in a Chinese population. Arch Med Res. 2012;43:154–8. [PubMed: 22475780]
18. Lim JH, Kim SY, Kim DJ, Park SY, Han HW, Han JY, et al. Genetic polymorphism of catechol-O-methyltransferase and cyto-chrome P450c17alpha in preeclampsia. Pharmacogenet Genomics. 2010;20:605–10. [PubMed: 20729792]
19. Roten LT, Fenstad MH, Forsmo S, Johnson MP, Moses EK, Austgulen R, et al. A low COMT activity haplotype is associated with recurrent preeclampsia in a Norwegian population cohort (HUNT2). Mol Hum Reprod. 2011;17:439–46. [PubMed: 21355050]
20. Taravati A, Tohidi F, Moniri M, Kamali K. Catechol-O-methyltransferase gene polymorphism (Val158Met) and development of pre-eclampsia. Arch Med Res. 2017;48:180–6. [PubMed: 28625321]
21. Gogos JA, Morgan M, Luine V, Santha M, Ogawa S, Pfaff D, et al. Catechol-O-methyltransferase-deficient mice exhibit sexually dimorphic changes in catecholamine levels and behavior. Proc Natl Acad Sci U S A. 1998;95:9991–6. [PubMed: 9707588]
22. Banerjee S, Randeva H, Chambers AE. Mouse models for preeclampsia: disruption of redox-regulated signaling. Reprod Biol Endocrinol. 2009;7:4. [PubMed: 19146669]

23. Liao S, Wu H, Chen R. Apolipoprotein A1 mimetic peptide ATI-5261 reverses arterial stiffness at late pregnancy and early postpartum in a COMT(−/−) mouse model of preeclampsia. Clin Hypertens. 2018;24:11. [PubMed: 30237900]
24. Parchem JG, Kanasaki K, Kanasaki M, Sugimoto H, Xie L, Hamano Y, et al. Loss of placental growth factor ameliorates maternal hypertension and preeclampsia in mice. J Clin Invest. 2018;128:5008–17. [PubMed: 30179860]
25. Poudel R, Stanley JL, Rueda-Clausen CF, Andersson IJ, Sibley CP, Davidge ST, et al. Effects of resveratrol in pregnancy using murine models with reduced blood supply to the uterus. PLoS One. 2013;8: e64401. [PubMed: 23667712]
26. Rueda-Clausen CF, Stanley JL, Thambiraj DF, Poudel R, Davidge ST, Baker PN. Effect of prenatal hypoxia in transgenic mouse models of preeclampsia and fetal growth restriction. Reprod Sci. 2014;21:492–502. [PubMed: 24084523]
27. Stanley JL, Andersson IJ, Poudel R, Rueda-Clausen CF, Sibley CP, Davidge ST, et al. Sildenafil citrate rescues fetal growth in the catechol-O-methyl transferase knockout mouse model. Hypertension. 2012;59:1021–8. [PubMed: 22392899]
28. Pertegal M, Fenoy FJ, Hernandez M, et al. Fetal Val108/158Met catechol-O-methyltransferase (COMT) polymorphism and placental COMT activity are associated with the development of preeclampsia. Fertil Steril. 2016;105:134–43 e131–133. [PubMed: 26450530]
29. Zhao X, Liu J, Zhao C, Ye P, Ji J, Wang J, et al. Association between COMT Val158Met polymorphism and preeclampsia in the Chinese Han population. Hypertens Pregnancy. 2016;35:565–72. [PubMed: 27646972]
30. Palmer K, Saglam B, Whitehead C, Stock O, Lappas M, Tong S. Severe early-onset preeclampsia is not associated with a change in placental catechol O-methyltransferase (COMT) expression. Am J Pathol. 2011;178:2484–8. [PubMed: 21531374]
31. Seol HJ, Cho GJ, Oh MJ, Kim HJ. 2-methoxyoestradiol levels and placental catechol-O-methyltransferase expression in patients with late-onset preeclampsia. Arch Gynecol Obstet. 2013;287:881–6. [PubMed: 23233290]
32. Perez-Sepulveda A, Monteiro LJ, Dobierzewska A, España-Perrot PP, Venegas-Araneda P, Guzmán-Rojas AM, et al. Placental aromatase is deficient in placental ischemia and preeclampsia. PLoS One. 2015;10:e0139682. [PubMed: 26444006]
33. Kaufmann P, Black S, Huppertz B. Endovascular trophoblast invasion: implications for the pathogenesis of intrauterine growth retardation and preeclampsia. Biol Reprod. 2003;69:1–7. [PubMed: 12620937]
34. Brosens I, Pijnenborg R, Vercruysse L, Romero R. The “great obstetrical syndromes” are associated with disorders of deep placentation. Am J Obstet Gynecol. 2011;204:193–201. [PubMed: 21094932]
35. Adamson SL, Lu Y, Whiteley KJ, Holmyard D, Hemberger M, Pfarrer C, et al. Interactions between trophoblast cells and the maternal and fetal circulation in the mouse placenta. Dev Biol. 2002;250:358–73. [PubMed: 12376109]
36. Ain R, Canham LN, Soares MJ. Gestation stage-dependent intrauterine trophoblast cell invasion in the rat and mouse: novel endocrine phenotype and regulation. Dev Biol. 2003;260:176–90. [PubMed: 12885563]
37. Soncin F, Natale D, Parast MM. Signaling pathways in mouse and human trophoblast differentiation: a comparative review. Cell Mol Life Sci. 2015;72:1291–302. [PubMed: 25430479]
38. Pijnenborg R Animal models of deep trophoblast invasion. In: Pijnenborg R., Brosens I., & Romero R. (eds.), Placental bed disorders: basic science and its translation to obstetrics. Cambridge: Cambridge University Press; 127–139 (2010).
39. Pijnenborg R, Robertson WB, Brosens I, Dixon G. Trophoblast invasion and the establishment of haemochorial placentation in man and laboratory animals. Placenta. 1981;2:71–91. [PubMed: 7010344]
40. Soares MJ, Chakraborty D, Karim Rumi MA, Konno T, Renaud SJ. Rat placentation: an experimental model for investigating the hemochorial maternal-fetal interface. Placenta. 2012;33:233–43. [PubMed: 22284666]

41. Capecchi MR. The new mouse genetics: altering the genome by gene targeting. Trends Genet. 1989;5:70–6. [PubMed: 2660363]
42. Jacob HJ, Lazar J, Dwinell MR, Moreno C, Geurts AM. Gene targeting in the rat: advances and opportunities. Trends Genet. 2010;26:510–8. [PubMed: 20869786]
43. Ain R, Konno T, Canham LN, Soares MJ. Phenotypic analysis of the rat placenta. Methods Mol Med. 2006;121:295–313. [PubMed: 16251750]
44. Chakraborty D, Cui W, Rosario GX, Scott RL, Dhakal P, Renaud SJ, et al. HIF-KDM3A-MMP12 regulatory circuit ensures trophoblast plasticity and placental adaptations to hypoxia. Proc Natl Acad Sci U S A. 2016;113:E7212–21. [PubMed: 27807143]
45. Kubota K, Cui W, Dhakal P, Wolfe MW, Rumi MAK, Vivian JL, et al. Rethinking progesterone regulation of female reproductive cyclicity. Proc Natl Acad Sci U S A. 2016;113:4212–7. [PubMed: 27035990]
46. Renaud SJ, Scott RL, Chakraborty D, Rumi MAK, Soares MJ. Natural killer-cell deficiency alters placental development in rats. Biol Reprod. 2017;96:145–58. [PubMed: 28395334]
47. Stanley JL, Sulek K, Andersson IJ, Davidge ST, Kenny LC, Sibley CP, et al. Sildenafil therapy normalizes the aberrant metabolomic profile in the Comt(−/−) mouse model of preeclampsia/fetal growth restriction. Sci Rep. 2015;5:18241. [PubMed: 26667607]
48. Erickson RP. Mouse models of human genetic disease: which mouse is more like a man? Bioessays. 1996;18:993–8. [PubMed: 8976156]
49. Threadgill DW, Dlugosz AA, Hansen LA, Tennenbaum T, Lichti U, Yee D, et al. Targeted disruption of mouse EGF receptor: effect of genetic background on mutant phenotype. Science. 1995;269:230–4. [PubMed: 7618084]
50. Burton GJ, Fowden AL, Thornburg KL. Placental origins of chronic disease. Physiol Rev. 2016;96:1509–65. [PubMed: 27604528]
51. Dilworth MR, Sibley CP. Review: transport across the placenta of mice and women. Placenta. 2013;34 Suppl:S34–9. [PubMed: 23153501]
52. Knipp GT, Audus KL, Soares MJ. Nutrient transport across the placenta. Adv Drug Deliv Rev. 1999;38:41–58. [PubMed: 10837745]

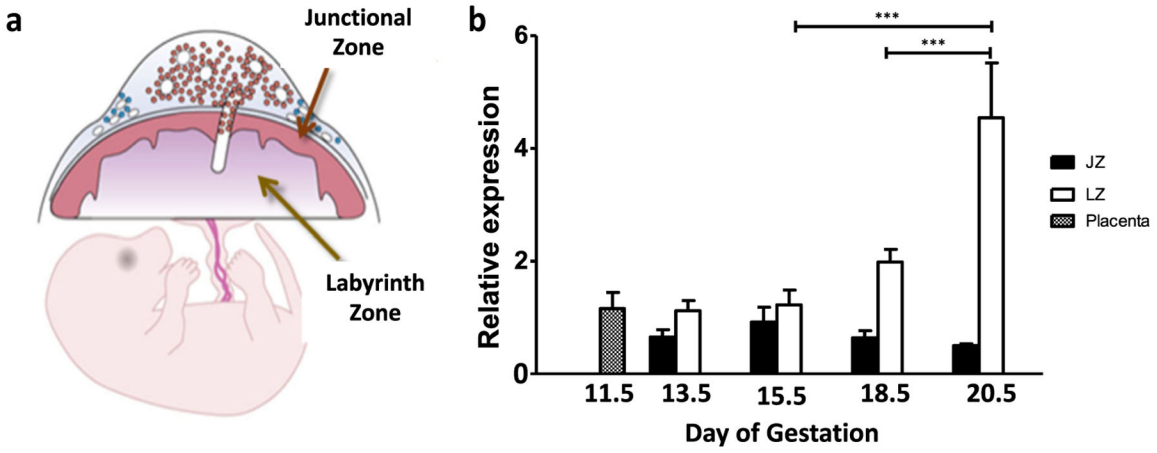

Fig. 1.

Expression profile of *Comt* transcripts in the rat placenta during the second half of gestation. **a** Schematic representation of the rat placenta. **b** RT-qPCR was used to measure *Comt* transcript levels in the entire placenta (gestation day 11.5) and in the junctional zone (JZ) and labyrinth zone (LZ) of the rat placenta from gestation days 13.5, 15.5, 18.5, and 20.5 (n = 6/group, ***$P < 0.001$, one-way analysis of variance followed by Tukey's post hoc test)

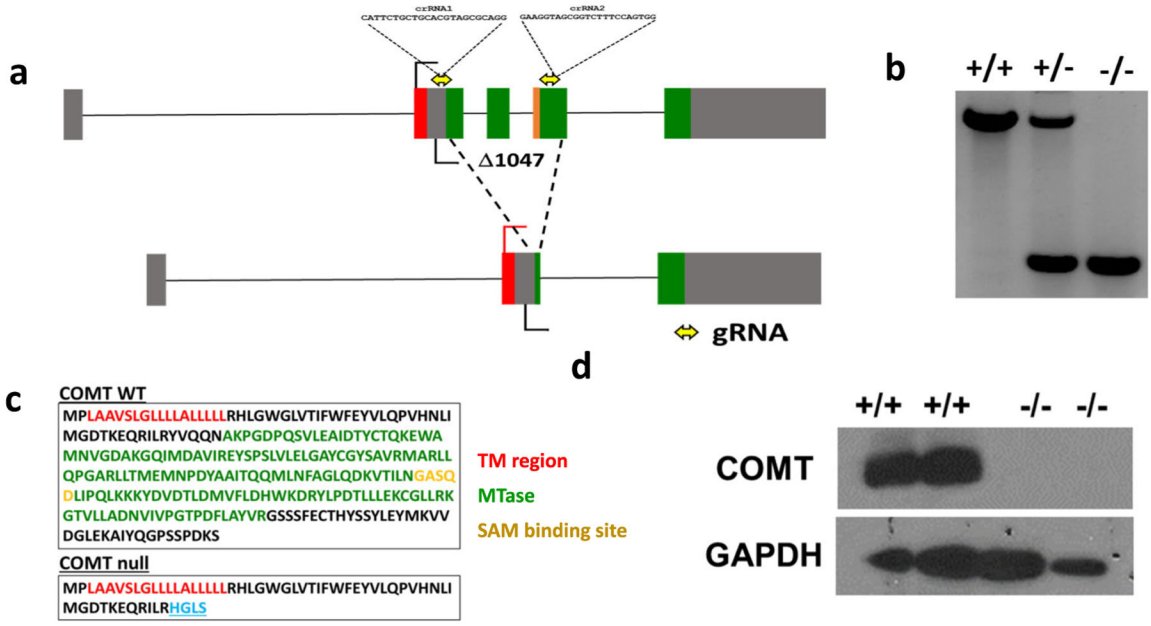

Fig. 2.

Generation of *Comt* null rat using CRISPR/Cas9. **a** Schematic representation of the organization of the *Comt* gene, the engineered deletion (Δ1047 bp), and location of two guide RNAs used to generate the *Comt* null rat. **b** Genotyping of wild type and *Comt* mutant alleles. Genomic DNA was isolated, PCR performed, and resolution of DNA fragments determined by agarose electrophoresis. **c** Wild type and COMT mutant amino acid sequences. The transmembrane (TM) is shown in red. The S-adenosylmethionine (SAM)-dependent methyltransferase domain (MTase) is shown in green. The SAM-binding site is shown in brown. The frameshift sequence in the COMT mutant sequence is shown in blue. **d** Western blot analysis of COMT protein in wild type and *Comt* null rat liver tissue

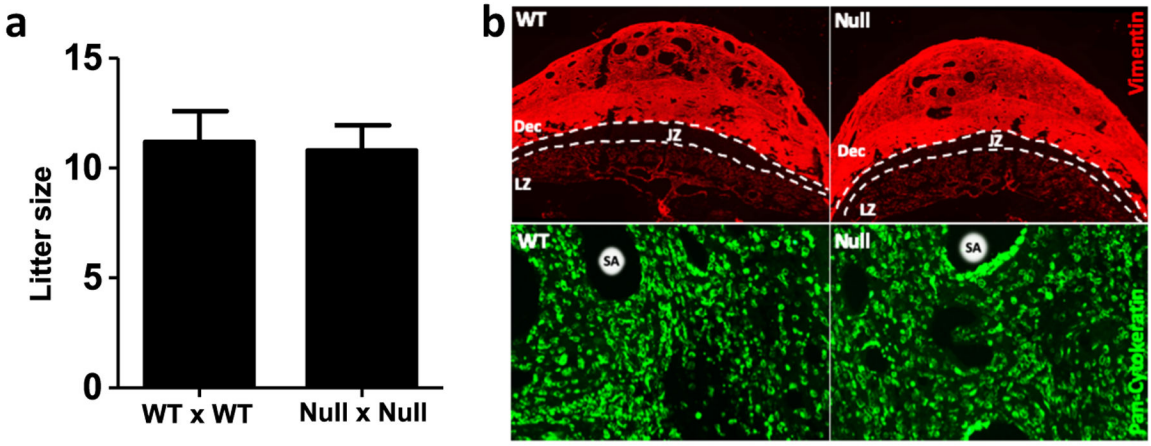

Fig. 3.

Pregnancy outcomes in wild type and *Comt* null rats. **a** Litter sizes from wild type males mated to wild type females (WT) and *Comt* null males mated to *Comt* null females (Null) (*n* = 6/group). **b** Histological structure of WT versus *Comt* null (Null) placentas. Vimentin staining of gestation day 13.5 placentation sites is shown in the upper panels, and pan-cytokeratin staining within the uterus proximal to the placenta at gestation day 18.5 is shown in the lower panels. Vimentin demarcates the placental compartments (JZ, junctional zone; LZ, labyrinth zone; Dec, decidua), whereas pan-cytokeratin staining within the uterus proximal to the placenta identifies invasive trophoblast cells (SA; spiral artery)

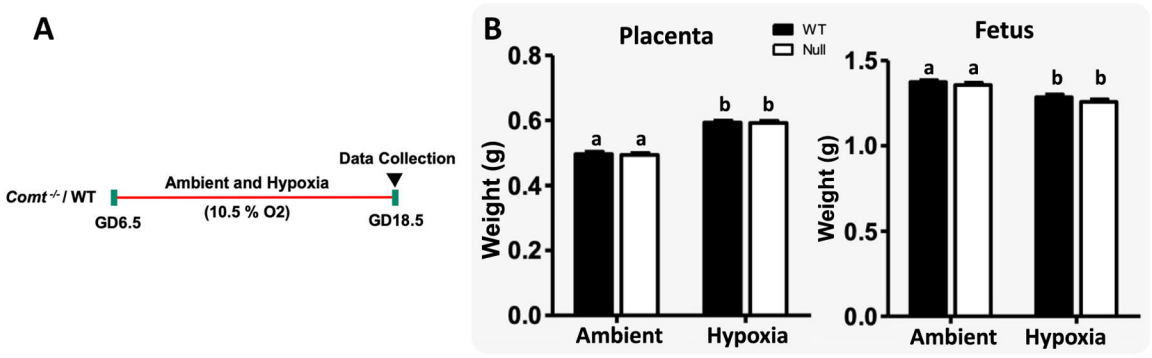

Fig. 4.

Effects of COMT deficiency on pregnancy-dependent adaptations to hypoxia. **a** Ambient and hypoxia exposure schemes for pregnancy outcome experiments. **b** Placental and fetal weights in ambient and hypoxic conditions. Bar values with different letters indicate statistically significant differences among the groups (one-way analysis of variance followed by Tukey's post hoc test: $P < 0.0001$), $n = 8–10$

Table 1

Mendelian ratio of *Comt*$^{+/-}$ × *Comt*$^{+/-}$ breeding outcomes

Outcome	*Comt*$^{+/+}$	*Comt*$^{+/-}$	*Com*$^{-/-}$
# observed	39 (22.67%)	92 (53.58%)	41 (23.83%)
# expected	43 (25%)	86 (50%)	43 (25%)