

Maternal Mthfr 677C>T genotype modifies folate responses to oxcarbazepine and is associated with developmental vulnerability in mice

Tiancai Huang

The Seventh Affiliated Hospital of Sun Yat-sen University

Jiahao Tian

The Seventh Affiliated Hospital of Sun Yat-sen University

Wenyao Huang

The Seventh Affiliated Hospital of Sun Yat-sen University

Jiaqi Zhang

The Seventh Affiliated Hospital of Sun Yat-sen University

Yubao Fang

The Seventh Affiliated Hospital of Sun Yat-sen University

Yinchao Li

The Seventh Affiliated Hospital of Sun Yat-sen University

Liemin Zhou

zhoulm@mail.sysu.edu.cn

The Seventh Affiliated Hospital of Sun Yat-sen University

Research Article

Keywords: Mthfr 677C > T, oxcarbazepine, one-carbon metabolism, folate, developmental vulnerability

Posted Date: October 14th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-10881268/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License. [Read Full License](#)

Additional Declarations: No competing interests reported.

Abstract

Background

Genetic variation in folate-dependent one-carbon metabolism may contribute to interindividual differences in developmental responses to antiseizure medications. We investigated whether maternal *Mthfr* 677C > T genotype modifies metabolic and developmental responses to gestational oxcarbazepine (OXC) exposure.

Methods

Pregnant *Mthfr* 677C > T knock-in mice (CC, CT, and TT; n = 67) received vehicle, 65 mg/kg OXC, or 130 mg/kg OXC at embryonic day 7.5. Maternal hepatic MTHFR activity, serum homocysteine (Hcy) and folate, and embryonic developmental outcomes were assessed.

Results

Hepatic MTHFR activity progressively decreased from CC to CT to TT dams. OXC increased Hcy and decreased folate. For Hcy, genotype and OXC showed significant main effects, but their interaction was not significant (P = 0.481). In contrast, folate showed a significant genotype × OXC interaction (F = 44.774, P < 0.0001), with the greatest reduction in TT dams. Compared with CC dams, TT dams had lower odds of fetal survival (OR = 0.202, 95% CI 0.041–0.991) and higher odds of resorption (OR = 4.945, 95% CI 1.009–24.245). Maternal genotype also affected mean fetal body weight per litter (P = 0.041), whereas OXC and genotype × OXC interactions were not significant for these developmental outcomes. Foot and dorsal congestion occurred only at 130 mg/kg OXC, without significant genotype-group differences.

Conclusion

Maternal *Mthfr* 677C > T genotype modifies the folate response to OXC and is associated with baseline developmental vulnerability, whereas genotype-dependent OXC developmental toxicity was not statistically established.

1. Introduction

Epilepsy is a common chronic neurological disorder among women of reproductive age, and continued treatment with antiseizure medications (ASMs) is often necessary during pregnancy to maintain seizure control. Consequently, balancing maternal seizure control against the potential developmental effects of prenatal ASM exposure remains an important issue in both clinical epilepsy management and reproductive toxicology. Developmental risks differ substantially among individual ASMs. Valproic acid (VPA) is consistently associated with a relatively high risk of major congenital malformations (MCMs), whereas lamotrigine, levetiracetam, and oxcarbazepine (OXC) are generally associated with lower observed risks. The 2024 practice guideline jointly issued by the American Academy of Neurology, American Epilepsy Society, and Society for Maternal-Fetal Medicine recommends considering lamotrigine, levetiracetam, or OXC, when clinically appropriate, to minimize the risk of MCMs while maintaining effective seizure control[1]. In the updated prospective EURAP registry, MCMs occurred in 13 of 443 pregnancies exposed to OXC monotherapy, corresponding to a prevalence of 2.9%[2]. A large population-

based study using Nordic national health registries likewise found no significant increase in MCM risk with OXC monotherapy compared with lamotrigine[3]. More recently, the North American Antiepileptic Drug Pregnancy Registry reported an MCM prevalence of 1.5% among 327 pregnancies exposed to OXC monotherapy during the first trimester[4]. A systematic review and meta-analysis also found no statistically significant increase in congenital malformations associated with OXC compared with either unexposed pregnancies or lamotrigine, although the available evidence was limited by heterogeneity and relatively modest numbers of OXC-exposed pregnancies[5]. Collectively, these data support a comparatively low observed population-level risk of major malformations associated with OXC. However, population-average estimates do not establish that susceptibility to OXC is uniform across different maternal genetic and metabolic backgrounds.

Despite its relatively favorable clinical teratogenic profile, experimental evidence indicates that the potential developmental effects of OXC warrant further investigation. Prenatal OXC exposure has been associated with developmental alterations in animal models that extend beyond major structural malformations. González-Maciel et al. reported that OXC exposure during gestational days 7–15 increased apoptosis in several hippocampal regions and altered hippocampal cytoarchitecture in rat offspring, accompanied by reduced litter size and lower fetal weight[6]. These experimental findings are not necessarily inconsistent with the comparatively low MCM risk observed in pregnancy registries. Clinical studies estimate average risks in genetically heterogeneous populations under therapeutic exposure conditions, whereas animal models permit controlled manipulation of genetic background, exposure level, and developmental timing. Accordingly, a relatively low population-level teratogenic risk may coexist with greater biological susceptibility under specific maternal genetic or metabolic conditions. Identifying such susceptibility factors is therefore relevant to understanding interindividual variability in developmental responses to OXC.

One biologically plausible determinant of this variability is folate-dependent one-carbon metabolism. This metabolic network provides one-carbon units required for purine and thymidylate biosynthesis, amino acid homeostasis, methyl-donor generation, and epigenetic regulation, all of which are particularly important during rapid embryonic growth[7]. Methylenetetrahydrofolate reductase (MTHFR) is a key regulatory enzyme in this pathway and catalyzes the reduction of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate (5-MTHF), which provides the methyl group required for the remethylation of homocysteine (Hcy) to methionine. The common MTHFR 677C > T variant (rs1801133) results in an Ala222Val substitution and produces a thermolabile enzyme with reduced catalytic activity[8]. Its metabolic consequences are genotype dependent. A systematic review and meta-analysis demonstrated a consistent gradient in circulating folate concentrations across MTHFR 677C > T genotypes, with an overall pattern of CC > CT > TT[9]. A separate meta-analysis of folic acid intervention studies similarly showed that the TT genotype was associated with lower serum folate and higher plasma Hcy concentrations and with a smaller serum folate response to supplementation[10]. These findings identify MTHFR 677C > T as a functional genetic determinant of one-carbon metabolic capacity, particularly under conditions in which folate metabolism is challenged by additional nutritional or pharmacological stress.

The relevance of maternal one-carbon metabolism to embryonic development is supported by both human and experimental evidence. Folate-dependent nucleotide synthesis and methylation are required for normal embryogenesis, and disruption of folate metabolism has long been implicated in neural tube defects (NTDs) and other congenital abnormalities. Contemporary reviews emphasize that NTDs arise through complex interactions between genetic susceptibility and environmental or nutritional exposures rather than through a single causal pathway[11]. A systematic review and meta-analysis showed that mothers of offspring affected by NTDs had modestly but significantly higher plasma Hcy concentrations than mothers of unaffected offspring[12]. Meta-

analysis of MTHFR C677T further demonstrated an association with NTD risk in both affected offspring and their mothers, whereas no corresponding association was detected for paternal genotype[13]. Experimental studies provide additional evidence for a specifically maternal contribution. In mice, maternal MTHFR deficiency, low dietary folate, or their combination increased fetal loss, developmental delay, intrauterine growth restriction, and congenital cardiac abnormalities[14]. These observations support the concept that genetically determined impairment of maternal folate–Hcy metabolism can alter the intrauterine metabolic environment and thereby contribute to developmental vulnerability.

A particularly relevant question is whether variation in this pathway modifies metabolic or developmental responses to ASMs. VPA provides an important precedent because it is a well-established developmental toxicant, and perturbation of folate-dependent metabolism has been proposed as one of several mechanisms contributing to its congenital and developmental effects[15]. More directly relevant to OXC, Zhu et al. examined 279 patients with epilepsy receiving VPA, OXC, lamotrigine, levetiracetam, or no ASM treatment and found that MTHFR rs1801133 was independently associated with serum Hcy concentrations. Within the OXC monotherapy subgroup, patients with the TT genotype had significantly higher Hcy concentrations than those with either the CC or CT genotype[16]. Importantly, this finding represents an association between MTHFR genotype and Hcy concentrations among OXC-treated patients rather than formal evidence of a genotype × OXC interaction. Moreover, an earlier systematic review and meta-analysis did not identify significant overall differences in Hcy, folate, or vitamin B12 concentrations between patients receiving OXC monotherapy and healthy controls[17]. Together, these apparently heterogeneous clinical findings raise the possibility that metabolic responses to OXC may vary according to host characteristics, including one-carbon metabolic genotype, rather than being uniform across all exposed individuals.

Experimental evidence provides further support for the concept of an MTHFR-dependent susceptibility background. Using the *Mthfr* 677C > T knock-in mouse model directly related to that used in the present study, Yang et al. showed that the variant was associated with reduced hepatic MTHFR activity and altered Hcy metabolism. Following VPA exposure at E7.5, higher rates of fetal abnormalities were observed in pregnancies from CT and TT dams than in those from CC dams, supporting the possibility that genetically altered one-carbon metabolism influences susceptibility to ASM-associated developmental toxicity[18]. More recently, Christensen et al. used a separate *Mthfr* 677C > T knock-in pregnancy model and reported lower maternal hepatic MTHFR expression, reduced circulating methylTHF, and higher Hcy in TT mice. Under low-folate dietary conditions, developmental delays were more pronounced in litters from TT dams[19]. These studies collectively suggest that the *Mthfr* 677C > T background may establish a metabolically vulnerable maternal state whose developmental consequences become more apparent when an additional nutritional or pharmacological stressor is present.

However, whether this susceptibility paradigm extends from VPA or folate restriction to OXC during pregnancy remains unclear. VPA and OXC differ substantially in their clinical teratogenic profiles, with OXC generally associated with a much lower population-level MCM risk. Nevertheless, the association between MTHFR C677T and Hcy concentrations among OXC-treated patients, together with experimental evidence that MTHFR-related metabolic vulnerability can influence developmental outcomes under VPA or low-folate stress, raises the possibility that maternal MTHFR functional status may also influence metabolic responses to OXC during gestation. It remains unknown whether such metabolic susceptibility is accompanied by altered embryonic developmental outcomes and whether these responses vary across OXC exposure levels.

Therefore, in the present study, we used the same established *Mthfr* 677C > T knock-in mouse line employed in the recent VPA study to investigate whether maternal *Mthfr* 677C > T genotype is associated with differential maternal metabolic responses and embryonic developmental outcomes following OXC exposure. Pregnant mice were exposed at E7.5 to vehicle, 65 mg/kg OXC, or 130 mg/kg OXC. We first characterized genotype-associated differences in maternal hepatic MTHFR activity and then evaluated the effects of maternal genotype, OXC exposure, and their interaction on serum Hcy and folate concentrations. Embryonic survival, resorption, fetal growth, and gross fetal abnormalities were subsequently assessed across maternal genotype groups and OXC exposure levels. We hypothesized that reduced MTHFR function associated with the maternal *Mthfr* 677C > T variant would create a metabolically susceptible maternal environment, leading to altered one-carbon metabolic responses to OXC and potentially increasing vulnerability to adverse embryonic developmental outcomes, particularly at higher exposure levels.

2. Materials and Methods

2.1. Animals and *Mthfr* 677C > T knock-in mice

A previously established and characterized *Mthfr* 677C > T knock-in mouse model was used in this study. The model was generated on a C57BL/6J genetic background using CRISPR/Cas9-mediated genome editing to introduce the amino acid substitution corresponding to the human MTHFR C677T variant into the endogenous *Mthfr* gene. The generation and initial characterization of this mouse model have been reported previously[18].

Experimental animals were obtained from subsequent generations of the established *Mthfr* 677C > T colony. No additional genome editing or de novo colony establishment was performed in the present study. Female mice were genotyped before experimentation and classified according to their maternal *Mthfr* 677C > T genotype as CC, CT, or TT. Female mice used for pregnancy experiments were 8–10 weeks old.

All mice were housed in a specific pathogen-free facility at the Laboratory Animal Center of Shenzhen Campus, Sun Yat-sen University, under controlled environmental conditions with appropriate temperature and humidity and a 12-h light/12-h dark cycle. Animals had free access to food and water. All procedures were approved by the Institutional Animal Care and Use Committee of Sun Yat-sen University (approval number: SYSU-IACUC-2024-002515).

2.2. Genotyping of *Mthfr* 677C > T mice

Genotyping was performed using PCR amplification followed by Sanger sequencing. Genomic DNA was extracted from mouse tail-tip tissue using the TaKaRa MiniBEST Universal Genomic DNA Extraction Kit (9765; Takara Bio Inc., Kusatsu, Japan).

PCR was performed using the following primers:

Forward primer: 5'-CTCTCTGATGTCTTCACTCCTTGA-3';

Reverse primer: 5'-AATAACTGTTCTCCGTACAAGCCC-3'.

The 25-μL PCR mixture contained genomic DNA template, primers, Green Taq Mix (P131; Vazyme Biotech Co., Ltd., Jiangsu, China), and ddH₂O. PCR cycling conditions were: 95°C for 3 min; 35 cycles of 95°C for 15 s, 60°C for 15 s,

and 72°C for 60 s/kb; followed by 72°C for 5 min. The 437-bp PCR products were separated on 2% agarose gels prepared in TBE buffer and subjected to Sanger sequencing.

As shown in Fig. 1, the *Mthfr* T/T genotype was identified by a homozygous C-to-T substitution at the target locus, represented by a single T peak (red peak). The *Mthfr* C/T genotype exhibited overlapping C and T peaks, whereas the *Mthfr* C/C genotype corresponded to the wild-type C allele (blue peak).

Only maternal mice were genotyped. E18.5 fetuses were not individually genotyped; therefore, all embryonic outcomes were analyzed according to maternal *Mthfr* genotype.

2.3. Mating and pregnancy determination

Genotyped female mice (8–10 weeks old) were paired overnight with male mice of the corresponding *Mthfr* genotype at a female-to-male ratio of 2:1. Vaginal plugs were examined the following morning, and the day of plug detection was designated as embryonic day 0.5 (E0.5).

2.4. OXC exposure and experimental design

After pregnancy confirmation, a 3 × 3 factorial design was established according to maternal *Mthfr* genotype and OXC exposure. Experimental factors included maternal genotype (CC, CT, TT) and treatment (vehicle control, OXC 65 mg/kg, OXC 130 mg/kg).

Oxcarbazepine (OXC; PHR1635, Sigma-Aldrich) was prepared in a vehicle containing 10% dimethyl sulfoxide, 40% polyethylene glycol 300, 5% Tween-80, and 45% sterile 0.9% sodium chloride (v/v). Because of limited solubility, 5 mg/mL was used for the 130 mg/kg group and 2.5 mg/mL for the 65 mg/kg group, resulting in an identical injection volume of 26 mL/kg.

OXC doses were selected based on the WHO ATC/DDD Index and the human-to-mouse dose conversion method described in Pharmacological Experimental Methodology. A human reference dose of 14.29 mg/kg/day was converted to approximately 130 mg/kg in mice using the body surface area conversion factor. The 65 mg/kg dose represented 50% of this equivalent exposure level.

Pregnant mice received a single intraperitoneal injection of OXC or vehicle on E7.5.

2.5. Maternal blood and liver sample collection

At E18.5, pregnant mice were anesthetized by intraperitoneal injection of 1% sodium pentobarbital (50 mg/kg). Blood samples were collected from the retro-orbital venous plexus for serum Hcy and folate measurements.

After blood collection, mice were euthanized by CO₂ inhalation. Blood samples were allowed to clot at 4°C for 30 min and centrifuged at 2000 rpm for 15 min. Serum samples were stored at 4°C and analyzed within 1 h.

Maternal livers were rapidly collected, washed with saline, and used for MTHFR activity determination.

2.6. Hepatic MTHFR activity assay

Hepatic MTHFR activity was measured in vehicle-treated pregnant mice. Liver samples (0.5 g) were homogenized in 4.5 mL saline and centrifuged at 5000 rpm for 20 min. The supernatant was collected for enzymatic analysis.

MTHFR activity was measured using a commercial MTHFR activity detection kit (ML2605; Shanghai Enzyme-linked Biotechnology Co., Ltd., Shanghai, China) according to the manufacturer's instructions using a colorimetric method.

2.7. Serum Hcy and folate measurements

Serum homocysteine was measured using an ELISA kit (YM-S3025; Yuanmu Biotech, Shanghai, China). Serum folate was measured using an ELISA kit (KGC1053-96; KeyGEN BioTECH, Jiangsu, China). All measurements were performed according to the manufacturers' instructions.

2.8. Embryonic developmental assessment and fetal morphology

At E18.5, uteri were examined and implantation sites, live fetuses, resorptions, and dead fetuses were recorded for each dam. Definitions of resorption and dead fetus followed the criteria used in the previous study[18].

All live fetuses were weighed individually, and the mean fetal body weight per litter was calculated. External morphological examination was performed using a stereomicroscope (MZ125; Leica, Wetzlar, Germany). Evaluations were performed by an observer blinded to maternal genotype and treatment group. Abnormalities recorded included foot congestion and dorsal congestion.

The incidence of fetal abnormalities was calculated using the number of live fetuses as the denominator. All fetal outcomes were classified according to maternal genotype.

2.9. Statistical analysis

Statistical analyses were performed using SPSS version 16.0 and R version 4.4.2. Continuous variables are presented as mean $\pm$ SD, and categorical variables are presented as n (%). Two-sided P values < 0.05 were considered statistically significant.

Hepatic MTHFR activity in vehicle-treated dams was analyzed using one-way ANOVA followed by Tukey's multiple-comparisons test.

Serum Hcy and folate levels were analyzed using Type III two-way ANOVA with maternal genotype, OXC treatment, and genotype $\times$ treatment interaction as fixed effects. Simple effects and Tukey-adjusted pairwise comparisons were performed using the pooled residual variance from the same ANOVA model.

Live fetus rate and resorption rate were analyzed using binomial generalized linear models with maternal genotype and OXC treatment as predictors. Mean fetal body weight per litter was analyzed using Type III two-way ANOVA with maternal dam/litter as the experimental unit.

Incidences of foot congestion and dorsal congestion were compared using Fisher–Freeman–Halton exact tests because these abnormalities occurred only in the 130 mg/kg OXC group and event numbers were limited.

3. Results

3.1. Genotyping of *Mthfr* 677C > T mice

Representative Sanger sequencing chromatograms confirmed the three *Mthfr* 677C > T genotypes. A single C peak (blue peak) was observed in *Mthfr*^{C/C} mice, overlapping C and T signals were detected in *Mthfr*^{C/T} mice, and a single T peak (red peak) was observed in *Mthfr*^{T/T} mice at the variant site (Fig. 1).

3.2. Effects of maternal *Mthfr* 677C > T genotype and OXC exposure on hepatic MTHFR activity and maternal serum homocysteine and folate concentrations

Hepatic MTHFR activity differed significantly among vehicle-treated pregnant mice with the three *Mthfr* 677C > T genotypes (Fig. 2A). MTHFR activity was highest in CC mice, intermediate in CT mice, and lowest in TT mice. MTHFR activity progressively decreased with increasing number of 677T alleles, with significant differences observed among CC, CT, and TT dams.

Serum homocysteine concentrations were analyzed using a Type III two-way ANOVA with genotype and OXC treatment as fixed factors. Significant main effects were observed for genotype ($F_{2,58} = 16.72P < 0.001$) and OXC treatment ($F_{2,58} = 41.81P < 0.001$), whereas the genotype-by-treatment interaction was not significant ($F_{4,58} = 0.88P = 0.481$) (Fig. 2B; Supplementary Table S1A). Thus, serum homocysteine concentrations varied according to both genotype and OXC exposure, but the overall treatment-associated pattern was not statistically different among genotypes.

Simple-effects analyses showed no significant difference among CC, CT, and TT mice in the vehicle control group ($P = 0.143$). In contrast, significant genotype effects were detected after treatment with 65 and 130 mg/kg OXC (Supplementary Table S1B). Following 65 mg/kg OXC treatment, serum homocysteine concentrations were higher in TT mice than in CC mice (23.90 ± 3.37 vs. 18.81 ± 2.05 $\mu\text{mol/L}$; Tukey-adjusted $P = 0.004$) and CT mice (23.90 ± 3.37 vs. 19.96 ± 2.16 $\mu\text{mol/L}$; adjusted $P = 0.026$), whereas CC and CT mice did not differ significantly. Following 130 mg/kg OXC treatment, TT mice also had higher concentrations than CC mice (28.53 ± 4.76 vs. 22.16 ± 2.80 $\mu\text{mol/L}$; adjusted $P < 0.001$) and CT mice (28.53 ± 4.76 vs. 23.66 ± 3.17 $\mu\text{mol/L}$; adjusted $P = 0.006$); no significant difference was observed between CC and CT mice.

Within-genotype comparisons further showed an increase in serum homocysteine concentrations with OXC exposure. In CC mice, the 130 mg/kg group had a higher concentration than the vehicle group (adjusted $P < 0.001$), whereas the other treatment comparisons were not significant. In CT mice, the 65 and 130 mg/kg groups both had higher concentrations than the vehicle group (adjusted $P = 0.044$ and $P < 0.001$, respectively), and the concentration was higher at 130 mg/kg than at 65 mg/kg (adjusted $P = 0.040$). In TT mice, all three treatment groups differed significantly from one another: 65 mg/kg versus vehicle, adjusted $P = 0.002$; 130 mg/kg versus vehicle, adjusted $P < 0.001$; and 130 versus 65 mg/kg, adjusted $P = 0.010$. Complete Tukey-adjusted pairwise comparisons and 95% confidence intervals are provided in Supplementary Table S1C. Because the genotype-by-treatment interaction was not significant, these simple-effects findings should not be interpreted as evidence that the OXC dose-response relationship differed significantly among genotypes.

For serum folate concentrations, Type III two-way ANOVA revealed significant effects of genotype ($F_{2,58} = 365.17P < 0.001$), OXC treatment ($F_{2,58} = 328.03P < 0.001$), and their interaction ($F_{4,58} = 44.77P < 0.001$) (Fig. 2C; Supplementary Table S1A). The significant interaction indicated that the effect of OXC treatment on serum folate concentrations differed according to *Mthfr* genotype.

In the vehicle control group, serum folate concentrations were highest in CC mice, followed by CT and TT mice (25.30 ± 0.88 , 24.16 ± 0.85 , and 22.30 ± 0.88 ng/mL, respectively). All pairwise genotype comparisons were significant: CC versus CT, adjusted $P = 0.045$; CC versus TT, adjusted $P < 0.001$; and CT versus TT, adjusted $P < 0.001$. Following 65 mg/kg OXC treatment, folate concentrations were higher in CC mice than in CT mice (23.40 ± 0.88 vs. 22.20 ± 0.89 ng/mL; adjusted $P = 0.034$), and both CC and CT mice had higher concentrations than TT mice (16.40 ± 0.92 ng/mL; both adjusted $P < 0.001$). Following 130 mg/kg OXC treatment, no significant difference was observed between CC and CT mice (20.60 ± 0.95 vs. 20.13 ± 0.88 ng/mL; adjusted $P = 0.574$), whereas both genotypes had markedly higher folate concentrations than TT mice (10.30 ± 0.97 ng/mL; both adjusted $P < 0.001$).

Within each genotype, serum folate concentrations decreased progressively with increasing OXC dose. All pairwise comparisons among the vehicle, 65 mg/kg, and 130 mg/kg groups were significant in CC, CT, and TT mice (all adjusted $P < 0.001$). The reduction was most pronounced in TT mice, in which the mean folate concentration decreased from 22.30 ± 0.88 ng/mL in the vehicle group to 16.40 ± 0.92 ng/mL in the 65 mg/kg group and to 10.30 ± 0.97 ng/mL in the 130 mg/kg group. Complete simple-effects tests and Tukey-adjusted pairwise comparisons are presented in Supplementary Tables S1B and S1D.

3.3. Embryonic development following maternal OXC exposure

Embryonic developmental outcomes are summarized in Table 1. The proportions of live fetuses from CC, CT, and TT dams were 97.1%, 92.9%, and 87.3%, respectively, in the vehicle control group; 96.8%, 91.9%, and 81.7%, respectively, following treatment with 65 mg/kg OXC; and 96.9%, 88.9%, and 75.0%, respectively, following treatment with 130 mg/kg OXC. Correspondingly, the resorption rates in pregnancies from CC, CT, and TT dams were 2.9%, 7.1%, and 12.7% in the vehicle group; 3.2%, 8.1%, and 18.3% in the 65 mg/kg group; and 3.1%, 11.1%, and 23.2% in the 130 mg/kg group. A single dead fetus was observed among fetuses from TT dams exposed to 130 mg/kg OXC. Maternal *Mthfr* genotype was significantly associated with embryonic survival outcomes, with TT dams showing lower odds of fetal survival and higher odds of resorption than CC dams, whereas neither OXC exposure nor the genotype–OXC interaction reached statistical significance (Supplementary Table S2). Mean fetal body weight per litter showed a numerical decline with increasing OXC dose across the maternal *Mthfr* genotype groups, with the lowest values observed in litters from TT dams. The lowest mean fetal body weight was observed in litters from TT dams treated with 130 mg/kg OXC (1.05 ± 0.07 g), compared with 1.18 ± 0.06 g in litters from vehicle-treated TT dams. Type III two-way ANOVA showed a significant effect of maternal *Mthfr* genotype on mean fetal body weight per litter ($F = 3.368$, $P = 0.041$), whereas neither OXC treatment ($F = 0.620$, $P = 0.541$) nor the genotype–OXC interaction ($F = 0.613$, $P = 0.655$) was significant (Supplementary Table S2).

Table 1
Embryonic development according to maternal Mthfr 677C > T genotype following vehicle or oxcabazepine exposure (65 or 130 mg/kg) on E7.5

	Vehicle control			OXC 65 mg/kg			OXC 130 mg/kg		
	CC	CT	TT	CC	CT	TT	CC	CT	TT
Number of pregnant mice treated, n	8	7	8	7	8	7	8	7	7
No. of implantation sites, n	70	56	63	62	62	60	65	63	56
No. of live fetuses, n (%)	68 (97.1%)	52 (92.9%)	55 (87.3%)	60 (96.8%)	57 (91.9%)	49 (81.7%)	63 (96.9%)	56 (88.9%)	42 (75.0%)
No. of resorptions, n (%)	2 (2.9%)	4 (7.1%)	8 (12.7%)	2 (3.2%)	5 (8.1%)	11 (18.3%)	2 (3.1%)	7 (11.1%)	13 (23.2%)
No. of dead fetuses, n (%)	0	0	0	0	0	0	0	0	1 (1.8%)
Mean fetal body weight per litter, g $\pm$ SD	1.25 $\pm$ 0.05	1.21 $\pm$ 0.05	1.18 $\pm$ 0.06	1.23 $\pm$ 0.05	1.19 $\pm$ 0.06	1.15 $\pm$ 0.07	1.20 $\pm$ 0.06	1.14 $\pm$ 0.07	1.05 $\pm$ 0.07
Foot congestion, n (%)	0	0	0	0	0	0	1 (1.6%)	2 (3.6%)	5 (11.9%)
Dorsal congestion, n (%)	0	0	0	0	0	0	0	1 (1.8%)	1 (2.4%)
Data are presented as n, n (%), or mean $\pm$ SD, as appropriate. Percentages of live fetuses, resorptions, and dead fetuses were calculated using the number of implantation sites as the denominator, whereas the incidences of foot congestion and dorsal congestion were calculated using the number of live fetuses as the denominator. No significant differences in the incidences of foot congestion or dorsal congestion were observed among maternal genotype groups within the 130 mg/kg OXC group (Fisher–Freeman–Halton exact test; P = 0.069 and P = 0.521, respectively). OXC, oxcabazepine; E7.5, embryonic day 7.5. CC, CT, and TT indicate maternal Mthfr 677C > T genotypes; fetal genotypes were not determined.									

3.4. Gross fetal abnormalities following maternal OXC exposure

No foot or dorsal congestion was observed in the vehicle control or 65 mg/kg OXC groups. In the 130 mg/kg OXC group, foot congestion was observed in fetuses from CC dams (1/63, 1.6%), CT dams (2/56, 3.6%), and TT dams (5/42, 11.9%). Although the incidence was numerically higher among fetuses from TT dams than among those from CT or CC dams, the overall difference among maternal genotype groups did not reach statistical significance (Fisher–Freeman–Halton exact test, P = 0.069). Dorsal congestion was observed in 0 of 63 fetuses from CC dams, 1 of 56 fetuses from CT dams (1.8%), and 1 of 42 fetuses from TT dams (2.4%), with no significant difference among maternal genotype groups (Fisher–Freeman–Halton exact test, P = 0.521; Table 1).

Representative gross images are presented in Fig. 3. Panels A and B show the left and right lateral views of a normal fetus, respectively. Panels C and D show the left and right lateral views of the same fetus with foot congestion, and panel E shows a representative fetus with dorsal congestion.

4. Discussion

The present study used a previously established *Mthfr* 677C > T knock-in mouse model to systematically evaluate the influence of maternal *Mthfr* 677C > T genotype on maternal one-carbon metabolism and embryonic developmental outcomes following gestational oxcarbazepine (OXC) exposure. Under vehicle conditions, hepatic MTHFR activity progressively decreased with increasing maternal 677T-allele burden. Following OXC exposure, maternal serum homocysteine (Hcy) concentrations generally increased, whereas serum folate concentrations decreased with increasing OXC dose. Notably, a significant maternal genotype $\times$ OXC treatment interaction was detected for serum folate, with TT dams showing the greatest reduction following OXC exposure. In contrast, although significant main effects of maternal genotype and OXC treatment were observed for Hcy, their interaction was not statistically significant. Analysis of developmental outcomes further showed that maternal *Mthfr* genotype was significantly associated with fetal survival, resorption, and mean fetal body weight per litter, whereas neither the OXC main effect nor the maternal genotype $\times$ OXC interaction reached statistical significance for these quantitative developmental outcomes. Foot and dorsal congestion were observed only in the 130 mg/kg OXC group. The incidence of foot congestion was numerically highest among fetuses from TT dams, although differences among maternal genotype groups were not statistically significant. Collectively, these findings support an association of the maternal *Mthfr* 677C > T genetic background with one-carbon metabolic status and baseline developmental vulnerability during pregnancy. They further suggest that OXC perturbs maternal folate–Hcy metabolism, with its effect on folate homeostasis being significantly modified by maternal *Mthfr* genotype. However, the present developmental data do not establish a statistically significant maternal genotype $\times$ OXC interaction for developmental toxicity.

MTHFR is a key enzyme in folate-dependent one-carbon metabolism and catalyzes the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate (5-MTHF), which provides the methyl group required for the remethylation of Hcy to methionine. One-carbon metabolism also contributes to purine and thymidylate synthesis, amino acid homeostasis, and cellular methylation reactions[7]. The MTHFR 677C > T variant results in an Ala222Val substitution and produces a thermolabile enzyme with reduced activity, with the greatest functional impairment generally observed in the TT genotype[8]. The association between the 677TT genotype and elevated Hcy is further influenced by folate status and becomes more pronounced under conditions of lower folate availability[20]. In addition, a systematic review and meta-analysis demonstrated a consistent genotype-dependent gradient in circulating folate concentrations, with an overall pattern of CC > CT > TT[9]. Therefore, the progressive reduction in hepatic MTHFR activity and corresponding metabolic differences observed among CC, CT, and TT dams in the present study are consistent with the established functional consequences of this variant and provide a biological basis for interpreting genotype-associated metabolic responses to OXC.

Our findings are also consistent with recent experimental studies using *Mthfr* 677C > T mouse models. Yang et al. used a directly related genetically modified mouse model and showed that reduced MTHFR activity and elevated Hcy were associated with the *Mthfr* 677C > T background. Following VPA administration at E7.5, pregnancies from CT and TT dams showed higher rates of fetal malformations, with the most pronounced susceptibility observed in the TT group[18]. The present study extends this model from VPA to OXC and further incorporates two OXC exposure levels, thereby allowing maternal genotype, drug exposure level, and their interaction to be evaluated

within the same experimental framework. In a separate *Mthfr* 677C > T knock-in pregnancy model, Christensen et al. reported lower hepatic MTHFR expression, lower circulating methylTHF, and higher Hcy concentrations in TT dams, together with more pronounced developmental delay in litters from TT dams under low-folate dietary conditions[19]. Taken together, these findings suggest that the metabolic background associated with the 677TT genotype may not necessarily produce severe developmental impairment under all conditions, but may confer reduced metabolic reserve that becomes more apparent when additional nutritional or pharmacological stress is imposed.

Compared with VPA, the gross fetal abnormalities observed following OXC exposure in the present study were considerably less frequent, and no foot or dorsal congestion was detected at 65 mg/kg. This observation is compatible with current pregnancy registry data indicating a relatively low overall risk of major congenital malformations associated with OXC. In the 2018 EURAP study, the prevalence of major congenital malformations among 333 pregnancies exposed to OXC monotherapy was approximately 3.0%[21]. Updated EURAP data identified 13 major congenital malformations among 443 OXC monotherapy pregnancies, corresponding to a prevalence of 2.9%[2]. More recently, the North American Antiepileptic Drug Pregnancy Registry reported a major congenital malformation prevalence of 1.5% among 327 pregnancies exposed to OXC monotherapy and did not identify a clinically meaningful increase relative to lower-risk comparator antiseizure medications[4]. Nevertheless, experimental studies indicate that OXC can exert developmental effects under specific exposure conditions. For example, González-Maciel et al. reported increased hippocampal apoptosis and altered hippocampal structure in rat offspring following maternal OXC exposure from gestational days 7–15, accompanied by reduced litter size and lower fetal weight at gestational day 19[6]. Thus, a relatively low population-level risk of major congenital malformations does not exclude the possibility of increased biological susceptibility under particular exposure conditions or genetic and metabolic backgrounds. Accordingly, the significance of the present study is not to redefine OXC as a strongly teratogenic agent, but rather to investigate whether a drug with a comparatively low population-level teratogenic risk may nevertheless produce heterogeneous metabolic and developmental responses according to host genetic background.

One of the most notable findings of the present study was the significant maternal *Mthfr* genotype × OXC treatment interaction for serum folate. Folate concentrations progressively decreased with increasing OXC exposure in CC, CT, and TT dams, with the greatest reduction observed in TT dams. Mean serum folate in TT dams declined from 22.30 ng/mL in the vehicle group to 16.40 ng/mL after 65 mg/kg OXC and further to 10.30 ng/mL after 130 mg/kg OXC. This interaction indicates that the magnitude of the OXC-associated reduction in circulating folate was not uniform across maternal *Mthfr* genotypes, but was significantly modified by genetic background. Previous clinical studies have suggested that OXC may affect folate–Hcy metabolism. In a prospective study of patients with newly diagnosed epilepsy, Kim et al. reported an approximately 32% increase in Hcy and a 21% decrease in serum folate after 6 months of OXC monotherapy[22]. Belcastro et al. also reported mean total Hcy concentrations above the physiological reference range in OXC-treated patients, together with lower folate concentrations among patients receiving newer antiseizure medications than among healthy controls[23]. More directly relevant to the present hypothesis, Zhu et al. found that MTHFR rs1801133 was significantly associated with Hcy concentrations in 279 patients with epilepsy and that OXC monotherapy was independently associated with higher Hcy. Within the OXC-treated subgroup, patients with the TT genotype had significantly higher Hcy concentrations than those with CC or CT genotypes[16]. This clinical observation provides direct support for the concept that the metabolic response to OXC may vary according to MTHFR genetic background.

However, clinical evidence regarding the overall effect of OXC on folate–Hcy metabolism has not been entirely consistent. In a systematic review and meta-analysis, Rezaei et al. found no statistically significant overall differences in total Hcy, folate, or vitamin B12 between patients receiving OXC monotherapy and healthy controls[17]. Such apparently discrepant findings may reflect differences in population characteristics, baseline nutritional status, treatment duration, and genetic background. If the metabolic effect of OXC is substantially heterogeneous across individuals, comparisons based solely on population-level mean values may fail to identify genetically susceptible subgroups. In this context, the significant maternal genotype × OXC interaction for folate observed in the present study, together with the MTHFR-related Hcy differences reported by Zhu et al. in OXC-treated patients, supports further consideration of one-carbon metabolic genetic background when evaluating metabolic responses to OXC[16, 17].

The mechanism underlying the genotype-dependent folate response observed here remains uncertain. Reduced MTHFR activity primarily alters the production of 5-MTHF and the distribution of folate derivatives within one-carbon metabolism; it does not imply that reduced MTHFR activity directly “consumes” or depletes total circulating folate. A more plausible interpretation is that TT dams, because of reduced MTHFR functional reserve, may have a diminished capacity to maintain folate homeostasis when challenged by additional metabolic stress associated with OXC exposure. Previous animal studies support this concept. Li et al. showed that maternal MTHFR deficiency, low dietary folate, or their combination increased fetal loss, intrauterine growth restriction, and congenital cardiac abnormalities in mice[14]. Pickell et al. further demonstrated that maternal MTHFR deficiency combined with low dietary folate increased embryonic developmental delay and was associated with placental abnormalities and fetal loss[24]. Mikael et al. reported that MTHFR deficiency and low folate intake reduced methylation potential and altered ApoA1- and IFN-γ-related signaling in the placenta and spleen[25]. More recently, a *Mthfr* 677C > T knock-in study demonstrated that low dietary folate had a greater developmental impact on litters from TT dams[19]. Together, these findings support the concept that genetically constrained one-carbon metabolism may become particularly vulnerable when subjected to additional nutritional or pharmacological stress.

In contrast to folate, serum Hcy showed significant main effects of both maternal genotype and OXC treatment, but no significant maternal genotype × OXC interaction. Under both 65 and 130 mg/kg OXC exposure, TT dams had higher Hcy concentrations than CC and CT dams, and Hcy concentrations generally increased with increasing OXC exposure within each maternal genotype. This direction of change is consistent with the biochemical consequence of reduced MTHFR activity, namely impaired Hcy remethylation[8, 20], and is also compatible with clinical observations reported by Kim et al., Belcastro et al., and Zhu et al. [16, 22, 23]. Nevertheless, because the interaction term was not significant, the present data do not demonstrate that TT dams have a statistically greater Hcy dose response to OXC. Rather, maternal genotype and OXC exposure were each associated with Hcy concentrations, but the magnitude of the OXC-associated increase did not differ significantly among maternal genotype groups.

The different interaction patterns observed for folate and Hcy may also reflect differences in the biological complexity of their regulation. In addition to MTHFR activity and folate status, Hcy concentrations are influenced by vitamin B12-dependent methionine synthase activity, vitamin B6-dependent transsulfuration, and other components of the methionine cycle[7]. These additional regulatory influences, together with greater interindividual variability in Hcy, may reduce statistical power to detect genotype × OXC interactions in relatively small experimental groups. Moreover, the absence of a consistent overall OXC effect on Hcy and folate in the meta-analysis by Rezaei et al. further indicates substantial heterogeneity across studies and individuals[17]. Therefore, serum folate may represent a more sensitive metabolic indicator of the joint influence of maternal *Mthfr* genotype and OXC exposure

in the present model. This interpretation remains provisional, however, and should be evaluated by direct measurement of 5-MTHF, methionine, S-adenosylmethionine (SAM), S-adenosylhomocysteine (SAH), vitamin B12, and other components of one-carbon metabolism.

Altered maternal one-carbon metabolism may in turn be relevant to developmental vulnerability. In the formal dam/litter-level analyses performed in the present study, maternal *Mthfr* genotype was significantly associated with fetal survival and resorption. Compared with CC dams, TT dams showed lower odds of fetal survival and higher odds of resorption. Maternal genotype was also significantly associated with mean fetal body weight per litter. These findings suggest that the *Mthfr* 677C > T genetic background itself may contribute to a baseline state of developmental vulnerability. Importantly, however, neither the OXC main effect nor the maternal genotype × OXC interaction reached statistical significance for live fetus rate, resorption rate, or mean fetal body weight per litter. Therefore, although Table 1 showed numerical trends toward reduced fetal survival, increased resorption, and lower mean fetal body weight with increasing OXC exposure, particularly among litters from TT dams, these patterns should not be interpreted as statistically established evidence that the TT maternal genotype confers a stronger developmental dose response to OXC. A more conservative interpretation is that maternal *Mthfr* genotype is associated with baseline developmental outcomes, whereas the current data do not demonstrate an additional statistically significant genotype-dependent effect of OXC on these quantitative developmental endpoints.

This baseline developmental vulnerability is biologically plausible. Folate-dependent one-carbon metabolism supplies one-carbon units required for purine and thymidylate synthesis in rapidly proliferating embryonic tissues and contributes to methyl-donor generation and epigenetic regulation[7]. In mice, folate deficiency has been shown to alter embryonic genomic methylation and suppress planar cell polarity signaling involved in embryonic development[26]. Maternal MTHFR deficiency and low folate status have also been associated with increased fetal resorption, developmental delay, intrauterine growth restriction, and placental abnormalities[14, 24, 25]. In human pregnancy, Furness et al. reported lower red blood cell folate and higher plasma Hcy concentrations among women with intrauterine growth restriction, with red blood cell folate independently associated with IUGR risk[27]. Thus, the concurrent occurrence of lower maternal folate, higher Hcy, and less favorable embryonic developmental outcomes in the present study is biologically coherent, although the association remains observational and does not establish a causal pathway.

Establishing such causality will require moving beyond association to mechanistic intervention. Future studies should characterize 5-MTHF, methionine, SAM/SAH ratios, and DNA methylation in maternal, placental, and embryonic tissues, together with markers of proliferation, oxidative stress, and apoptosis. More importantly, folate or 5-MTHF supplementation could be used as a rescue strategy to determine whether correction of one-carbon metabolic disturbances attenuates OXC-associated metabolic and developmental phenotypes. Previous studies of MTHFR deficiency and low-folate conditions have demonstrated substantial sensitivity of this metabolic system to folate availability[14, 19, 24]. Such intervention studies would therefore help determine whether altered folate metabolism acts as a mechanistic mediator of increased developmental susceptibility or primarily represents a biomarker of an underlying vulnerable metabolic state.

Another finding warranting further investigation was the occurrence of gross fetal abnormalities only in the 130 mg/kg OXC group. Foot and dorsal congestion were absent in both vehicle and 65 mg/kg groups. At 130 mg/kg, foot congestion occurred in 1.6%, 3.6%, and 11.9% of fetuses from CC, CT, and TT dams, respectively. Although the incidence was numerically highest among fetuses from TT dams, the overall difference among maternal genotype groups was not statistically significant. Dorsal congestion likewise did not differ significantly among maternal

genotype groups. The most appropriate interpretation is therefore that higher OXC exposure may be associated with the occurrence of these gross fetal abnormalities, whereas the numerically higher incidence of foot congestion among fetuses from TT dams represents a hypothesis-generating observation rather than evidence of significantly greater developmental susceptibility associated with the TT maternal genotype.

From a mechanistic perspective, both folate deficiency and elevated Hcy have been implicated in impaired vascular development at the maternal–fetal interface. Li et al. reported impaired decidual angiogenesis in folate-deficient pregnant mice, accompanied by reduced expression of VEGFA, VEGFR2, and PLGF[28]. Xu et al. found that Hcy exposure during early gestation reduced placental Nrp1 and Vegf expression in a mouse model and was associated with endothelial abnormalities[29]. These findings raise the possibility that the foot or dorsal congestion observed in the present study may be related to impaired maternal–fetal vascular development, placental dysfunction, or local circulatory disturbance. However, no placental histology, fetal vascular pathology, or vascular molecular markers were assessed in the present study. Accordingly, a causal sequence linking folate/Hcy disturbance to vascular abnormalities and fetal congestion cannot currently be established, and this interpretation should be regarded strictly as a hypothesis for future investigation.

The present study has several strengths. First, the use of a previously established and characterized *Mthfr* 677C > T knock-in mouse model enabled the relationship between maternal MTHFR functional status and OXC exposure to be evaluated within a defined genetic framework. Second, inclusion of two OXC exposure levels within a 3 × 3 factorial design allowed maternal genotype, OXC treatment, and their interaction to be assessed within a unified statistical framework. Third, the study integrated maternal MTHFR enzymatic activity, Hcy, and folate with dam/litter-level measures of fetal survival, resorption, and mean fetal body weight, as well as gross fetal morphology, thereby providing a continuous experimental framework linking maternal genetic background, metabolic phenotype, and developmental outcomes. In particular, the significant maternal genotype × OXC interaction for serum folate provides direct statistical evidence that genetically determined differences in MTHFR function can modify the maternal folate response to OXC exposure.

Several limitations should also be acknowledged. First, each experimental cell included only approximately seven to eight pregnant mice, limiting statistical power for interaction effects and especially for low-frequency fetal abnormalities. The small numbers of foot and dorsal congestion events mean that these findings should be considered exploratory. Second, hepatic MTHFR activity was measured only in vehicle-treated dams. Consequently, the present study cannot determine whether OXC itself further alters MTHFR activity or whether the OXC-associated reduction in folate is mediated through a direct effect on MTHFR. Third, maternal plasma concentrations of OXC and its major active metabolite, 10-monohydroxy derivative (MHD), were not measured. Differences in systemic drug exposure among maternal genotype groups therefore cannot be excluded. Fourth, individual E18.5 fetuses were not genotyped for *Mthfr*. Because CC females were mated with CC males, CT females with CT males, and TT females with TT males, fetuses from CC × CC and TT × TT pregnancies were expected to be CC and TT, respectively, whereas fetuses from CT × CT pregnancies could segregate into CC, CT, and TT genotypes. The present design therefore does not allow complete separation of maternal genotype effects from potential direct fetal genotype effects. Fifth, live fetus rate, resorption rate, and mean fetal body weight were analyzed at the dam/litter level, whereas foot and dorsal congestion, because of their low event frequencies, were evaluated primarily using exploratory fetus-level exact tests; residual within-litter clustering therefore could not be completely accounted for in these analyses. Sixth, gross fetal abnormalities were assessed morphologically without histopathological or vascular-marker confirmation. Finally, only Hcy and total serum folate were measured, which

cannot fully characterize the one-carbon metabolic network. Future assessment of 5-MTHF, methionine, SAM, SAH, vitamin B12, and tissue-specific metabolic profiles will be necessary to clarify the underlying mechanisms.

5. Conclusions

Using a previously established *Mthfr* 677C > T knock-in mouse model, this study systematically evaluated the influence of maternal *Mthfr* genetic background on maternal one-carbon metabolism and embryonic developmental outcomes following gestational oxcarbazepine (OXC) exposure. Maternal *Mthfr* 677C > T genotype was closely associated with MTHFR function and one-carbon metabolic status. Hepatic MTHFR activity progressively decreased with increasing maternal 677T-allele burden. Following OXC exposure, maternal serum homocysteine (Hcy) concentrations generally increased, whereas serum folate concentrations decreased with increasing OXC dose. Importantly, a significant maternal genotype × OXC treatment interaction was observed for serum folate, with TT dams showing the greatest folate reduction after OXC exposure, indicating that genetically determined differences in MTHFR function can modify the maternal folate response to OXC. In contrast, although significant main effects of maternal genotype and OXC treatment were observed for Hcy, their interaction was not statistically significant.

With respect to embryonic development, maternal *Mthfr* genotype was significantly associated with fetal survival, resorption, and mean fetal body weight per litter, suggesting that the *Mthfr* 677C > T genetic background itself may contribute to baseline developmental vulnerability. However, neither the OXC main effect nor the maternal genotype × OXC interaction reached statistical significance for these quantitative developmental outcomes. Therefore, the present data do not establish that the TT maternal genotype confers significantly greater susceptibility to OXC-associated developmental toxicity. Foot and dorsal congestion were observed only in the 130 mg/kg OXC group, and the incidence of foot congestion was numerically highest among fetuses from TT dams; however, differences among maternal genotype groups were not statistically significant and should therefore be regarded as exploratory findings.

Overall, the strongest evidence from this study supports the conclusion that maternal *Mthfr* 677C > T genetic background modifies folate metabolic responses to OXC exposure and is associated with baseline developmental vulnerability. These findings extend previous work on *Mthfr* 677C > T-associated susceptibility to valproic acid and suggest that genetic variation in one-carbon metabolism may represent an important host factor contributing to interindividual differences in metabolic and developmental responses to antiseizure medications during pregnancy. Further studies incorporating larger sample sizes, fetal genotyping, pharmacokinetic assessment of OXC and its active metabolite MHD, comprehensive profiling of one-carbon metabolites such as 5-MTHF and SAM/SAH, and mechanistic intervention with folate or 5-MTHF supplementation are warranted to determine whether maternal metabolic susceptibility translates into a definitive genotype-dependent developmental response to OXC.

Declarations

Declaration of competing interest

The authors declare no conflict of interest.

Ethics Approval

All procedures involving animals were reviewed and approved by the Institutional Animal Care and Use Committee of Sun Yat-sen University (approval no. SYSU-IACUC-2024-002515). All experiments were conducted in accordance with the relevant institutional guidelines and regulations for the care and use of laboratory animals.

Funding

This work was funded by the National Natural Science Foundation of China (file no. LMZ 82371456), the Shenzhen Municipal Science and Technology key projects of the Basic Research Program (file no. LMZ JCYJ20220818102007015).

Author Contribution

Tiancai Huang: Conceptualization, Methodology, Data curation, Writing - original draft. Jiahao Tian: Validation, Formal analysis. Wenyao Huang, Jiaqi Zhang, Yubao Fang and Yinchao Li: Resources. Liemin Zhou: Project administration, Data curation, Writing - review & editing.

Acknowledgments

The authors have no acknowledgements to declare.

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request. Relevant summary data and statistical results are provided in the article and its Supplementary Materials.

References

1. Pack AM, Oskoui M, Williams Roberson S, et al. Teratogenesis, perinatal, and neurodevelopmental outcomes after in utero exposure to antiseizure medication: Practice guideline from the AAN, AES, and SMFM. *Neurology* 2024;102:e209279. <https://doi.org/10.1212/WNL.0000000000209279>.
2. Battino D, Tomson T, Bonizzoni E, et al. Risk of major congenital malformations and exposure to antiseizure medication monotherapy. *JAMA Neurol* 2024;81:481. <https://doi.org/10.1001/jamaneurol.2024.0258>.
3. Cohen JM, Alvestad S, Cesta CE, et al. Comparative safety of antiseizure medication monotherapy for major malformations. *Annals of Neurology* 2023;93:551–62. <https://doi.org/10.1002/ana.26561>.
4. Hernandez-Diaz S, Quinn M, Conant S, et al. Use of antiseizure medications early in pregnancy and the risk of major malformations in the newborn. *Neurology* 2025;105:e213786. <https://doi.org/10.1212/WNL.0000000000213786>.
5. Athar F, Ehsan M, Farooq M, et al. Adverse fetal and neonatal outcomes following in-utero exposure to oxcarbazepine: A systematic review and meta-analysis. *British Journal of Clinical Pharmacology* 2022;88:3600–9. <https://doi.org/10.1111/bcp.15413>.
6. González- Maciel A, Romero-Velázquez R, Alfaro-Rodríguez A, et al. Prenatal exposure to oxcarbazepine increases hippocampal apoptosis in rat offspring. *Journal of Chemical Neuroanatomy* 2020;103:101729.

<https://doi.org/10.1016/j.jchemneu.2019.101729>.

7. Ducker GS, Rabinowitz JD. One-carbon metabolism in health and disease. *Cell Metabolism* 2017;25:27–42. <https://doi.org/10.1016/j.cmet.2016.08.009>.
8. Frosst P, Blom HJ, Milos R, et al. A candidate genetic risk factor for vascular disease: A common mutation in methylenetetrahydrofolate reductase. *Nat Genet* 1995;10:111–3. <https://doi.org/10.1038/ng0595-111>.
9. Tsang BL, Devine OJ, Cordero AM, et al. Assessing the association between the methylenetetrahydrofolate reductase (MTHFR) 677C > T polymorphism and blood folate concentrations: A systematic review and meta-analysis of trials and observational studies. *The American Journal of Clinical Nutrition* 2015;101:1286–94. <https://doi.org/10.3945/ajcn.114.099994>.
10. Colson NJ, Naug HL, Nikbakht E, et al. The impact of MTHFR 677 C/T genotypes on folate status markers: A meta-analysis of folic acid intervention studies. *Eur J Nutr* 2017;56:247–60. <https://doi.org/10.1007/s00394-015-1076-x>.
11. Finnell RH, Caiaffa CD, Kim S-E, et al. Gene environment interactions in the etiology of neural tube defects. *Frontiers in Genetics* 2021;12. <https://doi.org/10.3389/fgene.2021.659612>.
12. Yang M, Li W, Wan Z, et al. Elevated homocysteine levels in mothers with neural tube defects: A systematic review and meta-analysis. *The Journal of Maternal-Fetal & Neonatal Medicine* 2017;30:2051–7. <https://doi.org/10.1080/14767058.2016.1236248>.
13. Yang Y, Chen J, Wang B, et al. Association between MTHFR C677T polymorphism and neural tube defect risks: A comprehensive evaluation in three groups of NTD patients, mothers, and fathers. *Birth Defects Research Part A: Clinical and Molecular Teratology* 2015;103:488–500. <https://doi.org/10.1002/bdra.23361>.
14. Li D, Pickell L, Liu Y, et al. Maternal methylenetetrahydrofolate reductase deficiency and low dietary folate lead to adverse reproductive outcomes and congenital heart defects in mice. *Am J Clin Nutr* 2005;82:188–95. <https://doi.org/10.1093/ajcn.82.1.188>.
15. Reynolds EH, Green R. Valproate and folate: Congenital and developmental risks. *Epilepsy Behav* 2020;108. <https://doi.org/10.1016/j.yebeh.2020.107068>.
16. Zhu S, Ni G, Sui L, et al. Genetic polymorphisms in enzymes involved in one-carbon metabolism and anti-epileptic drug monotherapy on homocysteine metabolism in patients with epilepsy. *Front Neurol* 2021;12:683275. <https://doi.org/10.3389/fneur.2021.683275>.
17. Rezaei S, Shab-Bidar S, Abdulahi Abdurahman A, et al. Oxcarbazepine administration and the serum levels of homocysteine, vitamin B12 and folate in epileptic patients: A systematic review and meta-analysis. *Seizure* 2017;45:87–94. <https://doi.org/10.1016/j.seizure.2016.11.016>.
18. Yang M, Li Y, Lei L, et al. Influence of methylenetetrahydrofolate 677C > T variant in murine models following valproic acid exposure. *Reproductive Toxicology* 2025;137:108995. <https://doi.org/10.1016/j.reprotox.2025.108995>.
19. Christensen KE, Faquette M-L, Keser V, et al. Low dietary folate increases developmental delays in the litters of Mthfr677TT mice. *Nutrients* 2025;17:2536. <https://doi.org/10.3390/nu17152536>.
20. Relation between folate status, a common mutation in methylenetetrahydrofolate reductase, and plasma homocysteine concentrations n.d. <https://doi.org/10.1161/01.CIR.93.1.7>.
21. Tomson T, Battino D, Bonizzoni E, et al. Comparative risk of major congenital malformations with eight different antiepileptic drugs: A prospective cohort study of the EURAP registry. *The Lancet Neurology* 2018;17:530–8. [https://doi.org/10.1016/S1474-4422\(18\)30107-8](https://doi.org/10.1016/S1474-4422(18)30107-8).

22. Kim DW, Lee S-Y, Shon Y-M, et al. Effects of new antiepileptic drugs on circulatory markers for vascular risk in patients with newly diagnosed epilepsy. *Epilepsia* 2013;54:e146–9. <https://doi.org/10.1111/epi.12338>.
23. Belcastro V, Striano P, Gorgone G, et al. Hyperhomocysteinemia in epileptic patients on new antiepileptic drugs. *Epilepsia* 2010;51:274–9. <https://doi.org/10.1111/j.1528-1167.2009.02303.x>.
24. Pickell L, Li D, Brown K, et al. Methylenetetrahydrofolate reductase deficiency and low dietary folate increase embryonic delay and placental abnormalities in mice†. *Birth Defects Research Part A: Clinical and Molecular Teratology* 2009;85:531–41. <https://doi.org/10.1002/bdra.20575>.
25. Mikael LG, Pancer J, Jiang X, et al. Low dietary folate and methylenetetrahydrofolate reductase deficiency may lead to pregnancy complications through modulation of ApoA1 and IFN- γ in spleen and placenta, and through reduction of methylation potential. *Molecular Nutrition & Food Research* 2013;57:661–70. <https://doi.org/10.1002/mnfr.201200152>.
26. Geng Y, Gao R, Liu X, et al. Folate deficiency inhibits the PCP pathway and alters genomic methylation levels during embryonic development. *Journal of Cellular Physiology* 2018;233:7333–42. <https://doi.org/10.1002/jcp.26564>.
27. Furness D, Fenech M, Dekker G, et al. Folate, vitamin B12, vitamin B6 and homocysteine: Impact on pregnancy outcome. *Matern Child Nutr* 2011;9:155–66. <https://doi.org/10.1111/j.1740-8709.2011.00364.x>.
28. Li Y, Gao R, Liu X, et al. Folate deficiency could restrain decidual angiogenesis in pregnant mice. *Nutrients* 2015;7:6425–45. <https://doi.org/10.3390/nu7085284>.
29. Xu X, Yang X-Y, He B-W, et al. Placental NRP1 and VEGF expression in pre-eclamptic women and in a homocysteine-treated mouse model of pre-eclampsia. *European Journal of Obstetrics and Gynecology and Reproductive Biology* 2016;196:69–75. <https://doi.org/10.1016/j.ejogrb.2015.11.017>.

Figures

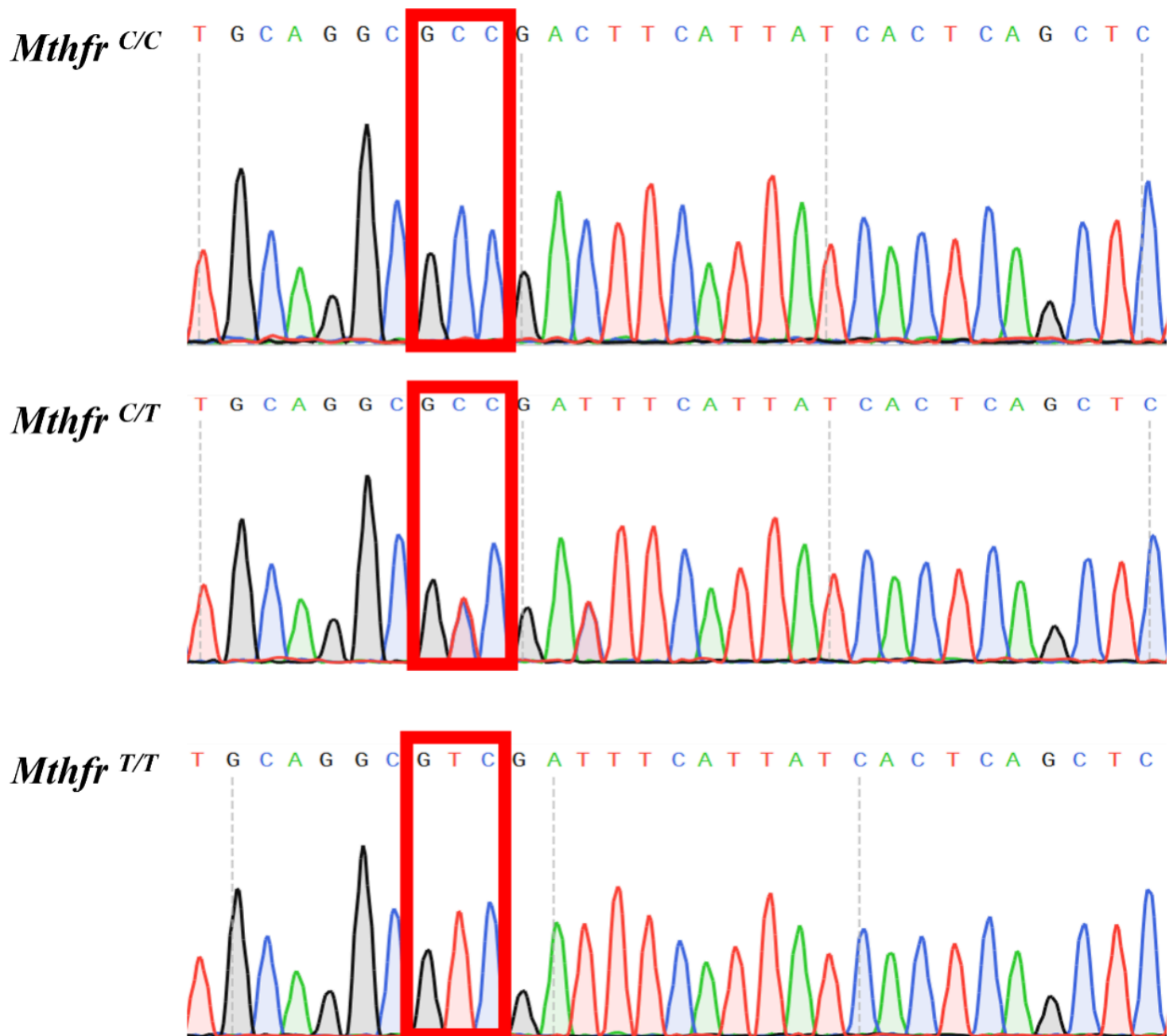


Figure 1

Identification of *Mthfr* 677C>T genotypes by Sanger sequencing. Representative Sanger sequencing chromatograms showing the *Mthfr*^{C/C}, *Mthfr*^{C/T}, and *Mthfr*^{T/T} genotypes. The nucleotide position corresponding to the 677C>T variant is highlighted by red boxes.

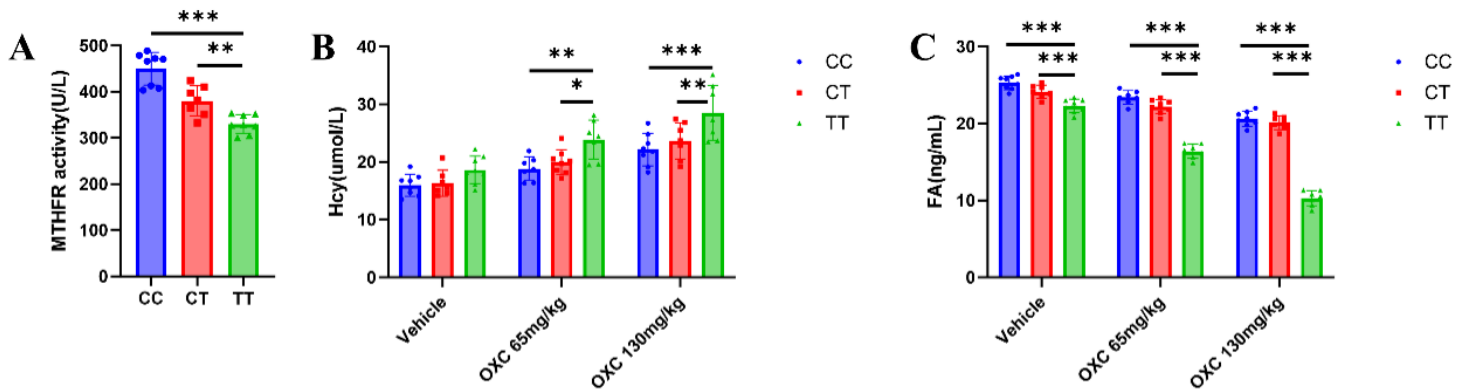


Figure 2

Effects of the *Mthfr* 677C>T genotype and oxcabazepine exposure on hepatic MTHFR activity and serum homocysteine and folate concentrations in pregnant mice. (A) Hepatic MTHFR activity in vehicle-treated pregnant mice with the CC, CT, and TT genotypes. (B) Serum homocysteine concentrations in pregnant CC, CT, and TT mice treated with vehicle, 65 mg/kg OXC, or 130 mg/kg OXC. (C) Serum folate concentrations in pregnant CC, CT, and TT mice treated with vehicle, 65 mg/kg OXC, or 130 mg/kg OXC. Each symbol represents an individual pregnant mouse, and bars represent the mean $\pm$ SD (n=8, 7, or 8 per genotype group as indicated). Data in panel A were analyzed using one-way ANOVA followed by Tukey's multiple-comparisons test. Data in panels B and C were analyzed using Type III two-way ANOVA with genotype, OXC treatment, and their interaction as fixed effects. Simple-effects analyses were performed using the pooled residual variance from the two-way ANOVA model, followed by Tukey-adjusted pairwise comparisons. Only pairwise genotype comparisons within each treatment group are shown in the figure; comparisons among treatment groups within each genotype and the complete statistical results are provided in Supplementary Table S1.

* $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$.

MTHFR, methylenetetrahydrofolate reductase; Hcy, homocysteine; FA, folate; OXC, oxcabazepine.

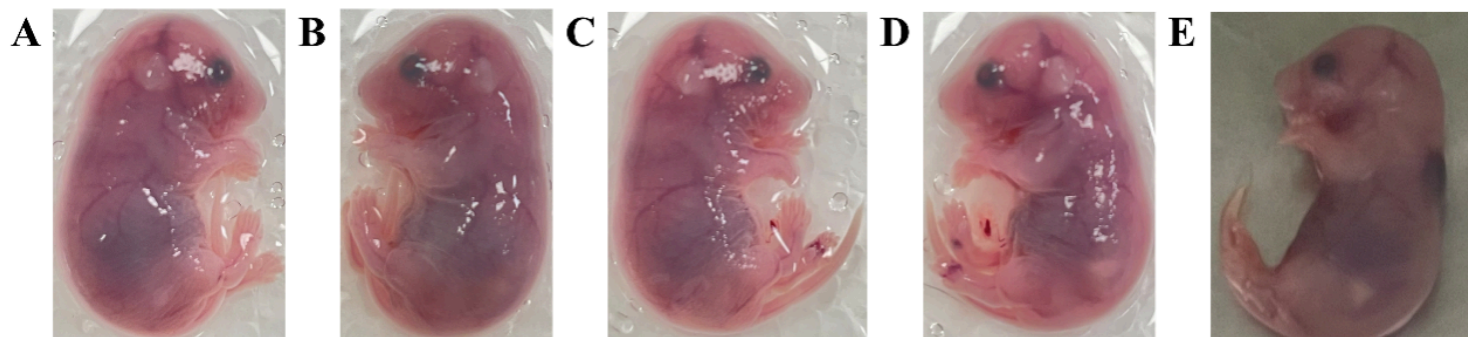


Figure 3

Representative gross appearance of normal and affected fetuses following maternal OXC exposure. (A, B) Left and right lateral views of a normal fetus. (C, D) Left and right lateral views of a fetus with foot congestion. (E) Representative fetus with dorsal congestion.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementaryMaterials.docx](#)