Supplementary Materials

Newborn *MTHFR* rs1801133 Variant and Extremely Low Birth Weight: A Case–Control Study and Meta-Analysis

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Supplemental Table S1. Comparison of clinical parameters between Study Cohort A and B.

Parameter		Study Cohort A (2009 - 2014) n=164	Study Cohort B (2015 - 2020) n=213	B *vs.* A p-value or OR (95% CI), p-value
Gestational age [weeks]	Mean (SD)	27.8 (1.8)	27.8 (2.3)	0.936
	Range	24 - 31.9	22-31.9	
	<28 weeks), n (%)	68 (41.5)	84 (39.4)	0.691
Birth weight [g]	Mean (SD)	1,145.7 (301.6)	1,067.3 (332.8)	0.018
	Range	490 – 1,960	432 – 2,340	
	<1,000 g), n (%)	68 (41.5)	81 (38.0)	0.499
	SGA (<10th), n (%)	48 (29.3)	51 (23.7)	0.288
	FGR/IUGR , n (%)	6 (3.7)	19 (8.9)	2.6 (1.0–6.6), 0.049
Male sex, n (%)		87 (53.0)	119 (55.9)	0.586
Risk factors at birth				
PPROM, n (%)		58 (35.4)	49 (23.0)	0.54 (0.34–0.86), 0.011
PPROM >3 d, n (%) 74		52 (31.7)	22 (10.3)	0.25 (0.14-0.43), <0.0001
Delivery by cesarean section, n (%)		95 (57.9)	109 (51.2)	0.377
Apgar 1, Median (Q1, Q3)		5 (2, 7)	5 (2, 7)	0.586
Apgar 5, Median (Q1, Q3)		7 (6, 8)	7 (6, 8)	0.451
Parameters related to respiratory failure				
Surfactant treatment, n (%)		83 (50.6)	98 (46.0)	0.406
Resuscitation, n (%)		133 (81.1)	188 (86.4)	0.164
Mech. vent., n (%)		124 (75.6)	134 (62.9)	0.64 (0.43–0.95), 0.009
Mech. vent. period [d], Median (Q1, Q3)		20 (6, 46)	11 (1, 30)	0.0001
Oxygen supply period [d], Median (Q1, Q3)		28 (1, 58)	13 (2, 59)	0.020
Blood transfusions, Mean (SD)		3.6 (3.4)	3.6 (3.2)	0.892
Complications of prematurity, n (%)				
NEC		27 (16.5)	52 (24.4)	0.062
BPD		59 (36.0)	90 (42.3)	0.217
IVH		92 (55.8)	126 (59.2)	0.551
RDS		78 (47.6)	173 (81.2)	4.8 (3.0-7.6), <0.0001
PVL		14 (8.5)	14 (6.6)	0.472
ROP		102 (62.2)	137 (64.3)	0.671
pROP		43 (26.2)	77 (36.2)	1.6 (1.0-2.5), 0.041
PDA		39 (23.8)	65 (30.5)	0.148
Death		3 (1.8)	5 (2.3)	0.730

Abbreviations and symbols: BPD – bronchopulmonary dysplasia; FGR – fetal growth restriction; IUGR - intrauterine growth retardation; IVH – intraventricular hemorrhage; NEC – necrotizing enterocolitis; PDA - patent ductus arteriosus; PPROM - preterm prelabor rupture of membranes; PVL - periventricular leukomalacia; RDS – respiratory distress syndrome; ROP – retinopathy of prematurity; SGA - small for gestational age.

Supplemental Table S2. Comparison of the impact of neonatal *MTHFR* rs1801133 genotypes on ELBW and ELGA in males and females. The table presents genotype (CT and TT vs CC) and allele-based comparisons (dominant and recessive models) with two-sided χ^2 test p-values. Statistically significant values are highlighted in bold.

Genotype and allele frequencies	ELBW				ELGA			
	Female		Male		Female		Male	
	no n=92	Yes n=79	no n=130	Yes n=76	no n=100	Yes n=71	no n=125	Yes n=81
MTHFR rs1801133C>T (Ala222Val)								
CC	50 (54.3)	35 (44.3)	75 (57.7)	33 (43.4)	50 (50.0)	35 (49.3)	72 (57.6)	36 (44.4)
CT	36 (39.1)	33 (41.8)	42 (32.3)	35 (47.4)	39 (39.0)	30 (42.3)	40 (32.0)	38 (46.9)
TT	7 (6.5)	11 (13.9)	13 (10.0)	7 (9.2)	11 (11.0)	6 (8.5)	13 (10.4)	7(8.6)
MAF	0.261	0.348	0.262	0.329	0.305	0.296	0.264	0.321
Statistical analysis: OR (95%CI), p								
Genotype								
CC	1.0 (reference)		1.0 (reference)		1.0 (reference)		1.0 (reference)	
CT	1.31 (0.69–2.48); p = 0.409		**1.89 (1.03–3.48); p = 0.038**		1.10 (0.58–2.09); p = 0.774		**1.90 (1.05–3.45); p = 0.034**	
TT	2.24 (0.79–6.36); p = 0.122		1.22 (0.45–3.35); p = 0.694		0.78 (0.26–2.30); p = 0.652		1.08 (0.40–2.93); p = 0.885	
Genetic model								
Dominant (CT+TT vs CC)	1.46 (0.80–2.67); p = 0.216		1.74 (0.98–3.08); p = 0.059		1.03 (0.56–1.89); p = 0.928		1.70 (0.97–2.98); p = 0.065	
Recessive (TT vs CT+CC)	1.99 (0.73–5.40); p = 0.172		0.93 (0.35–2.43); p = 0.877		0.75 (0.26–2.12); p = 0.583		0.81 (0.31–2.14); p = 0.677	

Supplemental Table S3. Meta-analysis results of association of *MTHFR* variant with LBW**.**

Variant/Analysis	Genetic model		Type of model	Heterogeneity		Statistical analysis				Publication bias; p_{Eggers}
				I^2 (%)	p_H	OR	95% CI	p_{crude}	p_{adj}	
MTHFR rs1801133T	Allele contrast	T vs. C	REM	79	0.001	1.10	0.80; 1.45	0.607	1.000	0.897
	Recessive	TT vs. TC+CC	REM	60	0.039	1.14	0.84; 1.95	0.245	1.000	0.719
	Dominant	TT+TC vs. CC	REM	70	0.010	0.97	0.70; 1.42	0.996	1.000	0.779
	Overdominant	TC vs. TT+CC	REM	68	0.013	0.83	0.59; 1.13	0.219	1.000	0.719
	Homozygotes 1	TT vs. CC	REM	59	0.046	1.18	0.77; 1.93	0.399	1.000	0.735
	Homozygotes 2	**TT vs. TC**	**FEM**	**3**	**0.395**	**1.41**	**1.08; 1.80**	**0.0097**	**0.068**	**0.949**
	Heterozygotes	TC vs. CC	REM	28	0.228	0.92	0.64; 1.30	0.856	1.000	0.152
MTHFR rs1801133T Developed countries	Allele contrast	T vs. C	FEM	0	0.617	0.85	0.71; 1.00	0.054	0.378	n/a
	Recessive	TT vs. TC+CC	FEM	0	0.886	0.88	0.61; 1.27	0.503	1.000	n/a
	Dominant	**TT+TC vs. CC**	**FEM**	**0**	**0.434**	**0.79**	**0.63; 0.99**	**0.038**	**0.267**	**n/a**
	Overdominant	TC vs. TT+CC	FEM	0	0.408	0.82	0.65; 1.03	0.086	0.599	n/a
	Homozygotes 1	TT vs. CC	FEM	0	0.885	0.79	0.54; 1.16	0.226	1.000	n/a
	Homozygotes 2	TT vs. TC	FEM	0	0.673	1.01	0.69; 1.50	0.947	0.068	n/a
	Heterozygotes	TC vs. CC	FEM	0	0.377	0.79	0.63; 0.99	0.049	0.346	n/a
MTHFR rs1801133T Developing countries and this study	Allele contrast	T vs. C	FEM	21	0.282	1.43	1.18; 1.73	<0.0001	0.002	0.027
	Recessive	**TT vs. TC+CC**	**FEM**	**0**	**0.558**	**1.85**	**1.34; 2.53**	**<0.0001**	**0.001**	**0.548**
	Dominant	TT+TC vs. CC	FEM	50	0.138	1.38	1.02; 1.85	0.034	0.237	0.041
	Overdominant	TC vs. TT+CC	REM	83	0.003	0.80	0.40; 1.62	0.535	1.000	0.876
	Homozygotes 1	TT vs. CC	FEM	0	0.763	1.87	1.24; 2.81	0.003	0.019	0.259
	Homozygotes 2	TT vs. TC	FEM	36	0.208	1.78	1.27; 2.49	0.001	0.005	0.885
	Heterozygotes	TC vs. CC	REM	67	0.049	1.05	0.58; 1.90	0.868	1.000	0.183

Abbreviations: FEM - the fixed effects model; REM - the random effects model.