

Supplementary Information

Placental expression of serotonin transporter (*SERT*) gene: associations with maternal overweight/obesity and neonatal anthropometry

Maja Perić, Marina Horvatiček, Maja Kesić, Maja Žutić, Lipa Čičin-Šain, Jasminka Štefulj

Table S1. Gene-specific primers used for amplification of cDNA fragments in RT-qPCR analyses.

Gene symbol	Gene name	Primer sequence (5' - 3')	Primer concentration (μM)	Amplicon (bp)	Sequence source
<i>TPH1</i>	<i>tryptophan hydroxylase 1</i>	f: TGCAAAGGAGAAGATGAGAGAATTTAC r: CTGTTATGCTCTTGGTGTCTTTC	0.50	114	[1]
<i>TPH2</i>	<i>tryptophan hydroxylase 2</i>	f: ACGGAGAGGGTTTTCCCTG r: GCCTTTGTCGTCATTTTGCCA	0.30	103	[2] ^c
<i>MAOA</i>	<i>monoamine oxidase A</i>	f: GAGCGGCTACATGGAAGGG r: TCACCTTCCCGAGACCATTTA	0.40	77	[3]
<i>MAOB</i>	<i>monoamine oxidase B</i>	f: CTTTTGGAGAGACATTTGCC r: TCACAAGTAGCCCCCTTTTGT	0.25	116	[2] ^c
<i>SERT</i> / <i>SLC6A4</i>	<i>serotonin transporter</i>	f: TGGTTCTATGGCATCACTCAGTTC r: GTTGTGGCGGGCTCATCAG	0.40	148	[1]
<i>OCT3</i> / <i>SLC22A3</i>	<i>organic cation transporter 3</i>	f: GAGGACCACAGTGGCTACAT r: ACAGACCTGAACAGAGCGAA	0.30	128	[4] ^a
<i>PMAT</i> / <i>SLC29A4</i>	<i>plasma membrane monoamine transporter</i>	f: CTGTCCTCCTGAACAACGTCC r: ACACGTCGCAGATGCTGATAA	0.50	111	[5] ^b
<i>HTR2A</i>	<i>5-hydroxytryptamine receptor 2A</i>	f: GCAAGATGCCAAGACAACAGATAA r: TCACACACAGCTCACCTTTTCAT	0.50	118	[6]
<i>YWHAZ</i>	<i>tyrosine 3-monooxygenase /tryptophan 5-monooxygenase activation protein zeta</i>	f: CCGTTACTTGGCTGAGGTTG r: AGTTAAGGGCCAGACCCAGT	0.30	143	[7]
<i>ACTB</i>	<i>actin beta</i>	f: TCCCTGGAGAAGAGCTACG r: GTAGTTTCGTGGATGCCACA	0.50	131	[8]
<i>UBC</i>	<i>ubiquitin C</i>	f: TCGCAGCCGGGATTTG r: GCATTGTCAAGTGACGATCACA	0.40	64	[7]

f – forward primer, r – reverse primer, bp – base pair

^a primer sequences were designed using GenScript software [4]

^b primer sequences were obtained from PrimerBank, ID: 100913033c2 [5]

^c primer sequences were obtained from RTPrimerDB, ID: 3165 [2]

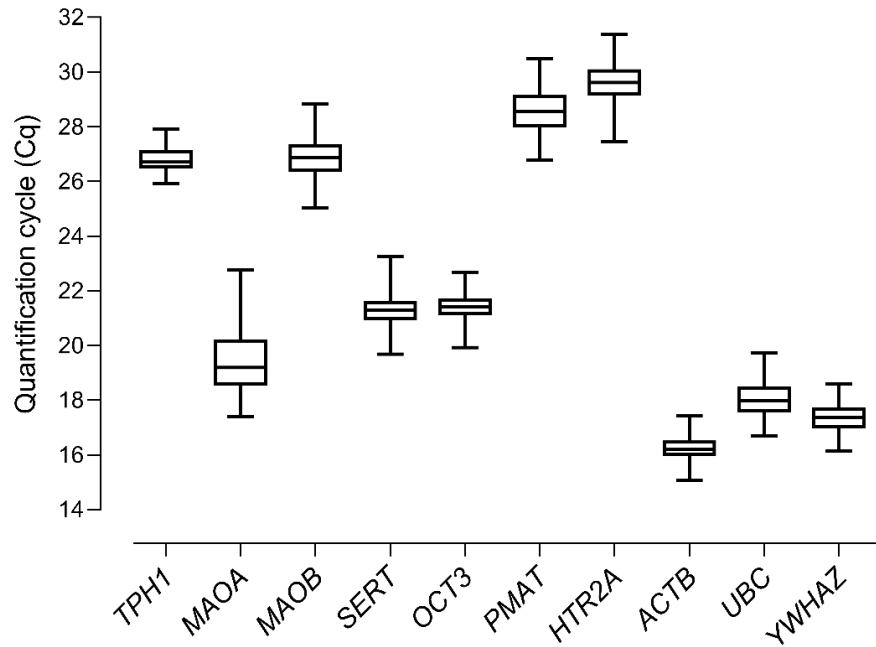


Figure S1. Expression levels of serotonin-regulating genes in human term placentas (N=90). Shown are quantification cycle (Cq) values for serotonin-regulating genes (*TPH1*, *MAOA*, *MAOB*, *SERT*, *OCT3*, *PMAT*, *HTR2A*) and reference genes (*ACTB*, *UBC*, *YWHAZ*), obtained by reverse transcription – quantitative real-time PCR (RT-qPCR) using 40 ng of cDNA per reaction. The boxes represent the interquartile range, with lines inside the boxes indicating the medians and whiskers extending from the minimum to the maximum values. No specific signal was detected for *TPH2*. *TPH1*, tryptophan hydroxylase 1; *MAOA*, monoamine oxidase A; *MAOB*, monoamine oxidase B; *SERT*, serotonin transporter; *OCT3*, organic cation transporter 3; *PMAT*, plasma membrane monoamine transporter; *HTR2A*, serotonin receptor type 2A; *ACTB*, actin beta; *UBC*, ubiquitin C; *YWHAZ*, tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein zeta.

Table S2. Spearman's correlation coefficients (95% confidence intervals) for associations between mRNA levels of serotonin-regulating genes in human term placentas (N=216).

	<i>TPH1</i>	<i>MAOA</i>	<i>SERT</i>	<i>OCT3</i>	<i>PMAT</i>
<i>MAOA</i>	-0.46 *** (-0.56, -0.34)				
<i>SERT</i>	0.05 (-0.09, 0.18)	-0.12 (-0.25, 0.02)			
<i>OCT3</i>	0.51 **** (0.40, 0.61)	-0.47 **** (-0.57, -0.36)	0.04 (-0.10, 0.18)		
<i>PMAT</i>	0.25 **** (-0.12, 0.38)	-0.34 **** (-0.45, -0.21)	-0.02 (-0.15, 0.12)	0.27 **** (0.14, 0.40)	
<i>HTR2A</i>	-0.02 (-0.16, 0.12)	0.12 (-0.02, 0.25)	0.07 (-0.06, 0.21)	0.01 (-0.13, 0.15)	0.12 (-0.02, 0.26)

Statistically significant results are shown in bold (**** $p < 0.001$).

Table S3. Correlation between maternal pre-pregnancy BMI and placental mRNA levels of serotonin-regulating genes in total sample (N=216) and in women with normal glucose tolerance (N=109) or gestational diabetes mellitus (N=107).

Gene	Total sample			Normal glucose tolerance			Gestational diabetes mellitus		
	r_s	95% CI	p -value	r_s	95% CI	p -value	r_s	95% CI	p -value
<i>TPH1</i>	0.05	-0.09, 0.18	0.481	0.10	-0.10, 0.29	0.303	-0.02	-0.22, 0.17	0.802
<i>MAOA</i>	-0.07	-0.20, 0.07	0.325	-0.15	-0.33, 0.05	0.120	0.05	-0.15, 0.24	0.614
<i>SERT</i>	-0.11	-0.24, 0.03	0.107	-0.20	-0.38, -0.01	0.035	-0.01	-0.21, 0.18	0.883
<i>OCT3</i>	0.07	-0.07, 0.21	0.301	0.15	-0.05, 0.33	0.130	-0.03	-0.22, 0.17	0.778
<i>PMAT</i>	0.23	0.09, 0.35	0.001	0.24	0.04, 0.42	0.014	0.21	0.01, 0.39	0.035
<i>HTR2A</i>	0.01	-0.17, 0.19	0.925	-0.08	-0.32, 0.167	0.510	0.09	-0.19, 0.35	0.524

Shown are Spearman correlation coefficients (r_s) with 95% confidence intervals (95% CI). Statistically significant results are indicated in bold.

Table S4. Association between maternal pre-pregnancy body weight status (pBWS; normal weight vs. overweight/obese) and mRNA levels of serotonin-regulating genes in term placentas, with glucose tolerance status (GTS; normal glucose tolerance vs. gestational diabetes mellitus) as a moderator (unadjusted analyses).

	B	95% CI	SE	t	p-value
<i>TPH1</i> mRNA					
pBWS	0.05	-0.01, 0.10	0.03	1.60	0.112
GTS	0.03	-0.02, 0.09	0.03	1.20	0.232
Interaction pBWS × GTS	-0.06	-0.14, 0.02	0.04	-1.55	0.123
$R^2=0.014$; $F(3, 212)=0.98$, $p=0.401$					
<i>MAOA</i> mRNA					
pBWS	-0.07	-0.21, 0.06	0.07	-1.07	0.288
GTS	-0.11	-0.24, 0.03	0.07	-1.56	0.121
Interaction pBWS × GTS	0.16	-0.03, 0.35	0.10	1.64	0.103
$R^2=0.014$; $F(3, 212)=1.01$, $p=0.389$					
<i>SERT</i> mRNA					
pBWS	-0.06	-0.10, -0.02	0.02	-3.20	0.002
GTS	-0.02	-0.06, 0.02	0.02	-0.83	0.406
Interaction pBWS × GTS	0.08	0.03, 0.14	0.03	2.87	0.005
$R^2=0.058$; $F(3, 212)=4.42$, $p=0.005$					
<i>OCT3</i> mRNA					
pBWS	0.07	-0.01, 0.16	0.04	1.69	0.092
GTS	0.05	-0.04, 0.13	0.04	1.10	0.273
Interaction pBWS × GTS	-0.12	-0.24, 0.00	0.06	-1.97	0.050
$R^2=0.020$; $F(3, 207)=1.40$, $p=0.245$					
<i>PMAT</i> mRNA					
pBWS	0.15	0.03, 0.27	0.06	2.49	0.014
GTS	0.05	-0.06, 0.17	0.06	0.91	0.363
Interaction pBWS × GTS	-0.07	-0.24, 0.09	0.09	-0.87	0.387
$R^2=0.038$; $F(3, 206)=2.73$, $p=0.045$					
<i>HTR2A</i> mRNA					
pBWS	-0.03	-0.11, 0.06	0.04	-0.65	0.516
GTS	-0.03	-0.11, 0.06	0.04	-0.62	0.533
Interaction pBWS × GTS	0.04	-0.08, 0.18	0.06	0.65	0.515
$R^2=0.003$; $F(3, 212)=0.19$, $p=0.904$					

Significant *p*-values are in bold. B, unstandardized beta coefficient; CI, confidence interval; SE, standard error of B.

Table S5. Association between maternal pre-pregnancy body weight status (pBWS; normal weight vs. overweight/obese) and mRNA levels of serotonin-regulating genes in term placentas, with glucose tolerance status (GTS; normal glucose tolerance vs. gestational diabetes mellitus) as a moderator (all analyses adjusted for parity, gestational age, and smoking status).

	B	95% CI	SE	t	p-value
<i>TPH1</i> mRNA					
pBWS	0.03	-0.02, 0.09	0.03	1.15	0.251
GTS	0.03	-0.03, 0.08	0.03	0.94	0.351
Interaction pBWS × GTS	-0.05	-0.13, 0.03	0.04	-1.13	0.262
Parity	0.06	0.01, 0.10	0.02	2.67	0.008
Gestational age	0.00	-0.03, 0.02	0.01	-0.17	0.863
Smoking status	-0.06	-0.10, -0.01	0.02	-2.44	0.016
$R^2=0.076$; $F(6, 201)=2.75$, $p=0.014$					
<i>MAOA</i> mRNA					
pBWS	-0.02	-0.15, 0.12	0.07	-0.23	0.820
GTS	-0.07	-0.21, 0.06	0.07	-1.06	0.290
Interaction pBWS × GTS	0.09	-0.10, 0.29	0.10	0.97	0.334
Parity	-0.17	-0.27, -0.07	0.05	-3.30	0.001
Gestational age	0.01	-0.05, 0.07	0.03	0.30	0.763
Smoking status	-0.01	-0.12, 0.10	0.06	-0.14	0.890
$R^2=0.061$; $F(6, 201)=2.16$, $p=0.048$					
<i>SERT</i> mRNA					
pBWS	-0.06	-0.10, -0.02	0.02	-3.22	0.002
GTS	-0.01	-0.05, 0.02	0.02	-0.76	0.449
Interaction pBWS × GTS	0.07	0.01, 0.12	0.03	2.42	0.017
Parity	0.01	-0.02, 0.04	0.01	0.87	0.385
Gestational age	-0.04	-0.05, -0.02	0.01	-4.66	<0.0001
Smoking status	0.02	-0.02, 0.05	0.02	1.01	0.315
$R^2=0.158$; $F(6, 201)=6.29$, $p<0.0001$					
<i>OCT3</i> mRNA					
pBWS	0.06	-0.03, 0.15	0.04	1.36	0.176
GTS	0.04	-0.05, 0.12	0.04	0.91	0.364
Interaction pBWS × GTS	-0.11	-0.23, 0.02	0.06	-1.72	0.087
Parity	0.07	0.01, 0.13	0.03	2.17	0.032
Gestational age	-0.01	-0.05, 0.02	0.02	-0.73	0.464
Smoking status	-0.06	-0.13, 0.01	0.04	-1.78	0.077
$R^2=0.061$; $F(6, 197)=2.13$, $p=0.051$					

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Table S5. (continued)

	B	95% CI	SE	t	<i>p</i>-value
<i>PMAT</i> mRNA					
pBWS	0.13	0.01, 0.25	0.06	2.10	0.037
GTS	0.05	-0.07, 0.17	0.06	0.77	0.444
Interaction pBWS × GTS	-0.05	-0.22, 0.12	0.09	-0.63	0.528
Parity	0.05	-0.04, 0.14	0.05	1.19	0.236
Gestational age	-0.02	-0.07, 0.03	0.03	-0.98	0.328
Smoking status	-0.08	-0.18, 0.02	0.05	-1.64	0.102
$R^2=0.062$; $F(6, 196)=2.17$, $p=0.048$					
<i>HTR2A</i> mRNA					
pBWS	-0.02	-0.11, 0.06	0.04	-0.53	0.597
GTS	-0.02	-0.11, 0.06	0.04	-0.53	0.594
Interaction pBWS × GTS	0.03	-0.09, 0.15	0.06	0.48	0.634
Parity	0.04	-0.03, 0.10	0.03	1.13	0.258
Gestational age	0.03	0.00, 0.07	0.02	1.77	0.079
Smoking status	0.00	-0.06, 0.07	0.03	0.11	0.910
$R^2=0.021$; $F(6, 201)=0.71$, $p=0.639$					

Significant *p*-values are in bold. B, unstandardized beta coefficient; CI, confidence interval; SE, standard error of B.

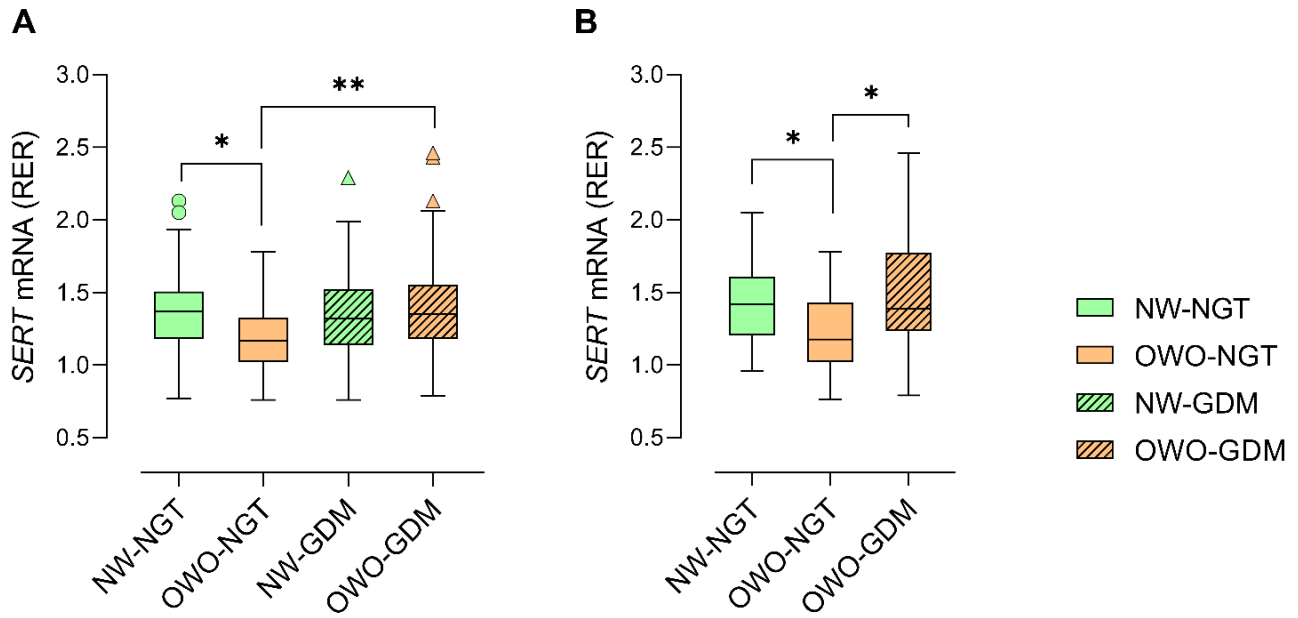


Figure S2. *SERT* mRNA levels in term placentas of women with normal weight and normal glucose tolerance (NW-NGT), overweight/obesity and gestational diabetes mellitus (OWO-NGT), normal weight and gestational diabetes mellitus (NW-GDM) and overweight/obesity and gestational diabetes mellitus (OWO-GDM). Shown are *SERT* mRNA levels for **(A)** entire cohort (NW-NGT, N=59; OWO-NGT, N=49; NW-GDM, N=50; OWO-GDM, N= 56) and **(B)** subset of participants included in the measurement of *SERT* protein levels (NW-NGT, N=21; OWO-NGT, N=22; OWO-GDM, N=22) (same data as in Figure 1A in the main text, shown here for ease of comparison). *SERT* mRNA levels were normalized to *YWHAZ* mRNA levels. Statistical analyses were performed using **(A)** Kruskal-Wallis test followed by Dunn's test or **(B)** one-way ANOVA followed by Fisher's LSD test. RER, relative expression ratio. * $p < 0.05$, ** $p < 0.01$.

Table S6. Spearman correlation coefficients (r_s) with 95% confidence intervals (95% CI) for associations of placental mRNA levels of serotonin-regulating genes and neonatal anthropometry.

Gene	Birth weight			Birth length			Ponderal index		
	r_s	95% CI	p -value	r_s	95% CI	p -value	r_s	95% CI	p -value
<i>TPH1</i>	0.03	-0.11, 0.17	0.634	0.13	-0.01, 0.26	0.062	-0.10	-0.23, 0.04	0.160
<i>MAOA</i>	0.05	-0.09, 0.18	0.499	0.04	-0.09, 0.18	0.519	0.04	-0.10, 0.17	0.591
<i>SERT</i>	-0.20	-0.33, -0.06	0.003	-0.16	-0.29, -0.06	0.019	-0.07	-0.21, 0.07	0.291
<i>OCT3</i>	0.12	-0.02, 0.25	0.094	0.07	-0.07, 0.21	0.295	0.07	-0.07, 0.21	0.308
<i>PMAT</i>	0.05	-0.092, 0.19	0.461	0.03	-0.113, 0.17	0.642	0.05	-0.09, 0.19	0.432
<i>HTR2A</i>	0.02	-0.11, 0.16	0.716	-0.07	-0.21, 0.07	0.308	0.11	-0.03, 0.25	0.100

Statistically significant results are shown in bold.

Table S7. Moderated mediation analysis examining placental *SERT* mRNA levels as a mediator between maternal pre-pregnancy body weight status (pBWS; normal weight vs. overweight/obesity) and neonatal birth weight, with glucose tolerance status (GTS; normal glucose tolerance vs. gestational diabetes mellitus) as a moderator (Hayes PROCESS Model 8).

	B	95% CI	SE (B)	t	p-value
a-path					
pBWS	-0.06	-0.10, -0.02	0.02	-3.15	0.002
GTS	-0.01	-0.05, 0.02	0.02	-0.75	0.452
Interaction pBWS × GTS	0.07	0.01, 0.12	0.03	2.40	0.017
$R^2=0.156$; $F(6, 201)=6.17$, $p<0.0001$					
b/c'-path					
pBWS (c'-path)	181	31, 331	76	2	0.018
<i>SERT</i> mRNA (b-path)	-574	-1099, -49	266	-2	0.032
GTS	147	3, 291	73	2	0.046
Interaction pBWS × GTS	-112	-322, 99	107	-1	0.296
$R^2=0.207$; $F(7, 200)=7.45$, $p<0.0001$					
Conditional indirect effect					
NGT	36	3, 79	19		^a
GDM	-3	-30, 21	12		^a
Conditional direct effect					
NGT	181	31, 331	76	2	0.018
GDM	69	-78, 216	74	1	0.353
Index of moderated mediation: -38 , 95% CI ^a : -94, -1					

The model was adjusted for maternal smoking, gestational age, and newborn sex. a-path represents the effect of predictor (pBWS) on mediator (*SERT* mRNA). c'-path represents the direct effect of predictor (pBWS) on outcome (birth weight). Statistically significant results are shown in bold. B, unstandardized beta coefficient; CI, confidence interval; SE, standard error ^a bootstrapped.

Supplementary references

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