

“Long non-coding RNAs in hypoxia and oxidative stress - novel insights investigating a piglet model of perinatal asphyxia published” in RNA biology by Benedicte Grebstad Tune, Maria Melheim, Monica Atneosen-Åsegg, Baukje Dotinga, Ola Didrik Saugstad, Rønnaug Solberg, and Lars Oliver Baumbusch at the Department of Pediatric Research, Division of Paediatric and Adolescent Medicine, Oslo University Hospital Rikshospitalet, Oslo, Norway.
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Supplementary Table S1. Piglet sample overview with clinical parameters.

Study arm	Piglet	Excluded	Sex (M/F)	Weight (g)	Hypoxia (min)	Autopsy after end hypoxia (min)	Hemoglobin (g/ml)		Base excess (mM)	Mean arterial blood pressure (mmHg)	
							Start study	End study	End hypoxia	End hypoxia	End study
21% hypoxia	01		F	2025	46	540	7.9	7.4	-12.4	19.7	43.0
Control	02		M	2190	35	540	6.9	7.8	-2.2	58.0	57.0
30 min 100%	03		M	2230	72	540	8.4	7.6	-21.6	21.0	37.0
21% hypoxia	04	**	M	2010	65	540	8.1	8.2	-22.2	22.2	28.0
Control	05		F	1920	45	570	8.1	7.5	6.7	46.0	59.0
30 min 100%	06		M	1990	45	540	9.2	8.0	-6.4	-22.0	44.0
Control	07		F	1965	45	570	8.7	8.2	5.3	56.0	48.0
21% hypoxia	08		F	1980	50	570	6.5	5.0	-11.7	32.0	32.0
30 min 100%	09		F	2120	32	570	7.8	8.1	0.3	44.4	61.0
21% hypoxia	10		M	2165	35	570	7.5	6.6	-20.3	31.4	81.0
30 min 100%	11		M	1900	20	570	6.0	4.3	-17.0	19.9	61.0
Control	12		M	1980	45	570	8.3	8.1	0.5	53.0	70.0
21% hypoxia	13		F	1900	41	570	9.1	4.8	-20.7	31.7	29.0
Control	14	**	M	2090	45	570	9.5	8.4	4.4	56.0	21.0
21% hypoxia	15	*	F	2080	170	156	7.5	8.6	-21.5	25.0	20.0
30 min 100%	16		M	2020	35	570	7.6	7.0	-21.2	22.3	41.0
30 min 100%	17		M	2020	40	570	9.0	8.4	-21.1	29.2	42.0
Control	18	**	F	2085	45	570	9.1	7.5	-1.6	59.0	67.0
30 min 100%	19		F	2065	20	570	8.9	8.1	-15.5	19.5	45.0
21% hypoxia	20		F	2050	15	570	8.8	8.1	-9.2	19.9	67.0
Control	21		F	2020	45	570	7.7	7.7	-3.3	59.0	47.0
3 min 100%	22	*	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
3 min 100%	23		F	1960	34	570	7.0	5.7	-22.3	31.5	46.9

Control	24		M	1805	45	570	6.9	6.2	0.7	54.0	54.0
Control	25		F	1870	45	570	5.3	5.5	5.9	50.0	41.0
3 min 100%	26		F	1910	20	570	6.1	4.8	-13.7	18.8	55.0
3 min 100%	27		M	2050	24	570	5.7	5.5	-14.1	24.9	24.9
3 min 100%	28		M	2050	28	570	6.4	6.6	-17.6	19.4	25.6
3 min 100%	29		F	1930	53	570	8.4	7.3	-18.6	20.3	42.6
3 min 100%	30		M	1940	50	570	6.6	6.7	-19.7	19.9	47.5
21% hypoxia	31		F	1890	28	570	6.8	6.1	-23.8	19.1	28.3
30 min 100%	32		F	1995	21	570	7.4	6.8	-10.7	19.8	50.6
3 min 100%	33		F	1910	28	570	6.5	6.4	-19.2	32.0	57.0
21% hypoxia	34	*	M	2190	17	210	6.3	5.8	-14.0	17.9	24.7
21% hypoxia	35		M	1830	44	570	7.5	5.7	-20.0	19.6	57.0
3 min 100%	36		M	1950	38	570	7.2	5.8	-25.2	27.2	57.0
Control	37	*	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
3 min 100%	38		M	2085	21	570	6.3	5.2	-10.7	19.5	49.7
21% hypoxia	39		M	1950	26	570	9.2	6.2	-17.6	19.7	60.1
30 min 100%	40		M	1975	21	570	5.2	5.0	-15.8	19.1	57.8
21% hypoxia	41	*	M	1870	50	360	8.2	9.0	-22.1	32.0	13.0
30 min 100%	42		F	1870	42	570	8.0	8.2	-21.7	22.3	28.5
Mean values											
Control				1992	44	567	7.8	7.4	1.8	50.1	51.6
21% hypoxia				1995	49	483	7.8	6.8	-18.0	24.2	40.3
3 min 100%				1976	33	570	6.7	6.0	-17.9	23.7	45.1
30 min 100%				2019	35	564	7.9	7.2	-15.1	19.6	46.8
Median values											
Control				1980	45	570	8.1	7.7	0.7	56.0	54.0
21% hypoxia				1995	43	570	7.7	6.4	-20.2	21.1	30.5
3 min 100%				1950	28	570	6.5	5.8	-18.6	20.3	47.5
30 min 100%				2008	34	570	7.9	7.8	-16.4	20.5	44.5

Supplementary Table S1. Thermocycler program for EvaGreen Supermix prior to ddPCR.

Cycling Step	Temp (°C)	Time	Ramp rate	# of cycles
Enzyme activation	95	5 min	50%	1
Denaturation	95	30 sec		40
Annealing/Extension	60	1 min		
Signal Stabilization	4	5 min		1
	90	5 min		
Hold	4	∞		

Supplementary Table S2. Mean Ct-values from qPCR of protein coding genes (mRNA) and long non-coding RNA (lncRNA). and simple overview of the analysis used for each brain region. Genes are either only analyzed on qPCR or analyzed on both qPCR and ddPCR (^a).

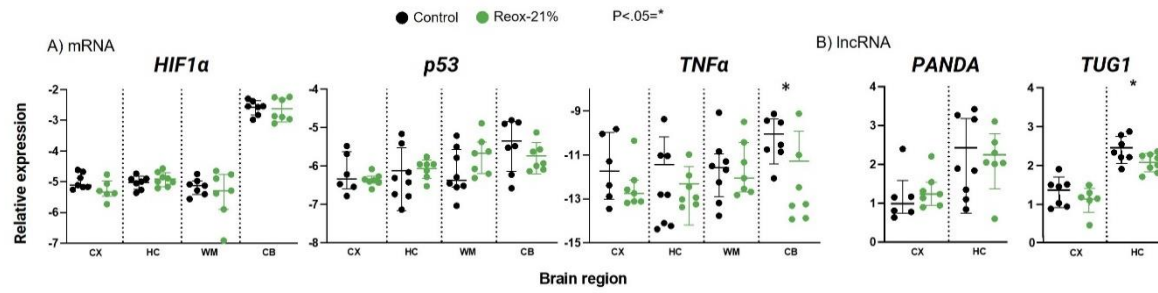
Brain region	Cortex				Hippocampus		White matter		Cerebellum	
Study arm	1	2	3	4	1	2	1	2	1	2
mRNA										
HIF1a	5.07	5.48	N/A	N/A	5.01	4.99	5.19	5.53	2.60	2.68
VEGFA	5.28	4.27	N/A	N/A	5.21	4.22	4.73	4.26	4.93	3.82
BDNF	11.12	9.72	N/A	N/A	7.89	7.60	11.68	11.85	8.22	8.14
p53	6.56	6.37	N/A	N/A	6.27	6.28	5.91	6.24	5.75	5.48
TNFA	12.47	12.07	N/A	N/A	12.62	12.59	11.63	11.83	12.23	10.55
LncRNA										
BDNF-AS	6.72 ^a	4.74 ^a	4.50 ^a	4.52 ^a	3.85	3.43	6.03	5.70	2.83	2.41
H19	6.24 ^a	4.11 ^a	4.85 ^a	4.30 ^a	5.63	4.80	5.19	3.83	3.74	3.86
MALAT1	-10.30 ^a	-10.89 ^a	-10.26 ^a	-10.10 ^a	-10.39	-10.69	-11.11	-11.41	-10.41	-10.69
ANRIL	4.16 ^a	3.44 ^a	3.99 ^a	4.10 ^a	2.32	2.54	2.36	1.65	3.99	3.54
PANDA	-1.26	-1.13	N/A	N/A	-2.18	-2.05	N/A	N/A	N/A	N/A
TUG1	-1.25	-0.91	N/A	N/A	-2.32	-2.02	N/A	N/A	N/A	N/A

Abbreviation: ^a, analyzed by both qPCR and ddPCR; N/A; not analyzed; 1, sham-operated control group; 2, Reox-21%; 3, Reox-100%(3'); 4, Reox-100%(30').

Supplementary Table S3. Mean values from ddPCR analysis of endogenous control (TBP) and selected lncRNAs associated with hypoxia and oxidative stress.

Brain region		Cortex			
Study arm		1	2	3	4
lncRNA					
TBP	Concentration (copies/ μ L)	272.74	84.56	92.14	120.49
	Relative quantity (RQ)	1.00	0.31	0.34	0.44
BDNF-AS	Concentration (copies/ μ L)	0.83	1.76	1.61	2.12
	Relative quantity (RQ)	1.00	2.12	1.94	2.56
	Normalized relative expression	1.00	6.85	5.75	5.79
H19	Concentration (copies/ μ L)	3.44	4.51	3.55	5.07
	Relative quantity (RQ)	1.00	1.31	1.03	1.47
	Normalized relative expression	1.00	4.22	3.05	3.33

Abbreviation: 1, sham-operated control group; 2, Reox-21%; 3, Reox-100%(3'); 4, Reox-100%(30').



Supplementary Figure S1. Differential expression of hypoxia- and oxidative stress-regulated mRNAs (A) and lncRNAs (B) in cortex (CX), hippocampus (HC), white matter (WM), cerebellum (CB) of the brain in a perinatal asphyxia piglet model, analyzed using qPCR. The control group was exposed to 21% O₂ during the entire procedure, Reox-21% group was exposed to hypoxia (8% O₂) followed by normoxic reoxygenation (21% O₂). Relative gene expressions ($2^{-\Delta C_t}$) are shown on a log scale and expressed as either mean \pm SD (p53, TNF α , TUG1, PANDA: HC, WM, CB; HIF1 α : CX, HC, WM) or median \pm Interquartile range (PANDA, p53, TNF α : CX; HIF1 α : CB). $P < .05 = *$. HIF1 α , Hypoxia-inducible factor α ; p53, Tumor suppressor gene p53; TNF α , Tumor necrosis factor α ; PANDA, P21-associated ncRNA DNA damage-activated; TUG1, Taurine Upregulated gene 1.