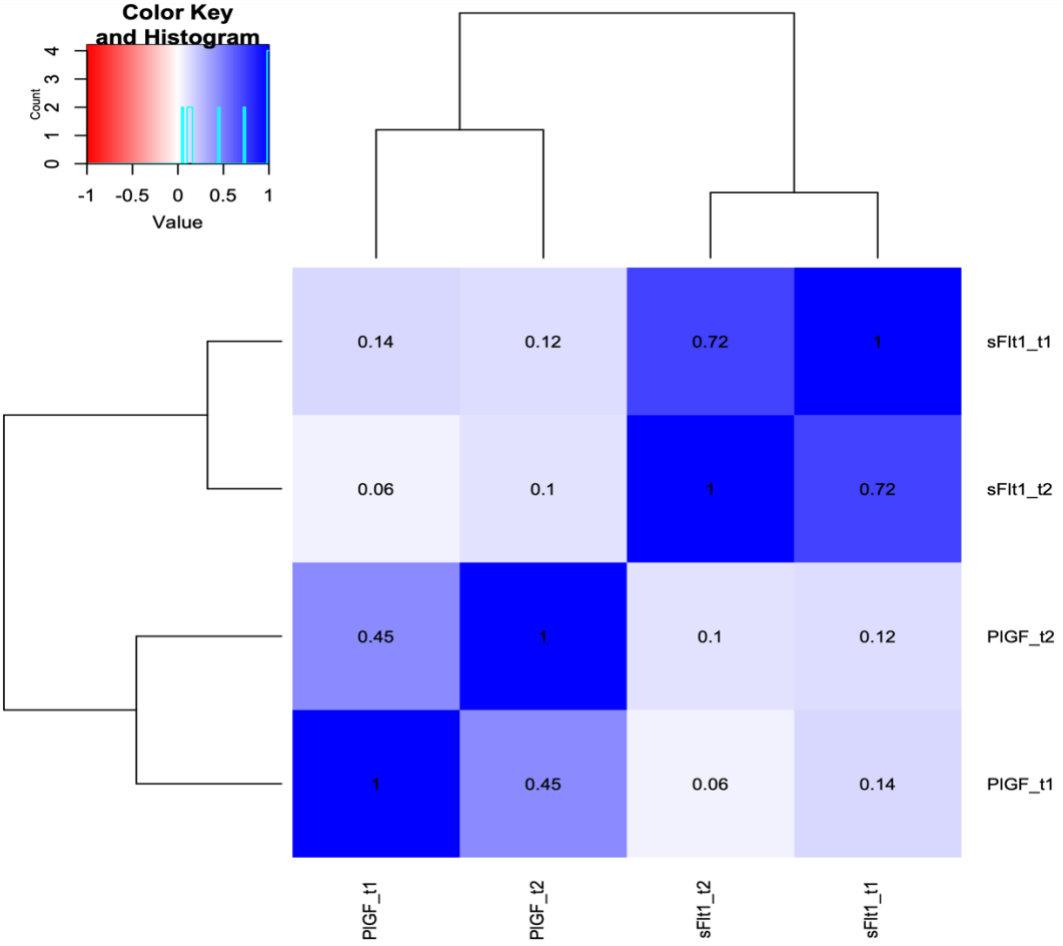
Supplementary Material

Supplementary Figure

Supplementary Figure 1: Heatmap of the pairwise correlations of all assayed placental markers in maternal plasma. All values are Pearson's correlation coefficients, significant at p<0.001. t1: 1st trimester measurement / t2: 2nd trimester measurement



Supplementary Material: Tables

***Supplementary Table 1**: Maternal characteristics in the entire Generation R cohort, their differences according to fetal sex (p-value for Mann Whitney U-test) and their association to z-scores of SRS Scores (values for univariate linear regression models). Further details in previous publications. *BMI at the start of pregnancy.*

	Age of child (months)	*Maternal age (years)*	*Maternal BMI**	*Maternal Education*	*Birth Weight (grams)*
Cohort	mean=74.02 SD=5.8	mean=31.36 SD=4.69	mean=24.89 SD=4.56	1: Primary or less, n=151 2: Secondary, n=1845 3: Higher, n=3063	mean=3409.5 SD=563.7
Males	mean=74.05 SD=5.87	mean= 31.41 SD=4.72	mean=24.80 SD=4.49	1: n=81 2: n=912 3: n=1547	mean= 3470.4 SD=580.14
Females	mean=73.98 SD=5.71	mean= 31.31 SD=4.66	mean=24.97 SD=4.63	1: n=78 2: n=929 3: n=1512	mean= 3347 SD= 539.3
p for sex	0.651	0.420	0.123	0.534	**<0.0001**
SRS Scores					
β to SRS	**0.10**	**-0.127**	**0.087**	**-0.248**	**-0.058**
p-value	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**

***Supplementary Table 2:** Maternal characteristics in the entire Generation R cohort, and their correlation to placental marker concentrations in the 1st and 2nd trimester (via Pearson's) . *BMI at the start of pregnancy.*

	*Gestational age**	*Maternal age*	*Maternal BMI**	*Placental Weight*	*Birth Weight*
1st trimester					
PlGF					
β	**0.713**	0.019	0.007	**0.059**	0.008
p-value	**<0.0001**	0.277	0.60	**0.0001**	0.644
Sflt-1					
β	**0.071**	-0.027	**-0.142**	**0.130**	**0.084**
p-value	**<0.0001**	0.123	**<0.0001**	**<0.0001**	**<0.0001**
2nd trimester					
PlGF					
β	**0.315**	-0.01	**-0.119**	**0.072**	**0.047**
p-value	**<0.0001**	0.541	**<0.0001**	**<0.0001**	**0.003**
Sflt-1					
β	**0.024**	**-0.084**	**-0.154**	**0.068**	0.018
p-value	**0.044**	**<0.0001**	**<0.0001**	**<0.0001**	0.264
Longitudinal					
PlGF - change					
β	NA	-0.008	**-0.10**	**0.047**	-0.004
p-value		0.645	**<0.0001**	**0.004**	0.835
sFlt-1 - change					
β	NA	**-0.080**	**-0.058**	**-0.064**	**-0.082**
p-value		**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**

***Supplementary Table 3**: Full linear Regression models for sex differences of placental marker concentrations, controlling for placental weight and gestational age. Marker levels have been log-transformed as the dependent variable.*

1st trimester	*N*	*Sex*	*Placental Weight*	*Gestational Age*	*MR Model*
PlGF	5963	β=-0.0461	β=0.0002	β=0.2389	Adj.R^2= 0.5174
		p=0.0011	p < 0.0001	p < 0.0001	p= <0.0001
sFlt-1	5951	β=-0.0859	β=0.0005	β=0.0227	Adj.R^2=0.0289
		p<0.0001	p<0.0001	p<0.0001	p<0.0001
2nd trimester					
PlGF	7294	β=0.055	β=0.0002	β=0.1408	Adj.R^2=0.098
		p=0.0001	p<0.0001	p<0.0001	p<0.0001
sFlt-1	7292	β=-0.0776	β=0.0003	β=0.0084	Adj.R^2=0.0081
		p<0.0001	p<0.0001	p=0.256	p<0.0001
Longitudinal					
PlGF - change	5250	β=1.6421	β=0.0058	N/A	Adj.R^2=0.0036
		p=0.0072	p=0.0051		p=0.0004
sFlt-1 - change	5236	β=0.0043	β=-0.00018	N/A	Ad.R2=0.0036
		p=0.743	p=<0.0001		p=0.0004

***Supplementary Table 4**: Sensitivity Analyses for PlGF at the 2nd trimester, in association with autistic traits (SRS z-scores). Model 1 covariates: age of child at SRS measurement. Model 2 covariates: age of child at SRS measurement, maternal age, maternal BMI in the beginning of the pregnancy, maternal ethnicity, birth weight adjusted for gestational age and maternal education level. Model 3 covariates as in Model 2 and in addition: placental weight at birth, and total birth weight-adjusted for gestational age at birth.*

**European ethnicities include the following categories: Dutch, American-western, Asian-western, Turkish, European, Oceanian.*

***Complications excluded include: Pregnancy-induced Hypertension, Preeclampsia, Born small for gestational age and Spontaneous Preterm Birth.*

PlGF - 2nd trimester to SRS	*FEMALES*			*MALES*			*BOTH*		
	N	β (SE)	p	N	β	p	N	β	p
European ethnicities									
Model 1	1224	**0.279 (0.13)**	**0.045**	1317	0.137 (0.17)	0.429	2541	**0.212** (0.11)	**0.050**
Model 2		**0.270 (0.14)**	**0.049**		0.085 (0.17)	0.619		0.187 (0.10)	0.086
Model 3		**0.367 (0.16)**	**0.025**		0.095 (0.21)	0.652		0.237 (0.13)	0.073
No autism									
Model 1	1657	**0.403 (0.12)**	**0.0008**	1760	**0.403 (0.13)**	**0.003**	3417	**0.206 (0.02)**	**<0.0001**
Model 2		0.221 (0.12)	0.061		0.184 (0.13)	0.157		**0.204 (0.08)**	**0.020**
Model 3		**0.292 (0.14)**	**0.037**		0.178 (0.16)	0.266		**0.227 (0.11)**	**0.032**
No complications									
Model 1	1233	**0.528 (0.14)**	**0.0001**	1179	**0.379** (0.18)	**0.0006**	2412	**0.451 (0.11)**	**<0.0001**
Model 2		**0.360 (0.14)**	**0.0089**		0.151 (0.18)	0.412		**0.249 (0.12)**	**0.031**
Model 3		**0.555 (0.17)**	**0.0015**		-0.049 (0.24)	0.838		0.262 (0.15)	0.076