



***GSTM1* genotypes modify the effect of maternal smoking during pregnancy on cognitive functioning in children**

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Background

- **Maternal smoking during pregnancy is associated with cognitive deficits in children**
- **Mechanisms and meaning remain unclear**
- **Socioeconomic and parental factors are proposed as an explanatory.**
- ***GSTM1* and *GSTT1* are two major enzymes involved in detoxification of tobacco smoke components**

Objective

To study the influence of *GSTM1* and *GSTT1* polymorphisms on the cognitive functioning effects induced by active maternal smoking during pregnancy

Methods

- **Participants, study design and setting.** Children (n=384) from a prospective population-based birth cohort set up in the island of Menorca (Spain) between 1997 and 1999
- **Main outcome of interest.** Cognitive function evaluation at age 4 years was assessed by McCarthy Scales of Children's Abilities (MCSA): 18 items derived from six scales:
 - general cognitive
 - verbal
 - perceptual-performance
 - quantitative
 - memory
 - motor

Exposure assessment: Paternal smoking habits

Interviewer-administered questionnaires during the third trimester of pregnancy and then yearly up to age 4 years of children

- Maternal smoking during pregnancy
 - 1) non-smokers
 - 2) smokers: at least 1 cig./day irrespective of whether they smoked in post-natal period
 - 3) Dose-response effects: number of cig./day

- Postnatal maternal smoking
 - non-smokers in any of the post-natal surveys
 - smokers: 1 cig./day at least once in a post-natal survey, but not during pregnancy

- Fathers
 - non-smokers in any survey
 - smokers: fathers reporting at least 1 cig./day in any of the surveys

- **Genotyping methods**

Genotyping for null alleles from *GSTM1* and *GSTT1*

- **Statistical analysis**

Multivariate linear regression models were used to examine the association between active maternal smoking during pregnancy and MCSA outcomes by *GSTM1* and *GSTT1* genotypes

Results

Characteristics of children according to *GSTM1* and *GSTT1* genotypes

	<i>GSTM1</i> genotype			<i>GSTT1</i> genotype		
	Present (n=162)	Null (n=222)	p-value*	Present (n=311)	Null (n=73)	p-value*
Parental smoking habits, n (%)						
Maternal smoking during pregnancy						
Yes	35 (21.6)	48 (21.6)	0.997	69 (22.2)	14 (19.2)	0.574
Maternal smoking only post-natal						
Yes	61 (37.6)	88 (39.6)	0.693	123 (39.5)	26 (35.6)	0.535
Paternal smoking, n (%)						
Yes	116 (72.5)	148 (67.6)	0.303	212 (69.1)	52 (72.2)	0.599
McCarthy areas, mean (sd)						
General cognitive	106.3 (20.9)	106.2 (19.3)	0.956 [†]	106.1 (19.8)	106.9 (21.0)	0.744 [†]
Perceptual-performance	40.9 (7.9)	39.9 (7.6)	0.190 [†]	40.3 (7.6)	40.5 (8.2)	0.851 [†]
Memory	23.2 (8.0)	23.1 (7.1)	0.891 [†]	23.1 (7.3)	23.3 (8.2)	0.833 [†]
Quantitative	17.9 (4.7)	17.9 (4.6)	0.949 [†]	17.9 (4.6)	17.8 (4.8)	0.855 [†]
Verbal	47.3 (12.6)	48.3 (10.8)	0.406 [†]	47.7 (11.5)	48.6 (12.0)	0.586 [†]
Motor	35.2 (6.9)	33.9 (6.7)	0.088 [†]	34.4 (6.7)	34.8 (7.2)	0.602 [†]
Maternal social class, n (%)						
	(n=159)	(n=216)		(n=303)	(n=72)	
Manual	100 (62.9)	143 (66.2)	0.507	195 (64.4)	48 (66.7)	0.712
Maternal education level, n (%)						
	(n=155)	(n=213)		(n=299)	(n=69)	
Less than secondary	87 (56.1)	125 (58.7)	0.624	176 (58.9)	36 (52.2)	0.311

*Otherwise indicate p value derived from χ^2 test; [†]p value derived from Student's *t* test.

Associations between the **general cognitive** outcome at 4 years and maternal smoking during pregnancy by *GSTM1* and *GSTT1* genotypes

	Adjusted ^a			Adjusted ^b		
	β	(95% CI)	p*	β	(95% CI)	p*
Smoking during pregnancy (yes)						
<i>GSTM1</i>						
Present	1.14	(-5.99, 8.28)	0.032	3.70	(-5.92, 13.34)	0.045
Null	-5.54	(-10.67, -0.40)		-2.67	(-9.19, 3.84)	
<i>GSTT1</i>						
Present	-1.43	(-5.70, 2.84)	0.797	0.27	(-5.37, 5.92)	0.773
Null	-6.40	(-17.99, 5.19)		-0.85	(-18.27, 16.56)	
Smoking as continuous (cig./day)						
<i>GSTM1</i>						
Present	-0.22	(-1.22, 0.78)	0.236	-0.19	(-1.37, 0.98)	0.374
Null	-0.64	(-1.26, -0.01)		-0.22	(-0.98, 0.55)	
<i>GSTT1</i>						
Present	-0.32	(-0.85, 0.21)	0.882	-0.13	(-0.78, 0.53)	0.909
Null	-1.54	(-3.42, 0.34)		-1.14	(-3.74, 1.45)	

For all comparisons the reference category is no smoking.

*p value for interaction.

^aAdjusted for sex, maternal education, mother's social class, mother's parity, child's birth weight and height, school season at testing, evaluator (psychologist), child's breastfeeding duration, maternal alcohol consumption during pregnancy and p,p' -DDT cord serum levels.

^bFurther adjusted for postnatal maternal smoking and father smoking.

Adjusted* associations between active maternal smoking during pregnancy and sub-areas of the global cognitive outcome at 4 years by *GSTM1* genotypes

	Maternal smoking during pregnancy (reporting, yes)				p [†]
	<i>GSTM1</i> Present		<i>GSTM1</i> Null		
	β	(95% CI)	β	(95% CI)	
Sub-areas of the global cognitive					
Perceptual-performance	1.42	(-5.56, 8.41)	-4.37	(-9.48, 0.74)	0.037
Memory	3.71	(-3.52, 10.93)	-2.91	(-8.43, 2.59)	0.050
Quantitative	-0.27	(-2.29, 1.75)	-4.77	(-11.43, 1.89)	0.027
Verbal	1.50	(-6.17, 9.18)	-4.36	(-9.58, 0.86)	0.126
Motor	3.92	(-3.01, 10.86)	-2.84	(-8.01, 2.33)	0.033
Executive function	0.68	(-6.79, 8.16)	-6.26	(-11.43, -1.09)	0.030
Working memory	-0.18	(-1.63, 1.27)	-1.65	(-4.19, 0.89)	0.061

For all comparisons the reference category is no smoking.

†p value for interaction.

*Adjusted for sex, maternal education, mother's social class, mother's parity, child's birth weight and height, school season at testing, evaluator (psychologist), child's breastfeeding duration, maternal alcohol consumption during pregnancy and *p,p'*-DDT cord serum levels.

Limitations

- Confusion by other genetic variants in linkage disequilibrium with the variant under study, or by population stratification
- Role of maternal genotypes in modifying the adverse effects of tobacco smoke?
- Maternal-fetal gene interaction?
- Examination of other genetic polymorphisms?

Strengths

- High participation rate, 80%
- Selection bias is likely to be minimal (included vs. non-included)
- A prospective population-based birth cohort
- Validated the MCSA new scale (executive function)
- Genetic variants assessed were independent of cognitive outcomes and smoking habits, thus confounding epidemiological associations are unlikely

Conclusions

- **Adverse effect of active maternal smoking on cognitive functioning was restricted to children having *GSTM1* null allele**
- **Results support the occurrence of neurotoxic effects of maternal smoking during pregnancy, and reduce the possibility that those effects were due to confounding for parental characteristics**
- **The interaction between active maternal smoking and *GSTM1* null allele adds to the plausibility of a biological interaction between GST enzymes and components of tobacco smoke in the detoxification process during fetal neurodevelopment**

THANK YOU FOR YOUR ATTENTION

