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Maternal prenatal smoking and offspring emotional problems: No moderating effect of maternal or child 5-HTTLPR genotype

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In a recent paper, Cents and others (2012) demonstrated a novel interaction effect of a polymorphism in the serotonin transporter gene (5-HTTLPR) and maternal prenatal smoking on offspring emotional (internalizing) problems at age 3 in a sample of 1,529 Dutch mother-child dyads. The 5-HTTLPR polymorphism has previously been shown to moderate effects of stressful life-events and childhood maltreatment on depression (Karg and others, 2011). Cents and others (2012) extended those findings to the period of fetal life using maternal prenatal smoking as the environmental risk factor, and offspring emotional problems at age 3 as the outcome measure. Cents and others (2012) did not find a significant main effect of the 5-HTTLPR genotype or maternal prenatal smoking on offspring emotional problems, but detected an interaction effect. Having the short allele of the 5-HTTLPR polymorphism in combination with maternal smoking during pregnancy was associated with increased emotional problems at age 3 as rated by the mother. A similar interaction was observed for maternal 5-HTTLPR genotype and this effect was independent of the child's genotype, suggesting that maternal serotonin levels influence fetal development. The interactions remained significant after correction for maternal educational level, maternal psychopathology, and age and sex of the child. Maternal rater bias was controlled for by alternatively using paternal ratings of offspring emotional problems which showed comparable results. Moreover, when Cents and others (2012) repeated the analyses using paternal prenatal smoking as a predictor instead of maternal prenatal smoking, no interaction with 5-HTTLPR was observed, providing additional support for a direct effect of prenatal tobacco exposure as opposed to confounding effects.

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Replication of this novel genotype x environment interaction effect is important, since the 5-HTTLPR x stress interaction effect on depression has been observed in some, but not all studies (Karg and others 2011; Risch and others 2009). Therefore, we repeat the analyses of Cents and others (2012) in an effort to replicate the interaction of 5-HTTLPR genotype and maternal prenatal smoking on emotional problems in another population-based sample of Dutch children (n=1,865).

The Netherlands Twin Register (NTR) was established in 1987 at the VU University in Amsterdam (Boomsma and others 2006). Data on young twins are collected by longitudinal surveys, starting with maternal reports on the pregnancy shortly after the twins are born. Parental reports on emotional problems are collected starting at age 3 years. Data collection and participation rates have been described in detail by Bartels and others (2007). Child 5-HTTLPR genotype and maternal reports on prenatal smoking and emotional problems at age 3 were available for 1,988 children born between 1986-2004. Ethnic outliers were identified based on genome-wide genotype data and excluded from the analyses (n=72). From the 17.1% of the children without genome-wide genotype data, 11 children were additionally excluded since one or both of their parents were born in a non-western country. This resulted in a sample of 1,905 children (53.0% girls). Data on prenatal maternal smoking were available for 1,865 of these children and maternal 5-HTTLPR genotype data for 612 children.

Maternal and child DNA were derived from blood and buccal samples. In line with Cents and others (2012), maternal/child 5-HTTLPR was included in the analysis as an additive effect (ll=0, ls=1, ss=2). Hardy-Weinberg equilibrium (HWE) was tested using Sib-pair, as this program allows for a test of HWE in related individuals (Duffy 2012).

Maternal reports on prenatal smoking were obtained on average 8.4 months after the children were born. Maternal prenatal smoking was coded as 'non-smoking' and 'smoking during pregnancy'. Following Cents and others (2012), mothers who reported having only smoked in the first trimester were excluded (n=33). Offspring emotional problems at age 3 were assessed with maternal reports on the internalizing subscale of the Child Behavior Checklist (CBCL/2-3; Achenbach and Rescorla 2000), which were corrected for positive skewness with a square root transformation, in line with the original study. Cents and others (2012) included maternal educational attainment, maternal psychopathology, child age and child sex as covariates. In the NTR sample, maternal educational attainment was assessed simultaneously with offspring emotional problems, in 5 categories (ranging from 'primary school' to 'university'), which were dichotomized into the same categories as Cents and others (2012) used ('primary/secondary school' and 'higher education'). No data on maternal prenatal psychopathology were available. Child age was reported when emotional problems were measured and child sex was assessed at enrollment in the NTR.

Effects of prenatal maternal smoking and 5-HTTLPR genotype on offspring emotional problems were examined with linear mixed models in SPSS 20 (IBM 2011). To account for familial clustering in the data, a random intercept was modeled over families, which was estimated separately for monozygotic and dizygotic twins. Main and interaction effects of prenatal maternal smoking and 5-HTTLPR genotype were tested without covariates first. Next, the covariates included by Cents and others (2012) were added (maternal educational attainment, child sex, child age). All tests were evaluated at $\alpha=.05$.

The distribution of 5-HTTLPR genotype was in Hardy-Weinberg equilibrium (empirical p-value .32). The prevalence of prenatal maternal smoking was 18.9%. Mothers were on average 30.7 years old when they gave birth to the twins (sd 3.7) and the majority had completed higher education (68.9%). Children were on average 39.6 months old when

emotional problems were assessed (sd 3.1). The average internalizing score was 1.9 (sd 1.0) and 8.6% of the children scored in the borderline/clinical range (defined as t-score ≥ 65).

The effects of prenatal maternal smoking and maternal/child 5-HTTLPR genotype are shown in Table 1.

Child 5-HTTLPR genotype was not significantly associated with emotional problems at age 3, nor was maternal 5-HTTLPR genotype (p-values between .359 and .439). Prenatal maternal smoking was only significantly associated with offspring emotional problems before covariates were included in the model ($p=.033$ without covariates versus $p=.175$ with covariates). This attenuation was mainly due to the effect of maternal education. The interaction effect between child 5-HTTLPR genotype and maternal prenatal smoking is illustrated in Figure 1.

The interaction effects between prenatal smoking and maternal/child 5-HTTLPR genotype were not significant, with or without covariates included (p-values between .288 and .635).

To summarize, the significant interaction between maternal/child 5-HTTLPR genotype and maternal smoking during pregnancy on emotional problems at age 3 was not replicated in this large, population-based sample of Dutch children. As previously noted, the study by Cents and others (2012) adds to a body of literature on gene x environment interactions at the serotonin transporter locus. Three meta-analyses have been performed on this topic, with inconsistent conclusions (Karg and others 2011; Munafò and others 2009; Risch and others 2009). It has been argued that positive results may be spurious due to the large number of possible interaction models that can be tested and the generally low statistical power to detect interactions (Duncan and Keller 2011; Hunter 2005; Munafò and others 2009). Munafò and others (2009) demonstrated that without a main effect of the genotype, an interaction effect is highly unlikely. As two meta-analyses have not confirmed a main effect of 5-HTTLPR on depression, one may question the existence of interaction effects at this locus (Munafò and others 2009; Risch and others 2009). However, others have argued that the contradictory findings are due to the varying quality of measurement instruments and differences in definitions of stressful life events (Caspi and others 2010; Karg and others 2011). To ensure that the current replication effort was not qualitatively different from the original study, we used the same instrument to measure emotional problems and similarly assessed and coded maternal prenatal smoking. The scoring method for the CBCL internalizing scale used in the NTR sample was slightly different from the original study (CBCL/2-3 versus CBCL/1,5-5), since more observations were available for the CBCL/2-3 scoring. However, the internalizing scores from both methods were highly correlated ($r=.814$) and when the analyses were repeated with the CBCL/1,5-5 scoring method, similar results were obtained. The NTR sample was quite similar to the original sample with respect to maternal prenatal smoking and educational attainment. Moreover, as both samples originate from the Netherlands and ethnic outliers were excluded, it is improbable that the lack of replication is due to genetic heterogeneity. Finally, statistical interactions can arise from anomalies in the data such as violations of distributional assumptions and heteroscedasticity. This problem, however, is not necessarily solved by replication as the replication dataset might suffer from the same distributional problems as the original report (Eaves 2006; Kendler and Gardner 2010).

In conclusion, a replication effort in a sample of Dutch children does not support a moderating effect of 5-HTTLPR genotype on the association between prenatal maternal smoking and offspring emotional problems at age 3. Considering the ferocious debates surrounding 5-HTTLPR genotype by environment interactions, it is important that after the original first study, both replications and non-replications find their way into the literature.

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References

- Achenbach, TM.; Rescorla, LA. Manual for the ASEBA Preschool Forms & Profiles. University of Vermont; Research Center for Children Youth & Families; Burlington, VT: 2000.
- Bartels M, van Beijsterveldt CEM, Derks EM, Stroet TM, Polderman TJC, Hudziak JJ, Boomsma DI. Young Netherlands Twin Register (Y-NTR): A longitudinal multiple informant study of problem behavior. *Twin Res Hum Genet.* 2007; 10(1):3–11. [PubMed: 17539360]
- Boomsma DI, de Geus EJC, Vink JM, Stubbe JH, Distel MA, Hottenga JJ, Posthuma D, van Beijsterveldt TCEM, Hudziak JJ, Bartels M. Netherlands Twin Register: From twins to twin families. *Twin Res Hum Genet.* 2006; 9(6):849–857. others. [PubMed: 17254420]
- Caspi A, Hariri AR, Holmes A, Uher R, Moffitt TE. Genetic sensitivity to the environment: the case of the serotonin transporter gene and its implications for studying complex diseases and traits. *Am J Psychiatry.* 2010; 167(5):509–27. [PubMed: 20231323]
- Cents RAM, Tiemeier H, Velders FP, Jaddoe VWV, Hofman A, Verhulst FC, Lambregtse-van den Berg MP, Hudziak JJ. Maternal smoking during pregnancy and child emotional problems: The relevance of maternal and child 5-HTTLPR genotype. *Am J Med Genet B Neuropsychiatr Genet.* 2012; 159B(3):289–297. [PubMed: 22259195]
- Duffy, DL. Sib-pair: A program for simple genetic analysis, version 1.0.0: User Manual. Queensland Institute of Medical Research; Herston, Australia: 2012.
- Duncan LE, Keller MC. A critical review of the first 10 years of candidate gene-by-environment interaction research in psychiatry. *Am J Psychiatr.* 2011; 168(10):1041–9. [PubMed: 21890791]
- Eaves LJ. Genotype Environment Interaction in Psychopathology: Fact or Artifact? *Twin Res Hum Genet.* 2006; 9(1):1–8. [PubMed: 16611461]
- Hunter DJ. Gene-environment interactions in human diseases. *Nat Rev Genet.* 2005; 6(4):287–98. [PubMed: 15803198]
- IBM. IBM SPSS Statistics 20 Command Syntax Reference. IBM; Chicago, IL: 2011.
- Karg K, Burmeister M, Shedden K, Sen S. The serotonin transporter promoter variant (5-HTTLPR), stress, and depression meta-analysis revisited: Evidence of genetic moderation. *Arch Gen Psychiatr.* 2011; 68(5):444–454. [PubMed: 21199959]
- Kendler KS, Gardner CO. Interpretation of interactions: guide for the perplexed. *Brit J Psychiatr.* 2010; 197(3):170–171.
- Munafò MR, Durrant C, Lewis G, Flint J. Gene X environment interactions at the serotonin transporter locus. *Biol Psychiatr.* 2009; 65(3):211–9. [PubMed: 18691701]
- Risch N, Herrell R, Lehner T, Liang KY, Eaves L, Hoh J, Griem A, Kovacs M, Ott J, Merikangas KR. Interaction between the serotonin transporter gene (5-HTTLPR), stressful life events, and risk of depression: a meta-analysis. *JAMA: J Am Med Assoc.* 2009; 301(23):2462–71.

Figure 1.

This attenuation was mainly due to the effect of maternal education. The interaction effect between child 5-HTTLPR genotype and maternal prenatal smoking is illustrated in Figure 1.

Table 1 Main and interaction effects of maternal prenatal smoking and 5-HTTLPR genotype on offspring emotional problems (maternal reports) at age 3

	Without covariates (n=1,865)		With covariates (n=1,794)	
	B (95% CI)	p-value	B (95% CI)	p-value
MAIN EFFECTS				
Smoking during pregnancy	.24 (.02 – .47)	.033	.17 (-.07 – .40)	.175
Child 5-HTTLPR	.03 (-.05 – .12)	.439	.04 (-.05 – .13)	.389
Maternal 5-HTTLPR	.07 (-.10 – .24)	.408	.08 (-.09 – .25)	.359
INTERACTION EFFECTS				
Child 5-HTTLPR x Smoking during pregnancy	-.06 (-.26 – .13)	.524	-.05 (-.26 – .16)	.635
Maternal 5-HTTLPR x Smoking during pregnancy	-.17 (-.52 – .18)	.346	-.20 (-.56 – .17)	.288

Note. Covariates were offspring sex and age and maternal educational attainment. Main and interaction effects of maternal 5-HTTLPR genotype were assessed in separate analyses (n=608 without covariates; n=585 with covariates).