LETTER TO EDITOR

AMERICAN JOURNAL OF medical genetics PART B
Neuropsychiatric Genetics

# Maternal Prenatal Smoking and Offspring Emotional Problems: No Moderating Effect of Maternal or Child 5-HTTLPR Genotype

**Lot M. Geels,[1,2]* Maria M. Groen-Blokhuis,[1,2] Catharina E.M. van Beijsterveldt,[1] Jacqueline M. Vink,[1,3] Christel M. Middeldorp,[1,3,4] Meike Bartels,[1,2,3] Kelly A. Nelson,[5] Patricia E. Huizenga,[5] Gareth E. Davies,[5] and Dorret I. Boomsma[1,2,3]**

[1]Department of Biological Psychology, VU University Amsterdam, Amsterdam, The Netherlands

[2]EMGO+ Institute for Health and Care Research, VU University Medical Center Amsterdam, Amsterdam, The Netherlands

[3]Neuroscience Campus Amsterdam, Amsterdam, The Netherlands

[4]Department of Child and Adolescent Psychiatry, GGZ in Geest/VU Medical Center, Amsterdam, The Netherlands

[5]Avera Institute for Human Genetics, Avera Behavioral Center, Sioux Falls, South Dakota

Manuscript Received: 14 September 2012; Manuscript Accepted: 2 October 2012

In a recent article, Cents et al. [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 et al., 2011]. Cents et al. [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 et al. [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 et al. [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.

Replication of this novel genotype × environment interaction effect is important, since the 5-HTTLPR × stress interaction effect on depression has been observed in some, but not all studies [Karg et al., 2011; Risch et al., 2009]. Therefore, we repeat the analyses of Cents et al. [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).

**How to Cite this Article:**
Geels LM, Groen-Blokhuis MM, van Beijsterveldt CEM, Vink JM, Middeldorp CM, Bartels M, Nelson KA, Huizenga PE, Davies GE, Boomsma DI. 2012. Maternal Prenatal Smoking and Offspring Emotional Problems: No Moderating Effect of Maternal or Child 5-HTTLPR Genotype.
Am J Med Genet Part B 159B:1009–1012.

Grant sponsor: European Research Council: Genetics of Mental Illness; Grant number: ERC-230374; Grant sponsor: Beyond the Genetics of Addiction; Grant number: ERC 284167; Grant sponsor: US National Institute of Mental Health; Grant number: 1RC2MH089995-01.
Lot M. Geels and Maria M. Groen-Blokhuis contributed equally to this work.
The authors have no conflicts of interest to declare.
*Correspondence to:
Lot M. Geels, Department of Biological Psychology, VU University Amsterdam, van der Boechorststraat 1, 1081 BT Amsterdam.
E-mail: l.m.geels@vu.nl
Article first published online in Wiley Online Library (wileyonlinelibrary.com): 22 October 2012
DOI 10.1002/ajmg.b.32110

© 2012 Wiley Periodicals, Inc.

The Netherlands Twin Register (NTR) was established in 1987 at the VU University in Amsterdam [Boomsma et al., 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 et al. [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 and 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 et al. [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 et al. [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 et al. [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 five categories (ranging from "primary school" to "university"), which were dichotomized into the same categories as Cents et al. [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 et al. [2012] were added (maternal educational attainment, child sex, and child age). All tests were evaluated at $\alpha = 0.05$.

The distribution of 5-HTTLPR genotype was in Hardy–Weinberg equilibrium (empirical *P*-value 0.32). 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 $\geq$65).

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

Child 5-HTTLPR genotype was not significantly associated with emotional problems at age 3, nor was maternal 5-HTTLPR genotype (*P* values between 0.359 and 0.439). Prenatal maternal smoking was only significantly associated with offspring emotional problems before covariates were included in the model ($P = 0.033$ without covariates vs. $P = 0.175$ 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 0.288 and 0.635).

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

**TABLE I. 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 | 0.24 (0.02–0.47) | 0.033 | 0.17 (−0.07–0.40) | 0.175 |
| Child 5-HTTLPR | 0.03 (−0.05–0.12) | 0.439 | 0.04 (−0.05–0.13) | 0.389 |
| Maternal 5-HTTLPR | 0.07 (−0.10–0.24) | 0.408 | 0.08 (−0.09–0.25) | 0.359 |
| Interaction effects | | | | |
| Child 5-HTTLPR × smoking during pregnancy | −0.06 (−0.26–0.13) | 0.524 | −0.05 (−0.26–0.16) | 0.635 |
| Maternal 5-HTTLPR × smoking during pregnancy | −0.17 (−0.52–0.18) | 0.346 | −0.20 (−0.56–0.17) | 0.288 |

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).

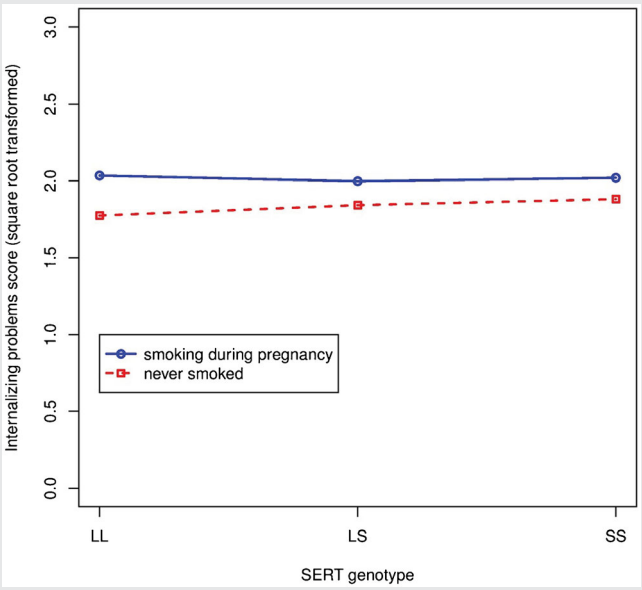


**FIG. 1. Mean internalizing scores by child 5-HTTLPR genotype and maternal prenatal smoking.**

large, population-based sample of Dutch children. As previously noted, the study by Cents et al. [2012] adds to a body of literature on gene × environment interactions at the serotonin transporter locus. Three meta-analyses have been performed on this topic, with inconsistent conclusions [Munafò et al., 2009; Risch et al., 2009; Karg et al., 2011]. 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 [Hunter, 2005; Munafò et al., 2009; Duncan and Keller, 2011]. Munafò et al. [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 et al., 2009; Risch et al., 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 et al., 2010; Karg et al., 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 vs. 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 ($r = 0.814$) 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.

## ACKNOWLEDGMENTS

The study was supported by European Research Council: Genetics of Mental Illness (ERC-230374) and Beyond the Genetics of Addiction (ERC 284167); Twin-family database for behavior genetics and genomics studies (NWO 480-04-004); US National Institute of Mental Health as part of the American Recovery and Reinvestment Act of 2009 (1RC2MH089995-01: Genomics of Developmental Trajectories in Twins); A twin-sib study of adolescent wellness (NWO-VENI 451- 04-034); and Genetic influences on stability and change in psychopathology from childhood to young adulthood (ZonMW 912-10-020).

## REFERENCES

Achenbach TM, Rescorla LA. 2000. Manual for the ASEBA Preschool Forms & Profiles. Burlington VT: University of Vermont; Research Center for Children Youth & Families.

Bartels M, van Beijsterveldt CEM, Derks EM, Stroet TM, Polderman TJC, Hudziak JJ, Boomsma DI. 2007. Young Netherlands twin register (Y-NTR): A longitudinal multiple informant study of problem behavior. Twin Res Hum Genet 10:3–11.

Boomsma DI, de Geus EJC, Vink JM, Stubbe JH, Distel MA, Hottenga JJ, Posthuma D, van Beijsterveldt TCEM, Hudziak JJ, Bartels M. et al. 2006. Netherlands twin register: From twins to twin families. Twin Res Hum Genet 9:849–857.

Caspi A, Hariri AR, Holmes A, Uher R, Moffitt TE. 2010. Genetic sensitivity to the environment: The case of the serotonin transporter gene and its implications for studying complex diseases and traits. Am J Psychiatry 167:509–527.

Cents RAM, Tiemeier H, Velders FP, Jaddoe VWV, Hofman A, Verhulst FC, Lambregtse-van denBerg MP, Hudziak JJ. 2012. Maternal smoking during pregnancy and child emotional problems: The relevance of maternal and child 5-HTTLPR genotype. Am J Med Genet Part B 159B:289–297.

Duffy DL. 2012. Sib-pair: A program for simple genetic analysis, version 1.0.0: User Manual. Herston, Australia: Queensland Institute of Medical Research.

Duncan LE, Keller MC. 2011. A critical review of the first 10 years of candidate gene-by-environment interaction research in psychiatry. Am J Psychiatry 168:1041–1049.

Eaves LJ. 2006. Genotype environment interaction in psychopathology: Fact or artifact? Twin Res Hum Genet 9:1–8.

Hunter DJ. 2005. Gene–environment interactions in human diseases. Nat Rev Genet 6:287–298.

IBM. 2011. IBM SPSS statistics 20 command syntax reference. Chicago, IL: IBM.

Karg K, Burmeister M, Shedden K, Sen S. 2011. The serotonin transporter promoter variant (5-httlpr), stress, and depression meta-analysis revisited: Evidence of genetic moderation. Arch Gen Psychiatry 68:444–454.

Kendler KS, Gardner CO. 2010. Interpretation of interactions: Guide for the perplexed. Br J Psychiatry 197:170–171.

Munafò MR, Durrant C, Lewis G, Flint J. 2009. Gene X environment interactions at the serotonin transporter locus. Biol Psychiatry 65: 211–219.

Risch N, Herrell R, Lehner T, Liang KY, Eaves L, Hoh J, Griem A, Kovacs M, Ott J, Merikangas KR. 2009. Interaction between the serotonin transporter gene (5-HTTLPR), stressful life events, and risk of depression: A meta-analysis. JAMA 301:2462–2471.