Psychiatry Research 245 (2016) 387–391

Contents lists available at ScienceDirect

Psychiatry Research

journal homepage: www.elsevier.com/locate/psychres

Brain derived neurotrophic factor moderates associations between maternal smoking during pregnancy and offspring behavioral disorders

Ardesheer Talati [a,b,e,*], Zagaa Odgerel [b], Priya J. Wickramaratne [a,b,d], Myrna M. Weissman [a,b,c,e]

[a] Department of Psychiatry, Columbia University Medical Center, New York, NY, USA
[b] Division of Epidemiology, New York State Psychiatric Institute, New York, NY, USA
[c] Departments of Epidemiology, Mailman School of Public Health of Columbia University, New York, NY, USA
[d] Departments of Biostatistics, Mailman School of Public Health of Columbia University, New York, NY, USA
[e] Sackler Institute for Developmental Psychobiology, Columbia University, New York, NY, USA

ARTICLE INFO

Article history:
Received 29 April 2016
Received in revised form
25 August 2016
Accepted 27 August 2016
Available online 28 August 2016

Keywords:
In utero exposure
val66met

ABSTRACT

Maternal smoking during pregnancy is associated with a number of adverse offspring outcomes. In the present study, based on 209 offspring from a 3-generation family study of depression, we show that the effects of prenatal exposure on offspring externalizing psychopathology (conduct, substance use disorder) is more pronounced in the presence of lower-expressing brain derived neurotrophic factor (BDNF) gene variants. BDNF plays an important role in the development and survival of neural circuits. Individuals with low-expressing variants who are further exposed to prenatal tobacco smoke may be most vulnerable to a spectrum of behavioral disorders that depend on these circuits.

1. Introduction

Smoking during pregnancy is a leading cause of preventable illness among pregnant mothers and their offspring (CDC, 2006a, 2006b). The fetus is particularly vulnerable, as numerous tobacco components not only traverse the placenta, but, with chronic exposure, can reach higher levels in the fetus than in the mother (Lambers and Clark, 1996; Rogers, 2009). Offspring problems begin early, in the form of pregnancy-related complications and lower birthweight (DiFranza and Lew, 1995; Kallen, 2001). They continue as the offspring age through childhood and adolescence, with reported increases in a range of behavioral problems and disorders (Nomura et al., 2011; O'Callaghan et al., 2009; Wakschlag et al., 2002). Not all exposed offspring go on to develop these problems, however. Searching for factors that moderate the risks conferred by prenatal exposure can help understand mechanistic pathways and identify offspring groups at greatest risk, so they can be targeted for early intervention.

Neural growth factors are attractive candidates within this framework as they, like the cholinergic receptors to which nicotine binds, are ubiquitously expressed in the brain and active during fetal development in promoting cell growth and survival (Groves, 2007; Lu et al., 2005). Brain derived neurotrophic factor (BDNF) is the most ubiquitous example, and is of particular interest from a genetic perspective given demonstrated functional consequences of variation within its gene: a polymorphism that substitutes a nucleotide encoding valine to one encoding methionine (*val66met*) results in decreased gene expression and activity-dependent secretion (Chen et al., 2006; Egan et al., 2003). Mice with BDNF deletions show impaired learning and spatial memory (Heldt et al., 2007, 2014), as well as disruptions in GABAergic and cholinergic neurons (Grosse et al., 2005). In human studies, lower BDNF levels have also been linked to reduced cortical thickness (Legge et al., 2015; Wang et al., 2014). Prenatal exposure to tobacco has been further associated with lower levels of BDNF mRNA and proteins in rodents (Yochum et al., 2014) and with higher gene methylation in humans (Toledo-Rodriguez et al., 2010). In adult smokers, chronic smoking is also correlated with BDNF protein levels (Bhang et al., 2010; Bus et al., 2011).

We use an existing longitudinal family study of depressive disorders to test whether BDNF moderates the effect of exposure to tobacco on childhood and adolescent psychopathology, hypothesizing that offspring with both *genetic* (*met*-encoding BDNF

* Correspondence to: Department of Psychiatry, New York State Psychiatric Institute, 1051 Riverside Drive, Unit 24, New York, NY 10032, USA.
E-mail addresses: talatia@nyspi.columbia.edu, at2071@cumc.columbia.edu (A. Talati), zdodgerel@yahoo.com (Z. Odgerel), wickramp@nyspi.columbia.edu (P.J. Wickramaratne), mmw3@columbia.edu (M.M. Weissman).

http://dx.doi.org/10.1016/j.psychres.2016.08.061
0165-1781/

variants) and *environmental* (exposure to maternal smoking in pregnancy) risks will have higher rates of internalizing and externalizing psychopathology than offspring with one or neither of these risks.

2. Methods

2.1. Sample

Design and clinical procedures have been detailed previously (Weissman et al., 1999, 2006, 2005). Briefly, depressed probands were selected from outpatient clinics for treatment of mood disorders; non-depressed probands were selected concurrently from an epidemiological sample from the same community. The probands, along with their children (G2) were followed longitudinally for six waves up to 30 years. At the third wave (year 10), grandchildren (G3) were also directly interviewed. Procedures were kept similar across waves to avoid method variation bias. Procedures were approved by the New York State Psychiatric Institute's Institutional Review Board, and informed consent was obtained from all study participants.

Prenatal histories were collected on 389 participants. Of these, 378 (97%) offspring were directly interviewed at least once, and 209 (55%) provided DNA. Participants with and without DNA did not vary by exposure status [among the genotyped subset, 27% were exposed; among the non-genotyped, 29% were exposed, $\chi^2=0.22$, $p=0.63$].

2.2. Assessments

Mothers (G1 mothers, for G2 offspring, and G2 mothers for G3 offspring) completed a report for each child that included questions about the course of pregnancy and delivery. For G2 offspring, They were asked whether they had smoked while pregnant, and if so, how frequently they had smoked ≥ 10 cigarettes per day: [1–2 times/3–5 times/6–10 times/every two weeks/weekly/daily or almost daily]. Because most ($>98\%$) mothers reported either not smoking at all or smoking daily, we generated a dichotomous variable based on whether or not the mother reported smoking ≥ 10 cigarettes/day, almost every day. Similar cutoffs are used in other studies (Cornelius et al., 2000; Hoff et al., 1986).

Offspring were assessed using the age-appropriate version of the semi-structured Schedule for Affective Disorders and Schizophrenia interview (Kaufman et al., 1997; Mannuzza et al., 1986), administered by trained doctoral- and masters-level mental health professionals, blind to prenatal exposure or parental history. The first interview assessed the lifespan until that interview; subsequent interviews assessed intervening time periods. The total assessment period, therefore, is cumulative to the year of final interview. Final diagnoses were made using the best-estimate procedure (Leckman et al., 1982). Inter-rater reliability was high (Weissman et al., 1999).

2.3. Genotyping

Val66met (rs6265) was genotyped using DNA from blood samples, with Taqman technology (Applied Biosystems, Foster City, CA). Assays were made as follows in 5 μl reaction volume: 10 ng/μl DNA dried down, 1X Applied Biosystems Taqman Universal PCR MasterMix, 0.5X Taqman assay mix. The reaction was cycled for 1 cycle of 95 °C for 10 min., and 50 cycles of 92 °C for 15 s, and 60 °C for 60 s on an Applied Biosystems GeneAmp PCR System 9700. Genotypes were read on Applied Biosystems 7900 using SDS 2.1 software.

2.4. Statistical analysis

Data were analyzed using Statistical Analysis Software (SAS 9.0/Carey NC). Outcome, genetic, and exposure variables were categorized as dichotomous variables. Group comparisons were conducted using chi-square tests. Consistent with other studies (Lang et al., 2007; Montag et al., 2008), we compared offspring with ≥ 1 copy of the *met*-encoding allele to those with *val/val*. To test the hypothesis that genotype moderates the association between prenatal smoke exposure and psychopathology, we used a Breslow-Day Test for Homogeneity of Odds Ratios (Breslow and Day, 1980). This tests the null hypothesis that the odds ratio representing the association between smoke exposure and disorder is the same across different genotypes. Finally, we modeled the effects of prenatal exposure and genotype on each significant outcome using logistic regression within a generalized estimating equations approach (Hardin and Hilbe, 2003) [PROC GENMOD] to account for potential within-family correlations. Exposure and offspring genotype were the predictor variables, and age, gender, and maternal depression and substance use history as covariates. To test whether the effects of exposure on outcome varied significantly by genotype, we included a gene-by-exposure interaction term in the model.

3. Results

Sample characteristics are summarized in e-Table 1. Mothers who smoked during pregnancy were more likely to have lifetime major depression or substance use disorder. However there were no other parental or demographic differences between offspring exposed versus unexposed offspring, or between the offspring with risk versus non-risk encoding genotypes. Genotype and exposure were also not independently associated with each other (43% exposed, as compared to 33% unexposed, offspring had ≥ 1 *met* allele ($\chi^2=2.17$, $p=0.14$)).

We examined associations between exposure and outcome within each genotype group (Table 1). Among offspring with *val/val* encoding genotypes, there was no significant association between exposure to maternal smoking and any offspring outcome (left columns), except substance use disorders, where exposure reduced the rates of substance use. Among offspring with ≥ 1 *met* alleles, however, prenatal exposure was associated with higher rates of offspring externalizing disorders, particularly conduct (52 vs 22%) and substance use (72 vs 41%) disorders (there were no associations with casual drug or alcohol use). Genotype × exposure comparisons to formally test whether associations between exposure and outcome varied by genotype revealed significant interactions for externalizing disorder (right-most column, $p=0.0097$), which were largely accounted for by substance use ($p=0.0009$) and conduct ($p=0.01$) disorders. There were no significant associations for internalizing disorders, although a trend was noted for major depression.

We next tested the above findings in a model that further accounted for offspring age and sex, maternal depression and substance use, and the potential non-independence of outcomes within families. As shown in Table 2, a significant overall genotype by exposure interaction was detected for externalizing disorders ($p=0.019$), which was driven by substance use ($p=0.0023$) and conduct ($p=0.017$), but not internalizing disorders. Among the other included covariates, having a familial history of depression, being female, and being older, each increased the overall risk for having an internalizing disorder; conversely, being male was associated with higher rates of having an externalizing disorder.

When we restricted the sample to the second generation offspring, exposure × genotype interactions remained significant