Genetic Instrumentation for Multiple Maternal Behavioral Effects on Infant Health Using Genome-Wide Association Study Data

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Background and Objective:

Maternal risk behaviors during pregnancy such as smoking, alcohol use and others have been reported to have significant effects on infant and child health. Understanding the role of such behaviors in child health is essential for developing policy and behavioral interventions that enhance child health. However, a major complication in studying these behaviors is maternal self-selection into those behaviors based on factors that are unobserved in observational data and that also affect child health. This self-selection can significantly bias the behavioral effects in classical estimation approaches in directions that can be inferred a-priori. One approach to account for this self-selection is to apply instrumental variables (IV) analysis. Instruments have typically involved area-level policy variables such as taxes and cigarette/alcohol access policies. However, these instruments have theoretical and empirical limitations, including but not limited to individuals selecting to live in areas based on the local policies and the fact that policies ignore variation in reasons predisposing to these behaviors between individuals living in the same area.

Genetic instruments have been proposed as a useful source of exogenous variation to identify behavioral effects. The idea is that genetic variants with strong evidence of being related in previous studies to a certain behavior and of otherwise being unrelated to the outcome of interest except through that behavior can be used as instruments to identify the behavior's effect. However, one limitation of this approach has been the lack of large samples with data on both maternal behaviors and child health on one hand, and genetic information on potential instruments on the other. Another limitation is the uncertainty about how the estimates of the behavioral effects may change with using different instruments that vary in their strength of association with the behavior and in evidence from the literature of being related to the behavior.

This paper has two main goals. First, we *simultaneously* evaluate the effects of three maternal behaviors during pregnancy – cigarette smoking, alcohol use and body weight – using as instruments genetic variants in genes with "unequivocal" evidence of affecting these behaviors and that have no evidence of being related to child health through other pathways. This first aim

makes significant contribution to the literature. To our knowledge, no previous study has applied this identification approach to simultaneously estimate these behavioral effects on infant health. This work provides significant advantage not only in the novel source of variation used to identify the behavioral effects, but to be able to simultaneously model and study multiple endogenous behaviors. Considering the effects under the first aim using the preferred-instrument set to be the "gold-standard" IV estimates based on genetic instruments, we then evaluate how the effects change with other genetic instruments that are either not as strongly related to the behavior in the sample or have weaker evidence from the literature of being involved in these behaviors. This second aim allows us to understand the "stability" of the behavioral effects under different conditions of instrument strengths, which allows us to understand potential biases of using "weaker" genetic instruments and provides a reference and framework for interpreting and evaluating the results of studies that employ such genetic instruments for these behaviors.

Data and Empirical Model:

We employ data from the prematurity GWAS sample (U01 HG-4423; Murray JC PI) of 2000 mother-baby pairs from the Danish National Birth Cohort (DNBC). The sample includes 1000 spontaneous preterm babies (≤36 weeks of gestation) and 1000 randomly selected full-term babies (40 0/7 and 40 6/7 weeks). The sample excluded multiple gestations and congenital anomalies. The DNBC included more than 101,000 pregnant women and their newborns (about 96,800) in Denmark in1996-2003 (Olsen et al, 2001; Andersen et al, 2002). Women were first recruited and interviewed at the first prenatal visit (usually in the first trimester) about their health, risk behaviors, and socioeconomic characteristics (reducing report bias based on observed birth outcomes). The women also provided a blood sample (for DNA extraction). Another interview occurred at the beginning of the third trimester. Data and DNA samples are of high quality. The GWAS genotyped the maternal and infant DNA samples for approximately 550,000 SNPs using a high density panel (Illumina Human660W-Quadv1_A platform) at the Center for Inherited Disease Research (CIDR). The final sample that included observations on all model variables and genetic instruments included 1,719 mother-baby pairs.

We identified from the literature the candidate genes for smoking, alcohol and obesity/body weight and the SNPs in these genes that were covered under the GWAS panel. The genes were ranked into those with unequivocal evidence of affecting the behaviors and those with weaker evidence based on the number and consistency of the studies that report significant associations with the study behaviors. We identified and analyzed more than 1,458 SNPs in 89 genes for these three behaviors. We first studied the correlations between the SNPs in the behavior-relevant genes and each behavior. We evaluated further those that were significantly related to the behavior at $p < 0.01$ for their correlations with observed confounders (other behaviors, maternal health risks, demographic characteristics) and excluded those that were found to be significantly correlated with these other variables.

The "gold-standard" instruments in unequivocally relevant genes for the behaviors were one SNP in *CHRNA3* for cigarette smoking, *ADH1B* for alcohol, and *FTO* for body weight. These instruments had F-statistics of 8.5 for cigarette smoking, 14.9 for body mass index (BMI), and 12.7 for alcohol use.

We next evaluate the effects of these behaviors in the same model on infant health, which we measure by birth weight, adjusting of demographic and health risk factors. We estimate the classical model using OLS, and the IV model with three-stage least squares (3SLS). After estimating the IV model with the preferred instrument specification, we then re-estimate the IV model while varying the instruments for each risk behavior, one at a time, to include instruments that have "statistically" significant effects on the behavior but have either small F-statistics (between 3 and 8) or large F-statistics but are in genes with weaker literature evidence of association with the behavior. In other words, when evaluating the stability of the effects of a certain behavior to the instrument conditions, we keep the preferred instruments for the other two endogenous behaviors in the model.

Preliminary Results:

In the classical model, the study behaviors have generally the expected effects. Cigarette smoking reduces body weight by about 20 grams per cigarette, while alcohol use increases body weight by about 200 grams. BMI has a small and insignificant positive effect (7 grams increase in birth weight with 1 unit increase in BMI).

In the IV model using the preferred instrument specification, the effects of cigarette smoking more than doubles (48 gram decrease per cigarette) but are insignificant. The alcohol effects increase by more than 2 times (about 485 gram increase in body weight) but are also significant. However, the effects of maternal BMI switch to negative (marginally significant at p=0.06) with an-88 gram decrease in birth weight with 1-unit increase in BMI.

The behavior effects under the non-preferred instrument specifications had a wide range, and some of these effects were statistically significant or marginally significant. For cigarettes, the IV effect ranged from about 164 gram decrease (marginally significant; p=0.055) to about 200 gram increase in birth weight per cigarette (statistically significant; p=0.03). The IV effects of BMI ranged from a decrease of 5 to 112 grams in birth weight (non-statistically significant). For alcohol, the effects under the non-preferred instruments ranged from a decrease of 76 grams to an increase of over 1,000 grams in birth weight. None of the alcohol effects were statistically significant.

Discussion and Conclusions:

This is the first study that employs genetic information on genetic variants that are unequivocally related to smoking, alcohol and body weight to simultaneously identify the effects of these maternal behaviors on birth weight. The study is also the first to provide estimates of the "causal" effects of maternal body weight on birth weight using an instrumental variables approach and finds a large detrimental effect of increasing body weight on infant's birth weight that is significantly underestimated in classical models, which may suggest positive effects. This may suggest "unobserved" characteristics of mothers of higher body weight that are positively correlated to birth weight, which may include social, economic and nutritional endowments.

The study provides a comparative analysis of behavioral effects under several instrument combinations that are suboptimal but that may be utilized in other studies due to the lack of data

on the preferred instruments with unequivocal evidence of being related to the behaviors and finds large deviances in effects from the estimates based on the preferred instruments (gold-standard estimates). The implausible yet statistically significant effects observed under some of the non-preferred instruments and the wide range of estimates clearly highlight the importance of utilizing only instruments that have a strong evidence of being unequivocally affecting the behaviors of interest and the potential large biases in inference that may result when utilizing those that are in genes with less consistent evidence from the literature.