GENES AS INSTRUMENTS FOR STUDYING RISK BEHAVIOR EFFECTS: AN APPLICATION TO MATERNAL SMOKING AND OROFACIAL CLEFTS

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Abstract

This study uses instrumental variable (IV) models with genetic instruments to assess the effects of maternal smoking on the child's risk of orofacial clefts (OFC), a common birth defect. The study uses genotypic variants in neurotransmitter and detoxification genes relateded to smoking as instruments for cigarette smoking before and during pregnancy. Conditional maximum likelihood and two-stage IV probit models are used to estimate the IV model. The data are from a population-level sample of affected and unaffected children in Norway. The selected genetic instruments generally fit the IV assumptions but may be considered "weak" in predicting cigarette smoking. We find that smoking before and during pregnancy increases OFC risk substantially under the IV model (by about 4–5 times at the sample average smoking rate). This effect is greater than that found with classical analytic models. This may be because the usual models are not able to consider self-selection into smoking based on unobserved confounders, or it may to some degree reflect limitations of the instruments. Inference based on weak-instrument robust confidence bounds is consistent with standard inference. Genetic instruments may provide a valuable approach to estimate the "causal" effects of risk behaviors with genetic-predisposing factors (such as smoking) on health and socioeconomic outcomes.

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1. INTRODUCTION

Risk behaviors such as smoking, alcohol use, drug use, poor nutrition/obesity, and others have major impact on health, psychosocial and economic status. Studies of these effects are common and span a wide range of outcomes and populations. A particular research area of major public health and policy implications is the study of maternal risk behavior and its effects on child health. Numerous studies have evaluated the impact of maternal smoking, alcohol use, obesity and other risk behaviors on infant and child health outcomes such as birth weight, infant mortality, birth defects and child development.

One major challenge in studying behavioral effects on health and other outcomes is non-random self-selection into behaviors based on unobserved or inadequately measured characteristics. Behaviors are a function of individual preferences for health and risk taking, perceptions of health risks, expectations of health outcomes and biological predispositions to behaviors (driven environmentally or genetically). In the case of maternal health behaviors, the mother's choice of prenatal risk behaviors is also a function of her preferences for child health, her perceptions of extent of fetal health risk factors and expectations of infant and child health outcomes. Preferences and risk perceptions are generally unobserved in data sources used for studying behavioral effects. Furthermore, direct adjustment using proxy measures is typically inadequate to account for such unobservable relevant factors.

When ignored, self-selection into behaviors based on unobserved factors can result in biased estimation of behavioral effects. The unobserved factors that affect behaviors may also affect the study outcomes through other pathways, resulting in classical omitted variable (confounding) bias. Preferences and risk perceptions likely affect the choice of several behaviors, some of which may not be adequately observed in available data. Furthermore, risk perceptions may be correlated with actual risk factors that may not be adequately observed and that affect the study outcomes. Therefore, standard regression models that involve direct adjustment for observable confounders only may not adequately account for the unobservable confounders.

Instrumental variable (IV) designs may be used to account for unobservable confounders when studying behavioral effects on health or other outcomes. Several risk behaviors have identified genetic risk factors that may serve as instruments. These genetic factors may serve as instruments that provide a useful source of behavior variation for identifying the "causal" behavioral effects on health and other outcomes. The paper proceeds as follows: Section 2 provides a background on instrumental variables, genetic instruments and study objectives; section 3 describes the data sample and methods; section 4 provides the results; and section 5 includes discussion and conclusions.

2. BACKGROUND

Instrumental variables (IV) have been used extensively to study the effects of prenatal care use, maternal smoking, alcohol use and other behaviors on infant health (Evans and Ringel 1999; Grossman and Joyce 1990; Lien 2005; Rosenzweig 1983; Wehby et al. 2009a). The IV design uses instruments that are strongly correlated with endogenous variables (behaviors), but are otherwise unrelated to the study outcomes either directly or indirectly through unobserved confounders (Angrist, Imbens, and Rubin 1996). Under these assumptions, the IV model uses variation in behaviors that is unrelated to the unobservable confounders (and is, therefore, free from self-selection and confounding) to estimate the behavioral effects on the outcomes.

Previous IV studies of risk behaviors such as smoking or alcohol use have typically employed prices/taxes or related policies such as state-level policies preventing public-area

smoking. However, there are several disadvantages with these instruments. Such aggregate-level instruments use area-level variation in risk behaviors and ignore behavioral variations among individuals living in the same area. Furthermore, the individual's self-selection into area of residence may introduce correlations between these instruments and other potentially relevant factors such as individual preferences and health risks. Finally, state-level policies and prices/taxes may also be reversely affected by the population's preferences and health risks.

In this study, we evaluate the utility of employing genetic variants as instruments in IV designs to assess risk behavior effects. This approach exploits variation in risk behaviors that is generated by exogenously inherited genetic factors for estimating the behavior effects on outcomes. The genetic variants are single nucleotide polymorphisms (SNPs), which are DNA base-pair variants, each of which has two alleles (variants). The strength of genetic variants as instruments is the "random" transmission of alleles from parents into their children. Each of the two SNP alleles has an equal chance of being transmitted during generation of germ cells (meiosis). This is the reason for describing the use of genetic instruments as "Mendelian Randomization" in epidemiology (Lawlor et al. 2008). However, the approach is a standard application of instrumental variables (Wehby, Ohsfeldt, and Murray 2008b). The "random" assignment/inheritance of alleles into an individual's genome seems to support the IV condition of exogenous instruments and suggests that unobserved confounders are unlikely to be correlated with genetic instruments when these do not affect the unobserved confounders and are not correlated with genetic variants that affect these confounders. (Smith et al. 2007) find that genetic variants are generally uncorrelated with behavioral and social variables when such associations are not a-priori expected or hypothesized.

"Random" inheritance does not mean that all individuals are at the same chance of inheriting a certain allele. Furthermore, "genetic instruments" may predict variation in an individual's risk behavior not only through the direct genetic effects, but also through other pathways that may or may not be correlated to the unobserved confounders. For example, consider a genetic variant x that increases smoking risks. Parents who have (more copies of) variant x are more likely to smoke and more likely to pass this variant to their children than parents without this variant. If parental smoking causes family-level economic and psychosocial effects that are also inherited either directly or indirectly through affecting their children's preferences, health risks, and other factors, and that may be related to the unobserved confounders of interest when using the children's genes as instruments for their own behaviors (such as for studying the effects of smoking on health outcomes), then the genetic instruments may no longer be endogenous. While it may be unlikely that smoking has such effects, such bias is theoretically possible. Therefore, it is important to evaluate the "assumption" of instrument exogeneity with the available tests described below. However, one clear-cut advantage in using genetic instruments is that they cannot be affected by risk behaviors – unlike other individual-level instruments such as income or distance to providers/supplies, or area-level instruments such as prices/taxes and policies. Health economic studies have begun to utilize genetic factors as instruments to identify health risk and behavior effects (Ding et al. 2009; Fletcher 2009a; Fletcher 2009b; von Hinke Kessler Scholder 2010)

2.1 Study Objectives and Significance

We evaluate the utility of genetic instruments for studying maternal smoking effects on the child's risk of being born with orofacial clefts (OFC). OFC are common birth defects occurring in about 1 per 700 births and are among the most prevalent birth defects in the US (about 20,400 births in 1999–2001) (Centers for Disease Control and Prevention 2006). OFC, including cleft lip with/without palate and cleft palate only, have a complex etiology

of genetic, environmental and behavioral risk factors (Mossey et al. 2009). OFC impose significant burdens on individual and family health and economic wellbeing due to the need for surgical, speech and behavioral treatments. These may significantly reduce individual and family quality of life (Wehby and Cassell 2010; Wehby, Ohsfeldt, and Murray 2006). OFC increase child hospitalization costs by more than five times (Boulet et al. 2009; Cassell, Meyer, and Daniels 2008), and may increase the risk of long term health problems including certain cancers (Bille et al. 2005), mental health problems (Christensen and Mortensen 2002), and mortality and suicide risks (Christensen et al. 2004).

Several studies suggest that maternal smoking moderately increases OFC risk, with some variation across studies in the magnitude of the effects and the affected cleft type (Bille et al. 2007; Chung et al. 2000; Honein et al. 2007; Khoury, Gomez-Farias, and Mulinare 1989; Lie et al. 2008; Lieff et al. 1999; Little et al. 2004a; MacLehose et al. 2009; Shaw et al. 2009; Werler et al. 1990). Two meta-analyses of earlier studies estimated 1.3 times increased odds for OFC (Little, Cardy, and Munger 2004b; Wyszynski, Duffy, and Beaty 1997).

Some variation in the smoking effect estimates may be due to self-selection bias. All previous studies of maternal smoking effects on OFC have assumed random maternal self-selection into risk behaviors after adjusting for observed/measured confounders. These studies use designs that cannot account for self-selection bias due to unobserved factors and therefore, may suffer from confounding bias.

Behavioral theories and empirical evidence for infant health outcomes strongly indicate that maternal behaviors during pregnancy are in part determined by unobserved factors that are also related to infant health (Mullahy 1997; Rosenzweig and Schultz 1983; Wehby et al. 2009a). Available data sources for studying maternal behavioral effects on infant health lack valid measures of the relevant unobserved factors such as preferences and risk perceptions described above. Also, good proxies for these unobservable confounders are usually unavailable. Therefore, it is virtually impossible to fully account for these unobservable factors using only direct adjustment multivariate methods.

Maternal risk behaviors prior to and during pregnancy are "endogenous" to infant health outcomes, including OFC status. The assumption of ignorable self-selection bias in previous studies of smoking effects on OFC is strong and unsupported. Further, the direction of bias (i.e. over- or underestimation) in estimates that ignore self-selection is theoretically ambiguous due to the potential role of several unobserved factors. Obtaining accurate estimates of smoking effects on OFC is important for developing effective counseling and prevention programs for OFC. If smoking effects are underestimated in classical models, identifying the real smoking effects may improve counseling of women of childbearing age and make it more effective for preventing OFC. Similarly, if smoking effects are overestimated in classical models, smoking-focused approaches may not be effective in preventing OFC. Therefore, identifying the causal smoking effects of OFC has important implications for improving child health outcomes and reducing birth defects.

This paper reports the results of a novel application of the genetic IV model to estimate maternal smoking effects on OFC. The IV analysis simultaneously models 1) smoking as a function of relevant observed characteristics and genetic variants that influence smoking, and 2) OFC risk as a function of smoking and relevant observed factors. The goal is to exploit variation in smoking that is generated only by the genetic instruments and to account for non-random self-selection and unobserved confounders that have been a common limitation of previous studies of the effects of maternal smoking on OFC. By doing this, we will evaluate the utility of employing genetic variants as instruments to study maternal

behavior effects on child health. This is one of the first studies to apply the genetic IV model to evaluate the effects of maternal risk behaviors on child health. This paper is also one of the first to apply genetic instrumental variables to data collected with case-cohort or case-control designs, and illustrates approaches for estimating and interpreting the behavior effects in such applications, which do not provide directly interpretable effect estimates, unlike classical models (such as standard logistic regression) that assume exogenous behaviors.

2.2 Genetics of Smoking

Smoking behaviors are well known to involve a complex etiology that involves genetic and environmental factors (Li 2006; Tyndale 2003), and there is a convergent evidence of strong links between specific genes and cigarette smoking. Twin and adoption studies find that genetic heritability is at least 50% for both smoking initiation and persistence (Carmelli et al. 1992; Heath and Martin 1993; Lessov et al. 2004; Maes et al. 2004).

Several nicotine, detoxification, and neurotransmitter genes have been implicated with smoking behaviors. Some of the main implicated genes are dopamine 2 receptor (*DRD2*) (Comings DE 1996; Noble et al. 1994; Spitz et al. 1998), dopamine beta hydroxylase (*DBH*) (McKinney et al. 2000), *DOPA* decarboxylase (*DDC*) (Ma et al. 2005; Yu et al. 2006), Cholecystokinin (*CCK*) (Comings et al. 2001), Tryptophan Hydroxylase (*TPH*) (Lerman et al. 2001; Sullivan 2001), gamma-aminobutyric acid type B receptor subunit 2 gene (*GABAB2*) (Beuten 2005; Li et al. 2009), nicotinic acetylcholine receptor a4 subunit (*CHRNA4*) (Li et al. 2005) and a3 subunit (rs1051730 in *CHRNA3*, (Thorgeirsson et al. 2008)) and Cytochrome P450 2A6 (*CYP2A6;* (Pianezza, Sellers, and Tyndale 1998; Sellers, Kaplan, and Tyndale 2000)).[1] Recent studies have found significant associations between smoking and SNPs in *GABRA4*, *GABRA2* and *GABRE* (Agrawal et al. 2008). Two recent Genome Wide Association Studies (GWAS) have identified correlations between various nicotine dependence measures and SNPs in *CHRNA3* and *CHRNA5* (Berrettini et al. 2008; Caporaso et al. 2009) and in *MAOA* and *ACTN1* (Caporaso et al. 2009). Recently, a large meta-analysis confirmed the previously reported *CHRNA3* and *CHRNA5* effects and identified two new SNPs within these genes (rs55853698 and rs6495308) that explain most of the locus *15q2* effects on smoking quantity (Liu et al. 2010).

3. METHODS

3.1 Data Source and Study Measures

This paper employs a sample from a study of oral clefts in Norway. The study is a population-level survey of infants born with OFC in Norway in 1996 through 2001 and their parents as well as a randomly selected control sample of infants born without birth defects in the same period (NIEHS. 2009). The main study sample includes 574 children with OFC who represent 88% of the total eligible OFC population and 763 unaffected children who represent 76% of the randomly selected 1,006 eligible live births. DNA samples were collected from parents and children, and extensive data on maternal behaviors and household factors and socioeconomics were provided by the mothers. Data on maternal behaviors and characteristics was completed through self-administered surveys and most variables have complete data for more than 97% of the survey sample. The sample in this paper includes 856 mother-child pairs with available maternal genotypic data on the employed instruments and all model variables -- 363 mothers of children with isolated oral clefts and 493 mothers of unaffected children. There are no significant differences in smoking and other relevant model variables between the analytical sample and those

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[1]The variant rs1051730 is also related to quitting and intensity of smoking during pregnancy (Freathy et al. 2009).

excluded due to incomplete data on genetic instruments or other variables. All study models include these 856 persons except for the additional models that exclude cases with cleft palate only, as described below.

The study outcome is isolated OFC without other malformations. Some of the previous studies summarized above report differences in smoking effects between cleft lip with/without palate and cleft palate only. In order to evaluate the potential heterogeneity in smoking effects by cleft type, we estimate an additional specification that excludes cleft palate only from the sample (106 affected children), leaving 257 cases and 493 controls. [2]

We measure maternal smoking by the average number of cigarettes smoked per day during the 12 months before pregnancy and alternatively during the first trimester, for which we identify instruments for the IV analysis as described below. Women were asked about whether they smoked at all during the last 12 months before pregnancy and during the first pregnancy trimester, and if so how much they smoked "on average" per day or month.[3] Smoking during the first trimester is the more biologically relevant and preferred measure of exposure for fetal development of OFC. However, we also evaluate smoking before pregnancy as a complementary measure given that some women may not become aware of their pregnancy and modify their smoking status until a few weeks after conception. OFC occur by the 9th gestational week (Sperber 2002) and in many cases before the mother becomes aware of the pregnancy, as several pregnancies are unplanned.[4] This raises the possibility that the report of quitting smoking right before pregnancy (i.e. reporting smoking before pregnancy but not during the first trimester) may not always be accurate, causing the embryo to be exposed to cigarette smoking. For these cases, smoking before pregnancy (as measured in this study) may also represent smoking during the early stages of pregnancy. About 47% of the sample mothers smoked during the last 12 months before pregnancy and about 37% reported smoking during the first pregnancy trimester (i.e. about 23% of the women who smoked before pregnancy quit smoking in the first trimester).

There is a decreased variation in smoking participation and intensity in the first pregnancy trimester compared to before pregnancy. In addition to decreasing participation, the average cigarettes smoked per day among smokers decreases from about 9 to 6 cigarettes. This decreases the power for observing significant instrument effects on cigarette smoking. Furthermore, women who choose to continue to smoke during pregnancy may be different from those who quit smoking during pregnancy based on their genetic risk factors for smoking. Some genetic variants that predict smoking during a more ordinary time (e.g., before pregnancy) may not have significant effects on smoking during pregnancy. We find most of the genetic instruments for smoking before pregnancy to have significant yet "weaker" effects on smoking during the first trimester, as described below.

3.2 Genetic Instruments and Instrument Validation

In this study, we use SNPs that had been genotyped as part of a large-scale genotyping project through the Center for Inherited Disease Research (CIDR) for studies of OFC. SNPs were selected to study the effects of smoking on OFC. We employ SNPs rs1435252, rs1930139 and rs1547272 in the gamma-aminobutyric acid type B receptor subunit 2 gene (*GABAB2*) and SNP rs2743467 in cytochrome P450 gene *CYP2D6* as instruments for

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[2]Cleft lip only and cleft lip with palate are commonly grouped in the literature given their similarities in development and recurrence risks.
[3]The specific questions on smoking and other survey questions can be found on http://www.niehs.nih.gov/research/atniehs/labs/epi/studies/ncl/question.cfm. *).* We do not have direct data on changes in smoking amounts within these periods, but these may be partly reflected in the responses to average smoking intensity as described above. We focus on estimating the overall average effects of cigarette quantity per day for the days when the women smoked in these periods.
[4]About 27% of the mothers in the study sample reported that the pregnancy was unplanned.

average cigarettes per day during the 12 months before pregnancy[5]. None of these genes is considered to be a candidate gene for OFC or is known to affect OFC through unobserved behaviors. There are numerous neurotransmitter and detoxification pathways with multiple genes that have different and specialized functions. The fact that a gene plays a role in one pathway and affects a certain risk behavior does not necessarily mean that it plays a role in all risky behaviors as many of the genetic effects are specific to certain behaviors and pathways. We use the same *GABAB2* SNPs (rs1435252, rs1930139 and rs1547272) as instruments for cigarette smoking during the first trimester and before pregnancy. The *CYP2D6* SNP has an insignificant effect on first trimester cigarette smoking, and is therefore not used as an instrument for cigarettes in the first trimester of pregnancy. Previous studies have reported variants in *GABAB2 and CYP2D6* to be associated with smoking behaviors (Beuten 2005; Li et al. 2009; Saarikoski et al. 2000).

Table S1 in the Supplementary Material summarizes the various instrument specifications that we employ. The main instrument specification for cigarettes before pregnancy includes, for each SNP, two indicators for the minor allele homozygote genotype and for the heterozygote genotype – the reference category is the major allele homozygote genotype. This does not impose the restriction that these two genotypes have the same effect. As sensitivity analysis, we evaluate alternative instrument specifications to gauge the sensitivity of the IV cigarette effect estimates to various instruments and to assumptions about gene-dosage instrument effects. These alternative specifications include one indicator per SNP that combines the minor allele homozygote and heterozygote genotypes.[6] Further, for smoking before pregnancy, we evaluate additional instrument specifications excluding the *CYP2D6* SNP as an instrument given that there is generally less evidence from previous studies for *CYP2D6* involvement in smoking dependence than *GABAB2*.[7]

We evaluate the extent to which the instruments fit the first IV assumption by testing the joint significance of their effects on the number of cigarettes adjusting for the other model variables. There is no statistical test to *fully* evaluate the second IV assumption – that instruments influence OFC only through smoking and observed variables – due to the role of unobservable factors (Wooldridge 2002). Therefore, it is important to select the instruments based on the theoretical (and in the case of genetic instruments biological) justification for their excludability from the outcome function (Wehby, Ohsfeldt, and Murray 2008a). To our knowledge, there is no evidence that the specific employed instruments or their genes are involved in the biological/genetic pathways for OFC other than through smoking (Jugessur, Farlie, and Kilpatrick 2009; Lidral, Moreno, and Bullard 2008). We evaluate the second IV assumption using the standard over-identification test (Baum 2007; Lee 1992), which is a partial test of the second IV assumption and evaluates whether the additional instruments used to identify the IV model (i.e. the over-identification restrictions) have no effects on OFC independent of smoking and are, therefore, excludable from the OFC function (a minimum of one instrument is needed to identify the effect of one endogenous variable).[8]

[5]These SNPs pass the Hardy-Weinberg Equilibrium (HWE) test at p=0.34–0.9. The HWE test evaluates whether the genotypic proportions at each SNP express deviations from an equilibrium/constant distribution due to non-random mating and other factors that may result in such deviations. Higher p values suggest more confidence in not rejecting the random mating hypothesis and in lack of deviation from the HWE equilibrium.
[6]We include one indicator because we find that the effects of the two indicators for each SNP are in the same direction and generally insignificant from each other, except for SNP rs1435252, for which these two effects are significantly different at p<0.05. Therefore, we keep two indicators for SNP rs1435252. Another advantage of reducing the number of instruments is that in the presence of "weak instruments", which is the case for these instruments as described below, the bias in the IV estimate increases with the number of instruments (Hahn and Hausman 2003).
[7]The Norway sample had also been genotyped independently of the CIDR project for several other variants in *GABAB2* as well as *DDC,* and *CHRNA4,* which are genes that have also been implicated in smoking behaviors (Ma et al. 2005; (Li et al. 2005). However, these variants had insignificant effects on smoking during the first pregnancy trimester. Supplementary Table S2 reports the results for the relationship between these additional variants and first trimester smoking.

3.3 Model Estimation

We estimate the following function for OFC status:

$$OFC_i = \alpha_0 + \beta CIGARETTES_i + \sum_{s=1}^{S} \delta_s SOCIOECONOMIC_{si} + \sum_{b=1}^{B} \lambda_b OTHER\_BEHAVIOR_{bi} + v_i.$$

(1)

For child i, OFC status is a function of maternal cigarettes smoked before pregnancy or alternatively in the first trimester (*CIGARETTES*), maternal socioeconomic and demographic factors that are relevant for OFC including age, education and income (*SOCIOECONOMIC*), and other risk and health behaviors (*OTHER_BEHAVIOR*) that may affect OFC. We include the following in *OTHER_BEHAVIOR*: participation in and intensity of alcohol drinking during the first pregnancy trimester, multivitamin intake in the first trimester, body weight indicators at pregnancy, average number of daily calories during the first trimester, and pregnancy intendedness. [9] Table 1 includes a description of the model variables.

As described above, we estimate this function using an IV model in order to account for the correlation between *CIGARETTES* and *v* (i.e. the correlation between CIGARETTES and the unobserved confounders that also affect OFC), which if ignored, may lead to a biased estimation of the effect (*β*) of *CIGARETTES* on OFC. The IV model involves an additional function for *CIGARETTES* which we specify as follows:

$$CIGARETTES_i = b_0 + \sum_{g=1}^{G} \pi_g GENES_{gi} + \sum_{s=1}^{S} \omega_s SOCIOECONOMIC_{si} + \sum_{b=1}^{B} \xi_b OTHER\_BEHAVIOR_{bi} + e_i,$$

(2)

where *GENES* include the genetic instruments for cigarettes as described above.

We estimate the IV model using conditional maximum likelihood (CML) which simultaneously estimates equations (1) and (2) with equation (1) estimated as a probit functional form. The model has several advantages over other estimation approaches (Wooldridge 2002). First, CML provides the most efficient estimator if the distributional assumptions are correctly specified. Second, the average partial effects of the model variables can be directly estimated without retransformation. Third, the hypothesis of exogenous cigarette selection (i.e. no confounding bias when ignoring unobserved factors) can be directly evaluated by testing the correlations between the error terms *v* and *e*. A significant correlation indicates that cigarettes are endogenous and that standard probit estimates may be biased.

In order to evaluate the sensitivity of the cigarette effect estimates to the CML assumptions, we also estimate the IV model using a two-stage IV probit model. This is the two-stage residual substitution method of Rivers and Vuong (1988), which includes the OLS residual term (e) of equation (2) as a regressor in equation (1), which is estimated by probit as follows (Rivers and Vuong 1988):

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[8]We evaluate the over-identification restrictions based on Newey's (1987) minimum chi-square distance estimator of the IV probit model (Newey 1987).

[9]Some studies report that maternal alcohol consumption, mainly excessive consumption, increases the risk of OFC (e.g. (Grewal et al. 2008; Romitti et al. 1999). However, other studies report insignificant (e.g. (Bille et al. 2007; Meyer et al. 2003). Some studies also report that maternal obesity increases OFC risk (e.g. (Stothard et al. 2009; Villamor, Sparen, and Cnattingius 2008) but others do not find significant effects (Shaw 2000). Food insecurity may also increase cleft palate risk (Carmichael et al. 2007). Low socioeconomic resources may increase OFC risk (Clark et al. 2003; Durning P 2007; Yang et al. 2008). We include pregnancy intendedness as it reflects maternal preferences for risk taking and may proxy for some unobserved confounders.

$$OFC_i = \alpha_0 + \beta CIGARETTES_i + \sum_{s=1}^{S} \delta_s SOCIOECONOMIC_{si} + \sum_{b=1}^{B} \lambda_b OTHER\_BEHAVIOR_{bi} + \gamma e_i + u_i$$

(3)

Testing the coefficient γ of e in equation (3) evaluates whether cigarettes are endogenous. One advantage of this method is that it does not impose the CML joint distributional assumptions. However, a disadvantage is that equation (1) coefficients are estimated only up to a scale (Wooldridge 2002). Obtaining the marginal effects requires rescaling the coefficients. We employ Wooldridge's (2002) approach for rescaling the coefficients.[10]

Estimating the marginal effects of cigarettes on the sample probability (proportions) of OFC alone is less intuitive and insufficient in case-cohort and case-control designs. Given that the outcome is a binary measure and the data is collected using with a case-cohort design, we also estimate the "odds ratio" (OR) for smoking effects as follows:

$$OR = \frac{\frac{p_s}{1-p_s}}{\frac{p_n}{1-p_n}},$$

(4)

where p_s is the average predicted OFC probability at the mean cigarette number among smokers and p_n is the average predicted OFC probability with no smoking. The OFC probabilities are predicted using the estimated regression coefficients for all observations based on their values for the model variables (except for cigarette number as explained above) and then averaged across the sample in order to estimate p_s and p_n. The OR is the standard effect estimate for case-control and case-cohort designs, and is a consistent estimate of the relative risk for rare outcomes such as OFC. The OR as defined in equation (4) based on a probit function is virtually similar to that obtained from a logistic regression[11]. However, logistic regressions are significantly harder to estimate in the case of endogenous regressors, especially using conditional maximum likelihood. The confidence intervals for the ORs are estimated using bootstrap with 2,000 replications.[12] As a reference model, we also estimate equation (1) using standard probit regression ignoring the problem of unobserved confounders.

Another way of interpreting the regression coefficients involves weighting the regression models by the sample probability weights, which provides an estimate of the effects of smoking on oral cleft incidence in the population. As mentioned above, the study sample includes most of the infants with OFC born in Norway during the study years and randomly selected unaffected births. This allows constructing probability weights for participation in the study. However, these probability weights are rather simplistic as they do not account for potential differences in study participation by factors that may affect oral clefts. Also, these weights make the CML model unstable and cause convergence problems. Therefore, as an

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[10]This approach averages the predicted probabilities from equation (3) across all observations i (so that the predicted effects are averaged across e_i).

[11]In models that include only smoking and no covariates, the OR as defined in equation (4) that is obtained from a probit function is *identical* to that from a logistic function, which is the standard regression model used in case-control or case-cohort designs. For example, both logistic regression and probit regression of OFC on smoking participation (yes/no) in the first trimester provide an OR estimate of 1.54. When the model includes additional covariates, the odds ratio from the probit model is expected to differ slightly from the logistic regression OR, because unlike the logistic regression OR, the probit OR is dependent on the covariate values. However, the ORs from the two models are still expected to be very close to each other. For example, when adding the covariates described above into the model, the OR for first trimester smoking participation is 1.379 and 1.370 in the probit and logistic regressions, respectively.

[12]For the bootstraps, we set the maximum number of iterations for each IV-probit CML regression to 10. Using the full study sample, The CML model converged at 5 iterations with cigarettes before pregnancy and 6 iterations for first trimester cigarettes.

alternative approach, we use these sample probability weights with the standard probit and the two-stage probit model to evaluate the smoking effects on OFC incidence.

The variables in *OTHER_BEHAVIOR* may be correlated with unobserved confounders, which may bias the model estimates. We do not have access to instruments to account for the endogenous maternal selection into all of these factors. In order to gauge their effects on the cigarette effect estimates, we estimate an additional specification of equation (1) by CML that excludes *OTHER_BEHAVIOR*. The expectation is that the IV cigarette effect estimates should be insensitive to any potential biases in these variables if it is insensitive to their exclusion.

3.4 Weak Instruments

As described below, the genetic instruments employed in this study have statistically significant effects on cigarette smoking (F statistic of 3.33 in the main instrument specification; p=0.0009). However, the magnitude of these effects suggests that they are "weak", or not strongly correlated with cigarette smoking[13]. If instruments are "exogenous", which is the required IV assumption, weak instruments are expected to bias the IV effect estimates towards the classical estimates and the IV variance estimates downward (Hahn and Hausman 2003). Therefore, standard inference approaches may be biased with weak instruments. In order to assess the weak-instrument effects on our inference, we re-estimate the IV probit function using Newey's minimum chi-square distance estimator (Newey 1987) with weak-instrument robust confidence bounds for the cigarette coefficients and compare these confidence bounds to the usual bounds that do not account for weak instruments (Finaly and Magnusson 2009). We show below that the cigarette marginal effects are comparable between Newey's IV probit estimator and the CML estimator, providing support for using the Newey's estimator to evaluate the weak-instrument effects on inference.[14]

Assuming exogenous instruments, weak-instruments are expected to result in over-rejection of the over-identification restrictions (Hahn and Hausman 2003). Therefore, given that our evaluations of the second IV condition suggest that the instruments are exogenous as described below, we do not expect that the weak-instrument problem is resulting in an inability to reject the over-identification restrictions.

4. RESULTS

4.1. Main Estimates

Table 2 reports the effects of smoking before pregnancy and during the first pregnancy trimester on OFC risk using the main instrument specifications. [15] Also listed are the results of testing the joint instrument effects on cigarettes (first IV assumption), the excludability of the instruments from the OFC function using the over-identification test (second IV assumption), and the exogenous selection of cigarettes.

Cigarette smoking before pregnancy or during the first trimester has a small and marginally significant effect on OFC using standard probit. Smoking 9 cigarettes per day before pregnancy (the average smoking rate among smokers) increases OFC risk by 1.2 times

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[13]The "threshold" for weak instruments varies with several parameters including sample size, number of instruments, and other parameters. However, F-statistics below 10 are generally considered to suggest weak-instruments in linear models (Staiger and Stock 1997). There are no rules of thumb for instrument strengths in the CML model, but the instruments may also be weak in that model.
[14]The weak-instrument robust confidence bounds are only available for the Newey's IV probit estimator and are not available for the other IV probit models (CML IV-Probit or the two-stage IV probit with residual substitution).
[15]Table S3 in the Supplementary Material reports the full CML regression results for the OFC functions in (Equation 1). Table S4 reports the full regression results for the cigarette functions in the CML model (Equation 2).

relative to non-smoking. A similar effect is observed for smoking 6 cigarettes per day during the first trimester, which is the average smoking rate among smokers.[16]

Using the main instrument specification and the CML-IV probit model, the cigarette smoking coefficients increase and become more strongly statistically significant. Average cigarette smoking (9 cigarettes per day) before pregnancy relative to non-smoking significantly increases OFC risk by about 4.2 times. Smoking during the first pregnancy trimester has a larger effect, increasing OFC risk by 5.4 times at the first trimester average smoking rate of 6 cigarettes. The hypothesis of the exogenous selection of cigarette smoking is rejected for both smoking measures (at p <0.05). As expected, the bootstrapped-based OR confidence intervals are generally less statistically significant compared with the cigarette regression coefficients, given that multiple variables (and variances) are averaged in the OR estimate and its variance.

The main instrument specification has statistically significant effects on cigarette smoking both before pregnancy and during the first pregnancy trimester (p <0.01 and <0.05, respectively). However, as mentioned above, the instruments may be considered "weak" based on the magnitude of their effects in the reduced-form model (F statistics of 3.33 and 2.63 for cigarettes before pregnancy and during the first trimester, respectively). The over-identification restrictions (instrument excludability from the OFC function) are not rejected at p=0.86 for cigarettes before pregnancy and p=0.77 for smoking in the first pregnancy trimester.

4.1.a Excluding Cleft Palate Only—Table 3 reports the smoking effects on cleft lip with/without palate (CL/P) excluding cleft palate only from the sample.[17] The same pattern of differences in effects between the probit and CML-IV probit is observed as in the total sample. However, the IV smoking effects are larger than in the combined sample. In the probit model, average smoking before pregnancy (9 cigarettes per day) or during the first pregnancy trimester (6 cigarettes per day) significantly increases OFC risk by about 1.3 times. Under CML-IV probit, average smoking before pregnancy increases OFC risk by 4.9 times. A larger effect is observed for first trimester smoking, with a 6.1 times increase in OFC risk. The cigarette coefficients are statistically significant for both periods under the CML-IV probit model, but only the OR for smoking before pregnancy is marginally significant. Similar to the total sample, the exogenous selection of smoking is also rejected for the CL/P sample (at p<0.05). The instruments have similar effects in the CL/P subsample as in the total sample, and the over-identification restrictions are not rejected (p=0.79 for smoking before pregnancy and 0.64 for first trimester smoking).

4.2 Sensitivity Analysis

4.2.1 Smoking before Pregnancy—Table 4 reports the effects of smoking before pregnancy on OFC under alternative model/instrument specifications and estimation approaches. Excluding the other potential endogenous variables from the model (*OTHER_BEHAVIOR*) has virtually no effect on the cigarette smoking effect or other test

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[16]We interpret the smoking effects using odds ratios based on the estimated cigarette number regression coefficients by simulating the effects of smoking the average number of cigarettes among smokers relative to not smoking. The average cigarette numbers (9 cigarettes per day before pregnancy and 6 per day during the first trimester) used in this simulation are conditional on being a smoker. Note that these effects may not be directly compared to odds ratios for smoking participation (yes/no) effects. One reason is that cigarette number is likely to be measured with some error, which attenuates the effect towards zero. We do not use binary smoking status or categorical indicators for cigarette number because of the limited instrument effects on these measures. Therefore, these effects should not be contrasted with odds ratio effects reported in previous studies using this sample (Lie et al. 2008). For instance, adjusting for the model covariates described above, we estimate an odds ratio of any smoking during the first trimester of 1.38 (95% CI: 1.02; 1.88) for OFC and 1.55 (95% CI: 1.1,2.17) for cleft lip without palate.
[17]Table S5 in the Supplementary Material reports the full regression results for the OFC function excluding cleft palate only. Table S6 reports the full regression results for the cigarette function.

results. Furthermore, the smoking effect is similar using the two-stage IV probit model (with residual substitution) to the CML-IV probit model, with also a 4.2 times higher OFC risk with average smoking.

The pattern of higher smoking effects under the IV model compared with the probit model is consistent across the alternative instrument specifications, and the OR estimate is slightly larger than the main instrument specification and ranges from 4.6 to 4.8 times across these specifications. The exogenous selection of cigarettes is rejected under these instrument specifications. Under all specifications, instruments have statistically significant effects on smoking (at $p < 0.01$) and the over-identification restrictions cannot be rejected.

4.2.2 First Trimester Smoking—Table 5 reports the first trimester smoking effects on OFC under alternative model and instrument specifications. Similar to smoking before pregnancy, the smoking effects are generally insensitive to excluding the *OTHER_BEHAVIOR* vector from the model (the effect increases slightly in the probit model). The effect in the two-stage IV probit model is virtually similar to that of the CML-IV probit model. The exogenous cigarette selection is rejected in these specifications.

Similar to the first instrument specification, the first trimester smoking effect under instrument specification 2 is larger than the probit effect with an OR of 5.9 (marginally significant). The exogenous selection of cigarettes is rejected. The instruments have significant effects on smoking ($p < 0.05$), and the over-identification restrictions cannot be rejected.

4.2.3 Weak-Instrument Effects—Table 6 reports the cigarette coefficients in the IV probit regression estimated using the minimum chi-square distance estimator (Newey 1987). Also included in Table 6 are the standard 95% confidence bounds for the coefficients, which do not assume weak-instruments, and confidence bounds that are robust for weak instruments. Evaluating the sensitivity of the inference to the instrument weakness involves comparing the standard and the weak-instrument robust confidence bounds for this estimator. Alternative weak-instrument robust confidence bounds are presented using the Conditional Likelihood Ratio (CLR), Anderson Rubin (AR), Lagrange Multiple (LM) and the LM-J statistics (Andrews, Moreira, and Stock 2006; Finaly and Magnusson 2009).[18] The CLR test is more powerful under weak-instrument identification than the AR or the LM test (Andrews et al. 2006). However, we report the AR and the LM bounds for comparison purposes.

Inference for the smoking regression coefficient using the classical asymptotic standard errors is overall comparable between Newey's IV probit estimator and the CML-IV probit estimator for both smoking measures. This provides support for evaluating the weak-instrument issue using Newey's estimator. The CLR, LM and LM-J weak-instrument robust confidence bounds result in similar inference for the smoking regression coefficient as the standard confidence bounds under all employed instrument specifications. However, the AR confidence bounds suggest insignificant smoking coefficients under all specifications. Given that the AR test suffers from lower power than the other statistics, which all result in similar inference, these results suggest that the weak-instrument problem is unlikely to have substantially biased the IV smoking effects presented above. Of course, this conclusion is conditional on the instruments being truly exogenous. Both the over-identification tests described above and the current knowledge of the biologic functions of the instrument genes and of the OFC genetic etiology support the assumption that the instruments are exogenous.

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[18]The regression coefficients cannot be directly compared between these models as they require transformation to obtain the variable effects.

4.2.4 Weighted Models and Cigarette Effects on Incidence—We estimate the population incidence of OFC based on the study sample and the employed probability weights to be about one affected birth per 500 births, which is similar to what has been previously estimated for Scandinavian populations.(Christensen 1999; Harville et al. 2007; Harville et al. 2005). Table 7 reports the smoking and quitting effects on the incidence OFC, which is estimated by weighting the models with probit and two-stage probit models with the sampling probability weights described above. When weighted, the standard probit model suggests that a population-level smoking rate before pregnancy of about 9 cigarettes per day (the average number of cigarettes among smokers) increases the population OFC incidence rate by one additional OFC birth per 2,500 births or by about 20% relative to no smoking (marginally significant). This model predicts that OFC incidence would decrease by about 8% if all current smokers quit (marginally significant). However, the weighted two-stage probit model predicts that a population-level smoking rate before pregnancy of about 9 cigarettes per day relative to no smoking increases OFC incidence by one additional affected birth per 200 births, or by about 245%, and that incidence would decrease by about 70% if all smokers quit (marginally significant). The weighted models for smoking during the first trimester show generally comparable effects on OFC incidence but the effects are overall statistically insignificant based on the bootstrap confidence bounds.

5. DISCUSSION

The study provides an application of genetic instruments for studying the effects of behaviors on health. The findings generally support the utility of employing genetic instruments for obtaining consistent estimates of maternal risk behavior effects on child health. Accurate estimates of the "causal" behavioral effects are needed for designing effective prevention programs for adverse child health outcomes and devising public policies to improve child health. To our knowledge, this application is among the first to use genetic instruments for assessing maternal behavior effects on infant health. The paper also illustrates an application of instrumental variables using data from case-cohort or case-control designs and ways of interpreting the behavior effects in such situations.

The employed instruments are predictive of cigarette smoking and do not appear to be related to health outcomes (OFC) through unobserved pathways based on the literature or statistical tests (including the inability to reject the over-identification restrictions at high critical values and the observation of similar smoking effects when excluding other relevant behavioral factors from the model). Of course, it is still possible that the instruments may somehow be related to OFC through unobserved, as is the case for any application using genetic instruments. As further knowledge is obtained on the genes' functions and pathways, it is important to reevaluate the evidence for the exogeneity of the instruments and its implications for the results. One limitation of the instruments employed in this study that may be relevant to other applications is that instruments may have "weak" (although statistically significant) effects on behaviors. This is not surprising given the complex etiologies of risk behaviors, which may involve several genetic, economic and psychosocial factors, and given that large datasets with genetic instruments are unavailable for many applications, especially for studying maternal behavior effects. In this case, it is important to employ weak-instrument robust inference methods such as those presented above.

Genetic instruments provide a unique source of behavior variation to identify "causal" behavioral effects. In applications where the genetic instruments may be "weak", the benefits of the genetic instrumental variables approach may outweigh the inference challenges due to "weak instruments" given that weak-instrument robust inference methods are available for most IV estimators. This is particularly relevant when non-genetic instruments are not available or suffer from theoretical limitations. While prices/taxes and

other area-level variables are commonly used as instruments for smoking and other risk behaviors, there are significant theoretical and empirical challenges in employing these instruments as described above. We are limited to a small set of already genotyped variants as instruments for smoking in this study. These instruments are considered "weak" as discussed above. Future studies are expected to have better access to stronger instruments as the genetic etiologies of behaviors are further revealed and genotypic data become more widely available. Recent research studies have identified new variants that have strong effects on smoking. Of these, variants in *CHRNA3* and *CHRNA5* are the most promising and are strong candidates to be used as instruments (Liu et al, 2010).[19] Future studies are needed to formally evaluate the utility of these variants as instruments for smoking.

The study results suggest that maternal cigarette smoking may substantially increase the child's OFC risk, and that this effect may be significantly underestimated in analyses that ignore unobserved confounders. If our estimates are correct, the prevention of all cigarette smoking at the population level may reduce OFC incidence by more than 50%. The study provides further evidence that direct adjustment for observed confounders may be insufficient for consistent estimation of maternal behavioral effects due to self-selection into these behaviors based on unobserved factors that also affect child health.

The results are consistent with favorable self-selection into smoking based on unobserved characteristics that may reduce the OFC risk. In other words, women who smoke at and during pregnancy may, on average, have higher rates of certain unobserved "baseline" characteristics that reduce OFC risk, and therefore result in underestimating the harmful smoking effects when ignored in classical single-equation models. These characteristics may include favorable family and child health history and lower maternal baseline health risks. Such factors may increase maternal propensity to smoke, but may relate to unobserved "health endowments" that reduce OFC risk such as favorable genetic, economic or psychosocial factors. This may seem counterintuitive given that most observable characteristics suggest adverse self selection into smoking with less education, unemployment, alcohol drinking, underweight, not using vitamins and not planning the pregnancy being positively correlated with cigarette smoking (see Table S3). The only exception is the positive correlation between income and cigarette smoking. We cannot evaluate further the hypothesis of adverse self-selection based on unobservables. However, the change in smoking effects on OFC with the IV estimation is consistent with several previous IV studies of smoking impacts on birth weight using cigarette tax rates and other non-genetic instruments (Evans and Ringel 1999; Grossman and Joyce 1990; Lien 2005; Permutt and Hebel 1989; Rosenzweig 1983) which find larger adverse smoking effects using the IV models and also with IV studies of other behavioral effects on child health such as prenatal care effects on birth weight, which also appear to be underestimated in classical models (Wehby et al. 2009a, 2009b).

One potential bias source that may contribute to underestimating adverse smoking effects in classical models is biased reporting of maternal smoking status depending on observed OFC status. Mothers of children with OFC may be less likely to report, post delivery, their participation in and intensity of smoking before and during pregnancy compared to mothers of unaffected children due to guilt feeling and avoiding blame. In this study, mothers reported smoking after the child's birth (by about 4 months). However, previous analyses of

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[19]Recent studies have provided several additional candidates for smoking, with confirmed associations for the gene encoding cholinergic receptor, nicotinic, alpha 5 (*CHRNA5* on chr 15q24) in multiple, independent studies (Hung et al. 2008; Liu et al. 2010; Saccone et al. 2010; Thorgeirsson et al. 2008; Thorgeirsson et al. 2010). Additional susceptibility loci have been identified through recent genome-wide association studies, including acetylcholine receptor genes *CHRNB3* and *CHRNA6* on chr 8p11, egl nine homolog 2 (*EGLN2*) on chr 9q13, brain-derived neurotrophic factor (*BDNF*) on chr 11p13, *CHRNA3* on chr 15q24, and cytochrome P450 genes *CYP2A6* and *CYP2B6* on chr 19q13 (2010; McKay et al. 2008; Thorgeirsson et al. 2010).

this data showed that differences between smoking status reported prospectively by the study women during their first prenatal visit (available through birth registry data), which was around 10.3 gestational weeks and for most cases likely before obtaining information about OFC status, and first trimester smoking status as reported post delivery, are similar between mothers of affected and unaffected children (Lie et al. 2008). Therefore, it is unlikely that biased smoking report based on observed OFC is resulting in underestimating smoking effects in the classical probit model. However, it is possible that the smoking effects are attenuated toward zero in the classical models due to random errors in smoking self-report or biases that are not related to OFC, which may partially explain the observed increase in the effects of smoking when treated as endogenous. Intuitively, the estimates of the IV model are expected to apply mainly to those whose behaviors change with the instruments[20], which may also contribute to the increase in smoking effects if these instruments affect smoking behaviors in specific ways (e.g., spacing or timing of cigarette use or smoking certain brands), or if those who smoke because of these instruments have different characteristics that intensify the smoking effects, although we cannot evaluate this in this study. It also remains theoretically possible that part of the increase is due to noise introduced by the instruments or unforeseen endogeneity issues with the instruments that may be aggravated by the instrument weakness. Therefore, it is important to replicate this study in the future with the recently identified and potentially stronger instruments for smoking.

One limitation of designs that condition sampling on the study outcome such as case-control or case-cohort designs is that they are at higher risk for sample selection bias. This bias occurs when the sampling frame or study participation is related to "unobserved" factors that also affect the outcome (Heckman 1979). Additional sample selection bias that is particularly relevant for IV applications using such data may occur if the sampling frame or study participation is related to unobserved factors that are related to both the outcome and the employed instruments. While such limitations are theoretically possible, we do not expect that either the sampling frame or maternal decision to participate in this study is related to unobserved factors that affect OFC or are related to the employed genetic instruments. The study case-cohort sample included the majority of the eligible OFC population in Norway during the study period and a random sample of unaffected children. When suspected, sample selection should be modeled and accounted for using approaches that explicitly account for the role of unobserved relevant factors that result in this bias such as Heckman's approach.

The IV model using genetic instruments may be applied in several other frameworks and research areas that so far have either relied solely on adjusting for observed confounders or utilized instruments that may not be effective in accounting for omitted variable bias. Studies have identified specific genetic risk factors for major behavioral risk factors such as alcohol use (Edenberg and Foroud 2006; Luo et al. 2006; Tolstrup et al. 2008) and obesity (Dina et al. 2007; Frayling et al. 2007; Loos et al. 2008; Qi et al. 2008; Willer et al. 2009). In the US, 34% of women of childbearing age are obese and 8% are extremely obese – another 25% are overweight (Flegal et al. 2010). The alarmingly high obesity rates increase the importance of obtaining consistent estimates of the "causal" maternal obesity effects on child health in order to forecast the impacts of changes in obesity rates on disease incidence and assess the returns of prevention programs.

In conclusion, the study provides a novel application using genetic instruments to assess the "causal" effects of maternal smoking before and during pregnancy on child health in the

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[20]Unlike for the local average treatment effect of two stage least squares (2SLS), this is not a strict but rather an intuitive interpretation of the effects in the CML and other IV probit models.

form of OFC status. The study finds that genetic instruments may be useful for such applications and highlights some potential limitations of this approach and ways for addressing them. Employing genetic instruments provides a valuable approach for accounting for unobserved confounders and obtaining consistent estimates of the "causal" behavior effects. The model may be used to study maternal risk behavior effects on various infant and child health outcomes such as birth weight, fetal growth, preterm birth, birth defects and child development and also to study long-term behavior effects on health, economic and psychosocial status.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Description of the Study Variables

Variable	Definition	Mean (SD)
Cigarettes before pregnancy	Number of cigarettes per day smoked in the 12 months before pregnancy	4.2 (6.4)
Cigarettes in the first trimester	Number of cigarettes per day smoked in the first trimester	2.3 (4.3)
Maternal age	Maternal age in years	29.3 (4.7)
Less than high school[a]	Binary (0,1) indicator for maternal education of less than completed high school	0.111 (0.314)
High school[a]	Binary (0,1) indicator for maternal education of completed high school	0.255 (0.436)
Technical college[a]	Binary (0,1) indicator for mother attending technical college	0.202 (0.402)
University[a]	Binary (0,1) indicator for mother completing university education	0.064 (0.245)
Maternal employment	Binary (0,1) indicator for maternal employment in the first trimester	0.839 (0.368)
Very low maternal income[b]	Binary (0,1) indicator for current gross maternal yearly income of less than 150,000 kr	0.359 (0.48)
Moderate maternal income[b]	Binary (0,1) indicator for current gross maternal yearly income between 201,000 and 250,000 kr inclusive	0.244 (0.43)
High maternal income[b]	Binary (0,1) indicator for current gross maternal yearly income of 251,000 kr or more	0.141 (0.349)
Binge drinking in the first trimester[c]	Binary (0,1) indicator for ≥ 4 drinks per drinking occasion in the first trimester	0.06 (0.237)
Moderate drinking in the first trimester[c]	Binary (0,1) indicator for 2–3 drinks per occasion in the first trimester	0.092 (0.29)
Low drinking in the first trimester[c]	Binary (0,1) indicator for 1 drink per occasion in the first trimester	0.189 (0.392)
Multivitamin use	Binary (0,1) indicator for using multivitamins before and during the first two months of pregnancy	0.359 (0.48)
Underweight[d]	Binary (0,1) indicator for maternal BMI prior to pregnancy of less than 18.5	0.03 (0.172)
Overweight[d]	Binary (0,1) indicator for maternal BMI prior to pregnancy between 25 and 29.9 inclusive	0.19 (0.393)
Obese[d]	Binary (0,1) indicator for maternal BMI prior to pregnancy of greater than 29.9	0.079 (0.271)
Calories	Average daily calorie intake during the first trimester	2244.6 (750.8)
Pregnancy planning	Binary (0,1) indicator for mothers who were planning to become pregnant	0.735 (0.442)
rs1930139_BB[e]	Binary (0,1) indicator of AB genotype of rs1930139	0.327 (0.469)
rs1930139_AB[e]	Binary (0,1) indicator of BB genotype of rs1930139	0.051 (0.221)
rs1547272_BB[e]	Binary (0,1) indicator of AB genotype of rs1930139	0.345 (0.476)
rs1547272_AB[e]	Binary (0,1) indicator of BB genotype of rs1930139	0.06 (0.237)
rs1435252_AA[f]	Binary (0,1) indicator of AB genotype of rs1435252	0.082 (0.274)
rs1435252_AB[f]	Binary (0,1) indicator of BB genotype of rs1435252	0.405 (0.491)
rs2743467_BB[e]	Binary (0,1) indicator of AB genotype of rs2743467	0.43 (0.495)
rs2743467_AB[e]	Binary (0,1) indicator of BB genotype of rs2743467	0.11 (0.313)

Note: The sample includes 856 observations.

[a]The reference category is 2–4 years of college;

[b]The reference category is 151,000–200,000 kr;

[c] The reference category is no drinking;

[d] The reference category is normal weight (18.5≤BMI<25);

[e] The reference category for the SNP genotype indicators is the AA genotype;

[f] The reference category for the SNP genotype indicator is the BB genotype.

Table 2

Cigarette Smoking Effects on OFC

Model	Probit Regression Coefficient	Odds Ratio [95% CI]	1st IV condition X^2 [df]	2nd IV condition X^2 [df]	Exogenous Cigarettes X^2 [1]
Cigarettes before Pregnancy					
Probit	0.013* (0.007) [0.005*]	1.2* [0.97,1.48]			
CML-IV probit	0.100*** (0.029) [0.033***]	4.16** [1.06,8.0]	26.87*** [8]	3.3 [7]; p=0.86	5.7**
Cigarettes in the first trimester					
Probit	0.020* (0.011) [0.008*]	1.21* [0.98,1.52]			
CML-IV probit	0.172*** (0.046) [0.057***]	5.41* [0.88,9.81]	15.54** [6]	2.52 [5]; 0.77	5.31**

Note: The models are based on a sample size of 856 observations. Standard errors of regression coefficients are in parentheses. The marginal effects of cigarettes, evaluated holding all model variables at their means, are listed in brackets under the regression coefficient column (the standard errors of the marginal effects are suppressed for simplicity but are available from the authors upon request). The 95% confidence bounds of the odds ratio estimates are in brackets. The first IV condition column reports a chisquare test of the joint significance of the instruments in the cigarettes function (degrees of freedom [df] in brackets). The second IV condition reports the over-identification chi-square test (df in brackets).

*
p < 0.1,

**
p < 0.05,

***
p < 0.01.

Table 3

Cigarette Smoking Effects on Cleft Lip with/without Cleft Palate

Model	Probit Regression Coefficient	Odds Ratio [95% CI]	1st IV condition X^2 [df]	2nd IV condition X^2 [df]	Exogenous Cigarettes X^2 [1]
Cigarettes before Pregnancy					
Probit	0.0156* (0.008) [0.006**]	1.25** [1.004,1.58]			
CML-IV probit	0.110*** (0.030) [0.034***]	4.92* [0.999,8.9]	22.44*** [8]	3.92 [7]; 0.79	5.65**
Cigarettes in the First Trimester					
Probit	0.024** (0.012) [0.009]***	1.27** [1.03,1.64]			
CML-IV probit	0.180*** (0.045) [0.057***]	6.13 [0.16,12.28]	14.98**	3.39 [5]; 0.64	5.33**

Note: The models are based on a sample size of 750 observations. Standard errors of regression coefficients are in parentheses. The marginal effects of cigarettes, evaluated holding all model variables at their means, are listed in brackets under the regression coefficient column (the standard errors of the marginal effects are suppressed for simplicity but are available from the authors upon request). The 95% confidence bounds of the odds ratio estimates are in brackets. The first IV condition column reports a chisquare test of the joint significance of the instruments in the cigarettes function (degrees of freedom [df] in brackets). The second IV condition reports the over-identification chi-square test (df in brackets).

*
 $p < 0.1$,

**
 $p < 0.05$,

***
 $p < 0.01$.

Table 4

Effects of Cigarette Smoking before pregnancy on OFC under Alternative Model and Instrument Specifications

Model	Probit Regression Coefficient	Odds Ratio [95% CI]	1st IV condition X^2/F [df]	2nd IV condition X^2 [df]	Exogenous Cigarettes X^2 [1]
Excluding OTHER_BEHAVIOR					
Probit	0.013* (0.007)	1.21* [0.99,1.51]			
CML-IV probit – Instrument Specification 1	0.099*** (0.030)	4.2** [1.01,9.13]	24.52*** [8]	3.08 [7]; p=0.88	5.33**
Two stage IV probit – Instrument Specification 1	0.11** (0.04)	3.82** [1.09,7.13]	3.3*** [8, 829]		4.98**
CML-IV probit under alternative instrument specifications					
Instrument Specification 2	0.11*** (0.03)	4.73** [1.13,8.17]	21.8*** [5]	2.45 [4]; p = 0.65	6.24**
Instrument Specification 3	0.11*** (0.03)	4.59** [0.94,8.19]	19.19*** [6]	2.88 [5]; p= 0.72	5.00**
Instrument Specification 4	0.11*** (0.03)	4.82** [0.96,8.32]	17.42*** [4]	2.45 [3]; p= 0.48	5.14**

Note: The models are based on a sample size of 856 observations. Standard errors of regression coefficients are in parentheses. The 95% confidence bounds of the odds ratio estimates are in brackets. The first IV condition column reports a chisquare test of the joint significance of the instruments in the cigarettes function (degrees of freedom [df] in brackets). The second IV condition reports the over-identification chi-square test (df in brackets).

*
$p < 0.1$,

**
$p < 0.05$,

***
$p < 0.01$.

Table 5

First Trimester Cigarette Smoking Effects on OFC under Alternative Model and Instrument Specifications

Model	Probit Regression Coefficient	Odds Ratio [95% CI]	1st IV condition X^2/F [df]	2nd IV condition X^2 [df]	Exogenous Cigarettes X^2 [1]
Excluding OTHER_BEHAVIOR					
Probit –	0.023** (0.011)	1.26** [1.02,1.56]			
CML-IV probit – Instrument Specification 1	0.172*** (0.046)	5.5* [0.65,11.5]	13.88** [6]	2.49 [5]; p=0.78	4.93**
Two stage IV probit – Instrument Specification 1	0.190** (0.081)	5.1* [0.99,11.26]	2.63** [6, 831]		4.51**
CML-IV probit under alternative instrument specifications					
Instrument Specification 2	0.181*** (0.045)	5.88* [0.97,9.95]	13.17** [6]	2.04 [3]; p=0.56	5.51**

Note: The models are based on a sample size of 856 observations. Standard errors of regression coefficients are in parentheses. The 95% confidence bounds of the odds ratio estimates are in brackets. The first IV condition column reports a chi-square test of the joint significance of the instruments in the cigarette function (degrees of freedom [df] in brackets). The second IV condition reports the over-identification chi-square test (df in brackets).

*
p < 0.1,

**
p < 0.05,

***
p < 0.01.

Table 6

Weak-Instrument Robust Confidence Bounds for the IV probit Cigarette Smoking Coefficients

Model	IV-Probit Coefficient (Newey, 1987)	Conditional Likelihood Ratio (CLR)	Anderson Rubin (AR)	Lagrange Multiplier (LM)	LM-J
Smoking before pregnancy					
Instrument Specification 1	0.105** (0.046) [0.015,0.196]	[0.018,0.28]	[−0.043,0.521]	[0.025,0.263]	[0.02, 0.273]
Instrument Specification 2	0.121** (0.053) [0.028,0.323]	[0.03,0.319]	[−0.012,0.485]	[0.033,0.308]	[0.028,0.323]
Instrument Specification 3	0.114** (0.055) [0.006,0.221]	[0.011,0.357]	[−0.041,0.657]	[0.018,0.333]	[0.013,0.352]
Instrument Specification 4	0.121** (0.058) [0.006,0.235]	[0.019,0.365]	[−0.01,0.512]	[0.021,0.36]	[0.016,0.38]
First Trimester Smoking					
Instrument Specification 1	0.19** (0.092) [0.01,0.371]	[0.018,0.703]	[−0.068,1.633]	[0.036,0.616]	[0.027,0.66]
Instrument Specification 2	0.21** (0.103) [0.009,0.411]	[0.038,0.765]	[−0.017,1.361]	[0.044,0.724]	[0.035,0.785]

Note: The table reports the IV probit smoking coefficients estimated using the minimum chi-square distance estimator (Newey, 1987) and 95% weak-instrument robust confidence bounds based on the CLR, AR, LM and LM-J statistics. The usual standard errors and confidence bounds for the smoking regression coefficients are also reported in parentheses and brackets, respectively before the regression coefficient. The models are based on a sample size of 856 observations.

Table 7

Cigarettes Effects on OFC Incidence Based on Weighted Models

Model	Probit Regression Coefficient	APE of smoking versus no smoking on incidence	APE of current smokers quitting on incidence
Cigarettes before pregnancy			
Probit	0.0068* (0.0039)	0.0004* [−0.00005, 0.00097]	−0.00017* [−0.00038, 0.00002]
Two stage IV probit – Instrument Specification 1	0.06** (0.0234)	0.0049* [−0.00004, 0.0141]	−0.0014* [−0.0029, 0.00002]
Cigarettes in the first trimester			
Probit	0.0098* (0.0058)	0.0004* [−0.00004, 0.001]	−0.00012 [−0.0003, 0.00001]
Two stage IV probit – Instrument Specification 1	0.0884* (0.0459)	0.0055 [−0.002, 0.0256]	−0.001 [−0.0028, 0.0008]

Note: The table reports the regression coefficients of cigarettes in the OFC functions that are weighted by the sampling probability weights. Standard errors of regression coefficients are in parentheses. The average partial effects (APE) of smoking the "weighted" averages of cigarettes among smokers (about 9 cigarettes per day for smoking before pregnancy and 6 cigarettes per day for smoking during the first pregnancy trimester) relative to not smoking on the incidence of OFC are reported. The APE of all current smokers quitting on OFC incidence is also reported. The 95% CI of the APE based on 2000 bootstrap replications are in brackets. The models are based on a sample size of 856 observations.

*
$p < 0.1$,

**
$p < 0.05$,

***
$p < 0.01$.