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Candidate Gene Study for Smoking, Alcohol Use, and Body Weight in a Sample of Pregnant Women

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Abstract

Objective—Prenatal smoking, alcohol use, and obesity have significant effects on maternal and fetal health. However, not much is known about the genetic contributions to these risk factors among pregnant women. We evaluate the associations between several candidate genes and smoking, alcohol use, pre-pregnancy body weight, and weight gain during pregnancy in a sample of pregnant women.

Methods—The study analyzes a sample of about 1,900 mothers from the Danish National Birth Cohort. We test the association between 1,450 SNPs in/near 117 genes/loci and various risk factor measures.

Results—Only a few SNPs in *FTO* were significantly associated with pre-pregnancy obesity and body mass index (4 and 2 SNPs, *respectively*) after SNP-level correction for multiple testing. A few loci were significantly related to various smoking measures (any smoking, quitting, cigarette number) with gene/locus-level correction for multiple testing, but not after SNP-level correction. Similarly, some loci were significant for the alcohol measures at the gene/locus-level but not at SNP-level correction.

Conclusion—The study suggests that the majority of the evaluated candidate genes may not play an important role in influencing these risk factors among pregnant women, highlighting the importance of other genetic factors and non-genetic contributors to their etiology.

Keywords

Fetal health; child health; maternal health; prenatal risks; pre-pregnancy weight

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Author Declarations

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Early child health and development are important determinants of subsequent health and human capital and are significantly influenced by perinatal risk factors such as smoking, alcohol use, and obesity. These risk factors have been associated with a variety of adverse infant and child health outcomes and conditions including mortality, low birth weight (LBW), birth defects, asthma, obesity, and developmental delays/problems [1–4].

Infants of mothers who smoke during pregnancy are at greater risk for fetal growth retardation/low birth weight [5, 6], sudden infant death syndrome, and behavioral and mental health problems complications later in childhood [3, 7]. Similarly, there is a strong evidence for increased risks for small for gestational age (SGA) [8, 9], LBW [10, 11], preterm birth [12, 13], and fetal alcohol syndrome with heavy alcohol consumption [14]. Alcohol use during pregnancy may impact fetal brain development through a variety of pathways [15]. These brain changes related to alcohol exposure are associated with long-term mental health outcomes such as increased anxiety and depression [16]. Finally, the growing rate of obesity in the United States has prompted an increased focus on the impact of pre-pregnancy maternal body weight on both maternal and fetal/child outcomes. Adverse perinatal outcomes associated with maternal obesity include stillbirth [17, 18], macrosomia, congenital anomalies, birth injury and lower rates of breastfeeding initiation as well as shorter breastfeeding duration [19].

Developing cost-effective prevention and counseling programs for these maternal risk factors requires accurate characterization of their etiology. It is well-known that these risk factors in the general population have a complex genetic and environmental etiology. Smoking initiation and persistence are estimated to be at least 50% heritable across multiple studies [20–23]. In addition, up to 50% of alcohol dependence can be attributed to genetic effects [24, 25]. Similarly, obesity and body mass index (BMI) also have a high heritability estimated at 73% and 80%, respectively [26]. While much of this genetic etiology remains unknown, several studies have identified loci that are associated with these risk factors in the general population such as loci in *FTO* for obesity [27, 28], *CHRNA3* for cigarette number [29], and *ADH1B* for alcohol consumption [30, 31] although these loci still explain a very small percentage of the genetic heritability of these risk factors.

In addition, several candidate genes have been proposed for smoking, alcohol use, and BMI/obesity, a few of which have already been supported in the general population. However, little is known about the association between these candidate genes and the same risk factors among pregnant women. Most previous studies are based on “general” samples and it is unknown to what extent their results apply to pregnant women. One could theorize that genetic effects on behavioral risk factors could change during pregnancy compared to a more ordinary time. Pregnancy involves multiple physiological changes including metabolic, cardiac, and respiratory changes, hormonal elevations (increases in estrogen and progesterone), weight gain, and changes in physical activity as well as in several psychological domains involving stress, anxiety, self-control, expectations, and motivation. These psychological changes may be partly driven by the physiological changes but also by the pregnancy experience and expectations. Several health behaviors such as smoking and use of multivitamins are well-known to change during pregnancy [32]. Furthermore, women during pregnancy have increased interactions with healthcare providers through seeking

prenatal care and their knowledge and attitudes towards health behaviors may change as supported in previous research [33]. All these changes may result in modifying health behaviors during pregnancy. Furthermore, the pregnancy-induced behavioral changes may further modify the effects of genetic factors that influence the same or related behaviors through an interaction mechanism to play a stronger or weaker role during pregnancy than at a more ordinary time. For example, the associations between variants in the melanin-concentrating hormone receptor 1 (*MCHRI*) and measures of body weight and adiposity have been reported to vary with physical activity and carbohydrate intake [34], both of which may change during pregnancy. Similarly, it is well recognized that stress and hormonal changes during adolescence may modify genetic effects and expression on risky behaviors such as alcohol consumption [35]. One such interaction is between the corticotrophin releasing hormone receptor (*CRCHRI*) and stressful events reported to influence heavy drinking among adolescents [36]. Such findings support the theory of changing genetic effects on health behaviors among pregnant women compared to general samples.

Women who are planning their pregnancy (about 90% of the women in our study sample described below reported that they have planned their pregnancy including 12% who reported that they had partly planned their pregnancy) may modify their health behaviors and risks before pregnancy in order to improve their prenatal health. One such risk factor is pre-pregnancy weight [37], which is one of the most important indicators of preconception health that has been linked to multiple child health outcomes including fetal growth, birth weight, and subsequent child obesity [38]. In such cases, pregnancy planning may modify genetic influences underlying weight through an interaction mechanism that could either attenuate or exacerbate certain genetic effects. For example, the pre-pregnancy weight levels of women who are planning on becoming pregnant and reducing their weight before pregnancy may be less influenced by carrying a genetic risk for weight gain than their weight at other times of their life.

More generally, many health behavior choices including those related to smoking, drinking, and body weight among women of childbearing age differ overall from the rest of the population whether they are planning a pregnancy or not. Therefore, genetic associations with health behaviors may differ among women of childbearing age from the rest of the population. Evaluating how candidate genes relate to major health behaviors and risk factors both among women of childbearing age and during pregnancy is useful for understanding their etiology and may subsequently facilitate developing behavioral therapy interventions and treatments for quitting risky behaviors and reinforcing healthy ones. Subsequent research may show that specific genetic risks modify treatment effectiveness and predispose women to be more (or less) responsive to particular interventions, whether medical or behavioral during pregnancy. Understanding genetic influences on important markers of preconception health such as pre-pregnancy weight is especially useful given the importance of preconception health for both the mother and child [39].

To the best of our knowledge, close to a handful of studies provide data on differences in genetic associations with maternal behaviors during pregnancy compared to an ordinary time [40, 41, 42]. Nonetheless, this small literature provides suggestive evidence consistent

with the theory that genetic associations with smoking and drinking may change during pregnancy compared to a more ordinary time and that they could both intensify or decrease. For example, three studies focusing on single genes/loci report suggestive evidence that the effects of *ADH1B* and *CHRNA3-CHRNA5* on alcohol and smoking are stronger during than before pregnancy [40, 41, 42]. One of these studies found that the effect of rs1229984 in *ADH1B* – a variant with unequivocal evidence of being implicated in alcohol behaviors – on any alcohol consumption is about three times stronger during than before pregnancy [42]. In contrast, another study found that the effects of variants in *GABAB2* were more strongly related to smoking before pregnancy than the first trimester, the only period during pregnancy with such data in that study [43].

In this paper, we evaluate the extent to which candidate genes for smoking, alcohol use, and BMI/obesity associate with smoking, alcohol consumption, and weight gain during pregnancy and with pre-pregnancy weight. To our knowledge, this is the first study that evaluates a large number of candidate genes for these outcomes in a sample of pregnant women.

METHODS

This study utilizes data from the prematurity genome wide association study (GWAS) sample from the Danish National Birth Cohort (DNBC). The DNBC included more than 101,000 pregnant women and their newborns (about 96,800) in Denmark in 1996–2003 [44]. The prematurity study sample included 2,000 mothers of preterm and term infants and excluded multiple gestations and congenital anomalies [45]. The women provided a blood sample (for DNA extraction) at eight weeks of pregnancy (time of consent). Four interviews were conducted with the mother, two during pregnancy (around the 12th and 30th gestational weeks) and two post-delivery (6 and 18 months after delivery). Data were collected via computer-assisted telephone interviews. Further information on the mothers was obtained from the Danish health registers. The DNBC has the advantage of measuring several maternal risk factors and avoiding potential maternal reporting bias after observing the birth outcomes as risk factors were assessed before delivery. The prematurity GWAS genotyped the maternal DNA samples for 550K SNPs using Human660W-Quadv1_A (Illumina 660W) platform.

Our study combined data on women of preterm and preterm infants in order to analyze the largest available sample. Combining these two groups would result in confounding if: 1- the maternal genetic variants we studied are related to preterm birth risk, and 2- smoking, alcohol, and BMI/obesity were associated with preterm birth status. The literature does not support the first condition, and the association between these risk factors and preterm birth status is also weak. Furthermore, these risk factors preceded the infant's birth; therefore, having a preterm or term infant could not have "caused" these risk factors. Therefore, combining the two groups should not confound the analysis of candidate genes for these risk factors.

We analyze currently known candidate genes covered in this GWAS panel. We follow a candidate gene approach since the study sample is limited to undertake a genome wide

analysis. Previous studies have suggested a variety of candidate genes for smoking, alcohol use, and obesity, which we identified from the literature [27, 46–55]. We evaluated 1,450 SNPs that are within or near genes or loci that are considered candidates for these three risk factors and covered in the GWAS panel including 79 genes/loci that are candidates for smoking, 29 genes/loci for alcohol, and 15 genes/loci for obesity and/or BMI. A few of these genes/loci are candidates for more than one risk of interest; these are evaluated in relation to both risk factors of interest independently. Supplementary Table A1 online provides a list of the SNPs and candidate genes/loci evaluated for each risk factor. All SNPs were non-imputed and passed the following criteria: genotyping efficiency ≥ 95%, HWE not rejected $p < 0.001$, and minor allele frequency ≥ 1%.

We evaluated the association of the maternal SNPs in the candidate genes and their related risk factor including smoking, alcohol use, pre-pregnancy body weight, and weight gain during pregnancy. We consider as much as possible the various aspects of smoking and alcohol decisions, which include engaging in these behaviors during pregnancy (which would include women continuing these behaviors during pregnancy and those who initiate them during pregnancy, admittedly a very small group), or abstaining from them. The last category includes both women who engaged in these behaviors before but quit during pregnancy and women who did not initiate these behaviors before and during pregnancy. Quitting is arguably the most relevant behavioral change specific to pregnancy followed by reducing the intensity of consumption (for example smoking fewer cigarettes instead of quitting). However, the group of abstainers includes two groups of women, those abstaining because of their habit to avoid these behaviors, and those (even if arguably a small group) who do not initiate these behaviors before (as part of pregnancy planning) or during pregnancy because of concerns for fetal health, but may choose to initiate these behaviors sometime after pregnancy. Since the group of abstainers includes some women who could initiate smoking or drinking sometime after pregnancy (in addition to those who will never initiate these behaviors), evaluating any engagement in these behaviors during pregnancy in the total sample in addition to quitting is useful for capturing as much as possible the multiple aspects of these behaviors.

Smoking was measured by an indicator for any smoking during pregnancy and by indicators for smoking separately for each of the three trimesters based on responses to detailed questions about durations and periods of smoking in the first three interviews in order to evaluate if genetic associations differed with changes in smoking behavior during pregnancy. We also studied the number of cigarettes smoked per day among smokers and separately for all women (including 0 for nonsmokers) both averaged throughout pregnancy and by trimester. We also evaluated genetic associations with quitting smoking throughout pregnancy (i.e. not smoking anytime during pregnancy) among those who were smokers at the time when they became pregnant.

Alcohol was measured by indicators for any alcohol consumption and bingeing (defined as reporting having five or more drinks per setting) during pregnancy and by trimester from responses to detailed questions about alcohol consumption from the first three interviews. We also evaluated alcohol quitting throughout pregnancy by an indicator for not consuming any alcohol during pregnancy among those who consumed alcohol before pregnancy.

Pre-pregnancy BMI was derived from self-reported pre-pregnancy weight and height during the first interview (around 12th gestational week) in response to direct questions about weight (in kilos) before pregnancy – “what was your weight before the pregnancy” – and current height (in centimeters) – “how tall are you” –, and by an indicator for obesity (BMI 30). We also studied weight gain during the entire pregnancy based on maternal self-report during the first interview after pregnancy (around 6 months); this measure was standardized to a gestational length of 40 weeks for pregnancies delivered before 40 weeks. In addition to analyzing weight gain as a continuous outcome, indicators for excessive and inadequate weight gain (relative to normal) based on Institute of Medicine (IOM) pregnancy-weight guidelines were analyzed [56].

We evaluated the association between the SNPs with binary risk factors such as any smoking using logistic regression and with continuous measures such as BMI using ordinary least squares (OLS) regression. We considered two Bonferroni-type corrections for multiple testing. Under the first SNP-based correction, the significance threshold for the tests of association with a certain risk factor was defined as $0.05/n$, where n is the number of SNPs evaluated for that risk factor. The second approach to correct for multiple testing was gene/locus-based and less restrictive, defining the significance threshold as $0.05/g$, where g is the number of genes/loci evaluated for a risk factor. The second approach acknowledges that SNPs within a certain gene are correlated, some of them highly so. Since the sample was highly homogeneous on race/ethnicity, we did not correct for population stratification.

RESULTS

Table 1 shows the descriptive statistics for the study risk factors. Smoking was relatively common with 28% of the women smoking about 6 cigarettes per day on average. About one in four smokers at pregnancy quit smoking during the pregnancy. Nearly 60% of women reported drinking sometime during pregnancy, and binge drinking was observed in about one in four women in the first trimester but declined substantially during the second and third trimesters. Thirty-two percent of women who drank alcohol before pregnancy abstained from using alcohol during the pregnancy. About 8% of the women were obese, and average BMI was 23.5 in the total sample. On average, women gained 14.6 kilograms throughout pregnancy. Excessive weight gain (according to IOM guidelines) was observed in about one-third (32.5%) of women, and 43.6% gained an inadequate amount of weight [56].

Tables 2, 3, and 4 report summaries of the SNP associations with the smoking, alcohol, and weight measures, respectively; detailed results for each SNP and risk factor are in Supplementary Table A1 online. There were several SNPs and genes related to the risk factors at $p < 0.05$. Of these, 23 SNPs and 9 genes were significantly related to the risk factors after the gene-based correction for multiple testing. However, only 4 of these loci were significant under the SNP-based correction threshold. We summarize the results below for each risk factor category.

Smoking

Of the 733 SNPs in 79 candidate genes for smoking, 50 SNPs in 19 genes/loci were found to be associated with any smoking during pregnancy at $p < 0.05$ (Table 2). However, none remained significantly associated with any smoking after SNP-based ($p < 6.8E-05$) or gene/locus-based ($p < 0.0006$) corrections for multiple testing. Similarly, none of the loci were significantly related to smoking status by trimester at the SNP-level corrected threshold; however, *ACTN1*(rs4902682), *DBH* (rs2519155), and rs3791729 (*CHRND*) were significantly associated with any smoking in the first, second, and third trimester respectively after gene/locus-level correction for multiple testing.

Several SNPs were associated with the various measures of number of cigarettes at $p < 0.05$; however, none of the genes/loci remained significant at the SNP-level corrected threshold (Table 2). The number of cigarettes smoked per day during pregnancy among smokers was associated with 27 SNPs in 18 genes/loci at $p < 0.05$ (Table 2). Of these, none were significant after adjustment for multiple testing (same thresholds as for any smoking). *ACTN1* (rs181484 and rs4902682) was significant at the gene/locus-level corrected threshold for the average number of cigarettes smoked in the total sample (with 0 for non-smokers), and the number of cigarettes smoked in the first (rs4902682, rs2268968, and rs181484) and third trimesters (rs4902682). *CHRNA3* (rs12914385) was significant at gene/locus-level threshold for the number of cigarettes among smokers in the first trimester.

Finally, 35 SNPs in 19 genes/loci were associated with quitting smoking among those who were smokers at the beginning of pregnancy, but none of these were significant at the SNP-level threshold. Only two SNPs in *FAAH* (rs3766246) and *DBH* (rs2519147) were significant at the gene/locus-level threshold.

Alcohol use

Of the 493 evaluated SNPs in 29 candidate genes/loci for maternal alcohol consumption, none were significantly related to any of the alcohol consumption measures at the SNP-level threshold (Table 3). 12 SNPs in 7 genes/loci were significantly related to any alcohol use during pregnancy at $p < 0.05$. Of these, one SNP (rs6035239) in *PDYN* remained significant after gene/locus-based correction for multiple testing but was insignificant after SNP-based correction ($p < 1.0E-04$).

ADH1C (rs11499823) was significant at the gene/locus-level threshold for any drinking in the first trimester. Several SNPs in/near *GRM8* were significantly related to any drinking in second trimester, any bingeing during pregnancy, and any first-trimester bingeing at the gene-level threshold.

Finally, 15 SNPs in 9 genes/loci were significantly related to quitting alcohol during pregnancy at $p < 0.05$; however, none of these SNPs were significant at SNP- or gene/locus-level corrected thresholds.

Body weight

Of the 285 SNPs in 15 genes/loci evaluated for maternal pre-pregnancy body weight, 22 variants in 5 genes/loci were related to maternal obesity at $p < 0.05$ (Table 4). Of these, 7 SNPs (rs9941349, rs3751812, rs8050136, rs10852521, rs7190492, rs8044769, and rs9930333) in *FTO* remained significant after gene/locus-level correction for multiple testing ($p < 0.0033$). Four of these (rs9930333, rs9941349, rs3751812, and rs8050136) remained significant after SNP-based correction for multiple testing ($p < 1.75E-04$).

Fourteen SNPs in 3 genes/loci were associated with BMI at $p < 0.05$ (Table 2). Of these, 6 SNPs (rs8050136, rs3751812, rs9941349, rs7190492, rs8044769, and rs9930333) in *FTO* were significant after gene/locus-based correction for multiple testing (same threshold as for obesity). Two SNPs (rs8050136 and rs3751812) were significant after SNP-based correction (Table 4).

Finally, 15 SNPs in 5 genes/loci were related to weight gain during pregnancy measured continuously at $p < 0.05$, but none of them remained significant after SNP- or gene/locus-level corrected thresholds. However, one SNP (rs2293084) in *TMEM18* and another (rs2540766) in *FTO* were significantly related to excessive and inadequate weight gain, respectively, after gene/locus-correction.

DISCUSSION

This is one of the first studies to evaluate a large number of candidate genes for association with smoking, alcohol, and body weight specifically among pregnant women. In accord with previous the genetic studies of smoking, alcohol, and body weight in general samples which were able to explain only a limited portion of the genetic heritability, only a handful of loci were significantly related to these factors during pregnancy after correction for multiple testing. This may be partly because of the relatively small sample size and low power. For example, the two SNPs in *TMEM18* (which is identified in meta-GWAS to be significantly related to BMI [52]) that are related to pre-pregnancy BMI at $p < 0.05$ in our study have about 10% power to be significant at the SNP-level corrected significance threshold and 30% power at the gene-level corrected threshold at the current sample size; however, the power increases to about 80% at a sample size of 6200 observations for the SNP-level threshold and 4200 observations for the gene-level threshold. Therefore, some associations could have become significant after correcting for multiple testing in a sample that is 3–4 times larger than the sample available for our study. Also, the GWAS panel did not include some of the specific SNPs with the strongest evidence in the literature for these genes such as rs1229984 in *ADH1B*. The linkage disequilibrium (LD) between the covered SNPs in *ADH1B* and rs1229984 ranged from 0 to 0.152. In contrast, even though the SNP in *FTO* with the largest effect on obesity (rs9939609) was not covered, the LD between that SNP and the significant SNPs in that gene after SNP-based correction for multiple testing was ~ 1 .

The study results suggest that the majority of the current candidate genes provide little insight into the genetics of these risk factors during pregnancy. Specifically, about 99 percent of the candidate genes and variants examined in this study are not significantly related to the maternal risk factors of interest. However, as mentioned above, our analysis

was only based on the SNPs covered in the GWAS panel and not on preselected SNPs based on the literature. Therefore, it is possible that the covered SNPs are not reflective of the association of other strongly linked SNPs in these genes. However, this is unlikely to be the reason for lack of effects for most of these genes which had overall good coverage in the GWAS. All candidate genes had multiple SNPs covered and included the haplotype blocks in which the strongest SNPs from published data were found, some to greater degree than others. Furthermore, even though all evaluated genes are considered candidates for these risk factors, most of these genes have been reported in very few studies and have not been replicated across many studies. Currently, only a few loci are considered to be unequivocally related to these risk factors. Of these, we confirm the association between *FTO* and maternal obesity and BMI at pregnancy. We also find significant association ($p=0.0012$) between *CHRNA3* and the number of cigarettes among smokers which has been confirmed in general samples in previous studies, although it did not pass the gene/locus- ($p<0.0006$) or SNP-based ($p<6.8E-05$) correction for multiple testing. However, the association between *CHRNA3* and first trimester number of cigarettes among smokers was significant after gene/loci-based correction ($p=0.0003$).

The limited sample size and power that may have prevented detecting significant loci highlights the importance of doing this study in a much larger sample. Also, we did not have access to a replication sample. To our knowledge, only a few existing datasets provide GWAS data and measures of prenatal risk factors for a population-based sample of pregnant women. An additional limitation of the study is that the sample is very homogeneous in geographic ancestry. This reduces the bias of population stratification, but limits the generalizability of the results to more diverse populations. Therefore, evaluating this question in other populations and ancestries is needed.

The results suggest that prevention of maternal risk factors before and during pregnancy should focus more on environmental contributors, as the currently known genetic loci explain a very small portion of the variation in these factors. Specifically, the significant SNPs after multiple testing in our study explain very small portions of the variation in these risk factors (for e.g., ~ 3.7% for any smoking during pregnancy, ~0.1% for number of cigarettes smoked during pregnancy, ~1.7% for any alcohol use during pregnancy, ~ 1.1% for pre-pregnancy obesity, and ~1.0% for pre-pregnancy BMI). These very small percentages of explained variation are not surprising given that the loci identified in meta-GWAS analyses collectively explain very small portions of the variation in these outcomes in their large study samples (for e.g. <2% of variation in cigarettes per day [29] and BMI [52]). The percentages explained by the significant loci in our study that have been confirmed in meta-GWAS and can be compared to previous estimates are broadly consistent with some estimates from these studies although some differences are observed and are noteworthy. For example, rs12914385 in *CHRNA3* explains 0.6% of the variation in cigarettes per day in first trimester, which is close to 0.5% explained variation by rs1051730 in *CHRNA3* in a meta-GWAS [29]; rs1051730 was not covered in the SNP panel under which our study sample was genotyped but it is in strong LD with rs12914385 ($r^2 = 0.84$). In contrast, rs8050136 in *FTO* explains about 0.8% of the variation in pre-pregnancy BMI in our analysis, which is about twice the fraction of variation (0.34%) explained by *FTO*

rs1558902 in a meta-GWAS [52]; rs1558902 was not measured in our dataset but it is in strong LD with rs8050136 ($r^2 = 0.9$). Also, we find a much larger fraction of variation in quitting smoking during pregnancy to be explained by *DBH* rs2519147 (~2.7%) than what has been reported for *DBH* rs3025343 which was the only significant SNP in the meta-GWAS for smoking cessation and explained about 0.19% of the variation [29]; however, these two SNPs are only weakly correlated ($r^2=0.1$) and none of the other *DBH* SNPs genotyped in our sample had a stronger LD with the meta-GWAS SNP. Although such differences could suggest a varying strength of genetic effects between pregnant women and the general samples included in meta-GWAS but could also be due to other factors such as differences in measurement or statistical variation of parameters. Given the small fraction of explained variation, it is unlikely that currently known genetic risk factors may be useful for developing population-wide screening and counseling women before pregnancy as part of prevention efforts in the near future. Rare variants may partly explain the lack of or limited associations with common variants in the candidate genes observed in this study [57, 58]. However, investigating rare variants contributing to these behavioral phenotypes may not necessarily have substantial implications for improving maternal and child health at a population level.

The fact that only a few variants were found to be significant in this study suggests that it is possible that genetic effects are modified in the population of pregnant women and are not as relevant during pregnancy. As mentioned above, the multiple physiological changes (such as in hormonal and metabolic pathways and body composition) that are drastically impacted by pregnancy may modify the genetic effects on risk factors during pregnancy. Therefore, studying the genetics of risk factors specifically for women of childbearing age and pregnant women in future research could reveal important heterogeneity and new interaction mechanisms that modify genetic influences.

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Table 1**Study Sample Characteristics**

Variable	N	Frequency (%)	Mean (SD)
Smoking			
Any smoking (anytime during pregnancy)	1900	28.0%	
Number of cigarettes (smokers)	445		6.28 (5.27)
Number of cigarettes	1813		1.54 (3.76)
Any 1st trimester smoking	1635	27.0%	
Any 2nd trimester smoking	1635	19.9%	
Any 3rd trimester smoking	1623	18.0%	
Number of cigarettes 1st Tri (smokers)	438		7.09 (5.07)
Number of cigarettes 2nd Tri (smokers)	321		8.25 (5.16)
Number of cigarettes 3rd Tri (smokers)	290		8.75 (5.08)
Number of cigarettes 1st Tri	1632		1.90 (4.10)
Number of cigarettes 2nd Tri	1634		1.62 (4.00)
Number of cigarettes 3rd Tri	1622		1.57 (3.98)
Quit smoking throughout pregnancy	517	25.9%	
Alcohol			
Any alcohol (anytime during pregnancy)	1937	58.8%	
Any alcohol 1st trimester	1837	46.1%	
Any alcohol 2nd trimester	1587	50.2%	
Any alcohol 3rd trimester	1431	45.7%	
Any bingeing (anytime during pregnancy)	1451	33.2%	
Any 1st trimester bingeing	1456	23.9%	
Any 2nd trimester bingeing	1449	3.8%	
Any 3rd trimester bingeing	1252	1.0%	
Quit alcohol throughout pregnancy	1565	32.3%	
Maternal Body Weight			
Weight gain during pregnancy	1346		14.57 (6.33)
Excessive weight gain	1273	32.5%	
Inadequate weight gain	1273	43.6%	
Maternal obesity	1811	8.7%	
Body mass index	1811		23.52 (4.31)

Notes: The number of observations with missing data on smoking and alcohol measures by trimester is greater than that for measures throughout pregnancy due to missing data on specific dates in some observations but not on any use or averages throughout pregnancy. Therefore, the summary statistics for trimester-specific measures (such as the frequencies of smoking, alcohol, and bingeing) may not exactly match the overall-pregnancy measures.

Table 2

Summary of SNP Associations with Prenatal Risk Factors - Smoking

Behavior	# Genes	# SNPs	# SNPs <0.05	# Genes <0.05	Significant SNPs after Gene-Level Correction # SNP (Gene)	Significant SNPs after SNP-Level Correction # SNP (Gene)
Any smoking	79	733	50	19	0	0
Number of Cigarettes (smokers)	79	733	27	18	0	0
Number of Cigarettes (with 0 for nonsmokers)	79	733	35	14	rs181484 (<i>ACTN1</i>) rs4902682 (<i>ACTN1</i>)	0
Any 1st trimester smoking	79	733	41	16	rs4902682 (<i>ACTN1</i>)	0
Any 2nd trimester smoking	79	733	46	19	rs2519155 (<i>DBH</i>)	0
Any 3rd trimester smoking	79	733	45	20	rs3791729 (<i>CHRNA3</i>)	0
Number of Cigarettes 1st Tri (smokers)	79	733	42	14	rs12914385 (<i>CHRNA3</i>)	0
Number of Cigarettes 2nd Tri (smokers)	79	733	25	20	0	0
Number of Cigarettes 3rd Tri (smokers)	79	733	32	25	0	0
Number of Cigarettes 1st Tri (with 0 for nonsmokers)	79	733	41	12	rs4902682 (<i>ACTN1</i>), rs2268968 (<i>ACTN1</i>), rs181484 (<i>ACTN1</i>)	0
Number of Cigarettes 2nd Tri (with 0 for nonsmokers)	79	733	33	12	0	0
Number of Cigarettes 3rd Tri (with 0 for nonsmokers)	79	733	38	14	rs4902682 (<i>ACTN1</i>)	0
Quit Smoking	79	733	35	19	rs3766246 (<i>FAAH</i>) rs2519147 (<i>DBH</i>)	0

Table 3

Summary of SNP Associations with Prenatal Risk Factors - Alcohol

Behavior	# Genes	# SNPs	# SNPs <0.05	# Genes <0.05	Significant SNPs after Gene-Level Correction # SNP (Gene)	Significant SNPs after SNP-Level Correction # SNP (Gene)
Any alcohol	29	493	12	7	rs6035239 (<i>PDYN</i>)	0
Any alcohol 1st trimester	29	493	15	8	rs11499823 (<i>ADH1C</i>)	0
Any alcohol 2nd trimester	29	493	19	8	rs11563766 (<i>GRM8</i>), rs3779538 (<i>MIR592</i> , <i>GRM8</i>)	0
Any alcohol 3rd trimester	29	493	22	8	0	0
Any binge	29	493	29	9	rs11563673 (<i>GRM8</i>), rs4141414 (<i>GRM8</i>), rs7776557 (<i>GRM8</i>)	0
Any 1st trimester binge	29	493	18	5	rs4141414 (<i>GRM8</i>), rs7776557 (<i>GRM8</i>)	0
Any 2nd trimester binge	29	493	28	9	0	0
Any 3rd trimester binge	29	493	11	5	0	0
Quitting Alcohol	29	493	15	9	0	0

Table 4

Summary of SNP Associations with Prenatal Risk Factors - Maternal Body Size

Behavior	# Genes	# SNPs	# SNPs <0.05	# Genes <0.05	Significant SNPs after Gene-Level Correction # SNP (Gene)	Significant SNPs after SNP-Level Correction # SNP (Gene)
Weight gain during pregnancy	15	285	15	5	0	0
Excessive weight gain ^a	15	285	4	3	rs2293084 (<i>TMEM18</i>)	0
Inadequate weight gain ^b	15	285	15	3	rs2540766 (<i>FTO</i>)	0
Maternal obesity	15	285	22	5	rs10852521 (<i>FTO</i>) rs9930333 (<i>FTO</i>) rs7190492 (<i>FTO</i>) rs3751812 (<i>FTO</i>) rs8050136 (<i>FTO</i>) rs9941349 (<i>FTO</i>) rs8044769 (<i>FTO</i>)	rs3751812 (<i>FTO</i>) rs8050136 (<i>FTO</i>) rs9930333 (<i>FTO</i>) rs9941349 (<i>FTO</i>)
Body Mass Index	15	285	14	3	rs9930333 (<i>FTO</i>) rs7190492 (<i>FTO</i>) rs3751812 (<i>FTO</i>) rs8050136 (<i>FTO</i>) rs9941349 (<i>FTO</i>) rs8044769 (<i>FTO</i>)	rs3751812 (<i>FTO</i>) rs8050136 (<i>FTO</i>)

Notes:

^a compared to normal and inadequate weight gain combined using logistic regression.

^b compared to normal and excessive weight gain combined using logistic regression.