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Brief report
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Brief report

Association Between rs1051730 and Smoking During Pregnancy in Dutch Women

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Abstract

Introduction: The common genetic variant (rs1051730) in the 15q24 nicotinic acetylcholine receptor gene cluster CHRNA5-CHRNA3-CHRNB4 was associated with smoking quantity and has been reported to be associated also with reduced ability to quit smoking in pregnant women but results were inconsistent in nonpregnant women. The aim of this study was to explore the association between rs1051730 and smoking cessation during pregnancy in a sample of Dutch women.
Methods: Data on smoking during pregnancy were available from 1337 women, who ever smoked, registered at the Netherlands Twin Register (NTR). Logistic regression was used to assess evidence for the association of rs1051730 genotype on smoking during pregnancy. In a subsample of 561 women, we investigated the influence of partner's smoking. Educational attainment and year of birth were used as covariates in both analyses.
Results: There was evidence for a significant association between having one or more T alleles of the rs1051730 polymorphism and the likelihood of smoking during pregnancy (p = .03, odds ratio = 1.28, 95% CI = 1.02 to 1.61). However, this association attenuated when adjusting for birth cohort and educational attainment (p = .37, odds ratio = 1.12, 95% CI = 0.87 to 1.43). In the subsample, smoking spouse was highly associated with smoking during pregnancy, even when educational attainment and birth cohort were included in the model.
Conclusions: Our results did not support a strong association between this genetic variant and smoking during pregnancy. However, a strong association was observed with the smoking behavior of the partner, regardless of the genotype of the women.
Implications: The present study emphasizes the importance of social influences like spousal smoking on the smoking behavior of pregnant women. Further research is needed to address the role of rs1051730 genetic variant in influencing smoking cessation and the interaction with important environmental factors like the smoking behavior of the partner.

Introduction

Maternal smoking during pregnancy continues to be a public health issue in developed countries and in an increasing number of developing countries.[1–5] Although the true prevalence of smoking during pregnancy is difficult to discern, in recent studies this prevalence varies worldwide with estimates well below 10% in high-income countries such as Australia, Canada, the United Kingdom,

and the United States[6] and higher prevalence in low- and middle-income countries like Uruguay (18.3%)[7] and Romania (15%).[8] Smoking during pregnancy appears to have decreased steadily in recent years. Numbers may vary reflecting some variation between studies in the way the questions concerning the prevalence were posed, but overall prevalence rates among pregnant women declined from the 1960s onwards. One reason for the decline in the rates of smoking during pregnancy is probably the increase in awareness of the health risks to both mother and child of smoking.

Many studies have shown that tobacco smoke during pregnancy can cause serious health and behavioral issues for a developing fetus and/or infant.[9–11] For example, maternal smoking increased the risk of stillbirths, preterm birth and low birth weight, and sudden infant death syndrome.[12] Later, these offspring were at increased risk for neurodevelopmental disorders such as ADHD (Attention Deficit Hyperactivity Disorder),[13] externalizing behavior, aggression, overactive and withdrawn behavior,[13,14] and obesity[15]. Thus, quitting smoking before pregnancy reduces the child's health risks and the risk of complications during delivery.

Smoking behavior aggregates in families, and heritability estimates for different stages of smoking behavior vary from 33% for smoking initiation to 86% for nicotine dependence[16–18] indicating a substantial genetic component. In 2010, the first large genome-wide association studies (GWAS) meta-analyses revealed that the strongest genetic contribution to smoking-related traits came from variation in the nicotinic acetylcholine receptor (nAChR) subunit genes,[19–21] as was first explained on a genome-wide significant level.[22] The *CHRNA5-CHRNA3-CHRNB4* gene cluster on chromosome 15q25.1, encoding the alpha5, alpha3, and beta4 subunits, has provided the best established genetic evidence; first in relation to the amount smoked (cigarette per day (CPD)) and subsequently in relation to other smoking-related phenotypes (eg, nicotine dependence, smoking cessation) as well as smoking-related diseases (eg, lung cancer and chronic obstructive pulmonary disease).[23–28] However, there is no association with smoking initiation.[20] The association between this gene cluster and smoking cessation treatment is inconsistent.[29–31]

Specifically, two SNPs rs1051730 (*CHRNA3*) and rs16969968 (*CHRNA5*), in perfect LD (D' = 1, r^2 = 1), have consistently been associated with nicotine dependence and CPD. Not only was this locus the top-result in the meta-analyses described above, but also it was strongly associated with nicotine dependence in other studies.[32] Notably, rs16969968 is a missense mutation, resulting in an amino acid change (aspartate to asparagine) in the resultant alpha-5 nicotinic receptor subunit protein. This change is associated with reduced receptor function in vitro.[33] Few studies have examined the association between this locus and smoking cessation during pregnancy. Freathy et al.[34] found an association of rs1051730 and smoking cessation in 2474 pregnant women, which was replicated in a case-control study of 1891 pregnant women[35].

In addition to genetic factors, demographic and contextual factors also seem to play a role in the smoking behavior of pregnant women. Smoking status of the partner has been shown to be important in smoking behavior[36,37] and in smoking cessation.[38] More recent research has pointed to a positive effect on smoking cessation in pregnant women of having no one in the vicinity smoke, and of having a smoking ban in the home.[39,40] Other factors that are often reported are maternal age[28] (pregnant women aged 15 to 24 had the highest rate of smoking compared with pregnant women aged 25 and older) and educational level[28] (the lowest smoking rates among pregnant women are for those with a bachelor's degree or more). However, age and education level have not been consistently associated with attempts to stop smoking, or the success of such attempts.[2,41,42]

The aim of this study was to explore the association between the rs1051730 risk allele (T) and smoking during pregnancy in a sample of Dutch women (N = 1337) registered at the Netherlands Twin Register (NTR). In addition, we investigated the influence of the partner's smoking behavior (N = 561 spouses) in combination with the genotype of the pregnant woman on smoking during pregnancy.

Methods

The Netherlands Twin Register—Participants

The Netherlands Twin Register (NTR) includes twins and their family members who participated in longitudinal research projects. Young twins (YNTR) were registered at birth by their parents. Demographic characteristics, recruitment, and data collection procedures in these samples have been described in detail elsewhere.[43] Adolescent and adult twins (ANTR) were recruited through City Councils in 1990–1993, and through additional efforts, such as newsletters and advertisements. ANTR participants (twins and their family members) took part in longitudinal survey studies in 1991, 1993, 1995, 1997, 2000, 2002, 2004/5, and 2009/11. All mothers of multiples were invited to complete a short survey in 2005 (Mother survey),[44] and a group of sisters who were both mothers of DZ twins were invited to participate in a "DZ twinning project." A large group of subjects also participated in the NTR Biobank study, between 2004 to 2008, during which DNA samples and other biological material were collected. Details were described elsewhere.[45]

Smoking During Pregnancy

The following NTR survey studies included questions on smoking during pregnancy: ANTR survey 1 (1991; n = 1588), ANTR survey 5 (2000; n = 4233), Mother survey (n = 1526), DZ twinning project (n = 673), and YNTR survey for mothers of twins (n = 818). Details about the questions in each survey and the genotype data available are outlined in the Supplementary Methods. In total, data on smoking during pregnancy were available for 1414 women. When women participated more than once, answers were checked for inconsistencies. Data of 77 women who answered at least once "yes" and once "no" to the question "Did you smoke during pregnancy?" were excluded from further analyses. The remaining dataset consisted of 1337 women who ever smoked, completed information on smoking during pregnancy and have genotype data. Information on the smoking behavior during pregnancy was available for 561 spouses of the 1337 women. We have focused on women who ever smoked, because smoking initiation itself is not associated with rs1051730. This SNP is known to be associated with smoking quantity and nicotine dependence. Here, we test whether it also contributes to the inability not to smoke during pregnancy in ever smokers.

Covariates

Educational attainment (EA) was measured longitudinally with the question "What is the highest educational level that you have finished?" The answer categories varied per survey, but all could be recoded into the following three categories. "Low": primary school only or lower vocational school and lower secondary school; "Middle": intermediate vocational school and intermediate or higher secondary school; "High": higher vocational school and university. EA was available for 1182 women.

Age of mother was not available for all women who participated in different surveys at different times. We used instead the birth cohort variable. Year of birth was recoded in a dichotomous variable "cohort" with 0 for women born before 1954 and 1 for women born in or after 1954 (1954 is the median birth year). We included the covariates EA and cohort as it has been demonstrated that the inclusion of nonconfounding covariates in the logistic regression of general population samples, in contrast to ascertained case-control samples, increases the power of association testing.[46,47]

Genotyping

The rs1051730 SNP was genotyped using standard methods, details of which were presented previously in Mbarek et al.[48] The observed genotyped counts in the present study did not differ significantly from those expected under the Hardy–Weinberg equilibrium (CC = 605, 45.3%, CT = 596, 44.6%, and TT = 136, 10.2%; for women who smoked during pregnancy and women who did not smoke during pregnancy $p > .1$).

Statistical Analyses

We performed logistic regression to test for the effects of rs1051730 genotype on smoking during pregnancy (counts were comparable for women who did and did not smoke during pregnancy). The genotype was coded as 0 versus 1 or 2 copies of the T (risk) allele because the number of participants with 2 copies of the T allele was relatively low. Analyses were performed with and without adjustment for birth cohort and educational attainment. However, we considered the analyses with covariate as the analyses of choice, because the covariates are nonconfounding and the sample is a population sample (ie, not ascertained as in a case-control study). In this specific setting, the analyses with covariates are more powerful than the analyses without the covariates.[46,47]

In a subsample, we explored the influence of partner's smoking with and without the risk alleles on smoking during pregnancy with adjustment for birth cohort and educational attainment. Analyses were done using SPSS version 21.0 (SPSS Inc., Chicago, IL).

Results

In the total sample of 1337 women who ever smoked, 461 smoked during pregnancy (34.5%) whereas the remaining women (65.5%) did not smoke during pregnancy. The median birth cohort was 1953 (range = 1929–1977) in the group who smoked during pregnancy and 1955 (range = 1921–1979) in the group who did not smoke during pregnancy. In a subsample of 561 women, information was available on the smoking behavior of their spouses. In this subsample, 204 women smoked during pregnancy. In total, 72.1% of the spouses of these 204 women also smoked during pregnancy, and 41.5% of the spouses of 357 women, who did not smoke during pregnancy, smoked during their partner's pregnancy. The difference in percentage (72.1 vs. 41.5) is significant: $\chi^2 = 48.76$ ($p < .0001$).

In the total sample of 1337 women who ever smoked, there was evidence for a significant association between having one or more T alleles of the rs1051730 polymorphism and the likelihood of smoking during pregnancy (OR = 1.28, 95% CI = 1.02 to 1.61, $p = .03$, Table 1). However, this association was attenuated when adjusting for birth cohort and educational attainment (OR = 1.12, 95% CI = 0.87 to 1.43, $p = .37$, Table 1). The probability of smoking during pregnancy was lower in women with higher education. This association remained significant in the present analyses. These variables together explain 4.6% of the variance in smoking cessation during pregnancy.

Next, we included smoking behavior of the spouse during pregnancy in the model. In the group of women who had a nonsmoking spouse the probability of smoking was almost two times higher (OR 1.92) for women with one or two risk alleles compared to women with no risk alleles, although the difference was not statistically significant ($p = .056$). When having a smoking spouse, the risk of smoking during pregnancy was more than five times higher (OR = 5.55 for group with no risk alleles and OR = 5.70 for group with one or two risk alleles) regardless of the genotype (Table 2). There was no significant difference between these last two groups with regard to the risk of smoking during pregnancy. So, having a smoking spouse was highly associated with smoking during pregnancy, even when

Table 1. Univariate Analyses: Logistic Regressions With Smoking During Pregnancy as Dependent Variable, and Each of the Following Variables as Independent Variable (1 by 1): Having Zero Versus One Or Two Risk Alleles for SNP rs1051730, Educational Level (Low, Middle, High), Birth Cohort (Year of Birth Before 1954 Versus In or After 1954)

	Smoking during pregnancy					
	Univariate analyses			Multivariate analyses		
	OR (95% CI)	*p* value	Nagelkerke R-square	OR (95% CI)	Adjusted *p* value	Nagelkerke R-square
rs1051730: no risk alleles (= ref)	1					
1 or 2 risk alleles	1.28 (1.02–1.61)	.032	0.5%	1.12 (0.87–1.43)	.368	
Educational level: low (= ref)	1	<.0001 (2df)		1	<.0001 (2df)	
middle	0.61 (0.46–0.82)	.001	3.9%	0.66 (0.49–0.90)	.008	4.3 %
high	0.42 (0.30–0.57)	< .0001		0.43 (0.32–0.60)	<.0001	
Year of birth: before 1954 (= ref)	1					
In or after 1954	0.79 (0.62–0.98)	.032	0.5%	0.82 (0.63–1.05)	.121	

The total sample consists of $N = 1337$ women who ever smoked; ref = reference category. Multivariate analyses: logistic regression analysis with smoking during pregnancy as dependent variable, and all previously described variables as independent variables. Nagelkerke R-square is used to assess the variance explained. It is an adjusted version of the Cox & Snell R-square that adjusts the scale of the statistic to cover the full range from 0 to 1.

Table 2. Logistic Regression Analysis With Smoking During Pregnancy as Dependent Variable, and the Following Variables as Independent Variables: Having Zero Versus One or Two Risk Alleles for SNP rs1051730, Having a Smoking Spouse During Pregnancy (Yes/No), Educational Level (Low, Middle, High), Birth Cohort (Year of Birth Before 1954 Versus In or After 1954)

		Smoking during pregnancy		
		OR (95% CI)	*p* value	Nagelkerke R-square
rs1051730 and spousal smoking behavior:	no risk alleles and nonsmoking spouse (= ref), *N* = 121	1	<.0001 (3 df)	17.5%
	1 or 2 risk alleles and nonsmoking spouse, *N* = 145	1.92 (0.98–3.75)	.057	
	no risk alleles and smoking spouse, *N* = 125	5.69 (2.88–11.25)	<.0001	
	1 or 2 risk alleles and smoking spouse, N = 170	5.78 (3.02–11.05)	< .0001	
Educational level:	low (= ref)	1	.003 (2df)	
	middle	0.67 (0.41–1.10)	.115	
	high	0.41 (0.24–0.68)	.001	
Year of birth:	before 1954 (= ref)	1		
	in or after 1954	1.67 (1.08–2.57)	.02	

N = 561 women who ever smoked; ref = reference category.

educational attainment and birth cohort were included in the model. We also tested for an interaction between genotype and education on cessation, and found a significant interaction for the low- and middle-education levels but not for the high-education level (results not shown). All variables together (Table 2) explained the 17.5% of the variance in smoking during pregnancy but the largest part of the explained variance came from having a smoking spouse. Finally, we performed a sensitivity analysis by imputing the missing data in the subsample, the results were similar before and after imputation with one exception for the effect year of birth which was not significant (see Supplementary Table S2).

Discussion

To our knowledge, our study included the largest replication sample in order to investigate the association of rs1051730 and smoking cessation during pregnancy taking into account birth cohort and educational attainment level, and in a subsample also partner's smoking status. We were not able to reproduce Freathy et al.[34] and Thorgeirsson et al.[35] findings of association between rs1051730 variant and smoking during pregnancy. We did observe an association without adjustment for birth cohort and educational attainment level (p = .03). However, adjusting for these covariates rendered the association not significant (p = .37). As the analyses with covariate has greater power[46,47] in the present setting, we conclude that there is no association.

This is in contrast with a meta-analyses (in 24 studies of European ancestry, *N* = 29 072) showing a lower likelihood of smoking cessation for the rs16969968 A allele. The AA genotype was also associated with a four-year delay in median age of quitting compared with the GG genotype.[27]

Interestingly, in our study, the smoking behavior of the spouse was strongly associated with smoking in pregnant women. The effect of genotype did not reach statistical significance (OR = 1.92, p = .056) on smoking during pregnancy in the group of women with a nonsmoking spouse; however, the possible genetic effect seemed totally overruled by having a smoking partner in the other groups (OR > 5 for women with and without the risk genotype).

Spousal similarity in smoking behavior has been shown in a Dutch study of current smoking behavior[49] but the present study emphasized the importance of social influences like spousal smoking on smoking behavior of pregnant women.

Our study did also have some limitations: 1) Some of our participants might have given up smoking years before they were pregnant. Therefore, the effect sizes are likely to be smaller than those observed in the paper of Freathy et al.,[34] who restricted analyses to women smoking regularly just prior to pregnancy. 2) We did not stratify the analysis by smoking period during pregnancy due to small sample size for each period. 3) there are many other nongenetic factors that influence smoking cessation during pregnancy, including environmental factors and psychological factors (eg, concurrent alcohol use, financial status, internalizing, and externalizing problems), which were not taken into account in the present study. 4) Although we used the largest sample to date to test the interaction between partner smoking and the rs1051730 genotype, it is likely that a larger sample is required in order to ensure sufficient power to detect an interaction effect.

To conclude, our results did not support a strong association between this genetic variant and smoking during pregnancy. However, a strong association was observed with smoking behavior of the partner, regardless of the genotype of the women. Further research is needed to address the role of this and other genetic factors in influencing smoking cessation and their interaction with important environmental factors like smoking behavior of the partner. A knowledge of these genetic factors will help to identify mothers at risk early on and reduce their risk for nicotine dependence and its related negative health consequences on the mother and the child.[50–53]

Supplementary Material

Supplementary Methods and Table S2 can be found online at https://academic.oup.com/ntr/

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Declaration of Interest

None declared.

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