

A common genetic variant in the 15q24 nicotinic acetylcholine receptor gene cluster (*CHRNA5–CHRNA3–CHRNB4*) is associated with a reduced ability of women to quit smoking in pregnancy

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Maternal smoking during pregnancy is associated with low birth weight and adverse pregnancy outcomes. Women are more likely to quit smoking during pregnancy than at any other time in their lives, but some pregnant women continue to smoke. A recent genome-wide association study demonstrated an association between a common polymorphism (rs1051730) in the nicotinic acetylcholine receptor gene cluster (*CHRNA5–CHRNA3–CHRNB4*) and both smoking quantity and nicotine dependence. We aimed to test whether the same polymorphism that predisposes to greater cigarette consumption would also reduce the likelihood of smoking cessation in pregnancy. We studied 7845 pregnant women of European descent from the South-West of England. Using 2474 women who smoked regularly immediately pre-pregnancy, we analysed the association between the rs1051730 risk allele and both smoking cessation during pregnancy and smoking quantity. Each additional copy of the risk allele was associated with a 1.27-fold higher odds (95% CI 1.11–1.45) of continued smoking during pregnancy ($P = 0.0006$). Adjustment for pre-pregnancy smoking quantity weakened, but did not remove this association [odds ratio (OR) 1.20 (95% CI 1.03–1.39); $P = 0.018$]. The same risk allele was also associated with heavier smoking before pregnancy and in the first, but not the last, trimester [OR for smoking 10+ cigarettes/day versus 1–9/day in first trimester = 1.30 (95% CI 1.13–1.50); $P = 0.0003$]. To conclude, we have found strong evidence of association between the rs1051730 variant and an increased likelihood of continued smoking in pregnancy and have confirmed the previously observed association with smoking quantity. Our data support the role of genetic factors in influencing smoking cessation during pregnancy.

INTRODUCTION

Maternal smoking in pregnancy is associated with a number of adverse outcomes including fetal growth restriction and various pregnancy complications (1,2). Clinical trials of interventions to promote smoking cessation have effectively reduced smoking and the prevalence of low birth weight and

preterm birth (3), but despite a strong and direct public health message, many pregnant women continue to smoke. In the year 2000, prevalence estimates for the USA, Sweden and UK, were 12, 13 and 20%, respectively (2,4).

Observational data suggest that 20–40% of female smokers quit during pregnancy (2). Smoking cessation during pregnancy is influenced by multiple factors, including maternal

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age, socioeconomic position, parity and partner's smoking (5,6). In addition, genetic susceptibility to the addictive properties of nicotine is likely to be important. Evidence from twin studies suggests that up to 70% of the variance in nicotine dependence is due to genetic factors (7). Consistent with this, a study of 840 adoptive families demonstrated high concordance between the smoking behaviour of adoptees and their biological full siblings (8).

Recently, a genome-wide association study of 15 771 Europeans showed that a single nucleotide polymorphism (SNP rs1051730) on chromosome 15q24 was associated with smoking quantity and nicotine dependence [assessed by questionnaire incorporating the Fagerstrom test (9)] among smokers (10). The polymorphism was equally prevalent in smokers and never-smokers, suggesting that it does not predispose to smoking initiation, but to dependence among those who smoke. Each additional copy of the minor, T, allele of the rs1051730 variant was associated with an increase of 0.095 (95% CI 0.075–0.115) smoking quantity units, approximately equal to one cigarette per day ($P = 6 \times 10^{-20}$). The SNP lies within the nicotinic acetylcholine receptor gene, *CHRNA3*, in a linkage disequilibrium block containing two other strong candidate genes, *CHRNA5* and *CHRNA4* (10). A recent study showed that the minor allele of the missense polymorphism, D398N, in *CHRNA5* (rs16969968), which is highly correlated with rs1051730 ($r^2 > 0.79$), conferred a reduced response to a nicotinic agonist *in vitro* ($P < 0.0001$) (11). This polymorphism may therefore be the functional variant responsible for the association with smoking quantity.

We considered that pregnancy would be a situation characterized by considerable health and social pressure to stop smoking and proposed that the same genetic factor that leads individual smokers to consume more cigarettes could also influence smoking cessation in pregnancy. We aimed to test the hypothesis that the rs1051730 risk allele is associated with an increased likelihood of continued smoking in pregnancy.

RESULTS

The basic characteristics of the study subjects are presented in Table 1.

Association between rs1051730 genotype and the odds of continued smoking in pregnancy

We observed a strong association between the rs1051730 risk allele and the odds of continuing to smoke in pregnancy. Each additional copy of the risk allele conferred a 1.27-fold higher odds (95% CI 1.11–1.45) of continuing to smoke during the first trimester ($P = 0.0006$). The association based on third trimester smoking was very similar ($P = 0.0003$; Table 2 and Supplementary Material, Table S1). Adjustment for pre-pregnancy smoking quantity weakened, but did not remove the associations (Table 2). The genetic associations were little altered by adjustment for covariates of smoking cessation in pregnancy (Supplementary Material, Table S1). These variables were not themselves associated with the rs1051730 risk allele (Supplementary Material, Table S2).

Table 1. Basic characteristics of study subjects

Study	ALSPAC	EFSOCH
Total number of pregnant women ^a	6998	847
Median age in years (IQR)	28 (25–31)	31 (27–34)
Median pre-pregnancy BMI in kg/m ² (IQR)	22.19 (20.47–24.55)	22.98 (21.12–25.52)
Percentage of women who had ever smoked regularly	49	29
Percentage of women who smoked regularly just before pregnancy	32	28
Percentage of women who smoked regularly during the first trimester of pregnancy ^b	23	18
Percentage of women who smoked regularly during the third trimester of pregnancy ^c	19	14

IQR, inter-quartile range.

^aIncludes women of white ethnicity with rs1051730 genotype and data available on whether or not they smoked regularly during the first 3 months of pregnancy.

^bWomen were questioned during the 18th week (ALSPAC) or the 28th week (EFSOCH) of pregnancy about their smoking behaviour during the first 3 months of pregnancy.

^cWomen were questioned during the 32nd week (ALSPAC) or the 28th week (EFSOCH) of pregnancy about their current smoking behaviour.

Association between rs1051730 genotype and smoking quantity

Within smokers ($N = 1361$ – 2379), the rs1051730 genotype was associated with smoking quantity before pregnancy [odds ratio (OR) for heavy (10+ cigarettes/day) versus light smoking (1–9 cigarettes/day) = 1.28 (95% CI 1.12–1.47); $P = 0.0004$] and during the first trimester [OR = 1.30 (95% CI 1.13–1.50); $P = 0.0003$], but not the third trimester [OR = 1.12 (95% CI 0.96–1.32); $P = 0.15$] (Table 3 and Supplementary Material, Table S3). Using all 7845 subjects with data on pre-pregnancy smoking status, we found no difference in the frequency of the risk allele between smokers and non-smokers [OR for being a smoker = 1.02 (95% CI 0.95–1.10); $P = 0.57$].

Association between maternal rs1051730 genotype and offspring birth weight

We hypothesized that each copy of the rs1051730 risk allele carried by the mother would be associated with reduced offspring birth weight in women who smoked during the first and/or third trimester of pregnancy. In this group ($n = 1829$), we observed a trend in the expected direction [per-risk allele change in birth weight = -28 g (95% CI -59 to 2 g); $P = 0.07$]. We additionally used a triangulation approach and first trimester data to estimate the expected effect size of the association between maternal genotype and offspring birth weight, assuming that it is mediated through the polymorphism's association with smoking quantity. The 95% CIs of our observed effect size estimate in this group included the expected

Table 2. Association between smoking cessation in pregnancy and the rs1051730 risk allele: combined analysis of data from the ALSPAC and EFSOCH studies

Time period	Number with questionnaire data available on smoking status (out of 2474 women who smoked regularly just before pregnancy)	% of women who had stopped smoking since pre-pregnancy	Risk allele frequency in women who continued to smoke/stopped smoking	Per-risk allele OR (95% CI) for continuing to smoke	P-value	Per-risk allele OR (95%CI) for continuing to smoke, adjusted for pre-pregnancy smoking quantity	P-value, adjusted for pre-pregnancy smoking quantity
First trimester	2474	28	0.35/0.30	1.27 (1.11–1.45)	0.0006	1.20 (1.03–1.39)	0.018
Third trimester	2249	43	0.36/0.30	1.27 (1.12–1.44)	0.0003	1.21 (1.05–1.39)	0.009

effect size, estimated using the birth weight–smoking quantity and smoking quantity–rs1051730 associations (Supplementary Material, Fig. S1). After adjustment for first trimester smoking quantity, the association with offspring birth weight was -21 g (95% CI -53 to 10 g) per maternal risk allele ($P = 0.19$). There was no association with offspring birth weight in the 5446 women who did not smoke in either the first or third trimester ($P = 0.86$).

DISCUSSION

Using data from 2474 European women, who smoked regularly before becoming pregnant, we have shown that the risk allele of the rs1051730 SNP in the *CHRNA5–CHRNA3–CHRNA4* gene cluster is associated with a 1.27-fold higher odds (95% CI 1.11–1.45) of continuing to smoke during pregnancy. We have also shown that the same risk allele is associated with the quantity of cigarettes smoked before pregnancy and in the first trimester. The association of the risk allele with continued smoking in pregnancy was reduced after adjustment for pre-pregnancy smoking quantity, but was not removed. This is consistent with the risk allele having two related effects, each reflecting a predisposition to nicotine dependence: (i) an effect on the likelihood of quitting through a primary effect on smoking quantity, whereby carriers find it harder to quit because they smoke in greater quantities and (ii) an effect on the likelihood of quitting, regardless of smoking quantity at the time of attempting to quit.

Previously, Thorgeirsson *et al.* (10) observed that the same variant was associated with smoking quantity in smokers, but not with smoking prevalence, implicating the variant in nicotine dependence rather than smoking initiation. Our data are consistent with this and with other studies reporting associations between SNPs in linkage disequilibrium with rs1051730 and nicotine dependence or smoking quantity (12–14). The lack of association with smoking quantity in our third trimester smokers may reflect this group being enriched with the most nicotine-dependent subjects. Two additional studies have demonstrated highly significant associations between rs1051730 and lung cancer (OR ≈ 1.3 ; $P < 10^{-8}$) but found either weak or no evidence of association with smoking behaviour, suggesting that further work is necessary to determine whether the variant has a role in disease susceptibility independently of smoking behaviour (15,16).

We observed a trend to reduced birth weight with each copy of the rs1051730 risk allele carried by the mother ($P = 0.07$). The size and direction of effect were consistent with those expected, given the smoking quantity–birth weight and smoking quantity–risk allele associations. However, the sample size was limited. Further well-powered studies are needed to investigate more thoroughly the hypothesized associations with fetal growth.

Our finding is the first robust association between a common genetic variant and smoking cessation. Previous candidate gene association studies investigating smoking cessation have used much smaller sample sizes and have either shown inconsistent results or require replication in independent larger studies (17,18). While our statistical evidence does not meet generally accepted criteria for ‘genome-wide

Table 3. Association between daily cigarette smoking quantity and the rs1051730 risk allele before and during pregnancy: combined analysis of data from the ALSPAC and EFSOCH studies

Time period	Smoking quantity category (cigarettes per day)	Number of smokers	Risk allele frequency	Overall per-risk allele increase in smoking quantity (SE) ^a	<i>P</i> -value	Overall per-risk allele OR (95% CI) for heavy (10+ cigarettes per day) versus light smoking (one to nine cigarettes per day) ^b	<i>P</i> -value
Pre-pregnancy	1–9	700	0.30	0.099 (0.054, 0.143)	1×10^{-5}	1.28 (1.12–1.47)	0.0004
	10–19	1026	0.34				
	20+	653	0.38				
	Total	2379 ^c	0.34				
First trimester	1–9	821	0.32	0.088 (0.040, 0.137)	0.0004	1.30 (1.13–1.50)	0.0003
	10–19	674	0.38				
	20+	246	0.39				
	Total	1741	0.35				
Third trimester	1–9	551	0.33	0.042 (–0.012, 0.097)	0.13	1.12 (0.96–1.32)	0.15
	10–19	605	0.36				
	20+	205	0.37				
	Total	1361	0.35				

^aFrom linear regression of smoking quantity (three categories: 0 = '1–9', 1 = '10–19', 2 = '20+') on number of risk alleles, adjusted for study.

^bFrom logistic regression of heavy (=1) versus light (=0) smoking on number of risk alleles, adjusted for study.

^cOf the 2474 women who said they smoked regularly pre-pregnancy, 2379 women gave details of smoking quantity.

significance' ($P < 5 \times 10^{-8}$), the prior genome-wide association with smoking quantity ($P = 6 \times 10^{-20}$) (10), coupled with the strong evidence for association in our study ($P = 0.0003–0.0006$), suggests that our result is unlikely to be a false-positive.

Motivated by concern for the health of their babies (2), women are more likely to quit smoking in pregnancy than at any other time (17). However, many pregnant women continue to smoke. Our data support the role of genetic factors in predisposing to this detrimental behavioural phenotype. The association is not deterministic: approximately one-third of women carrying two copies of the risk allele did quit smoking in pregnancy. However, the 1.66-fold (95% CI 1.21–2.26) higher odds of continued smoking in women with two risk alleles (11% of the total) versus women with none (44%) is a strong evidence that the polymorphism is a susceptibility factor for an important human behavioural trait. This may have implications for the design of interventions to help women quit smoking in pregnancy, and possibly for smoking cessation strategies more generally. It will be important to investigate this possibility.

There are some limitations to our study. First, data on smoking behaviour were self-reported. Multiple validation studies using biochemical markers such as cotinine have demonstrated that pregnant women may not admit to smoking (2), so our prevalence figures are likely to be underestimates. In addition, examination of our data suggests that non-responders in the third trimester in the Avon Longitudinal Study of Parents and Children (ALSPAC) study were more likely to be pre-pregnancy smokers than were responders (Supplementary Material, Fig. S2). However, such sources of error in the phenotypic data are likely to result in increased noise and reduced power rather than confounding (19). Importantly, the associations we observed are unlikely to be due to bias in self-reporting of smoking cessation. Any bias in our study of pregnant women is more likely to be towards a null result than a false-positive result. This is because while pregnant smokers may falsely report that they have quit, the reverse situation, in which pregnant women falsely report

smoking, is extremely unlikely. Women who continue to smoke in pregnancy are expected to have a higher frequency of the risk allele due to higher nicotine dependence. If a proportion of these women falsely declared that they had quit smoking, this would cause the group of 'quitters' to be enriched with the risk allele, causing the association to be biased towards the null. Another limitation is that our study participants may not fully represent the general population. We have previously shown that Exeter Family Study of Childhood Health (EFSOCH) study participants had a lower-than-average level of socioeconomic deprivation and were more likely to be non-smokers (20). However, the rs1051730 SNP is not associated with these factors, so this will not introduce bias.

To conclude, our study provides further evidence for a role of the rs1051730 SNP in the *CHRNA5–CHRNA3–CHRNA4* gene cluster in smoking quantity and demonstrates an association with smoking cessation in pregnancy. Further studies are needed to assess the association between the SNP and smoking cessation in other situations. Our finding provides an example of how genes can influence what is perceived by many to be a purely behavioural phenotype. There are parallels between our results and those of genetic association studies which have implicated appetite-regulatory pathways in obesity (21,22). Both phenotypes are thought by many scientists, health-care professionals and policy-makers to be a matter of 'self-control' and have much social stigma attached. Twin and other studies have previously shown that these traits have a heritable component, but the identification of robust associations with common genetic variants may help a little to emphasize that physiology plays an important role in 'socially unacceptable' phenotypes.

MATERIALS AND METHODS

Study subjects

We studied pregnant women of white, European ancestry from two studies (Table 1). The ALSPAC study (23) is a

prospective study, which recruited pregnant women from Bristol, UK, with expected delivery dates between April 1991 and December 1992. The EFSOCH study (20) is a prospective study of children born between 2000 and 2004, and their parents, from a geographically defined region of Exeter, UK. All women gave informed consent and ethical approval was obtained from the ALSPAC Law and Ethics Committee and the local review committee for each study.

Data collection on smoking behaviour and covariates of smoking cessation in pregnancy

Smoking behaviour of women before and during pregnancy was determined from questionnaires (Supplementary Material, Fig. S3). In the ALSPAC study, a questionnaire was administered in the 18th gestational week, asking about lifetime, pre-pregnancy and first-trimester smoking behaviour (whether or not the woman smoked and, for smokers, the quantity of cigarettes per day). Women were questioned again about current smoking behaviour during the 32nd week of pregnancy. In the EFSOCH study, a questionnaire was administered during the 28th gestational week, asking about lifetime, pre-pregnancy, first trimester and current smoking quantity. At each time point, the data on smoking quantity from both studies were categorized into 1–9, 10–19 and 20+ cigarettes per day. Data on known covariates of smoking cessation in pregnancy (5,6) were also collected via questionnaire: age, Townsend deprivation score (24) (EFSOCH only), occupational position, educational level (ALSPAC only), parity, partner's smoking status and age started smoking (ALSPAC only).

Genotyping

The rs1051730 polymorphism was genotyped in subjects from both studies using standard methods, details of which are presented in the Supplementary Material.

Statistical methods

Analysis of the association between rs1051730 genotype and the odds of continued smoking in pregnancy. We selected women reporting smoking regularly immediately before becoming pregnant (ALSPAC: $n = 2241$; EFSOCH: $n = 233$). We pooled subjects from both studies for analysis, after verifying that there was no difference in allele frequency ($P = 0.35$). Patients were classified, using data collected on first trimester smoking, as 'stopped smoking' or 'continued to smoke'. A similar dichotomous variable was created using data on third trimester smoking (assessed in ALSPAC at 32 weeks and EFSOCH at 28 weeks gestation). We performed logistic regression (multiplicative model) to assess the association between each dichotomized variable and number of copies of the rs1051730 risk allele carried, adjusting for study. In the separate studies, we repeated the logistic regression analysis including known covariates of smoking cessation in pregnancy.

It is possible that any association observed in our study between rs1051730 and smoking cessation in pregnancy would be secondary to the known association with smoking

quantity. To test this hypothesis, we repeated the analysis with pre-pregnancy smoking quantity included as a covariate.

Analysis of the association between rs1051730 genotype and smoking quantity. In the pooled data set, we assessed the association between the pre-pregnancy, first trimester and third trimester smoking quantity (cigarettes per day) and the rs1051730 polymorphism, by performing linear regression of smoking quantity level on number of risk alleles (10). We also dichotomized smoking quantity to reflect 'light' (1–9 cigarettes per day) and 'heavy' (10+ cigarettes per day) smoking, because the previous study found the largest difference in allele frequency between equivalent categories (10). We assessed the association between this variable and the number of risk alleles using logistic regression.

Analysis of the association between maternal rs1051730 genotype and offspring birth weight. We hypothesized that each copy of the risk allele carried by the mother would be associated with reduced offspring birth weight, via its association with smoking quantity, in women who smoked during pregnancy. To test this, we selected subjects who said that they smoked during the first and/or the third trimester of pregnancy (excluding multiple births and babies born before 36 full weeks' gestation). We performed linear regression of birth weight on the number of maternal risk alleles carried, with sex, gestational age and study as covariates. We then repeated this analysis including first trimester smoking quantity as an additional covariate. We additionally used a triangulation approach to estimate the expected effect size of the association between maternal genotype and offspring birth weight. We hypothesized that this effect size would be determined by (A) the effect size of the association between maternal genotype and smoking quantity and (B) the effect size of the association between smoking quantity and birth weight (see Supplementary Material, Fig. S1). We estimated (A) and (B) in the 1649 mothers with data available on offspring birth weight and first trimester smoking quantity, using linear regression, with sex, gestational age and study as covariates. The expected effect size was then estimated by multiplying (A) \times (B). We previously used the same approach to investigate associations between a common variant in the *FTO* gene, BMI and related metabolic traits (25).

SUPPLEMENTARY MATERIAL

Supplementary Material is available at *HMG* online.

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Conflict of Interest statement. None declared.

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