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Interplay of Genetic Risk (*CHRNA5*) and Environmental Risk (partner smoking) on Cigarette Smoking Reduction*

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Abstract

Background—This study tests whether the genetic predictor (*CHRNA5* nicotine receptor gene variants) and an environmental risk factor (partner smoking) interact in the prediction of smoking reduction.

Methods—Subjects were from a community-based, longitudinal study of women (N=1,856) who smoked before pregnancy, and a randomized comparative effectiveness smoking cessation trial (N=1,065). Smoking reduction was defined as the trajectory of self-reported smoking quantities over time in the observational study, and as the trajectory of alveolar CO levels in the cessation trial.

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Contributors: Authors Li-Shiun Chen, Timothy Baker, George Davey Smith, Marcus Munafo, and Laura Bierut designed the study. Authors Li-Shiun Chen, Timothy Baker, Marcus Munafo, and Laura Bierut wrote summaries of previous related work. Authors Charles Gu, Megan Piper, Steven Smith, and Rick Grucza advised on the analysis designs and plans. Authors Li-Shiun Chen undertook the statistical analysis, and author Li-Shiun Chen, Timothy Baker, Marcus Munafo, and Laura Bierut wrote the first draft of the manuscript. All authors contributed to and have approved the final manuscript.

Conflict of Interest: Laura J. Bierut is listed as an inventor on issued U.S. Patent 8,080,371, “Markers for Addiction” covering the use of certain SNPs in determining the diagnosis, prognosis, and treatment of addiction. All other authors declare no potential conflict of interest.

Results—In the pregnancy study, rs16969968 genotype and partner smoking status interacted such that the smoking reduction was lowest for expectant mothers with high genetic risk and partner smoking, and highest for those with high genetic risk but not partner smoking (interaction of genotype*partner smoking on smoking quantity trajectory slope β=0.071, 95%CI=0.013, 0.13, p=0.017). In the clinical trial, a similar interaction was found (interaction β=0.20, 95%CI=0.049, 0.36, p=0.010). Furthermore, these associations were moderated by pharmacotherapy such that the interactive relation of genetic and environmental factors occurred in the placebo group, but not in the active pharmacotherapy group (interaction of genotype*partner smoking*pharmacotherapy on CO trajectory slope β=-0.25, 95%CI=-0.42, -.091, p=0.0023).

Conclusions—The *CHRNA5* genetic risk synergized the effect of partner smoking, producing an especially low likelihood of successful smoking reduction in two complementary studies. This suggests that the genetic vulnerability may be mitigated by altering environmental factors. In addition, cessation pharmacotherapy neutralizes the increase in cessation failure associated with combined genetic and environmental risks, which has possible relevance to treatment algorithms.

Keywords

Smoking Reduction; *CHRNA5*; Partner Smoking; ALSPAC; UW-TTURC

1. Introduction

Tobacco smoking is a continuing global public health concern despite effective smoking cessation treatments and public health policies (Jha et al., 2013; Schroeder, 2013; Thun et al., 2013), and rates of smoking cessation failure remain high in both clinical and general populations (Baker et al., 2007; Breslau and Johnson, 2000; West, 2005). Identification of the genetic and environmental predictors of quitting success is critical in understanding the causes of smoking cessation outcomes and developing more effective clinical interventions and health policies.

Growing evidence suggests that genetic variants predict cessation success (Baker et al., 2009; Breitling et al., 2010; Conti et al., 2008; Freathy et al., 2009; King et al., 2012; Munafo et al., 2011; Rose et al., 2010; Sarginson et al., 2011; Uhl, 2009, 2008, 2012). Specifically, rs16969968, a non-synonymous coding variant in the nicotinic receptor gene (*CHRNA5)*, is not only unequivocally associated with heavy smoking in multiple large scale meta-analyses, but also is associated with a functionally significant change in nicotinic receptor binding to agonist (Bierut et al., 2008; Liu et al., 2010; Saccone et al., 2010; TAG, 2010; Thorgeirsson et al., 2010; Ware et al., 2011). This *CHRNA5* variant has been shown to predict smoking cessation success and response to cessation pharmacotherapy in multiple studies. Individuals with the rs16969968 risk variant (A) are less likely to be abstinent at the end of treatment and more likely to benefit from cessation pharmacotherapy such as nicotine replacement (Bergen et al., 2013; Chen et al., 2012b; Munafo et al., 2011).

Having a partner who smokes is a well-established risk factor for low motivation to quit smoking and failure to quit smoking successfully (Bolt et al., 2009; Harmer and Memon, 2013; Homish and Leonard, 2005; Okechukwu et al., 2012; Ruge et al., 2008). This may be because partner smoking allows immediate access to cigarettes and greater exposure to

smoking cues. It is currently unknown how this major environmental risk affects smoking cessation in the context of the major genetic risk (i.e., *CHRNA5* risk alleles). It is possible that the two factors merely produce additive effects, or they interact such that one amplifies the risk posed by the other. For instance, it is possible that partner smoking affects only those low in genetic risk; i.e., those high in genetic risk will likely relapse regardless of cigarette availability and exposure. Conversely, it is possible that environmental risk is most damaging to those high in genetic risk; i.e., partner smoking is especially challenging to those with a strong genetic vulnerability to cessation failure. The current research aims to address a clinically significant question: i.e., Do major genetic and environmental risks synergize to produce individuals with an especially high risk of cessation failure?

Using data from a community-based study, the Avon Longitudinal Study of Parents and Children (ALSPAC(Golding et al., 2001)), and a University of Wisconsin Transdisciplinary Tobacco Use Research Center (UW-TTURC; Piper et al., 2009)) smoking cessation clinical trial, we examine the main and interactive effects of partner smoking and *CHRNA5* genetic risk on smoking reduction likelihood. The two studies differ in type of participants, study duration, and design. However, complementary hypotheses are developed for these two research designs.

The ALSPAC study includes pregnant women smokers who are likely to limit their smoking or quit completely during pregnancy with health and social concerns (Cnattingius, 2004; Triche et al., 2008). In the ALSPAC study, the primary outcome is smoking reduction defined by a trajectory of decreasing self-reported smoking quantity during pregnancy. In the Wisconsin smoking cessation trial, a biomarker for smoking heaviness (alveolar CO level) was assessed both before and after the quit date through 8 weeks post-quit. CO level over time constitutes an objective biomarker of smoking reduction. In sum, smoking reduction is assessed by trajectories of self-reported smoking quantity in a community sample and alveolar CO level in a treatment trial. Use of continuous measures of smoking outcomes provides a more sensitive index of outcome than does a binary measure such as point prevalence abstinence (Baker et al., 2011).

Analyses address these questions: 1) Whether the *CHRNA5* effect on smoking reduction is moderated by partner smoking in the observational community study; 2) Whether the *CHRNA5* effect on smoking reduction is moderated by partner smoking in the cessation trial; and 3) Given evidence that the *CHRNA5* risk for smoking cessation failure occurs primarily amongst individuals not using pharmacotherapy, does the gene × environmental risk interaction occur only amongst individuals receiving placebo? Given evidence that CHRNA5 risk for smoking cessation varies with pharmacotherapy (Bergen et al., 2013; Chen et al., 2012b), we will also test whether the gene x environmental risk interaction varies with pharmacotherapy.

2. Methods

2.1 Avon Longitudinal Study of Parents and Children (ALSPAC)

The ALSPAC study (Fraser et al., 2012; Golding et al., 2001) is a prospective study that recruited pregnant women from Avon, UK, with expected delivery dates between April

1991 and December 1992 (known as Phase I enrollment). The study website contains details of all the data that are available through a fully searchable data dictionary (http://www.bris.ac.uk/alspac/researchers/data-access/data-dictionary/). All women gave informed consent and ethical approval was obtained from the ALSPAC Law and Ethics Committee and the local review committee.

Smoking behavior of women before and during pregnancy was determined from questionnaires. A questionnaire was administered in the 18th gestational week, asking about pre-pregnancy and first-trimester smoking behavior (whether or not the woman smoked and, for smokers, the quantity of cigarettes per day). Women were questioned again about current smoking behavior during the 32nd week of pregnancy. At each time point, the data on smoking quantity were categorized into 0, 1–9, 10–19, and 20+ cigarettes per day. Data on known covariates of smoking cessation in pregnancy (Ebert and Fahy, 2007; Lu et al., 2001) were also collected via questionnaire: age and partner's smoking status. Smoking cessation was defined as the trajectory of smoking quantity over 3 time points: pre-pregnancy, first-trimester, and third trimester. Cessation pharmacotherapy was not provided as part of this observational study and likely very rare given the risk of most medication use during pregnancy. The proxy variant for *CHRNA5* rs16969968, rs1051730 (r^2=1, 1000 Genome CEU, http://www.1000genomes.org/), was genotyped. Genetic and phenotypic data are available on 1,856 subjects of European ancestry.

2.2 University of Wisconsin Transdisciplinary Tobacco Use Research Center (UW-TTURC)

The UW-TTURC study was a randomized, placebo-controlled smoking cessation trial (Piper et al., 2009). The University of Wisconsin-Madison IRB approved this trial, and all subjects provided written informed consent. Participants were 18 years of age or older, smoked 10 or more cigarettes per day, and were motivated to quit smoking. Prior to randomization, participants completed baseline assessments of demographics, smoking history (including cigarettes smoked per day), and environmental risks (e.g., living with a partner who smoked). Participants provided a breath sample for alveolar carbon monoxide (CO) analysis to verify their smoking status and estimate their smoking heaviness at 6 time points: pre-quit, quit-date, and 1, 2, 4 and 8 weeks post-quit during the trial. Smoking reduction was defined as the linear trajectory of CO level over time.

Participants (N=1,065 of European ancestry with genetic data) were randomly assigned to either placebo (n = 134) or active pharmacotherapy ((n=931): (nicotine patch (n = 187); nicotine lozenge (n = 179); bupropion SR (n = 183); nicotine patch and nicotine lozenge (n = 192); or bupropion and nicotine lozenge (n = 190)) for 2 months. All participants received six brief (10 minute) individual counseling sessions.

Genotyping of the UW-TTURC sample was performed by the Center for Inherited Disease Research at Johns Hopkins University using the Illumina Omni2.5 microarray (www.illumina.com). Data cleaning was led by the GENEVA Coordinating Center at the University of Washington.

2.3 Analysis

We examined the association between the *CHRNA5* variant rs16969968, coded additively, and smoking cessation in both studies.

In the ALSPAC study, we used a standard series of mixed models to analyze smoking outcome: the linear trajectory of smoking quantity during pregnancy. Cigarettes smoked per day (CPD) were coded as 4 levels (0, 1–9, 10–19, 20+). Self-reported cigarettes smoked per day for 3 time points (pre-pregnancy, 1st trimester, 3rd trimester, with the repeated measures coded as 0, 1, and 2) was analyzed with mixed models for repeated measures. The β coefficient for 'time' was the slope characterizing the trajectory of smoking quantity change over time. For example, the interaction term of 'time' and rs16969968 was a test of genetic effect on the slope, i.e., the trajectory of smoking quantity. In secondary analyses, we examined dichotomous outcomes (abstinence and reduction) in the 32nd week of pregnancy with logistic regressions.

In the UW-TTURC study, we used a standard series of mixed models to analyze smoking outcome: the linear trajectory of alveolar CO levels during the trial. Because the distribution of CO levels was skewed to the right, it was square root transformed. Alveolar CO level for 6 time points (pre-quit, quit date, and 1, 2, 4 and 8 weeks post-quit, with repeated measure coded as 0-5) was analyzed with mixed models for repeated measures. Covariates included age, gender, and pharmacotherapy (placebo versus active pharmacotherapy in the UW-TTURC study). In secondary analyses, we examined the dichotomous outcome (abstinence) in the end of treatment at 2 months post-quit with logistic regressions.

3. Results

3.1 Avon Longitudinal Study of Parents and Children (ALSPAC)

Subjects were of European descent, identified as smokers pre-pregnancy (defined as active smoking ≥1 cigarettes per day (CPD)), and had genotype data (N=1,856). Demographic data, pre-pregnancy smoking quantity, genotype frequencies are given in Table S1(A)[1] and 62.0% of women reported living with a partner who smoked cigarettes. We found a robust association between *CHRNA5* rs16969968 and smoking heaviness defined by CPD adjusted for age (β=0.081, 95%CI=0.044 to 0.118, $p=1.47\times10^{-5}$).

During pregnancy, these women had a trajectory of decreased smoking quantity over time (pre-pregnancy, first trimester, 3rd trimester) (β=-0.53, 95% CI=-0.55 to -0.50, p<0.0001). Both genetic risk (rs16969968 (A)) and partner smoking predicted an overall higher level of smoking quantity during pregnancy (β=0.070, 95% CI=0.033-0.11, $p=1.8\times10^{-4}$ for rs16969968 (A); β=0.23, 95% CI=0.19 to 0.29, p<0.0001 for partner smoking; Table 1, Model 1). Genetic risk of variant rs16969968 interacted with partner smoking in the prediction of smoking quantity. The trajectory of smoking quantity remained especially elevated amongst women who had both a smoking partner and high risk in rs16969968 (interaction of genotype*partner smoking on smoking quantity trajectory slope β=0.071, 95% CI=0.013 to 0.13, p=0.017; Table 1, Model 2).

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Figure 1 displays the relations of the genetic and environmental risk factors with trajectory of smoking quantity. Figure 1(A) displays the decreasing level of smoking quantity during pregnancy and the pattern of heavier smoking for individuals with the high-risk rs16969968 genotype (AA) compared with those with the low-risk genotype (GG). Further, the right panel shows that the effects of the risk environment (partner smoking) are not equivalent across levels of genetic risk. The risk of partner smoking is increased markedly in the subjects with AA genotypes.

In secondary analyses, we found consistent results with dichotomous cessation outcomes at the 32nd week of pregnancy. Many pregnant women either reduced their smoking quantity (26%) or became abstinent (42%) at the 32nd week of pregnancy. These results suggested possible interactions of rs16969968 and partner smoking on whether they were abstinent at the 32nd week during pregnancy (interaction of genotype*partner smoking on abstinence OR=0.49, p=0.078) or reduced their smoking quantity (interaction OR=0.60, p=0.14).

3.2 University of Wisconsin Transdisciplinary Tobacco Use Research Center (UW-TTURC) Study

Subjects of European ancestry with genotype data and alveolar CO data were included in the analysis (N=1,065). The sample characteristics and genotype frequencies are shown in Table S1(B) and 27.7% of the participants lived with someone who smoked. In this treatment-seeking sample, CO was associated with *CHRNA5* rs16969968 adjusted for age and gender (β=0.13, 95% CI=0.041 to 0.23, p=0.0050), a modest effect in this sample of heavy smokers.

In this trial, 134 participants were randomized to the placebo group and thus, like the women in the ALSPAC study, did not use smoking cessation pharmacotherapy. During the cessation trial, these smokers showed decreasing alveolar CO level over time (pre-quit, quit date, and 1, 2, 4 and 8 weeks post-quit) (β=-0.29, 95% CI=-0.34 to -0.23, p<0.0001, Table 2, Model 1). The rs16969968 high-risk allele (A) predicted an increased level of alveolar CO level (β=0.36, 95% CI=0.050 to 0.67, p=0.023; Table 2, Model 1). Genetic risk of variant rs16969968 interacted with partner smoking in the prediction of smoking quantity as estimated by CO; the trajectory of CO remained especially elevated amongst subjects who had both partner smoking and high rs16969968 genetic risk (interaction of genotype*partner smoking on CO trajectory slope β=0.20, 95% CI=0.049 to 0.36, p=0.0101; Table 2, Model 2).

Figure 1(B) displays the decreasing alveolar CO level during the trial among placebo participants, and the pattern of heavier smoking for individuals with the high-risk rs16969968 genotype (AA) compared with those with the low-risk genotype (GG). Further, the right panel shows that the risk of partner smoking is increased in the subjects with AA genotypes. In secondary analyses, we found similar interaction results of partner smoking and rs16969968 on the dichotomous outcome, cross-sectional abstinence at 8 weeks post-quit (interaction of genotype*partner smoking on abstinence OR=4.53, df=1, p=0.049). Thus, the effect of partner smoking is most prominent in subjects with AA genotypes: those with a partner who smoked were less likely to be abstinent (0% vs. 25%) and had higher CO levels (21.7 vs. 15.3) at this time point (8 weeks) than those without a partner who smoked.

3.3 Pharmacotherapy neutralizes the increase in cessation failure associated with combined genetic and environmental risks

Using the same subjects in the TTURC study, we previously showed that pharmacotherapy benefit only those at high *CHRNA5* genetic risk (Chen et al., 2012b). That research, however, used a cross-sectional abstinence outcome. In the present study, we attempted to replicate that finding using the CO smoking trajectory outcome. Thus, we compared the genetic effect on smoking trajectory in the placebo and active pharmacotherapy groups. In subjects receiving placebo, there was a clear *CHRNA5* rs16969968 effect on smoking cessation trajectory, while in subjects receiving active pharmacotherapy, there was no such effect. There was an interaction between genetic risk and pharmacotherapy (interaction of genotype*pharmacotherapy on CO trajectory slope β=-0.092, 95% CI=-0.17 to -0.016, p=0.018; Table S2 Model 2[2]; Figure 1(B) and 1(C): left panel).

Given that pharmacotherapy appears to mitigate the genetic effects of *CHRNA5* on smoking cessation, we deemed it important to determine whether pharmacotherapy also mitigates the gene X environment interaction observed in the placebo group. Therefore, we tested and found a significant 3-way interaction involving genetic risk, environmental risk, and pharmacotherapy (interaction of genotype*partner smoking*pharmacotherapy on CO trajectory slope β=-0.25, 95% CI=-0.42 to -0.091, p=0.0023; Table 3 Model 2; Figure 1(A) and 1(B): right panel). In other words, the interaction of genetic and environmental risks was observed only in the placebo group (β=0.20, 95% CI=0.049 to 0.36, p=0.0101), but not in the active pharmacotherapy group (β=-0.14, 95% CI=-0.36 to 0.075, p=0.20). This gene * environment * pharmacotherapy interaction did not differ across the different active treatment arms (nicotine patch, nicotine lozenge, bupropion, nicotine patch and lozenge, bupropion and nicotine lozenge, F=1.2, df=4, p=0.31).

To further illustrate the effect of pharmacotherapy on CO trajectory, we show in Figure 2 how the pharmacotherapy effect differs as a function of the combined genetic and environmental risks. The strongest pharmacotherapy effect was seen in participants with both high-risk genotype (AA) and high-risk environment (living with someone who smokes), compared with the other three groups who have 0 or 1 risk factor. In secondary analyses, we found similar interaction results of partner smoking, rs16969968, and active pharmacotherapy when modeling a dichotomous outcome, cross-sectional abstinence at end of treatment at 2 months (interaction of genotype*partner smoking*pharmacotherapy on abstinence OR=0.20, df=1, p=0.039).

4. Discussion

Our study represents an initial evaluation of the complex interplay of gene, environment, and pharmacotherapy in smoking behaviors. We found, as in prior studies, that the risk variant rs16969968 in *CHRNA5* decreases the likelihood of smoking cessation success, as does living with a partner who smokes (Bergen et al., 2013; Bolt et al., 2009; Chen et al., 2012b; Harmer and Memon, 2013; Homish and Leonard, 2005; Munafo et al., 2011; Okechukwu et al., 2012; Ruge et al., 2008). However, across two complementary studies,

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we found a quantitative interaction between partner smoking and rs16969968 such that relative to other individuals, those with both risk factors were especially unlikely to quit or reduce their smoking successfully. In the ALSPAC community-based observational study of smoking cessation during pregnancy, expectant mothers decreased their smoking quantity over time while receiving no cessation pharmacotherapy. Both the genetic risk (rs16969968 (A)) and environmental risk (partner smoking) independently predicted less smoking reduction during pregnancy. In addition, an interaction was found between the variant rs16969968 and partner smoking: women who possessed the risk allele and lived with a partner who smoked were especially unlikely to reduce their smoking. Conversely, women with the risk allele who lived with a non-smoking partner had the greatest smoking reduction. This same pattern of interaction was found in the UW-TTURC smoking cessation trial; i.e., smokers possessing both risk factors who did not receive active pharmacotherapy were especially unlikely to quit or reduce their smoking, as evidenced by elevated CO levels. Despite differences in study population, motivation and support for smoking cessation, follow up duration, and type of outcome measure, both studies revealed the same interaction between the genetic and environmental risks. The risk associated with *CHRNA5* is moderated by partner smoking, a known risk for smoking cessation difficulty.

This gene-environment interaction suggests that the environmental risk effect is not constant, but variable depending on the individual';s genetic makeup. Partner smoking predicts cessation failure, and this effect is more prominent for individuals with the high-risk genotype compared to those with the low-risk genotype. One possible relapse mechanism might be that individuals with the high-risk genotype experience more craving and withdrawal symptoms while trying to quit (Chen et al., 2012a), and they are more likely to relapse if cigarettes are more accessible and triggers are more common when they live with someone who smokes.

This research extends existing evidence that *CHRNA5* increases the risk of cessation failure, and this increased risk is ameliorated by cessation pharmacotherapy (Bergen et al., 2013; Chen et al., 2012b). Our prior study evaluated these effects using a cross-sectional abstinence outcome. This study used the longitudinal assessment of a tobacco exposure biomarker (alveolar CO), a quantitative measure over time that should reflect smoking outcomes more sensitively than would a binary measure such as point-prevalence abstinence (Baker et al., 2011). Using repeated assessments of this tobacco exposure biomarker, we find that *CHRNA5* predicts higher CO levels, and there is also an interaction between *CHRNA5* and pharmacotherapy, with the genetic risk being ameliorated by cessation pharmacotherapy.

The results of this research have potential clinical relevance. First, they show that heightened risk for poor smoking outcomes, whether due to *CHRNA5* genotype or the environmental risk of partner smoking, can be mitigated through the use of smoking cessation pharmacotherapy (e.g., see Figure 1(B) and 1(C)). This observation agrees with recent suggestions that an optimal smoking cessation treatment algorithm should comprise both environmental and genetic risk factors (Bough et al., 2013). Second, the results suggest that in the absence of cessation pharmacotherapy, reducing exposure to smoking cues and opportunities may be especially important for individuals with high *CHRNA5* genetic risk.

The results of this study should be interpreted in the context of several limitations. First, the placebo group in the cessation trial is fairly small. However, the results obtained in a different study, the ALSPAC observational study of pregnant women, support the validity of the clinical trial results. These results should be treated with caution until further replication by independent studies or meta-analyses. Second, the smoking reports in the ALSPAC sample were not confirmed by biochemical confirmation. Although research shows that self-report is a valid indicant of current smoking when there are no strong incentives to deceive (SRNT, 2002), pregnant women may or may not misreport their smoking status (Dietz et al., 2011; Kvalvik et al., 2012). However, an objective biomarker of tobacco exposure (alveolar CO level) was obtained in the UW-TTURC trial to define smoking cessation. Third, we specifically examined a longitudinal smoking reduction outcome instead of abstinence, a commonly used dichotomous cross-sectional cessation outcome. Another limitation is the choice of a linear model in analyzing these repeated smoking quantity measures instead of others (e.g., quadratic model) which may capture the dynamic fluctuation of smoking quantity over time. Furthermore, this work only studied one genetic locus, and it is clear that multiple genes contribute to smoking cessation success. Fourth, this study took a targeted approach by examining the hypothesized interaction between *CHRNA5* and partner smoking in two complementary samples without exploring other possible interactions (Keller, 2014). Multiple differences exist across the two studies including the motivation to reduce of quit smoking, the level of cessation treatment, the demographic distribution, and the prevalence of partner smoking (62.0% in the pregnancy study, and 27.7 % in the clinical trial). These differences are important in the interpretation of these results. Environmental risk levels were not randomly assigned in this research, and so there might have been other factors correlated with environmental risk that were causally active in affecting smoking cessation and reduction. In addition, it is possible that the genetic and environmental effects are not independent; smokers with heightened genetic risk may be more likely to marry another smoker. Finally, this study only included subjects of European descent; therefore, findings may not generalize to other populations. We need more independent treatment studies and investigation of other environmental risks which may be partially correlated with partner smoking (Davey Smith, 2011).

While acknowledging the limitations of our study, we note that this work complements and builds upon existing research on the genetic, environmental, and treatment determinants of smoking cessation. Using diverse samples, this work underscores the importance of incorporating both genetic and environmental factors in order to understand smoking cessation failure and to design and apply smoking cessation treatments in an optimal manner. In addition to theoretical relevance, these results suggest that there is a population of smokers for whom medication, and perhaps environmental change, is especially important in order for them to achieve successful smoking cessation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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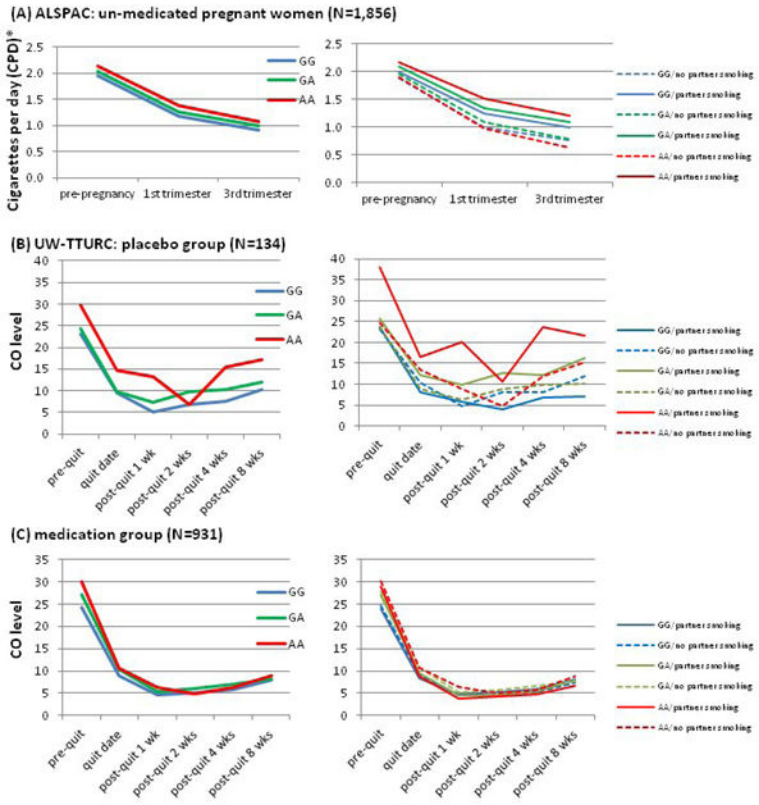

Figure 1.

Convergent results in two independent samples: Environmental effect (partner smoking) on quitting is stronger in individuals with *CHRNA5* risk allele: Convergent results in two independent samples of un-medicated smokers

(A) and (B)

Interaction of rs16969968 and partner smoking on quitting (decrease of smoking quantity over time) is significant.

(b=0.071, 95% CI 0.013-0.13, p=0.017 in ALSPAC, and b=0.20, 95%CI 0.049-0.36, p=0.010 in TTURC).

(B) and (C)

Medication neutralizes the G effect (b=-0.092, 95% CI=-0.17 to -0.016, 0.018).

Medication neutralizes the G*E effect (b=-0.25, 95% CI=-0.42 to -0.091, p=0.0023)

Reported data points indicate means in each group for that time points.

*CPD coding for 4 levels (0-1, 1=1-9, 2=10-19, 3=20 or more).

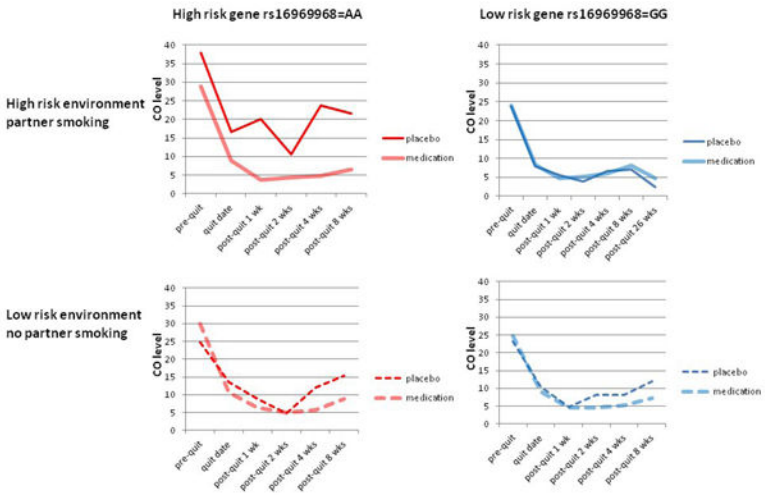

Figure 2.

Medication effect on post-quit smoking quantity is moderated by both *CHRNA5* rs16969968 genotypes and partner smoking status

Sample size	AA	GG
partner smoking	33	127
no partner smoking	87	304

Table 1

ALSPAC: The effect of *CHRNA5* rs16969968[a] on the trajectory of smoking quantity during pregnancy is moderated by partner smoking (n=1856)

		95% Confidence Interval		
Predictor	**B**	**Lower Bound**	**Upper Bound**	**P value**
Model 1				
Age	-0.0029	-.047	.041	0.90
Time	-.53	-.55	-.50	<0.0001
Partner smoking	0.23	0.19	0.29	<0.0001
rs16969968[a]	.070	.033	0.11	1.8×10^{-4}
Model 2				
Age	-0.0030	-0.047	0.041	0.90
Time	-0.51	-0.56	-0.46	<0.0001
Partner smoking	0.16	0.082	0.24	<0.0001
rs16969968[a]	0.078	0.021	0.14	7.8×10^{-3}
rs16969968[a] * Time	0.017	-0.032	0.066	0.50
Partner smoking* Time	0.032	-0.10	0.04	0.38
Partner smoking* rs16969968[a] * Time[a]	0.071	0.013	0.13	0.017

[a] rs1051730 was used as the proxy for rs16969968 (r^2=1.0 in 1000G CEU).

Table 2

UW-TTURC Placebo Group: The effect of *CHRNA5* rs16969968 on the trajectory of exhaled CO level after quitting is moderated by living with someone who smokes (n=134)

Parameter	β	95% Confidence Interval		P value
		Lower Bound	Upper Bound	
Model 1				
Age	0.0076	-0.0086	0.024	0.36
Gender	-0.29	-0.69	0.10	0.15
Time	-0.29	-0.34	-0.23	<0.0001
rs16969968	0.36	0.050	0.67	0.023
Partner smoking	0.22	-0.20	0.64	0.31
Model 2				
Age	0.0070	-0.0090	0.023	0.39
Gender	-0.29	-0.68	0.11	0.15
Time	-0.28	-0.37	-0.18	<0.0001
rs16969968	0.25	-0.12	0.62	0.18
Partner smoking	0.19	-0.31	0.69	0.45
rs16969968 * Time	-0.021	-0.12	0.081	0.69
Partner smoking * Time	-0.12	-0.28	0.033	0.12
Partner smoking * rs16969968 * Time	0.20	0.049	0.36	0.010

Table 3

UW-TTURC: The interactive effects of genetic effect (rs16969968) and environment (partner smoking) on the trajectory of exhaled CO level after quitting is moderated by cessation pharmacotherapy.

		95% CI		
Parameter	**β**	**LB**	**UB**	**Sig.**
Model 1				
Age	0.0024	-0.0030	0.0079	0.38
Gender	-0.27	-0.40	-0.15	<.0001
Time	-0.47	-0.49	-0.45	<.0001
rs16969968	0.17	0.043	0.23	0.0041
Medication	-0.39	-0.58	-0.20	<0.0001
Partner smoking	0.0037	-0.13	0.14	0.96
Model 2				
Age	0.0026	-0.0029	0.0080	0.35
Gender	-0.28	-0.40	-0.15	<0.0001
Time	-0.47	-0.51	-0.44	<0.0001
rs16969968_A	0.22	0.10	0.34	2.3×10^{-4}
Medication	0.074	-0.16	0.31	0.54
Partner smoking	-0.075	-0.25	0.098	0.39
rs16969968_A * Time	-0.033	-0.068	0.0021	0.065
Medication * Time	-0.18	-0.28	-0.089	1.5×10^{-4}
Partner smoking * Time	0.060	-0.0015	0.12	0.056
Medication * rs16969968 * Time	-0.012	-0.11	0.081	0.81
Partner smoking * rs16969968 * Time	-0.043	-0.10	0.014	0.14
Medication * Partner smoking * Time	0.15	-0.0027	0.30	0.054
Medication * Partner smoking * rs16969968 * Time	-0.25	-0.42	-0.091	2.3×10^{-3}

[a] rs16969968_A is coded additively as 0,1,2 copy of the minor risk allele.