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Short communication

Genetic variation (*CHRNA5*), medication (combination nicotine replacement therapy vs. varenicline), and smoking cessation

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ABSTRACT

Objective: Recent evidence suggests that the efficacy of smoking cessation pharmacotherapy can vary across patients based on their genotypes. This study tests whether the coding variant rs16969968 in the *CHRNA5* nicotinic receptor gene predicts the effects of combination nicotine replacement therapy (cNRT) and varenicline on treatment outcomes.
Method: In two randomized smoking cessation trials comparing cNRT vs. placebo, and varenicline vs. placebo, we used logistic regression to model associations between *CHRNA5* rs16969968 and abstinence at end of treatment.
Results: For abstinence at end of treatment, there was an interaction between cNRT and rs16969968 ($X^2 = 8.15$, d$f = 2$, omnibus-$p = 0.017$ for the interaction); individuals with the high-risk AA genotype were more likely to benefit from cNRT. In contrast, varenicline increased abstinence, but its effect did not vary with *CHRNA5*. However, the genetic effects differed between the placebo control groups across two trials (wald = 3.94, d$f = 1$, $p = 0.047$), this non-replication can alter the interpretation of pharmacogenetic findings.
Conclusions: Results from two complementary smoking cessation trials demonstrate inconsistent genetic results in the placebo arms. This evidence highlights the need to compare the most effective pharmacotherapies with the same placebo control to establish pharmacogenetic evidence to aid decisions on medication choice for patients trying to quit smoking.

1. Introduction

Smoking is a leading cause of preventable death and disability worldwide (Knopik et al., 2012; Schroeder, 2013; Thun et al., 2013; Whiteford and Baxter, 2013), and smoking cessation greatly diminishes the increased risk of mortality (Jha et al., 2013). Two pharmacotherapies have been shown to be more effective than others for smoking cessation: combination Nicotine Replacement Therapy (cNRT) and varenicline (Fiore and Baker, 2011; Fiore et al., 2008; Piper et al., 2009).

Cessation treatment may be improved via personalized medicine: i.e., using individual genetic markers to maximize efficacy and minimize side effects (McMahon and Insel, 2012; Vaidyanathan, 2012). Smokers vary greatly in the benefit they derive from particular pharmacotherapies, and genotypes may predict their response to a specific pharmacotherapy (Chen et al., 2014; David et al., 2013; Gold and Lerman, 2012; McGeary et al., 2012; Ray et al., 2009; Rose et al., 2010; Uhl et al., 2010). Large scale genome-wide association (GWA) meta-analyses (Liu et al., 2010; TAG, 2010; Thorgeirsson et al., 2010) have confirmed an association between nicotine dependence and the genetic variant rs16969968, which results in an amino acid change (D398N) in the nicotinic receptor

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gene *CHRNA5* (Saccone et al., 2007). *CHRNA5* encodes the α5 nicotinic receptor subunit and plays a role in the pharmacodynamic pathway of nicotine dependence (Fowler et al., 2011). Further studies have demonstrated change in the receptor function in response to nicotine agonists given this amino acid change in the α5 nicotinic receptor subunit (Bierut et al., 2008; Brown et al., 2007). Growing evidence indicates that rs16969968 in *CHRNA5* is a predictor for smoking cessation in some circumstances and predicts a delay in successful smoking cessation (Baker et al., 2009; Bergen et al., 2013; Chen et al., 2012, 2015; Freathy et al., 2009; Munafo et al., 2011; Sarginson et al., 2011; Zhu et al., 2014).

Using data from the University of Wisconsin Transdisciplinary Tobacco Use Research Center (UW-TTURC), we have previously shown that *CHRNA5* gene modulates clinical response to NRT. Specifically, smokers with the high risk genotypes were less likely to quit while receiving placebo medication (Chen et al., 2012). Whether the effects of these two effective pharmacotherapies (cNRT and varenicline) vary with *CHRNA5* is of clinical interest because these results may potentially inform medication choice.

Using data from both the UW-TTURC trial and the varenicline smoking cessation trial by Pfizer (Gonzales et al., 2006; Jorenby et al., 2006), we can examine the effects of *CHRNA5* and the two most effective cessation pharmacotherapies (varenicline and cNRT) on smoking abstinence. These two studies differ in type of participants, medication comparator, study duration, and design. However, complementary analyses can be developed for these two research designs. Our primary aim was to obtain and compare evidence on how the genetic effect of *CHRNA5* predicts smoking abstinence when subjects receive different treatments.

2. Methods

2.1. University Of Wisconsin Transdisciplinary Tobacco Use Research Center (Uw-Tturc) trial

Participants were eligible if they were 18 years of age or older, and smoked 10 or more cigarettes per day (Piper et al., 2009). The University of Wisconsin-Madison IRB approved this trial, and all subjects provided written informed consent. Participants ($N = 328$) were randomly assigned to either placebo ($n = 135$) or cNRT (nicotine patch and nicotine lozenge, $n = 193$) for eight weeks. Additional arms in this trial, not analyzed in this analysis, included nicotine patch, nicotine lozenge, bupropion SR, and bupropion/nicotine lozenge ($n = 747$). All participants received individual counseling.

The primary outcome was the biochemically confirmed seven-day abstinence at end of treatment (EOT, eight weeks post-quit). All self-reports of abstinence were confirmed by an expired carbon monoxide (CO) level of less than 10 parts per million (ppm). Individuals with missing data were considered to be smoking. This sample was previously examined by Chen et al. (2012) who reported the interaction of *CHRNA5* and medication on smoking abstinence (Chen et al., 2012). This paper presents new analyses targeting the effect of *CHRNA5* rs16969968 in the cNRT and placebo arms.

Genotyping 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.

2.2. Varenicline Randomized Controlled Trial

Participants were eligible if they were 18–75 years of age, smoked 10 or more cigarettes per day, and had <3 months of abstinence in the past year (Gonzales et al., 2006; Jorenby et al., 2006). This trial was conducted at 19 centers in the US and the IRB at each site approved this trial. All subjects provided written informed consent including genetic analyses. Participants ($N = 790$) were randomly assigned to either placebo ($n = 376$) or varenicline ($n = 414$) for 12 weeks. An additional study arm not included in this analysis was bupropion ($n = 345$). All participants received individual counseling.

The primary endpoint was four-week continuous abstinence for the last four weeks of treatment (weeks 9–12). Abstinence at each visit was defined as a self-report of no smoking since the previous visit, confirmed by an expired CO level of 10 ppm or less. In the case of missed visits, if at the next visit there was a self-report of no smoking and no use of other tobacco products, a status of not smoking was imputed. This sample was previously examined by King et al who reported association of multiple nicotinic receptor genes including *CHRNA5* (reported variant rs518425 has low correlation with rs16969968) and abstinence in the varenicline arm (King et al., 2012). This paper presents new analyses targeting the comparison of cNRT vs. placebo arms focusing on the *CHRNA5* rs16969968 genotype.

Genotyping was performed on the Illumina Golden Gate platform, ABI Taqman and SNPlex methods. Genotyping call rates and Hardy–Weinberg equilibrium were previously published (King et al., 2012).

2.3. Analysis

The primary phenotype was smoking abstinence at EOT, and the primary predictor was the rs16969968 genotype. We used logistic regression models. Subjects of European ancestry were examined. The following covariates were tested: gender, age, cigarettes per day (in four levels: ≤10, 11–20, 21–30, ≥31), and treatment (placebo vs. cNRT in the UW-TTURC trial; placebo vs. varenicline in the varenicline trial). Age did not differ across treatment arms and was not significantly associated with cessation outcomes, so it was not included in the final models.

3. Results

3.1. CHRNA5 and pharmacotherapy are associated with abstinence

3.1.1. UW-TTURC trial (N = 328). Table S1[1] shows demographics and genotype frequencies. 48.5% of participants were abstinent at EOT (eight weeks post-quit). Receiving cNRT, compared to placebo, increased abstinence (60.1% in cNRT $n = 193$; 31.9% in placebo $n = 135$; OR = 3.17, 95% CI = 1.99–5.05, d$f = 1$, $p = 8.1 \times 10^{-6}$).

Consistent with our previous findings, the association of rs16969968 genotypes with abstinence depended upon treatment condition. Rs16969968 genotypes were associated with abstinence in the placebo group ($X^2 = 6.54$, d$f = 2$, $p = 0.038$), but not in the cNRT group ($X^2 = 2.55$, d$f = 2$, $p = 0.28$). The effect of cNRT differed across genotype groups (GG: OR = 1.57, 95% CI = 0.79–3.16, d$f = 1$, $p = 0.20$; GA: OR = 4.45, 95% CI = 2.21–8.98, d$f = 1$, $p = 3.1 \times 10^{-5}$; AA: OR = 14.7, 95% CI = 2.57–84.3, d$f = 1$, $p = 0.0025$). These results reflect a significant interaction between treatment (placebo versus cNRT) and rs16969968 genotypes (Table 1; Fig. 1). We found a similar pharmacogenetic interaction result after adjusting for cigarettes per day (CPD; Table S3[2]).

We identified the utility of *CHRNA5* genotypes in predicting response to cNRT (Fig. 1A): the clinical impact of this interaction

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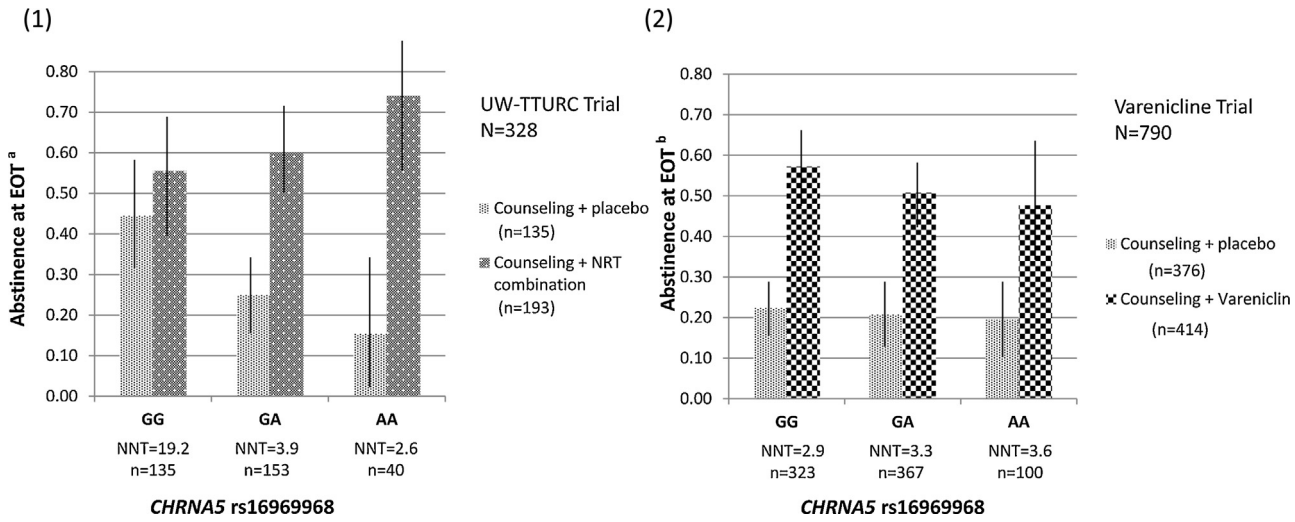

Fig. 1. Medication efficacy on abstinence EOT and *CHRNA5* rs16969968 genotypes: (1) UW-TTURC trial shows that combination NRT increases abstinence EOT in genotypes GA and AA, but not GG. Varenicline trial shows that varenicline increases abstinence across genotypes. (2) Genotypic effects in the placebo groups differ between these two trials. [a]UW-TTURC trial: Combination NRT increases abstinence(OR = 3.17, 95% CI = 1.99–5.05, df = 1, $p = 8.1 \times 10^{-6}$). A significant interaction between treatment (placebo versus combination NRT) and rs16969968 genotypes ($X^2 = 8.15$, df = 2, omnibus-p = 0.017 for the interaction). [b]Varenicline trial: Varenicline increases abstinence (OR = 4.18, 95% CI = 3.05–5.72, df = 1, $p = 5.5 \times 10^{-19}$). The *CHRNA5* rs16969968 genotypic effects on abstinence in the placebo groups differ significantly between the two studies (rs16969968 × Study Interaction Wald = 3.94, df = 1, p = 0.047). There is a genotypic effect in the UW-TTURC trial placebo arm, but not in the varenicline trial placebo arm. NNT: number needed to treat.

Table 1
CHRNA5 rs16969968 and the medication effect on abstinence at end-of-treatment.

UW-TTURC trial (N = 328)	Abstinence at end of treatment		
	Odds ratio	95% C.I.	P-value
Genotypes (rs16969968)			
GG	Reference		
GA	0.42	(0.19, 0.90)	0.025
AA	0.23	(0.05, 1.15)	0.073
Treatment Status			
Placebo	Reference		
Combination NRT (cNRT)	1.57	(0.78, 3.13)	0.21
Interaction of genotypes and intervention			(a)
GG × cNRT	Reference		
GA × cNRT	2.92	(1.09, 7.82)	0.033
AA × cNRT	9.79	(1.51, 63.6)	0.017
Varenicline trial (N = 790)			
Genotypes (rs16969968)			
GG	Reference		
GA	0.82	(0.59, 1.13)	0.23
AA	0.75	(0.45, 1.24)	0.26
Treatment status(b)			
Placebo	Reference		
Varenicline	4.18	(3.05, 5.72)	5.5×10^{-19}

All models were adjusted for gender. C.I. = Confidence Interval.
(a) chi squared = 8.15, df = 2, omnibus p = 0.017 for the overall interaction effect.
(b) Test of gene-treatment interaction is not significant with chi square = 0.32, df = 2, omnibus p = 0.85.

was exemplified by the number needed to treat (NNT), which varied with genotypes. Smokers with AA and GA genotypes were more likely to respond to cNRT (NNT = 2.6 and 3.9, respectively). Conversely, smokers with the GG genotype received less benefit from cNRT (NNT = 19.2).

*3.1.2. Varenicline trial (*N = *790).* In the trial, 38.0% of participants had four-week continuous abstinence at EOT (weeks 9–12 post-quit). Receiving varenicline, compared to placebo, increased abstinence (53.1% in varenicline arm n = 414; 21.3% in placebo arm n = 376; OR = 4.18, 95% CI = 3.05–5.72, df = 1, $p = 5.5 \times 10^{-19}$). Varenicline was associated with abstinence across all three genotype groups (GG: OR = 4.65, CI = 2.85–7.57, $p = 7.0 \times 10^{-10}$; GA: OR = 3.89, 95% CI = 2.44–6.20, df = 1, $p = 1.0 \times 10^{-8}$; AA: OR = 3.80, 95% CI = 1.56–9.27, df = 1, p = 0.0033).

Unlike the UW-TTURC trial results, rs16969968 genotype was not associated with abstinence in the placebo group or the varenicline group (Table 1; Fig. 1), and there is no pharmacogenetic interaction. Given the sample size (n = 403 in placebo; n = 428 in varenicline), two-sided α of 0.05, and baseline abstinence rate of 0.21, we had power to detect a genotypic association with an OR of 0.66 or lower. In the varenicline trial, NNT for varenicline did not vary much by genotype (NNT = 3.6, 3.3, 2.9 for smokers with AA, GA GG genotypes, respectively).

3.2. Placebo arms differ between trials

Both trials include a placebo arm and the genotypic associations in the placebo arms significantly differed between these two trials (interaction wald = 3.94, df = 1, p = 0.047). In the UW-TTURC trial, *CHRNA5* rs16969968 genotypes were associated with abstinence at EOT in the placebo group ($X^2 = 6.54$, df = 2, p = 0.038). Unlike the UW-TTURC trial results, rs16969968 genotype was not associated with abstinence at EOT in the placebo group ($X^2 = 0.21$, df = 2, p = 0.90) in the varenicline trial.

4. Discussion

Our findings demonstrate the significant difference in the *CHRNA5* genetic effect on abstinence at end of treatment in the placebo groups in two cessation trials. This difference across the two trials is likely due to heterogeneity in study design such as ascertainment, placebo conditions, and counseling effects. This difference in placebo groups substantially alters the interpretation and comparison of pharmacogenetic effects of two highly effective smoking cessation pharmacotherapies (cNRT and varenicline). Comparison of results from these two important trials highlights the need for a head-to-head comparison trial with a uniform placebo control to compare the pharmacogenetic effects related to both pharmacotherapies. No randomized trial has yet compared the efficacy of varenicline and cNRT with the same placebo control

(Rigotti, 2013). Thus, the current results are intriguing and suggest caution in extrapolating research findings into clinical practice.

Combination NRT (cNRT) and varenicline have been identified as the two most effective smoking cessation medications (Cahill et al., 2013; Fiore et al., 2008). In the UW-TTURC trial, the efficacy of cNRT varied with rs16969968 genotypes, representing a pharmacogenetic interaction. In contrast, in the varenicline trial, we found that the efficacy of varenicline did not vary with *CHRNA5* genotypes, which then leads to the conclusion that all subjects respond to varenicline regardless of *CHRNA5* rs16969968 genotype. These results are based on retrospective analyses of existing trials testing different medications, using dissimilar designs, and showing markedly different clinical success in the placebo arms (e.g., 44% vs. 22% abstinence for GG genotypes in the UW-TTURC and varenicline trials), all of which suggest caution in the interpretation of these pharmacogenetic effects as well as raise the question about why the placebo responses are differing significantly between the two studies.

The results of this study should be interpreted in the context of several limitations. First, the power was limited in specific treatment or placebo arms due to sample sizes. Thus, the observed association or lack of association between *CHRNA5* and smoking cessation should be considered preliminary. Second, the genetic risk for cessation cannot be directly compared across the different pharmacotherapy conditions (cNRT vs. varenicline) due to dissimilar study designs. Third, this work studies only one gene and it is clear that multiple genes contribute to smoking cessation success. Finally, this study only included subjects of European descent.

In summary, this work highlights the challenges and needed evidence to translate genetic research findings in the area of precision medicine. Using two complementary trials, the genetic effect on placebo response differs significantly between the two trials and this difference then influences the interpretation of pharmacogenetic response of smoking cessation. If the results in the placebo groups were reversed in the two trials, a completely different interpretation of the pharmacogenetic results would follow. This finding highlights the importance of having a head-to-head comparison with the same placebo control in pharmacogenetic research in order to achieve the goal of precise smoking cessation treatments (Rutter, 2006).

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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. Nancy Saccone's spouse is also listed as an inventor on the above issued U.S. Patent 8,080,371. Robert M. Carney or a member of his family owns stock in Pfizer, Inc. Douglas Jorenby has received research support from Pfizer. All other authors declare no potential conflict of interest.

Contributors

Authors Li-Shiun Chen, Timothy Baker, Douglas Jorenby, Megan Piper, and Laura Bierut designed the study. Authors Li-Shiun Chen, Timothy Baker, and Laura Bierut wrote summaries of previous related work. Authors Douglas Jorenby, Megan Piper, Nancy Saccone, Eric Johnson, Naomi Breslau, Dorothy Hatsukami, and Robert Carney advised on the analysis designs and plans. Author Li-Shiun Chen undertook the statistical analysis, and authors Li-Shiun Chen, Timothy Baker, and Laura Bierut wrote the first draft of the manuscript. All authors contributed to and have approved the final manuscript.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.drugalcdep.2015.06.022

References

Baker, T.B., Weiss, R.B., Bolt, D., von Niederhausern, A., Fiore, M.C., Dunn, D.M., Piper, M.E., Matsunami, N., Smith, S.S., Coon, H., McMahon, W.M., Scholand, M.B., Singh, N., Hoidal, J.R., Kim, S.Y., Leppert, M.F., Cannon, D.S., 2009. Human neuronal acetylcholine receptor A5-A3-B4 haplotypes are associated with multiple nicotine dependence phenotypes. Nicotine Tob. Res. 11, 785–796.

Bergen, A.W., Javitz, H.S., Krasnow, R., Nishita, D., Michel, M., Conti, D.V., Liu, J., Lee, W., Edlund, C.K., Hall, S., Kwok, P.Y., Benowitz, N.L., Baker, T.B., Tyndale, R.F., Lerman, C., Swan, G.E., 2013. Nicotinic acetylcholine receptor variation and response to smoking cessation therapies. Pharmacogenet. Genomics 23, 94–103.

Bierut, L.J., Stitzel, J.A., Wang, J.C., Hinrichs, A.L., Grucza, R.A., Xuei, X., Saccone, N.L., Saccone, S.F., Bertelsen, S., Fox, L., Horton, W.J., Breslau, N., Budde, J., Cloninger, C.R., Dick, D.M., Foroud, T., Hatsukami, D., Hesselbrock, V., Johnson, E.O., Kramer, J., Kuperman, S., Madden, P.A., Mayo, K., Nurnberger Jr., J., Pomerleau, O., Porjesz, B., Reyes, O., Schuckit, M., Swan, G., Tischfield, J.A., Edenberg, H.J., Rice, J.P., Goate, A.M., 2008. Variants in nicotinic receptors and risk for nicotine dependence. Am. J. Psychiatry 165, 1163–1171.

Brown, R.W., Collins, A.C., Lindstrom, J.M., Whiteaker, P., 2007. Nicotinic alpha5 subunit deletion locally reduces high-affinity agonist activation without altering nicotinic receptor numbers. J. Neurochem. 103, 204–215.

Cahill, K., Stevens, S., Perera, R., Lancaster, T., 2013. Pharmacological interventions for smoking cessation: an overview and network meta-analysis. Cochrane Database Syst. Rev. 5, 1–44 (CD009329).

Chen, L.S., Baker, T.B., Piper, M.E., Breslau, N., Cannon, D.S., Doheny, K.F., Gogarten, S.M., Johnson, E.O., Saccone, N.L., Wang, J.C., Weiss, R.B., Goate, A.M., Bierut, L.J., 2012. Interplay of genetic risk factors *(CHRNA5-CHRNA3-CHRNB4)* and cessation treatments in smoking cessation success. Am. J. Psychiatry 169, 735–742.

Chen, L.S., Bloom, J.A., Baker, T.B., Smith, S.S., Piper, M.E., Martinez, M., Saccone, N.L., Hatsukami, D., Goate, A.M., Bierut, L.J., 2014. Pharmacotherapy effects on smoking cessation vary with nicotine metabolism gene *(CYP2A6)*. Addiction 109, 128–137.

Chen, L.S., Hung, R.J., Baker, T., Horton, A., Culverhouse, R., Saccone, N., Cheng, I., Deng, B., Han, Y., Hansen, H.M., Horsman, J., Kim, C., Lutz, S., Rosenberger, A., Aben, K.K., Andrew, A.S., Breslau, N., Chang, S.C., Dieffenbach, A.K., Dienemann, H., Frederiksen, B., Han, J., Hatsukami, D.K., Johnson, E.O., Pande, M., Wrensch, M.R., McLaughlin, J., Skaug, V., van der Heijden, H.F., Wampfler, J., Wenzlaff, A., Woll, P., Zienolddiny, S., Bickeboller, H., Brenner, H., Duell, E.J., Haugen, A., Heinrich, J., Hokanson, J.E., Hunter, D.J., Kiemeney, L.A., Lazarus, P., Le Marchand, L., Liu, G., Mayordomo, J., Risch, A., Schwartz, A.G., Teare, D., Wu, X., Wiencke, J.K., Yang, P., Zhang, Z.F., Spitz, M.R., Kraft, P., Amos, C.I., Bierut, L.J., 2015. *CHRNA5* risk variant predicts delayed smoking cessation and earlier lung cancer diagnosis-a meta-analysis. J. Natl. Cancer Inst. 107 (5), 1–9.

David, S.P., Strong, D.R., Leventhal, A.M., Lancaster, M.A., McGeary, J.E., Munafo, M.R., Bergen, A.W., Swan, G.E., Benowitz, N.L., Tyndale, R.F., Conti, D.V., Brown, R.A., Lerman, C., Niaura, R., 2013. Influence of a dopamine pathway additive genetic efficacy score on smoking cessation: results from two randomized clinical trials of bupropion. Addiction 108, 2202–2211.

Fiore, M.C., Baker, T.B., 2011. Clinical practice. Treating smokers in the health care setting. N. Engl. J. Med. 365, 1222–1231.

Fiore, M.C., Jaen, C.R., Tb, B., et al., 2008. Treating Tobacco Use And Dependence: 2008 Update. U.S. Department Of Health And Human Services, U.S. Public Health Service, Rockville, MD.

Fowler, C.D., Lu, Q., Johnson, P.M., Marks, M.J., Kenny, P.J., 2011. Habenular alpha5 nicotinic receptor subunit signalling controls nicotine intake. Nature 471, 597–601.

Freathy, R.M., Ring, S.M., Shields, B., Galobardes, B., Knight, B., Weedon, M.N., Smith, G.D., Frayling, T.M., Hattersley, A.T., 2009. 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. Hum. Mol. Genet. 18, 2922–2927.

Gold, A.B., Lerman, C., 2012. Pharmacogenetics of smoking cessation: role of nicotine target and metabolism genes. Hum. Genet. 131 (6), 857–876.

Gonzales, D., Rennard, S.I., Nides, M., Oncken, C., Azoulay, S., Billing, C.B., Watsky, E.J., Gong, J., Williams, K.E., Reeves, K.R., 2006. Varenicline, an alpha4beta2 nicotinic acetylcholine receptor partial agonist, vs. sustained-release bupropion and placebo for smoking cessation: a randomized controlled trial. JAMA 296, 47–55.

Jha, P., Ramasundarahettige, C., Landsman, V., Rostron, B., Thun, M., Anderson, R.N., McAfee, T., Peto, R., 2013. 21st-century hazards of smoking and benefits of cessation in the United States. N. Engl. J. Med. 368, 341–350.

Jorenby, D.E., Hays, J.T., Rigotti, N.A., Azoulay, S., Watsky, E.J., Williams, K.E., Billing, C.B., Gong, J., Reeves, K.R., 2006. Efficacy of varenicline, an alpha4beta2 nicotinic acetylcholine receptor partial agonist, vs. placebo or sustained-release bupropion for smoking cessation: a randomized controlled trial. JAMA 296, 56–63.

King, D.P., Paciga, S., Pickering, E., Benowitz, N.L., Bierut, L.J., Conti, D.V., Kaprio, J., Lerman, C., Park, P.W., 2012. Smoking cessation pharmacogenetics: analysis of varenicline and bupropion in placebo-controlled clinical trials. Neuropsychopharmacology 37, 641–650.

Knopik, V.S., Maccani, M.A., Francazio, S., McGeary, J.E., 2012. The epigenetics of maternal cigarette smoking during pregnancy and effects on child development. Dev. Psychopathol. 24, 1377–1390.

Liu, J.Z., Tozzi, F., Waterworth, D.M., Pillai, S.G., Muglia, P., Middleton, L., Berrettini, W., Knouff, C.W., Yuan, X., Waeber, G., Vollenweider, P., Preisig, M., Wareham, N.J., Zhao, J.H., Loos, R.J., Barroso, I., Khaw, K.T., Grundy, S., Barter, P., Mahley, R., Kesaniemi, A., McPherson, R., Vincent, J.B., Strauss, J., Kennedy, J.L., Farmer, A., McGuffin, P., Day, R., Matthews, K., Bakke, P., Gulsvik, A., Lucae, S., Ising, M., Brueckl, T., Horstmann, S., Wichmann, H.E., Rawal, R., Dahmen, N., Lamina, C., Polasek, O., Zgaga, L., Huffman, J., Campbell, S., Kooner, J., Chambers, J.C., Burnett, M.S., Devaney, J.M., Pichard, A.D., Kent, K.M., Satler, L., Lindsay, J.M., Waksman, R., Epstein, S., Wilson, J.F., Wild, S.H., Campbell, H., Vitart, V., Reilly, M.P., Li, M., Qu, L., Wilensky, R., Matthai, W., Hakonarson, H.H., Rader, D.J., Franke, A., Wittig, M., Schafer, A., Uda, M., Terracciano, A., Xiao, X., Busonero, F., Scheet, P., Schlessinger, D., St Clair, D., Rujescu, D., Abecasis, G.R., Grabe, H.J., Teumer, A., Volzke, H., Petersmann, A., John, U., Rudan, I., Hayward, C., Wright, A.F., Kolcic, I., Wright, B.J., Thompson, J.R., Balmforth, A.J., Hall, A.S., Samani, N.J., Anderson, C.A., Ahmad, T., Mathew, C.G., Parkes, M., Satsangi, J., Caulfield, M., Munroe, P.B., Farrall, M., Dominiczak, A., Worthington, J., Thomson, W., Eyre, S., Barton, A., Mooser, V., Francks, C., Marchini, J., 2010. Meta-analysis and imputation refines the association of 15q25 with smoking quantity. Nat. Genet. 42, 436–440.

McGeary, J.E., Knopik, V.S., Hayes, J.E., Palmer, R.H., Monti, P.M., Kalman, D., 2012. Predictors of relapse in a bupropion trial for smoking cessation in recently-abstinent alcoholics: preliminary results using an aggregate genetic risk score. Subst. Abuse 6, 107–114.

McMahon, F.J., Insel, T.R., 2012. Pharmacogenomics and personalized medicine in neuropsychiatry. Neuron 74, 773–776.

Munafo, M.R., Johnstone, E.C., Walther, D., Uhl, G.R., Murphy, M.F., Aveyard, P., 2011. *CHRNA3* rs1051730 genotype and short-term smoking cessation. Nicotine Tob. Res. 13, 982–988.

Piper, M.E., Smith, S.S., Schlam, T.R., Fiore, M.C., Jorenby, D.E., Fraser, D., Baker, T.B., 2009. A randomized placebo-controlled clinical trial of 5 smoking cessation pharmacotherapies. Arch. Gen. Psychiatry 66, 1253–1262.

Ray, R., Tyndale, R.F., Lerman, C., 2009. Nicotine dependence pharmacogenetics: role of genetic variation in nicotine-metabolizing enzymes. J. Neurogenet. 23, 252–261.

Rigotti, N.A., 2013. Smoking cessation in patients with respiratory disease: existing treatments and future directions. Lancet Respir. Med. 1, 241–250.

Rose, J.E., Behm, F.M., Drgon, T., Johnson, C., Uhl, G.R., 2010. Personalized smoking cessation: interactions between nicotine dose, dependence and quit-success genotype score. Mol. Med. 16, 247–253.

Rutter, J.L., 2006. Symbiotic relationship of pharmacogenetics and drugs of abuse. AAPS J. 8, E174–E184.

Saccone, S.F., Hinrichs, A.L., Saccone, N.L., Chase, G.A., Konvicka, K., Madden, P.A., Breslau, N., Johnson, E.O., Hatsukami, D., Pomerleau, O., Swan, G.E., Goate, A.M., Rutter, J., Bertelsen, S., Fox, L., Fugman, D., Martin, N.G., Montgomery, G.W., Wang, J.C., Ballinger, D.G., Rice, J.P., Bierut, L.J., 2007. Cholinergic nicotinic receptor genes implicated in a nicotine dependence association study targeting 348 candidate genes with 3713 SNPs. Hum. Mol. Genet. 16, 36–49.

Sarginson, J.E., Killen, J.D., Lazzeroni, L.C., Fortmann, S.P., Ryan, H.S., Schatzberg, A.F., Murphy Jr., G.M., 2011. Markers in the 15q24 nicotinic receptor subunit gene cluster *(CHRNA5-A3-B4)* predict severity of nicotine addiction and response to smoking cessation therapy. Am. J. Med. Genet. B. Neuropsychiatr. Genet. 156B, 275–284.

Schroeder, S.A., 2013. New evidence that cigarette smoking remains the most important health hazard. N. Engl. J. Med. 368, 389–390.

TAG, 2010. Genome-wide meta-analyses identify multiple loci associated with smoking behavior. Nat. Genet. 42, 441–447.

Thorgeirsson, T.E., Gudbjartsson, D.F., Surakka, I., Vink, J.M., Amin, N., Geller, F., Sulem, P., Rafnar, T., Esko, T., Walter, S., Gieger, C., Rawal, R., Mangino, M., Prokopenko, I., Magi, R., Keskitalo, K., Gudjonsdottir, I.H., Gretarsdottir, S., Stefansson, H., Thompson, J.R., Aulchenko, Y.S., Nelis, M., Aben, K.K., den Heijer, M., Dirksen, A., Ashraf, H., Soranzo, N., Valdes, A.M., Steves, C., Uitterlinden, A.G., Hofman, A., Tonjes, A., Kovacs, P., Hottenga, J.J., Willemsen, G., Vogelzangs, N., Doring, A., Dahmen, N., Nitz, B., Pergadia, M.L., Saez, B., De Diego, V., Lezcano, V., Garcia-Prats, M.D., Ripatti, S., Perola, M., Kettunen, J., Hartikainen, A.L., Pouta, A., Laitinen, J., Isohanni, M., Huei-Yi, S., Allen, M., Krestyaninova, M., Hall, A.S., Jones, G.T., van Rij, A.M., Mueller, T., Dieplinger, B., Haltmayer, M., Jonsson, S., Matthiasson, S.E., Oskarsson, H., Tyrfingsson, T., Kiemeney, L.A., Mayordomo, J.I., Lindholt, J.S., Pedersen, J.H., Franklin, W.A., Wolf, H., Montgomery, G.W., Heath, A.C., Martin, N.G., Madden, P.A., Giegling, I., Rujescu, D., Jarvelin, M.R., Salomaa, V., Stumvoll, M., Spector, T.D., Wichmann, H.E., Metspalu, A., Samani, N.J., Penninx, B.W., Oostra, B.A., Boomsma, D.I., Tiemeier, H., van Duijn, C.M., Kaprio, J., Gulcher, J.R., McCarthy, M.I., Peltonen, L., Thorsteinsdottir, U., Stefansson, K., 2010. Sequence variants at *CHRNB3-CHRNA6* and *CYP2A6* affect smoking behavior. Nat. Genet. 42, 448–453.

Thun, M.J., Carter, B.D., Feskanich, D., Freedman, N.D., Prentice, R., Lopez, A.D., Hartge, P., Gapstur, S.M., 2013. 50-year trends in smoking-related mortality in the United States. N. Engl. J. Med. 368, 351–364.

Uhl, G.R., Drgon, T., Johnson, C., Ramoni, M.F., Behm, F.M., Rose, J.E., 2010. Genome-wide association for smoking cessation success in a trial of precessation nicotine replacement. Mol. Med. 16, 513–526.

Vaidyanathan, G., 2012. Redefining clinical trials: the age of personalized medicine. Cell 148, 1079–1080.

Whiteford, H.A., Baxter, A.J., 2013. The Global Burden of Disease 2010 study: what does it tell us about mental disorders in Latin America? Rev. Bras. Psiquiatr. 35, 111–112.

Zhu, A.Z., Zhou, Q., Cox, L.S., David, S.P., Ahluwalia, J.S., Benowitz, N.L., Tyndale, R.F., 2014. Association of *CHRNA5-A3-B4 SNP* rs2036527 with smoking cessation therapy response in African-American smokers. Clin. Pharmacol. Ther. 96, 256–265.