Are Serotonergic System Genes Associated to Smoking Cessation Therapy Success in Addition to CYP2A6?

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Abstract

Despite progress made in the treatment of tobacco dependence, currently available treatments are effective for only a fraction of smokers. The aim of this study was to evaluate the association between the effectiveness of treatment with nicotine or bupropion in heavy smokers (n = 70), and 6 candidate polymorphisms in CYP2A6, 5-HTT and HTR2A genes. Analysis revealed a significant association between ‘favourable’ genotype combination carriers (CYP2A6 “slow metabolizer” or SHIT L-allele or HTR2A-1438GG) and nicotine treatment outcome (OR=2.69, 95% CI=1.28–5.64). Genetic variations in CYP2A6 gene or genotypes associated with reduced synaptic serotonin activity may influence the success of smoking cessation treatment.

Key words
	nicotine · bupropion · CYP2A6 · 5-HTT LPR · HTR2A-1438A>G

Introduction

Smoking is the single biggest preventable cause of death in contemporary societies. Unfortunately, available treatments against tobacco dependence are only effective for a minority of smokers. Current guidelines recommend nicotine patches as the first line of treatment, yet ~70–80% of smokers under this treatment relapse in the long-term [1]. Although bupropion produces higher quitting rates than nicotine substitutive treatment (NST), yet ~70–80% of smokers under this treatment. Approximately 90 people were screened in the randomized manner from the Hospital Gregorio Marañón (Spain). Inclusion criteria were: (i) to be enrolled in a ‘standard’ smoking cessation program between 2007 and 2010 but only 76 individuals were included in the study. Patients were motivated to stop smoking and went there to try to stop smoking on their own without any kind of advertisement or recruitment method. Inclusion criteria were: (i) to be enrolled in a ‘standard’ (12-week duration) smoking cessation program with nicotine substitutive treatment (NST) or bupropion following medical criteria, (ii) smoking history > 10 cigarettes/day and > 10 packs/year, and (iii) > 3 scores in the Fagerstrom test for nicotine dependence (FTND).

It was an open label trial and the doses of bupropion SR provided were (150 mg once/day for days 1–6, followed by 150 mg twice/day for days 7–84), 12 weeks. For NRT the doses were: 1) for smokers ≥20 cigarettes/day, Nicotinell TTS 30 (4 weeks), Nicotinell TTS 20 (4 weeks) and Nicotinell TTS 10 (4 weeks), 2) for smokers <20 cigarettes/day, Nicotinell TTS 20 (4 weeks), Nicotinell TTS 20 (4 weeks) and Nicotinell TTS 10 (4 weeks).

Approval was obtained from the local Ethics Committee (Hospital Gregorio Marañón) and all patients provided written informed consent. The study was in accordance with the Helsinki Declaration.

Treatment effectiveness was defined as prolonged abstinence for 12 months post-treatment and the abstinence rate was measured at 3, 6, 9 and 12 months during the follow-up visits.
We selected the aforementioned genetic polymorphisms according to their prevalence in the Spanish population and phenotypic effects. Genotyping was performed from blood samples using an allele-specific polymerase chain reaction as detailed elsewhere [18].

We compared smoking phenotypes between the 2 treatment groups (nicotine or bupropion) with the unpaired Student’s t-test. We used the χ² test to assess deviations of genotype distributions from the Hardy-Weinberg equilibrium (HWE). We also used a logistic regression to analyze the association between treatment outcome and genotypes or genotype combinations (see below). We combined CYP2A6 genotypes in 3 different groups based on expected enzymatic activity levels, i.e.: ‘slow’ (50% activity), ‘medium’ (80–100% activity) and ‘fast metabolizers’ (>100% activity) [19]. All statistical analyses were corrected for multiple comparisons using the Bonferroni method, in which the threshold P value is obtained by dividing 0.05 by the number of tests. All analyses were performed with the PASW/SPSS Statistics 18.0 (SPSS Inc, Chicago, IL) program.

Results

The study included 76 patients demanding smoking cessation treatment between May 2006 and July 2009. 70 of the 76 people completed the followed up period. All participants were at the preparation or contemplation stage in the process of quitting smoking. The number of subjects in the NST and bupropion preparation or contemplation stage in the process of quitting smoking. The number of subjects in the NST and bupropion groups, being successful in 39% and 38% of subjects (P = 0.575).

Table 1  Genotype frequency distributions (%).

<table>
<thead>
<tr>
<th>Genotype</th>
<th>M/M</th>
<th>M/m</th>
<th>m/m</th>
<th>HWE (P-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP2A6*2</td>
<td>95.7</td>
<td>4.3</td>
<td>0.0</td>
<td>0.800</td>
</tr>
<tr>
<td>CYP2A6*9</td>
<td>87.1</td>
<td>12.9</td>
<td>0.0</td>
<td>0.490</td>
</tr>
<tr>
<td>CYP2A6*12</td>
<td>47.1</td>
<td>40.0</td>
<td>12.9</td>
<td>0.347</td>
</tr>
<tr>
<td>CYP2A6<em>1</em>2</td>
<td>95.7</td>
<td>4.3</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5-HTT LPR</td>
<td>27.1</td>
<td>47.1</td>
<td>25.8</td>
<td>0.563</td>
</tr>
<tr>
<td>HTR2A-1438 A&gt;G</td>
<td>38.6</td>
<td>47.1</td>
<td>14.3</td>
<td>0.585</td>
</tr>
</tbody>
</table>

HWE = Hardy-Weinberg equilibrium, M = major allele, m = minor allele; see text for gene abbreviations

Discussion

Although preliminary, the results of our study provide evidence that several genetic variations, especially in CYP2A6 gene and thus leading to different phenotypes in terms of rate of nicotine metabolism may influence the success of smoking cessation.

Table 2  Odds ratio for the association between genotypes in left column (vs. the other genotypes for each polymorphism), and nicotine or bupropion treatment effectiveness.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Nicotine Group</th>
<th>Bupropion Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP2A6 ‘slow metabolizer’</td>
<td>3.86 (1.93–7.72)</td>
<td>0.86 (0.79–0.93)</td>
</tr>
<tr>
<td>5-HTT LPR, L</td>
<td>1.90 (1.19–3.03)</td>
<td>0.78 (0.37–1.64)</td>
</tr>
<tr>
<td>HTR2A-1438 S/S</td>
<td>1.44 (0.81–2.55)</td>
<td>0.77 (0.46–1.27)</td>
</tr>
<tr>
<td>‘Favourable’ genotype combination</td>
<td>2.69 (1.28–5.64)</td>
<td>0.47 (0.16–1.40)</td>
</tr>
</tbody>
</table>

OR = odds ratio, 95% CI = 95% confidence interval

a  CYP2A6 genotype combinations were: normal (‘1’/‘1’, ‘1’/‘2’, ‘1’/‘12’, ‘12’/‘12’), slow (‘2’/‘2’, ‘1’/‘2’, ‘1’/‘9’) and fast metabolizers (‘1’>‘2’).

b ‘Favourable’ genotype combination: CYP2A6 ‘slow metabolizer’ genotype or 5HTT L-allele carriers or HTR2A-1438GG carriers (‘unfavourable genotype combination’: fast/normal metabolizer genotype or 5HTT S/S genotype or HTR2A-1438 A-allele carriers)

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treatment. It is possible that anxiety (altered serotonin activity), a common symptom of nicotine withdrawal, may be a critical factor in the maintenance of smoking and decrease smoking cessation success. We believe that our findings are of clinical relevance because up to date there is no clear consensus or criteria on when to select nicotine or bupropion for inducing tobacco cessation. Yet several genetic factors identified here have a strong influence on treatment effectiveness, at least in the case of nicotine, and could be used to predict treatment effectiveness. Combining variants resulted in a pronounced increase of cessation rates. In slow metabolizers (based on CYP2A6 genotype) treatment is likely to be clearly more successful with nicotine than with bupropion, and thus the former could be the selected treatment. This is consistent with the finding that nicotine slow-metabolizers have higher plasma nicotine levels compared with normal/fast metabolizers [22]. Thus, the higher treatment-related plasma nicotine levels among slow metabolizers compared with normal metabolizers may contribute to greater quitting success during nicotine therapy [24]. NTS success was also higher in those participants carrying the ‘high-activity’ L-allele of the 5HTT LPR gene or the HTR2A 1438GG genotype. Nicotine increases brain serotonin secretion (with serotonergic tone therefore playing a role in nicotine effects) while nicotine withdrawal has the opposite effects [25]. Thus, genotypes associated with reduced synaptic serotonin activity (e.g., decreased serotonin synthesis, decreased serotonin receptor activity, or increased serotonin transporter activity) might be associated with higher success rates of nicotine treatment for smoking cessation, given the need to self-medicate nicotine withdrawal [11]. To the best of our knowledge, there are no previous studies on the HTR2A-1438A>C polymorphism and smoking cessation in spite of the fact that this polymorphism has been associated with a better response to antidepressant treatment [26]. Approximately 40% of our cohort had the ‘favourable’ genotype combination (for CYP2A6, 5HTT LPR or HTR2A). Such a genetic endowment is expected to result in considerably higher smoking cessation rates after 12 months (>63%) than the actual value of 38% reported for the nicotine group. As for bupropion treatment, only the CYP2A6 genotype provided a certain predictive value for treatment effectiveness. Thus, more research in genetic factors is needed to predict the success of other alternative treatments for nicotine cessation, e.g., antidepressants such as nortriptyline [21].

We believe this is the first association study in a Spanish population in the field of genetics and tobacco cessation performed with heavy smokers which takes into account the most important polymorphisms that are strong candidates to influence smoking behaviour, i.e., those involved in nicotine metabolism, as well as in the brain effects of nicotine. The results of our study are overall valid, as all the following criteria were met [27]: the smoking phenotypes and the study outcome were properly defined and accurately recorded by a researcher who was blind to the genetic information; we adjusted all statistical inferences for multiple comparisons (Bonferroni’s criteria for the p values); and the results are overall consistent with previous research in the field. A weakness of our study was the low sample size of the 2 treatment groups, yet we believe this can be partly overcome by the fact that both groups were homogeneous and well defined in terms of phenotype assessment. Keeping in mind the values of large-scale population nicotine dependence association studies, the limitation of their interpretation and also their usefulness in the clinical (and pharmacological) innovations are suggested. In accordance with this, studies with more sophisticated designs (including more appropriate phenotype measures, representative population) are required even with the risk of smaller sample size. In summary, we provided preliminary evidence supporting that genetic screening can be useful to predict success of nicotine treatment against smoking cessation and thus can contribute to reduce the mortality caused by smoking.

Acknowledgements
The research was supported by the UEM 03-2006 Internal Project of European University of Madrid and by the 035-2006 Project of The Spanish Lung Foundation (SEPAR).

Conflict of Interest
No competing interests.

References
1 Fiore MC. US public health service clinical practice guideline: treating smoking use and dependence. Respir Care 2000; 45: 1200–1262
2 Gold PB, Rubey RN, Harvey RT. Naturalistic, self-assignment comparative trial of bupropion SR, a nicotine patch, or both for smoking cessation treatment in primary care. Am J Addict 2002; 11: 315–331
3 Fiore MC, Jann CR. A clinical blueprint to accelerate the elimination of tobacco use. JAMA 2008; 299: 2083–2085
16 Dreumuller N, Tadic A, Dragicovic A et al. The serotonin transporter promoter polymorphism (5-HTTLPR) affects the relation between antidepressant serum concentrations and effectiveness in major depression. Pharmacopsychiatry 2012; 45: 108–113