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Genetic variation in the serotonin pathway and smoking cessation with nicotine replacement therapy: New data from the Patch in Practice trial and pooled analyses

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Contributors: Dr. David and Dr. Munafò wrote the first draft of the manuscript, and planned and conducted the data analyses. All authors commented and revised the draft and it was agreed by all authors. The research protocol for the Patch in Practice trial was designed and written by authors Dr. Murphy and Dr. Munafò.

Conflict of interest

Dr. David has received travel grants from the Society for Research on Nicotine and Tobacco, a speaking fee from Pfizer, Inc., and was a recipient of an unrestricted research grant from Addex Pharmaceuticals. Dr. Aveyard has received free nicotine replacement products from Novartis and nortriptyline from King Pharmaceuticals for distribution to trial participants. Dr. Aveyard has received personal income for advice to Xenova, a biotechnology company investigating a nicotine vaccine and had served as a consultant and received funding from McNeill AB and Pfizer for work on smoking cessation. He has received small gifts and had numerous meals paid for by drug companies, including those producing medications for smoking cessation. He has received travel grants to attend conferences from the Society for Research in Nicotine and Tobacco. Dr. Munafò has received fees for invited lectures from the National Health Service, GlaxoSmithKline, Novartis, the Moffitt Cancer Research Center and the Karolinska Instituet, and received benefits in kind (hospitality, etc.) from various pharmaceutical companies. He has received research and travel support from the European Research Advisory Board, GlaxoSmithKline, Pfizer Consumer Healthcare and Novartis. Consultancy has been provided to the National Audit Office and G-Nostics Ltd. Dr. Johnstone has received consultancy income from European Network for Smoking Prevention. Dr. Murphy has provided scientific consultancy services through University of Oxford ISIS Innovation to G-Nostics Ltd. He also has intellectual property interests in G-Nostics which is trying to exploit the application of molecular biological diagnostics to smoking cessation (patents: Genetic indicators of tobacco consumption, European Patent Application 00977718.6; US Patent Application 10/130,907; Canadian Patent Application 2390772; and Japanese Patent Application 539909/2001. He has received no income from these patents, and should he do so, such income will not be used as personal income. Dr. Lerman has served as a consultant and has received research funding from companies that market smoking cessation medications including GlaxoSmithKline, Pfizer, and Astra Zeneca. The Childhood Cancer Research Group and the Cancer Research UK General Practice Research Group have received unrestricted educational grants, research project grants, and consultancy fees from Ciba Geigy/Novartis, Glaxo Smith Kline, Pharmacia/Pfizer, Ares-Serono, Sanofi-Synthelabo, Third Wave Technologies, Astra-Zeneca, and G-Nostics.

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Abstract

The serotonin pathway has been implicated in nicotine dependence and may influence smoking cessation. Therefore, 792 cigarette smokers from the Patch in Practice trial were genotyped for the tryptophan hydroxylase (*TPH1* A779C), serotonin transporter (*SLC6A4 5-HTTLPR*), and 5-HT_{1A} (*HTR1A* C-1019G) polymorphisms. Cox regression analysis did not demonstrate significant effects of any of the three genotypes on relapse to smoking: *TPH1* (Reference AA; AC: hazard ratio (HR) 0.99, 95% confidence interval (CI) 0.78, 1.24, $p = 0.90$; CC: HR 0.93, 95% CI 0.73, 1.18, $p = 0.55$); *5-HTTLPR* (Reference LL; SL: HR 1.01, 95% CI 0.85, 1.20, $p = 0.90$; SS: HR 1.13, 95% CI 0.91, 1.39, $p = 0.27$); *HTR1A* (Reference CC; CG: HR 1.04, 95% CI 0.86, 1.25, $p = 0.70$; GG: HR 1.01, 95% CI 0.82, 1.24, $p = 0.93$). Moreover, pooled analyses of data from all three extant pharmacogenetic NRT trials ($N = 1398$) found no significant effect of *5-HTTLPR* genotype on continuous abstinence at 12-week (Reference LL; SL: odds ratio (OR) = 1.25, 95% CI 0.89, 1.74, $p = 0.19$; SS: OR= 1.31, 95% CI 0.86, 1.98, $p = 0.21$) or 26-week follow-up (Reference LL; SL: OR= 0.93, 95% CI 0.64, 1.33, $p = 0.68$; SS: OR= 1.00, 95% CI 0.63, 1.58, $p = 1.00$). These data do not support a statistically or clinically significant moderating effect of these specific 5-HT pathway genetic variants on smoking cessation. However, the possibility remains that other variants in these or other 5-HT genes may influence NRT efficacy for smoking cessation in treatment seeking smokers.

Keywords

Smoking cessation; Tryptophan hydroxylase; Serotonin transporter; 5-HT_{1A} receptor; Nicotine replacement therapy; Pharmacogenetics

1. Introduction

With converging data from multiple lines of investigation, it has become clear that nicotine dependence (ND) is under genetic influence (Li et al., 2003; Munafò et al., 2004), and that subgroups defined by sex and genotype (David et al., 2007a,c; Johnstone et al., 2004; Lerman et al., 2003, 2006a; Malaiyandi et al., 2006) may derive differential efficacy from pharmacological smoking cessation therapies. Based on the neurobiology of reward and the pharmacokinetics of nicotine, the predominance of research into genetic influences on tobacco use has focused on the dopamine (DA) and oxidative hepatic nicotine metabolism pathways [(i.e., cytochrome P450 (CYP2A6)] (Munafò et al., 2004). Despite mixed results and frequent lack of replication in case–control studies of some smoking-related phenotypes (e.g., smoking initiation, persistence, cigarettes/day) (Munafò et al., 2004), some relatively consistent findings are emerging regarding the influence of genetic variants in the DA pathway (e.g., *ANKK1*, *COMT*) and *CYP2A6* for treatment response to sustained-release bupropion (David et al., 2007a; Lerman et al., 2003; Swan et al., 2005) and nicotine replacement therapy(NRT) (Johnstone et al., 2004; Lerman et al., 2006b). However, few pharmacogenetic smoking cessation studies have investigated serotonergic candidate genes.

Centrally, tryptophan enters neurons via active transport and is converted by tryptophan hydroxylase (TPH) to 5-hydroxytryptophan and then decarboxylated to 5-hydroxytryptamine (serotonin; 5-HT) (Rang et al., 2003). The conversion of tryptophan to 5-HT by TPH is the rate-limiting step in 5-HT synthesis. Nicotine administration stimulates release of 5-HT in the dorsal raphe nucleus (DRN) (Cheeta et al., 2001). When 5-HT is released in the DRN, it binds to inhibitory 5-HT_{1A} autoreceptors (Cheeta et al., 2001), which appear to mediate synaptic release of 5-HT in terminal fields including the ventral striatum (Sprouse and Aghajanian, 1987) and hippocampus (Balfour et al., 1986a,b; Benwell and Balfour, 1979; Benwell et al., 1990). Tryptophan depletion and neurotoxic 5-HT depletion are associated with increased compulsive use of drugs of abuse including nicotine in rats (Engel et al., 1992; Roberts et al., 1994). Pharmacological 5-HT depletion and stimulation of 5-HT_{1A} receptors induce behavioral sensitization to nicotine (Olausson et al., 1999, 2001a,b), while interventions that enhance 5-HT neurotransmission decrease compulsive drug use of amphetamine (Porrino et al., 1989), heroin (Higgins et al., 1994), alcohol (LeMarquand et al., 1994), and nicotine (Opitz and Weischer, 1988); and 5-HT_{1A} receptor stimulation inhibits behavioral sensitization to nicotine (Olausson et al., 1999, 2001a) and may mediate nicotine withdrawal symptoms (Cheeta et al., 2000, 2001; Engberg et al., 2000; Irvine et al., 2001; Mihailescu et al., 2001; Olausson et al., 2001b; Tucci et al., 2003).

In humans, tryptophan depletion is associated with increased smoking intensity and desire to smoke (Hitsman et al., 2005), higher attentional salience to smoking cues (Hitsman et al., 2007), and higher negative affect in smokers; however, conversely, while pharmacological interventions that increase synaptic 5-HT – such as use of SSRIs – are efficacious for treatment of depression, meta-analyses of the use of serotonergic medications including SSRIs and buspirone (5-HT_{1A} agonist) have not demonstrated robust evidence of efficacy for smoking cessation (Hughes et al., 2000). Finally, nicotine-induced 5-HT-x-DA interactions contribute to neuroadaptations throughout the extended amygdala (Balfour, 2002; Olausson et al., 2002). Thus, converging data from animal and human studies suggest that TPH, the 5-HT transporter, and 5-HT_{1A} receptors are important neurological substrates for nicotine dependence.

Two genes have been identified that code for TPH (*TPH1; TPH2*), both of which code for different isoforms of the TPH enzyme with differential expression in the brain (Abumaria et al., 2008) and somatic tissues (Walther et al., 2003). The only published genetic association studies of nicotine dependence have examined variants in TPH1 (11p15.3-p14; GeneID: 7166)—which is approximately 20,000 base-pairs (bp) and contains 10 exons. Primarily two variants (C218A, A779C; both within intron 7) of the *TPH1* gene have been the subject of multiple studies. The C218A and A779C are in tight linkage disequilibrium and have been associated with reduced levels of cerebrospinal fluid 5-hydroxyindoleacetic acid (a metabolite of 5-HT) (Nielsen et al., 1994) and with impulsive and aggressive traits, suicide, and alcoholism (Chung et al., 2005; Nielsen et al., 1998) and smoking behavior (David et al., 2007b; Lerman et al., 2001; Reuter and Hennig, 2005; Sullivan et al., 2001).

The 5-HTT gene (*SLC6A4*; 17q11.1-q12; Gene ID: 6532) is approximately 38,000 bp, is composed of 14 exons and encodes a transmembrane transporter responsible for reuptake of serotonin at the synapse and regulation of the magnitude and duration of serotonergic

signaling. A 44 bp insertion/deletion polymorphism located approximately 1000 bp from transcription initiation site of the *SLC6A4* gene results in a polymorphism known as the serotonin transporter gene-linked polymorphic region (*5-HTTLPR*) (Heils et al., 1996). The *5-HTTLPR* short (S) allele is associated with decreased expression of the *SLC6A4* gene relative to the long (L) form, and S allele carriers (SS or SL) genotypes have been associated with relatively decreased reuptake of 5-HT from the medium *in vitro* (Heils et al., 1997; Lesch et al., 1996; Little et al., 1998) and reduced 5-HT_{1A} binding potential *in vivo* (David et al., 2005). The *5-HTTLPR* has been associated with anxiety-related and compulsive behavior traits (Bengel et al., 1999; Munafò et al., 2003; Sen et al., 2004), suicide (Du et al., 1999), and greater attention to smoking-related cues (Munafò et al., 2005a). There are three additional variants in untranslated regions of the *SLC6A4* gene in the promoter which may affect its expression (Lasky-Su et al., 2005; Murphy et al., 2004). However, to our knowledge, only the *5-HTTLPR* has demonstrated associations with smoking-related phenotypes (Lerman et al., 2000; Munafò et al., 2004, 2005a).

The gene encoding 5-HT_{1A} receptor proteins (*HTR1A*; 5q11.2-q13; Gene ID: 3350), consists of 1268 bp and is without introns. The *HTR1A* gene codes for a G-protein coupled receptor with seven transmembrane domains (Wu and Comings, 1999). There are at least nine single nucleotide polymorphisms (SNPs) present in the *HTR1A* gene, but only one SNP appears to be common in Europeans (C-1019G) (David et al., 2005). The *HTR1A*-1019G allele has been associated with decreased transcriptional efficiency (Lemonde et al., 2003), anxiety and depression (Lemonde et al., 2003; Strobel et al., 2003), schizophrenia, substance use disorder, and panic attacks (Huang et al., 2004), and there have been mixed results in terms of association with suicide.

Therefore, converging evidence from animal, preclinical and clinical human studies indicates that the 5-HT pathway is implicated in nicotine dependence—potentially contributing to neuroadaptations that moderate behavioral sensitization to nicotine, nicotine-induced alterations in mood, and nicotine withdrawal (Balfour, 2002; Cheeta et al., 2000, 2001; Engberg et al., 2000; Irvine et al., 2001; Mihailescu et al., 2001; Olausson et al., 2001b; Tucci et al., 2003). Furthermore, TPH enzymes, 5-HTTs, and 5-HT_{1A} receptors compose an integrated circuit that regulates synaptic 5-HT throughout the mesocorticolimbic system and functional genetic variants in the genes that encode these proteins have been identified (i.e., *TPH1* A779C, *SLC6A4 5-HTTLPR*, *HTR1A* C-1019G) and thus they are strong candidates for examining smoking-related phenotypes. To date however, there have been only two other pharmacogenetic studies of genes in the serotonin (5-HT) pathway and both studies included only one genetic variant (*5-HTTLPR*) (David et al., 2007b; Munafò et al., 2006); moreover neither study demonstrated significant main effects or interactions with NRT for smoking cessation.

We hypothesized that genotypes associated with reduced synaptic 5-HT activity would be associated with greater efficacy of NRT for smoking cessation, given the theoretical need to self-medicate nicotine withdrawal (Lerman et al., 1996), and therefore wished to explore whether this larger sample size of treatment seeking smokers might provide evidence for a small effect of *5-HTTLPR* and other 5-HT pathway genotypes (i.e., *TPH1* A779C, *HTR1A* C-1019G) on smoking cessation. In addition, we sought to examine whether we could detect

main effects of genotype on smoking cessation by pooling data from all extant pharmacogenetic trials of NRT incorporating serotonergic genotypes.

2. Materials and methods

2.1. Patch in Practice study

2.1.1. Participants—Participants were eligible if they were aged 18 years and over and smoked 10 cigarettes/day or more, and were recruited from 26 general practices in Buckinghamshire and Oxfordshire in the United Kingdom (UK). Physicians recruited patients attending for other reasons (*N*= 60, 6.5%), or patients volunteered having seen posters or heard about the study (*N*= 15, 1.6%), while in some practices every registered smoker was contacted offering trial entry (*N*= 850, 91.9%). Blood samples were successfully collected and DNA extracted on *N*= 908 (98%) participants at trial entry. All participants provided informed consent prior to being included in the study. Ethical approval was obtained from the relevant local Research Ethics Committees at the University of Oxford and the University of Pennsylvania.

2.1.2. Treatment—Participants were randomly assigned to one of two levels of smoking cessation behavioral support: usual care or recommended best practice, the latter typically including a higher level of support than is usually offered in primary care in the United Kingdom. In addition, all participants received 8 weeks of 15 mg nicotine replacement therapy transdermal patch. The details and results of the main trial are reported elsewhere (Aveyard et al., 2007).

Participants were visited at 1 week, 2 weeks and 4 weeks from quit day to provide ongoing smoking cessation support and to assess smoking status, which was verified by exhaled carbon monoxide (CO) monitoring. Participants were phoned at 12 weeks, 26 weeks, and 52 weeks from quit day to assess smoking status, and those claiming at least 7-day abstinence were posted a saliva collection device to measure salivary cotinine.

2.1.3. Assessments

2.1.3.1. Demographics: Self-reported sex, ancestry, and age were assessed at a baseline interview

2.1.3.2. Smoking cessation outcomes: Abstinence was defined as continuous confirmed abstinence at 12 and 26-weeks from start of treatment. Two standard definitions of abstinence have been proposed (Hughes et al., 2003; West et al., 2005), which recommend a 2-week grace period at the beginning of cessation, during which lapses to smoking do not count against abstinence. Confirmation of abstinence was defined as an exhaled CO less than 10 parts per million (ppm), or salivary cotinine concentration less than 15 ng/ml on each occasion (Society for Research on Nicotine and Tobacco, 2002). Participants who were withdrawn (which was commonly due to reverting to smoking) or who were lost to follow-up (unless they moved to an untraceable address or had died) were counted as smokers, as is standard (West et al., 2005).

2.1.3.3. Nicotine dependence: Nicotine dependence severity was assessed with the Fagerström Test of Nicotine Dependence (FTND) (Heatherton et al., 1991), which is a validated, 6-item self-reported measure of nicotine dependence.

2.1.3.4. Genotyping: Blood samples were processed upon receipt or after overnight storage at 4 °C (up to a maximum of 7 days after collection depending on delivery method to laboratory). Whole blood was separated by centrifugation and DNA immediately extracted from buffy coat lymphocytes using a standard salting out procedure (Miller et al., 1988). Plasma was stored at −80 °C until required for analysis. Participants were genotyped for the *5-HTTLPR* polymorphism, described in detail elsewhere (Munafò et al., 2005b) and briefly here. Genotyping for the *TPH1* A779C (Lerman et al., 2001) and *HTR1A* C-1019G polymorphisms was completed using methods described below.

Oligonucleotide primers (Heils et al., 1996) flanking the *5-HTTLPR* from the *SLC6A4* gene (GenBank accession number X76753) were used to amplify the DNA template using allele-specific PCR (Munafò et al., 2005b) and the amplified product resolved by agarose gel electrophoresis and stained with ethidium bromide. This enabled determination of the long (L: 528 bp) and/or short (S: 484 bp) allele in each individual. Genotyping for *TPH1* A779C and *5HT1A* C-1019G was completed using the ABI Prism 7900HT Sequence Detection System (Applied Biosystems, Warrington, UK). Each Taqman reaction was performed using 10 ng DNA, 2.5 μl of 2× ABI Taqman Universal Mastermix, 0.375 μl water, and 0.125 μl of 40× Assay by Design SNP assay for the relevant gene target. Primers and probes for Taqman assays are shown in Table 1. The 5 μl reactions were performed in a 384-well plate (Abgene, Epsom, UK). Thermal cycling was completed in a MJ Research PTC225 Tetrad DNA engine (GRI, Braintree, UK) using the following conditions: (1) activation of reaction mix with a 50 °C hold for 2 min, (2) denaturation for 10 min at 95 °C, 40 cycles of 95 °C for 15 s and 60 °C for 1 min and (3) 4 °C hold. The plates were scanned using the ABI Prism 7900HT Allelic Discrimination End-Point analysis and data were analysed by the SDS V1.2 software (Applied Biosystems, Warrington, UK). The assays were initially validated by 48 samples of European origin. Two investigators blinded to subject smoking cessation outcomes reviewed PCR results independently.

2.2. Pooled analysis of PiP, UPenn, and Patch II trials

2.2.1. Description of extant studies—The first published pharmacogenetic trial of a serotonergic candidate gene, by Munafò et al. (2006), was conducted using samples from *N*= 393 smokers of European ancestry who participated in a randomized clinical trial of transdermal nicotine patch vs. nicotine nasal spray for 8 weeks of treatment. Participants were recruited from Washington, DC and Philadelphia, PA, USA in a study conducted at the University of Pennsylvania (UPenn trial). Additional details of this study design have been previously reported (Lerman et al., 2006a). Verification of abstinence was confirmed by exhaled carbon monoxide (<10 ppm) at the end of treatment (EOT) and 6-month follow-ups. The mean age of participants was 46 years 7 months (standard deviation (S.D.) = 11 years 5 months; range = 20–78 years) and 47% of participants were female. Of the 393 participants randomized to nicotine nasal spray or transdermal patch, confirmed abstinence data was available for 182 smokers randomized to NRT patch who were of European ancestry.

The second study, by David et al. (2007b), included *N*= 741 smokers of European ancestry from the Patch II trial who participated in a double-blind, randomized, placebo-controlled trial of the nicotine transdermal patch between June 1991 and March 1992 (the Patch II trial) (Imperial Cancer Research Fund General Practice Research Group, 1993, 1994) and were re-contacted in 1999–2000 for DNA collection. Participants were recruited from general practice surgeries in Oxfordshire, UK. The duration of treatment with active nicotine (*N*= 341) and placebo patches (*N*= 340) was 12 weeks. Details regarding the Patch II trial (Imperial Cancer Research Fund General Practice Research Group, 1993, 1994), and the 8-year follow-up (Patch II study) have been comprehensively described elsewhere (Johnstone et al., 2004; Yudkin et al., 2003, 2004). The mean age of participants was 42 years 11 months (S.D. = 9 years 11 months; range = 25–65 years) and 60% of participants were female. Only participants from the treatment arm (i.e., active nicotine patch) of the Patch II trial were included in the analyses.

The third (present) study is the PiP trial described above.

2.3. Statistical analysis

2.3.1. Survival analysis—Cox regression survival analysis was used to test for main effects of genotype and genotype-x-sex and genotype-x-genotype interaction terms on time to relapse to smoking. Using a backwards conditional model, *TPH1* genotype (AA, AC or CC), *5-HTTLPR* genotype (LL, SL or SS), and *HTR1A* genotype (CC, CG or GG) were entered into the model, controlling for the effects of age, FTND and time spent using NRT, since not all participants used the full course of NRT that they were prescribed. The genotype-x-sex and genotype-x-genotype interaction terms were each tested and removed if non-significant, in which case the effect of sex was controlled.

2.3.2. Pooled analyses—Next, binary logistic regression analysis of the PiP study participants combined with samples from the UPenn and Patch II trials was performed using biochemically verified point prevalence of abstinence at end of treatment and 26-week follow-up. Data were not available for days of continuous abstinence from all three studies and therefore survival analyses could not be performed with the pooled data. There were 845 participants of European ancestry with point prevalence data available from the PiP trial, which were combined with data from the UPenn trial (*N*= 182) and the Patch II trial (*N*= 371) for a pooled sample size of *N*= 1398.

All analyses were conducted using the Statistical Software for the Social Sciences (v. 12.0) (SPSS Inc., Chicago, IL). An α level of 0.05 was maintained throughout the analyses.

2.4. Statistical power

The sample size of the PiP Study is adequate to detect an OR of 1.8 for *TPH1* A779C, 1.6 for *5-HTTLPR*, and 2.2 for *HTR1A* C-1019G at $\alpha = 0.05$ and power of 80% for main effects of genotype on 12-week abstinence outcomes using binary logistic regression. When the samples from the PiP, UPenn, and Patch II trials are combined, the sample size is more than sufficient to detect an OR of 1.4 for association of *5-HTTLPR* with smoking cessation at $\alpha = 0.05$, and power of 80%.

3. Results

3.1. Patch in practice (PiP) study

3.1.1. Characteristics of participants—Recruitment took place between July 2002 and March 2005 and a total of *N*= 925 participants were recruited into the main trial (Aveyard et al., 2007) of which *N*= 908 (98%) provided a DNA sample and *N*= 886 were successfully genotyped for all three (*TPH1* A779C, *5-HTTLPR* and *HTR1A* C-1019G) genetic variants. Nicotine dependence data were missing in 70 participants. Of the remaining 816 participants, 792 were of European ancestry and had complete data at all time points—thus constituting our final study sample. Basic characteristics and genotype frequencies for the study participants can be seen in Table 2. Frequencies for each genetic variant did not deviate from Hardy–Weinberg Equilibrium ($\chi^2 < 2.5$; *p*s > 0.1).

3.1.2. Smoking cessation outcomes—Survival analyses of the PiP trial: Cox regression analysis (*N*= 792) did not demonstrate significant effects of any of the three genotypes on relapse to smoking: *TPH1* (Reference AA; AC: hazard ratio (HR) 0.99, 95% confidence interval (CI) 0.78, 1.24, $p = 0.90$; CC: HR 0.93, 95% CI 0.73, 1.18, $p = 0.55$); *5-HTTLPR* (Reference LL; SL:HR 1.01, 95% CI 0.85, 1.20, $p = 0.90$; SS: HR 1.13, 95% CI 0.91, 1.39, $p = 0.27$); *HTR1A* (Reference CC; CG: HR 1.04, 95% CI 0.86, 1.25, $p = 0.70$; GG: HR 1.01, 95% CI 0.82, 1.24, $p = 0.93$). There was no evidence of interaction effects between any of the genotypes, or between the genotypes and sex.

3.2. Pooled analysis of PiP, UPenn, and Patch II trials

Logistic regression analyses (*N*= 1398) did not indicate a significant main effect of *5-HTTLPR* genotype on continuous abstinence at 12-week (Reference LL; SL: odds ratio (OR) = 1.25, 95% CI 0.89, 1.74, $p = 0.19$; SS: OR= 1.31, 95% CI 0.86, 1.98, $p = 0.21$) or 26-week follow-up (Reference LL; SL: OR= 0.93, 95% CI 0.64, 1.33, $p = 0.68$; SS: OR= 1.00, 95% CI 0.63, 1.58, $p = 1.00$). The sex-x-genotype study-x-genotype interaction terms were non-significant at both time points. The main effect of study was also significant at both time points, reflecting lower abstinence rates in the larger studies (PiP and Patch II). These results did not alter substantially when point prevalence abstinence data were analysed. Abstinence rates stratified by genotype for all three studies are presented in Table 3.

4. Discussion

Despite the putative role of 5-HT in nicotine dependence, our pharmacogenetic analyses do not suggest that any of the three specific genetic variants from the 5-HT pathway (TPH1 C779A, 5-HTTLPR, HTR1A C-1-19G) exert a substantial influence on smoking cessation outcomes in patients seeking treatment with NRT. There are a number of possible explanations for why these candidate genes produced null results.

These data do not preclude moderating effects of other polymorphisms in the *TPH1*, *SLC6A4* or *HTR1A* genes on smoking cessation. First, without fine-grained SNP mapping of the *TPH1*, *HTR1A*, and *SLC6A4* genes it is possible that other genetic variants associated with NRT efficacy for smoking cessation may be present but were not included in our analyses.

Secondly, the phenotypes for which significant associations between 5-HT pathway genes have been demonstrated are very different constructs from NRT efficacy for smoking cessation per se even though such phenotypes frequently co-occur with nicotine dependence. For example, the *TPH1* C779A polymorphism has been associated with smoking initiation (Sullivan et al., 2001) and age of onset of smoking (Lerman et al., 2001), and the *5-HTTLPR* has been associated with selective processing of smoking-related cues (Munafò et al., 2005a) or traits that frequently co-occur with smoking such as anxiety traits, mood disorders, and alcohol consumption (Curran et al., 2005; Lasky-Su et al., 2005; Lesch et al., 1996; Munafò et al., 2005b; Murphy et al., 2004). Likewise, there are no published studies demonstrating associations between the *HTR1A* gene and smoking-related traits, even though the *HTR1A* C-1019G polymorphism has been associated depression, anxiety-related and depression-related traits, and substance use disorder (Huang et al., 2004; Lemonde et al., 2003; Strobel et al., 2003).

Previous studies demonstrating significant associations with smoking cessation on NRT have incorporated genes within the dopamine pathway and the *CYP2A6* gene—candidate genes whose role is directly or indirectly related to nicotine administration. There is as yet no evidence indicating that 5-HT neurotransmission is a critical neurological substrate for the process of smoking cessation per se or for a putative role in suppressing nicotine withdrawal in smokers administered NRT. Although our results do not support a moderating role of these specific serotonergic candidate gene variants on smoking cessation in treatment seeking smokers using NRT, it remains to be seen whether or not these genetic variants influence the efficacy of other agents such as SSRIs for smoking cessation, which have been associated with decreased craving and negative affect during quit attempts and may portend a modest benefit for smoking cessation (Cook et al., 2004; Killen et al., 2000; Niaura et al., 2002). Indeed, Killen et al. (2000) demonstrated that paroxetine reduced tobacco craving, and Cook et al. (2004) observed that fluoxetine was associated with lower negative and positive affect during periods of abstinence in smokers undergoing quit attempts.

Therefore, to date, there is no evidence to support a substantial influence of serotonergic genotypes on NRT efficacy for smoking cessation. The present study (PiP) represents the largest within-treatment pharmacogenetic study of NRT to date (N= 792), and the pooled analyses the largest sample size yet reported for analyses of pharmacogenetic smoking cessation studies utilizing repeated-measures clinical trial data with biochemical verification of abstinence (N= 1398). Despite adequate power to detect a modest effect size in the pooled analyses, there was no evidence for association between the *5-HTTLPR* and smoking cessation. Given the statistical power of the pooled analyses, if an association exists between the *5-HTTLPR* and smoking cessation, any effect would likely be a small one (OR < 1.4), consistent with evidence for the effects of other single gene polymorphisms on behavioral phenotypes.

The prospect of potentially tailoring NRT based on serotonergic genotypes appears unlikely to materialize. Nevertheless, it has yet to be established whether or not genetic variation in the 5-HT pathway influences smoking cessation efficacy for other pharmacological agents. It remains a possibility that serotonergic pharmacotherapies such as SSRIs or buspirone, which have not demonstrated efficacy for smoking cessation may be efficacious for

subgroups defined by genetic variants in the 5-HT pathway. There is currently no evidence suggesting a genetic influence on serotonergic agents for smoking cessation and thus this remains a potential area for future research.

In conclusion, pharmacogenetic analyses of three key 5-HT pathway genotypes failed to detect associations with smoking cessation in a practice-based clinical trial of NRT. In the one candidate gene variant with data available in other pharmacogenetic studies (*5-HTTLPR*), pooled analyses of three studies, including new data presented here, demonstrated no significant association with smoking cessation at either end of treatment or 6 months. As these data represent all known pharmacogenetic datasets of 5-HT genotypes in clinical trials of NRT, and are robust when combining three independent samples from the U.S. and the U.K., the results add to a growing body of literature suggesting that genetic variation in the 5-HT pathway is unlikely to serve an important role in the moderation of treatment response to NRT for smoking cessation.

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References

Abumaria N, Ribic A, Anacker C, Fuchs E, Flugge G. Stress upregulates TPH1 but not TPH2 mRNA in the rat dorsal raphe nucleus: identification of two TPH2 mRNA splice variants. Cell Mol Neurobiol. 2008; 28 (3):331–342. [PubMed: 18197473]

Aveyard P, Brown K, Saunders C, Alexander A, Johnstone E, Munafo MR, Murphy M. Weekly versus basic smoking cessation support in primary care: a randomised controlled trial. Thorax. 2007; 62:898–903. [PubMed: 17483139]

Balfour DJ. Neuroplasticity within the mesoaccumbens dopamine system and its role in tobacco dependence. Curr Drug Targets CNS Neurol Disord. 2002; 1:413–421. [PubMed: 12769613]

Balfour DJ, Benwell ME, Graham CA, Vale AL. Behavioural and adrenocortical responses to nicotine measured in rats with selective lesions of the 5-hydroxytryptaminergic fibres innervating the hippocampus. Br J Pharmacol. 1986a; 89:341–347. [PubMed: 2430657]

Balfour DJ, Graham CA, Vale AL. Studies on the possible role of brain 5-HT systems and adrenocortical activity in behavioural responses to nicotine and diazepam in an elevated X-maze. Psychopharmacology (Berl). 1986b; 90:528–532. [PubMed: 2433708]

Bengel D, Greenberg BD, Cora-Locatelli G, Altemus M, Heils A, Li Q, Murphy DL. Association of the serotonin transporter promoter regulatory region polymorphism and obsessive-compulsive disorder. Mol Psychiatry. 1999; 4:463–466. [PubMed: 10523819]

Benwell ME, Balfour DJ. Effects of nicotine administration and its withdrawal on plasma corticosterone and brain 5-hydroxyindoles. Psychopharmacology (Berl). 1979; 63:7–11. [PubMed: 112625]

Benwell ME, Balfour DJ, Anderson JM. Smoking-associated changes in the serotonergic systems of discrete regions of human brain. Psychopharmacology (Berl). 1990; 102:68–72. [PubMed: 1697418]

Cheeta S, Irvine EE, Kenny PJ, File SE. The dorsal raphe nucleus is a crucial structure mediating nicotine's anxiolytic effects and the development of tolerance and withdrawal responses. Psychopharmacology (Berl). 2001; 155:78–85. [PubMed: 11374339]

Cheeta S, Kenny PJ, File SE. The role of 5-HT1A receptors in mediating the anxiogenic effects of nicotine following lateral septal administration. Eur J Neurosci. 2000; 12:3797–3802. [PubMed: 11029650]

Chung IW, Kim H, Sribney W, Hong JB, Lee CH, Lee KY, Nan HM, Kim YS, Manowitz P. Tryptophan hydroxylase polymorphism is associated with age of onset of alcoholism related behaviors. Alcohol. 2005; 36:1–3. [PubMed: 16257348]

Cook JW, Spring B, McChargue DE, Borrelli B, Hitsman B, Niaura R, Keuthen NJ, Kristeller J. Influence of fluoxetine on positive and negative affect in a clinic-based smoking cessation trial. Psychopharmacology (Berl). 2004; 173:153–159. [PubMed: 14727000]

Curran S, Purcell S, Craig I, Asherson P, Sham P. The serotonin transporter gene as a QTL for ADHD. Am J Med Genet B Neuropsychiatr Genet. 2005; 134:42–47. [PubMed: 15719397]

David SP, Brown RA, Papandonatos GD, Kahler CW, Lloyd-Richardson EE, Munafo MR, Shields PG, Lerman C, Strong D, McCaffery J, Niaura R. Pharmacogenetic clinical trial of sustained-release bupropion for smoking cessation. Nicotine Tob Res. 2007a; 9:821–833. [PubMed: 17654295]

David SP, Munafò MR, Murphy MF, Walton RT, Johnstone EC. The serotonin transporter 5-HTTLPR polymorphism and treatment response to nicotine patch: follow-up of a randomized controlled trial. Nicotine Tob Res. 2007b; 9:225–231. [PubMed: 17365753]

David SP, Murthy NV, Rabiner EA, Munafo MR, Johnstone EC, Jacob R, Walton RT, Grasby PM. A functional genetic variation of the serotonin (5-HT) transporter affects 5-HT1A receptor binding in humans. J Neurosci. 2005; 25:2586–2590. [PubMed: 15758168]

David SP, Strong DR, Munafo MR, Brown RA, Lloyd-Richardson EE, Wileyto PE, Evins EA, Shields PG, Lerman C, Niaura R. Bupropion efficacy for smoking cessation is influenced by the DRD2 Taq1A polymorphism: analysis of pooled data from two clinical trials. Nicotine Tob Res. 2007c; 9:1251–1257. [PubMed: 18058343]

Du L, Faludi G, Palkovits M, Demeter E, Bakish D, Lapierre YD, Sotonyi P, Hrdina PD. Frequency of long allele in serotonin transporter gene is increased in depressed suicide victims. Biol Psychiatry. 1999; 46:196–201. [PubMed: 10418694]

Engberg G, Erhardt S, Sharp T, Hajos M. Nicotine inhibits firing activity of dorsal raphe 5-HT neurones in vivo. Naunyn Schmiedebergs Arch Pharmacol. 2000; 362:41–45. [PubMed: 10935531]

Engel JA, Fahlke C, Hard E, Johannessen K, Svensson L, Soderpalm B. Serotonergic and dopaminergic involvement in ethanol intake. Clin Neuropharmacol. 1992; 15 (Suppl 1 Pt A):64A–65A.

Heatherton TF, Kozlowski LT, Frecker RC, Fagerstrom KO. The Fagerstrom test for nicotine dependence: a revision of the Fagerstrom Tolerance Questionnaire. Br J Addict. 1991; 86:1119–1127. [PubMed: 1932883]

Heils A, Mossner R, Lesch KP. The human serotonin transporter gene polymorphism—basic research and clinical implications. J Neural Transm. 1997; 104:1005–1014. [PubMed: 9503253]

Heils A, Teufel A, Petri S, Stober G, Riederer P, Bengel D, Lesch KP. Allelic variation of human serotonin transporter gene expression. J Neurochem. 1996; 66:2621–2624. [PubMed: 8632190]

Higgins GA, Wang Y, Corrigall WA, Sellers EM. Influence of 5- HT3 receptor antagonists and the indirect 5-HT agonist, dexfenfluramine, on heroin self-administration in rats. Psychopharmacology (Berl). 1994; 114:611–619. [PubMed: 7855223]

Hitsman B, Spring B, Pingitore R, Munafò MR, Hedeker D. Effect of tryptophan depletion on the attentional salience of smoking cues. Psychopharmacology (Berl). 2007; 192:317–324. [PubMed: 17479308]

Hitsman B, Spring B, Wolf W, Pingitore R, Crayton JW, Hedeker D. Effects of acute tryptophan depletion on negative symptoms and smoking topography in nicotine-dependent schizophrenics and nonpsychiatric controls. Neuropsychopharmacology. 2005; 30:640–648. [PubMed: 15647750]

Huang YY, Battistuzzi C, Oquendo MA, Harkavy-Friedman J, Greenhill L, Zalsman G, Brodsky B, Arango V, Brent DA, Mann JJ. Human 5-HT1A receptor C(−1019)G polymorphism and psychopathology. Int J Neuropsychopharmacol. 2004; 7:441–451. [PubMed: 15469667]

Hughes JR, Keely JP, Niaura RS, Ossip-Klein DJ, Richmond RL, Swan GE. Measures of abstinence in clinical trials: issues and recommendations. Nicotine Tob Res. 2003; 5:13–25. [PubMed: 12745503]

Hughes JR, Stead LF, Lancaster T. Anxiolytics for smoking cessation. Cochrane database of systematic reviews (Online). 2000:CD002849.

Imperial Cancer Research Fund General Practice Research Group. Effectiveness of a nicotine patch in helping people stop smoking: results of a randomised trial in general practice. Imperial Cancer Research Fund General Practice Research Group BMJ. 1993; 306:1304–1308.

Imperial Cancer Research Fund General Practice Research Group. Randomised trial of nicotine patches in general practice: results at 1 year. Imperial Cancer Research Fund General Practice Research Group BMJ. 1994; 308:1476–1477.

Irvine EE, Cheeta S, File SE. Development of tolerance to nicotine's anxiogenic effect in the social interaction test. Brain Res. 2001; 894:95–100. [PubMed: 11245819]

Johnstone EC, Yudkin PL, Hey K, Roberts SJ, Welch SJ, Murphy MF, Griffiths SE, Walton RT. Genetic variation in dopaminergic pathways and short-term effectiveness of the nicotine patch. Pharmacogenetics. 2004; 14:83–90. [PubMed: 15077009]

Killen JD, Fortmann SP, Schatzberg AF, Hayward C, Sussman L, Rothman M, Strausberg L, Varady A. Nicotine patch and paroxetine for smoking cessation. J Consult Clin Psychol. 2000; 68:883–889. [PubMed: 11068974]

Lasky-Su JA, Faraone SV, Glatt SJ, Tsuang MT. Meta-analysis of the association between two polymorphisms in the serotonin transporter gene and affective disorders. Am J Med Genet B Neuropsychiatr Genet. 2005; 133:110–115. [PubMed: 15578606]

LeMarquand D, Pihl RO, Benkelfat C. Serotonin and alcohol intake, abuse, and dependence: findings of animal studies. Biol Psychiatry. 1994; 36:395–421. [PubMed: 7803601]

Lemonde S, Turecki G, Bakish D, Du L, Hrdina PD, Bown CD, Sequeira A, Kushwaha N, Morris SJ, Basak A, Ou XM, Albert PR. Impaired repression at a 5-hydroxytryptamine 1A receptor gene polymorphism associated with major depression and suicide. J Neurosci. 2003; 23:8788–8799. [PubMed: 14507979]

Lerman C, Audrain J, Orleans CT, Boyd R, Gold K, Main D, Caporaso N. Investigation of mechanisms linking depressed mood to nicotine dependence. Addict Behav. 1996; 21:9–19. [PubMed: 8729703]

Lerman C, Caporaso NE, Audrain J, Main D, Boyd NR, Shields PG. Interacting effects of the serotonin transporter gene and neuroticism in smoking practices and nicotine dependence. Mol Psychiatry. 2000; 5:189–192. [PubMed: 10822347]

Lerman C, Caporaso NE, Bush A, Zheng YL, Audrain J, Main D, Shields PG. Tryptophan hydroxylase gene variant and smoking behavior. Am J Med Genet. 2001; 105:518–520. [PubMed: 11496367]

Lerman C, Jepson C, Wileyto EP, Epstein LH, Rukstalis M, Patterson F, Kaufmann V, Restine S, Hawk L, Niaura R, Berrettini W. Role of functional genetic variation in the dopamine D2 receptor (DRD2) in response to bupropion and nicotine replacement therapy for tobacco dependence: results of two randomized clinical trials. Neuropsychopharmacology. 2006a; 31:231–242. [PubMed: 16123753]

Lerman C, Shields PG, Wileyto EP, Audrain J, Hawk LH Jr, Pinto A, Kucharski S, Krishnan S, Niaura R, Epstein LH. Effects of dopamine transporter and receptor polymorphisms on smoking cessation in a bupropion clinical trial. Health Psychol. 2003; 22:541–548. [PubMed: 14570538]

Lerman C, Tyndale R, Patterson F, Wileyto EP, Shields PG, Pinto A, Benowitz N. Nicotine metabolite ratio predicts efficacy of transdermal nicotine for smoking cessation. Clin Pharmacol Ther. 2006b; 79:600–608. [PubMed: 16765148]

Lesch KP, Bengel D, Heils A, Sabol SZ, Greenberg BD, Petri S, Benjamin J, Muller CR, Hamer DH, Murphy DL. Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. Science. 1996; 274:1527–1531. [PubMed: 8929413]

Li MD, Cheng R, Ma JZ, Swan GE. A meta-analysis of estimated genetic and environmental effects on smoking behavior in male and female adult twins. Addiction. 2003; 98:23–31. [PubMed: 12492752]

Little KY, McLaughlin DP, Zhang L, Livermore CS, Dalack GW, McFinton PR, DelProposto ZS, Hill E, Cassin BJ, Watson SJ, Cook EH. Cocaine, ethanol, and genotype effects on human midbrain serotonin transporter binding sites and mRNA levels. Am J Psychiatry. 1998; 155:207–213. [PubMed: 9464199]

Malaiyandi V, Lerman C, Benowitz NL, Jepson C, Patterson F, Tyndale RF. Impact of CYP2A6 genotype on pretreatment smoking behaviour and nicotine levels from and usage of nicotine replacement therapy. Mol Psychiatry. 2006; 11:400–409. [PubMed: 16402128]

Mihailescu S, Guzman-Marin R, Drucker-Colin R. Nicotine stimulation of dorsal raphe neurons: effects on laterodorsal and pedunculopontine neurons. Eur Neuropsychopharmacol. 2001; 11:359–366. [PubMed: 11597822]

Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res. 1988; 16:1215. [PubMed: 3344216]

Munafò M, Clark T, Johnstone E, Murphy M, Walton R. The genetic basis for smoking behavior: a systematic review and meta-analysis. Nicotine Tob Res. 2004; 6:583–597. [PubMed: 15370155]

Munafò MR, Clark TG, Moore LR, Payne E, Walton R, Flint J. Genetic polymorphisms and personality in healthy adults: a systematic review and meta-analysis. Mol Psychiatry. 2003; 8:471–484. [PubMed: 12808427]

Munafò MR, Johnstone EC, Mackintosh B. Association of serotonin transporter genotype with selective processing of smoking-related stimuli in current smokers and ex-smokers. Nicotine Tob Res. 2005a; 7:773–778. [PubMed: 16191748]

Munafò MR, Johnstone EC, Wileyto EP, Shields PG, Elliot KM, Lerman C. Lack of association of 5-HTTLPR genotype with smoking cessation in a nicotine replacement therapy randomized trial. Cancer Epidemiol Biomarkers Prev. 2006; 15:398–400. [PubMed: 16492936]

Munafò MR, Lingford-Hughes AR, Johnstone EC, Walton RT. Association between the serotonin transporter gene and alcohol consumption in social drinkers. Am J Med Genet B Neuropsychiatr Genet. 2005b; 135:10–14. [PubMed: 15729746]

Murphy DL, Lerner A, Rudnick G, Lesch KP. Serotonin transporter: gene, genetic disorders, and pharmacogenetics. Mol Interv. 2004; 4:109–123. [PubMed: 15087484]

Niaura R, Spring B, Borrelli B, Hedeker D, Goldstein MG, Keuthen N, DePue J, Kristeller J, Ockene J, Prochazka A, Chiles JA, Abrams DB. Multicenter trial of fluoxetine as an adjunct to behavioral smoking cessation treatment. J Consult Clin Psychol. 2002; 70:887–896. [PubMed: 12182272]

Nielsen DA, Goldman D, Virkkunen M, Tokola R, Rawlings R, Linnoila M. Suicidality and 5-hydroxyindoleacetic acid concentration associated with a tryptophan hydroxylase polymorphism. Arch Gen Psychiatry. 1994; 51:34–38. [PubMed: 7506517]

Nielsen DA, Virkkunen M, Lappalainen J, Eggert M, Brown GL, Long JC, Goldman D, Linnoila M. A tryptophan hydroxylase gene marker for suicidality and alcoholism. Arch Gen Psychiatry. 1998; 55:593–602. [PubMed: 9672049]

Olausson P, Akesson P, Engel JA, Soderpalm B. Effects of 5-HT1A and 5-HT2 receptor agonists on the behavioral and neurochemical consequences of repeated nicotine treatment. Eur J Pharmacol. 2001a; 420:45–54. [PubMed: 11412838]

Olausson P, Akesson P, Petersson A, Engel JA, Soderpalm B. Behavioral and neurochemical consequences of repeated nicotine treatment in the serotonin-depleted rat. Psychopharmacology (Berl). 2001b; 155:348–361. [PubMed: 11441424]

Olausson P, Engel JA, Soderpalm B. Behavioral sensitization to nicotine is associated with behavioral disinhibition; counteraction by citalopram. Psychopharmacology (Berl). 1999; 142:111–119. [PubMed: 10102762]

Olausson P, Engel JA, Soderpalm B. Involvement of serotonin in nicotine dependence: processes relevant to positive and negative regulation of drug intake. Pharmacol Biochem Behav. 2002; 71:757–771. [PubMed: 11888567]

Opitz K, Weischer ML. Volitional oral intake of nicotine in tupaias: drug-induced alterations. Drug Alcohol Depend. 1988; 21:99–104. [PubMed: 3262051]

Porrino LJ, Ritz MC, Goodman NL, Sharpe LG, Kuhar MJ, Goldberg SR. Differential effects of the pharmacological manipulation of serotonin systems on cocaine and amphetamine self-administration in rats. Life Sci. 1989; 45:1529–1535. [PubMed: 2586218]

Rang, HP.; Dale, MM.; Ritter, JM.; Moore, PK., editors. Pharmacology. 5. Elsevier Science; London, UK: 2003.

Reuter M, Hennig J. Pleiotropic effect of the TPHA779C polymorphism on nicotine dependence and personality. Am J Med Genet B Neuropsychiatr Genet. 2005; 134:20–24. [PubMed: 15635702]

Roberts DC, Loh EA, Baker GB, Vickers G. Lesions of central serotonin systems affect responding on a progressive ratio schedule reinforced either by intravenous cocaine or by food. Pharmacol Biochem Behav. 1994; 49:177–182. [PubMed: 7816870]

Sen S, Burmeister M, Ghosh D. Meta-analysis of the association between a serotonin transporter promoter polymorphism (5-HTTLPR) and anxiety-related personality traits. Am J Med Genet B Neuropsychiatr Genet. 2004; 127:85–89. [PubMed: 15108187]

Society for Research on Nicotine and Tobacco. Biochemical verification of tobacco use and cessation. Nicotine Tob Res. 2002; 4:149–159. [PubMed: 12028847]

Sprouse JS, Aghajanian GK. Electrophysiological responses of sero-toninergic dorsal raphe neurons to 5-HT1A and 5-HT1B agonists. Synapse. 1987; 1:3–9. [PubMed: 3505364]

Strobel A, Gutknecht L, Rothe C, Reif A, Mossner R, Zeng Y, Brocke B, Lesch KP. Allelic variation in 5-HT1A receptor expression is associated with anxiety- and depression-related personality traits. J Neural Transm. 2003; 110:1445–1453. [PubMed: 14666415]

Sullivan PF, Jiang Y, Neale MC, Kendler KS, Straub RE. Association of the tryptophan hydroxylase gene with smoking initiation but not progression to nicotine dependence. Am J Med Genet. 2001; 105:479–484. [PubMed: 11449402]

Swan GE, Valdes AM, Ring HZ, Khroyan TV, Jack LM, Ton CC, Curry SJ, McAfee T. Dopamine receptor DRD2 genotype and smoking cessation outcome following treatment with bupropion SR. Pharmacogenomics J. 2005; 5:21–29. [PubMed: 15492764]

Tucci S, Genn RF, Marco E, File SE. Do different mechanisms underlie two anxiogenic effects of systemic nicotine? Behav Pharmacol. 2003; 14:323–329. [PubMed: 12838038]

Walther DJ, Peter JU, Bashammakh S, Hortnagl H, Voits M, Fink H, Bader M. Synthesis of serotonin by a second tryptophan hydroxylase isoform. Science. 2003; 299:76. [PubMed: 12511643]

West R, Hajek P, Stead L, Stapleton J. Outcome criteria in smoking cessation trials: proposal for a common standard. Addiction. 2005; 100:299–303. [PubMed: 15733243]

Wu S, Comings DE. A common C-1018G polymorphism in the human 5-HT1A receptor gene. Psychiatr Genet. 1999; 9:105–106. [PubMed: 10412191]

Yudkin P, Hey K, Roberts S, Welch S, Murphy M, Walton R. Abstinence from smoking eight years after participation in randomised controlled trial of nicotine patch. BMJ. 2003; 327:28–29. [PubMed: 12842953]

Yudkin P, Munafò M, Hey K, Roberts S, Welch S, Johnstone E, Murphy M, Griffiths S, Walton R. Effectiveness of nicotine patches in relation to genotype in women versus men: randomised controlled trial. BMJ. 2004; 328:989–990. [PubMed: 15033882]

Table 1

SNP's primers and probes

Gene	SNP site	Primer sequences	Probe sequences
TPH	A779C	CGGGACATGACCTAAGAGTTCA	AACTTGTACCTCT***C***TTTCAG
		AGCACTGCAGCGTGACA	CTTGTACCTCT***A***TTTCAG
5HT1A	C-1019G	GCGAGAACGGAGGTAGCTT	TGTGTCTTC***G***TTTTTAAA
		GGGTCAGTCTCCCAATTATTGCTAA	TGTGTCTTC***C***TTTTTAAA

Bold/italic = variable base.

Table 2

Basic characteristics and genotype frequencies

Basic characteristics & FTND	**Mean (S.D.) or *n* (%) (*N*= 792)**
Age	44 (12)
Sex (female)	410 (58%)
Nicotine dependence	5 (2)
Genotype frequency (%)	
TPH1 A779C	
AA	102 (13%)
AC	392 (50%)
CC	298 (38%)
5-HTTLPR	
LL	234 (30%)
LS	397 (50%)
SS	161 (20%)
HTR1A C-1019G	
CC	179 (23%)
CG	398 (50%)
GG	215 (27%)

Nicotine dependence = Fagerström Test of Nicotine Dependence (Heatherton et al., 1991). S.D. = standard deviation.

Table 3

Abstinence data from the PiP, UPenn and Patch II trials by 5-HTTLPR genotype

Study	5-HTTLPR genotype											
	SS			SL			LL			SS	SL	LL
	1	2	3	1	2	3	1	2	3	Studies 1–3 combined		
12-week												
Succeeded	16 (9%)	17 (55%)	21 (29%)	54 (13%)	50 (53%)	41 (24%)	25 (10%)	25 (45%)	31 (25%)	54 (20%)	145 (21%)	81 (19%)
Failed	153 (91%)	14 (45%)	51 (71%)	372 (87%)	45 (47%)	132 (76%)	225 (90%)	31 (55%)	95 (75%)	218 (80%)	549 (79%)	351 (81%)
Total	169	31	72	426	95	173	250	56	126	272	694	432
26-week												
Succeeded	13 (8%)	10 (34%)	15 (21%)	38 (9%)	35 (37%)	30 (17%)	21 (8%)	20 (37%)	30 (24%)	38 (14%)	103 (15%)	71 (17%)
Failed	156 (92%)	19 (66%)	57 (79%)	388 (91%)	60 (63%)	143 (83%)	229 (92%)	34 (63%)	96 (76%)	232 (86%)	591 (85%)	359 (83%)
Total	169	29	72	426	95	173	250	54	126	270	694	430

Note. Abstinence data were missing for two participants with the SS genotype and two participants with the LL genotype in the UPenn trial. Study 1 (PiP, *N*= 845); Study 2 (UPenn, *N*= 182 at 12 weeks; *N*= 178 at 26 weeks); Study 3 (Patch II, *N*= 371); pooled analyses (*k*=3; *N*= 1,398).

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