

Dopamine transporter polymorphisms are associated with short-term response to smoking cessation treatment

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Objectives To examine the association between polymorphisms in the dopamine transporter gene (SLC6A3, DAT1) and treatment outcome in smokers attempting to quit using either nicotine replacement therapy or bupropion.

Methods The sample consisted of 583 smokers recruited from a smoking cessation clinic, and followed throughout the 4 weeks of post-cessation treatment with behavioural support and either nicotine replacement therapy or bupropion.

Results At 1 week after smoking cessation, the 3' untranslated region (3'UTR) variable number of tandem repeats (VNTRs) and the 30-bp intron 8 VNTR DAT1 genotypes were associated with the ability to stop smoking (3'UTR VNTR, odds ratio=2.0, 95% confidence interval=1.2–3.5, novel intron 8 VNTR, odds ratio=1.8, 95% confidence interval=1.0–2.9), controlling for potential confounders. The results were weaker and no longer significant at a 4-week follow-up.

Conclusions We find evidence, although modest, of a medium-sized effect of DAT1 genotype on the ability to

stop smoking early in a smoking cessation attempt. If the effect is real, and is strongest in the very early stages of smoking cessation, this suggests that the primary utility of DAT1 screening in this field will be in the identification of those most at risk of early relapse after quitting. *Pharmacogenetics and Genomics* 17:61–67 © 2007 Lippincott Williams & Wilkins.

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Introduction

The enormous toll of tobacco smoking on mortality and morbidity has prompted considerable research into pharmacological treatments to overcome dependence on inhaled nicotine in cigarettes and thereby to aid smoking cessation. Nicotine replacement therapy (NRT) and bupropion (Zyban, Glaxo Smith Kline, London, UK) are currently the only medicines widely accepted and licensed for smoking cessation treatment. Both have proven efficacy and approximately double the likelihood of cessation relative to behavioural treatments alone [1,2]. NRT is the standard recommended treatment for smoking cessation and is used by the majority of smokers treated in clinical settings in the UK. It partially replaces the nicotine obtained from cigarettes, but in a more slowly absorbed form, without the high arterial bolus doses achieved by tobacco smoke inhalation. Regardless of the NRT preparation (patch, gum, lozenge, nasal spray, buccal inhalator, sublingual tablet), smokers typically obtain venous nicotine levels about half those from their smoking. This is sufficient to partially relieve withdrawal symptoms and desire to smoke, promoting a staged progression away from smoking. The action by which

bupropion relieves nicotine withdrawal symptoms and aids cessation is not clearly understood. It appears to increase dopamine and noradrenaline concentrations, both of which are implicated in the maintenance of smoking [3].

As even the best specialist behavioural counselling achieves only ~10% long-term abstinence, however, the marginal effectiveness of these medicines is similarly modest (<10%) and there remains an urgent need to improve treatments. One option is to tailor existing treatments by matching them more effectively to the differing individual characteristics of smokers. Evidence from twin studies suggests that inherited factors explain about 50% of variation in smoking status and possibly 70% of the variation in smoking maintenance [4,5]. Hence, the possibility of using the genetic make-up of individuals in tailoring treatment plans has recently received considerable interest [6].

The reinforcing properties of nicotine have been attributed to effects on dopamine transmission [7,8]. By binding to acetylcholine receptors nicotine has been

shown to stimulate dopamine release and to inhibit reuptake, and the resultant pleasure induced is believed to be at least partly responsible for smoking maintenance [9,10]. It is therefore plausible that genes affecting dopamine function and resultant endogenous dopamine levels affect the degree of nicotine dependence and the ability to stop smoking.

The SLC6A3 gene regulates synaptic dopamine through coding for the reuptake protein known as the dopamine transporter (DAT1) [11,12]. Genetic studies of DAT1 and smoking have concentrated on the variable number of tandem repeats (VNTRs) polymorphism in the 3' untranslated region of the gene (3'UTR) [13–15]. The initial two studies used retrospective self-reports of smoking status as the target phenotype. Lerman *et al.* [16] found that people with a variant 9-repeat allele were less likely to be smokers and that those who did smoke were less likely to have started before the age of 16 years and to have had longer periods of abstinence. Ex-smokers and smoking cessation rates were not recorded in this study. In a study that also included former smokers, Sabol *et al.* [17] found that among those who had ever smoked, those with a 9-repeat allele compared with those with 10/10 genotype were 1.5 times more likely to be ex-smokers. Jorm *et al.* [18] reported a similar, but non-significant, trend with an odds ratio (OR) of 1.2 in favour of quitting with the 9-repeat allele. Vandenberg *et al.* [19], however, observed a significant trend in the opposite direction and an OR of 0.7. In a meta-analysis of the three studies, Munafo *et al.* [20] concluded that there was insufficient evidence for an influence of 3'UTR VNTR on smoking and cited somewhat disparate and potentially imprecise phenotypes as a possible cause of the excessive variation in results. More recently, Lerman *et al.* [21] conducted the first prospective study of the 3'UTR VNTR in relation to smoking cessation. In a trial of bupropion, there was no evidence of a main effect on cessation, although there was evidence that an interaction with the dopamine D2 receptor (DRD2) was predictive [21]. To date, there are no studies of the influence of DAT1 polymorphisms on outcome among those treated with NRT or on differences between NRT and bupropion.

We conducted a prospective study of the influence of DAT1 polymorphisms on the outcome of smoking cessation treatment with either NRT or bupropion among dependent smokers treated at a specialist clinic. Hence, we were able to examine whether there is a different relationship between DAT1 and smoking cessation according to whether NRT or bupropion had been taken to aid cessation, as has recently been observed in relation to the DRD2 locus, –141C ins/del [22]. Besides the 3'UTR VNTR, we also studied the intron 8 30-bp VNTR, which has been found to be associated with cocaine abuse [23], and three other haplotype tagging single nucleotide polymorphisms (SNPs) between them. We also examined

the confounding influence of prognostic characteristics such as history of alcohol or drug problems, and history of depression, for which there is either heuristic or direct empirical evidence of a genetic link with tobacco dependence [24].

Methods

Study participants and procedures

Smokers attending a National Health Service smoking cessation clinic at the Maudsley Hospital, London, between October 2001 and June 2004 were invited to take part in the study; 583 out of 1181 (49.4%) smokers consented to give a DNA sample before stopping smoking (Maudsley Smoking Cessation Clinic phase 1 sample). The treatment course was 7 weekly 1-h group or individual support sessions over 6 weeks, plus NRT or bupropion. Either NRT (all formulations) or bupropion was chosen by the smoker, subject to contraindications and clinical advice, 2 weeks (session 1) before attempting to stop smoking, and were used according to the manufacturers' recommendations (for 12 and 8 weeks, respectively). Smokers undertook to stop smoking from session 3 (quit day), with previous sessions used for assessment and preparation for quitting. DNA samples were collected via buccal cell swabs at session 2. Clinic staff were unaware of genotyping results.

All other data collection and treatment procedures were as for normal clinic attendees. All participants completed a baseline questionnaire giving details on demographics, smoking history, degree of tobacco dependence and medical history before DNA sampling. At all post-quit sessions, participants reported number of cigarettes smoked in the previous week and, if attending in person, had expired air CO measured to verify self-reported abstinence (< 10 p.p.m. was confirmation).

Marker selection, DNA sampling and genotyping

The 3'UTR 40-bp repeat and the 30-bp intron 8 VNTR [23] were genotyped. Following characterization of linkage disequilibrium across the DAT1 gene [25], we also selected three SNPs: I1 + 1036 (rs2963238; C/A), I1 + 478 (rs11564752; G/T) and I8 + 2086 (rs27048; A/G), two located in intron 1 and one in intron 8, respectively. Genomic DNA was extracted from buccal mucosa cells [26]. The VNTRs were amplified by polymerase chain reaction: 5 min at 95°C, 35 cycles of 1 min at 95°C, 1 min at 60°C and 1 min at 72°C. The oligonucleotide primers used were 3'UTR-F, 5'TGGCACGCACCTGAGAG3'; 3'UTR-R, 5'GGCATTGGAGGATGGGG3'; Int8-F, 5'CTTGGGGAAGGAAGGG3'; and Int8-R, 5'TGTGTGCGTGCATGTGG3'. Alleles of the 3'UTR were coded according to the number of repeats they contained, whereas alleles of the intron 8 were coded according to their relative size. All SNP genotyping was performed by K-Biosciences, Cambridge, UK [27], using an amplifluor assay (assay details are available upon request).

Measures

Smoking cessation outcome

Two short-term measures of smoking cessation were used. The primary 'intent-to-treat' measure, as outlined in the Department of Health smoking cessation monitoring guidelines [28], was consistent with the Society for Research on Nicotine and Tobacco guidelines. To be classified as 'abstinent', participants had to report, in person or by telephone or letter, not smoking during the final 2 weeks of the course, from 2 weeks after quit day onwards, and have this confirmed by CO verification. In this cohort, no participant who self-reported abstinence subsequently failed CO verification. Therefore, the verified abstinent group consisted of all self-report abstinent participants who also attended the final session. Those who did not attend the last scheduled session and who failed to respond to repeated requests to attend an individual verification session were considered to be smoking [29]. A second outcome measure was based on the ability to quit for 1 week following quit day. Only those smokers who attended session 4 (1 week after quit day) were included in the sub-sample for this analysis because, unlike for the final 2 weeks of treatment, during this initial week all were encouraged to attend regardless of whether they had yet achieved abstinence.

Genotype

Following all previous studies of DAT1 showing evidence of a prognostic effect on smoking cessation, we tested in our primary analyses the dominant variant allele model, comparing two copies of the common allele with all other genotypes. The co-dominant three-genotype model was considered in secondary analyses. Genotypes for the five markers were dichotomized for comparison according to possession of one or more variant alleles (3'UTR VNTR: 10/10 vs. 10/9, 9/9; intron 8 VNTR: 3/3 vs. 3/2, 2/2; rs115 SNP: G/G vs. G/T, T/T; rs270 SNP: G/G vs. G/A, A/A; rs296 SNP: C/C vs. C/A, A/A). We excluded from the initial analyses the small number of participants with rare VNTR genotypes for which there is no *a priori* evidence of association in an attempt to gain a more precise comparison of 10 vs. 9 and 3 vs. 2 repeats. Their effect was considered separately in subsequent analyses.

Nicotine dependence

The Heaviness of Smoking Index (HSI) is a two-item, 60% subset of the Fagerstrom Test for Nicotine Dependence (FTND), combining the daily number of cigarettes (scored 0–3) and latency to the first cigarette of the day (scored 3–0). The HSI performs similarly to the FTND in reliably predicting smoking cessation [30]. It was administered at session 1, 2 weeks before quit day.

Demographic and other background measures

Relevant demographics, smoking history, mental health history and drug use history were recorded on a self-completion questionnaire at session 1. Responses were

checked by a clinician during an individual assessment at the same session. Histories of alcohol and drug use problems were not recorded separately, only as a single combined checklist item.

Data processing and statistical analysis

Initial univariate comparisons of cessation rates and background characteristics according to genotype were made by the χ^2 test or the *t* test. As there are no existing studies of the three SNPs in this context, to control for multiple testing we used an effective significance level of 0.02 for each individual SNP. Confidence intervals (CIs) for differences or ORs were also calculated. SPSS (v12) software (Chicago, Illinois, USA) was used for statistical analyses. Logistic regression models were fitted to control for confounders, study interaction effects on cessation between genotypes and type of medication used, and between genotype and potentially linked prognostic characteristics such as depression and drug use history.

Sample size

The sample size for the study was based on the results observed among women by Yudkin *et al.* [31] when studying DRD2 genotypes and smoking cessation. A sample of at least 230 women using NRT was required and this resulted in a total sample of 583 smokers (men and women, NRT and bupropion), for the analysis of DAT1. For the principal marker (3'UTR VNTR), the genotyped sample gave 75% power to detect an OR of 1.5 in favour of stopping smoking with the 10/9 or 9/9 genotype, as observed in the original study by Sabol *et al.* [17]. No viable power calculations were undertaken regarding the direction of a possible interaction between genotype and drug, given that this was the first study of this type and because the action of bupropion is not clearly understood.

Ethics

Ethical approval was obtained from the South London and Maudsley and Institute of Psychiatry Research Ethics Committee and all participants gave signed, informed consent.

Results

Of the 583 patients who gave consent and DNA samples, 578 were treated with either NRT or bupropion and were considered for analysis. Participants with rare 3'UTR VNTR alleles [17] or intron 8 VNTR alleles [20] were excluded. Genotype frequencies for the analyses were 3'UTR VNTR ($n = 541$; 10/10 = 306; 9/10 = 202; 9/9 = 33), intron 8 VNTR ($n = 530$; 3/3 = 292; 2/3 = 188; 2/2 = 50), rs115 SNP ($n = 525$; G/G = 433; G/T = 84; T/T = 8), rs270 SNP ($n = 516$; G/G = 184; G/A = 231; A/A = 101) and rs296 SNP ($n = 501$; C/C = 182; C/A = 233; A/A = 86).

The genotype distribution of the 3'UTR VNTR and the three SNPs were in Hardy-Weinberg equilibrium in the whole sample (3'UTR: $\chi^2 = 0.0019$, $P = 0.964$; rs115: $\chi^2 = 2.70$, $P = 0.101$; rs270: $\chi^2 = 3.37$, $P = 0.066$; rs296: $\chi^2 = 0.59$, $P = 0.441$). For the intron 8 VNTR, there was evidence of disequilibrium in the whole sample ($\chi^2 = 5.5$, $P = 0.019$), but not when the subgroups with European ($\chi^2 = 1.87$, $P = 0.171$) and African ($\chi^2 = 0.65$, $P = 0.420$) ancestry were analysed separately. There was good evidence of significant linkage disequilibrium for four of the 10 marker-marker pairs (Table 1).

The demographic and key participant characteristics are given individually by genotype in Table 2. For the 3'UTR VNTR cohort, the mean age was 43.7 years, 41.6% were male, 86% were of European ancestry, 44.4% had a history of depression, 12.8% had a history of either alcohol or drug problems, 15% were smoking cannabis, the mean HSI score was 3.3 and 73.4% used NRT. Frequencies were similar for the other markers under study and did not differ across genotypes.

Relationship between genotypes and smoking cessation

Smoking cessation abstinence rates according to genotype are displayed in Table 3. In simple univariate analyses, there was no evidence of an association between smoking cessation and SNP genotype. There was also no evidence to suggest that the VNTR genotypes were associated with the rate of complete cessation during the final 2 weeks of treatment. Cessation during the first week, however, was clearly related to both VNTR genotypes. For the 3'UTR VNTR, a 10% higher cessation rate was observed among those carrying a 9-repeat allele (9/10, 9/9) than among those with 10/10 repeats (86 vs. 76%, $\chi^2 = 6.3$, $P = 0.012$). When the 17 participants with minor genotypes were also included with those possessing a 9-repeat allele, the results were virtually identical. A similar, but slightly smaller difference was observed for the intron 8 VNTR. Those carrying a 2-repeat allele

Table 1 Pair-wise linkage disequilibrium measures for the DAT1 markers

	Intron 8 VNTR	rs115654752	rs27048	rs2963238
3'UTR VNTR	$r^2 = 0.246$ $\chi^2 = 102$ $P < 0.001$	$r^2 = 0.005$ $\chi^2 = 2.3$ $P = 0.13$	$r^2 = 0.017$ $\chi^2 = 7.3$ $P = 0.007$	$r^2 = 0.004$ $\chi^2 = 1.7$ $P = 0.19$
Intron 8 VNTR		$r^2 = 0.006$ $\chi^2 = 2.6$ $P = 0.11$	$r^2 = 0.075$ $\chi^2 = 31.2$ $P < 0.001$	$r^2 = 0.000$ $\chi^2 = 0.06$ $P = 0.80$
rs11564752			$r^2 = 0.015$ $\chi^2 = 6.2$ $P = 0.013$	$r^2 = 0.109$ $\chi^2 = 45.4$ $P < 0.001$
rs27048				$r^2 = 0.008$ $\chi^2 = 3.45$ $P = 0.063$

Analysis sample consists of 416 study participants with all markers typed. Results for the maximum sample available for each pair were similar (mean $n = 487$). DAT1, dopamine transporter 1; 3'UTR, 3' untranslated region; VNTR, variable number of tandem repeat.

Table 2 Demographics, mental health history, drug use history, severity of tobacco dependence, and treatment medication by DAT1 genotypes

	Variant allele	Common allele	Difference/odds ratio (95% CI)
3'UTR VNTR	9/10, 9/9 ($n = 235$)	10/10 ($n = 306$)	
Age, mean years (SD)	43.9 (12.2)	43.5 (12.9)	0.33 (-1.8, 2.5)
% Male (n)	38.7 (91)	43.8 (134)	0.83 (0.59, 1.40)
% European (n)	90.6 (213)	82.4 (252)	2.1 (1.2, 3.6)
% Hx depression (n)	45.1 (106)	43.8 (134)	1.1 (0.77, 1.49)
% Hx alcohol/drug use (n)	10.2 (24)	14.7 (45)	0.67 (0.38, 1.1)
% Using cannabis (n)	13.2 (31)	16.3 (50)	0.74 (0.45, 1.2)
HSI score, mean (SD)	3.4 (1.6)	3.3 (1.6)	0.13 (-0.14, 0.41)
% Treated with NRT (n)	69.8 (164)	76.1 (233)	0.71 (-0.5, 1.1)
Intron 8 VNTR	2/3, 2/2 ($n = 238$)	3/3 ($n = 292$)	
Age, mean years (SD)	43.4 (12.1)	44.0 (13.0)	-0.67 (-2.8, 1.5)
% Male (n)	40.3 (142)	42.1 (123)	0.91 (0.67, 1.3)
% European (n)	75.6 (180)	91.1 (266)	0.30 (0.19, 0.50)
% Hx depression (n)	44.1 (105)	44.5 (130)	1.0 (0.71, 1.4)
% Hx alcohol/drug use (n)	12.6 (30)	13.0 (38)	1.0 (0.59, 1.6)
% Using cannabis (n)	12.6 (30)	15.4 (45)	0.77 (0.45, 1.3)
HSI score, mean (SD)	3.4 (1.6)	3.3 (1.7)	0.10 (-0.18, 0.38)
% Treated with NRT (n)	72.3 (172)	75.7 (221)	0.83 (0.55, 1.20)
rs115 SNP	G/T, T/T ($n = 92$)	G/G ($n = 433$)	
Age, mean years (SD)	43.8 (12.7)	43.5 (12.4)	0.28 (-2.5, 3.1)
% Male (n)	38.0 (35)	42.3 (183)	0.83 (0.53, 1.30)
% European (n)	91.3 (84)	82.4 (357)	2.2 (1.0, 4.80)
% Hx depression (n)	51.1 (47)	43.0 (186)	1.4 (0.91, 2.2)
% Hx alcohol/drug use (n)	18.5 (17)	12.2 (53)	1.6 (0.90, 2.9)
% Using cannabis (n)	20.7 (19)	14.3 (62)	1.7 (0.97, 3.1)
HSI score, mean (SD)	3.1 (1.8)	3.4 (1.6)	-0.25 (-2.5, 3.1)
% Treated with NRT (n)	76.1 (70)	73.9 (320)	1.1 (0.67, 1.9)
rs270 SNP	G/A, A/A ($n = 332$)	G/G ($n = 184$)	
Age, mean years (SD)	44.9 (13.0)	42.2 (11.6)	2.7 (-0.46, 4.9)
% Male (n)	41.6 (138)	40.8 (75)	1.0 (0.71, 1.5)
% European (n)	86.7 (288)	78.3 (144)	1.8 (1.1, 2.9)
% Hx depression (n)	47.6 (158)	42.9 (79)	1.2 (0.83, 1.7)
% Hx alcohol/drug use (n)	13.9 (46)	12.5 (23)	1.1 (0.67, 1.9)
% Using cannabis (n)	16.0 (53)	14.1 (26)	1.2 (0.71, 2.0)
HSI score, mean (SD)	3.3 (1.6)	3.3 (1.7)	0.02 (-0.27, 0.32)
% Treated with NRT (n)	75.0 (249)	75.5 (139)	1.0 (0.63, 1.5)
rs296 SNP	C/A, A/A ($n = 319$)	CC ($n = 182$)	
Age, mean years (SD)	43.8 (12.6)	44.5 (12.2)	-0.75 (-3.0, 1.5)
% Male (n)	39.5 (126)	43.4 (79)	0.83 (0.59, 1.2)
% European (n)	80.3 (256)	91.2 (166)	0.39 (0.22, 0.70)
% Hx depression (n)	47.0 (150)	45.1 (82)	1.1 (0.77, 1.6)
% Hx alcohol/drug use (n)	13.8 (44)	12.6 (23)	1.1 (0.63, 1.9)
% Using cannabis (n)	15.4 (49)	15.9 (29)	0.91 (0.55, 1.6)
HSI score, mean (SD)	3.2 (1.7)	3.5 (1.5)	-0.20 (-0.50, 0.09)
% Treated with NRT (n)	77.1 (246)	74.2 (135)	1.2 (0.77, 1.8)

DAT1, dopamine transporter 1; CI, confidence interval; 3'UTR, 3' untranslated region; VNTR, variable number of tandem repeat; HSI, Heaviness of Smoking Index; SNP, single nucleotide polymorphism; NRT, nicotine replacement therapy.

Table 3 Percentage and numbers of smokers stopping smoking by DAT1 genotypes

Outcome measure	Variant allele	Common allele	Odds ratio (95% CI)
3'UTR VNTR	9/10, 9/9	10/10	
% DH week 3/4 successes ^a	56.2 (132/235)	53.3 (163/306)	1.1 (0.77, 1.6)
% week 1 successes ^b	85.7 (144/168)	75.8 (188/248)	1.9 (1.1, 3.2)*
Intron 8 VNTR	2/3, 2/2	3/3	
% DH week 3/4 successes	55.0 (131/238)	52.4 (153/292)	1.1 (0.77, 1.6)
% week 1 successes	84.7 (150/177)	76.1 (172/226)	1.7 (1.0, 2.9)*
rs115 SNP	G/T, T/T	G/G	
% DH week 3/4 successes	47.8 (44/92)	55.0 (238/433)	0.75 (0.48, 1.2)
% week 1 successes	79.5 (62/78)	79.7 (259/325)	1.0 (0.53, 1.8)
rs270 SNP	G/A, A/A	G/G	
% DH week 3/4 successes	54.5 (181/332)	53.8 (99/184)	1.0 (0.72, 1.5)
% week 1 successes	79.3(203/256)	79.4 (112/141)	1.0 (0.59, 1.6)
rs296 SNP	C/A, A/A	C/C	
% DH week 3/4 successes	54.9 (175/319)	53.3 (97/182)	1.1 (0.71, 1.5)
% week 1 successes	80.1 (197/246)	78.4 (105/134)	1.1 (0.67, 1.9)

* $P < 0.05$. DAT1, dopamine transporter 1; CI, confidence interval; 3'UTR, 3' untranslated region; VNTR, variable number of tandem repeat; SNP, single nucleotide polymorphism.

^aDepartment of Health primary outcome measure. Self-report of no smoking during last 2 weeks of treatment, validated by expired air CO < 10 p.p.m. at week 4. The intent-to-treat model with those failing to respond/attend regarded as continuing smokers.

^bSelf-report of no smoking during the first week of the quit attempt, validated by expired air CO < 10 p.p.m., only among those who attended the treatment session 1 week after quit day, with those not attending the session excluded from the base sample.

(2/3, 2/2) had an 8.5% improved cessation rate during week 1 (84.7 vs. 76.1%, $\chi^2 = 4.7$, $P = 0.03$). Inclusion of the 20 participants with rare genotypes gave virtually identical results.

Of the uncontrolled participant characteristics shown in Table 2, age, HSI and history of alcohol/drug problems were the only predictors of treatment responses at week 1 and weeks 3–4 (results not shown). Adjustment of the relationship between genotypes and the outcomes for these characteristics had no material effect. At week 1, there remained evidence of an effect for the 3'UTR VNTR (OR = 2.0, 95% CI = 1.2–3.5, $P = 0.011$) and for the intron 8 VNTR (OR = 1.8, 95% CI = 1.0–2.9) and no evidence of an effect for the three SNPs (rs115: OR = 0.959, $P = 0.896$; rs270: OR = 1.136, $P = 0.635$; rs296: OR = 1.098, $P = 0.728$).

In extended multiple logistic regressions, there was no evidence of predictive interactions between genotype and age, sex, ethnic ancestry, depression history, alcohol/drug problems, cannabis use and severity of tobacco dependence. In the same models, there was also no evidence for a differential effect of genotype according to whether NRT or bupropion had been used for any marker on either outcome measure. When modelling the joint effect of all DAT1 markers and their interactions in a

backward-elimination step-wise logistic model, only the main effect for the 3'UTR VNTR remained in the model. Owing to the linkage between the 3'UTR and intron 8 VNTRs, however, a competing model containing the intron 8 VNTR main effect is equally viable.

Alternative genotype model

In addition to our primary dominant variant allele model, we also considered the prognostic characteristics of the three-category co-dominant genotype model. For cessation during the first week, the results were weaker than previously seen in the primary model (3'UTR VNTR: $\chi^2 = 6.1$, $P = 0.047$; intron 8 VNTR: $\chi^2 = 5.6$, $P = 0.060$; rs115 SNP: $\chi^2 = 0.661$, $P = 0.719$; rs270 SNP: $\chi^2 = 1.49$, $P = 0.474$; rs296 SNP: $\chi^2 = 3.5$, $P = 0.168$). Although marginally significant in the case of the two VNTRs, the abstinence rates for each genotype indicated support for the dominant variant allele model (3'UTR VNTR: 10/10 = 76%, 10/9 = 86%, 9/9 = 84%; intron 8 VNTR: 3/3 = 76%, 3/2 = 86%, 2/2 = 80%). When looking at cessation after 4 weeks, there was no evidence of an association between the three-category model and success (3'UTR VNTR: $P = 0.328$; intron 8 VNTR: $P = 0.822$; rs115 SNP: $P = 0.456$; rs270 SNP: $P = 0.474$; rs296 SNP: $P = 0.811$).

Discussion

To our knowledge, this is the first prospective study of the influence of DAT1 polymorphisms on the outcome of smoking cessation treatment with either NRT or bupropion in dependent smokers treated at a specialist smoking cessation clinic. Hence, we were able to examine whether there is a different relationship between DAT1 and cessation according to whether NRT or bupropion had been taken to aid cessation, as recently reported for the DRD2 – 141C ins/del [22].

We found modest but significant positive associations between DAT1 3'UTR VNTR and intron 8 30-bp VNTR genotypes and smoking cessation at 1 week after smoking cessation. We found that the intron 8 VNTR was in linkage disequilibrium with the commonly studied 3'UTR VNTR, and although the observed association between these two markers was not strong enough to indicate a co-linearity of effect, the two markers did give similar significant results with regard to OR of cessation. We were unable to construct a combined prognostic measure from the two VNTRs better than considering genotypes of either alone. As we were able to control for the well-known demographic, smoking history and health history determinants of smoking cessation, it is unlikely that the relationships we observed between genetic markers and outcome are purely statistical correlations resulting from confounding factors. The possibility remains, however, that a previously unidentified prognostic characteristic might also be associated with DAT1

genotypes, and might be responsible for the association we have attributed to DAT1. Perhaps more plausible is the possibility that both VNTRs are associated with cessation through linkage disequilibrium with one or more other polymorphisms that were not studied.

Our cessation results for the previously studied 3'UTR VNTR varied according to the time since quit day. The OR in favour of the 9-repeat allele was 2.0 (1.20–3.50) at the end of the first week of the cessation attempt, but declined to 1.12 (0.77–1.60) by the end of the fourth week. Alone, the latter result is not particularly compelling in terms of clinical utility. It is close to the average for previous studies [17–19,21], however, and supports the suggestion that less difficulty stopping smoking is associated with the 9-repeat allele. From a clinical tailoring perspective, we could find no evidence that the relationship between 3'UTR VNTR (or intron 8) and initial cessation was modified by whether NRT or bupropion had been used to aid stopping. Although this is only the first study of DAT1 smoking pharmacogenetics involving two therapeutic drugs, it raises questions regarding the prospect of tailoring these popular treatments according to DAT genotypes. There was no suggestion of a relationship between any of the genotypes under study and the severity of nicotine dependence as measured by the HSI before stopping, although the HSI was associated with cessation. This lack of an association between genotype and HSI might indicate a weakness in subjective questionnaire measures of tobacco dependence and that only when someone tries to stop smoking do we get a true measure of the degree of their dependence.

In conclusion, we found modest statistical evidence of a medium-sized effect of DAT1 genotype on the ability to stop smoking. If the effect is real, it is at its strongest in the very early stages of smoking cessation, which suggests that the primary utility of DAT1 pharmacogenetic testing in this field will be in the identification of those most at risk of relapse following initial abstinence.

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