Supplementary Materials

Materials and Methods

1 Participants

Two hundred and eleven eligible participants took part in this study, and were randomly divided into active or placebo treatment groups in a double-blind manner. The characteristics of all the participants with sublingual nicotine tablet status and smoking severity are shown in Table S1. Inclusion criteria were: (1) northern Han Chinese aged 20–65 years living in the Haidian District of Beijing; (2) motivated to stop smoking; (3) smoked ≥10 cigarettes/day for ≥1 year; (4) presented with a carbon monoxide (CO) level ≥10 parts per million in exhaled air. Participants were excluded if they had a family history of psychiatric disorders or neurological diseases. Individuals with internal medical disorders or additional systemic or central nervous system diseases were excluded. The study protocol was approved by the Review Board of Beijing Anding Hospital. Each participant gave written informed consent. All participants were divided into two groups based on smoking status as well as scores on the Fagerstrom test for nicotine dependence (FTND) and the Visual Analogue Scale (VAS). Moderate dependence (MD) was defined as FTND <7 and severe dependence (SD) was set at FTND ≥7. No participants had smoked <100 cigarettes during their lifetime. Former smokers were defined as persons who had previously smoked >1 cigarette/day but had quit smoking for ≥1 year. Current smokers were defined as individuals who smoked >1 cigarette/day and had smoked for ≥1 year.

2 Assessments

A self-administered questionnaire at baseline was used to provide the sociodemographic characteristics age, gender, years of cigarette smoking, daily cigarette consumption, marital status, alcohol consumption, education level, occupation, and body mass index. All participants were visited on day 4, at the end of 2, 4, 6, and 8 weeks, and after 3 months. Self-reported average daily cigarette consumption was verified at every visit by analysis of CO using a monitor (Bedfont Smokerlyzer, Technical Instruments Ltd., Kent, UK) to assess end-expiratory air after holding the breath for 15 sec. Urinary cotinine concentration was determined by gas chromatography at baseline, and at the end of 1, 2, and 3 months. Smoking status was evaluated and verified at the end of treatment (end of the 2nd month) and at one month follow-up (end of the 3rd month). Participants who had smoked for 7 consecutive days during the period were considered to be abstinence failures. Only those participants with cotinine levels <15 ng/mL and CO <8 ppm were defined as abstinent. After the 12-week treatment period, all participants were

given face-to-face counseling for ~10 min by a doctor trained in smoking-cessation therapy.

3 Treatment administration

All participants received 8 weeks of either placebo or sublingual nicotine tablets (Beijing Zesh-eng Biotechnology Institute), which are 6 mm in diameter, soluble, and contain 2 mg nicotine bound to β-cyclodextrin. The tablet dissolution time was ~20 min. The placebo tablet was identical in appearance but only contained 3 μg capsaicin. The medication was free of charge. Smokers were encouraged to use one or two tablets (4 mg nicotine) per hour, a minimum of 15 and a maximum of 20 tablets per day. The treatment lasted 2 months and was followed up for 1 month. During the 4-week follow-up phase, no further medication was dispensed. The administration of medication was the same as in our previous study [1].

4 DNA extraction and genetic analysis

Genomic DNA was extracted from 5 ml peripheral blood using a salting-out method [2]. The two polymorphisms were genotyped using polymerase chain reaction restriction (PCR). The PCR product of rs1051740 (Tyr113His T>C) and rs2234922 (His139Arg A>G) was digested with fragment length polymorphism as described below. The genotypes of 50% of the samples were checked by two independent researchers to confirm the results. The primers and PCR conditions for rs1051740 and rs2234922 were as provided in the literature [3-5]. According to Genbank® (http://www.ncbi.nlm.nih.gov/nuccore/NG_009776.1?from=4980&to=40468&report=genbank), exon 3 ranges from 21705 to 21885, and rs1051740 is located at 21858; exon 4 ranges from 28580 to 28807, and rs2234922 is located at 28631. A previous study has reported that these two SNPs are associated with alcohol-dependence in an Indian population [6].

The primer pairs for rs1051740 were 5’-GATCGATAAGTTCCGTTTCACC-3’ and 5’-ATCTTAGTCTTGAAGTGAGGAT-3’. The 163-bp PCR product of rs1051740 was digested with 5U EcoRv restriction enzyme (New England Biolabs, Beverly, MA) and then underwent electrophoresis in 3% agarose gel stained with ethidium bromide; this product remained intact (allele C) at 163 bp, while the T allele displayed two fragments of 140 and 23 bp. The primer pairs 5’-GGGGTACCAGAGCCTGACCGT-3’ and 5’-AACACCGGGCCCACCCTTGGC-3’ were used to assay for rs2234922. The PCR product was digested with 5U RsaI restriction enzyme (New England Biolabs). The digestion products were separated on 2.5% agarose gel stained with ethidium bromide. The A allele remained at 295 and 62 bp, while the G allele displayed three fragments of 174, 121, and 62 bp.

5 Statistical analysis

The allele and genotype frequencies, Hardy-Weinberg equilibrium, and differences in allele

and genotype frequencies between groups were calculated by direct counting and the χ^2 test. The χ^2 test or analysis of variance (ANOVA) was used to calculate interactions between genotypes, age, and continuous variables. To evaluate the association between these factors and the success of smoking cessation, analyses were performed at the end of the 2nd and 3rd months using multivariate logistic regression analyses. The Java software THESIAS was used for statistical analysis of the two loci [7], including linkage disequilibrium, haplotype-based association analysis, the simultaneous estimation of haplotype frequencies, and their associated effects on the phenotype of interest. Generalized multifactor dimensionality reduction (GMDR) was used to calculate the interactions of polymorphisms and continuous variables; this is a nonparametric and genetic model-free alternative to linear or logistic regression to detect and characterize nonlinear interactions among discrete genetic and environmental attributes [8]. The *p*-values were calculated for interaction models on the basis of 1,000 permutations. All of the analyses were carried out in all samples and then in the active treatment group. $P < 0.05$ was reported as statistically significant.

References

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Table S1. Characteristics of all the participants with sublingual nicotine tablet status and smoking severity

Baseline characteristics	According to administration			
	Placebo	**Nicotine sublingual**	**MD**	**SD**
	***n*=110 (*n*,%)**	***n*=101 (*n*,%)**	***n*=104 (*n*,%)**	***n*=107 (*n*,%)**
Age (years)	39.6±11.3	43.2±11.4*	39.4(11.8)	43.2(10.9)*
Gender (M/F)	106/4	94/7	98/6	102/5
Years of smoking	20.7±11.2	23.5±10.6	20.1±11.4	23.8±10.4**
No. of cigarettes/day	23.6±10.0	24.0±10.4	18.9±7.5	28.0±9.9**
Marital status				
Single	38(34.5)	29(28.7)	37(35.6)	30(28.0)
Married	68(61.8)	66(65.3)	63(60.0)	71(66.4)
Divorced or lost spouse	4(3.7)	6(6.0)	4(0.04)	6(0.06)
Education				
Up to middle school	6(5.5)	4(4.0)	4(3.8)	6(5.6)
High school	79(71.8)	71(70.3)	69(66.3)	81(75.7)
College or higher	25(22.7)	26(25.7)	31(29.8)	20(18.7)
Occupation				
Labor worker	47(42.7)	43(42.6)	47(45.2)	43(40.2)
Office worker	47(42.7)	45(44.5)	45(43.3)	49(45.8)
Unemployed	14(12.6)	13(12.9)	12(11.5)	15(14.0）
Alcohol use				
Yes	54(49.1)	36(35.6)	47(45.2)	43(40.2)
BMI (kg/m^2)	24.3±3.4	24.48±3.1	24.2±3.0	24.6±3.4
FTND	6.3(2.15)	5.92(2.51)		
VAS	7.1±1.9	7.23±1.98		
Expired CO (ppm)	15.45(6.7)	15.84(7.9)	14.8(7.0)	16.5(7.6)
Urinary cotinine (μg/L)	22.7±11.5	23.0±13.8	21.6(12.0)	24.0(13.2)

MD, moderate dependence; FTND, Fagerstrom test for nicotine dependence; SD, severe dependence; BMI, body mass index; VAS, visual analogue scale; *P <0.05; **P ≤0.01.

Table S2. Ages of participants (n = 211) with rs1051740 and rs223492 genotypes

	rs1051740			rs223492		
	C/C	**C/T**	**T/T**	**A/A**	**A/G**	**G/G**
Group (placebo/nicotine)	23/33	40/32	47/36	66/56	24/32	20/13
Age (years)	44.32 ± 11.37	41.82 ± 11.89	38.93±10.76	41.33 ± 11.81	41.00 ± 10.59	42.00±11.93

Table S3. VAS scores and urinary cotinine concentrations at baseline and the end of the 2nd and 3rd months for groups and genotypes

		End of 2nd month				End of 3rd month			
		VA		Cotinin		VA		Cotinin	
		F	*P*	F	*P*	F	*P*	F	*P*
rs1051740									
Main	Age	2.0	0.1	0.02	0.8	2.61	0.11	0.21	0.6
	Group	2.44	0.1	1.86	0.1	2.61	0.11	8.98	0.0
	rs1051740	2.45	0.0	0.05	0.9	2.09	0.1	0.21	0.8
	Time	0.14	0.7	0.02	0.9	0.37	0.5	0.05	0.8
Interaction	Group × rs1051740	0.07	0.52	0.34	0.71	0.58	0.56	0.43	0.65
	Time × Age	3.56	0.0	0.40	0.5	2.44	0.1	1.90	0.1
	Time × Group	0.00	0.9	3.12	0.0	0.01	0.9	11.43	0.0
	Time × rs1051740	1.23	0.30	1.05	0.35	1.78	0.17	1.01	0.37
	Time × Group × rs1051740	0.91	0.40	5.20	0.01	1.46	0.24	4.33	0.01
rs223492									
Main effect	Age	1.81	0.18	0.07	0.70	2.22	0.14	0.42	0.52
	Group	1.38	0.2	3.77	0.0	1.75	0.1	7.47	0.0
	rs223492	0.42	0.6	0.86	0.4	0.45	0.6	1.82	0.1
	Time	0.07	0.7	0.01	0.9	0.17	0.6	0.26	0.6
Interaction	Group × rs223492	0.86	0.43	2.45	0.09	0.78	0.46	0.31	0.74
	Time × Age	4.13	0.0	0.45	0.5	3.22	0.0	3.15	0.0
	Time × Group	0.05	0.8	2.28	0.1	0.23	0.6	4.13	0.0
	Time × rs223492	0.08	0.9	0.95	0.3	0.15	0.8	0.66	0.5
	Time × Group × rs223492	0.53	0.59	1.95	0.15	0.60	0.55	2.33	0.10