ORIGINAL ARTICLE

Molecular Genetics of Successful Smoking Cessation

Convergent Genome-Wide Association Study Results

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Context: Smoking remains a major public health problem. Twin studies indicate that the ability to quit smoking is substantially heritable, with genetics that overlap modestly with the genetics of vulnerability to dependence on addictive substances.

Objectives: To identify replicated genes that facilitate smokers' abilities to achieve and sustain abstinence from smoking (hereinafter referred to as quit-success genes) found in more than 2 genome-wide association (GWA) studies of successful vs unsuccessful abstainers, and, secondarily, to nominate genes for selective involvement in smoking cessation success with bupropion hydrochloride vs nicotine replacement therapy (NRT).

Design: The GWA results in subjects from 3 centers, with secondary analyses of NRT vs bupropion responders.

Setting: Outpatient smoking cessation trial participants from 3 centers.

Participants: European American smokers who successfully vs unsuccessfully abstain from smoking with biochemical confirmation in a smoking cessation trial using NRT, bupropion, or placebo (N=550).

Main Outcome Measures: Quit-success genes, reproducibly identified by clustered nominally positive single-nucleotide polymorphisms (SNPs) in more than 2 independent samples with significant *P* values based on Monte Carlo simulation trials. The NRT-selective genes were nominated by clustered SNPs that display much larger *t* values for NRT vs placebo comparisons. The bupropion-selective genes were nominated by bupropion-selective results.

Results: Variants in quit-success genes are likely to alter cell adhesion, enzymatic, transcriptional, structural, and DNA, RNA, and/or protein-handling functions. Quit-success genes are identified by clustered nominally positive SNPs from more than 2 samples and are unlikely to represent chance observations (Monte Carlo $P<.0003$). These genes display modest overlap with genes identified in GWA studies of dependence on addictive substances and memory.

Conclusions: These results support polygenic genetics for success in abstaining from smoking, overlap with genetics of substance dependence and memory, and nominate gene variants for selective influences on therapeutic responses to bupropion vs NRT. Molecular genetics should help match the types and/or intensity of antismoking treatments with the smokers most likely to benefit from them.

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Cigarette smoking continues to contribute substantially to US morbidity and mortality, despite increasingly stringent public health measures that make it more difficult and expensive to smoke.[1] Smokers may thus now include many who have considerable difficulty in achieving sustained abstinence from tobacco, despite the availability of therapeutic drugs that act at sites that include nicotinic receptors and monoamine transporters.[2] Understanding the mechanisms that facilitate or impede smokers' abilities to achieve and sustain abstinence from smoking could thus inform a major current US public health problem and augment understanding of mnemonic and other brain mechanisms that might influence abstinence.[3] Understanding mechanisms that contribute to the abstinence-enhancing efficacies of specific therapeutic drugs could help match smokers with treatments that would be more likely to be effective for them.

Twin studies suggest that 40% to 60% of individual differences in the ability to successfully quit smoking are heritable.[4] Nicotine dependence is also heritable in ways that overlap substantially with vul-

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nerability to dependence on other addictive substances.[5-8] Many of the heritable influences on successful smoking cessation, however, appear to differ from those that influence aspects of nicotine dependence.[4]

Several pharmacological approaches increase smoking cessation success. Nicotine replacement therapies (NRTs) and bupropion hydrochloride can each double effective rates of smoking cessation.[9,10] These 2 approaches use different pharmacological mechanisms and might conceivably be expected to work differently in different individuals.[11]

Candidate gene and genome-wide association (GWA) studies can detect genes that harbor allelic variants with modest effects on disorders that display complex genetic architectures (http://www.niehs.nih.gov/oc/news/gei.htm). Some, although not all, candidate gene studies have associated variants in genes related to dopamine and opioid neurotransmission with success in smoking cessation.[12-17] Genome-wide association studies can detect the effects of allelic variants in genes that were unanticipated, and have already identified previously unanticipated addiction-vulnerability genes that are likely to contain variants that contribute to interindividual differences in vulnerability to substance dependence.[18-23] Convergence between addiction vulnerability and genes that facilitate smokers' abilities to achieve and sustain abstinence from smoking (hereinafter referred to as quit-success genes) would provide enhanced confidence in the genes identified by both comparisons. Confidence in correct identification of quit-success genes for smoking that are not also addiction vulnerability genes, however, requires replicated results from multiple samples.

We herein report the results of such convergent, replicate GWA studies of smoking cessation. We included the study participants in independent clinical trials from 3 centers whose success in smoking cessation has been rigorously assessed by self-report and biochemical test results. We describe (1) substantial convergence of the results from these independent samples that is unlikely to be due to chance alone; (2) more modest, but significant, overlap with GWA data for dependence on nicotine and other substances of abuse; and (3) initial evidence of specific genetic influences on therapeutic responses to NRT vs bupropion.

METHODS

SAMPLES 1 THROUGH 3

European American individuals who smoked responded to newspaper, flyer, and television advertisements and/or to physician referrals for help in quitting smoking.[16,24] Subjects aged 18 to 65 years who provided informed consent, were not pregnant or lactating, had no *DSM-IV* Axis I psychiatric disorder, reported no current use of study medications (eg, bupropion or nicotine-containing products other than cigarettes), and described no contraindications for use of these medications were included. Individuals who were dependent on other addictive substances, current users of psychotropic medications, or diagnosed as having cancer in the last 6 months were excluded.

Subjects were enrolled in 1 of 4 randomized clinical trials for smoking cessation that were approved by the appropriate institutional review boards. Each participant received standardized behavioral counseling.

Sample 1

Sample 1 consisted of subjects enrolled in (1) a double-blind placebo-controlled trial of bupropion hydrochloride, 300 mg/d, or matching placebo for 10 weeks (bupropion trial) or (2) an open-label trial of a nicotine nasal spray vs a nicotine patch for 8 weeks (NRT trial).[16] Smoking status was assessed by telephone interview at 0, 8, and 24 weeks after the targeted quit date using validated timeline follow-back methods.[25] Abstinence was also assessed by measuring levels of cotinine (<15 μg/L [to convert to nanomoles per liter, multiply by 5.675]; bupropion trial) or carbon monoxide (NRT trial). The 126 individuals with biochemically confirmed abstinence for at least the 7 days before the end of treatment (8 weeks) and at the 24-week assessment were contrasted with the 140 individuals who were not abstinent at either measurement. Sample 1 was 55% female, averaged 45 years of age, reported smoking an average of 21 cigarettes a day, and displayed average Fagerström Test for Nicotine Dependence (FTND) scores of 5.4 before treatment. Eighty-nine percent described at least 1 previous effort to quit smoking.

Sample 2

Sample 2 consisted of subjects enrolled in a trial of the clinical effectiveness of NRT. Participants received active nicotine skin patches, 21 mg/d, or placebo patches for 2 weeks before the targeted quit date. Participants also received oral mecamylamine hydrochloride, 10 mg/d, before the targeted quit date to attenuate the reinforcing effects of smoking.[26] After the quit date, participants were randomly assigned to groups that received mecamylamine hydrochloride, 10 mg/d, vs matching placebo and nicotine skin patches at dosages of 21 or 42 mg every 24 hours. Fifty-five individuals reported continuous abstinence from smoking at assessment 6 weeks after the quit date with confirmation by means of carbon monoxide levels; 79 were not abstinent. Sample 2 was 48% female, averaged 44 years of age, reported smoking an average of 30 cigarettes a day, and displayed average pretreatment FTND scores of 6.4. Most of these individuals reported at least 1 previous attempt to quit smoking (average, 4.4 attempts). Initial analysis of the data from these individuals has been reported previously.[19]

Sample 3

Sample 3 consisted of individuals enrolled in a 10-week double-blind, placebo-controlled trial of placebo or bupropion hydrochloride (150 mg/d for the first 3 days, then 300 mg/d) with a targeted quit date 1 week after initiation of drug or placebo therapy.[24] Smoking cessation was assessed using point abstinence, defined by self-report and a saliva cotinine levels of less than 15 μg/L. The 60 individuals with biochemically confirmed abstinence for at least the 7 days before the end of treatment and at the 24-week assessment were contrasted with the 90 individuals who were not abstinent at either measurement. Sample 3 was 51% female, averaged 45 years of age, reported smoking an average of 25 cigarettes a day, and displayed an average pretreatment FTND score of 7.5. Most of these individuals reported at least 1 previous attempt to quit smoking (average, 5 attempts).

DNA PREPARATION, POOLING, AND ANALYSIS

Genomic DNA was prepared from blood samples,[20] carefully quantitated, combined into pools representing 13 to 20 sub-

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jects with successful or unsuccessful attempts to quit smoking (eTable 1; available at: http://www.archgenpsychiatry.com), and analyzed as described by scientists blinded to the quit success phenotype[20] (described in the supplementary text; available at: http://www.archgenpsychiatry.com). Allele frequencies for each single-nucleotide polymorphism (SNP) in each DNA pool were assessed on the basis of hybridization to the perfect-match cells from replicate experiments as described elsewhere.[18,22] Nominally positive SNPs with 2-tailed *t* values for successful vs unsuccessful abstainer differences of $P<.01$ were identified as described.[18,21,22]

Analyses focused on nominally positive SNPs that cluster in small chromosomal regions in at least 2 samples because this pattern of results would not be anticipated by chance but would be anticipated if haplotype blocks that contained multiple SNPs were present at differing frequencies in successful vs unsuccessful abstainers. The **Table** thus identifies SNPs that (1) display *t* values of $P<.01$ in comparing successful vs unsuccessful abstainers; (2) cluster so that at least 3 such SNPs on at least 2 array types (sample 1) or at least 2 such SNPs (samples 2 and 3) lie within 0.1 megabase (Mb) of each other; (3) identify annotated genes; and (4) identify genes that contain clustered SNPs with $P<.01$ compared with at least 1 other sample. Genes that provide replicated results based on clustered SNPs with nominal $P<.01$ findings and that lie within the annotated exons of the genes listed, ±10 kilobase, are listed in eTable 1 and eTable 2. The location of clustered SNPs within the genes' exons and introns, within the genes' flanks, and outside the annotated genes are listed in eTable 2 and eTable 3.

Using Monte Carlo simulation trials, we calculated *P* values for (1) the clustering of nominally positive SNPs from a single sample that lie within annotated genes and (2) the convergence between these clusters from a single sample and similar data from at least 1 other sample based on 10 000 or 100 000 simulation trials, as noted in the presented *P* values. Each trial sampled a random set of SNPs from the database that contains the results from these studies and applied the same procedure that had been followed for the actual data analysis. The number of trials for which the results from the randomly selected set of SNPs matched or exceeded the results actually observed from the SNPs identified in the present study was tabulated. We calculated empirical *P* values by dividing the number of trials for which the observed results were matched or exceeded by the total number of Monte Carlo simulation trials performed. This method examines the properties of the SNPs in the present data set and thus should be relatively robust, despite the uneven distribution of SNP markers across the genome in the Affymetrix microarrays (Affymetrix Inc, Santa Clara, California), the slightly different complement of SNPs represented on the early access and commercial versions of the arrays, and the differing criteria for clustering applied to the larger sample 1 and smaller samples 2 and 3.

We estimated statistical power using the observed standard deviations and mean abuser/control differences from each sample using the program PS, version 2.1.31[32] ($\alpha=.05$).

To control for the possibility that differences between successful and unsuccessful abstainers were due to occult ethnic differences, we compared the clustered SNPs that displayed nominally positive results in the present study with SNPs that displayed the largest differences among African American vs European American control individuals previously studied in this laboratory, or the largest differences among subsets of self-reported white controls recruited from different sites within the United Kingdom.[33] To control for the possibility that differences were due to noisy assays, we compared the largest variation between pools in data from this and other studies that used the same arrays, as described previously.[18]

Secondary analyses pooled data from placebo-, bupropion-, and NRT-treated individuals from all 3 samples. For each SNP, *t* values for allele frequency differences between successful vs unsuccessful abstainers in bupropion vs placebo and NRT vs placebo comparisons were plotted (**Figure 1**). Although there is no single statistical approach to such data, we sought SNPs that displayed an effect on responses to at least 1 of these treatments that was of nominally high significance, and divided these SNPs into those that displayed bupropion-selective, NRT-selective, or nonselective effects. We identified SNPs that displayed *t* values corresponding to $P<.005$ for NRT ($t>3.69$), bupropion ($t>3.58$), or both. We calculated the differences between the *t* values for NRT vs placebo and the *t* values for the differences between bupropion vs placebo for each SNP. We defined as *NRT specific* the one-third of SNPs for which *t* values for NRT provided the largest positive differences from those for bupropion; as *nonspecific* the one-third of SNPs for which *t* values for nicotine replacement were similar to those for bupropion; and as *bupropion specific* the one-third of SNPs for which *t* values for bupropion provided the largest positive differences from those for NRT. We then tallied the genes that were identified by at least 2 such SNPs. Genes for which most SNPs were NRT specific, those for which most SNPs were bupropion specific, and those for which there was no clear treatment specificity are listed in eTable 4.

To test whether these nicotine-specific, nonspecific, and bupropion-specific SNPs were clustered on chromosomal regions in ways that we would anticipate if they identified haplotypes that contained such gene variants but not if they represented random noise, we sought clustering of the SNPs that appeared to identify these bupropion- or NRT-selective genes. Tests for the significance of the clustering of the bupropion- and NRT-selective SNPs were performed as noted for tests of the clustering of the quit-success SNPs described in this section.

RESULTS

VARIABILITY AND CORRESPONDING POWER CALCULATIONS

There was modest variability in the allele frequency assessments that were based on hybridization to the perfect-match cells on each of 4 arrays for DNA from each pool.[18,22] Standard errors of the mean (SEMs) for the variation among the 4 replicate studies of each DNA pool were 0.037, 0.035, and 0.048 for samples 1 through 3, respectively. The SEMs for the variation among the pools studied for each phenotype group were 0.029, 0.028, and 0.034. These results support modest variation. They are consistent with results of validation studies that show correlations of 0.95 between pooled and individually determined genotype frequencies using these arrays[18,20,21,27-29] (see also the supplementary text). Based on these variances, the power to detect allele frequency differences in samples 1, 2, and 3 was 0.71, 0.45, and 0.46, respectively, for 5% allele frequency differences, and 0.99, 0.95, and 0.96, respectively, for 10% differences.

SUCCESSFUL VS UNSUCCESSFUL ABSTAINERS IN EACH OF THE SAMPLES

In comparing data from successful vs unsuccessful abstainers from samples 1, 2, and 3, we identified 5411, 4539,

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Table. Genes Harboring Allelic Variants That Distinguish Successful vs Unsuccessful Quitters in at Least 2 Samples[a]

Class/Gene	Description	Cluster SNPs[b] 1	2	3	*P* Value[c]
Cell adhesion					
DAB1	Disabled homologue 1	7		2	.0117
ASTN	Astrotactin		2	1	.0334
USH2A	Usher syndrome 2A		2	3	.0421
CTNNA2	Catenin α2		2	2	.1254
CLSTN2	Calsyntenin 2	1	4	3	.0045
SEMA5A	Semaphorin 5A	5		1	.0087
TRIO	Triple functional domain/PTPRF interacting protein		2	2	.0375
PTPRN2	Receptor protein tyrosine phosphatase N2	5	5		.0056
CSMD1	Cub and Sushi multiple domains 1		10	5	.0049
SGCZ	Sarcoglycan zeta		3	5	.0191
PTPRD	Receptor protein tyrosine phosphatase D		2	8	.0028
TEK	TEK receptor tyrosine kinase	2	2	3	.0011
PCDH15	Protocadherin 15	3		4	.0175
NRG3	Neuregulin 3		4	1	.0561
DSCAML1	Down syndrome cell adhesion molecule like 1		2	2	.0365
NRXN3	Neurexin 3		2	3	.1002
ITGA11	Integrin α11		2	1	.0166
CDH13	Cadherin 13	8	3	7	.0020
LAMA1	Laminin α1		2	2	.0186
PTPRT	Receptor protein tyrosine phosphatase T	5	6	5	.0024
DSCAM	Down syndrome cell adhesion molecule	10	2		.0016
Enzymes					
ST6GALNAC3	ST6 (α-*N*-acetyl-neuraminyl-2,3-β-galactosyl-1,3)-*N*-acetylgalactosaminide α-2,6-sialyltransferase 3		2	4	.0143
REN	Renin		1	2	.0067
CERKL	Ceramide kinase-like		1	3	.0079
NEK11	NIMA (never in mitosis gene a)–related kinase 11	7		7	.0016
TNIK	TRAF2 and NCK interacting kinase		2	2	.0377
LEPREL1	Leprecan-like 1		4	2	.0060
GALNT17	Polypeptide *N*-acetylgalactosaminyltransferase 17		4	2	.0580
ADCY2	Adenylate cyclase 2	3		1	.0175
PPP2R2B	Protein phosphatase 2 regulatory subunit B β isoform		5	8	.0007
DPP6	Dipeptidyl peptidase 6		2	1	.0310
PBK	PDZ binding kinase		1	3	.0038
ST3GAL1	ST3 β-galactoside α-2,3-sialyltransferase 1		2	1	.0158
MOBKL2B	MOB1-like 2B	1	2		.0082
DAPK1	Death-associated protein kinase 1		2	3	.0105
LYZL1	Lysozyme-like 1		2	2	.0075
PRKG1	cGMP-dependent protein kinase I	7	3	4	.0041
ADAM12	ADAM metallopeptidase domain 12		1	2	.0457
FDX1	Ferredoxin 1		2	1	.0100
SERPINA2	Serpin peptidase inhibitor A2		1	2	.0078
SERPINA1	Serpin peptidase inhibitor A1		1	2	.0080
AKAP13	A kinase anchor protein 13		5	2	.0065
ALDH3A2	Aldehyde dehydrogenase 3 family, member A2	1	1		.0033
ATP9A	Adenosine triphosphatase class II, type 9A	1	1	2	.0026
LARGE	Like-glycosyltransferase		4	4	.0067
Transcriptional regulation					
TDRD5	Tudor domain containing 5	2		1	.0064
RARB	Retinoic acid receptor β		1	2	.0218
SUPT3H	Suppressor of Ty 3 homologue		3	2	.0306
CREB5	cAMP-responsive element binding protein 5	5		2	.0082
GLIS3	GLI-similar family zinc finger 3		3	2	.0174
BNC2	Basonuclin 2		3	6	.0027
TACC2	Transforming acidic coiled-coil containing protein 2	1	1		.0141
SOX5	SRY box 5	2		2	.0514
NPAS3	Neuronal PAS domain protein 3		5	1	.0239
PAX9	Paired box gene 9	1		2	.0026
ZNF423	Zinc finger protein 423	3	3		.0044
SALL4	Sal-like 4	1		1	.0039
ERG	V-ets erythroblastosis virus E26 oncogene like		2	2	.0293
BCL2L13	Bcl2-like 13	3	1		.0037

(*continued*)

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Table. Genes Harboring Allelic Variants That Distinguish Successful vs Unsuccessful Quitters in at Least 2 Samples[a] (cont)

Class/Gene	Description	Cluster SNPs[b] 1	2	3	*P* Value[c]
Receptors					
GPR116	G protein–coupled receptor 116		2	1	.0114
CD109	CD109 molecule	1		1	.0113
GRIK2	Inotropic glutamate receptor kainate 2		2	2	.0648
MTUS1	Mitochondrial tumor suppressor 1		3	3	.0049
GRIN2A	Inotropic glutamate receptor *N*-methyl D-aspartate 2A		2	2	.0427
GRIK1	Inotropic glutamate receptor kainate 1	3	4		.0028
Channels					
KCNN3	N-type potassium channel 3		1	2	.0224
KCNK2	K-type potassium channel 2		4	2	.0069
CACNA2D3	Voltage-dependent calcium channel $\alpha 2/\delta$ subunit 3	3	8		.0031
KCNIP4	Kv channel interacting protein 4		6	2	.0095
CACNB2	Voltage-dependent calcium channel $\beta 2$		2	2	.0416
ITPR2	Inositol 1,4,5-triphosphate receptor 2		6	3	.0030
Transporters					
ATP8A1	Aminophospholipid transporter class type 8A 1	1		1	.0169
SLC1A2	Solute carrier family 1 high-affinity glutamate transporter 2		3	3	.0045
MATE2	H^+/organic cation antiporter	1	1		.0047
DNA/RNA processing					
RBM19	RNA-binding motif protein 19	5		2	.0033
A2BP1	Ataxin 2–binding protein 1	14		12	.0015
Ligands for receptors					
OLFM3	Olfactomedin 3	1	7		.0006
FGF12	Fibroblast growth factor 12		3	2	.0321
TMTC2	Transmembrane and tetratricopeptide repeat containing 2	1		2	.0234
THSD4	Thrombospondin type I domain containing 4	3	2	4	.0035
Protein processing					
PARK2	Parkin		6	2	.0236
SORCS1	Sortilin-related VPS10 domain containing receptor 1		2	4	.0168
Intracellular signaling pathways					
CHN2	Chimerin 2		2	2	.0314
PEBP4	Phosphatidylethanolamine-binding protein 4	3		2	.0065
NELL1	NEL-like 1		2	2	.0911
EFCBP2	EF-hand calcium binding protein 2	1		1	.0064
BIK	Bcl2-interacting killer		1	2	.0087
Structural proteins					
KIAA1026	Kazrin	3		2	.0130
NPHS2	Podocin	1		2	.0038
PKP1	Plakophilin 1		1	1	.0153
ERC2	ELKS/RAB6-interacting/CAST family member 2		5	3	.0129
MAGI1	Membrane-associated guanylate kinase, WW and PDZ domain containing 1	7		2	.0060
PARD3	Partitioning defective 3 homologue	4	2	2	.0077
UNC13C	Unc13 homologue C		3	2	.0354
SNAP25	Synaptosomal-associated protein		1	3	.0068
MYO18B	Myosin XVIIIB		5	3	.0033

(*continued*)

and 4894 SNPs, respectively, whose allele frequencies differ between the 2 groups with nominal $P<.01$ (**Figure 2**). The nominally positive SNPs from comparisons between successful vs unsuccessful abstainers in each of these samples cluster together to extents much greater than expected by chance if their allelic frequencies were independent of each other (Monte Carlo $P<.00001$). For sample 1, 1434 of these 5411 nominally positive SNPs lay in 308 clusters in which each positive SNP lay within 0.1 Mb of at least 2 other positive SNPs, with representation from SNPs on both array types (Figure 2). For samples 2 and 3, 2258 of the 4539 nominally positive SNPs and 2184 of the 4894 nominally positive SNPs, respectively, lie in 820 and 861 clusters in which each positive SNP lies within 0.1 Mb of at least 1 other positive SNP (Figure 2).

In each of these independent samples, we thus observed clustering that we would anticipate if many of these nominally positive SNPs identified haplotypes that were present in different frequencies in our samples of successful vs unsuccessful abstainers but not if they represented chance independent observations.

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Table. Genes Harboring Allelic Variants That Distinguish Successful vs Unsuccessful Quitters in at Least 2 Samples[a] (cont)

Class/Gene	Description	Cluster SNPs[b] 1	2	3	*P* Value[c]
Unknown functions					
TMEM108	Transmembrane protein 108	8	1		.0009
C4orf22	Chromosome 4 open reading frame 22		1	1	.0836
FLJ20184	Hypothetical protein FLJ20184		2	1	.0139
C10orf64	Chromosome 10 open reading frame 64	1		2	.0078
BTBD16	BTB (POZ) domain containing 16	1	1		.0059
LUZP2	Leucine zipper protein 2	3	1		.0217
CCDC73	Coiled-coil domain containing 73	3	3		.0031
FLJ42220	FLJ42220 protein	2		2	.0030
CCBE1	Collagen- and calcium-binding EGF domains 1	1		2	.0134

Abbreviations: cAMP, cyclic adenosine monophosphate; cGMP, cyclic guanosine monophosphate; SNP, single-nucleotide polymorphism.

[a] Chromosome number and initial chromosomal position for the genes come from National Center for Biotechnology Information Map Viewer Build 36.1 coordinates (http://www.ncbi.nlm.nih.gov/projects/mapview/F2). Monte Carlo *P* values for each gene come from 10 000 simulation trials that each begins with random sampling from a database that contains all gene segments ±10 kilobases (kb). These simulations assess the frequency of trials in which at least the observed numbers of nominally positive SNPs identified in each of the 3 samples studied herein was recorded to provide an empirical *P* value. Eight genes (noted in boldface) receive clustered, nominally highly significant association signals from each of the 3 samples studied herein.

[b] Data are expressed as numbers of clustered SNPs from samples 1, 2, and 3 that display nominally highly significant ($P < .01$) allele frequency differences between individuals with successful and unsuccessful attempts to quit smoking within the gene's exons or introns or in 10-kb 3′ or 5′ flanks of annotated exons. Empty cells reflect absence of clustered nominally significant SNPs in the sample.

[c] Indicates the *P* values for each gene, based on Monte Carlo simulations.

NOMINALLY POSITIVE SNPs THAT MIGHT RESULT FROM OCCULT RACIAL/ETHNIC STRATIFICATION OF THE SAMPLES OR FROM NOISY SNP ASSAYS

Controls for occult racial/ethnic stratification and poor technical quality do not explain the nominally positive SNPs from these comparisons of successful vs unsuccessful abstainers. The SNPs that display the largest allele frequency differences between European and African American controls in previous assessments[18] (G.R.U., T.D., and C.J., unpublished data, 2006), and between self-reported white samples from the United Kingdom[33] and the SNPs that display the largest between-pool variances in the present data set do not overlap with those that distinguish comparisons of successful vs unsuccessful abstainers more than anticipated by chance (40 vs 135, 0 vs 0, and 103 vs 105, respectively [sample 1]; 39 vs 114, 0 vs 0, and 115 vs 120, respectively [sample 2]; and 35 vs 122, 0 vs 0, and 112 vs 95, respectively [sample 3]).

GENES IDENTIFIED BY REPLICATED GWA RESULTS FROM MORE THAN 2 INDEPENDENT SAMPLES

Genes in which 3 or more (sample 1) or 2 or more (samples 2 and 3) nominally positive SNPs cluster and where clustered nominally positive SNPs from at least 1 other sample are also present are listed in eTable 2.

Nominally positive clustered SNPs from comparisons of successful vs unsuccessful abstainers in samples 1 through 3 thus cluster together on small chromosomal regions to extents much greater than those expected by chance. The Monte Carlo values for the replication/convergence for samples 1 vs 2, 1 vs 3, and 2 vs 3 are $P = .00054$, $P = .0016$, and $P = .00063$, respectively.

Eight genes are identified by clustered positive SNPs that come from samples 1, 2, and 3 (boldfaced in the Table), 23 from samples 1 and 2, 29 from samples 1 and 3, and 61 from samples 2 and 3 (Monte Carlo $P = .0003$ for overall convergence).

PROBABILITIES AND CLASSES FOR INDIVIDUAL GENES

Simulation studies support the idea that many of the genes identified by the clustered positive results in more than 2 samples are unlikely to represent chance observations (Table). The probabilities that all of the genes identified in this study are random are substantially lower than the probability that any individual gene is listed in eTables 1 and 2 by chance. Nevertheless, most of these gene-by-gene *P* values indicate nominal statistical significance. Twenty-one of the genes relate to cell adhesion, 24 are enzymes, and 14 regulate transcription. Six encode receptors, 6 encode channels, 3 encode transporters, and 4 encode receptor ligands. Two genes are involved with RNA processing, 2 with protein processing, 5 with intracellular signaling, 9 with cell structure, and 9 with unknown functions. Clustered positive SNPs from several samples also nominate chromosomal areas in which no gene is currently annotated (eTable 3).

SECONDARY ANALYSES SEEKING CANDIDATE GENES WITH TREATMENT-SPECIFIC EFFECTS

We combined data from successful and unsuccessful abstainers from the groups treated with bupropion, NRT, or placebo from all 3 centers. As noted in Figure 1, the distribution of *t* values for the differences between bupropion vs placebo and the differences between NRT vs placebo support the idea that some SNPs identify gene variants that do

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not influence responses to each of these treatments in the same way. We identified SNPs that displayed *t* values corresponding to $P < .005$ for NRT, bupropion, or both and calculated the differences between the *t* values for NRT vs placebo and the *t* values for the differences between bupropion vs placebo for each SNP. The one-third of SNPs for which *t* values for NRT provided the largest positive differences from those for bupropion were considered NRT specific; the one-third for which *t* values for NRT were similar to those for bupropion were considered nonspecific; and the one-third for which *t* values for bupropion provided the largest positive differences from those for NRT were considered bupropion specific. Genes that are identified by at least 2 SNPs in these groups are listed in eTable 4. The SNPs in the 41 NRT-specific, 66 nonspecific, and 26 bupropion-specific genes had mean *t* values of 4.28 vs 1.71, 3.58 vs 2.67, and 1.71 vs 4.28 for NRT vs bupropion, respectively.

We sought chromosomal clustering of the SNPs that appeared to identify treatment-specific genes in ways that we would anticipate if they identified haplotypes that contained treatment-specific gene variants, but not if they represented random noise. Bupropion- and NRT-selective SNPs each clustered in small chromosomal regions with Monte Carlo $P < .00001$.

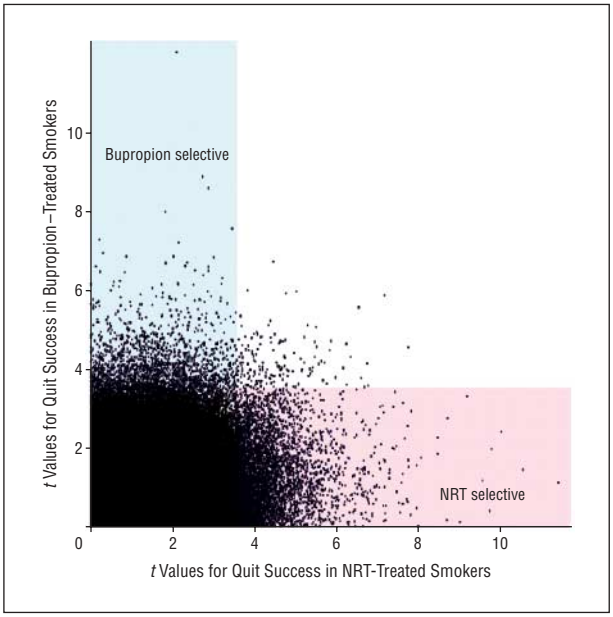

Figure 1. Scatterplot of the distributions of *t* values for comparisons between individuals with successful vs unsuccessful attempts to quit smoking in pooled samples from those receiving nicotine replacement therapy (NRT) (x-axis) vs bupropion hydrochloride (y-axis) for secondary analyses seeking candidate genes with treatment-specific effects. If no genes provided treatment-specific effects, values would cluster on a 45° line from the origin. We highlight single-nucleotide polymorphisms (SNPs) that provide NRT-selective (pink shading) or bupropion-selective (blue shading) effects (see also eTable 4). The *t* values of 3.6 and 3.7 for NRT and bupropion, respectively, correspond to $P < .005$. These data combine individuals from samples 1 and 2 who received NRT and individuals from samples 1 and 3 who received bupropion.

COMMENT

Convergent, replicated observations from these 3 GWA studies of smoking cessation are consistent with the substantial heritabilities for successful vs unsuccessful abstinence that are supported by classical genetic studies.[4,7,34-37] The overlap between some of these quit-success genes with substance-dependence vulnerability genes provides an interesting list that we discuss in the following paragraphs (eTables 1 and 2). Nevertheless, this overlap between quit-success and substance-dependence genes is more modest than the overlaps that we have previously identified in GWA studies of dependence on different addictive substances.[18,19,22,23]

Cadherin 13 (*CDH13*) (Entrez Gene 1012) is a glycosyl-phosphatidylinositol–anchored cell adhesion molecule identified herein and also in multiple comparisons between substance-dependent vs control individuals. The *CDH13* gene is expressed in neurons in interesting brain regions,[38] can inhibit neurite extension,[39] and can activate several signaling pathways,[40-43] rendering it a strong candidate for roles in brain mechanisms important for both developing and quitting addictions.

Neurexin 3 (*NRXN3*) (Entrez Gene 9369) is a single-transmembrane domain cell adhesion molecule that has also been identified in association- and linkage-based comparisons between controls and individuals dependent on legal and illegal substances.[21,44] Localization of this molecule to presynaptic aspects of classical synapses[45] and to interesting brain regions provides sites at which *NRXN3* gene variants could affect addictive processes.

Down syndrome cell adhesion molecule (*DSCAM*) (Entrez Gene 1826) is a single-transmembrane domain cell adhesion molecule that is expressed strongly in the brain[46] in ways that are required for appropriate neuronal connections to form in memory-associated circuits in model organisms.[47,48] Altered *DSCAM* expression in fruit flies changes memories for rewarded and punished behaviors.[48]

Cyclic guanosine monophosphate (cGMP)–dependent protein kinase 1 (*PRKG1*) (Entrez Gene 5592) is expressed in hippocampal and other neurons.[49,50] Nitric oxide dramatically modulates brain cGMP systems; *PRKG1* thus provides a major target for the products of nitric oxide synthases. Mnemonic and addictive functions can each be altered by changes in cGMP-dependent protein kinase and/or nitric oxide synthases.[51-53]

The genes for triple functional domain/PTPRF interacting protein (Entrez Gene 7204), Cub and Sushi multiple domains 1 (Entrez Gene 64478), sarcoglycan zeta (Entrez Gene 137868), and receptor protein tyrosine phosphatase D (Entrez Gene 5789) are other cell adhesion genes that appear to contain variants that influence both vulnerability to addiction and success in quitting. Although the functions of none of these genes is well understood, each of these genes is highly expressed in the hippocampus (http://www.brain-map.org).

Several of the genes identified by both quit-success and addiction vulnerability GWA approaches are likely to alter protein disposition and half-lives. Peptidase activities are regulated by inhibitory proteins that include the products of serpin peptidase inhibitor A1 (Entrez Gene 5265) and A2 (Entrez Gene 390502) genes, which are identified by results from addiction vulnerability and smoking cessation GWA data. These genes are ex-

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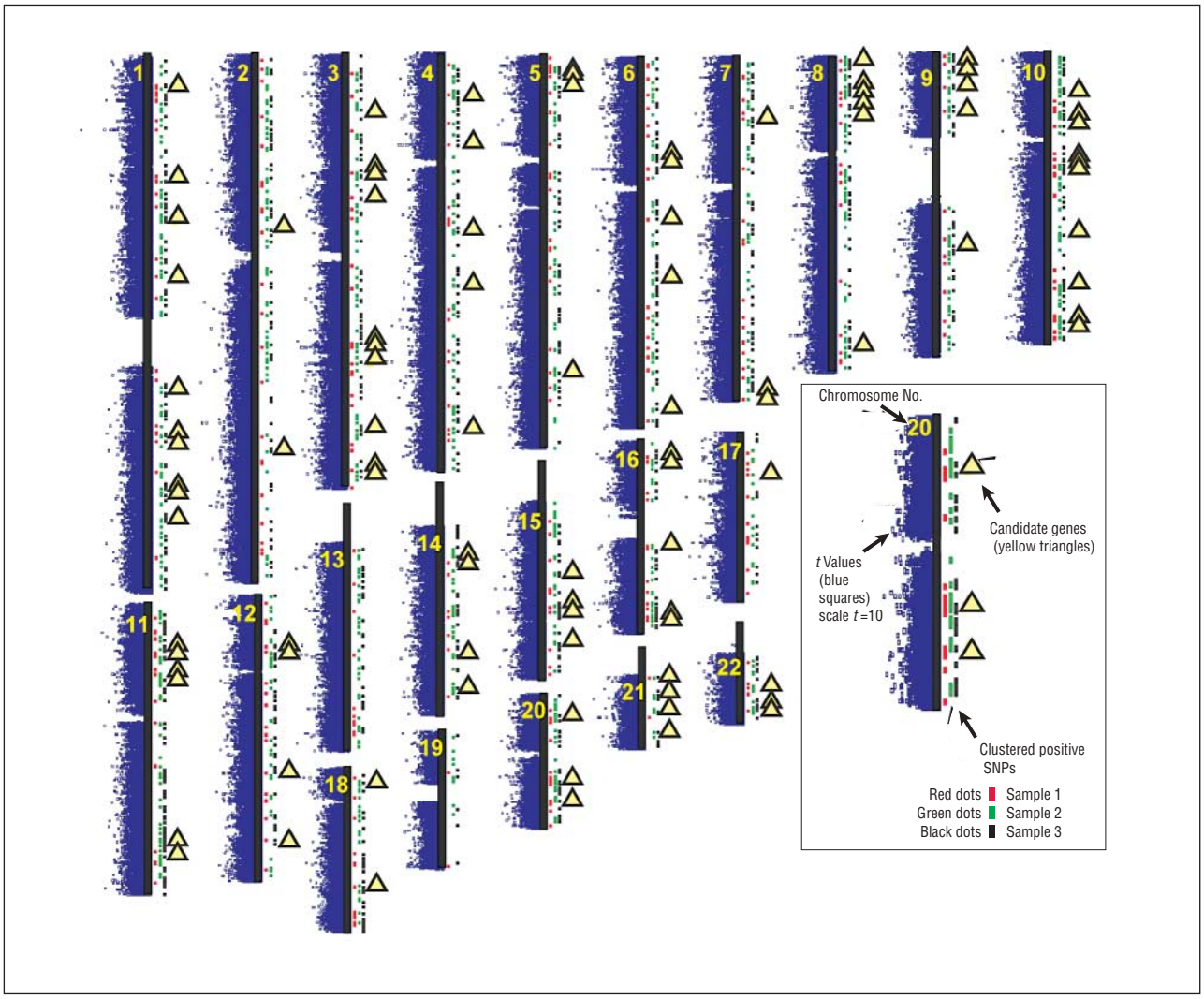

Figure 2. Distributions of data from the present study mapped onto cartoons of human chromosomes 1 through 10 (top row), 11 through 17 (second row), and 18 through 22 (third row). Blue dots to the left of the (black) main axis for each chromosome represent the *t* values for all single-nucleotide polymorphisms (SNPs) in the comparison of allele frequencies in individuals with successful vs unsuccessful attempts to quit smoking from sample 1 with the chromosomal position of each corresponding SNP. Red dots to the right of the (black) main axis for each chromosome mark sites for clustered positive SNPs from sample 1 for which successful vs unsuccessful abstainer differences display $P<.01$, lie within 0.1 megabase (Mb) of at least 1 other SNP that displays $P<.01$, and cluster so that at least 3 clustered outlier SNPs from 2 array types (*StyI, NspI*) are found in the cluster. Green dots to the right of the black main axis for each chromosome mark sites for clustered positive SNPs from sample 2 for which successful vs unsuccessful abstainer differences display $P<.01$, lie within 0.1 Mb of at least 1 other SNP that displays $P<.01$, and cluster so that at least 2 clustered outlier SNPs are found in the cluster. Black dots to the right of the black main axis for each chromosome mark sites for clustered positive SNPs from sample 3 for which successful vs unsuccessful abstainer differences display $P<.01$, lie within 0.1 Mb of at least 1 other SNP that displays $P<.01$, and cluster so that at least 2 clustered outlier SNPs are found in the cluster. Yellow triangles to the right of the black main axis for each chromosome mark sites for genes identified by clustered positive SNPs from at least 2 samples (see the Table). Chromosomal positions are based on National Center for Biotechnology Information Map Viewer Build 36.1 coordinates (http://www.ncbi.nlm.nih.gov/mapview/) and supplemental data from NetAffx Analysis Center (http://www.science.fau.edu/genechip/NetAffx_analysis_center.html).

pressed in the cerebral cortex, hippocampus, and other brain regions of interest. Cellular protein trafficking is likely to be altered by variants in the SorCS1 sortilin-related Vps10p domain containing receptor 1, which is expressed in interesting brain regions that include the cortex and hippocampus.

Signaling within neurons is likely to be altered by variants in a number of the genes that are identified for both quit success and addiction vulnerability. Variants in the A kinase anchor protein 13 gene (*AKAP13*) (Entrez Gene 11214) are likely to alter cyclic adenosine monophosphate–related signaling pathways, whereas variants in the chimerin 2 gene (*CHN2*) (Entrez Gene 1124) are also likely to alter signaling. Both of these genes exhibit abundant brain expression in interesting regions.

The neuronal PAS domain protein 3 gene (*NPAS3*) (Entrez Gene 64067) is expressed in developing and adult brains in ways that suggest that its variants could play important roles.

The gene for ataxin 2–binding protein 1 (*A2BP1*) (Entrez Gene 54715) is highly expressed in neurons in brain regions that include the hippocampus (http://www.brain-map.org). The *A2BP1* gene binds to a UGCAUG splicing enhancer element that borders a substantial number of neuron-specific exons and thus acts to regulate splicing processes that form mature messenger RNAs.[54]

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The *A2BP1* gene itself contains a number of splicing variants that are likely to alter its functions.

In addition to these genes that are identified in repeated comparisons between successful vs unsuccessful abstainers and in repeated comparisons between substance-dependent vs control individuals, we have also identified other interesting genes (and intragenic regions) in at least 2 samples of successful vs unsuccessful abstainers that are not consistently identified in comparisons of substance-dependent vs control individuals. These genes represent a large fraction of the total number of genes identified herein, consistent with classic genetic data that suggest modest overlap between the genetics of developing a dependence on tobacco and the genetics of successful cessation. Although detailed discussion of each of these genes is beyond the scope of the present report, the gene for calsyntenin 2 (*CLSTN2*) (Entrez Gene 64084) falls into this group and was also identified by recent association and linkage studies of individual differences in memory and executive function.[30,55] The *CLSTN2* gene is heavily expressed in brain regions that include the hippocampus (http://www.brain-map.org) and appears to function as a single transmembrane domain cell adhesion molecule.

It is important to consider several limitations for these convergent, replicated genome-wide data for smoking cessation. The sample sizes available for this work provide moderate power to detect gene variants. False-negative results are likely, because we require positive data from each of 2 samples and also require positive results from several SNPs that cluster within small chromosomal regions. We focus only on data from autosomal regions herein, allowing us to combine data from male and female smokers in ways that will miss potentially important contributions from genes on sex chromosomes. These subjects volunteered for demanding clinical protocols, potentially rendering them not totally representative of all smokers or of all smokers who seek to quit.[56] We used a preplanned analytic approach that represents only 1 of many current approaches to analyzing GWA data (the analysis and technical limits of GWA studies are discussed in the supplemental text available online and at http://www.nhlbi.nih.gov/resources/listserv/number35.htm [RFA-HL-07-010]).[33,57] Despite these limitations, the replicated positive results obtained herein and the failure of control experiments to support alternative hypotheses provide substantial confidence in roles for most of the genes reported.

Comparing data from successful vs unsuccessful abstainers from those treated with bupropion, NRT, or placebo provides samples that allow us to ask whether molecular genetic results are consistent with the idea that each gene variant identified in this work provides identical influences on responses to NRT and bupropion. There is a pharmacological rationale for differential effects because bupropion's relatively high affinity as a dopamine transporter blocker contrasts with nicotine's relatively high affinity for nicotinic acetylcholine receptors[58] (but bupropion also has moderate affinities for nicotinic acetylcholine receptors[59]). The results reported herein provide initial evidence that the genetics underlying successful smoking cessation with bupropion are not identical to the genetics of successful smoking cessation with NRT. There should be caution because the NRT- and bupropion-selective genes are identified in a single analysis that is not undergirded by twin study data. Nevertheless, these results (1) provide sets of clustered, nominally positive SNPs that converge with the SNPs where allele frequencies distinguish nondependent smokers from dependent smokers (Monte Carlo $P < .001$),[23] and (2) identify the *CYP2B7P1/CYP2B6* gene cluster in which cluster products are important for generating bupropion's pharmacologically active metabolite hydroxybupropion.[58] Replication of these data will allow use of such SNPs to individualize therapeutic approaches by selecting better candidates for NRT vs bupropion therapies and allow us to consider whether each gene's effect is additive or might depend on allelic variants at other loci (epistasis).

The data reported herein provide molecular genetic support for the idea that a smoker's ability to abstain from nicotine has polygenic genetic components. The data support modest overlap between these heritable components and the genes that contribute to vulnerability to dependence on addictive substances. Several of the genes identified in this work are consistent with emerging views of the importance of mnemonic mechanisms for the development of and remission from addictions.[3] The convergent, replicated results presented herein are supplemented by initial data that nominate differential genetic influences on quit success depending on which smoking cessation treatment is used. Taken together, the current data provide promise that we may soon use molecular genetics to match the type and/or intensity of antismoking treatments with the smokers most likely to benefit from them.

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