Addiction
RESEARCH REPORT
doi:10.1111/j.1360-0443.2008.02445.x

Further evidence for an association between the gamma-aminobutyric acid receptor A, subunit 4 genes on chromosome 4 and Fagerström Test for Nicotine Dependence

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ABSTRACT

Aims A previous association analysis identified polymorphisms in gamma-aminobutyric acid receptor A, subunit 4 (*GABRA4*) and *GABRA2* to be associated with nicotine dependence, as assessed by a score of 4 or more on the Fagerström Test for Nicotine Dependence (FTND). In the present report, we extend the previous study by expanding our genotyping efforts significantly for these two genes. **Design** In 1049 cases (FTND of 4 or more) and 872 controls (smokers with FTND of 0) from the United States and Australia, we examine the association between 23 *GABRA4* and 39 *GABRA2* recently genotyped single nucleotide polymorphisms (SNPs) and nicotine dependence using logistic regression-based association analyses using the genomic analysis package PLINK. **Results** Two and 18 additional SNPs in *GABRA4* and *GABRA2*, respectively, were associated with nicotine dependence. The SNPs identified in *GABRA4* (P-value = 0.002) were restricted to introns 1 and 2, exon 1 and the 5′ end of the gene, while those in *GABRA2* localized to the 3′ end of the gene and spanned introns 9–3, and were in moderate to high linkage disequilibrium (as measured by r^2) with each other and with previously studied polymorphisms. **Conclusion** Our findings demonstrate consistently the role of *GABRA4* and *GABRA2* in nicotine dependence. However, further research is needed to identify the biological influence of these intronic variations and to isolate functionally relevant polymorphisms neighboring them.

Keywords Association, GABRA2, nicotine dependence, NICSNP.

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Submitted 15 September 2008; initial review completed 7 October 2008; final version accepted 20 October 2008

INTRODUCTION

Currently, several efforts are under way to identify the genetic determinants of nicotine dependence [1–13]. We have reported previously on one such large-scale case–control study of nicotine dependence, the Nicotine Single Nucleotide Polymorphism (NICSNP) study, which included a genome-wide association and a candidate gene component [4,5,14]. Among the top association signals identified by the candidate gene component was a polymorphism in the gamma-aminobutyric acid receptor A, subunit 4 (*GABRA4*) gene. Detailed analyses of the entire family of GABA (receptors A and B) genes revealed evidence for association between nicotine dependence (FTND of 4 or more) and polymorphisms in both *GABRA4* and *GABRA2*, both on chromosome 4p [14].

*Drs Bierut and Madden are joint senior authors.

Due to the potential biological importance of this large family of inhibitory neurotransmitter receptors in addiction [15–17] and support for synonymous (rs279858, in *GABRA2*, *P*-value = 0.005) and non-synonymous single nucleotide polymorphisms (SNPs) (rs2229940, previously rs16859834, in *GABRA4*, *P*-value = 0.03) that were associated with nicotine dependence, we investigated these genes further. The aim of this study is to localize and delineate more clearly the association signal between nicotine dependence and *GABRA2* and *GABRA4* using the strategy of additional genotyping.

METHODS

Sample

The study design has been described in detail in previous publications [4,5]. Briefly, NICSNP participants include 1050 (1049 for the present study) unrelated cases and 879 (872 for the present study) unrelated controls, selected from two independent and ongoing studies: (i) the Collaborative Genetic Study of Nicotine Dependence (COGEND), which recruited from US sites in St Louis, Detroit and Minneapolis [4], and (ii) the Nicotine Addiction Genetics (NAG) [6] study, which included families ascertained for heavy smoking that were identified using two cohorts of the Australian Twin Panel. From both studies, only participants who had reported a history of smoking (100 or more cigarettes in their life-time), when queried by telephone interview, were eligible for inclusion in the NICSNP study. Current and life-time smokers from COGEND and NAG, respectively, with an FTND score of '0' for a self-reported period of smoking were eligible as controls. Participants reporting themselves to be current smokers with a current FTND score of 4 or greater (COGEND) or meeting this criterion for a self-reported period of heaviest smoking (NAG) were eligible as cases. Approximately 24% and 8% of the cases and controls, respectively, were drawn from the Australian sample. All subjects were of European descent. The sample included more women (52% and 66% of cases and controls, respectively) than men. Cases had a mean age of 37.7 years (range 25–82) while controls were, on average, aged 36.7 years (range 25–82). The Institutional Review Boards approved the protocol for both studies. Blood samples collected for DNA extraction were submitted along with electronic phenotypic and genetic data for both studies to the National Institute on Drug Abuse (NIDA) Center for Genetic Studies, which manages the sharing of research data according to guidelines of the National Institutes of Health.

Genotyping

During the first phase, genotyping was conducted by Perlegen Sciences using custom-designed high-density oligonucleotide assays [4]. During that phase of genotyping, six and 27 SNPs were typed in *GABRA2* and *GABRA4*, respectively. Through a competitive application to the Center for Inherited Diseases Research (CIDR), we further genotyped 1049 (of 1050) cases and 872 (of 879) controls. A total of 62 new SNPs were typed, including 39 in *GABRA2* (of 42 attempted) and 23 in *GABRA4*. Only SNPs with call rates of 98% and greater were included in this phase of data analysis, thus asserting a higher level of quality control on these data (Table 1).

Association analyses

Association analyses were conducted on the 62 CIDR SNPs. A logistic regression model, which has been described previously by Saccone *et al.* [5], was implemented in the genomic analysis package PLINK [18], with controls for sex (0 = male, 1 = female) and site (1 = Australia, 0 = United States, as a disproportionate number of cases and controls come from each site). The model tested the influence of an additive genotypic model, where 0 = no copies of the minor allele, 1 = one copy of the minor allele and 2 = both copies of the minor allele, on a dichotomous measure of case status. As in our previous study, an interaction between genotype and sex was also included. The Benjamini–Hochberg false discovery rate (FDR-BH, [19]) *P*-value was also computed for the effect of genotype.

HAPLOVIEW [20] was used to examine the extent of linkage disequilibrium, as indexed by r^2, across the combined CIDR and previously typed Perlegen SNPs [14].

RESULTS

Association results are presented in Table 2 (*GABRA2*) and 3 (*GABRA4*)—we focus upon results emerging from the newly genotyped SNPs, but to provide a comprehensive view of this association finding, results from previous analyses (originally published in [14]) are also presented in the tables. Sex (males being more likely to be cases) and site (Australia: this sample provided nearly three times as many cases as controls) had important main effects.

GABRA2

Of the 39 newly genotyped SNPs, 19 SNPs (Table 2) were associated with case status (FTND scores of 4+) at *P*-values less than 0.01. Even after correcting for multiple testing, the FDR-BH *P*-values were less than 0.05. An additional five SNPs were associated marginally at *P*-values less than 0.05. Association extended from the 3′ end of the gene to intron 3. Overall, the SNPs contributing to the association signal, including newly and previously typed polymorphisms, were in moderate to high linkage disequilibrium (LD) (r^2 ranging from 0.75–0.99,

Table 1 Single nucleotide polymorphisms (SNPs) newly genotyped by the Centers for Inherited Diseases Research (CIDR) in gamma-aminobutyric acid receptor A, subunit 2 (GABRA2) and GABRA4 in 1049 cases and 872 controls.

Gene	SNP (rs no.)	Position (basepair)	Minor allele	Major allele	Minor allele frequency		Hardy–Weinberg P-value	
					Case	Control	Case	Control
GABRA2	497068	45945434	G	A	0.446	0.414	0.492	1.000
GABRA2	541418	45947973	G	A	0.445	0.414	0.532	1.000
GABRA2	548260	45963241	G	A	0.446	0.413	0.617	0.889
GABRA2	2119183	45967563	A	G	0.098	0.107	0.021	0.475
GABRA2	1822016	45970533	G	A	0.446	0.413	0.617	0.889
GABRA2	12510993	45973499	A	G	0.012	0.016	1.000	1.000
GABRA2	532363	45977323	A	C	0.446	0.413	0.662	0.889
GABRA2	519869	45980119	C	A	0.446	0.413	0.617	0.834
GABRA2	17459039	45981638	A	G	0.033	0.039	0.322	0.380
GABRA2	537787	45983300	A	G	0.453	0.422	0.663	0.835
GABRA2	17537141	45997181	G	A	0.165	0.158	0.433	0.375
GABRA2	279866	46004521	G	A	0.453	0.421	0.709	0.781
GABRA2	279864	46005792	A	G	0.455	0.425	0.619	0.782
GABRA2	279861	46008082	C	G	0.453	0.421	0.663	0.781
GABRA2	279849	46015514	A	C	0.454	0.425	0.575	0.729
GABRA2	279847	46019387	A	C	0.453	0.427	0.663	0.581
GABRA2	279843	46019961	A	G	0.455	0.425	0.950	0.782
GABRA2	13131717	46020234	A	G	0.028	0.020	1.000	0.039
GABRA2	203654	46027226	A	C	0.480	0.442	0.757	0.537
GABRA2	1440130	46028010	G	A	0.479	0.442	0.951	0.537
GABRA2	279828	46029567	C	A	0.483	0.443	0.711	0.450
GABRA2	279829	46030412	C	A	0.272	0.249	0.101	0.204
GABRA2	279841	46035520	A	G	0.452	0.423	1.000	0.781
GABRA2	17537359	46035863	G	A	0.052	0.057	0.760	1.000
GABRA2	279821	46041027	C	A	0.456	0.423	0.901	0.627
GABRA2	279822	46045919	C	A	0.454	0.423	0.852	0.627
GABRA2	17537380	46048910	A	G	0.021	0.019	1.000	1.000
GABRA2	12647055	46050236	A	G	0.493	0.456	0.758	0.539
GABRA2	4540087	46050953	A	G	0.451	0.422	0.950	0.729
GABRA2	1442059	46051709	G	A	0.454	0.423	0.852	0.627
GABRA2	1442060	46060824	A	G	0.485	0.495	0.578	0.310
GABRA2	3849591	46063398	A	C	0.192	0.178	1.000	0.203
GABRA2	1442061	46065977	G	C	0.270	0.255	0.183	0.721
GABRA2	17459386	46069932	A	G	0.034	0.029	0.334	0.164
GABRA2	1442062	46071833	A	G	0.268	0.255	0.182	0.721
GABRA2	4695152	46076414	G	C	0.043	0.040	0.717	0.645
GABRA2	11503015	46082801	G	A	0.058	0.064	0.157	0.776
GABRA2	10013922	46084118	C	A	0.272	0.260	0.275	0.660
GABRA2	3756007	46085821	G	A	0.050	0.052	0.317	0.723
GABRA4	10004359	46615546	A	G	0.287	0.278	0.366	0.500
GABRA4	6447517	46623503	A	T	0.419	0.416	1.000	1.000
GABRA4	17599158	46628747	G	A	0.287	0.278	0.366	0.500
GABRA4	17599165	46634972	A	T	0.108	0.106	1.000	0.471
GABRA4	11946433	46641663	A	C	0.080	0.075	0.672	0.622
GABRA4	1512130	46647228	A	G	0.322	0.339	0.724	0.366
GABRA4	7685553	46648254	G	A	0.132	0.137	0.892	0.776
GABRA4	16859761	46648387	A	G	0.031	0.029	0.013	0.154
GABRA4	10015891	46652062	A	G	0.474	0.482	0.757	0.635
GABRA4	1512134	46655487	C	A	0.473	0.482	0.853	0.735
GABRA4	2280074	46662245	G	A	0.473	0.483	0.853	0.684
GABRA4	2055942	46662807	G	A	0.282	0.270	0.446	0.931
GABRA4	16859794	46666888	G	A	0.475	0.483	0.804	0.684
GABRA4	2271429	46667656	C	A	0.475	0.483	0.805	0.684
GABRA4	17599416	46668195	G	A	0.117	0.114	0.552	0.866
GABRA4	1512135	46668645	G	A	0.117	0.114	0.552	0.866
GABRA4	3792208	46668795	A	C	0.094	0.099	0.471	0.705
GABRA4	10517173	46677253	C	A	0.085	0.110	0.691	0.162
GABRA4	13117953	46679819	A	G	0.066	0.054	0.212	1.000
GABRA4	7694035	46680507	A	C	0.230	0.239	0.434	0.352
GABRA4	16851647	46685574	G	A	0.085	0.110	0.691	0.162
GABRA4	11735333	46688319	C	G	0.473	0.469	0.386	0.634
GABRA4	3792211	46689252	A	C	0.357	0.327	0.737	0.488

Table 2 Results from logistic regression-based association analyses between single nucleotide polymorphisms (SNPs) in gamma-aminobutyric acid receptor A, subunit 2 (*GABRA2*) and case status [Fagerström Test for Nicotine Dependence (FTND) score of 4+, controls are smokers with an FTND = 0] in the Nicotine Single Nucleotide Polymorphism (NICSNP) project.

			Genotype				*Genotype × sex*		
SNP (rs no.)	*Allele*	*Location*	*OR*	*95% CI*	*Observed P value*	*FDR–BH P-value*	*OR*	*95% CI*	*Observed P-value*
16859227	A	3′ near gene	1.12	0.87–1.43	0.38	0.717	0.95	0.70–1.30	0.767
497068	*G*	*3′ near gene*	*1.43*	*1.14–1.79*	*0.002*	*0.018*	*0.70*	*0.53–0.93*	*0.013*
573400	G	3′ UTR	1.44	1.15–1.80	0.002	0.018	0.70	0.53–0.92	0.011
541418	*G*	*Int 9*	*1.43*	*1.14–1.79*	*0.002*	*0.018*	*0.70*	*0.53–0.93*	*0.012*
548260	*G*	*Int 8*	*1.42*	*1.13–1.78*	*0.002*	*0.018*	*0.72*	*0.54–0.95*	*0.019*
2119183	*A*	*Int 8*	*0.80*	*0.58–1.11*	*0.19*	*0.425*	*1.21*	*0.79–1.85*	*0.385*
1822016	*G*	*Int 8*	*1.42*	*1.13–1.78*	*0.002*	*0.018*	*0.72*	*0.54–0.95*	*0.019*
12510993	*A*	*Int 8*	*0.85*	*0.27–2.71*	*0.78*	*0.860*	*0.92*	*0.24–3.50*	*0.906*
532363	*A*	*Int 8*	*1.42*	*1.13–1.78*	*0.003*	*0.019*	*0.72*	*0.54–0.95*	*0.021*
519869	*C*	*Int 8*	*1.41*	*1.13–1.77*	*0.003*	*0.019*	*0.72*	*0.55–0.95*	*0.022*
17459039	*A*	*Int 8*	*0.56*	*0.33–0.95*	*0.03*	*0.081*	*2.04*	*1.01–4.12*	*0.046*
537787	*A*	*Int 8*	*1.39*	*1.11–1.74*	*0.004*	*0.021*	*0.73*	*0.55–0.96*	*0.026*
17537141	*G*	*Int 8*	*1.19*	*0.88–1.60*	*0.265*	*0.542*	*0.83*	*0.57–1.21*	*0.328*
279866	*G*	*Int 6*	*1.39*	*1.11–1.74*	*0.004*	*0.021*	*0.73*	*0.55–0.96*	*0.027*
279864	*A*	*Int 6*	*1.35*	*1.08–1.69*	*0.009*	*0.031*	*0.76*	*0.57–1.00*	*0.049*
279861	*C*	*Int 5*	*1.39*	*1.11–1.74*	*0.004*	*0.021*	*0.73*	*0.55–0.97*	*0.027*
279849	*A*	*Int 4*	*1.36*	*1.09–1.70*	*0.007*	*0.030*	*0.75*	*0.58–0.99*	*0.040*
279847	*A*	*Int 4*	*1.32*	*1.06–1.65*	*0.015*	*0.045*	*0.77*	*0.58–1.01*	*0.059*
279843	*A*	*Int 4*	*1.36*	*1.09–1.71*	*0.007*	*0.030*	*0.74*	*0.56–0.98*	*0.038*
13131717	*A*	*Int 4*	*1.39*	*0.67–2.89*	*0.378*	*0.717*	*1.02*	*0.41–2.55*	*0.960*
203654	*A*	*Int 4*	*1.38*	*1.10–1.72*	*0.005*	*0.024*	*0.77*	*0.58–1.01*	*0.061*
1440130	*G*	*Int 4*	*1.37*	*1.10–1.72*	*0.005*	*0.024*	*0.76*	*0.58–1.01*	*0.057*
279828	*C*	*Int 3*	*1.40*	*1.17–1.74*	*0.003*	*0.021*	*0.76*	*0.58–1.00*	*0.057*
279829	*C*	*Int 3*	*1.35*	*1.05–1.74*	*0.019*	*0.055*	*0.73*	*0.54–1.00*	*0.052*
279841	*A*	*Int 3*	*1.35*	*1.08–1.69*	*0.009*	*0.031*	*0.76*	*0.58–1.00*	*0.053*
17537359	*G*	*Int 3*	*1.22*	*0.74–2.02*	*0.436*	*0.717*	*0.60*	*0.32–1.11*	*0.104*
279821	*C*	*Int 3*	*1.37*	*1.09–1.71*	*0.007*	*0.030*	*0.76*	*0.57–1.00*	*0.048*
279822	*C*	*Int 3*	*1.35*	*1.08–1.69*	*0.009*	*0.031*	*0.76*	*0.58–1.01*	*0.055*
17537380	*A*	*Int 3*	*1.29*	*0.49–3.45*	*0.614*	*0.757*	*0.81*	*0.26–2.53*	*0.722*
6833452	G	Int 3	1.35	1.08–1.69	0.009	0.031	0.76	0.58–1.01	0.054
12647055	*A*	*Int 3*	*1.29*	*1.03–1.61*	*0.024*	*0.064*	*0.84*	*0.64–1.11*	*0.221*
4540087	*A*	*Int 3*	*1.33*	*1.06–1.67*	*0.012*	*0.039*	*0.77*	*0.58–1.01*	*0.063*
1442059	*G*	*Int 3*	*1.35*	*1.08–1.69*	*0.009*	*0.031*	*0.76*	*0.58–1.01*	*0.056*
1442060	*G*	*Int 3*	*0.99*	*0.80–1.23*	*0.917*	*0.937*	*0.90*	*0.69–1.19*	*0.473*
3849591	*A*	*Int 3*	*1.24*	*0.94–1.64*	*0.135*	*0.320*	*0.82*	*0.58–1.17*	*0.282*
1442061	*G*	*Int 3*	*0.97*	*0.76–1.24*	*0.797*	*0.863*	*1.17*	*0.86–1.60*	*0.311*
17459386	*A*	*Int 3*	*1.57*	*0.79–3.09*	*0.196*	*0.431*	*0.70*	*0.31–1.58*	*0.389*
1442062	*A*	*Int 3*	*0.95*	*0.74–1.22*	*0.679*	*0.794*	*1.20*	*0.88–1.63*	*0.256*
4695152	*G*	*Int 3*	*1.17*	*0.68–2.02*	*0.576*	*0.729*	*0.96*	*0.48–1.90*	*0.895*
11503015	*G*	*Int 3*	*0.74*	*0.49–1.14*	*0.171*	*0.395*	*1.39*	*0.80–2.40*	*0.238*
10013922	*C*	*Int 2*	*0.97*	*0.76–1.24*	*0.824*	*0.873*	*1.12*	*0.83–1.53*	*0.456*
11503014	C	Int 1	0.96	0.76–1.21	0.704	0.802	1.08	0.80–1.45	0.626
3756007	*G*	*Int 1*	*1.17*	*0.71–1.95*	*0.538*	*0.717*	*0.69*	*0.36–1.30*	*0.245*
894269	A	Footprint	1.25	0.94–1.67	0.132	0.320	0.77	0.53–1.11	0.156

Rows in italic type represent the newly genotyped SNPs. CI: confidence interval; OR: odds ratio; FDR-BH: Benjamini–Hochberg false discovery rate. NOTE: For previously genotyped SNPs, minor allele notation in this publication may vary from prior based on plus/minus strand selection.

Fig. S1; see Supporting Information) with each other, even though they spanned the gene. Several significant genotype × sex interactions were noted, although these interactions did not seem to be as significant for SNPs in introns 3 and 4, that showed significant main effects of genotype. In all instances, the association signal was more likely in men than women [i.e. odds ratio (OR) less than 1.0].

Table 3 Results from logistic regression-based association analyses between single nucleotide polymorphisms (SNPs) in gamma-aminobutyric acid receptor A, subunit 4 (*GABRA4*) and case status [Fagerström Test for Nicotine Dependence (FTND) score of 4+, controls are smokers with an FTND = 0] in the Nicotine Single Nucleotide Polymorphism (NICSNP) project.

			Genotype				*Genotype × sex*		
SNP (rs no.)	*Allele*	*Location*	*OR.*	*95% CI*	*Observed P value*	*FDR–BH P-value*	*OR.*	*95% CI*	*Observed P-value*
10004359	*A*	*3′ near gene*	*1.09*	*0.87–1.38*	*0.487*	*0.717*	*0.90*	*0.66–1.22*	*0.484*
7691100	A	3′ UTR	1.09	0.86–1.38	0.499	0.717	0.90	0.66–1.22	0.503
10033500	A	3′ UTR	1.10	0.87–1.40	0.435	0.717	0.87	0.64–1.18	0.366
9291296	G	3′ UTR	1.11	0.87–1.40	0.411	0.717	0.89	0.66–1.21	0.468
956378	A	3′ UTR	1.09	0.77–1.53	0.641	0.759	0.95	0.61–1.47	0.809
12506608	G	3′ UTR	1.01	0.78–1.30	0.955	0.966	0.98	0.71–1.36	0.912
1512139	A	3′ UTR	0.93	0.75–1.15	0.499	0.717	1.11	0.84–1.46	0.468
9291298	G	3′ UTR	0.92	0.74–1.14	0.434	0.717	1.14	0.86–1.50	0.362
3920214	T	3′ UTR	1.10	0.77–1.56	0.603	0.754	0.92	0.59–1.43	0.697
17599074	G	3′ UTR	1.10	0.87–1.40	0.437	0.717	0.89	0.65–1.21	0.449
10004905	G	3′ UTR	0.93	0.76–1.16	0.542	0.717	1.11	0.84–1.45	0.476
4637372	A	3′ UTR	0.93	0.75–1.15	0.498	0.717	1.12	0.85–1.47	0.436
6447517	*A*	*3′ UTR*	*1.04*	*0.84–1.30*	*0.703*	*0.802*	*0.93*	*0.71–1.23*	*0.613*
17599102	A	3′ UTR	1.08	0.85–1.37	0.531	0.717	0.93	0.68–1.26	0.631
16859700	G	3′ UTR	1.47	0.75–2.89	0.261	0.542	0.64	0.28–1.45	0.282
7660336	C	3′ UTR	0.92	0.74–1.15	0.462	0.717	1.12	0.85–1.48	0.406
17599158	*G*	*Int 8*	*1.09*	*0.86–1.38*	*0.487*	*0.717*	*0.90*	*0.66–1.22*	*0.484*
17599165	*A*	*Int 8*	*1.09*	*0.77–1.54*	*0.627*	*0.759*	*0.92*	*0.59–1.43*	*0.718*
17599186/1028005	G	Int 8	1.00	0.77–1.29	0.978	0.978	0.99	0.71–1.37	0.928
11946433	*A*	*Int 8*	*1.03*	*0.70–1.53*	*0.876*	*0.906*	*1.07*	*0.65–1.78*	*0.791*
1160093	C	Int 8	1.04	0.83–1.29	0.735	0.827	0.94	0.71–1.24	0.661
1512130	*A*	*Int 8*	*0.93*	*0.74–1.16*	*0.516*	*0.717*	*1.01*	*0.76–1.35*	*0.929*
7685553	*G*	*Int 8*	*0.95*	*0.68–1.32*	*0.761*	*0.845*	*1.02*	*0.68–1.54*	*0.911*
16859761	*A*	*Int 8*	*1.47*	*0.75–2.89*	*0.261*	*0.542*	*0.64*	*0.28–1.45*	*0.284*
10015891	*A*	*Int 8*	*0.94*	*0.76–1.16*	*0.565*	*0.728*	*1.08*	*0.83–1.42*	*0.571*
1512134	*C*	*Int 8*	*0.93*	*0.75–1.16*	*0.536*	*0.717*	*1.08*	*0.83–1.42*	*0.567*
2280074	*G*	*Int 7*	*0.93*	*0.75–1.15*	*0.494*	*0.717*	*1.08*	*0.83–1.42*	*0.564*
2055942	*G*	*Int 7*	*1.06*	*0.83–1.35*	*0.637*	*0.759*	*0.96*	*0.71–1.30*	*0.787*
17599367	A	Int 7	1.14	0.82–1.60	0.438	0.717	0.87	0.57–1.34	0.538
16859794	*G*	*Int 7*	*0.93*	*0.75–1.15*	*0.505*	*0.717*	*1.09*	*0.83–1.43*	*0.545*
2271429	*C*	*Int 7*	*0.93*	*0.75–1.15*	*0.505*	*0.717*	*1.09*	*0.83–1.42*	*0.556*
17599416	*G*	*Int 6*	*1.15*	*0.82–1.60*	*0.424*	*0.717*	*0.87*	*0.57–1.33*	*0.508*
1512135	*G*	*Int 6*	*1.15*	*0.82–1.60*	*0.424*	*0.717*	*0.87*	*0.57–1.33*	*0.508*
3792208	*A*	*Int 6*	*0.95*	*0.65–1.40*	*0.807*	*0.865*	*0.96*	*0.60–1.54*	*0.857*
10517173	*C*	*Int 2*	*0.54*	*0.37–0.79*	*0.002*	*0.018*	*1.60*	*1.00–2.56*	*0.053*
13117953	*A*	*Int 2*	*1.74*	*1.05–2.89*	*0.031*	*0.079*	*0.58*	*0.31–1.06*	*0.076*
7694035	*A*	*Int 2*	*0.98*	*0.76–1.26*	*0.875*	*0.906*	*1.00*	*0.73–1.37*	*0.996*
16851647	*G*	*Int 2*	*0.54*	*0.37–0.79*	*0.002*	*0.018*	*1.60*	*1.00–2.56*	*0.053*
11735333	*C*	*Int 2*	*1.06*	*0.86–1.32*	*0.575*	*0.729*	*0.90*	*0.69–1.18*	*0.442*
11731576	A	Int 2	0.54	0.37–0.79	0.002	0.018	1.59	0.99–2.55	0.054
3792211	*A*	*Int 2*	*1.32*	*1.04–1.67*	*0.020*	*0.057*	*0.79*	*0.59–1.05*	*0.105*
2280072	G	Int 1	0.54	0.37–0.79	0.002	0.018	1.61	1.00–2.58	0.05
16859834/2229940	A	Exon 1	1.30	1.04–1.64	0.023	0.064	0.77	0.58–1.03	0.077
3762607	G	Footprint	0.47	0.30–0.73	0.0007	0.018	1.77	1.04–3.02	0.037
3762611	A	Footprint	0.48	0.31–0.74	0.0009	0.018	1.61	0.95–2.75	0.079

Rows in italic type represent newly genotyped SNPs. CI: confidence interval; OR: odds ratio; FDR-BH: Benjamini–Hochberg false discovery rate. NOTE: For previously genotyped SNPs, minor allele notation in this publication may vary from prior based on plus/minus strand selection.

GABRA4

Only two of the newly genotyped SNPs, rs10517173, rs16851647, were associated with nicotine dependence (observed *P*-values of 0.002, corrected FDR-BH *P*-values of 0.02). However, these SNPs were in complete LD ($r^2 = 1$, Fig. S2; see Supporting Information) with each other and with rs11731576. Thus, the signal tagged by the newly genotyped SNPs represented association at the same underlying locus identified by us previously. Association was restricted to introns 2 and 1 and the intervening exon and extended toward the 5′ end of the gene. Interactions with sex were marginally significant, suggesting again that these SNPs may exert a more potent influence in men versus women.

DISCUSSION

The current analyses provide compelling additional evidence for the association between SNPs in the *GABRA2* and *GABRA4* genes and nicotine dependence as assessed by the FTND. Some caveats and limitations of our study should be considered: first, these results are specific to our case–control definition—the intention of this study was to examine genes associated with progression in regular smokers and, thus, the controls in this study are regular smokers without a history of nicotine dependence (not even one symptom reported in a telephone interview) as assessed by the FTND. Secondly, our results are from a sample of European descent, and further study is needed to determine whether they extend to other ethnicities. Thirdly, it is important to consider the issue of multiple testing. In the context of the larger candidate gene study, these signals may not meet criteria for statistical significance. Within the context of these analyses, the FDR-BH *P*-values presented in Tables 2 and 3 suggest that a reasonable proportion of our signals are likely to be true positives.

Some have argued that additional genotyping of SNPs in moderate to high LD with previously genotyped markers is not an efficient strategy. However, evidence for LD is not necessarily synonymous with correlated evidence for association. Nielsen *et al.* [21] have demonstrated that in the presence of an observed signal, the relationship between association test results and inter-marker LD is related to several factors, including LD between each SNP and the functional site, multi-SNP LD and the genetic model. Due to the complexities inherent in the etiology of polygenic, multi-factorial phenotypes, such as nicotine dependence, we opted to proceed to the stage of additional genotyping.

This is, undoubtedly, only the first step towards understanding how genes constituting the GABAergic system influence vulnerability to smoking. Our next steps will include efforts to identify functional variants within these genes (and in neighboring regions), their impact on gene expression and, importantly, how environmental influences interact with these genomic variations. We also look forward to replication of our findings via independent efforts allowing for greater confidence in the role of *GABRA2* and *GABRA4* in the etiology of nicotine dependence.

Declarations of interest

Drs L.J. Bierut, A.M. Goate, A.J. Hinrichs, J.P. Rice and S.F. Saccone are listed as inventors on a patent (US 20070258898) held by Perlegen Sciences, Inc., covering the use of certain SNPs in determining the diagnosis, prognosis and treatment of addiction. Dr Bierut has acted as a consultant for Pfizer, Inc. in 2008.

Acknowledgements

The NICSNP project is a collaborative research group and part of the NIDA Genetics Consortium. Subject collection was supported by NIH grants CA89392 (Principle Investigator: L. Bierut) from the National Cancer Institute and DA012854 (Principle Investigator: P. Madden) from the National Institute on Drug Abuse. Genotyping work at Perlegen Sciences was performed under NIDA Contract HHSN271200477471C. Phenotypic and genotypic data are stored in the NIDA Center for Genetic Studies (NCGS) at http://nidagenetics.org/ under NIDA contract no. HHSN271200477451C (Principle Investigators: J. Tischfield and J. Rice). Genotyping services were provided by the Center for Inherited Disease Research (CIDR). CIDR is funded fully through a federal contract from the National Institutes of Health to the Johns Hopkins University, contract number HHSN268200782096. L.J.B. is also supported by DA21237. A.A. is also supported by DA023668. M.L.P. is supported by DA019951. S.F.S. is supported by ACS grant IRG5801050. N.L.S. is supported by K01DA015129. G.W.M. is supported by NHMRC339446.

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Fig. S1. r^2 values of linkage disequilibrium for gamma-aminobutyric acid receptor A, subunit ‘ (GABRA2) in 1049 cases and 872 controls. Boxes represent newly genotyped single nucleotide polymorphisms.
Fig. S2. r^2 values of linkage disequilibrium for gamma-aminobutyric acid receptor A, subunit 4 (GABRA4) in 1049 cases and 872 controls. Boxes represent newly genotyped single nucleotide polymorphisms.

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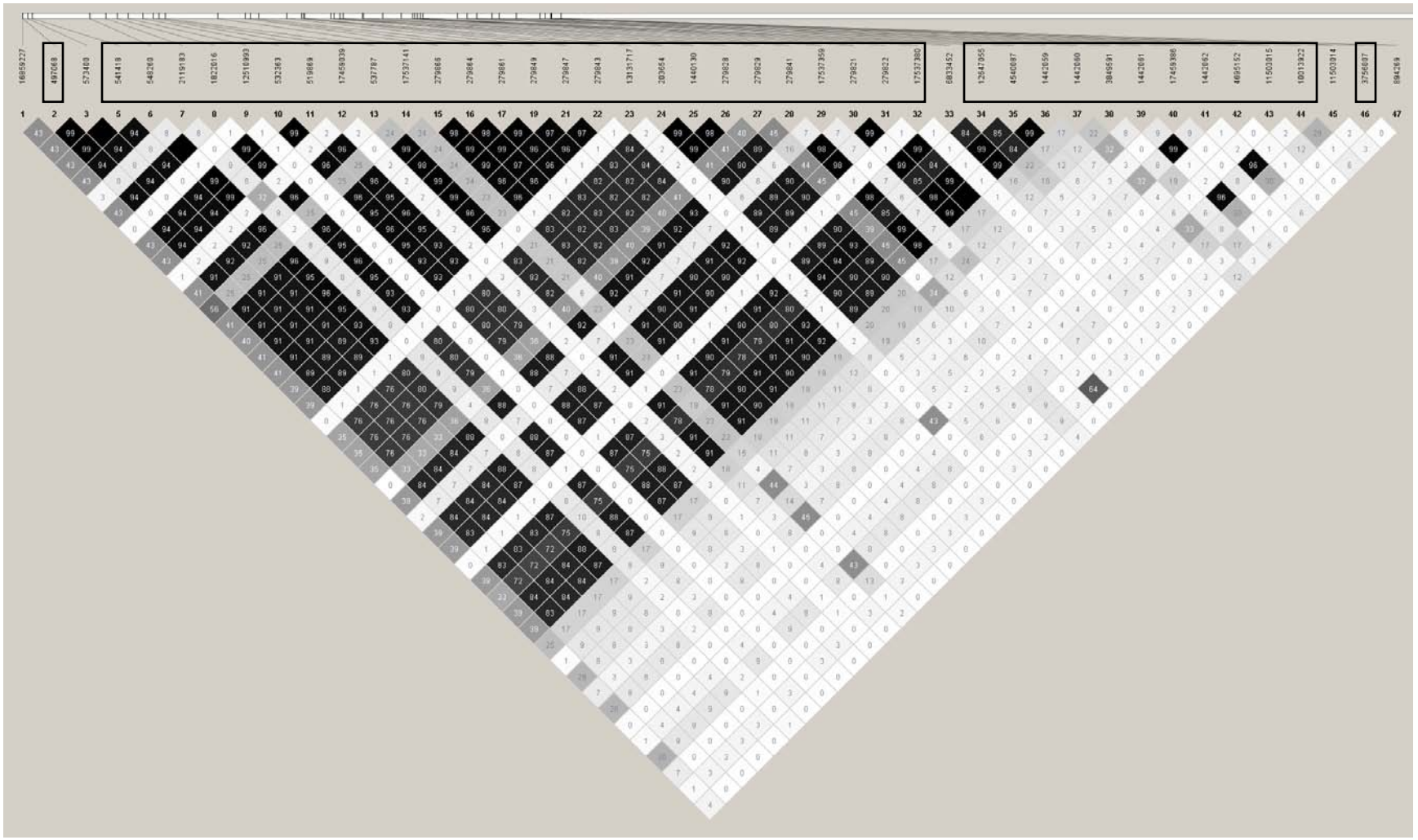

FIGURE S1 r^2 values of linkage disequilibrium for GABRA2 in 1049 cases and 872 controls. Boxes represent newly genotyped SNPs.

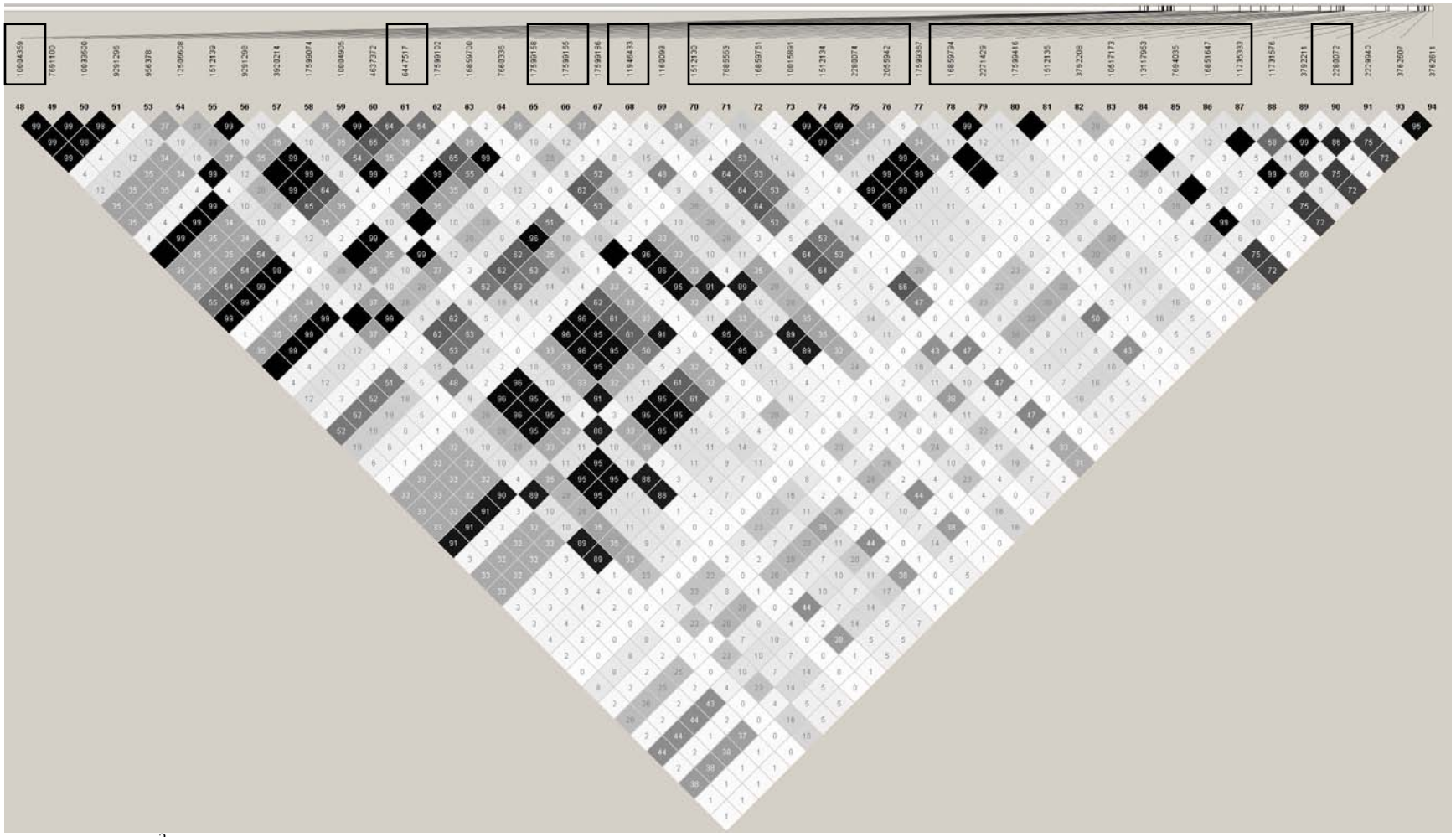

FIGURE S2 r^2 values of linkage disequilibrium for GABRA4 in 1049 cases and 872 controls. Boxes represent newly genotyped SNPs.