Genetic Variation in the Alpha Synuclein Gene (SNCA) Is Associated With BOLD Response to Alcohol Cues

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ABSTRACT. Objective: Preclinical studies implicate the gene encoding the alpha synuclein protein (SNCA) in the pathophysiology of alcohol dependence and dopamine neuron function. Results from clinical studies are less conclusive. Using neurobiological phenotypes in genetic studies, rather than typical heterogeneous diagnostic categories derived from self-report, may improve reliability across studies. This study aimed to examine whether polymorphisms in the SNCA gene were associated with alcohol taste cue–elicited responses in the brain, one such intermediate phenotype. **Method:** A total of 326 heavy drinkers who underwent an alcohol taste task during functional magnetic resonance imaging (fMRI) also were genotyped. Analyses focused on two previously identified SNCA variants (rs2583985 and rs356168) as well as 27 other single nucleotide polymorphisms from the Illumina Human1M BeadChip that were used in an exploratory analysis of the whole gene.

Neurobiological phenotypes were defined as fMRI blood oxygenation level–dependent (BOLD) responses to alcohol taste cue (vs. a control cue) in seven regions of interest known to be involved in cue processing and rich in dopaminergic axon terminals. **Results:** Polymorphisms in the SNCA gene were significantly correlated with BOLD activation. Specifically, the largest effect sizes and significance were seen for rs2583985 in paracingulate and caudate (focused analysis) and for rs1372522 in paracingulate (exploratory analysis). Activation in all regions of interest was correlated with alcohol-dependence severity. **Conclusions:** SNCA genotype was found to be associated with the degree of fMRI BOLD response during exposure to the taste of alcohol versus a control taste. This study also further validates the use of this alcohol taste task as an intermediate phenotype for alcohol-dependence severity. (*J. Stud. Alcohol Drugs*, 74, 233–244, 2013)

ALCOHOL DEPENDENCE (AD) is approximately 50% heritable (Köhnke, 2008), and recently progress has been made in elucidating the genes contributing to AD risk and severity. Unfortunately, findings across genetic studies of AD are mixed. Possible reasons for this lack of replication include the presence of multiple, genetically influenced predisposing factors for AD and the heterogeneity of the AD diagnosis phenotype (Kendler et al., 2012). One solution is to use a potentially more reliable objective intermediate neurobiological phenotype, such as alcohol taste cue–evoked blood oxygenation level–dependent (BOLD) response during functional magnetic resonance imaging (fMRI) (Hutchison, 2008). We used this approach in this study and investigated the association between polymorphisms in the gene encoding alpha synuclein (SNCA) and the evoked BOLD response to the alcohol taste cue compared with the control taste cue.

The SNCA gene and overexpression of the alpha synuclein protein have been implicated in alcohol preference and alcohol and cocaine self-administration in preclinical

studies (Boyer and Dreyer, 2007; Liang and Carr, 2006). Underexpression of alpha synuclein also may play a role in AD and is associated with stress-related increases in drinking (Le-Niculescu et al., 2008). Alpha synuclein is particularly abundant in dopamine neurons, and overexpression of or mutations in the protein can result in dopamine neuron dysfunction and cell death (Kurz et al., 2010; Nemani et al., 2010). Altered brain dopamine function is likely one of the primary causal factors in the pathophysiology of substance use disorders, regulating reward learning, pleasure, motivation, and conditioned drug seeking (Fields et al., 2007; Volkow et al., 2011).

Human studies also implicate the alpha synuclein protein and the SNCA gene in AD, although results are less conclusive. AD has been linked to a region on chromosome 4q, which includes the SNCA gene (Bönsch et al., 2005b). Further work has demonstrated associations between AD and longer alleles at a polymorphic complex repeat site 9 kb upstream of SNCA, which regulates alpha synuclein expression (Bönsch et al., 2005b; Fuchs et al., 2008). Alpha synuclein expression also has been found to be associated with cocaine dependence (Mash et al., 2003), alcohol craving, and AD (Bönsch et al., 2005a). More recent human studies have shown an association between polymorphisms in single nucleotide polymorphisms (SNPs) of the SNCA gene and alcohol craving but not AD (Clarimon et al., 2007; Foroud et al., 2007).

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Using neurobiological objective phenotypes in genetic studies of AD may improve reliability. One such neurobiological phenotype is the degree to which exposure to alcohol cues recruits brain areas known to have a critical role in AD risk and severity. Alcohol cues increase BOLD signal in the striatum, amygdala, anterior cingulate cortex (ACC), and thalamus with some consistency, and the degree of activation is associated with AD diagnosis, AD risk, craving, AD severity, and relapse (Claus et al., 2011; Filbey et al., 2008a; Grüsser et al., 2004; Heinz et al., 2004; Hermann et al., 2006; Kareken et al., 2004; Myrick et al., 2008).

In this study, we aimed to identify the neurobiological mechanism of the effect of SNCA on craving by examining the influence of two previously identified SNPs (rs2583985 and rs356168) (Foroud et al., 2007) on evoked response to the taste of alcohol versus juice using a taste cue paradigm that has been previously described (Claus et al., 2011; Filbey et al., 2008a) in 326 heavy drinking individuals. The participants overlap with those used in a previous study on the main effects of alcohol cues (Claus et al., 2011). We focused our analysis on seven *a priori* regions of interest (ROIs) (nucleus accumbens [NAC], caudate, putamen, thalamus, amygdala, anterior cingulate, paracingulate), because activation in the striatum, thalamus, amygdala, and ACC has been found to be associated with alcohol taste cue in our previous work (Claus et al., 2011) and because these structures mediate conditioned drug seeking and have a high density of dopamine nerve terminals (Cools and D'Esposito, 2011; Fields et al., 2007; Sanchez-Gonzalez et al., 2005; Volkow et al., 2007). In addition, we explored the association between the evoked response to taste cues and genotype for all SNPs in the Illumina Human1M BeadChip (Illumina, Inc., San Diego, CA) that were within the gene or within 20 kb in the 3' and 5' directions of the SNCA gene in order to include all potential important promoter SNPs. Although associations between AD severity measures and neural activation to alcohol taste in selected ROIs have been demonstrated by previous work in this laboratory (Claus et al., 2011; Filbey et al., 2008a), we expanded on this work by exploring associations between the same three AD severity measures and BOLD activation in the particular ROIs used in this study.

Method

Participants

A total of 326 heavy drinking men ($n = 225$) and women ($n = 101$) were recruited through flyers and media advertisements and consented to participate in the study, with approval from the University of New Mexico's institutional review board. For inclusion, men had to have drunk five or more drinks per drinking occasion (and four or more for women) at least five times in the past month. They were excluded for a history of severe alcohol withdrawal (delirium

tremens or seizures), previous brain injury, or loss of consciousness greater than 5 minutes. They were excluded from the MRI scan if they were in withdrawal (Clinical Institute Withdrawal Assessment of Alcohol Scale, Revised, score > 8) (Sullivan et al., 1989) or had recently been drinking (breath alcohol concentration $> .0$). All participants spoke fluent English, were free of MRI contraindications, and were compensated for their participation.

Materials

To evaluate AD severity, the following questionnaires were used: the Alcohol Use Disorders Identification Test (AUDIT; Babor et al., 2011), the Alcohol Dependence Scale (ADS; Skinner and Horn, 1984), and the failed control subscale of the Impaired Control Scale (ICSfc; Heather et al., 1998). Other alcohol and drug use measures also were obtained, specifically the Timeline Followback method (cigarette, marijuana, alcohol) and the Structured Clinical Interview for DSM-IV (First et al., 1995) for alcohol abuse and dependence. Ethnicity and race were determined by asking, "Are you from Hispanic ethnicity?" and "What is your race?"

DNA collection, extraction, storage, and analysis

Participants were instructed to generate 5 ml of saliva. DNA was extracted from the sample using published methods (Hutchison et al., 2008). The Illumina 1M Duo whole genome array was used to genotype rs356168 and rs2583985 for the primary analysis, as well as SNPs within 20 kb of the SNCA gene for the secondary analysis. In addition, we used principal components analysis (PCA) to control for population stratification (e.g., ethnicity) (Price et al., 2006).

For the whole-gene analysis, we identified all Illumina-genotyped SNPs within the SNCA gene and 20 kb in the 3' and 5' direction ($n = 43$) and then eliminated all SNPs with minor allele frequencies of less than 5% ($n = 13$). We then eliminated all SNPs that were not in Hardy-Weinberg equilibrium (HWE $P < .05$) ($n = 3$), leaving us with 27 SNPs (see Figure 1, for HapMap, Barrett et al., 2005; see Table 1 for marker statistics). An online interface (SNPSpD) for performing a correction for multiple comparisons was used to determine the effective number of marker loci in our 27 SNP analysis (Nyholt, 2004) (effective no. of independent marker loci = 14.3; significance threshold required to keep type I error rate at 5% = .003). Therefore, we used a $p < .003$ as our significance threshold for the exploratory analysis of all 27 SNPs, which corrected for the number of SNPs tested in the exploratory ROI by genotype analysis (see below).

fMRI task

We used a taste cue paradigm previously reported to elicit BOLD response in mesocorticolimbic areas (Claus et al.,

FIGURE 1. Genomic structure of the SNCA gene (± 20 kb; $n = 326$ individuals; 27 single nucleotide polymorphisms [SNPs] obtained from the Illumina Human1M BeadChip), generated with Haploview software. Dark gray = LOD #2, $D = 1$. Light gray = LOD #2, $D < 1$. White = LOD < 2, $D < 1$. D scores are recorded in the figure, but D scores of 1.0 are not recorded (for blank boxes, $D = 1.0$).

2011; Filbey et al., 2008a). In brief, participants received small amounts of two different beverages pseudorandomly presented through Teflon tubing while they were in the scanner by a computer-controlled gustometer. The two beverages included small amounts (1 ml) of their preferred alcoholic beverage (wine, alcohol, mixed drinks) alternated with a control beverage (lychee juice) that was selected for its appetitive and novel qualities.

fMRI data acquisition

All MRI data were collected on a 3T Siemens Trio (Erlangen, Germany) whole-body scanner. Participants were placed in the scanner, and a piece of tape was placed across the forehead to serve as feedback for movement reduction. An echo-planar gradient-echo pulse sequence [TR (repetition time) = 2000 ms, TE (echo time) = 29, flip angle = 75°] was acquired with an 8-channel head coil, and images were acquired parallel to the ventral surface of a participant's orbitofrontal cortex to reduce signal dropout and distortion. Each volume acquired consisted of 33 axial slices (64×64 matrix, 3.75×3.75 mm², 3.5 mm thickness, 1 mm gap). In

addition, a high-resolution T1-weighted Magnetization Prepared Rapid Gradient Echo (MP-RAGE) anatomical image was acquired (TR = 2530 ms, TE = 1.64 ms, flip angle = 7° , 192 sagittal slices, 256×256 matrix, slice thickness = 1 mm, no gap) for each participant (Claus et al., 2011).

Image analysis

All preprocessing, first-, and second-level analyses were identical to methods described in detail in other articles (Claus et al., 2011). All analyses were completed using tools from the FMRIB Software Library (FSL) (Smith et al., 2004). The first seven volumes of each functional run were discarded. Motion correction using FMRIB's Linear Image Registration Tool (Jenkinson et al., 2002) was used to realign images to the first volume within a run. Images were deskulled using the brain extraction tool, spatially smoothed with an 8-mm full-width half-max Gaussian kernel, temporally filtered using a high-pass filter of 100 seconds, and grand mean intensity normalized; all of these steps were performed using FMRIB Expert Analysis Tool (FEAT; Smith et al., 2004). Regressors of interest were created for

TABLE 1. Marker statistics for 27 single nucleotide polymorphisms analyzed

Marker	Position	Minor allele	Major allele	HWE <i>p</i>	Homozygous minor	Heterozygous	Homozygous major	Missing genotype
rs356174	90849924	C	A	.193	26	115	183	2
rs356220	90860363	T	C	.521	61	153	111	1
rs168552	90862167	C	T	.093	23	105	196	2
rs356204	90882565	A	G	.471	84	156	85	1
rs356200	90887637	A	G	.407	83	155	87	1
rs356168	90893454	G	A	.406	84	155	86	1
rs2736990	90897564	C	T	.346	84	154	87	1
rs356191	90907143	T	C	.546	24	120	178	4
rs356188	90910560	G	A	.529	19	107	183	17
rs3775434	90921804	G	A	.129	20	100	199	7
rs2737033	90926970	G	A	.405	17	101	196	12
rs3775439	90928764	A	G	.185	21	106	199	0
rs2197120	90948625	A	G	.611	24	122	179	1
rs2619372	90955750	A	G	.112	55	140	129	2
rs1812923	90958562	A	C	.059	62	140	122	2
rs10005233	90962354	C	T	.225	77	151	97	1
rs2583978	90969349	A	C	.599	23	120	182	1
rs1471483	90973315	T	C	.444	73	152	94	7
rs2583985	90974962	G	A	.495	16	103	207	0
rs3756059	90976295	G	A	.294	77	153	96	0
rs2619361	90976758	A	C	.458	16	102	207	1
rs2245801	90976863	A	G	.340	24	116	184	2
rs2619362	90976868	T	C	.495	16	103	207	0
rs2301134	90977968	T	C	.243	78	152	96	0
rs1023777	90980435	C	T	.069	36	122	165	3
rs1372522	90984303	G	A	.553	64	144	93	25
rs10516848	90994235	G	A	.065	36	122	166	2

Note: HWE = Hardy–Weinberg equilibrium.

the following conditions—alcohol cue, alcohol baseline, juice cue, and juice baseline—according to the timing scheme used in previous studies (Claus et al., 2011; Filbey et al., 2008a). The primary contrast of interest in this study compared BOLD response during the alcohol cue versus the juice cue. Statistical analyses were performed using the general linear model as implemented in FEAT. Customized square waveforms representing the condition of interest and the duration of stimulus presentation were convolved with a double gamma hemodynamic response function. Time-series analyses were conducted using FMRIB Improved Linear Model with local autocorrelation estimation (Woolrich et al., 2004). This first-level analysis generated parameter estimates for each condition of interest, and contrast maps were computed for each participant. Contrast maps were registered to the Montreal Neurological Institute (MNI) 152-brain template using a two-step registration process with the FMRIB Linear Image Registration Tool (Jenkinson et al., 2002). First, an average echo planar image was registered to the participant's high-resolution anatomical image. Each participant's high-resolution T1-weighted image was then registered to the MNI 152-brain template. Finally, contrast maps were registered to the MNI 152-brain template using parameters from the previous registration steps. Before computing group-level statistics, all second-level contrast images were registered to the MNI template using parameters from the two-step registration process described above.

Image X genotype analysis

The results from the second-level analyses were used in a third-level group analysis using higher-level analyses in FEAT (Smith et al., 2004). Specifically, we examined the correlation between genotype values and the alcohol–lychee contrast maps (e.g., difference maps of the beta values for the alcohol cue and the juice cue derived in the first-level multiple regression). Individuals were assigned a 0 if they were homozygous for the more common allele (major allele), 1 if they were heterozygous, and 2 if they were homozygous for the less common allele (minor allele). Mean deviated genotype scores were used as the covariate for the raw genotypes and PCA-corrected genotypes (Price et al., 2006). Both positive and negative relationships between BOLD response and genotype were examined. When there were demographic or clinical differences between genotype groups, we entered these in independent analyses as covariates of no interest. A priori ROIs (NAC, caudate, and putamen, amygdala, thalamus, cingulate and paracingulate) were derived using the Harvard–Oxford probabilistic atlas in FSL (Figure 2). All ROIs were used as masks in the group-level analyses; in each case, statistical maps were thresholded using Gaussian random field theory (Worsley et al., 1996) as implemented in cluster-based thresholding in FSL (voxel threshold $z = 2.3$, $p = .01$; cluster threshold $p = .05$).

FIGURE 2. Anatomical locations of the seven a priori regions of interest (ROIs) used in this study by color. All ROIs were derived using the Harvard–Oxford probabilistic atlas in the FMRIB Software Library (FSL; Smith et al. 2004).

To correct for multiple comparisons, we used a Bonferroni approach (Table 4, Table 6). Specifically, for a focused analysis of rs356168 and rs2583985, the corrected significance level was calculated to be $.05 / 2$ (no. of SNPs tested) $\times 2$ (no. of directions of effect) $\times 7$ (no. of ROIs) = .00179. For an exploratory analysis of the whole gene, the corrected significance level was calculated to be .003 (corrected p value for no. of SNPs tested from SNPSpD; see above) $/ 2$ (no. of directions of effect) $\times 7$ = .000214.

Validity of the neurobiological phenotype

Using a method previously published on an overlapping sample of participants (Claus et al., 2011), we derived the mean percentage signal change for alcohol taste–control taste for each ROI (caudate, putamen, cingulate, paracingulate, and thalamus) using Featquery. Then we used linear regression to determine the relationship between scores on clinical severity measures and mean percentage signal change. Although scores on the three self-report measures were intercorrelated, rather than creating a composite measure, we chose to investigate these individually because they focus on slightly different aspects of the dependence syndrome (loss of control vs. withdrawal severity vs. quantity of use). We did not calculate the association between the amygdala and NAC and scores on clinical severity measures because results of these analyses have been reported elsewhere (Claus et al., 2011). We also used linear regression

to determine the relationship between scores on clinical severity measures (i.e., AUDIT, ADS, ICSfc) and both raw and PCA-corrected genotype for our main SNPs of interest (rs2583985 and rs356168).

Results

Sample characteristics

Demographics for a very similar sample (differing only by six subjects) have been previously reported (Claus et al., 2011). In brief, males were more numerous ($n = 225$) than females ($n = 101$), and approximately half the sample was treatment seeking. The mean score on the ICSfc was 18.99 ($SD = 9.64$), the mean ADS score was 13.52 ($SD = 8.21$; $n = 325$), the mean AUDIT score was 18.96 ($SD = 7.69$; $n = 324$), the mean number of drinking days over the previous 30 days was 17.42 days ($SD = 9.52$), and the mean number of drinks per day over the previous 30 days was 7.20 drinks ($SD = 4.12$). A total of 40.49% of the participants stated that they were of Hispanic ethnicity. In response to questions about race, 45.4% identified themselves as White, 1.84% as Black, 0.61% as Asian, 25.46% as Latino, 5.21% as Native American, 21.47% mixed, and none as Native Hawaiian/Pacific Islander. There were marginally significant different frequencies of self-reported ethnicity and race by genotype group for rs356168 and a trend ($p = .077$) for whether they reported being a marijuana smoker in the last 90 days, but there were no other significant differences in clinical and demographic variables between genotype groups for the two main SNPs of interest (rs2583985, rs356168) (Tables 2a and 2b).

Association between genotype and BOLD activation to alcohol taste cue

For rs2583985, the major allele (A) was associated with significantly ($p < .05$) greater BOLD response to alcohol compared with control taste cue in all ROIs (Table 3) using both raw and PCA-corrected genotypes. For rs356168, the major allele (A) was associated with significantly ($p < .05$) greater BOLD response to alcohol compared with control taste cue in the NAC, cingulate, and paracingulate (Table 3). Rerunning these analyses using PCA-corrected genotypes did not result in any large changes in significance or effect size of associations, making it unlikely that ethnicity was driving results (Table 3). After correction for multiple comparisons using Bonferroni methods, the association between the rs2583985 genotype and the BOLD response in the caudate and paracingulate remained significant ($p < .05$) (Table 3). For observed demographic and clinical differences between genotype groups for rs356168 (Table 2B), we secondarily entered these as covariates of no interest into supplemental analyses, showing no evidence that these differences were driving results (Table 4).

TABLE 2A. Associations between rs2583985 genotype (genotype group) and potential confounds

Variable	(n)	AA (0) (n = 207) M	AG (1) (n = 103) M	GG (2) (n = 16) M	R	p
Age, years	(326)	31.94	31.96	31.06	.0112	.839
Days since last drink	(322)	2.99	2.66	2.38	.0539	.335
Cigarettes smoked in last 24 hours	(256)	4.39	4.90	4.71	.0262	.677
Cigarettes smoked in last 24 hours, smokers only	(170)	6.58	7.18	7.33	.034	.657
Days cigarettes smoked over last 90 days, smokers only	(177)	53	53	65	.082	.279
Average times per month in last year smoked marijuana, marijuana smokers only	(103)	25.39	14.18	9.40	.077	.438
Days marijuana smoked over last 90 days, marijuana smokers only	(122)	21.58	30.32	21.17	.092	.313
	(n)	%	%	%	χ^2	p
Male	(326)	72	65	63	1.92	.382
Treatment seeking	(326)	47	43	31	1.90	.387
Current alcohol abuse or dependence	(326)	86	81	79	1.768	.413
Race/ethnicity						
White race	(325)	42	50	63	4.07	.131
Latino race	(325)	24	29	25	1.03	.597
Hispanic ethnicity	(326)	43	37	25	2.91	.233
Cigarette smoker, any days on 90-day TLFB	(326)	55	54	56	0.020	.990
Marijuana smoker, any days on 90-day TLFB	(326)	43	38	40	0.218	.897

Notes: TLFB = Timeline Followback.

TABLE 2B. Associations between rs356168 genotype (genotype group) and potential confounds

Variable	(n)	AA (0) (n = 86) M	AG (1) (n = 155) M	GG (2) (n = 84) M	R	p
Age, years	(325)	32.84	31.23	32	.0317	.568
Days since last drink	(321)	2.61	3.05	2.73	.0120	.830
Cigarettes smoked in last 24 hrs, smokers only	(169)	6.92	7.51	4.87	.078	.311
Days cigarettes smoked over last 90 days, smokers only	(176)	53.12	58.25	46.95	.064	.399
Average times per month in last year smoked marijuana, smokers only	(102)	37.17	12.41	21.61	.092	.357
Days marijuana smoked over last 90 days, smokers only	(121)	23.41	22.48	30.91	.076	.408
	(n)	%	%	%	χ^2	p
Male	(325)	70	72	65	0.971	.615
Treatment seeking	(325)	50	40	49	2.927	.231
Current alcohol abuse or dependence	(325)	87	81	86	1.219	.544
Race/ethnicity						
White race	(324)	60	42	38	10.578	.005
Latino race	(324)	14	29	30	7.994	.018
Hispanic ethnicity	(325)	30	47	38	6.768	.034
Cigarette smoker, any days on 90-day TLFB	(325)	58	56	47	2.229	.328
Marijuana smoker, any days on 90-day TLFB	(325)	40	42	27	5.121	.077

Notes: Linear regression was used to determine the relationship between genotype and continuous variables such as age. Chi-square analyses were used to determine the relationship between genotype and dichotomous variables such as marijuana smoker. TLFB = Timeline Followback.

Association between genotype and alcohol dependence severity measures

Genotype was found to be significantly ($p < .05$) associated with ICSfc for rs2583985 (raw genotype) and

rs356168 with the major allele (A) predicting greater illness severity. Presence of the A allele for both SNPs was associated with greater severity scores on the ADS and the AUDIT as well, despite these associations not meeting significance (Table 5). Rerunning these analyses

TABLE 3. Association between genotype (single nucleotide polymorphisms [SNPs] rs2583985, rs356168) and evoked response to alcohol versus control taste in a priori regions of interest (ROIs)

Variable	Max Z	<i>p</i>	Cluster size (voxels)	X	Y	Z
rs2583985						
Caudate	4.07	.00485	72	9	18	6
	3.73	.00136*	108	-15	0	15
Putamen	3.27	.0491	29	-24	0	12
	3.42	.0296	56	3	-9	9
Nucleus accumbens	2.74	.0423	3	9	21	-3
Amygdala	3.28	.0287	24	-12	-6	-15
	3.35	.0263	26	18	-9	-15
Cingulate	3.73	.00718	230	18	42	18
Paracingulate	3.78	.000685*	449	18	39	18
Thalamus	3.35	.00282	186	-12	-3	12
rs2583985, PCA-corrected						
Caudate	3.42	.00270	88	-15	3	15
	4.04	.00659	64	9	18	6
Putamen	3.23	.0366	44	30	-9	9
Nucleus accumbens	2.6	.0422	3	12	21	-6
Amygdala	3.15	.0314	22	-15	-3	-15
	2.96	.0485	14	18	-6	-12
Cingulate	3.39	.0135	175	18	39	15
Paracingulate	3.6	.00121*	388	-3	33	42
Thalamus	3	.046	55	0	-21	0
rs356168						
Nucleus accumbens	2.59	.0424	3	-6	6	-9
Cingulate	3.27	.0141	172	-3	33	12
Paracingulate	3.26	.0333	100	-9	45	-3
rs356168, PCA-corrected						
Nucleus accumbens	2.9	.0341	5	-6	6	-6
Cingulate	3.48	.00683	235	-3	24	24
Paracingulate	3.43	.0178	145	-9	48	0
	3.2	.0148	159	-3	30	30

Notes: A priori ROIs are the caudate, putamen, nucleus accumbens, amygdala, cingulate, paracingulate, and thalamus. Max Z is the maximum test statistic value in the cluster; location (x,y,z) for Max Z is in Montreal Neurological Institute coordinates. Only clusters significant at $p < .05$ reported. PCA = principal component analysis.

* p values that remain significant after correcting for multiple comparisons using the Bonferroni method. Corrected significance level = $.05 / 2$ (no. of SNPs) $\times 2$ (no. of directions of effect) $\times 7$ (no. of ROIs) = .00179.

TABLE 4. Association between genotype (rs356168) and evoked response to alcohol versus control taste in a priori regions of interest (ROIs) corrected for potential confounds

Variable	ROI	Max Z	<i>p</i>	Cluster size (voxels)
Race	Nucleus accumbens	2.63	.0379	4
	Cingulate	3.4	.00534	258
	Paracingulate	3.17	.0117	178
		3.53	.0141	163
Ethnicity	Nucleus accumbens	2.52	.0424	3
	Cingulate	3.37	.00652	238
	Paracingulate	3.37	.0212	131
		3.09	.0254	118
Marijuana or no	Nucleus accumbens	2.51	.0481	2
	Cingulate	3.25	.0126	181
	Paracingulate	3.34	.0301	107

Notes: Ethnicity (Hispanic or Latino vs. not), race (dummy variables for White, Latino, mixed, Native American), and marijuana use (use in last 90 days or no) as covariates. Max Z is the maximum test statistic value in the cluster.

using PCA-corrected genotypes did not result in any large changes in the significance or the effect size of associations, making it unlikely that ethnicity was driving results (Table 5).

Exploratory whole gene analysis

Results from the whole gene analysis ($n = 27$ SNPs) are presented in Table 6. Notably, SNCA SNP genotype was found

TABLE 5. Association between genotype (rs2583985, rs356168) and clinical severity measures (AUDIT, ADS, ICSfc) for raw and PCA-corrected genotypes

Variable	Raw genotype		PCA-corrected	
	R	p	R	p
rs2583985				
AUDIT (n = 324)	-.102	.0660	-.0885	.112
ADS (n = 325)	-.0565	.310	-.0416	.455
ICSfc	-.117	.0352	-.0955	.0851
rs356168				
AUDIT (n = 324)	-.0342	.534	-.0599	.283
ADS (n = 325)	-.0272	.625	-.0539	.333
ICSfc	-.110	.0468	-.144	.00930

Notes: AUDIT = Alcohol Use Disorders Identification Test; ADS = Alcohol Dependence Scale; ICSfc = failed control subscale of Impaired Control Scale; PCA = principal component analysis. Individuals were assigned a 0 if they were homozygous for the more common allele (major allele), 1 if they were heterozygous, and 2 if they were homozygous for the less common allele (minor allele).

to be significantly ($p < .003$) associated with BOLD activation in the caudate, paracingulate, cingulate, and thalamus for a number of SNPs spanning the gene and present in all haplotype blocks (Figure 1). In all cases except for rs1372522, the major allele was associated with greater BOLD activation to taste cue (Table 1; Table 6). After correction for multiple comparisons using the Bonferroni method, only rs1372522 had significant associations with BOLD activation in the paracingulate.

Further validation of taste paradigm as an intermediate phenotype

BOLD activation to the alcohol versus the control taste cue in the amygdala and NAC was found to be associated with illness severity for all three self-report measures (AUDIT, ICSfc, ADS) in previously published work (Claus et al., 2011). Further exploration using the caudate, putamen, cingulate, thalamus, and paracingulate ROIs demonstrated significant associations between the ADS and BOLD activa-

TABLE 6. Exploratory analysis of 27 SNCA single nucleotide polymorphisms (SNPs) meeting criteria for inclusion in selected regions of interest (ROIs)

SNP	Direction of effect	ROI	Max Z	p	Cluster size (voxels)	X	Y	Z
rs2583985	A > G	Caudate	3.73	.00136	108	-15	0	15
	A > G	Paracingulate	3.78	.00069	449	18	39	18
rs1372522	G > A	Caudate	3.60	.00223	91	-18	0	21
	G > A	Cingulate	3.76	.00101	430	-6	-6	33
	G > A	Paracingulate	4.19	.00016*	622	-9	33	36
rs2619361	C > A	Caudate	3.57	.00232	93	-15	3	15
	C > A	Paracingulate	3.81	.00061	461	-9	45	27
rs2619362	C > T	Caudate	3.51	.00286	87	-15	0	15
	C > T	Paracingulate	3.72	.00083	429	-9	45	27
rs2737033	A > G	Paracingulate	3.61	.00157	364	18	48	9
rs168552	T > C	Paracingulate	3.77	.00216	330	-9	51	12
rs356174	A > C	Paracingulate	3.22	.00186	345	-9	51	9
rs2301134	C > T	Caudate	4.01	.00228	93	12	18	12
		Caudate	4.13	.00292	86	-18	0	21
	C > T	Thalamus	3.23	.00037	310	-6	-3	-6
rs3756059	A > G	Thalamus	3.29	.00079	262	-6	-3	-6
	A > G	Caudate	3.98	.00228	93	12	18	12

Notes: ROIs are the caudate, putamen, nucleus accumbens, amygdala, cingulate, paracingulate, and thalamus. Max Z is the maximum test statistic value in the cluster; location (x,y,z) for Max Z is in Montreal Neurological Institute coordinates. Only significant ($p < .003$) findings recorded.

*p values that remain significant after correcting for multiple comparisons using the Bonferroni method. Corrected significance level = .003 (corrected p value for no. of SNPs tested) / 2 (no. of directions of effect) $\times$ 7 = .000214.

TABLE 7. Associations between percentage signal change (alcohol taste-control taste) in selected regions of interest (ROIs) and clinical severity measures (AUDIT, ADS, ICSfc)

Variable	Caudate		Putamen		Cingulate		Paracingulate		Thalamus	
	R	p	R	p	R	p	R	p	R	p
AUDIT (n = 324)	.0496	.373	.104	.0622	.116	.0367	.113	.0420	.0911	.102
ADS (n = 325)	.0859	.122	.0896	.107	.136	.0139	.135	.0150	.0817	.142
ICSfc	.107	.0543	.186	.0007	.176	.0013	.151	.0064	.155	.0051

Notes: AUDIT = Alcohol Use Disorders Identification Test; ADS = Alcohol Dependence Scale; ICSfc = failed control subscale of Impaired Control Scale. ROIs are the caudate, putamen, cingulate, paracingulate, and thalamus. Amygdala and nucleus accumbens were excluded from this analysis because these analyses have been run in prior work (Claus et al. 2011), and significant associations were found between all three clinical severity measures and the amygdala and nucleus accumbens.

FIGURE 3. PANEL A: Effect of rs2583985 genotype on BOLD response to alcohol minus lychee taste cues in a voxelwise whole brain analysis, cluster corrected at $Z = 2.3$, $p = .05$, for visualization purposes. Additive genotype model assumed (e.g., AA = 0, AG = 1, GG = 2). Results for the AA > AG > GG contrast presented; $n = 326$. Main effects of genotype are seen in cingulate, paracingulate, striatum, and thalamus as well as posterior cingulate, and occipital cortices. PANEL B: Effect of rs1372522 genotype on BOLD response to alcohol minus lychee taste cues in a voxelwise whole brain analysis, cluster corrected at $Z = 2.3$, $p = .05$, for visualization purposes. Additive genotype model assumed (e.g., AA = 0, AG = 1, GG = 2). Results for the GG > AG > AA contrast presented; $n = 301$. Main effects of genotype are seen in cingulate, paracingulate, striatum, and thalamus as well as precentral and paracentral gyri.

tion in the paracingulate and cingulate as well as between the ICSfc and BOLD activation in the cingulate, paracingulate, thalamus, and putamen (Table 7).

Whole brain analysis

Figure 3 presents results for a whole brain analysis for rs2583985 and rs1372522, respectively.

Discussion

In this study, we found that two SNPs previously associated with alcohol craving (rs2583985, rs356168) were associated with the degree of evoked BOLD response during exposure to the taste of alcohol as compared with an appetitive control taste. Associations were identified for the BOLD response in all ROIs (NAC, amygdala, thalamus, cingulate,

paracingulate, caudate, putamen) for rs2583985 and in the NAC, cingulate, and paracingulate for rs356168. After correction for multiple comparisons, significant effects were seen for rs2583985 in the caudate and paracingulate. In addition, in the exploratory whole gene analysis, multiple SNPs within 20 kb of the SNCA gene were found to be associated with activation in the caudate, thalamus, cingulate, and paracingulate, with the association between the rs1372522 genotype in the paracingulate remaining significant after correction for multiple comparisons. In turn, activation in all ROIs (except for the caudate) was found to be significantly associated with the ICSfc, a self-report measure for AD severity. This study thus demonstrates that variation in the SNCA gene may moderate activation to an alcohol taste cue in important mesocorticolimbic areas involved in alcohol cue processing, which may be markers for AD severity. In particular, the effect of polymorphisms in the SNCA gene on AD severity may be best explained by the taste-elicited activation in the paracingulate because this region was strongly correlated with both polymorphisms in the SNCA gene and AD severity.

A variety of other genes have been implicated in AD severity and disease risk, some of which also are involved in brain dopamine signaling (Filbey et al., 2008b; Köhnke, 2008). However, findings across studies for many of these genes have been mixed and are not always replicated (Köhnke, 2008). Our results support the use of neurobiological intermediate phenotypes for genetic studies of AD to resolve these mixed findings. Indeed, our study demonstrates that the association between genotype and self-report severity measures was less robust than the association between genotype and the intermediate neurobiological phenotype (Table 5; Table 7), indicating increased penetrance at the neurobiological level and the utility of the fMRI taste paradigm in genetic studies of AD (Claus et al., 2011). However, as in many genetic studies, our effect sizes were still small. Other than its association with AD severity, the clinical significance of the increased BOLD response to alcohol taste is still unclear. It may represent craving, or it may represent some other aspect of AD severity. Cue reactivity may precede the development of AD, or it could result from it. Future studies might aim to clarify some of these issues.

Interestingly, the strongest associations between genotype or BOLD activation and self-report measures were seen for the ICSfc (compared with the other self-report measures). Previous work has shown that there are three genetic factors underlying the AD phenotype (tolerance and heavy use; loss of control with alcohol-associated social dysfunction; withdrawal and continued use despite problems) (Kendler et al., 2012). As opposed to the AUDIT and the ADS, the ICSfc questionnaire inquires more about loss of control (e.g., difficulty limiting quantity of use, using when having decided not to, irresistible urges). Notably, of the three self-report measures, ICSfc scores had the most robust associations

with the BOLD response to alcohol and with genotype. Our data imply that polymorphisms in the SNCA gene may have more of an influence on the loss-of-control dimension of the AD phenotype rather than on other symptom clusters. This is consistent with previous work showing lower levels of impulsive responding in SNCA knockout mice (Peña-Oliver et al., 2012). Further investigations may consider focusing studies on the role of SNCA in AD-related impulsivity.

Alpha synuclein expression and alterations in the SNCA gene are known to affect dopamine neuron function. Alpha synuclein is ubiquitously expressed in the human brain (Fuchs et al., 2008) but is particularly abundant in presynaptic nerve terminals (Mori et al., 2002) and nuclei (Tamo et al., 2002) of dopamine neurons. Studies show that alpha synuclein is involved in dopamine synthesis, storage, release, and reuptake (Kurz et al., 2010; Perez et al., 2002). Although the wild-type form of this protein may be neuroprotective, overexpression of alpha synuclein, or mutations in the protein, can result in decreased dopamine synthesis and release, as well as dopamine neuron death (Kurz et al., 2010; Nemani et al., 2010). In fact, abnormal alpha synuclein expression and mutations in the SNCA gene are likely key players in Parkinson's disease via neurotoxicity and subsequent degeneration of dopamine neurons in the substantia nigra (Kurz et al., 2010; Nemani et al., 2010).

Alterations in the SNCA gene may moderate AD severity by altering striatal dopamine function. Low striatal presynaptic dopamine synthesis capacity and dopamine release have been found to be associated with AD risk and severity (Heinz et al., 2005; Martinez et al., 2005; Volkow et al., 2007). Decreased striatal dopamine function (dopamine release and D2 receptor density) also is associated with increased BOLD response to a drug-related cue in ACC (Heinz et al., 2004). It is reasonable to hypothesize that the presence of an SNCA risk allele increases disease risk or severity (and associated activation throughout the brain) by decreasing striatal dopamine function. Although rs2583985 is located in an intron, rs1372522 is in the 5' untranslated region. Future work could aim to identify their functional significance, or whether they are in linkage disequilibrium with an SNP in a functional region. Future work also could consider testing effects of genetic variation in human subjects on presynaptic dopamine using 6-[18F]-Fluoro-m-tyrosine positron emission tomography (PET) (Eberling et al., 2007) or 6-[18F]-fluorodopa (Heinz et al., 2005) PET while also measuring evoked response to alcohol using fMRI.

That lower presynaptic dopamine neuron density or function in the striatum could confer an increased risk of alcohol use disorder severity is further supported by what is known about the biological mechanisms of conditioned cue responses. Dopamine is released tonically into the striatum and sets the systems responsiveness threshold, mediated predominantly by D2 receptors. Specifically, D2 binding decreases glutamate-induced excitatory postsynaptic currents

(André et al., 2010; Hernandez-Echeagaray et al., 2004; Higley and Sabatini, 2010). Cue-elicited glutamate release from PFC neurons into the striatum is believed to play a major role in conditioned responses (Reichel et al., 2011). Low tonic dopamine release resulting from dopamine neuron dysfunction may raise sensitivity to drug cue-induced glutamate release, decreasing the threshold for cues to elicit conditioned responses (André et al., 2010). In addition to the striatum, dopaminergic projections to other subcortical (amygdala, thalamus) and cortical (ACC) structures also are presumed to be of major importance in regulating reward learning, pleasure, motivation, conditioned drug seeking, habit formation, and decision making (Fields et al., 2007; Hermann et al., 2006; Narita et al., 2010; Sanchez-Gonzalez et al., 2005). For example, dopamine projections to the ACC play an important role in decision making, and dopamine neuron loss in this pathway could potentially inhibit the ability to resist impulsive responding upon presentation of alcohol or other drug cues (Assadi et al., 2009; Cools and D'Esposito, 2011).

In summary, our results support the possibility that genetic polymorphisms in the SNCA gene are associated with the degree of evoked BOLD response during exposure to the taste of alcohol in ROIs known to be rich in dopaminergic axon terminals. Future studies should aim to explore whether these findings are replicated in other samples and to define the specific effect of polymorphisms in these SNPs on dopamine neuron function.

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