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Cognitive Flexibility is Associated with *KIBRA* Variant and Modulated by Recent Tobacco Use

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The kidney and brain expressed protein gene (*KIBRA*) and the calsyntenin 2 gene (*CLSTN2*) are reportedly involved in synaptic plasticity. Single nucleotide polymorphisms (SNPs) rs17070145 (*KIBRA*) and rs6439886 (*CLSTN2*) have been found to affect memory performance measures. This study examined the association of *KIBRA* SNP rs17070145 and *CLSTN2* SNPs rs6439886 and rs17348572 (a nonsynonymous variant) with cognitive flexibility in 674 African Americans (AAs; 526 current smokers) and 419 European Americans (EAs; 318 current smokers). The subjects' cognitive flexibility was assessed using the Wisconsin Card Sorting Test. The effects on cognitive flexibility of sex, age, education, and tobacco recency (a possible mediator of gene effects in smokers), the three SNPs, and the interaction of each SNP with tobacco recency were analyzed using multivariate analysis of variance. In AAs, there were no main or interaction effects of the SNPs on cognitive flexibility. In EAs, the two *CLSTN2* SNPs showed no main effect on cognitive flexibility. However, among EAs, individuals with the *KIBRA* rs17070145 T allele made significantly more perseverative responses ($P = 0.002$) and perseverative errors ($P = 0.002$) than those with no T allele. Furthermore, among EAs with the rs17070145 T allele, current smokers made significantly fewer perseverative responses ($P < 0.001$) and perseverative errors ($P < 0.001$) than past smokers. Nongenetic factors (age, education, and tobacco recency) had substantial effects on cognitive flexibility in both AAs and EAs. We conclude that variation in *KIBRA* influences cognitive flexibility in a population-specific way, and that current smoking status moderates this effect.
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INTRODUCTION

Cognition is a set of high-level brain functions that vary substantially among individuals. The variability of cognition is largely attributable to genetic influence. Twin studies have demonstrated that the heritability is about 60% for general cognitive ability and about 50% for memory (McClearn *et al*, 1997). Moreover, heritability for working memory was estimated to be 33–64% (Ando *et al*, 2001; Wright *et al*, 2001; Chen *et al*, 2009) and heritability for episodic memory was shown to vary from 0 to 57% (Johansson *et al*, 1999; Taylor, 2007; Chen *et al*, 2009). Twin studies also indicated a high heritability (above 60%) for attention problems (Polderman *et al*, 2006). As cognition is a complex trait, it may also be modulated by environment and gene-environment interaction.

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Genes involved in synaptic signaling or plasticity have been implicated in cognitive variability. Recently, two genes have received particular attention for these effects. One is the WW and C2 domain containing 1 gene (*WWC1*or *KIBRA*) on chromosome 5q34-q35.2 and the other is the calsyntenin 2 gene (*CLSTN2*) on chromosome 3q23. *KIBRA* encodes the kidney and brain expressed protein (or KIBRA), which is highly expressed in the kidney and liver (Kremerskothen *et al*, 2003). As a postsynaptic scaffold protein connecting cytoskeletal and signaling molecule, KIBRA is also found in memory-related brain structures including the hippocampus and the temporal lobe (Johannsen *et al*, 2008; Yoshihama *et al*, 2009). KIBRA may function in memory performance through interaction with protein kinase Mzeta (PKMζ), which participates in synaptic plasticity and memory storage (Buther *et al*, 2004; Pastalkova *et al*, 2006; Shema *et al*, 2007). KIBRA and PKMζ colocalize in brain regions such as the hippocampal CA1, CA2, and the dentate gyrus (key regions for memory functions; Yoshihama *et al*, 2009). *CLSTN2* encodes the synaptic protein calsyntenin 2 or CLSTN2. This protein appears exclusively in the brain, with high levels in cortical

gamma-aminobutyric acid (GABA)ergic interneurons and in medial temporal lobe regions (Hintsch *et al*, 2002).

Variation in *KIBRA* and *CLSTN2* may covary with cognitive performance. Papassotiropoulos *et al* (2006) found that carriers of the *KIBRA* (rs17070145) T allele or the *CLSTN2* (rs6439886) T allele performed better on multiple episodic memory tasks than those homozygous for the C allele at either rs17070145 or rs6439886. Furthermore, using functional magnetic resonance imaging, they observed that hippocampal activation was significantly greater in *KIBRA* (rs17070145) T allele noncarriers than in T allele carriers during an episodic memory task. This implied that T allele noncarriers had poorer memory ability, requiring that their hippocampi had to work harder to accomplish the same memory task. Based on these findings, several other studies further examined the role of *KIBRA* and *CLSTN2* in cognitive function. Almeida *et al* (2008) also noticed that *KIBRA* SNP rs17070145 was associated with episodic memory. Schaper *et al* (2008) found that the influence of *KIBRA* SNP rs17070145 was restricted to hippocampal-related episodic memory. Nevertheless, inconsistent results have been reported as well. Nacmias *et al* (2008) found that individuals with the *KIBRA* (rs17070145) T allele performed more poorly on long-term memory tests than individuals with no T allele. Need *et al* (2008) found no association between SNP rs17070145 and multiple verbal memory tasks.

As memory impairment is a major component of Alzheimer's disease (AD), *KIBRA* variants were hypothesized to confer susceptibility to the development of AD. One study showed that the *KIBRA* (rs17070145) C allele was significantly associated with late-onset AD (Corneveaux *et al*, 2008). However, another study showed that the *KIBRA* (rs17070145) T allele was associated with an increased risk for very-late-onset AD (Rodriguez-Rodriguez *et al*, 2009). Compared to *KIBRA*, the association of *CLSTN2* variants and AD is less well studied. In addition to the findings of Papassotiropoulos *et al* (2006) mentioned above, Jacobsen *et al* (2009) reported that *CLSTN2* SNP rs6439886 had a significant main or interactive effect with prenatal or adolescent exposure to smoking on verbal or visuospatial memory. Moreover, Uhl *et al* (2008) reported that variation in *CLSTN2* was associated with smokers' ability to achieve and sustain abstinence from smoking.

As the relationship between *KIBRA* or *CLSTN2* and cognitive function is not clear, we examined whether variation in *KIBRA* or *CLSTN2* affects cognitive flexibility. Additionally, cognitive flexibility may be affected by tobacco use. There is evidence that nicotine administration can produce short-term enhancement of attention and memory (Maggio *et al*, 1998; Ernst *et al*, 2001) and smoking cessations can lead to acute impairment of verbal and working memory (Jacobsen *et al*, 2005). Therefore, we also analyzed the effect of smoking and gene-smoking interaction on cognitive flexibility.

MATERIALS AND METHODS

Subjects

A total of 674 unrelated African Americans (AAs) and 419 unrelated European Americans (EAs)—originally recruited for genetic association studies of drug or alcohol dependence (Zhang *et al*, 2009)—participated in this study. They were interviewed using an electronic version of the Semi-Structured Assessment for Drug Dependence and Alcoholism (SSADDA) instrument (Pierucci-Lagha *et al*, 2005; Pierucci-Lagha *et al*, 2007). Information on sex, age, years of education, and the recency of tobacco use was collected at the baseline interview. All 674 AAs and 419 EAs reported a lifetime history of tobacco use, which was quantitated as a tobacco recency score (1: last smoked within 2 weeks; 2: last smoked in the past 2–4 weeks; 3: last smoked in the past 1–6 months; 4: last smoked in the past 6–12 months; 5: last smoked over 1 year ago). The majority of AAs (526; 78.0%) were current tobacco users (based on last tobacco use $\leqslant$2 weeks), and among them, 404 had a lifetime DSM-IV (American Psychiatric Association, 1994) diagnosis of nicotine dependence (ND). A similar proportion of EAs (318; 75.9%) were current tobacco users, and among them, 260 had a lifetime DSM-IV diagnosis of ND. Subjects affected with major psychotic disorders (ie, schizophrenia, schizoaffective disorder, or bipolar disorder I) were excluded. Characteristics of the participants in this study are presented in Table 1. They were recruited at the University of Connecticut Health Center (Farmington, CT, USA) or the Yale University School of Medicine (APT Foundation, New Haven, CT, USA). The study protocol was approved by the institutional review board at each institution, and a certificate of confidentiality for the work was obtained from NIH (NIDA). Before study participation, all subjects provided written informed consent after receiving a complete description of the study.

Table 1 Characteristics of Study Subjects and Recency of Tobacco Use

	African Americans	European Americans
Number of subjects	674	419
Males (%)	354 (52.5%)	248 (59.2%)
Age (years ± SD)	41 (± 10)	40 (± 12)
Education (years ± SD)	12 (± 3)	13 (± 3)
Recency of Tobacco Use		
1 $\leqslant$2 weeks (current user)	526 (78.0%)	318 (75.9%)
2 2–4 weeks	4 (0.6%)	4 (0.9%)
3 1 month–6 months	6 (0.9%)	9 (2.1%)
4 6 months–1 year	10 (1.5%)	1 (0.2%)
5 >1 year	128 (19.0%)	87 (20.8%)
Nicotine dependence (%)	404 (59.9%)	260 (62.0%)

WCST Assessment of Cognitive Flexibility

Cognitive flexibility is the human ability to adapt one's cognitive processing strategies to face new and unexpected conditions in the environment (Canas *et al*, 2003). It is characterized by learning, memory, set shifting, etc. To evaluate whether cognitive flexibility is influenced by

specific genetic factors and/or tobacco use, we used the 128-card computerized version of the Wisconsin Card Sorting Test (WCST; Heaton *et al*, 1999). The WCST is a complex test that involves multiple cognitive processes (eg, problem solving, set shifting, working memory, and attention). During the test, subjects were required to match response cards to four stimulus cards on three dimensions (color, form, or number) by pressing one of four number keys (1–4) on the computer keyboard. The participant was required to determine which sorting principle was correct and when the principle would shift during the test. The computerized version of the WCST continues until all 128 cards are sorted, which differs from the traditional WCST in which the test ends after six correct categories are completed (Robinson *et al*, 1980).

In this study, three indices of the WCST were used to assess each individual's cognitive flexibility: percentage of perseverative responses (%PR), percentage of perseverative errors (%PE), and percentage of nonperseverative errors (%N-PE). Factor analysis of the WCST has shown that perseverative errors could be the most useful outcome measure in assessing executive function (Greve *et al*., 2005). Higher values of %PR, %PE, and/or %N-PE are indicative of poorer WCST performance and less cognitive flexibility.

DNA Sample and SNP Genotyping

In most cases, DNA was obtained from immortalized lymphoblastoma cell lines, but for a small number of subjects, DNA was obtained directly from blood or saliva. In addition to the two SNPs that have been studied previously (ie, rs17070145 in *KIBRA* intron 9 and rs6439886 in *CLSTN2* intron 1), we examined a rare nonsynonymous variant (rs17348572, Thr(C)/Ile(T)) in *CLSTN2* exon 7. SNPs were genotyped with a fluorogenic 5′ nuclease assay (TaqMan) method (Shi *et al*, 1999), using the ABI PRISM 7900HT Sequence Detection System (ABI, Foster City, CA, USA).

Statistical Analysis

The main effect of the three gene variants and their interactive effects with recent tobacco use on cognitive flexibility were analyzed in AAs and EAs separately. A multivariate analysis of variance was performed using the general linear model (GLM) procedure in the SPSS16.0 software package (SPSS Inc., Chicago, Illinois). WCST indices (%PR, %PE, and %N-PE) were treated as dependent variables, SNP genotypes as independent variables, and nongenetic factors (sex, age, tobacco recency, and years of education) as covariates. Interactive effects of genotypes and tobacco recency on cognitive flexibility were examined as well. Bonferroni corrections were used to avoid inflating type 1 error due to multiple testing. The influence of continuous variables (age and years of education) on cognitive flexibility was analyzed by correlational analyses in SPSS 16.0.

RESULTS

The influence of nongenetic factors on cognitive flexibility as measured by the three WCST indices (%PR, %PE, and %N-PE) is presented in Tables 2 and 3. Age was strongly inversely correlated with cognitive flexibility (including working memory and set shifting) in both AAs and EAs, such that older individuals made more perseverative responses (AAs: $r=0.235$, $P<0.001$; EAs: $r=0.200$, $P<0.001$), perseverative errors (AAs: $r=0.245$, $P<0.001$; EAs: $r=0.210$, $P<0.001$), and nonperseverative errors (AAs: $r=0.135$, $P<0.001$; EAs: $r=0.190$, $P<0.001$). Years of education were directly correlated with cognitive flexibility in both AAs (%PR: $r=-0.114$, $P=0.003$; %PE: $r=-0.123$, $P=0.001$; %N-PE: $r=-0.164$, $P<0.001$) and EAs (%PR: $r=-0.094$, $P=0.054$; %PE: $r=-0.096$, $P=0.056$; %N-PE: $r=-0.092$, $P=0.061$), but the effect of education on cognitive flexibility was stronger in AAs. Well-educated subjects made fewer perseverative responses, perseverative errors, and nonperseverative errors. Recent tobacco use was associated with poorer performance on two WCST domains (ie, significantly greater perseverative responses and perseverative errors) in AAs (%PR: $F_{(1,673)}=7.70$, $P=0.006$; %PE: $F_{(1,673)}=6.94$, $P=0.008$). In contrast, recent tobacco use was associated with slightly better performance on two WCST domains (ie, fewer perseverative responses and perseverative errors) in EAs (%PR: $F_{(1,418)}=3.72$, $P=0.055$; %PE: $F_{(1,418)}=2.26$, $P=0.133$). Other nongenetic factors (sex and nicotine dependence) did not show a significant effect on cognitive flexibility in either AAs or EAs.

There were no significant main effects of the three SNPs (*KIBRA* SNP rs17070145 and *CLSTN2* SNPs rs6439886 and rs17348572; Table 4) or interactions of the SNPs with recent tobacco use (data not shown) on cognitive flexibility in AAs. However, in EAs, SNP rs17070145 (*KIBRA*) significantly influenced two domains of cognitive flexibility (%PR: $F_{(2,412)}=5.14$, $P=0.006$; %PE: $F_{(2,412)}=5.09$, $P=0.006$). Carriers of the rs17070145 T allele made significantly more perseverative responses ($F_{(1,412)}=9.75$, $P=0.002$) and perseverative errors ($F_{(1,412)}=9.78$, $P=0.002$) than those homozygous for the C allele (Table 5). These significant P values can withstand Bonferroni correction (at the level of $\alpha=0.05/(3*3)=0.006$, with three SNPs tested for their effect on three WCST domains). Nonperseverative errors (one of the three WCST domains examined in this study) were not significantly affected by SNP rs17070145 in EAs.

To determine whether recent tobacco use moderates the genetic effect of SNP rs17070145 in EAs, we examined the interaction of this SNP with recent tobacco use. As shown in Figure 1a, EA subjects with the rs17070145 T allele (genotypes CT and TT) who were current smokers had markedly better performance than past smokers on two WCST measures (%PR: $F_{(1,206)}=25.03$, $P<0.001$; %PE: $F_{(1,206)}=23.41$, $P<0.001$). In EA subjects homozygous for the rs17070145 C allele (genotype: CC), cognitive flexibility did not differ as a function of current tobacco use (Figure 1b). As in AA subjects, the two *CLSTN2* SNPs (rs6439886 and rs17348572) showed neither a main effect (Table 5) nor an interactive effect with current tobacco use on cognitive flexibility (data not shown).

DISCUSSION

Human cognition is a polygenic trait. Genes participating in synaptic signaling or plasticity in brain regions such as the

Table 2 Influence of Nongenetic Factors on Cognitive Flexibility Measured by Wisconsin Card Sorting Tests in African Americans (AAs)

		%PR	%PE	%N-PE
Effects of continuous nongenetic variables (correlation analyses)				
Age		**$P<0.001$** ($r=0.235$)	**$P<0.001$** ($r=0.245$)	**$P<0.001$** ($r=0.135$)
Education		**$P=0.003$** ($r=-0.114$)	**$P=0.001$** ($r=-0.123$)	**$P<0.001$** ($r=-0.164$)
	n	**%PR (mean ± SEM)**	**%PE (mean ± SEM)**	**%N-PE (mean ± SEM)**
Effects of categorical nongenetic variables (multivariate GLM procedure)				
Sex				
Male	354	21.8 ± 0.8	19.3 ± 0.6	17.9 ± 0.6
Female	320	22.2 ± 0.8	19.4 ± 0.7	18.8 ± 0.6
		$F_{(1,673)}=0.06$, $P=0.800$	$F_{(1,673)}=0.01$, $P=0.909$	$F_{(1,673)}=1.17$, $P=0.280$
Tobacco recency[a]				
⩽2 weeks	526	22.9 ± 0.7	20.0 ± 0.5	18.4 ± 0.5
>2 weeks	148	18.9 ± 1.3	17.1 ± 1.0	18.1 ± 0.9
		$F_{(1,673)}=7.70$, $P=0.006$	**$F_{(1,673)}=6.94$, $P=0.008$**	$F_{(1,673)}=0.05$, $P=0.817$
Nicotine dependence[b]				
ND−	270	21.3 ± 0.9	19.0 ± 0.7	18.0 ± 0.7
ND+	404	22.5 ± 0.8	19.6 ± 0.6	18.5 ± 0.5
		$F_{(1,673)}=0.97$, $P=0.324$	$F_{(1,673)}=0.52$, $P=0.472$	$F_{(1,673)}=0.23$, $P=0.629$

[a]Difference in cognitive flexibility between recent tobacco users (⩽2 weeks) and former tobacco users (>2 weeks).
[b]Difference in cognitive flexibility between subject with nicotine dependence (ND+) and without ND (ND−).
% PR, percentage of perseverative responses; %PE, percentage of perseverative errors; %N-PE, percentage of nonperseverative errors; *r*, correlation coefficient.
P values that are in bold indicate statistical significance.

prefrontal cortex (PFC) and the hippocampus (likely to be the anatomic brain structures related to memory) have been implicated in cognition. A number of such genes have been identified, including the brain-derived neurotrophic factor gene (*BDNF*; Egan *et al*, 2003), the serotonin receptor 2A gene (5-*HT2α*; de Quervain *et al*, 2003), the catechol-O-methyltransferase gene (*COMT*; Caldu *et al*, 2007), the dopamine receptor D1 gene (*DRD1*; Rybakowski *et al*, 2005), the dopamine transporter gene (*DAT*; Caldu *et al*, 2007), the prion protein gene (*PRNP*; Papassotiropoulos *et al*, 2005), the Reelin gene (*RELN*; Wedenoja *et al*, 2008), and the kallikrein 8 gene (*KLK8*; Lu *et al*, 2009). Recently, two new genes (*KIBRA* and *CLSTN2*) were added to this group due to their impact on memory performance.

The aim of this study was to increase the specificity of the findings on the association of *KIBRA* and *CLSTN2* with cognition. Different from other studies, we assessed cognitive function with the WCST. Three major findings were obtained. First, as observed in most previous studies, variation within *KIBRA* was associated with cognitive function, though we found evidence of a different mechanism of the effect. In the paper by Papassotiropoulos *et al* (2006), the *KIBRA* rs17070145 T allele was reported to have a beneficial effect on episodic memory performance. In contrast, in this study, the *KIBRA* rs17070145 T allele was associated with less cognitive flexibility (ie, significantly more perseverative responses and perseverative errors) in EAs (Table 5). A plausible explanation for the discrepancy in these results is that different aspects of cognition were assessed in the studies, which may reflect the function of different memory-related brain regions. Papassotiropoulos *et al* (2006) evaluated episodic memory of subjects using 5-min and 24-h delayed free recall performance, whereas in this study, multiple cognitive functions (problem solving, set shifting, working memory, and attention) of subjects were examined by the WCST. The working memory and set shifting (two major aspect of cognitive flexibility) are controlled mainly by the prefrontal cortex and they act jointly to enable the individual to adapt to a changing environment (Konishi *et al*, 1999). We hypothesize that the *KIBRA* rs17070145 T allele differentially modulates memory-related activities in the hippocampus (ie, where it has a beneficial effect on long-lasting memory) and the prefrontal cortex (ie, where it compromises short-term memory or working memory). Additionally, in this study, neither *CLSTN2* SNP rs6439886 (in intron 1) nor *CLSTN2* SNP rs17348572 (a nonsynonymous variant in exon 7) was found to be associated with significantly altered cognitive flexibility in AAs or EAs (Tables 4 and 5). These findings differ from those obtained by Papassotiropoulos *et al* (2006) and Jacobsen *et al* (2009). The inconsistent results may reflect different genetic effects of variation in *CLSTN2* (and *KIBRA*) on different memory-related phenotypes (episodic

Table 3 Influence of Nongenetic Factors on Cognitive Flexibility Measured by Wisconsin Card Sorting Tests in European Americans (EAs)

		%PR	%PE	%N-PE
Effects of continuous nongenetic variables (correlation analyses)				
Age		**$P<0.001$** ($r=0.200$)	**$P<0.001$** ($r=0.210$)	**$P<0.001$** ($r=0.190$)
Education		$P=0.054$ ($r=-0.094$)	$P=0.056$ ($r=-0.096$)	$P=0.061$ ($r=-0.092$)
	n	**%PR (mean ± SEM)**	**%PE (mean ± SEM)**	**%N-PE (mean ± SEM)**
Effects of categorical nongenetic variables (multivariate GLM procedure)				
Sex				
Male	248	16.4 ± 0.8	14.7 ± 0.6	14.1 ± 0.6
Female	171	16.3 ± 1.0	14.3 ± 0.8	14.1 ± 0.7
		$F_{(1,418)}=0.01$, $P=0.933$	$F_{(1,418)}=0.05$, $P=0.816$	$F_{(1,418)}=0.00$, $P=0.992$
Tobacco recency[a]				
⩽2 weeks	318	15.6 ± 0.7	14.2 ± 0.6	14.3 ± 0.5
>2 weeks	101	18.6 ± 1.3	16.0 ± 1.0	13.4 ± 1.0
		$F_{(1,418)}=3.72$, $P=0.055$	$F_{(1,418)}=2.26$, $P=0.133$	$F_{(1,418)}=0.66$, $P=0.419$
Nicotine dependence[b]				
ND−	159	17.6 ± 1.0	15.5 ± 0.8	13.2 ± 0.8
ND+	260	15.5 ± 0.8	14.0 ± 0.6	14.6 ± 0.6
		$F_{(1,418)}=2.58$, $P=0.109$	$F_{(1,418)}=2.16$, $P=0.143$	$F_{(1,418)}=1.99$, $P=0.159$

[a]Difference in cognitive flexibility between recent tobacco users (⩽2 weeks) and former tobacco users (>2 weeks).
[b]Difference in cognitive flexibility between subject with nicotine dependence (ND+) and without ND (ND−).
%PR, percentage of perseverative responses; %PE, percentage of perseverative errors; %N-PE, percentage of nonperseverative errors; *r*, correlation coefficient.
P values that are in bold indicate statistical significance.

Table 4 Association of SNPs rs17070145 (*KIBRA*), rs6439886 (*CLSTN2*), and rs17348572 (*CLSTN2*) with Cognitive Flexibility in African Americans (AAs)

	n	**%PR (mean ± SEM)**	**%PE (mean ± SEM)**	**%N-PE (mean ± SEM)**
rs17070145				
CC	102	21.7 ± 1.5	19.4 ± 1.2	17.8 ± 1.1
CT	315	21.6 ± 0.8	18.9 ± 1.6	18.4 ± 0.6
TT	251	22.4 ± 1.0	19.6 ± 0.7	18.4 ± 0.7
		$F_{(2,667)}=0.25$, $P=0.781$	$F_{(2,667)}=0.27$, $P=0.760$	$F_{(2,667)}=0.14$, $P=0.870$
rs6439886				
CC	96	23.2 ± 1.5	20.0 ± 1.2	17.3 ± 1.1
CT	221	22.4 ± 1.0	19.6 ± 0.8	18.4 ± 0.7
TT	350	21.3 ± 0.8	18.9 ± 0.6	18.6 ± 0.6
		$F_{(2,666)}=0.71$, $P=0.492$	$F_{(2,666)}=0.47$, $P=0.627$	$F_{(2,666)}=0.55$, $P=0.578$
rs17348572				
CC+CT[a]	29	19.8 ± 2.8	17.5 ± 2.2	18.4 ± 2.0
TT	626	22.2 ± 0.6	19.5 ± 0.5	18.4 ± 0.4
		$F_{(1,654)}=0.68$, $P=0.410$	$F_{(1,654)}=0.80$, $P=0.372$	$F_{(1,654)}=0.00$, $P=0.997$

[a]Only one subject had *CLSTN2*17348572 CC genotype.
%PR, percentage of perseverative responses; %PE, percentage of perseverative errors; %N-PE, percentage of nonperseverative errors.

Table 5 Association of SNPs rs17070145 (*KIBRA*), rs6439886 (*CLSTN2*) and rs17348572 (*CLSTN2*) with Cognitive Flexibility in European Americans (EAs)

	n	%PR (mean ± SEM)	%PE (mean ± SEM)	%N-PE (mean ± SEM)
rs17070145 (*KIBRA*)				
CC	206	16.4 ± 1.6	14.5 ± 1.3	12.9 ± 1.3
CT	171	21.8 ± 1.6	18.7 ± 1.3	14.8 ± 1.3
TT	36	19.3 ± 3.1	15.6 ± 2.4	12.3 ± 2.4
		$F_{(2,412)} = 5.14$, $P = 0.006$	**$F_{(2,412)} = 5.09$, $P = 0.006$**	$F_{(2,412)} = 1.00$, $P = 0.370$
CC	206	16.4 ± 1.6	14.5 ± 1.3	12.9 ± 1.3
CT+TT	207	21.8 ± 1.6	18.6 ± 1.2	14.2 ± 1.2
		$F_{(1,412)} = 9.75$, $P = 0.002$	**$F_{(1,412)} = 9.78$, $P = 0.002$**	$F_{(1,412)} = 0.58$, $P = 0.446$
rs6439886 (*CLSTN2*)				
GG	28	14.1 ± 2.4	12.5 ± 1.9	11.2 ± 1.8
AG	111	16.5 ± 1.2	14.8 ± 0.9	15.0 ± 0.9
AA	275	16.5 ± 0.8	14.7 ± 0.6	14.1 ± 0.6
		$F_{(2,413)} = 0.44$, $P = 0.643$	$F_{(2,413)} = 0.68$, $P = 0.506$	$F_{(2,413)} = 1.81$, $P = 0.666$
rs17348572 (*CLSTN2*)				
CT[a]	44	14.5 ± 1.9	13.2 ± 1.5	13.8 ± 1.4
TT	370	16.6 ± 0.6	14.8 ± 0.5	14.2 ± 0.5
		$F_{(1,413)} = 1.10$, $P = 0.296$	$F_{(1,413)} = 1.04$, $P = 0.309$	$F_{(1,413)} = 0.8$, $P = 0.777$

[a]No subject had *CLSTN2* rs17348572 CC genotype.
%PR, percentage of perseverative responses; %PE, percentage of perseverative errors; %N-PE, percentage of nonperseverative errors.
P values that are in bold indicate statistical significance.

memory performance measured in the previous studies and working memory performance measured in this study). In addition, the discrepancies may be due to different smoking status of the study participants included in previous and current studies (given the high prevalence rate of smoking in the general population). Genotype × tobacco exposure (or withdrawal) interactions can exert a striking role in cognitive performance (Loughead *et al*, 2008). This study, in contrast to the previous one, took into consideration the interactive effect of smoking and genetics on cognitive flexibility. Therefore, it is not unusual to obtain these different results.

Second, the genetic effect of *KIBRA* on cognitive flexibility was population specific. As shown in Tables 4 and 5, the *KIBRA* (rs17070145) T allele appeared to be a risk factor for cognitive flexibility in EAs. However, this allele did not show a noticeable effect on cognitive flexibility in AAs. As this SNP is located in intron 9 of *KIBRA*, it is unlikely that it represents a causal variant with a substantial effect on cognitive flexibility. Possibly, SNP rs17070145 is in close linkage disequilibrium (LD) with a functional variant that can change the expression level of *KIBRA*, leading to altered cognitive flexibility. However, as LD between SNP rs17070145 and other potentially functional variants in *KIBRA* may vary by population, the genetic effect of SNP rs17070145 may be evident in one population but not another. In addition, the allele frequency of the T allele of SNP 17070145 was significantly higher in AAs (~61%) than in EAs (~29%). The significant difference in *KIBRA* (rs17070145) T allele frequency is consistent with a population-specific effect of *KIBRA* variants.

Third, the influence of recent smoking on cognitive flexibility was population specific, and recent smoking appeared to offset the genetic effect of the deleterious *KIBRA* variant on cognitive flexibility in EAs. When both AAs and EAs were examined jointly (irrespective of genotype information), no difference in cognitive flexibility was seen between current tobacco users (tobacco use $\leqslant 2$ weeks) and past tobacco users (tobacco use > 2 weeks; data not shown). However, we found a population-specific effect of recent smoking on cognitive flexibility when we examined AAs and EAs separately. Although recent smoking was associated with significantly less cognitive flexibility (ie, more perseverative responses and perseverative errors) in AAs (Table 2), it was associated with greater cognitive flexibility (ie, fewer perseverative responses and perseverative errors) in EAs (Table 3). This result may partially explain the findings by Vega and Gil (2005) of an ethnic/racial difference in rates and progress of tobacco use. In a 10-year study, these investigators found that AAs were less likely than EAs and Latinos to begin smoking in early adolescence and were least likely to be smokers as young adults, whereas EAs were most likely to still be smoking at 20 years of age. This is consistent with a negative effect of smoking on cognitive flexibility in AAs that may deter AAs from smoking, but a positive effect of smoking on cognitive flexibility in EAs may attract and sustain EA smokers. An interactive effect of variation in *KIBRA* and tobacco recency on cognitive flexibility was observed in EAs only. Although those EA subjects with the *KIBRA* (rs17070145) T allele had significantly less cognitive flexibility (ie, more perseverative responses and perseverative errors) than EAs without the T

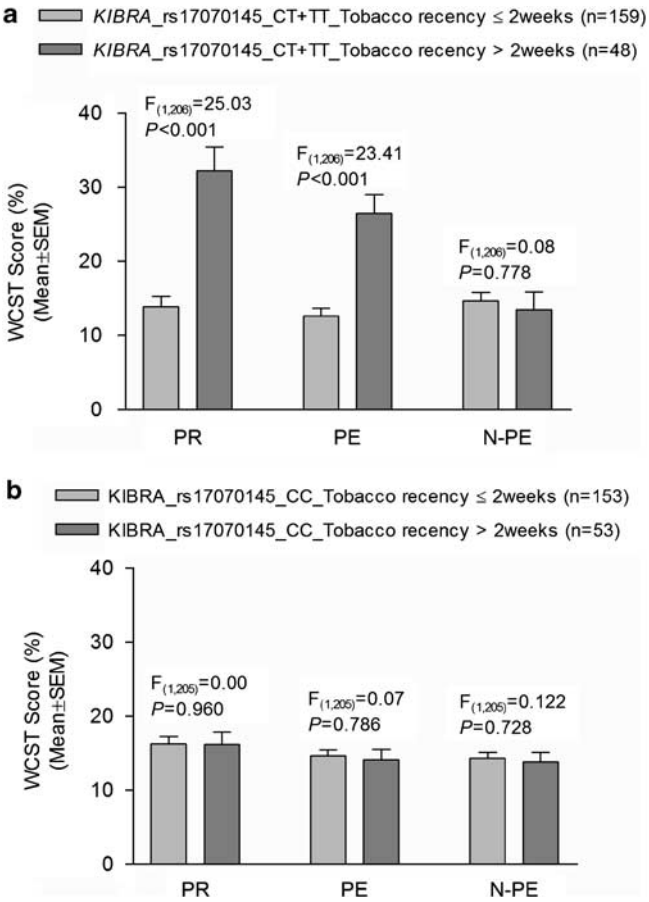

Figure 1 Interactive effects of *KIBRA* SNP rs17070145 and tobacco recency on cognitive flexibility in European Americans (EAs). (a) Interactive effects of rs17070145 genotypes CT + TT and tobacco recency on cognitive flexibility. (b) Interactive effects of rs17070145 genotype CC and tobacco recency on cognitive flexibility. WCST Score (%) (mean ± SEM): Wisconsin Cart Sorting Test Score (%) (mean ± SEM) obtained by the general linear model (GLM) multivariate analysis of variance; %PR: percentage of perseverative responses; %PE: percentage of perseverative errors; %N-PE: percentage of nonperseverative errors.

allele (Table 5), current smokers failed to show this detrimental effect (Figure 1). This finding implies that nicotine can improve cognitive flexibility (eg, attention, working memory, and set shifting) in EA subjects whose cognitive performance is diminished by certain risk gene variants such as the *KIBRA* (rs17070145) T allele.

Additionally, this study indicated that cognitive flexibility was not only moderated by specific genetic factors, but also by nongenetic factors. One nongenetic factor was age, an effect that is well known. Another nongenetic factor was education. More education is correlated with better decision making, problem solving, and a higher general cognitive function. Therefore, when analyzing genetic effects on cognitive flexibility, the confounding effect of nongenetic factors such as age and education cannot be ignored. This study has several strengths. First, to our knowledge, it is the largest study to examine the effect of genes on specific aspects of cognitive function. Second, the WCST is the most frequently used measure of cognitive flexibility. It is a complex test that draws on various components of executive function. The use of three major domains (perseverative responses, perseverative errors, and nonperseverative errors) of the WCST provides a measure of different aspects of cognitive flexibility. An increase in the number of perseverative errors (resulting from a poor working memory) has been associated with frontal lobe dysfunction (Monchi *et al*, 2001). Moreover, a relatively greater increase in perseverative versus nonperseverative errors may occur either when impairments in working memory are severe or cognitive inflexibility is present (Hartman *et al*, 2003). Third, we used the percentage of WCST responses or errors to assess cognitive flexibility, rather than the absolute number of WCST responses or errors. The percentage of WCST responses or errors may more accurately reflect the difference in cognitive flexibility among individual subjects (Rybakowski *et al*, 2005).

The major drawback of this study was that only one variant in *KIBRA* and two variants in *CLSTN2* were analyzed for their association with cognitive flexibility. This decision was based on our wish to extend previous observations and avoid excessive multiple testing. Both *KIBRA* and *CLSTN2* are large genes. *KIBRA*, which is about 180 kb long, has 23 exons, and *CLSTN2*, which is about 630 kb, has 17 exons. SNPs in *KIBRA* or *CLSTN2* are not in substantial LD; *KIBRA* SNP rs17070145 (or *CLSTN2* SNPs rs6439886 and rs17348572) cannot fully capture the genetic information of other SNPs in *KIBRA* (or *CLSTN2*). Need *et al* (2008) identified a *KIBRA* SNP that was weakly associated with delayed recall in the Auditory Verbal Learning Test (AVLT) task ($P = 0.03$) but not in LD with SNP rs17070145. Thus, increasing the density of markers in *KIBRA* or *CLSTN2* could increase the chance of identifying one or more loci that affect cognitive flexibility, but only findings with very high statistical significance would survive Bonferroni correction, ie, there would be a marked reduction in power. Another weakness of this study is that there were no data available for current exposure to nicotine on the day of testing. Thus, it is not known whether immediate nicotine exposure has a stronger effect on cognition than past nicotine exposure (within 2 weeks). Additionally, current smokers under abstinence may experience nicotine withdrawal, leading to deleterious effect on cognitive performance. However, this problem was not tackled in this study.

In summary, this work supports an association of a *KIBRA* variant with cognitive flexibility, though differently than previously reported and in a population-specific manner. To understand completely the potential role of *KIBRA* or *CLSTN2* variants in cognitive flexibility, additional genetic association studies using a fine mapping strategy are warranted.

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