Published in final edited form as:
Hum Genet. 2009 June ; 125(5-6): 605–613. doi:10.1007/s00439-009-0647-4.

Population admixture modulates risk for alcohol dependence

Lingjun Zuo,
Department of Psychiatry, Yale University School of Medicine, VA Psychiatry 116A2, 950 Campbell Avenue, West Haven, CT 06516, USA joel.gelernter@yale.edu

Lingjun.Zuo@yale.edu

VA Connecticut Healthcare System, West Haven Campus, West Haven, CT, USA

Xingguang Luo,
Department of Psychiatry, Yale University School of Medicine, VA Psychiatry 116A2, 950 Campbell Avenue, West Haven, CT 06516, USA joel.gelernter@yale.edu

VA Connecticut Healthcare System, West Haven Campus, West Haven, CT, USA

Jennifer B. Listman,
Department of Anthropology, New York University, New York, NY, USA

Henry R. Kranzler,
Department of Psychiatry, University of Connecticut School of Medicine, Farmington, CT, USA

Shuang Wang,
Department of Biostatistics, Mailman School of Public Health, Columbia University, New York, NY, USA

Raymond F. Anton,
Department of Psychiatry and Behavioral Sciences, Medical University of South Carolina, Charleston, SC, USA

Hilary P. Blumberg,
Department of Psychiatry, Yale University School of Medicine, VA Psychiatry 116A2, 950 Campbell Avenue, West Haven, CT 06516, USA joel.gelernter@yale.edu

VA Connecticut Healthcare System, West Haven Campus, West Haven, CT, USA

Murray B. Stein,
Departments of Psychiatry and Family and Preventive Medicine, University of California, La Jolla, San Diego, CA, USA

Godfrey D. Pearlson,
Department of Psychiatry, Yale University School of Medicine, VA Psychiatry 116A2, 950 Campbell Avenue, West Haven, CT 06516, USA joel.gelernter@yale.edu

Jonathan Covault,
Department of Psychiatry, University of Connecticut School of Medicine, Farmington, CT, USA

Dennis S. Charney,
Department of Psychiatry, Mount Sinai School of Medicine, New York, NY, USA

Daniel P. van Kammen,
Clinical Development, ACADIA Pharmaceuticals, San Diego, CA, USA

Lawrence H. Price,
Department of Psychiatry and Human Behavior, Butler Hospital, Warren Alpert Medical School of Brown University, Providence, RI, USA

Jaakko Lappalainen,

Department of Psychiatry, Yale University School of Medicine, VA Psychiatry 116A2, 950 Campbell Avenue, West Haven, CT 06516, USA joel.gelernter@yale.edu

VA Connecticut Healthcare System, West Haven Campus, West Haven, CT, USA

Joyce Cramer,
Department of Psychiatry, Yale University School of Medicine, VA Psychiatry 116A2, 950 Campbell Avenue, West Haven, CT 06516, USA joel.gelernter@yale.edu

VA Connecticut Healthcare System, West Haven Campus, West Haven, CT, USA

John H. Krystal, and
Department of Psychiatry, Yale University School of Medicine, VA Psychiatry 116A2, 950 Campbell Avenue, West Haven, CT 06516, USA joel.gelernter@yale.edu

VA Connecticut Healthcare System, West Haven Campus, West Haven, CT, USA

Joel Gelernter
Department of Psychiatry, Yale University School of Medicine, VA Psychiatry 116A2, 950 Campbell Avenue, West Haven, CT 06516, USA joel.gelernter@yale.edu

VA Connecticut Healthcare System, West Haven Campus, West Haven, CT, USA

Department of Genetics, Yale University School of Medicine, New Haven, CT, USA

Department of Neurobiology, Yale University School of Medicine, New Haven, CT, USA

Abstract

The admixture of different ancestral populations in America may have important implications for the risk for psychiatric disorders, as it appears to have for other medical disorders. The present study investigated the role of population admixture in risk for several psychiatric disorders in European-Americans (EAs) and African-Americans (AAs). This is a multisite study with 3,792 subjects recruited from across the United States, including 3,119 EAs and 673 AAs. These subjects included healthy controls and those with substance dependence (SD) (including alcohol dependence (AD), cocaine dependence, and opioid dependence), social phobia, affective disorders, and schizophrenia. In addition, DNA was included from 78 West Africans. The degree of admixture for each subject was estimated by analysis of a set of ancestry-informative genetic markers using the program STRUCTURE, and was compared between cases and controls. As noted previously, the degree of admixture in AAs was higher than EAs. In EAs, the degree of admixture (with African ancestry) was significantly lower in patients with SD (mainly AD) than controls ($P = 0.009$ for SD; $P = 0.008$ for AD). This finding suggests that population admixture may modulate risk for alcohol dependence. Population admixture might protect against alcohol dependence by increasing average heterozygosity and reducing the risk of deleterious recessive alleles. We cannot exclude the possibility that the results might have been influenced by selection bias due to the multisite nature of the study.

Introduction

Psychiatric disorders are multifactorial and genetically complex. They are influenced by both genetic and environmental factors, which can also be differentiated into internal factors (e.g., genetic, inborn, and biochemical factors) and external factors (e.g., trauma, infection of CNS, and stress). Environmental factors, including psychosocial, cultural, and socioeconomic factors, have long been accepted as influencing the risk for psychiatric disorders. In addition, family, twin, and adoption studies demonstrate that genetic factors are important components of the causes for psychiatric disorders. The universal prevalence of psychiatric disorders in different founding populations supports the idea that a biological basis underlies the cause for these diseases. The diversity of prevalence of psychiatric disorders in admixed populations

might be attributable in part to admixture. The focus of the present study is the effects of population admixture on the risk of substance dependence (SD), schizophrenia, affective disorders (AFD), and social phobia (SP).

Current populations are often divided by researchers for the purposes of medical studies into six major continental ancestral populations, i.e., African, European, Middle Eastern, Central/South Asian, East Asian, and Oceania (Rosenberg et al. 2002), although these populations are not entirely distinct (Hunley et al. 2009; Rosenberg et al. 2005). As a result of migrations during the past 500 years, admixture between these ancestral populations has occurred on a large scale in many parts of the world while gene flow was previously more restricted between them. In pre-Columbian times, indigenous or Amerindian peoples were the only populations in the United States. After Columbus arrived in the New World in 1492, Europeans began to migrate to North America, leading to their descendants becoming the largest modern US population, EA (69.1%). At approximately the same time, Africans (mainly from West Africa) were brought to North America as slaves [and their descendants as well as more recent immigrants from the West Indies and all parts of Africa are now the major ancestral group for the third largest modern US population, AA (12.1%)]; Hispanics (admixed mainly by Spanish-Europeans, Africans and Native Americans) constitute the second largest modern US population (12.5%); these majority and other minority populations continue to immigrate to the US. The migrations brought new opportunities for population admixture, and today, most modern populations in the US are admixed. Ancestry proportions vary among individuals in these admixed populations. This variation in the individual degree of admixture has many potential causes, including the number of generations during the admixture history and continued gene flow from the ancestral populations. Population-based differences in disease risk have been observed and admixture between high-risk and low-risk populations has occurred. The two most common genetically distinguishable populations in the US, EAs and AAs, have their origins in ancestral populations that migrated from multiple geographic regions of Europe and Africa, respectively. As reported previously (Hoggart et al. 2003; Parra et al. 1998), AAs are admixed primarily with Europeans, and some EA individuals have (usually small) proportions of African ancestries in addition to gene flow into these two populations from other major continental ancestral populations. The degree of European admixture in African-American populations varies greatly by geographical region, ranging from 6.8 to 22.5% with a standard error of the mean (SEM) of 1.3–2.7% (Parra et al. 1998). The estimated African/non-African ancestry crossover rate of 2.4 per 100 cM suggests that the average time since admixture between Africans and non-Africans is at least five generations, consistent with historical evidence (Molokhia et al. 2003). The extent of African admixture in EAs is around 2%. Moreover, there is considerable evidence of admixture *within* European populations (Wellcome Trust Case Control Consortium 2007).

The proportion of EA admixture in AA populations is much higher than the proportion of AA admixture in EA populations, however there can only be one rate of mixture between the two ancestral populations. This discrepancy is a result of a self-imposed retention of the one drop rule under which, individuals with some African ancestry are often identified, by themselves, their parents, and others as AA, regardless of their % African ancestry (Roth 2005; Waters 1991). This is borne out both in studies of census data and genetic data and is a phenomenon specific to the US (Goncalves et al. 2007). It has also been shown that European admixture in African Americans is sex-biased with up to four times greater male than female European gene flow into African American populations (Hammer et al. 2006; Lind et al. 2007; McLean et al. 2003; Parra et al. 2001).

In spite of continuing controversy, self-identification of population group is widely acceptable clinically, but is insufficient to evaluate admixture effects. Hanis et al. first used a genetic method to measure the degree of admixture in Mexican-Americans (Hanis et al. 1986).

McKeigue suggested that 2,000 SNPs, each with information content for ancestry (f) > 0.3, can extract 70–90% of the ancestry information for each locus to measure admixture between EAs and AAs, when admixture has occurred 2–10 generations earlier (McKeigue 1998). Rosenberg et al. demonstrated that 377 short tandem repeats (STRs) were sufficient to measure admixture among 52 world populations (Rosenberg et al. 2002). Hoggart et al. used 32 ancestry-informative markers (AIMs) to measure admixture between African-Caribbeans, Hispanics and AAs (Hoggart et al. 2003). Our previous studies demonstrated that 39 AIMs, including 1 SNP (FY) and 38 STRs, were sufficient for measuring admixture between EAs and AAs (Luo et al. 2005b; Stein et al. 2004; Yang et al. 2005), and thus these AIMs (excluding those two disease-associated STRs) were used in the present study (see "Methods").

Population-based genetic association studies are vulnerable to admixture effects. Population admixture could result in a spurious observation of association between gene and disease, violation of Hardy–Weinberg equilibrium (HWE) in the genotype frequency distribution, or LD between unlinked loci. Even when the statistical analysis is conducted separately within EAs and AAs, population stratification could still have effects on the analysis, because admixture within these two populations affects observation of LD block size, HWD tests, or phenotype associations. Several studies reported that, after controlling for admixture effects, relationships between risk for diseases and candidate genes were dramatically changed. For example, before controlling for admixture effects, 11, 7 and 5 markers of 32 AIMs (in AAs, African-Caribbeans and Hispanics, respectively) were shown to be associated with skin pigmentation. However, after controlling for admixture effects, only 2, 3 and 1, respectively, of 32 AIMs showed positive associations with skin pigmentation ($P < 0.05$) (Hoggart et al. 2003).

We also reported that 19 of 38 STRs were associated with alcohol dependence before controlling for admixture effects in a mixed EA and AA sample, but only two of them were associated with disease after controlling for admixture effects ($P < 0.05$) (Luo et al. 2005b). LD between these AIMs and known risk genes has been excluded. Thus, it is possible that there are correlations between these AIMs and risk for diseases that obscure the associations between risk for diseases and the candidate genes. The theoretical basis for studying the direct relationship of disease risk to individual admixture was set out some years ago (Chakraborty and Weiss 1986). Subsequently, Hanis et al. reported that the degree of admixture was marginally related to gallbladder disease in Mexican-Americans, with individuals having the most Amerindian affinity being at increased risk (Hanis et al. 1986). Molokhia et al. reported that the risk for systemic lupus erythematosus (SLE) increased with the degree of West African admixture in a Caribbean population (Molokhia et al. 2003). Brutsaert et al. found Quechua ancestry (as measured by Native American (NA) versus European ancestry proportion) was significantly inversely related to sustained hypoxic ventilatory response measured at sea level, and exercise ventilation measured at high altitude (Brutsaert et al. 2005). Studies examining the relationship between body mass index and European Ancestry in admixed American populations including AA, Hispanic, and NA have had conflicting results (Fernandez et al. 2003; Klimentidis et al. 2009; Reiner et al. 2007). The present study examined the relationship of psychiatric disease risk to the proportionate admixture of individuals in EA and AA populations.

Materials and methods

Subjects

A total of 3,870 subjects (3,119 self-reported European-Americans (EAs), 673 self-reported African-Americans (AAs) and 78 West Africans) were included in the present study, including healthy controls and subjects with substance (alcohol, cocaine, and/or opioid) dependence, social phobia, AFDs, or schizophrenia. The sample sizes, sex distribution, and mean ages of

these subjects are shown in Table 1. The cases met lifetime DSM-III-R or DSM-IV criteria (American Psychiatric Association 1987,1994) for these psychiatric disorders. DSM-III-R and DSM-IV criteria are highly consistent in the diagnosis of psychiatric disorders (e.g., cross system agreement ≥ 92% for alcohol dependence) (Rounsaville et al. 1993), so subjects diagnosed by these two instruments were combined in our study. Diagnoses were made using the Structured Clinical Interview for DSM-III-R (SCID) (Spitzer et al. 1992) or DSM-IV, the computerized Diagnostic Interview Schedule for DSM-III-R (C-DIS-R) (Blouin et al. 1988) or DSM-IV, the Semi-Structured Assessment for Drug Dependence and Alcoholism (SSADDA) (Gelernter et al. 2005;Pierucci-Lagha et al. 2005), or a checklist comprises DSM-III-R or DSM-IV symptoms. The control subjects were screened using an unstructured psychiatric history, the SCID, the C-DIS-R, or the Schedule for Affective Disorders and Schizophrenia (SADS) (Spitzer and Endicott 1975), to exclude major axis I mental disorders, including alcohol or drug abuse or dependence, psychotic disorders (including schizophrenia or schizophrenia-like disorders), AFDs, and major anxiety disorders.

The subjects were recruited at the University of Connecticut Health Center, the Connecticut Mental Health Center, the VA Connecticut Healthcare System-West Haven Campus, 14 other Veterans Affairs medical centers (Rosenheck et al. 1997), the Yale-New Haven Medical Center, the Medical University of South Carolina, the University of California at San Diego, Mount Sinai School of Medicine, Brown University School of Medicine, and Pennsylvania State University, or from the community (via word of mouth and advertisements). All subjects signed informed consent, which was approved by the IRB at each of the participating institutions. There was an exception to this approval process for subjects from Project MATCH (n = 862) (Project MATCH Research Group 1998) and a subsample of subjects collected at the Highland Drive VA (Pittsburgh), which were determined by the Office for Protection from Research Risks and the Yale Institutional Review Board (IRB), respectively, to be exempt from review because the research involved use of existing de-identified or anonymous samples. The study was approved by the IRB at each of the participating institutions.

Ancestry proportion estimation

To measure the degree of admixture, we estimated the different ancestry proportions for each individual, using a molecular genetic approach. This model-based clustering method examined ancestry proportions by utilizing the ancestry information content of a set of AIMs. The algorithm used for clustering is a Bayesian approach that is implemented in the program STRUCTURE (Pritchard et al. 2000).

Sources—This set of 38 AIMs includes 36 STRs (two disease-associated STRs were excluded from the original marker set), one highly ancestry-informative FY marker (rs2814778) [details were listed in tables by (Luo et al. 2005b; Yang et al. 2005)] and one highly ancestry-informative *SLC24A5* marker (rs1426654) (Lamason et al. 2005). The STR markers were selected from two sources. One set of markers was from the AmpFLSTR Identifiler PCR Amplification Kit, which is used for forensic and paternity determination purposes [Applied Biosystems, Inc. (ABI), Foster City, CA]. The second set of markers was chosen for their high δ as reported previously (Smith et al. 2001) [reflects the ancestral information content of a marker (Rosenberg et al. 2003) and was provided in tables by (Luo et al. 2005b)]. A sample of West Africans was included as well. This sample has a low admixture rate and serves as a parental (or reference) population (i.e., a genetic background control) in the ancestry proportion estimation.

Genotyping—The Duffy antigen gene (FY) marker (rs2814778) and *SLC24A5* marker (rs1426654) were genotyped by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) techniques as described previously (Luo et al. 2004); or via the

Taqman method. The 36 STR markers were assigned to two genotyping panels. Each panel was genotyped in a single capillary by a fluorescence capillary electrophoresis (FCE) technique using the ABI PRISM 3100 semiautomated capillary fluorescence sequencer as described in detail elsewhere (Yang et al. 2005).

Characteristics—The 38 AIMs are unlinked to each other; additionally, there was no linkage disequilibrium (LD) between any two of these AIMs. All markers were in HWE within EAs or AAs and were not associated with any phenotype tested in the present study.

Procedure—To measure the ancestry proportions of the subjects more accurately, all subjects including EAs, AAs and West Africans were studied together as one single "admixed" sample. All of the AIM genotype data were input into the program STRUCTURE to derive parameter estimates–ln[Pr(X|K)], conditioning on different *Ks* ($K = 1, 2, \ldots, 5$), where X denoted the genotypes of the sampled individuals, and K was the putative genetic ancestral population number. We ran 100,000 repetitions of simulations before collecting data to minimize the effect of the starting configuration (burn-in period length), and then 100,000 Markov Chain Monte Carlo repetitions to measure ln[Pr(X|K)] accurately. The optimal K is usually the one with the highest posterior probability Pr(K|X), and since it contains the major structure in the sample, this optimal K was selected as the most appropriate number of the subpopulation groups in our sample. The ancestry proportion for each of the K subpopulations in each subject was predicted using STRUCTURE.

Feasibility—The number of AIMs required for STRUCTURE depends on the number of generations since admixture, and on the information content for ancestry of the markers reflected by δ values (δ is a measure for difference of allele frequencies between two founding populations) (Yang et al. 2005). Admixture of EAs and AAs has occurred over the past five generations. We demonstrated previously that our original 39 AIMs (with δ values between 0.10 and 0.83) are sufficient to identify the ancestries of EAs and AAs (Yang et al. 2005). The suitability of this set of AIMs to detect the presence of population structure, the adequacy of this set of AIMs to provide information to assign all individuals to different genetic ancestral populations, and the feasibility with which they are validly analyzed by the program STRUCTURE and provide the ancestry proportions of different genetic subpopulations for each subject have already been demonstrated previously (Kaufman et al. 2004; Luo et al. 2005a; b; Stein et al. 2004; Yang et al. 2005).

Admixture degree (ancestry proportion) calculation and comparison, and correction for multiple testing

In EAs (or AAs), we define the degree of admixture as the sum of the weight of African (or European) ancestry of each subject divided by the sample size. A higher proportion of African (European) ancestry in EAs (or AAs) represents a greater degree of admixture.

The mean admixture degree of each phenotype group is listed in Table 1. The difference in the degree of admixture between patients and controls was compared using the *t* test and *P* values are shown in Table 1. Age does not affect the admixture degree in individuals (Reiner et al. 2007;Reiner et al. 2005), so this comparison needs no adjustment for age.

European-Americans and AAs were analyzed separately. Six phenotype groups [including alcohol dependence (AD), cocaine dependence (CD), and opioid dependence (OD), AFD, SP and schizophrenia] were tested in the comparisons. Some phenotypes (e.g., AD, CD, and OD) were strongly dependent on each other; correction for six tests would be too conservative. We, thus, applied a correction by treating these six phenotype groups as five independent groups,

i.e., drug dependence (DD) (including CD and OD), AD, AFD, SP and schizophrenia, and the significance level (α) was corrected for five comparisons, i.e., α = 0.05/5 = 0.010.

Data simulation and power analysis

The normality of distributions of the degree of admixture was checked by the one-sample Kolmogorov-Smirnov test implemented in SPSS, which showed that the degree of admixture was normally distributed both in EAs and AAs. We simulated the ancestry proportions (i.e., admixture rates) in each phenotype group by generating random numbers using R language, given the sample sizes and the ancestry proportion difference between cases and controls shown in Table 1. This simulation was based on the normal distribution that was defined by the mean admixture rates and the standard deviations shown in Table 1. The simulation procedure was repeated 10,000 times, and then we performed a *t* test between each simulated case group and control group and generated 10,000 *P* values. The power would be the proportion of times that the *P* value was equal to or smaller than 0.05, i.e., the proportion of times that the null hypothesis, i.e., the case group and the control group had the same mean admixture rates, was rejected at the significance level of 0.05.

Results

1. Two main ancestries were detected in our sample and the ancestry proportions in each subject were examined after analysis with STRUCTURE. When $K = 2$, the posterior probability $\Pr(K|X)$ reaches its highest value, and all subjects were assigned to two subpopulations. That is, two main ancestries, i.e., European and African, were detected in our sample, which accords with the demography of our sample and is consistent with a previous analysis of the ancestry of AAs (Hoggart et al. 2003). The two ancestry proportions in each individual were highly asymmetric; only three controls, nine SD subjects (of which 6 had a diagnosis of AD, 5 CD, and 1 OD), and eight schizophrenic subjects had European or African ancestry proportions of 0.5±0.1. Theoretically, these subjects might potentially have admixture with other populations, e.g., Asians or intra-European subpopulations. However, $K \geq 3$ resulted in decreasing posterior probability $\Pr(K|X)$ values. Thus, these other populations were ignored in the present study. Subjects with European ancestry proportions >0.5 had higher probability to be self-reported EAs than self-reported AAs, and thus were assigned to "ancestry validated" EAs; subjects with European ancestry proportions <0.5 had higher probability to be self-reported AAs than self-reported EAs, and thus were assigned to "ancestry validated" AAs. Among the self-reported EAs, 99.6% (= 3119/3131) were "ancestry validated" EAs. A somewhat smaller proportion (95.7% (= 751/785)) of self-reported AAs were "ancestry validated" AAs. Based on these criteria we excluded the questionable subjects whose self-reported ethnicities and genetic ancestries were unmatched (12 self-reported EAs and 34 self-reported AAs) to assure the sample quality.
2. The degree of admixture in AAs was higher than in EAs (Table 1). In EAs, the degree of admixture was between 1.37 and 2.41%; in AAs, the degree of admixture was between 3.15 and 7.26%. The degree of admixture in any phenotype subgroup was higher in AAs than in EAs. The degree of admixture in any phenotype subgroup in AAs was 5–12 times that in West Africans.
3. In EAs, the degree of admixture (with African ancestry) was significantly lower in patients with SD (mainly AD) ($P = 0.009$ for SD, 95% CI 0.106–0.727%; $P = 0.008$ for AD, 95% CI 0.112–0.736%) and nominally higher in patients with AFD ($P = 0.057$ for total; $P = 0.040$ when excluding comorbid AD which occupies 13%) than in controls (see Table 1); in AAs, the degree of admixture (with European ancestry) was

nominally higher in patients with schizophrenia than in controls ($P = 0.015$) (Table 1). After correcting for multiple testing (α=0.010), only in EAs, the degree of admixture remained significantly lower in patients with SD (mainly AD) than in controls. However, if we apply a conservative correction for multiple testing by the numbers of six diagnoses and two populations, this difference between cases and controls becomes suggestive only, possibly due to information loss. Given the sample sizes of SD (n = 1,527), AD (n = 1,450) and healthy controls (n = 811) in EAs (see Table 1), and given the admixture rate differences between SD and controls (δ = 0.41%) and between AD and controls (δ = 0.42%), power was 75 and 76%, respectively; to detect the small admixture rate difference (δ = 0.41, 0.42%, respectively) from controls; we had 75 and 76% probability, respectively, to get the significant results using our SD and AD samples. Our sample sizes of SD and AD have 80% power to detect the admixture rate difference from controls down to 0.44 and 0.43%, respectively.

Discussion

In the present study, we found that African ancestry was significantly lower in EA SD subjects (mainly AD subjects) than EA controls. This finding could suggest that population admixture might protect against alcohol dependence. However, before we draw this conclusion, two alternate explanations should be considered. First, because our samples were not randomly sampled from all over the US and were not ascertained by a completely consistent strategy across all locations, admixture history was not randomized or matched between cases and controls, so that the asymmetry in the degree of admixture between cases and controls could be attributable to sampling bias. The degree of admixture (3.15–7.26%) in AAs is lower than the reported estimates [(6.8–22.5%) ± SEM (1.3–2.7%); (Parra et al. 1998)], which might reflect this sampling bias, in view of the variance of immigrant history of AAs in different geographic areas. Sampling bias is very hard to avoid, because it is not feasible to randomize subjects on admixture history during sampling (there usually is bias in the tracking back to non-admixed parental populations) and because there are no reliable clinical methods to measure admixture during the sampling process. However, exactly as there is no clinical way to measure the degree of admixture, it is also impossible for us to intentionally sample patients with higher or lower degree of admixture. Although the degree of admixture in AAs detected is somewhat lower than what has typically been seen in the literature previously, it is still reasonably within the ranges of 95% CI of the previously reported ranges. The degree of admixture in AAs was 5–12 times that in West Africans. These reasonable ratios reflect the relative sufficiency and validity of our approach in detecting admixture, although it might be not sufficient to derive the absolute value of admixture degree. Second, the missing genotyping rates for the 38 AIMs may vary between different phenotype groups due to differences in DNA quality, so that the asymmetry between the predicted marker-based European and African ancestry proportions varies between phenotype groups. For example, 96.4, 97.8, 97.9, 97.3, 97.6, 92.6, 93.7, and 96.3% of subjects have >90% European ancestry proportions in our EA controls and EA patients with substance dependence, alcohol dependence, cocaine dependence, opioid dependence, social phobia, AFDs, and schizophrenia, respectively. Because variation in the rate of missing genotypes for the same marker between different phenotype groups is inevitable during high-throughput genotyping, the possibility that this influenced the association of admixture with SD cannot be completely excluded. However, whereas the genotyping was performed under the same conditions, in the same laboratory, using the same genotyping system, set of primers, and methods, the lack of conformity in genotyping between subgroups should not be great. Therefore, we conclude that there is an association between admixture and risk for SD.

Population admixture may influence risk of disease either via some specific environmental factors or by decreasing the genome-wide homozygosity rate, or via both mechanisms. Psychiatric disorders have been hypothesized to be influenced by both environmental and genetic factors that could bridge the association between psychiatric disorders and population admixture. Chakraborty and Weiss claimed that an association between disease risk and individual admixture is the most direct way to demonstrate that genetic, not environmental, factors predispose to disease risk, especially when the disease risk differs by population group (Chakraborty and Weiss 1986), which could most reasonably explain the case for psychiatric disorders. Usually, if disease risk is largely attributable to a specific environmental factor, the ethnic difference in disease risk would be expected to subside somewhat within a few generations of migration and admixture (Reid 1971). After at least five generations (>200 years) of admixture and living together in the same country, the current prevalence rates of phobic disorders (16.2%) and cocaine dependence (0.25%) of AAs still differ somewhat from those of EAs (9.1% and 0.10%, respectively), and the lifetime prevalence rates of schizophrenia (2.1%) and phobic disorders (23.4%) of AAs differ somewhat from those of EAs as well (1.4 and 9.7%, respectively) (Degenhardt et al. 2008; Singleton-Bowie 1995; Williams 1995). This is consistent with the above claim that the genetic factors may play an important role in their etiology. However, throughout the long-history of living together in the same country, there have been differences in socioeconomic status between AAs and EAs, so the influence of environmental factors still cannot be neglected; in other words, they might also play a role in the etiology of psychiatric disorders.

The expected risk of a disease, especially a recessive one, should decrease in admixed populations when compared to their ancestral populations, because the rate of inbreeding declines. This may explain for the present study why population admixture significantly decreases the risk for AD in EAs. We studied much larger sample size of AD than other phenotypes in the present study, which could explain why admixture was detected to affect AD risk. Increasing the sample sizes to reach sufficient power might help to detect the associations between population admixture and more psychiatric disorders. In addition, in the first admixed generation, all loci (including the disease risk loci) are ancestry-heterozygous (i.e., one chromosome is derived from one population and the other chromosome from the other population), which has the highest degree of admixture of 50%, so the disease risk rate in the first admixed generation might be significantly different from those in other generations. A similar situation might also occur for some psychiatric disorders.

The association between population admixture and risk for disease may obscure the association between diseases and other factors, such as candidate genes (Luo et al. 2005a). In addition, population admixture could result in violation of Hardy-Weinberg Equilibrium (HWE) in the genotype frequency distribution of a non-disease-associated genetic marker, or in linkage disequilibrium (LD) between unlinked loci (Luo et al. 2005b). Thus, an admixture effect should be considered in association studies, especially in those with a population-based design, when testing for HWE and in the analysis of LD. One way to consider admixture in association analysis is to condition the analysis on the degree of admixture, which we have done previously (Luo et al. 2005a; b). Another approach involves a family-based design—by typing parents of cases, and using the two untransmitted parental alleles as controls for the two alleles transmitted to each affected individual, to eliminate confounding by population admixture.

Acknowledgments

Ann Marie Lacobelle and Greg Kay provided excellent technical assistance. Thomas Hudak, Ellen Koucky, Michelle McKain, Michelle Slivinsky, Konstantin Voronin and Carolyn Wenner assisted with patient interviews. This work was supported in part by National Institute on Drug Abuse (NIDA) grants R01-DA12849, R01-DA12690, K24-DA15105, and DA020709, National Institute on Alcohol Abuse and Alcoholism (NIAAA) grants R01-AA11330, R01-AA016015, P50-AA12870, P50-AA03510, K24-AA13736, R01-AA015615 and K05-AA14906, National

Institute on Mental Health grants R01-MH43775 and R01-MH70902, National Center for Research Resources (NCRR) grant M01-RR06192, Alcoholic Beverage Medical Research Foundation (ABMRF) grant award R06932 (X. Luo); and by the U.S. Department of Veterans Affairs (the VA Alcohol Research Center, the VA Medical Research Program, the VA Connecticut–Massachusetts Mental Illness Research, Education and Clinical Center [MIRECC], and the VA Research Enhancement Award Program [REAP] research center). Drs. Mark D. Shriver and Robert Rosenheck provided some of the DNA samples. We thank the Department of Veterans Affairs Cooperative Study Group on Clozapine in Refractory Schizophrenia (members of the study group are listed in Rosenheck et al, 1997) and some investigators from Project MATCH, a collaborative clinical trial sponsored by NIAAA, who provided phenotypic data and blood samples for DNA extraction and genotyping.

References

American Psychiatric Association. Diagnostic and statistical manual of mental disorders. Vol. third edition, revised ed.. Washington, DC: American Psychiatric Press; 1987.

American Psychiatric Association. Diagnostic and statistical manual of mental disorders. Vol. fourth edition ed.. Washington, DC: American Psychiatric Press; 1994.

Blouin AG, Perez EL, Blouin JH. Computerized administration of the diagnostic interview schedule. Psychiatry Res 1988;23:335–344. [PubMed: 3387505]

Brutsaert TD, Parra EJ, Shriver MD, Gamboa A, Rivera-Ch M, Leon-Velarde F. Ancestry explains the blunted ventilatory response to sustained hypoxia and lower exercise ventilation of Quechua altitude natives. Am J Physiol Regul Integr Comp Physiol 2005;289:R225–R234. [PubMed: 15802561]

Chakraborty R, Weiss KM. Frequencies of complex diseases in hybrid populations. Am J Phys Anthropol 1986;70:489–503. [PubMed: 3766715]

Degenhardt L, Bohnert KM, Anthony JC. Assessment of cocaine and other drug dependence in the general population: "gated" versus "ungated" approaches. Drug Alcohol Depend 2008;93:227–232. [PubMed: 18065161]

Fernandez JR, Shriver MD, Beasley TM, Rafla-Demetrious N, Parra E, Albu J, et al. Association of African genetic admixture with resting metabolic rate and obesity among women. Obes Res 2003;11:904–911. [PubMed: 12855761]

Gelernter J, Panhuysen C, Weiss R, Brady K, Hesselbrock V, Rounsaville B, et al. Genomewide linkage scan for cocaine dependence and related traits: significant linkages for a cocaine-related trait and cocaine-induced paranoia. Am J Med Genet B Neuropsychiatr Genet 2005;136B:45–52. [PubMed: 15909294]

Goncalves VF, Prosdocimi F, Santos LS, Ortega JM, Pena SD. Sex-biased gene flow in African Americans but not in American Caucasians. Genet Mol Res 2007;6:156–161.

Hammer MF, Chamberlain VF, Kearney VF, Stover D, Zhang G, Karafet T, et al. Population structure of Y chromosome SNP haplogroups in the United States and forensic implications for constructing Y chromosome STR databases. Forensic Sci Int 2006;164:45–55. [PubMed: 16337103]

Hanis CL, Chakraborty R, Ferrell RE, Schull WJ. Individual admixture estimates: disease associations and individual risk of diabetes and gallbladder disease among Mexican-Americans in Starr County, Texas. Am J Phys Anthropol 1986;70:433–441. [PubMed: 3766713]

Hoggart CJ, Parra EJ, Shriver MD, Bonilla C, Kittles RA, Clayton DG, et al. Control of confounding of genetic associations in stratified populations. Am J Hum Genet 2003;72:1492–1504. [PubMed: 12817591]

Hunley KL, Healy ME, Long JC. The global pattern of gene identity variation reveals a history of long-range migrations, bottlenecks, and local mate exchange: Implications for biological race. Am J Phys Anthropol. 2009[Epub ahead of print]

Kaufman J, Yang BZ, Douglas-Palumberi H, Houshyar S, Lipschitz D, Krystal JH, et al. Social supports and serotonin transporter gene moderate depression in maltreated children. Proc Natl Acad Sci U S A 2004;101:17316–17321. [PubMed: 15563601]

Klimentidis YC, Miller GF, Shriver MD. The relationship between European genetic admixture and body composition among Hispanics and Native Americans. Am J Hum Biol. 2009[Epub ahead of print]

Lamason RL, Mohideen MA, Mest JR, Wong AC, Norton HL, Aros MC, et al. SLC24A5, a putative cation exchanger, affects pigmentation in zebrafish and humans. Science 2005;310:1782–1786. [PubMed: 16357253]

Lind JM, Hutcheson-Dilks HB, Williams SM, Moore JH, Essex M, Ruiz-Pesini E, et al. Elevated male European and female African contributions to the genomes of African American individuals. Hum Genet 2007;120:713–722. [PubMed: 17006671]

Luo X, Klempan TA, Lappalainen J, Rosenheck RA, Charney DS, Erdos J, et al. NOTCH4 gene haplotype is associated with schizophrenia in African Americans. Biol Psychiatry 2004;55:112–117. [PubMed: 14732589]

Luo X, Kranzler HR, Zuo L, Wang S, Blumberg HP, Gelernter J. CHRM2 gene predisposes to alcohol dependence, drug dependence and affective disorders: results from an extended case-control structured association study. Hum Mol Genet 2005a;14:2421–2434. [PubMed: 16000316]

Luo X, Kranzler HR, Zuo L, Yang BZ, Lappalainen J, Gelernter J. ADH4 gene variation is associated with alcohol and drug dependence: results from family controlled and population-structured association studies. Pharmacogenet Genomics 2005b;15:755–768. [PubMed: 16220108]

McKeigue PM. Mapping genes that underlie ethnic differences in disease risk: methods for detecting linkage in admixed populations, by conditioning on parental admixture. Am J Hum Genet 1998;63:241–251. [PubMed: 9634509]

McLean DC Jr. Spruill I, Gevao S, Morrison EY, Bernard OS, Argyropoulos G, et al. Three novel mtDNA restriction site polymorphisms allow exploration of population affinities of African Americans. Hum Biol 2003;75:147–161. [PubMed: 12943155]

Molokhia M, Hoggart C, Patrick AL, Shriver M, Parra E, Ye J, et al. Relation of risk of systemic lupus erythematosus to West African admixture in a Caribbean population. Hum Genet 2003;112:310–318. [PubMed: 12545274]

Parra EJ, Kittles RA, Argyropoulos G, Pfaff CL, Hiester K, Bonilla C, et al. Ancestral proportions and admixture dynamics in geographically defined African Americans living in South Carolina. Am J Phys Anthropol 2001;114:18–29. [PubMed: 11150049]

Parra EJ, Marcini A, Akey J, Martinson J, Batzer MA, Cooper R, et al. Estimating African American admixture proportions by use of population-specific alleles. Am J Hum Genet 1998;63:1839–1851. [PubMed: 9837836]

Pierucci-Lagha A, Gelernter J, Feinn R, Cubells JF, Pearson D, Pollastri A, et al. Diagnostic reliability of the semi-structured assessment for drug dependence and alcoholism (SSADDA). Drug Alcohol Depend 2005;80:303–312. [PubMed: 15896927]

Pritchard JK, Stephens M, Donnelly P. Inference of population structure using multilocus genotype data. Genetics 2000;155:945–959. [PubMed: 10835412]

Project MATCH Research Group; Project MATCH Research Group. Matching alcoholism treatments to client heterogeneity: treatment main effects and matching effects on drinking during treatment. J Stud Alcohol 1998;59:631–639. [PubMed: 9811084]

Reid DD. The future of migrant studies. Isr J Med Sci 1971;7:1592–1596. [PubMed: 5144604]

Reiner AP, Carlson CS, Ziv E, Iribarren C, Jaquish CE, Nickerson DA. Genetic ancestry, population sub-structure, and cardiovascular disease-related traits among African-American participants in the CARDIA Study. Hum Genet 2007;121:565–575. [PubMed: 17356887]

Reiner AP, Ziv E, Lind DL, Nievergelt CM, Schork NJ, Cummings SR, et al. Population structure, admixture, and aging-related phenotypes in African American adults: the Cardiovascular Health Study. Am J Hum Genet 2005;76:463–477. [PubMed: 15660291]

Rosenberg NA, Li LM, Ward R, Pritchard JK. Informativeness of genetic markers for inference of ancestry. Am J Hum Genet 2003;73:1402–1422. [PubMed: 14631557]

Rosenberg NA, Mahajan S, Ramachandran S, Zhao C, Pritchard JK, Feldman MW. Clines, clusters, and the effect of study design on the inference of human population structure. PLoS Genet 2005;1:e70. [PubMed: 16355252]

Rosenberg NA, Pritchard JK, Weber JL, Cann HM, Kidd KK, Zhivotovsky LA, et al. Genetic structure of human populations. Science 2002;298:2381–2385. [PubMed: 12493913]

Rosenheck R, Cramer J, Xu W, Thomas J, Henderson W, Frisman L, et al. A comparison of clozapine and haloperidol in hospitalized patients with refractory schizophrenia. Department of Veterans Affairs Cooperative Study Group on Clozapine in Refractory Schizophrenia. N Engl J Med 1997;337:809–815. [PubMed: 9295240]

Roth W. The end of the one-drop rule? Labeling of multiracial children in black intermarriages. Sociological Forum 2005;20:35–67.

Rounsaville BJ, Bryant K, Babor T, Kranzler H, Kadden R. Cross system agreement for substance use disorders: DSM-III-R, DSM-IV and ICD-10. Addiction 1993;88:337–348. [PubMed: 8461851]

Singleton-Bowie SM. The effect of mental health practitioners' racial sensitivity on African Americans' perceptions of service. Soc Work Res 1995;19:238–244. [PubMed: 10152600]

Smith MW, Lautenberger JA, Shin HD, Chretien JP, Shrestha S, Gilbert DA, et al. Markers for mapping by admixture linkage disequilibrium in African American and Hispanic populations. Am J Hum Genet 2001;69:1080–1094. [PubMed: 11590548]

Spitzer, R.; Endicott, J. Schedule for affective disorders and schizophrenia: lifetime version. New York: New York Biometrics Research Division, New York State Psychiatric Institute; 1975.

Spitzer RL, Williams JB, Gibbon M, First MB. The structured clinical interview for DSM-III-R (SCID). I: history, rationale, and description. Arch Gen Psychiatry 1992;49:624–629. [PubMed: 1637252]

Stein MB, Schork NJ, Gelernter J. A polymorphism of the beta1-adrenergic receptor is associated with low extraversion. Biol Psychiatry 2004;56:217–224. [PubMed: 15312808]

Waters MC. The role of lineage in identity formation among black Americans. Qualitative Sociology 1991;14:57–76.

Wellcome Trust Case Control Consortium. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. Nature 2007;447:661–678. [PubMed: 17554300]

Williams D. African American mental health: persisting questions and paradoxical findings. Afr Am Res Perspect 1995;2:8–16.

Yang BZ, Zhao H, Kranzler HR, Gelernter J. Practical population group assignment with selected informative markers: characteristics and properties of Bayesian clustering via STRUCTURE. Genet Epidemiol 2005;28:302–312. [PubMed: 15782414]

Table 1

Demographics data and admixture rates of our sample

	Ancestry-validated European-Americans (n=3119)						Ancestry-validated African-Americans (n=751)				
	Size (n)	**Male (%)**	**Mean Age (years)**	**European ancestry ≥90%[a] (%)**	**Admixture (%)**	***P* value[b]**	**SizeMale (n)(%)**	**Mean Age (years)**	**African ancestry ≥90%[a] (%)**	**Admixture (%)**	***P* value[b]**
West Africans							7838.5		98.7	0.61±1.71	
Healthy Controls	811	45.9	29.7±11.8	96.4	1.79±3.94		13539.1	33.9±12.1	91.1	3.50±7.90	
Substance Dependence	1527	67.1	41.5±9.9	97.8	1.38±2.99	**0.009**	39765.5	39.2±8.1	89.7	4.27±8.56	0.355
Alcohol Dependence	1450	67.0	42.0±9.8	97.9	1.37±2.98	**0.008**	33066.7	40.7±8.1	90.0	4.17±8.27	0.418
Cocaine Dependence	259	69.9	36.9±7.9	97.3	1.62±4.00	0.534	20066.5	38.7±8.1	90.0	4.16±8.98	0.489
Opioid Dependence	123	57.7	37.5±9.2	97.6	1.39±2.59	0.269	7258.3	41.5±8.6	87.5	4.58±9.43	0.384
Social Phobia	122	45.2	39.4±14.2	92.6	2.23±4.64	0.326	650.0	36.5±12.8	83.3	5.22±8.28	0.604
Affective Disorders	363	44.9	39.8±14.2	93.7	2.41±5.58	0.057	3756.8	41.4±7.9	94.6	3.15±5.20	0.799
Schizophrenia	296	20.9	40.4±11.6	96.3	1.73±3.60	0.814	9818.4	41.5±11.9	82.7	7.26±1.35	0.015

Admixture rates in European-Americans (or African-Americans) represent the African (or European) ancestry proportions in those subjects.

[1] The percentages of subjects with European (or African) ancestry proportions ≥90%.

[b] α=0.010.

Hum Genet. Author manuscript; available in PMC 2009 November 17.