

A Functional Neuropeptide Y *Leu7Pro* Polymorphism Associated With Alcohol Dependence in a Large Population Sample From the United States

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Background: Quantitative trait locus studies, and observations in animals manipulated for the neuropeptide Y (NPY) gene suggest that variation within this gene may contribute to alcoholism. A recent population study suggested that the *Pro7* allele of a functional NPY polymorphism (*Leu7Pro*) may be associated with increased alcohol consumption. We tested whether the *Pro7* allele is associated with alcohol dependence in European Americans (EA).

Methods: The design was a population study comparing the *Leu7Pro* allele frequencies in alcohol-dependent subjects and controls. Population stratification potential and diagnostic specificity was studied by genotyping individuals from additional populations and psychiatric diagnostic classes. We studied 2 independently collected samples of EA alcohol-dependent subjects (sample 1, $n=307$; sample 2, $n=160$) and a sample of psychiatrically screened EA controls ($n=202$); 8 population samples, including African Americans and European Americans (total $n=551$); and 4 samples of individuals with Alzheimer disease, schizophrenia, post-traumatic stress disorder, and major depression (total $n=502$). The main outcome measure was the difference

in *Leu7Pro* allele frequencies between alcohol-dependent subjects and controls.

Results: The frequency of the *Pro7* allele was higher in the alcohol-dependent subjects (sample 1, 5.5%; sample 2, 5.0%) compared with the screened EA controls (2.0%) (sample 1 vs controls, $P=.006$; sample 2 vs controls, $P=.03$). The attributable fraction (excess morbidity) in similarly affected populations, owing to the *Pro7* allele, was estimated to be 7.3%. The frequency of the *Pro7* allele was equal or lower in the population samples, as compared with the screened EA controls (0%-2.2%), with 1 exception (Bedouins). We found no significant evidence that the association of the *Pro7* allele with alcohol dependence was due to an association with a comorbid psychiatric disorder.

Conclusions: These results suggest that the NPY *Pro7* allele is a risk factor for alcohol dependence. This is only the second specific genetic mechanism ever identified that modulates risk for alcohol dependence.

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ALCOHOLISM is a genetically influenced disorder. The largest twin studies have established an estimated heritability of 50% to 60% for alcoholism,^{1,2} which is comparable to, or higher than, the heritability estimates for some other common disorders of major public health concern, such as non-insulin-dependent diabetes mellitus³ and cancer.⁴ The only genes unambiguously associated with risk for developing alcoholism are certain genes coding for alcohol metabolizing enzymes, such as alcohol dehydrogenase and acetaldehyde dehydrogenase.⁵ However, variation within these genes accounts for only a small proportion of the risk for individuals of European or African ancestry, who constitute the great majority of the US population.

Neuropeptide Y (NPY) is a 36-amino acid peptide neurotransmitter. Animal studies have suggested that NPY is involved in the regulation of appetite,⁶ reward,⁷ anxiety,⁸ and energy balance.⁹ Recently, quantitative trait locus (QTL) linkage mapping studies and behavioral observations of mice manipulated for the NPY gene have suggested that genetic variation at this locus may contribute to heritability in animal models of alcoholism. Thiele et al¹⁰ showed that mice deleted for the NPY gene prefer drinking alcohol-water solutions vs water. These mice were also less sensitive to the hypnotic and sedative effects of alcohol than nondeleted mice. In contrast, mice that were manipulated to overexpress NPY had a low preference for alcohol and were more sensitive to the sedative and hypnotic effects of ethanol. Quantitative trait locus map-

ping studies in alcohol-preferring and nonpreferring rats obtained the highest logarithm-of-odds (lod) score to rat chromosome 4. Marker *D4Mit7*, which is located in an intron of the NPY gene, was in the center of the identified 12.5cM critical region.^{11,12}

In humans, a functional *Leu7Pro* polymorphism in the NPY gene has been described.¹³ This polymorphism resides in the signal peptide part of the pre-proNPY and has been demonstrated to affect intracellular processing of the pre-proNPY and release of the mature NPY. For example, individuals with the *Pro7/Leu7* genotype have an average of 42% higher maximal increases in the plasma concentration of NPY in response to maximal physiological stress as compared with *Leu7/Leu7* individuals.¹⁴ Large population studies conducted in Northern Europe, particularly in Finland, have associated the *Pro7* allele with higher cholesterol levels¹³ and atherosclerosis.¹⁵ Consistent with the studies in animals, a recent large population study suggested that this polymorphism might also influence alcohol consumption in the normal range. In that study, Kauhanen et al¹⁶ reported that the *Pro7* allele is associated with an average of 34% higher alcohol consumption in a cohort of 889 Finnish middle-aged men.

Thus, a possible relationship between genetic variation at the NPY locus and alcohol dependence is supported by studies of genetically manipulated animals,¹⁰ by animal QTL linkage studies,^{11,12} by demonstration of functional concomitants of genetic variation at the locus,¹³⁻¹⁵ and by a relationship with alcohol consumption in a Finnish population,¹⁶ although not necessarily in a pathological range in that study.

In this study, we tested whether the NPY *Pro7* allele is associated with a greater risk of developing DSM-III-R or DSM-IV alcohol dependence in a large population sample from the United States. Two independent samples of European American alcohol-dependent subjects and a sample of psychiatrically screened European American controls were studied. Potential for population stratification was evaluated by determining the *Leu7Pro* allele frequencies in 8 additional population samples, including European Americans, European Ashkenazi Jews, and African Americans, and by determining the allele frequencies of the *FY(+/-)* polymorphism in our control sample. The *FY(-)* allele denotes a promoter polymorphism in the *FY* gene, which abrogates the expression of Duffy receptor on erythroid cells. The frequency of the *FY(-)* allele in modern day African Americans is 80% and only 0.9% in European Americans.¹⁷ Owing to this difference, the frequency of *FY* alleles may be used to evaluate the degree of admixture between these 2 major US ethnic groups. Diagnostic specificity was tested by determining *Leu7Pro* allele frequencies in European American individuals with schizophrenia, affective disorder, posttraumatic stress disorder (PTSD), and Alzheimer disease.

SUBJECTS AND METHODS

PATIENTS AND SAMPLES

All subjects in each group described here gave informed consent as approved by the relevant institutional review board.

Alcohol-Dependent Subjects

Two independent populations of alcoholics were studied. The initial sample was recruited from a combination of treatment-seeking and non-treatment-seeking patient populations through clinics and advertisements in the community. All subjects in the initial alcohol-dependent sample ($n=307$, termed hereafter "EA alcohol-dependent sample 1") were recruited in Connecticut, at either the University of Connecticut Health Center (Farmington, Conn), or the Veterans Administration (VA) Connecticut Healthcare System, West Haven campus. All alcohol-dependent subjects were diagnosed using the DSM-III-R¹⁸ or DSM-IV.¹⁹ The diagnosis was made with the Structured Clinical Interview for DSM-III-R or for DSM-IV (SCID),^{20,21} the computerized Diagnostic Interview Schedule for DSM-III-R (C-DIS-R)²² or a checklist composed of DSM-III-R symptoms. The second alcohol-dependent sample ($n=160$, termed hereafter "EA alcohol-dependent sample 2") was obtained through a multicenter VA study on the efficacy of naltrexone in the treatment of alcohol dependence (VA Cooperative Study 425, "Naltrexone in the Treatment of Alcoholism"). Subjects were recruited at 15 VA hospitals nationwide. The DSM-IV diagnosis was made using the SCID. All subjects in these 2 study populations were European Americans diagnosed with alcohol dependence. Individuals with psychotic disorders were excluded. In all analyses, these 2 alcohol-dependent groups were tested separately, and no pooled data are presented.

Screened Control Subjects

Subjects were recruited from the local communities through advertisement. All subjects were screened to exclude major Axis I disorders, including substance use disorders (such as alcohol dependence), psychotic disorders, anxiety disorders, and mood disorders. A total of 267 screened controls were collected at the VA Connecticut Healthcare System, West Haven Campus, and at the University of Connecticut Health Center. Screening was done using the SCID, the Schedule for Affective Disorders and Schizophrenia—Lifetime Version²³ (SADS-L), the C-DIS-R, or via a nonstructured interview with a psychiatrist or research assistant. There were both European American ($n=202$) and African American ($n=65$) screened control subjects. The screened African American controls were used as a population sample. For clarity, the screened European American control subjects will be termed the "screened EA controls" hereafter, and the screened African American controls will be termed "African American sample." Twenty-two percent of the screened EA controls were assessed using an unstructured interview.

Population Samples

DNA from unrelated Ashkenazi Jews ($n=90$), Ethiopian Jews ($n=47$), Bedouins ($n=32$), Moroccans ($n=92$), and Druze ($n=92$) was obtained from the National Laboratory for the Genetics of Israeli Populations, Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel. Samples from unrelated Japanese subjects ($n=66$) were provided by Hiroshi Ichinose, PhD, and Toshiharu Nagatsu, PhD, at Fujita Health University, Toyoake, Japan. A sample of unrelated elderly European American control subjects ($n=67$) (without Alzheimer disease diagnosis) was recruited at the Alzheimer's Disease Research Unit at the Yale University School of Medicine, New Haven, Conn.

Alzheimer Disease Subjects

The study population was recruited at the Alzheimer's disease Research Unit at the Yale University School of Medicine, and

Table 1. Frequency of NPY *Leu7Pro* Alleles in 2 Independent Populations of EA Alcohol-Dependent Subjects and Screened EA Controls*

	No. of Subjects	<i>Pro7</i>	<i>Leu7</i>	No. of Chromosomes	χ^2_1	P Value	OR (95% CI)
Sample 1	307	34 (5.5)	580 (94.5)	614
Sample 2	160	16 (5.0)	304 (95.0)	320
Controls	202	8 (2.0)	396 (98.0)	404
Sample 1 vs controls	7.80	.006	2.9 (1.3-6.3)
Sample 2 vs controls	5.08	.03	2.6 (1.1-6.2)

*Data in the *Pro7* and *Leu7* columns refer to the number (percentage) of chromosomes with these alleles. NPY indicates neuropeptide Y; EA, European American; OR, odds ratio; CI, confidence interval; Sample 1, EA alcohol-dependent sample 1; Sample 2, EA alcohol-dependent sample 2; and Controls, screened EA controls.

consisted of patients with probable Alzheimer disease (n=137), which was diagnosed according to standard criteria.²⁴ Other causes of dementia were excluded through a comprehensive evaluation, including medical history, physical and neurological examinations, extensive serum chemistry evaluations, and brain computed tomography scans or magnetic resonance imaging scans. All subjects with Alzheimer disease were European American.

PTSD Subjects

All subjects (n=77) with PTSD were Vietnam-era combat veterans who were recruited at the VA Connecticut Healthcare System, West Haven. Consensus diagnoses were made with SCID. Individuals with major psychotic disorders were excluded. All subjects with PTSD were European American.

Major Depression

Patients (n=122) were recruited at either the Connecticut Mental Health Center or the VA Connecticut Healthcare System, West Haven Campus. The diagnosis of *DSM-III-R* major depression, past or current, single episode or recurrent, was established by SCID or by consensus of 2 psychiatrists. Individuals with major psychotic disorders were excluded. All subjects with major depression were European American.

Schizophrenia

Patients (n=166) were recruited at 12 VA hospitals nationwide through VA Cooperative Study 17 comparing the efficacy and cost-effectiveness of haloperidol and clozapine, or through a separate research protocol at the VA Connecticut Healthcare System, West Haven Campus. The diagnoses were made with SCID. All subjects with schizophrenia were European American.

GENOTYPING

DNA was extracted from whole blood using standard methods, or from cell lines in the case of the samples obtained from Israel. The NPY *Leu7Pro* polymorphism was genotyped as previously described.¹³ Briefly, genomic DNA was amplified using standard polymerase chain reaction (PCR) techniques. The PCR product was digested with 10 Units of *BsiE1* enzyme (New England Biolabs, Beverly, Mass) and analyzed on a 3% Meta-phor gel (BioWhittaker Molecular Applications, Rockland, Me). Randomly chosen samples (approximately 8% of the total) were reamplified, digested, and reanalyzed for quality control purposes with 100% confirmation of previously assigned genotypes. Among the 1722 subjects genotyped for the *Leu7Pro* polymorphism in this study, only one *Pro7/Pro7* homozygote was found. That individual was diagnosed with PTSD and alcohol dependence. The *FY* polymorphism was genotyped as de-

Table 2. Frequency of the NPY *Leu7Pro* Alleles in the Population Samples, EA Alcohol Dependent Sample 1 and Screened EA Controls*

	No. of Subjects	<i>Pro7</i>	<i>Leu7</i>	No. of Chromosomes
Bedouins	32	5 (7.8)	59 (92.2)	64
Sample 1†	307	34 (5.5)	580 (94.5)	614
Moroccans	92	4 (2.2)	180 (97.8)	184
Ashkenazi	90	4 (2.2)	176 (97.8)	180
Elderly EA controls	67	3 (2.2)	131 (97.8)	134
Controls	202	8 (2.0)	396 (98.0)	404
Ethiopian Jews	47	1 (1.1)	93 (98.9)	94
Druze	92	1 (0.5)	183 (95.5)	184
African Americans	65	0 (0)	130 (100)	130
Japanese	66	0 (0)	132 (100)	132

*Data in the *Pro7* and *Leu7* columns refer to the number (percentage) of chromosomes with these alleles. NPY indicates neuropeptide Y; EA, European American; Sample 1, EA alcohol-dependent sample 1; and Controls, screened EA controls. For heterogeneity analysis, $\chi^2_8 = 21.2$; $P = .008$.

†This sample was not included in the χ^2 analysis. The *Leu7Pro* frequencies in sample 1 were included in this table to allow visual comparison to the population samples.

scribed previously.¹⁷ Briefly, genomic DNA was amplified using standard PCR techniques. The PCR product was digested with 10 Units of *RsaI* enzyme and analyzed on a 3% Metaphor gel.

STATISTICAL ANALYSIS

Contingency tables were used to compare allele frequencies between groups. In the first set of analyses, EA alcohol-dependent sample 1 was compared with the screened EA controls. This was followed by a comparison of EA alcohol-dependent sample 2 with the screened EA controls using a similar analysis (**Table 1**). In the second set of analyses, potential for population stratification was tested using heterogeneity analysis of all population samples (**Table 2**). In the third set of analyses, diagnostic specificity and potential for confounding by an association to a comorbid disorder was tested using heterogeneity analysis of the neuropsychiatric disorders (subjects with PTSD, major depressive disorder, schizophrenia, Alzheimer disease, and EA alcohol-dependent sample 1) and screened EA controls (**Table 3**). We performed 2×2 post hoc analyses to compare subjects with PTSD, major depressive disorder, schizophrenia, and Alzheimer disease with the screened EA controls (Table 3). Comparison of the EA alcohol-dependent samples with the screened EA controls was done using 2×2 tables. Larger contingency tables were used for heterogeneity analyses of both the population samples (2×9) and the set of samples with psychiatric disorders (2×6). Het-

Table 3. Frequency of the NPY *Leu7Pro* Alleles Among Alcohol-Dependent Subjects and Healthy Controls in Relation to the Subjects With Psychiatric Disorders*

Disorder†	No. of Subjects	<i>Pro7</i>	<i>Leu7</i>	No. of Chromosomes
Sample 1	307	34 (5.5)	580 (94.5)	614
Schizophrenia	166	14 (4.2)	318 (95.8)	332
PTSD	77	6 (3.9)	148 (96.1)	154
Alzheimer disease	137	10 (3.6)	264 (96.4)	274
Major depression	122	6 (2.5)	238 (97.5)	244
Controls‡	202	8 (2.0)	396 (98.0)	404

*Data in *Pro7* and *Leu7* columns are presented as the number (percentage) of chromosomes with these alleles. NPY indicates neuropeptide Y; Sample 1, European American alcohol-dependent sample 1; PTSD, posttraumatic stress disorder; and controls, screened European American controls.

†For heterogeneity analysis, $\chi^2_5 = 9.95$; $P = .08$. For sample 1 vs controls, $\chi^2_1 = 7.80$; $P = .006$. The rate of alcohol dependence among the individuals with PTSD was greater than 50%. The rate of comorbid alcohol dependence in the other psychiatric disorders (major depression, schizophrenia, Alzheimer disease) was not available.

‡ 2×2 post hoc comparisons of those with PTSD, schizophrenia, Alzheimer disease, or major depression vs controls were not significant.

erogeneity analysis tested whether the samples (cells) were drawn from a uniform distribution. In all cases, calculations were done using the χ^2 test. We used the CLUMP program,²⁵ which uses Monte Carlo simulation to estimate the significance of the χ^2 statistic. In each analysis, 10 000 to 100 000 simulations were performed to obtain P values for the χ^2 statistic. Results of the CLUMP T1 statistic are presented. Genotype frequencies were tested for Hardy-Weinberg equilibrium (HWE) using the HWSim program, which uses Monte Carlo simulation to test for departure from HWE.²⁶ Genotypes in all populations and for all psychiatric disorders were in HWE. Attributable fraction (also referred to as etiologic fraction) was calculated using the formula $F_c(R-1)/R$, where F_c is the fraction of cases with the risk factor and R is the incidence-density ratio (when using incidence cases) or prevalence odds ratio (when using prevalent cases).²⁷ The calculations for the attributable fraction were done using genotypes, counting the presence of the *Pro7* allele as a risk factor. Power analysis for transmission disequilibrium test was calculated using the method described by Risch and Merikangas²⁸ (implementation available at: <http://www.mds.qmw.ac.uk/statgen/dcurtis/software.html>).

RESULTS

COMPARISON OF NPY *Leu7Pro* ALLELE FREQUENCIES IN ALCOHOL-DEPENDENT SUBJECTS AND SCREENED EA CONTROLS

The frequency of the *Pro7* allele was significantly higher in EA alcohol-dependent sample 1 than in the screened EA controls ($\chi^2_1 = 7.80$, $P = .006$). The frequency of the *Pro7* allele was also significantly higher in EA alcohol-dependent sample 2 as compared with the screened EA controls ($\chi^2_1 = 5.08$, $P = .03$). These results are presented in Table 1.

COMPARISON OF NPY *Leu7Pro* ALLELE FREQUENCIES IN POPULATION SAMPLES

Analysis of *Leu7Pro* allele frequencies in the population samples (Bedouins, Moroccans, Ashkenazi Jews, elderly European American controls, Ethiopian Jews, Druze,

African Americans, and Japanese) and screened EA controls revealed significant heterogeneity ($\chi^2_8 = 21.2$, $P = .008$). These results are presented in Table 2.

COMPARISON OF NPY *Leu7Pro* ALLELE FREQUENCIES IN PSYCHIATRIC DISORDERS

Analysis of *Leu7Pro* allele frequencies in psychiatric disorders (EA alcohol-dependent sample 1, PTSD, and subjects with schizophrenia, Alzheimer disease, and major depression) and screened EA controls revealed suggestive heterogeneity ($\chi^2_5 = 9.95$, $P = .08$); 2×2 comparisons of subjects with PTSD, schizophrenia, Alzheimer disease, and major depression with the screened EA controls were not significant. These results are presented in Table 3.

FY(+/-) POLYMORPHISM IN THE SCREENED EA CONTROL SUBJECTS AND IN THE AFRICAN AMERICAN SAMPLE

The FY(+/-) polymorphism was genotyped in 75 screened EA control subjects (37% of individuals in the screened EA control sample) and 41 individuals from the African American sample (63% of individuals in the African American sample). All of the individuals belonging to the screened EA control group were homozygous for the FY(+) allele. The frequencies of the FY(+) and FY(-) alleles in the African American sample were 0.20 and 0.80, respectively.

COMMENT

In this study, we tested whether the NPY *Leu7Pro* polymorphism is associated with alcohol dependence. We derived the hypothesis from previous findings in animals and humans suggesting that genetic variation within the NPY locus may be associated with an altered risk to develop alcoholism-related traits and behaviors.^{10-12,16} We discovered an increased *Pro7* allele frequency among EA alcohol-dependent subjects compared with screened EA controls. This was observed in 2 independently collected samples of alcoholics. This suggests that the *Pro7* allele is associated with alcohol dependence.

A case-control strategy is vulnerable to confounding by population stratification, and we sought to address this by genotyping the NPY *Leu7Pro* in additional population samples, including African Americans, Ashkenazi Jews, and in a second sample of European Americans (elderly EA controls) collected in the same geographical region as the first study sample. Confounding by population stratification refers to a situation in which a noncausal association is observed because of differences in allele frequency for the marker of interest that occur based on population differences alone. Such differences by population are observed for most genetic markers, and we report here that they are indeed present for NPY *Leu7Pro* (Table 2). Thus, it is demonstrated to be important for case-control studies of this NPY polymorphism to establish that the case and control samples are well matched in terms of their respective population ancestries. Our population observations allow us to identify certain specific kinds of stratification (admixture) that could confound the results. In this study, ex-

cept for Bedouins, the *Pro7* allele frequency was lower in all populations studied as compared with the alcohol-dependent samples. Previously, Karvonen et al¹³ have reported that the *Pro7* frequency in Finns is approximately 6%. If these groups (Bedouins or Finns) were greatly over-represented in the alcohol-dependent groups, but not in the control groups, the population difference (rather than a physiological difference) could be driving the observed association. However, because the individuals residing in the geographical region where EA alcohol-dependent sample 1 and the screened EA controls were collected are not predominantly of Finnish or Bedouin descent, we have no reason to believe that population stratification explains the elevated *Pro7* frequency among alcohol-dependent individuals. Our study design does not fully eliminate the possibility that population stratification is confounding the elevated *Pro7* frequency in alcohol dependence; there could still be admixture that is not readily observable (eg, between different Northern European populations). Such admixture is much less likely to account for an association like that reported here because European populations tend to have similar marker allele frequencies. While there are no data to support the assumption that NPY allele frequencies are similar in the full range of European populations that might be ancestral for our sample, we do demonstrate that 3 different, and differently-ascertained, European populations (our screened EA controls, elderly controls, and Ashkenazi Jews) have similar *Pro7* allele frequency, and this markedly decreases the likelihood that our positive result is accounted for by occult stratification within European populations. Replication attempts to confirm the association between the *Pro7* allele and alcohol dependence in US populations are clearly needed, but it should be noted, however, that assuming similar *Leu7Pro* allele frequencies, a sample of at least 640 individuals needs to be tested to gain a statistical power of 0.8 ($\alpha = .01$). Studies that use either the transmission disequilibrium test or a linkage approach would be useful but relatively difficult to do, as it would be difficult to ascertain sufficient numbers of families segregating the *Pro7* allele, and because of loss of statistical power if flanking genetic markers are used (since presumably, all “real” association information would be derived from linkage disequilibrium with the functional *Leu7Pro* marker). We estimate that more than 200 parent-offspring trios would be required to confirm this finding using transmission disequilibrium testing (again, for power of 0.8; $\alpha = .01$).

As the frequency of the *Pro7* allele was 0% in the African American sample, it was remotely possible that the *Pro7* frequency among the screened EA controls was deflated due to admixture with the African American population. Several lines of evidence argue against occurrence of this particular kind of admixture in our sample. First, as mentioned earlier, in the 2 other European populations that we studied (Ashkenazi Jews and elderly EA controls), the *Pro7* allele frequency was similar to the observed frequency in the screened EA controls. Second, to further test for the presence of African American admixture in the screened EA controls, we determined the frequency of the *FY*(-) allele of the *FY*(+/-) polymorphism in part of the screened EA control group. The frequency of the *FY*(-) allele in modern-

day African Americans is 80% and only 0.9% in European Americans.¹⁷ We found that the frequency of the *FY*(-) allele in the screened EA control group was 0%, suggesting that no significant African American admixture was present. It is unlikely that the low *Pro7* allele frequency in the screened EA controls is a result of admixture with other populations, such as Asian Americans, since African Americans are the only sizeable minority population in this geographical region.

We next examined the specificity of the elevated *Pro7* frequency for alcohol dependence. Earlier reports that used plasma and cerebrospinal fluid NPY levels and yohimbine-stimulated NPY release as indices of NPY function implicated this neurotransmitter system in schizophrenia,²⁹ depression,³⁰ and PTSD.³¹ All of these disorders are also highly comorbid with alcohol dependence.³² It was therefore possible that the association of the *Pro7* allele to alcohol dependence was due to an association with a comorbid psychiatric disorder. We tested whether the *Leu7Pro* polymorphism is associated with schizophrenia, PTSD, major depression, and Alzheimer disease. These populations were collected independently of the alcohol-dependent and control subjects through separate research protocols focusing on recruiting individuals suffering primarily from these disorders. As compared with the alcohol-dependent samples, the absolute *Pro7* allele frequency was lower in each of the other psychiatric disorders studied, but higher than the frequency in the screened EA controls. The heterogeneity analysis that included all the subjects with disorders and screened EA controls revealed a suggestive significance ($P = .08$). Post hoc 2×2 comparisons revealed that the *Pro7* allele was not significantly associated with psychiatric disorders other than alcohol dependence. The sample sizes were smaller for the other disorders, and we cannot exclude that a positive association would be observed in larger samples. It may be concluded, however, that it is unlikely that the association between the *Pro7* allele and alcohol dependence is driven by an association of the *Pro7* allele with any of the other disorders we studied. The elevated *Pro7* allele frequency showed some specificity to alcohol dependence, but there was also a general trend toward higher *Pro7* frequency in the other disorders. This was most notable in those with PTSD and schizophrenia. We hypothesize that this may be driven by the high rate of comorbidity between alcohol dependence and these psychiatric disorders. For example, in the PTSD sample, the prevalence of alcohol dependence was higher than 50%, and the frequency of the *Pro7* allele among these individuals (ie, subjects with PTSD who had known comorbid alcohol dependence) was 5%. Another possibility is that the genetic risk for these different psychiatric disorders is partially shared. For example, one may speculate that the *Pro7* allele is related to poor stress tolerance,³³ which in some individuals may contribute to alcohol dependence, while in others it may contribute to psychotic disorders or to a tendency to respond maladaptively to traumatic events.

What is the significance of the *Pro7* allele in the development of alcohol dependence among European Americans? The attributable fraction, which is the proportion of the cases in the study population that would

not have occurred had the risk factor (eg, a risk allele) not been present, is a useful measure to quantify the impact of the *Pro7* allele on the development of alcohol dependence.²⁷ We used the recent census³⁴ and the reported 14.1% DSM-III-R lifetime population prevalence of alcohol dependence³⁵ to estimate the attributable fraction in European Americans who are 16 years of age or older. The attributable fraction due to the *Pro7* allele is 7.3%. Due to the high prevalence of alcohol dependence in the United States, the estimated number of cases that the *Pro7* allele accounts for among European Americans is higher than 1 800 000, assuming that our sample is representative of all cases in the population.

How does altered NPY function affect the risk to develop alcohol dependence? Kallio et al¹⁴ suggested that *Pro7/Leu7* individuals have an enhanced capacity to synthesize and release mature NPY. They showed that such individuals have an average of 42% higher maximal increases in the plasma concentrations of NPY under maximal physiological stress. Kallio et al¹⁴ also demonstrated that human umbilical vein endothelial cells from *Pro7/Leu7* individuals abundantly contain more mature NPY as compared with the human umbilical vein endothelial cells from *Leu7/Leu7* individuals. Based on these findings and the findings from animal studies showing an inverse correlation between NPY expression and alcohol preference, one would assume that the *Pro7/Leu7* individuals have a lower risk of developing alcoholism. The present findings and those of Kauhanen et al¹⁶ suggest the opposite. It is possible, however, that an exaggerated NPY release in *Pro7/Leu7* individuals may lead to a rapid depletion of NPY stores causing a prolonged nadir in the baseline NPY levels. This mechanism was recently postulated by Morgan et al.³³ They demonstrated that NPY is released in response to stress (military survival training). Interestingly, a subpopulation of their study subjects had significantly lower NPY plasma concentrations 24 hours after the stress, as compared with their prestress baseline. Other possible intermediate phenotypes may involve differences in alcohol sensitivity³⁶ and differences in alcohol's reward properties⁷ between the individuals with and without the *Pro7* allele. More research is needed to understand the pathways that connect the functional NPY *Leu7Pro* polymorphism and the risk of developing alcoholism.

Our findings suggest that the NPY *Pro7* allele contributes to the heritability of alcohol dependence. If confirmed, this would be only the second known specific mechanism by which genetic variation leads to differing risk for alcohol dependence. Although independent replication is needed, we note that our present finding was consistent across 2 differently ascertained alcohol-dependent samples. Also, although we have no control sample that can be strictly considered a replication sample, our results in elderly controls and unselected Ashkenazi Jews (of European origin) suggest that the low *Pro7* allele frequency in our screened EA control sample was not caused by a sampling error. Our findings are consistent with recent studies in animals and humans, which indicate that genetic variation within the NPY system may account for a proportion of the heritability of volitional alcohol intake. It is interesting that the *Pro7* allele seems to

contribute to a spectrum of phenotypic variation, ranging from increased average alcohol consumption¹⁶ to pathological drinking, as defined by DSM-III-R or DSM-IV criteria of alcohol dependence. Our findings are limited to European Americans, mostly due to the lack of access to alcohol-dependent samples from other populations. The *Pro7* allele was absent in the African American or Japanese samples, suggesting that this polymorphism is unlikely to play a significant role in the risk for alcohol dependence in these populations. Studies aiming to elucidate the pathways that connect the NPY *Leu7Pro* polymorphism and risk to develop alcoholism will be important. These studies may shed light on some of the neurobiological mechanisms and pathways that lead to the development of alcohol dependence, and may thereby facilitate the development of novel treatments and prevention strategies for this common and debilitating disorder.

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