Neuropeptide Y rs16147 single nucleotide polymorphism is associated with heavy drinking and severity of alcohol dependence

Derick Vergne  
*Medical University of South Carolina*

Raymond Anton  
*Medical University of South Carolina*

Konstantin Voronin  
*Medical University of South Carolina*

Abraham Tiffany  
*Medical University of South Carolina*

Hugh Myrick  
*Medical University of South Carolina*

See next page for additional authors

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Authors
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Caleb Canders, Garrick Klaybor, Patrick Randall, Joe Schacht

Center for Drug and Alcohol Programs.
Medical university of South Carolina, Charleston, SC

Abstract

Introduction: A wide array of preclinical animal work has established a link between a malfunctioning NPY system, anxiety, depression and alcohol dependence. In animals, neuropharmacological and neuroanatomical studies have consistently shown the NPY system to be 1) dysregulated in limbic areas strongly related to the stress system, and 2) in behavioral animal models of excessive alcohol drinking. In humans the 485C>T rs16147 SNP in the NPY promoter region, has been shown to increase plasma neuropeptide Y. We wished to evaluate the relationship between NPY genotype and alcohol consumption as well as to investigate whether this relationship is influenced by levels of anxiety.

Methods: 191 non-treatment seeking alcoholics receiving DSM-IV criteria for alcohol dependence (average age 48.8, 80% male, 90% Caucasian; alcohol dependence scale mean score of 14) were assessed with the Beck Anxiety Inventory (BAI), and also for drinking (using the timeline follow-back calendar method). In the 90 days prior to a blood draw for analysis of the NPY rs16147 SNP. DNA samples were genotyped for the SNP rs16147 using a Sanger genotyping assay (TaqMan®; Applied Biosystems, Foster City, CA). Amplification was performed via PCR (StepOne™ Real-Time PCR System; Applied Biosystems). Four clusters were identified, representing CC and TT homozygotes, CT heterozygotes, and no-DNA-template controls.

Results: The genotype frequencies were CC (25.7%), CT (49.2%) and TT (25.1%), CC and CT were compared to TT genotypes. Individuals with two copies of the T allele showed significantly less heavy drinking days (p=0.017). The TT genotype was also associated with lower alcohol severity, that became more significant when anxiety was taken into account, suggesting that the tendency for NPY genotype to be associated with alcohol severity is partially dependent on the presence of anxiety. To our knowledge this is the first human genetics study showing an association between rs16147 and alcohol severity. Further studies will elaborate on the relation of the NPY system to human alcoholism, anxiety, depression and their interactions.

Discussion: Our results show less heavy drinking when 2 copies of the minor allele (important to increase NPY production) are present, consistent with animal models showing an inverse relationship between NPY expression and drinking behavior. The TT genotype was associated with lower anxiety severity, that became more significant when anxiety was taken into account, suggesting that the tendency for NPY genotype to be associated with alcohol severity is partially dependent on the presence of anxiety. To our knowledge this is the first human genetics study showing an association between rs16147 and alcohol severity. Further studies will elaborate on the relation of the NPY system to human alcoholism, anxiety, depression and their interactions.

Subjects

Methods - NPY rs16147 DNA ISOLATION

DNA was extracted from peripheral blood mononuclear cells using a Puregene DNA Purification kit (Genta Systems, Minneapolis, MN). The amount of DNA extracted was quantified by absorbance spectrophotometry (260 and 280 nm) and diluted to 5-20 ng/µl in 1X TE buffer. DNA solutions were stored at 4°C

DNA samples were genotyped for the SNP rs16147 using a Sanger genotyping assay (TaqMan®; Applied Biosystems, Foster City, CA). TaqMan genotyping assays were presdesigned (Applied Biosystems, identification No. C_2826725_10) and contained locus-specific primers and fluorescent allele-specific probes.

The 3 µL reaction mixture consisted of 2.5 µL of 2X TaqMan Universal PCR Master Mix, 0.25 µL of 20X assay mix, 8 µL detection probe for each allele, 36 µM forward and reverse primer each (all reagents supplied as TaqMan Bioassays®; Applied Biosystems). 1.75 µL nuclease-free water (QIAGEN Sciences, Germantown, MD), and 5-20 ng genomic DNA. Amplification was performed via PCR (StepOne™ Real-Time PCR System; Applied Biosystems) using 48-well plates under the following conditions: 60°C for 30 seconds and 95°C for 10 minutes followed by 40 cycles of 92°C for 15 seconds and 60°C for 1 minute. During amplification, PCR software (StepOne v2.2; Applied Biosystems) measured SNP specific fluorescence and genotyped each sample. Four clusters were identified, representing CC and TT homozygotes, CT heterozygotes, and no-DNA-template controls. No significant deviation from Hardy-Weinberg equilibrium was found (CC 25.7%, CT 49.2% and TT 25.1%).

Discussion/Future Directions

Discussion/future directions: Our results show less heavy drinking in TT NPY genotype individuals. Since this genotype is purported to have increased NPY production these findings are consistent with animal models showing an inverse relationship between NPY expression and drinking behavior. Notably, NPY rs16147 allele carriers (TT genotype) had less alcohol dependence symptoms. This genotype effect on alcohol severity was mediated, at least to some degree, by the genotype effect on anxiety.

Our results should be interpreted in light of the following:

- Since a large amount of preclinical and clinical data suggest that NPY has independent effects on anxiety and on alcohol consumption, our data might begin to suggest how some of these data might converge.
- Since NPY is expressed in areas of the brain involved with reward perception as well as stress/anxiety modulation, it is appealing to consider various brain mechanisms whereby alcohol and NPY might interact to produce their clinical effects.

Future studies will clarify the role of the NPY system on drinking behavior and the nature of the relationship with stress/anxiety to modulate this behavior. This aim could be achieved by combining brain-imaging paradigms and genetic differences in clinical investigation and by evaluating drugs that work on the NPY system in clinical laboratory and treatment trials.

Introduction

A wide array of preclinical animal work has established a link between a malfunctioning NPY system, anxiety, depression and alcohol dependence. In animals, neuropharmacological and neuroanatomical studies have consistently shown the NPY system to be 1) dysregulated in limbic areas strongly related to the stress system, and 2) in behavioral animal models of excessive alcohol drinking. In fact, NPY gene knock out animals drink more than wild type controls, and rats selectively bred for high ethanol preference decreased their intake when ICV NPY was administrated. Analogically, the amygdala appears to be a key site for NPY action, as animals bred for high alcohol intake after alcohol extinction showed a diminished alcohol drinking behavior with intra-amygdalar NPY injection. Clinical studies linking NPY to mood and anxiety have shown decreased NPY CSF levels in patients with diagnosed PTSD and depression, as well as a hormonal cortisol decrease in NPY in post-mortem studies of depressive patients who committed suicide. NPY genetic studies evaluating single nucleotide polymorphisms (SNP) of the NPY gene have found an association between changes in NPY expression and drinking parameters in humans. The 485C>T rs16147 SNP in the NPY gene promoter region, has been shown to increase plasma neuropeptide Y levels. The presence of this SNP has also been associated with anxiety and response to antidepressants in depressive subjects. We wished to evaluate the relationship between NPY genotype and alcohol consumption as well as to investigate whether this relationship is influenced by levels of anxiety in non-treatment seeking alcoholics.

Results

Figure 1 Presence of 2 copies of the T allele is associated with decreased heavy drinking.

Figure 2 The presence of two copies of the T allele confers an almost statistically significant decrease in alcohol severity.

Figure 3 Relationship of Alcohol Severity and Anxiety.

Putative NPY circuit-rodent brain

Discussion/Future Directions

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