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

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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 meeting DSM-IV criteria for alcohol dependence (average age about 29, 80% male, 90% Caucasian, alcohol dependence scale score mean 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 5' nuclease genotyping assay (*Taq*Man®; 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 Alcoholism Severity (ADS score)(p=0.061), and those with TT showed less of an association between anxiety and alcohol symptoms (ADS score) compared to the CC and CT genotypes where the association was greater(p=0.012).

Discussion: Our results show less heavy drinking when 2 copies of the minor allele (purported 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 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 to human alcohol consumption in dependent drinkers. Further studies will elaborate on the relation of the NPY system to human alcoholism, anxiety, depression and their interactions
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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 administered. Anatomically, the amygdala appears to be a key site for NPY action, as animals bred for high alcohol intake after alcohol deprivation show 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 frontal cortical 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**.

Subjects

-Subjects (average age about 29, 80% male, 90% Caucasian, alcohol) recruited from advertisements and assessed prior to participation in a bar-lab and brain imaging study.
-Alcohol dependence by DSM-IV criteria (SCID-interview) but not seeking treatment.
-No Axis 1 psychiatric disorders, no other substance dependence except nicotine.
-Drinking during the 30 days prior ascertained with timeline followback procedure.
-Beck Anxiety Inventory (BAI) used to measure anxiety
- Alcohol Drinking Scale (ADS) used to measure perception of alcohol drinking effects
Blood drawn for DNA.

Methods

METHODS - NPY rs16147

DNA ISOLATION

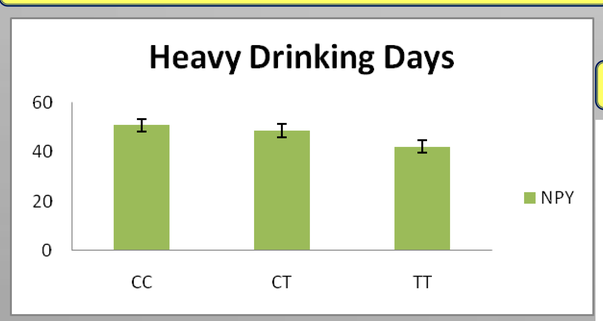
DNA was extracted from peripheral blood mononuclear cells using a Puregene DNA Purification kit (Gentra Systems, Minneapolis, MN). The amount of DNA extracted was quantified by absorbance spectroscopy (260 and 280 nm) and diluted to 5-20 ng/µL in 1X TE buffer. DNA solutions were stored at 4°C.

5' NUCLEASE GENOTYPING

DNA samples were genotyped for the SNP rs16147 using a 5' nuclease genotyping assay (*Taq*Man®; Applied Biosystems, Foster City, CA). *Taq*Man genotyping assays were predesigned (Applied Biosystems, identification No. C_2267279_10) and contained locus-specific primers and fluorogenic allele-specific probes.

The 5 µL reaction mixture consisted of 2.5 µL of 2X *Taq*Man® Universal PCR Master Mix, 0.25 µL of 20X assay mix, 8 µM detection probe for each allele, 36 µM forward and reverse primer each (all reagents supplied as *Taq*Man, Applied Biosystems), 1.75 µL nuclease-free water (QIAGEN Sciences, Germantown, MD), and 5-20 ng of 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™ v2.0; 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%).

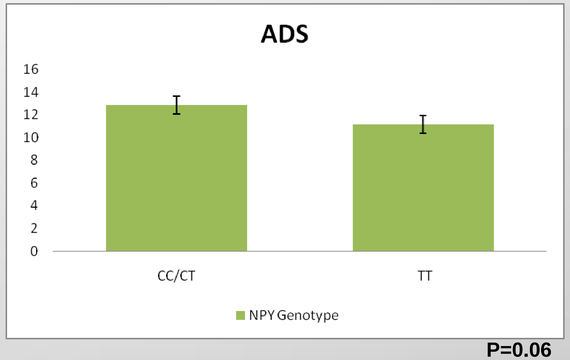
Results



P=0.017

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

Results



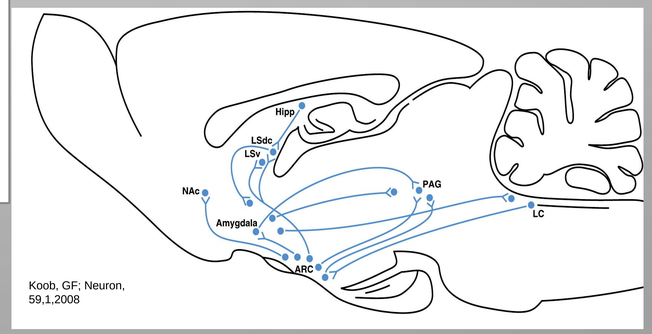
P=0.06

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

NPY GENOTYPE	ADS x BAI SLOPE
CC	0.64
CT	0.62
TT	0.55
	P=0.01

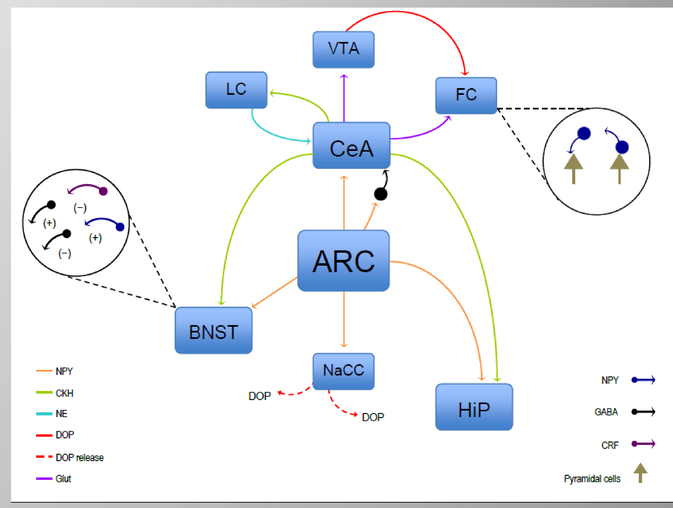
Figure 3. Relationship of Alcohol Severity and Anxiety**.**

Putative NPY circuit-rodent brain



Koob, GF; Neuron, 59,1,2008

NPY hypothetical role in Alcoholism-Stress Diathesis



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. We also found a main effect of NPY genotype on alcohol severity (ADS scores) such that the TT genotype individuals 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.