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Addiction Biology

HUMAN GENETIC STUDY

doi:10.1111/adb.12277

Genetic variation of the growth hormone secretagogue receptor gene is associated with alcohol use disorders identification test scores and smoking

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ABSTRACT

The multifaceted gut-brain peptide ghrelin and its receptor (GHSR-1a) are implicated in mechanisms regulating not only the energy balance but also the reward circuitry. In our pre-clinical models, we have shown that ghrelin increases whereas GHSR-1a antagonists decrease alcohol consumption and the motivation to consume alcohol in rodents. Moreover, ghrelin signaling is required for the rewarding properties of addictive drugs including alcohol and nicotine in rodents. Given the hereditary component underlying addictive behaviors and disorders, we sought to investigate whether single nucleotide polymorphisms (SNPs) located in the pre-proghrelin gene (*GHRL*) and GHSR-1a gene (*GHSR*) are associated with alcohol use, measured by the alcohol use disorders identification test (AUDIT) and smoking. Two SNPs located in *GHRL*, rs4684677 (Gln90Leu) and rs696217 (Leu72Met), and one in *GHSR*, rs2948694, were genotyped in a subset ($n = 4161$) of a Finnish population-based cohort, the Genetics of Sexuality and Aggression project. The effect of these SNPs on AUDIT scores and smoking was investigated using linear and logistic regressions, respectively. We found that the minor allele of the rs2948694 SNP was nominally associated with higher AUDIT scores ($P = 0.0204$, recessive model) and smoking ($P = 0.0002$, dominant model). Furthermore, *post hoc* analyses showed that this risk allele was also associated with increased likelihood of having high level of alcohol problems as determined by AUDIT scores ≥ 16 ($P = 0.0043$, recessive model). These convergent findings lend further support for the hypothesized involvement of ghrelin signaling in addictive disorders.

Keywords Candidate gene association study, gastrointestinal hormones, substance use disorder.

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INTRODUCTION

Recent pre-clinical and clinical studies suggest that the orexigenic ghrelin signaling system may be of importance for addictive behaviors such as alcohol use disorder and compulsive over-eating (for review, see Dickson *et al.* 2011; Leggio *et al.* 2011). Pre-clinical studies have shown that ghrelin, just as addictive drugs (Imperato & Di Chiara 1986; Engel *et al.* 1988; Larsson & Engel 2004; Larsson *et al.* 2005) and natural rewards (Hernandez & Hoebel 1988; Rada *et al.* 2000), activates the cholinergic-dopaminergic reward link in rodents (Jerlhag *et al.* 2007). Ghrelin has thus been suggested to increase the incentive salience of motivated behaviors such as those associated with drug taking. This hypothesis has been confirmed in several of our pre-clinical studies in which pharmacological suppression of the growth hormone secretagogue receptor subtype 1a (GHSR-1a), also known as the ghrelin receptor, was shown to attenuate the rewarding properties of alcohol and nicotine (Jerlhag *et al.* 2009; Jerlhag & Engel 2011). Further studies involving rodents have shown that ghrelin increases alcohol consumption while a GHSR-1a antagonist decreases alcohol intake, operant self-administration of alcohol and prevents relapse drinking (Jerlhag *et al.* 2009; Kaur & Ryabinin 2010; Landgren *et al.* 2012; Bahi *et al.* 2013; Suchankova *et al.* 2013b).

Plasma and serum ghrelin levels have been investigated in several studies in alcohol-dependent individuals with varying outcomes possibly relating to inherent differences between study samples (for reviews, see Wurst *et al.* 2007; Kenna *et al.* 2012). Nevertheless, some studies suggest a positive correlation between ghrelin levels and alcohol craving in abstinent alcohol-dependent individuals (Addolorato *et al.* 2006; Hillemacher *et al.* 2007; Koopmann *et al.* 2012; Leggio *et al.* 2012), possibly implicating the ghrelin signaling system as a potential target in the pharmacological treatment of alcohol use disorders in humans. A recent study reports a dose-dependent increased alcohol craving in heavy-drinking alcohol-dependent subjects receiving exogenous ghrelin intravenously (Leggio *et al.* 2014). Furthermore, clinical studies investigating the effect of smoking on ghrelin levels report conflicting results. Some show no difference in baseline levels between smokers and non-smokers or with craving in acute nicotine withdrawal (Kokkinos *et al.* 2007; Mutschler *et al.* 2012), while others report the decrease of ghrelin levels in saliva after acute smoking (Kaabi & Khalifa 2014) as well as, contradictory, increase in plasma ghrelin levels (Fagerberg, Hulten & Hulthe 2003; Bouros *et al.* 2006). The short-term effect of smoking has also been shown to cause suppression in ghrelin plasma levels in non-smoker but not in smokers (Kokkinos *et al.* 2007).

We have previously shown in human genetic studies that variants of the pre-proghrelin gene (*GHRL*) and GHSR-1a gene (*GHSR*) are associated with various factors related to alcohol, nicotine and amphetamine dependence (Landgren *et al.* 2008, 2010, 2011a; Suchankova *et al.* 2013a). On the basis of these preclinical and clinical observations, we sought to investigate whether single nucleotide polymorphisms (SNP) located in the *GHRL* and *GHSR* gene are associated with alcohol use assessed by the alcohol use disorders identification test (AUDIT) as well as smoking in a large Finnish cohort.

MATERIALS AND METHODS

Subjects

The Genetics of Sexuality and Aggression sample is a population-based cohort of Finnish twins and siblings of twins. The subsample used in the present study stems from the second data collection completed in 2006 (for data collection procedure, see Johansson *et al.* 2013). DNA was extracted from saliva samples (for characteristics, see Table 1). Some genotyping failure was seen for each of the analyzed SNPs resulting in a slight variation in *n*. The genotypes were imputed in cases where only one of the monozygotic twins was genotyped. A number of subjects with at least one successfully genotyped SNP in the resulting sample were 4161 subjects from a total of 1756 families (581 monozygotic and 380 dizygotic twin-pairs, 910 siblings and 1329 singletons).

Alcohol use assessment

The participants were asked to fill out the AUDIT, a self-report questionnaire used in the health care to identify individuals with hazardous and harmful patterns of alcohol consumption (Babor *et al.* 2001). It consists of 10 questions with a 5- and 3-point response format. The response is scored to give a total AUDIT score ranging from 0 to 40. According to the World Health Organization, an AUDIT score of ≥ 16 or above represents a high

Table 1 Sample characteristics for genotyped individuals.

	Overall	*Females*	*Males*
n[a]	4161 (100 percent)	2463 (59.2 percent)	1698 (40.8 percent)
Age	26.39 (5.07)	26.40 (5.25)	26.38 (4.80)
AUDIT			
n	4133 (100 percent)	2451 (59.3 percent)	1682 (40.7 percent)
AUDIT sum items 1–10	6.63 (4.99)	5.48 (4.37)	8.31 (5.36)
AUDIT ≥ 16	256 (6.2 percent)	89 (3.6 percent)	167 (9.9 percent)
AUDIT ≤ 1	563 (13.6 percent)	405 (16.5 percent)	158 (9.4 percent)
Cigarettes per day			
n	4112 (100 percent)	2441 (59.4 percent)	1671 (40.6)
none	3085 (75.0 percent)	1903 (77.6 percent)	1196 (71.3 percent)
≤ 10	556 (13.5 percent)	339 (13.8 percent)	218 (13.0 percent)
11–20	394 (9.6 percent)	185 (7.5 percent)	210 (12.6 percent)
21–30	75 (1.8 percent)	25 (1.0 percent)	50 (3.0 percent)
> 30	2 (0.05 percent)	0	2 (0.1 percent)

[a]Number of subjects with information on genotype for at least one of the analyzed single nucleotide polymorphisms. Data presented as number of cases (percentage) or mean (standard deviation). AUDIT = Alcohol Use Disorder Identification Test.

Table 2 Studied single nucleotide polymorphisms (SNPs).

Gene	*SNP*	*Position*[a]	*Alleles*	*SNP location*	*SNP type*
GHRL	rs4684677	10328453	A/T	Exon 3	Missense (Gln90Leu)
	rs696217	10331457	G/T	Exon 2	Missense (Leu72Met)
GHSR	rs2948694	172165163	A/G	Intron 1	Intron

[a]Position on chromosome 3 for the studied SNPs in *GHRL* and *GHSR*. *GHRL* = pre-proghrelin gene; *GHSR* = growth hormone secretagogue receptor gene.

level of alcohol problems, whereas an AUDIT score of ≤ 1 is reported by alcohol abstainers (Babor *et al.* 2001).

Smoking

The present study also aimed to investigate potential association between the studied SNPs and smoking status reported using questions about current smoking habits. The participants were asked how many cigarettes they smoke on an average day with a 5-point response format: not at all; ≤ 10; 11–20; 21–30; > 30 (Table 1). This outcome was significantly correlated to AUDIT scores in the whole sample (partial correlation controlling for gender and age, $r = 0.32$, $n = 4071$, $P < 0.001$). According to the Fagerström Test for Nicotine Dependence (Heatherton *et al.* 1991), smoking fewer than 11 cigarettes per day does not add to the final score of nicotine dependence. Subjects in this group were thus excluded from all smoking-related analyses while individuals that reported to smoke more than 10 cigarettes per day ($n = 472$) were grouped together in order to capture individuals that were more likely to be nicotine-dependent.

DNA extraction, SNP selection and genotyping

The DNA collection and extraction procedure has been described in detail previously (Jern *et al.* 2012). Briefly, Oragene™ DNA self-collection kits (DNA Genotek, Inc., Kanata, ON, Canada) were used when collecting saliva samples from participants. The kits were posted to the participants along with the manufacturer's instructions on collecting the samples (DNA Genotek, Inc.) and participants were instructed to deposit approximately 2 ml of saliva into the collection cup before returning it by mail. DNA was stabilized in the cup upon closure and stored at –20°C until used.

Two missense SNPs in the *GHRL* (rs4684677 or Gln90Leu and rs696217 or Leu72Met) and one SNPs in the *GHSR* (rs2948694) were selected on the basis of previous results (Landgren *et al.* 2008, 2010, 2011a,c). For closer description of the initial selection of these SNPs, see Landgren *et al.* (2008). SNPs with a previously reported minor allele frequency of < 5 percent were not included in the present study.

Genotyping of the SNPs was made by the LGC Genomics in the UK (http://www.lgcgenomics.com) using the KASPar chemistry—a competitive allele-specific polymerase chain reaction (PCR) SNP genotyping system performed with fluorescence resonance energy transfer (FRET) quencher cassette oligos. For details on the studied SNPs, see Table 2.

Statistical analyses

Deviation from Hardy-Weinberg equilibrium (HWE) ($P < 0.01$) was assessed for all genotyped SNPs using Haploview (Barrett *et al.* 2005).

Primary analysis involved genotypic effects on the main outcome variables AUDIT and smoking. Additive, dominant and recessive models of inheritance for the minor allele were all evaluated. Age and sex were included as covariates. Total sum of AUDIT items 1–10 was investigated using linear regression. Individuals smoking more than 10 cigarettes per day were grouped together and compared against the group that reported to be non-smokers using logistic regression. Due to the fact that a large part of the present cohort consists of twins and siblings, gene-dropping was used to maintain the between-subjects dependence when evaluating the significance of the effects by repeated simulations (MacCluer *et al.* 1986).

We also investigated the effect of genotype on AUDIT scores and smoking in models adjusting for not only age and gender but also smoking and AUDIT scores, respectively. Finally, a logistic regression analysis was performed for the associated SNP to estimate odds ratios (ORs) for individuals with high level of alcohol problems, i.e. AUDIT ≥ 16 ($n = 256$) compared with alcohol abstainers, i.e. AUDIT ≤ 1 ($n = 563$).

A significance level of 0.05 was used and all presented *P*-values are empirical estimates unless otherwise specified. *P*-values for main outcome variables (i.e. AUDIT and smoking) were Bonferroni corrected for three models, three SNPs and two primary endpoints (i.e. 18) and designated p_c. The statistical analysis was carried out using SPSS for Mac (Version 19.0.0.1, SPSS, Chicago, IL, USA) and R (Team RDC 2011).

RESULTS

All studied SNPs had a HWE *P*-value > 0.01 and a minor allele frequency of > 5 percent (Table 3). The minor allele

Table 3 Marker statistics for the genotyped SNPs in *GHRL* and *GHSR* genes.

Gene	*SNP*	*MAF*	*HWE p*	*dd*	*Dd*	*DD*	*Call rate*[a]
GHRL	rs4684677	13.1 percent	0.25	71 (1.8 percent)	867 (22.5 percent)	2923 (75.7 percent)	93.3 percent
	rs696217	12.1 percent	0.68	69 (1.6 percent)	891 (21.0 percent)	3282 (77.4 percent)	95.3 percent
GHSR	rs2948694	20.4 percent	0.02	192 (4.8 percent)	1241 (31.1 percent)	2558 (64.1 percent)	96.2 percent

[a]Not including imputed genotypes for monozygotic twins. rs4684677 and rs2948694 were genotyped in a samples of 4039 subjects, rs696217 was genotyped in a sample of 4325. *GHRL* = pre-proghrelin gene; *GHSR* = growth hormone secretagogue receptor gene; HWE = Hardy-Weinberg equilibrium; MAF = minor allele frequency; SNP = single nucleotide polymorphism; minor homozygotic, heterozygotic and major homozygotic genotype groups dd/Dd/DD.

Table 4 Associations between rs2948694, AUDIT scores and smoking.

	n	*b*	*OR*	P	p_c[d]
AUDIT[a]	3966	0.96		0.0204	0.3672
AUDIT ≤ 1 versus ≥ 16[b]	789		3.37	0.0043	
Smoking[b]	3411		1.48	0.0002	0.0036
Smoking[b] adjusted for AUDIT	3410		1.46	0.0008	
AUDIT[a] in subset[c]	3410	0.81		0.0475	
AUDIT[a] adjusted for smoking	3410	0.78		0.0489	

Data presented as empirical estimates obtained by permutation (50 000 gene-dropping simulations) of either [a]linear regression or [b]logistic regression. A recessive model was used for all analyses concerning AUDIT and a dominant model was used for smoking. The b represents increased AUDIT and the odds ratio (OR) represents increased risk of smoking or having an AUDIT ≥ 16. All analyses were performed by controlling for age and gender. AUDIT was reanalyzed in a matched [c]subset for comparison when controlling for smoking. Smoking variable is coded as 0 (non-smokers), 1 (smokers > 10 cigarettes/day). [d]Bonferroni corrected. AUDIT = Alcohol Use Disorder Identification Test.

of the rs2948694 SNP (G) located in intron 1 of the *GHSR* gene was associated with increased AUDIT scores ($\Delta_{AUDIT} = 1.2$; $P = 0.0204$; $p_c = 0.3672$) in a recessive model as well as with smoking in an dominant model (OR = 1.48; $P = 0.0002$; $p_c = 0.0036$; Table 4). See the Supporting Information for results on the primary outcomes using the other models (Supporting Information Table S1). When a subset of the subjects where selected and classified according to abstainers (AUDIT ≤ 1) and high consumers (AUDIT ≥ 16), the homozygotes for the risk allele had OR = 3.4 to be high consumers ($P = 0.0043$). Smokers (reporting to smoke > 10 cigarettes per day) had higher AUDIT scores [Mean(standard deviation, SD) AUDIT score in non-smokers = 5.63(4.35), Mean(SD) AUDIT score in smokers = 10.18(5.76)] and 61.6 percent of the subjects with AUDIT ≥ 16 were smokers compared with 9.2 percent in the AUDIT ≤ 1 group. Nevertheless, when the primary analyses involving AUDIT scores or smoking were adjusted for smoking or AUDIT scores, respectively, the association between the outcome variables and the rs2948694 SNP remained significant ($P = 0.0489$, $P = 0.0008$, respectively; Table 4). Neither of the investigated *GHRL SNPs* was associated with AUDIT scores or smoking in any of the genetic models (Gln90Leu: $P \geq 0.12$; Leu72Met: $P \geq 0.17$; Supporting Information Table S1).

Using available data from 1000GENOMES:phase_3_FIN confirmed that the rs2948694 has a higher minor allele frequency in the Finnish in Finland subpopulation (22 percent) compared with the European (11 percent) and was further found to capture 11 percent of alleles across the *GHSR* gene at $r^2 \geq 0.8$ using the Haploview Tagger function. Submitting the *GHSR* gene to the online alternative splice site predictor tool (http://wangcomputing.com/assp) predicted 39 sites as confident (≥ 0.9) putative splice sites and one of these sites included the rs2948694 as a cryptic donor alternative splice site. The G-allele of the SNP was found to increase the confidence score of this splice site prediction. We further explored the possible association between rs2948694 and *GHSR* expression using the publicly available BrainCloud (Colantuoni *et al*. 2011), an application consisting of post-mortem human brains collected at ages from fetal development to senescence ($n = 269$). Expression of *GHSR* in prefrontal cortex was analyzed against a proxy SNP for rs2948694 (not genotyped in this sample), namely rs16845548 (1000GENOMES:phase_3_FIN; distance = 20137 bp, $r^2 = 1.0$, $D' = 1.0$), in a linear regression controlling for age, gender, postmortem interval, pH, RNA integrity number and ancestral descent. Using a dominant model [heterozygotes ($n = 49$) pooled with the rare homozygous group ($n = 2$)], we found that this SNP was significantly associated with *GHSR* expression with

lower expression in the pooled groups compared with the more common homozygous group ($\beta = -0.110$, $P = 0.020$).

DISCUSSION

The present study is the first to report associations between the SNP rs2948694 in the *GHSR* gene and AUDIT scores as well as smoking. The association to smoking was not mediated by the association to AUDIT and vice versa. The data are in line with our previous studies in which the minor allele of rs2948694 was associated with increased risk of amphetamine dependence (Suchankova *et al.* 2013a) as well as increased body mass index in heavy alcohol-consuming individuals (Landgren *et al.* 2008). The present study further reports a significant association between a proxy SNP for rs2948694 and *GHSR* expression in the prefrontal cortex of the human brain, providing the first evidence for a possible functional role of this SNP. The previous association between the rs4684677 SNP and composite score of drug use from the addiction severity index (Suchankova *et al.* 2013a) was not backed up by the current study. Nevertheless, current and previous association studies report that the rs2948694 may be of importance for reward- and dependence-related phenotypes.

Genetic variation of the ghrelin signaling system has previously been associated with paternal alcohol dependence, reported withdrawal symptoms, smoking and type 2 alcohol dependence (i.e. early-onset and more heredity driven type of alcohol dependence) (Landgren *et al.* 2010). There is also a report of associations with personality traits such as self-directedness and self-transcendence in a sample consisting of controls and type 1 alcohol-dependent individuals (Landgren *et al.* 2011a). Interestingly, low self-directedness characterizes various kinds of addictive behaviors, raising the possibility that the system is involved in normal mental functions, which in turn may render a person susceptible to addictive behaviors.

Preliminary data on the possible functional value of the rs2948694 SNP reveal that it may affect *GHSR* expression in the prefrontal cortex of the human brain. Moreover, its location in intron 1 of *GHSR* leads the authors to speculate on a possible effect of this variant on the splicing of the gene especially given the prediction of a cryptic donor alternative splice site at the SNPs location. The *GHSR* gene is in fact subject to alternative splicing leading to two different mRNAs, GHSR-1a containing two exons forming the G-protein coupled receptor with high affinity for acylated-ghrelin and GHSR-1b encoded by exon 1 and the first 74 bases of intron 1. The physiological role of GHSR-1b is still unknown (Liu, Garcia & Korbonits 2011). An *in vitro* study reported heterodimerization between GHSR-1a and GHSR-1b resulting in a decrease in the constitutive signaling of GHSR-1a possibly due to the receptors translocation from the membrane to the nucleus (Leung *et al.* 2007). The rs2948694 SNP, although located in a non-coding region of either GHSR transcripts, could tentatively affect the splicing of the gene and cause a shift in GHSR-1a and GHSR-1b transcription, however, this remains to be confirmed. Furthermore, the SNP is in close proximity to a previously studied two-base pair deletion (Gueorguiev *et al.* 2009), rs10618418, and although we were not able to find any data on the linkage disequilibrium (LD) between these two SNPs, the previous study report strikingly similar genotype frequencies as seen for rs2948694 in the present study. It is thus possible that the markers are in strong LD and it is rather the effect of the deletion that is being studied here. Nevertheless, the functional value of this deletion remains to be determined.

The present findings showing associations between AUDIT scores, smoking and a SNP in *GHSR* gene are supported by pre-clinical data showing role for the GHSR-1a in drug reward. Pre-clinical studies have shown that pharmacological or genetic suppression of the GHSR-1a in mice attenuated the ability of alcohol to induce locomotor stimulation, increase accumbal dopamine release and induce a conditioned place preference (Jerlhag *et al.* 2009). In rodents, GHSR-1a antagonism reduces alcohol intake and operant self-administration of alcohol and prevents relapse drinking (Jerlhag *et al.* 2009; Kaur & Ryabinin 2010; Landgren *et al.* 2012; Bahi *et al.* 2013; Suchankova *et al.* 2013b). Similarly, the rewarding properties of several psychostimulant drugs, including nicotine, cocaine as well as amphetamine, are attenuated by GHSR-1a antagonists in rodents (Jerlhag *et al.* 2010; Jerlhag & Engel 2011; Wellman *et al.* 2011; Wellman, Clifford & Rodriguez 2013), which further supports our hypothesis that ghrelin signaling system regulates reward in general.

Clinical studies involving alcohol-dependent subjects have shown a reversible increase in plasma levels of ghrelin during the initial phase of abstinence (Wurst *et al.* 2007), as well as correlations between elevated ghrelin levels and craving during alcohol withdrawal (Addolorato *et al.* 2006; Leggio *et al.* 2012). However, the circuitry and mechanism by which ghrelin modulates these cravings in humans remain to be elucidated. In light of published pre-clinical results, a possible route could involve direct actions on the cholinergic-dopaminergic reward link. Firstly, *Ghsr* are expressed in nodes of this link including the nucleus accumbens (NAc) (Landgren *et al.* 2011b), on dopaminergic neurons in the ventral tegmental area (VTA) (Abizaid *et al.* 2006) and on cholinergic neurons in the laterodorsal tegmental area (LDTg) (Dickson *et al.* 2010). Secondly, local administration of ghrelin into either the VTA or LDTg increases the locomotor activity,

releases accumbal dopamine and increases alcohol consumption in mice (Jerlhag *et al.* 2007, 2009). Another possible route by which the ghrelin signaling system may alter the sensitivity of the mesolimbic dopamine system and the ability of addictive drugs to activate this system is by interacting with dopamine receptors. This was demonstrated recently *in vivo* and *in vitro* studies where GHSR-1a were found to regulate the activity of tegmental dopamine neurons by heterodimerizing with dopamine D1-like (Jiang, Betancourt & Smith 2006) and D2 receptors (DRD2) (Kern *et al.* 2012). Interestingly, GHSR-1a has been hypothesized to act as an allosteric modulator of dopamine-DRD2 signaling as effects are seen even in the absence of ligand (i.e. ghrelin) (Kern *et al.* 2012). This further suggests that GHSR-1a may function in areas with no ghrelin production and affect neurobiological processes involved in reward. This was further supported by a recently published study showing that when peripherally circulating endogenous ghrelin is pharmacologically hindered from entering the brain (via the high affinity compound Spiegelmer NOX-B11-2), there is no subsequent attenuation of the rewarding properties of alcohol in rodents (Jerlhag *et al.* 2014). Taken together with previous experiments, the importance of central GHSR-1a receptors in drug-induced reward was raised. It is thus possible that the minor allele of the rs2948694 alters the sensitivity of the cholinergic-dopaminergic reward link, which in turn may lead to a predisposition to smoking and increased alcohol consumption, reflected by the higher AUDIT scores in the present study.

Previous studies show that alcohol and nicotine dependence share a genetic background (True *et al.* 1999; Funk, Marinelli & Le 2006; von der Pahlen *et al.* 2008). This is further supported by the well-documented co-use of alcohol and nicotine—also observed in the present study—and the neurobiological interaction between alcohol and nicotine within the mesolimbic dopamine system (for review, see Larsson & Engel 2004; Soderpalm, Lof & Ericson 2009). Given the present results, it is possible that variations in *GHSR* contribute to the shared genetic background seen for these addictions.

The limitations of the current study include the low number of SNPs investigated and the fact that the genetic variation across the whole *GHRL* and *GHSR* genes are not taken into account. The best genetic models were recessive for AUDIT scores and dominant for smoking. A possible explanation to this discrepancy may be a threshold effect of the SNP on the various parameters i.e. while heterozygosity is enough to infer an increased risk in smoking, one needs to be homozygote for the risk allele in order to have an increased probability of having higher AUDIT scores. As nicotine is more addictive than alcohol (van Amsterdam *et al.* 2010), it is not farfetched to suggest that fewer genetic risk factors are needed to become addicted to nicotine. The poor fit with a recessive model for smoking explains why effect sizes for AUDIT are unchanged when controlling for smoking and a similar argument holds when controlling for AUDIT.

In summary, the minor allele of the rs2948694 in the *GHSR* gene was associated with increased AUDIT scores and smoking possibly reflecting an association with alcohol use disorder and nicotine dependence, respectively. These disorders share common reward mechanisms, involving the mesolimbic dopamine system. The current study suggests that the ghrelin signaling system may be a common denominator and thus a possible target in the development of new treatment strategies for addictive behaviors and disorders.

Acknowledgements

The Genetics of Sexuality and Aggression project has been funded by the following major grants: the Academy of Finland (grant no. 210298 and 212703) and Center of Excellence (grant no. 21/22/05) from the Åbo Akademi Foundation. The research was supported by grants from the Swedish Research Council (grant no. K2006-21X-04247-33-3, 2009-2782 and K2010-80X-21496-01-6), The Swedish brain foundation, LUA/ALF (grant no. 148251) from the Sahlgrenska University Hospital, Alcohol research council of the Swedish alcohol retailing monopoly and the foundations of Adlerbertska, The Society of Swedish Literature in Finland, Fredrik and Ingrid Thuring, Tore Nilsson, Längmanska, Torsten and Ragnar Söderberg, Wilhelm and Martina Lundgren, NovoNordisk, Knut and Alice Wallenberg, Magnus Bergvall, Anérs, Jeansons, Åke Wiberg, the Swedish Society of Medicine, Swedish Society for Medical Research and Torsten Söderberg Foundation. Neither of the funding sources had any further role in study design, collection, analysis and interpretation of data, in writing of the report and in the decision to submit the paper for publication.

Authors Contribution

PSu, JAE and EJ designed the study. PSu wrote the first draft of the manuscript and managed the literature search. SN and PSu undertook the statistical analyses. EJ supervised the manuscript preparation. BP, PSa, KS, PJ and AJ were in charge of project design, subject recruitment and data collection. PSu, SN, JAE and EJ contributed to the interpretation of data and manuscript preparation. All authors critically reviewed contents and approved final version for publication.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Table S1 Associations between investigated SNPs, AUDIT scores and smoking.