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Variation in Genes Encoding the Neuroactive Steroid Synthetic Enzymes 5α-Reductase Type 1 and 3α-Reductase Type 2 is Associated with Alcohol Dependence

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Abstract

Background—Studies of alcohol effects in rodents and *in vitro* implicate endogenous neuroactive steroids as key mediators of alcohol effects at $GABA_A$ receptors. We used a case-control sample to test the association with alcohol dependence (AD) of single nucleotide polymorphisms (SNPs) in the genes encoding two key enzymes required for the generation of endogenous neuroactive steroids: 5α–reductase, type I (5α-R) and 3α-hydroxysteroid dehydrogenase, type 2 (3α-HSD), both of which are expressed in human brain.

Methods—We focused on markers previously associated with a biological phenotype. For 5α-R, we examined the synonymous *SRD5A1* exon 1 SNP rs248793, which has been associated with the ratio of dihydrotestosterone to testosterone. For 3α-HSD, we examined the non-synonymous *AKR1C3* SNP rs12529 (H5Q), which has been associated with bladder cancer. The SNPs were genotyped in a sample of 1,083 non-Hispanic Caucasians including 552 controls and 531 subjects with AD.

Results—The minor allele for both SNPs was more common among controls than subjects with AD: *SRD5A1* rs248793 C-allele ($\chi^2(1)=7.6$, p=0.006) and *AKR1C3* rs12529 G-allele ($\chi^2(1)=14.6$, p=0.0001). There was also an interaction of these alleles such that the "protective" effect of the minor allele at each marker for AD was conditional on the genotype of the second marker.

Conclusions—We found evidence of an association with AD of polymorphisms in two genes encoding neuroactive steroid biosynthetic enzymes, providing indirect evidence that neuroactive steroids are important mediators of alcohol effects in humans.

Keywords

psychiatric genetics; neuroactive steroids; 5α-reductase; 3α-HSD; alcohol dependence; polymorphism

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Introduction

Alcohol dependence (AD) is a highly prevalent, chronic disease that has adverse effects on the health and quality of life of alcoholic individuals and their families. Although alcohol has multiple effects on neural cell physiology and signaling, the full set of factors contributing to the risk of AD is not yet known. One of ethanol's most potent effects on neural signaling is its allosteric modulation of γ-aminobutyric acid type A ($GABA_A$) receptors, where it increases the chloride channel open time (for a review see Koob (2004). Whether alcohol directly affects $GABA_A$ receptor function, however, is still unclear (Krystal et al. 2006). Alcohol may indirectly modulate $GABA_A$ receptor function by increasing levels of neuroactive steroids in plasma (Van Doren et al. 2000) and brain (Sanna et al. 2004). Neuroactive steroids are endogenous, highly potent (i.e., at nanomolar concentrations), positive allosteric modulators of $GABA_A$ receptor function (Paul and Purdy 1992). They increase the frequency and the duration of the open state of the GABA-gated chloride channel (Belelli and Lambert 2005), contributing to their anticonvulsant (Belelli et al. 1989; Reddy 2004), antinociceptive (Nadeson and Goodchild 2001), antidepressant (Khisti et al. 2000; Uzunova et al. 2006) and anxiolytic (Bitran et al. 1991; Wieland et al. 1991; Akwa et al. 1999) properties.

Neuroactive steroids have been shown to be involved in the acute effects of alcohol, as well as alcohol tolerance and dependence (Morrow et al. 2001). Alcohol increased levels of neuroactive steroids in plasma and brain of intact animals and in brain slice preparations (Barbaccia et al. 1999; Morrow et al. 1999; Van Doren et al. 2000; O'Dell et al. 2004; Sanna et al. 2004). Blockade of neuroactive steroid production by the 5-α-reductase (5α-R) inhibitor finasteride attenuated acquisition of alcohol preference in mice (Ford et al. 2008) and injection of the neuroactive steroid allopregnanolone into the hippocampus of alcohol-exposed rats decreased chronic voluntary alcohol consumption (Martin-García et al. 2007). Data from human studies supporting neuroactive steroids as mediators of alcohol effects are limited. In humans, the plasma concentration of allopregnanolone was increased following severe intoxication (Torres and Ortega 2003; Torres and Ortega 2004), but not moderate intoxication (Nyberg et al. 2005; Holdstock et al. 2006; Pierucci-Lagha et al. 2006). However, finasteride pretreatment in humans reduced the subjective effects of alcohol in some, but not all, healthy subjects (Pierucci-Lagha et al. 2005).

The most abundant neuroactive steroids in humans include the progesterone metabolite (3α, 5α)-3-hydroxypregnan-20-one (3α5α-tetrahydroprogesterone, allopregnanolone), the testosterone metabolite (3α,5α,17β)-androstane-3,17-diol (3α,5α-androstanediol), and the dihydroepiandrosterone metabolite (3α,5α)-3-hydroxyandrostan-17-one (3α,5α-androsterone). These compounds share a common A-ring structure containing a 3α-hydroxy group; in contrast 3β-isomers are inactive as $GABA_A$ receptor agonists. As depicted in Figure 1, generation of these compounds involves the sequential action of the enzymes 5α-R and 3α-hydroxysteroid dehydrogenase (3α-HSD) (Mellon and Griffin 2002). The neuroactive steroid 3α,5α-androsterone can also be converted to 3α,5α-androstanediol by the 17β-hydroxysteroid dehydrogenase (17β-HSD) enzyme activity of 3α-HSD type 2.

We used a case-control genetic association design to test the hypothesis that neuroactive steroids contribute to the effects of alcohol in humans. Specifically, we tested the association of AD with variation in genes encoding enzymes required for the synthesis of endogenous neuroactive steroids. We focused on the isoforms of the neurosteroid synthetic enzymes that are expressed in brain: 5α-R type I (*SRD5A1*), 3α-HSD type 2 (*AKR1C3*), 3α-HSD type 3 (*AKR1C2*), and 20α (3α)-HSD (*AKR1C1*). We limited the study to all common (reported frequency >0.05) single nucleotide polymorphisms (SNPs) that are in coding regions and

have previously been associated with a biological phenotype. This resulted in the selection of three SNPs, rs248793, rs12529, and rs11474, one in each of the genes (*SRD5A1*, *AKR1C3* and *AKR1C2*, respectively). For *SRD5A1*, the exon 1 synonymous SNP rs248793 (G>C) has been associated with the ratio of dihydrotestosterone to testosterone, such that individuals homozygous for the minor C-allele had a higher ratio than G-allele homozygotes and heterozygotes (Ellis et al. 2005). The *AKR1C3*2* SNP rs12529 (C>G; His5Gln) in exon 1 has been associated with bladder cancer in a large case-control sample (n=2299) of Spanish subjects, with the G-allele (Gln) being protective (Figueroa et al. 2008). For 3α-HSD type 3, it was reported that the 172Gln variant of the *AKR1C2*3* SNP rs11474 \ (T>A; Leu172Gln), when expressed *in vitro* from a synthetic gene construct, had a lower Vmax and Km for the reduction of dihydrotestosterone to 3α,5α-androstanediol (Takahashi et al. 2009). As secondary hypotheses we examined a) whether there were sex × genotype interactions based on the sex difference in serum levels of progesterone and testosterone neuroactive steroid precursors, and b) whether there were gene × gene interactions between the 5α-reductase and 3α-HSD gene polymorphisms in view of their sequential action in the production of neuroactive steroids.

Materials and Methods

Subjects

To reduce risk of population genetic stratification, we limited our analysis to a sample of self-identified non-Hispanic Caucasians. Alcohol dependent subjects (n=531) were recruited as part of ongoing studies of the genetics of AD or from treatment trials for AD at the University of Connecticut Health Center (UCHC), Farmington, CT. Psychiatric diagnoses were made using the Structured Clinical Interview for DSM-III-R or DSM-IV (SCID) (First et al., 2001), or the Semi-Structured Assessment for Drug Dependence and Alcoholism (SSADDA) (Pierucci-Lagha et al. 2005; Pierucci-Lagha et al. 2007). Control subjects (n=552) were screened using the SCID or the SSADDA to exclude individuals with a substance use disorder or other major Axis I psychiatric disorder. All subjects provided written, informed consent. All protocols and informed consent documents were approved by the institutional review board of the UCHC.

Genotyping

DNA was extracted from peripheral blood samples using a commercial kit (Gentra Puregene, Qiagen, Valencia, CA). The 5α-reductase rs248793 Hinf I restriction length polymorphism was analyzed by melting curve analysis. DNA was PCR amplified using primers TCGCCTACCTGCAGTGCGCC and TCGGAGCCTGTGGCTGGGCA in the presence of 1M Betaine for 40 cycles at 95°C for 30 seconds, 60°C for 15 seconds and 72°C for 60 seconds. 10uL of the PCR product was digested with 1 unit of Hinf I restriction endonuclease (New England Biolabs) overnight at 37°C. Following the addition of SYBR Green I and 6-Carboxy-X-Rhodamine (ROX), melting curve analysis was performed on a 7500 Real Time PCR System (Applied Biosystems).

The *AKR1C3* SNP (rs12529; Q5H) was genotyped using a closed-tube fluorescent TaqMan 5′-nuclease allelic discrimination assay using MGB-probes (Vic-ATTCCAAACA**G**CAGTGTG and Fam-ATTCCAAACA**C**CAGTGTG) and primers (GCTAGTCAGACAAGTGACAGGGAAT and CATGAAGTGGCCATCATTTAGCT) designed using Primer Express v3.0 software [Applied Biosystems Inc. (ABI) Foster City, CA]. The *AKR1C2* rs11474 SNP was examined using MGB-probes (Vic-CAGGC**A**GCTGGAG and Fam-CAGGC**T**GCTGGAG) and primers (ACATTTATCTTGcTCTTCagtcg and TACTTGAGCCCTGGCTTGTT) that were selected to amplify the *AKR1C2* flanking region but not the highly homologous *AKR1C1* gene

(primer sequence unique to *AKR1C2* shown in lower case). Fluorescence plate reads and genotype calls were made using ABI 7900 and 7500 Sequence Detection Systems following PCR amplification for 40 cycles at 95°C for 15 seconds followed by 60°C for 60 seconds.

Genotyping reactions failed for 12 samples for the *AKR1C3* TaqMan assay and for 47 samples for the *SRD5A1* Hinf I polymorphism. Fifteen percent of samples were re-assayed for the *AKR1C3* SNP with a single discrepancy observed. Ten percent of samples were re-assayed for the *SRD5A1* Hinf I polymorphism and two discrepancies were observed. Samples with discrepant results were not included. There was no evidence of deviation from Hardy-Weinberg expectations for *SRD5A1* or *AKR1C3* genotypes in AD or control subjects (all p's > 0.05). The *AKR1C2* SNP was monomorphic in all samples.

Data Analysis

Statistical comparisons were made using SPSS v15. Control and AD groups were compared on age using the t-test and on sex using the chi-square test. Associations between genotype and sex and genotype and AD, and deviation from Hardy-Weinberg expectations were examined using the chi-square test. Association between marker genotype and age was examined with ANOVA. Binary logistic regression was used to examine sex × genotype and gene × gene interactions with males coded as 1 and females as 2, and the number of minor alleles coded as 0, 1 or 2. The effect size per minor allele on AD relative risk for each marker conditioned on the genotype at the second marker was estimated using binary logistic regression. Age was not included in the binary logistic regression analyses as it was highly collinear with diagnostic group (cases and controls were independently collected and not matched for age). When age was included in a logistic regression model there was a 5.3-fold increase in risk per decade of age, and an initial Wald score of 265 for age. This was approximately 10 times that of sex and 100 times that of genotype and the main effect of *SRD5A1* genotype and interactions were no longer evident due to the shared variance that was accounted for by the age term and no longer attributable to genotype.

Results

Demographic characteristics of the study subjects are displayed in Table 1. All subjects were European-American. The control group was significantly younger than the AD group [$t(df=687) = -18.0$, $p<0.001$] and included more females [χ^2 $(df=1) = 102.8$, $p<0.001$]. Genotype was not significantly associated with age or sex in either the control or AD samples ($p>0.1$). Typical of clinical samples, a sizable number of alcohol dependent subjects had a history of cocaine or opioid use disorder (27.2%) and antisocial personality disorder (12.1%).

As shown in Table 2, controls and subjects with AD differed significantly on both genotype [χ^2 $(df=2) = 7.83$, $p=0.02$) and allele ($\chi^2(df=1) = 7.604$, $p=0.006$) frequencies for the *SRD5A1* exon 1 SNP rs248793. The minor C-allele was more frequent in controls than in subjects with AD (0.47 vs. 0.41). The C-allele frequency for the subset of AD subjects without a lifetime history of cocaine or opioid use disorder (0.42) did not differ significantly from that of the entire AD sample ($\chi^2(df=1) = 0.19$, $p=0.66$).

As shown in Table 3, for the *AKR1C3*2* exon 1 SNP rs12529 the phenotype groups differed significantly on both genotype ($\chi^2(df=2) = 15.1$, $p=0.0005$) and allele ($\chi^2(df=1) =14.64$, $p=0.0001$) frequencies.. The minor G-allele was more frequent in controls than in subjects with AD (0.45 vs. 0.37). Thus, for both the 5α-R type I and 3α-HSD type 2 genes, the minor allele may have a protective effect with respect to AD risk. The *AKR1C3*2* G-allele frequency for the subset of AD subjects without a lifetime history of cocaine or opioid use disorder (0.39) did not differ significantly from the entire AD sample ($\chi^2(df=1) = 0.63$,

p=0.43). The *SRD5A1* rs248793 and *AKR1C3* rs12529 genotypes (chr. 5 and chr. 10) were not correlated (r=0.013; p=0.667).

Examination of the putative (reported) *AKR1C2*3* L172Q variant (MAF 0.3 for CEPH population dbSNP build 130) indicated that it is likely a laboratory artifact related to the high degree of sequence identity between the *AKR1C1* and *AKR1C2* genes flanking the putative L172Q polymorphism (Figure 2). When gene specific forward primers were designed and used (*AKR1C1* - TcACATTTATCTTGaTCTT….CCA….CA and *AKR1C2* - ACATTTATCTTGcTCTTCagtcg) 100% of 96 Caucasian and 90 African American DNA samples were read as homozygous for T or leucine-172 for *AKR1C2* and A or glutamine-172 for *AKR1C1*. Direct sequencing of the region for 6 DNA samples using PCR amplicons generated with the gene-specific primers also showed homozygosity for all samples with CTG at codon 172 in *AKR1C2* and CAG at codon 172 in *AKR1C1*.

To examine potential sex × genotype and gene × gene interactions of the 5α-R type I and 3α-HSD type 2 gene polymorphisms, we used binary logistic regression to examine a model including sex, *SRD5A1* and *AKR1C3* genotypes in Block 1 and each 2-way interaction in Block 2 followed by backward elimination of non-significant interaction terms. As shown in Table 4, Block 2, sex × genotype interactions were not significant but there was a significant interaction of genotype for the *SRD5A1* and *AKR1C3* markers p=0.03.

The form of the *SRD5A1* × *AKR1C3* interaction is shown in Figure 3, where the AD relative risk estimates from binary logistic regression for each of the minor alleles as a function of genotype for the second marker are shown. As illustrated in Figure 3A, the reduced AD risk for the *AKR1C3* rs12529 minor G-allele was only observed among carriers of the *SRD5A1* rs248793 “protective” minor C-allele. The relative risk for AD per *AKR1C3*2* C-allele was 0.9 (95%CI=0.64–1.26; p=.54), 0.68 (95%CI=0.52–0.88; p=0.004), and 0.49 (95%CI=0.31–0.77; p=0.002), for subjects with *SRD5A1* G/G (n=311), G/C (n=520) and C/C (n=196) genotypes, respectively. A similar pattern was observed for the *SRD5A1* rs248793 SNP (Figure 3B), where a decreased AD risk for the *SRD5A1* minor C-allele was seen only among carriers of the *AKR1C3* rs12529 “protective” G-allele. The relative risk per *SRD5A1* rs248793 G-allele for AD was 0.98 (95%CI=0.72–1.35; p=0.92), 0.76 (95%CI=0.58–0.99; p=0.04), and 0.5 (95%CI=0.31–0.83; p=0.007), respectively, for subjects with *AKR1C3*2* rs12529 C/C (n=353), C/G (n=505) and G/G (n=169) genotypes.

Discussion

These findings support the hypothesis that neuroactive steroids play a role in mediating the effects of alcohol in humans by showing that polymorphic variation in enzymes required for the biosynthesis of neuroactive steroids are associated with risk of AD. We examined polymorphisms in genes encoding the brain expressed enzymes 5α-R type I (*SRD5A1*, containing 5 exons extending 36 kb on chromosome 5p15.31) and 3α-HSD type 2 (*AKR1C3*, containing 9 exons extending 13 kb onchromosome 10p15.1). These variants were previously associated with non-alcohol-related phenotypes. We found allelic and genotypic association with AD for each gene with the minor allele being more common in controls. A third marker that we examined based on a reported MAF of 0.3 and functional effects in vitro (Takahashi et al. 2009), *AKR1C2*3* rs11474 (L172Q), was monomorphic and likely represents a false SNP call in the human genome dataset generated by the high degree of sequence identity between the *AKR1C1* and *AKR1C2* genes.

To our knowledge, this is the first examination of the genetic association of AD with genes encoding enzymes involved in neuroactive steroid biosynthesis. Recently, (Joslyn et al. 2010) reported that a chromosomal region at 10p15-p14 containing the adjacent genes

AKR1C1, *AKR1C3* and *AKR1C4*, was associated with three measures of acute response to alcohol, adding further indirect (i.e., chromosomal localization) genetic support to the hypothesis that neuroactive steroids contribute to the effects of alcohol in humans.

We found that the minor C-allele of *SRD5A1* rs248793 was lower in frequency among alcoholics than controls. Although this exon 1 SNP does not encode an amino acid change, it could affect RNA stability or be in linkage disequilibrium with other polymorphisms that affect *SRD5A1* gene expression [rs248793 is contained in a 14 kb linkage-disequilibrium (LD) region comprising 2.5 kb upstream sequence, exon 1, and 11 kb of intron 1, (HapMap Phase III)].

In contrast to 5α-reductase, 3α-HSD type 2 has multiple enzyme activities. The 3α-HSD type 2 gene, *AKR1C3*, is a member of the aldo-keto reductase (AKR) superfamily with comparatively broad substrate specificity including estrogenic and androgenic steroid hormones (Penning et al. 2000), prostaglandins (Matsuura et al. 1998), drugs, and polycyclic aromatic hydrocarbons (Palackal et al. 2002). In addition to its 3-keto reductase function, the *AKR1C3* gene product displays prominent 17-keto reductase activity [the *AKR1C3* gene product is also known as 17β-HSD type 5 (Adamski and Jakob 2001)]. This 17-keto reductase activity is involved in the formation of testosterone from androstenedione (Dufort et al. 1999). Testosterone is then converted by 5α-R and 3α-HSD activities to the neuroactive steroid 3α-androstanediol, which is a potent anticonvulsant (Reddy 2004; Reddy 2008) and $GABA_A$ receptor modulating compound (Frye et al. 1996), whose clinical importance has not been as thoroughly studied as that of allopregnanolone. Finally, the *AKR1C3* gene product also has 3β-HSD activity, producing the inactive steroid 3β-androstanediol (Steckelbroeck et al. 2004). It is plausible that the polymorphism in the *AKR1C3* gene examined here affects the relative 17β-, 3α- and 3β-HSD activities of the gene product, thereby regulating the relative abundance of various steroids, only some of which positively modulate $GABA_A$ receptor function. Alternatively, the SNP may be in linkage disequilibrium with nearby promoter polymorphisms that influence the level of gene expression or an intronic polymorphism that affects RNA processing [rs12529 is contained in a 20 kb LD region encompassing the *AKR1C3* gene and 7 kb of upstream sequence (HapMap Phase III)].

Finally, our results suggest that there is a gene × gene interaction for the polymorphisms examined in *SRD5A1* and *AKR1C3*. Among subjects homozygous for the major allele in *SRD5A1*, there was no protective effect of the *AKR1C3* minor G-allele. Similarly, among subjects homozygous for the major *AKR1C3* allele, we observed no protective effect of the *SRD5A1* minor C-allele. Subjects homozygous for the *SRD5A1* minor C-allele have previously been reported to have a higher dihydrotestosterone/testosterone ratio (Ellis et al. 2005), suggesting that the minor C-allele may be associated with greater 5α-R Type I enzyme activity, and by extension potentially greater production of neuroactive steroids in response to alcohol. Others have hypothesized that a low level of response to alcohol is a risk factor for AD (Schuckit et al. 2007). Thus, if neuroactive steroids mediate some of alcohol's acute effects, increased neuroactive steroid response to alcohol could be protective and suggests that the *AKR1C3*2* protective minor allele may be associated with enhanced enzyme activity similar to the *SRD5A1* minor allele. Because the two gene products act in series to produce neuroactive steroids, it is possible that the minor (protective) allele for each gene is associated with effects (enhanced activity) that are reduced or silent when the second gene is homozygous for the ancestral major allele.

Strengths of this study are that it focused on polymorphisms previously reported to be associated with non-alcohol-related phenotypes, suggesting that they are functional or linked to functional polymorphisms in genes encoding the brain-expressed enzymes 5α-R type I

and 3α-HSD type 2. Second, the comparatively large samples of cases and controls provided good statistical power to detect association. Limitations of the study include that the cases and controls were not balanced on sex or age (however there was no association of either of these variables with genotype among cases or controls, arguing against their being major confounders). A second limitation is that we examined only one SNP in each of the two genes. Finally, because the case-control sample was limited to Caucasian subjects, the study of other populations is warranted. Replication of these results in additional case-control samples would be important.

In summary, this study provides support for a modulating role of neuroactive steroids in alcohol effects in humans and suggests that neuroactive steroid response patterns may differ among individuals, resulting in differential risk for AD. Future studies should examine haplotype-tagging SNPs in these genes in association with AD and the association of these markers with acute alcohol effects.

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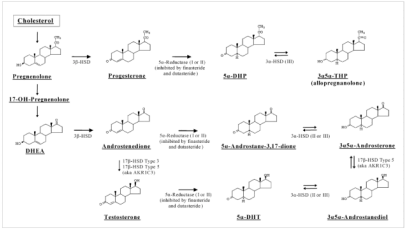

Figure 1.

Synthesis pathway of the neuroactive steroids allopregnanolone, androsterone and androstanediol. Abbreviations: 5α-DHP: 5α-dihydroprogesterone; 3α5α-THP: (3α,5α)-3-hydroxypregnan-20-one, 3α5α-tetrahydroprogesterone; DHEA: dehydroepiandrosterone; 5α-DHT: 5α-dihydrotestosterone; HSD: hydroxysteroid dehydrogenase

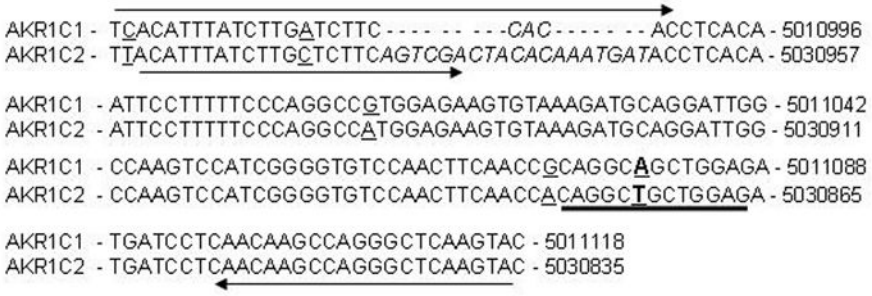

Figure 2.

Illustration of the high degree of sequence identity between *AKR1C1* and *AKR1C2* genes on chromosome 10 flanking the annotated polymorphism rs11474 (AKR1C2*3 L172Q). Base variations between the two genes are indicated by underscoring and inserted *AKR1C2* specific sequences in italics. The position of the annotated rs11474 *AKR1C2* T515A SNP is noted by bold text. The location of TaqMan allele specific probe is indicated by a solid line and the location of the common reverse and gene specific forward primers are indicated by arrows.

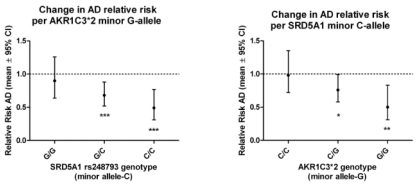

Figure 3.

Logistic regression relative risk estimates for AD with 95% confidence intervals for each marker stratified by the second genotype. Panel A. AD risk per *AKR1C3*2* minor G-allele as a function of the number of *SRD5A1* rs248793 minor C-alleles. Panel B. AD risk per *SRD5A1* minor C-allele as a function of the number of *AKR1C3*2* minor G-alleles. The protective effect of the minor allele for each gene was not evident in the setting of homozygosity for the major allele in the second gene. * $p<0.05$; ** $p<0.01$; *** $p<0.005$.

Table 1

Demographic Characteristics of the Samples.

	Controls n=552	Alcohol Dependent n=531
Age (years ± SD)	29.2 ± 9.8*	46.1 ± 9.4
Sex (% male)	38.8	69.5 #
Lifetime Cocaine Abuse or Dependence (%)	0	24.9
Lifetime Opioid Abuse or Dependence (%)	0	5
Antisocial Personality Disorder (%)	0	12.1

* $t = -18.0$; $p < 0.001$

χ^2 (df=1) = 102.8; $p < 0.001$

Table 2

Genotype and Allele Counts of *SRD5A1* rs248793 in Control and AD Subjects.

Group	Genotype count (frequency)			Genotype χ^2(df=2), *P*-value	Allele count (frequency)		Allele χ^2(df=1), *P*-value
	GG	GC	CC		G	C	
Controls n=524	141 (0.269)	270 (0.515)	113 (0.216)	χ^2 =7.83, P=0.02	552 (0.527)	496 (0.473)	χ^2 =7.604, P=0.006
AD n=512	174 (0.340)	253 (0.494)	85 (0.166)		601 (0.587)	423 (0.413)	

Table 3

Genotype and Allele Counts of *AKR1C3*2* rs12529 in Control and AD Subjects.

Group	Genotype count (frequency)			Genotype χ^2(df=2), *P*-value	Allele count (frequency)		Allele χ^2 (df=1), *P*-value
	CC	GC	GG		C	G	
Controls n=545	161 (0.295)	272 (0.499)	112 (0.206)	χ^2 =15.1, P=0.0005	594 (0.545)	496 (0.455)	χ^2 =14.64, P=0.0001
AD n=526	202 (0.384)	255 (0.485)	69 (0.130)		659 (0.626)	393 (0.374)	

Table 4

Logistic regression model results.

Factor	Initial Model			Final Model		
	b (SE)	Wald	*P*-value	b (SE)	Wald	*P*-value
Block 1 results						
Sex	−1.26 (0.13)	90.2	<0.001			
SRD5A1 rs248793	−0.24 (0.10)	6.40	0.012			
AKR1C3 rs12529	−0.36 (0.10)	13.9	<0.001			
Block 2 results						
Sex	−1.58 (0.27)	33.2	<0.001	−1.26 (0.13)	89.4	<0.001
SRD5A1 rs248793	−0.18 (0.32)	0.3	0.58	0.0 (0.15)	0	1.0
AKR1C3 rs12529	−0.46 (0.32)	2.1	0.15	−0.10 (0.16)	0.37	0.54
Sex × *SRD5A1*	0.13 (0.19)	0.42	0.52	-	-	-
Sex × *AKR1C3*	0.26 (0.20)	1.71	0.19	-	-	-
SRD5A1 × *AKR1C3*	−0.31 (0.14)	4.6	0.032	−0.31 (0.14)	4.54	0.033

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