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Genetic Markers of Co-Morbid Depression and Alcoholism in Women

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Abstract

Background—Alcohol Dependence (AD) is often accompanied by co-morbid depression. Recent clinical evidence supports the benefit of subtype specific pharmacotherapy in treating the population of AD subjects with co-morbid major depressive disorder (MDD). However, in many AD subjects, depression is a reactive response to chronic alcohol use and withdrawal, and abates with a period of abstinence. Genetic markers may distinguish alcohol dependent subjects with MDD not tied chronologically and etiologically to their alcohol consumption. In this work we investigated the association of adenylyl cyclase genes (*ADCY1–9*), which are implicated in both AD and mood disorders, with alcoholism and co-morbid depression.

Methods—Subjects from Vienna, Austria (n = 323) were genotyped and SNPs (1,152) encompassing the genetic locations of the nine *ADCY* genes were examined. The Vienna cohort contained alcohol dependent subjects differentiated using the Lesch Alcoholism Typology. In this typology subjects are segregated into four types. Type III alcoholism is distinguished by co-occurrence of symptoms of depression and by affecting predominantly females.

Results—We identified four haplotypes associated with the phenotype of Type III alcoholism in females. One haplotype was in a genomic area in proximity to *ADCY2*, but actually within a lincRNA gene, two haplotypes were within *ADCY5*, and one haplotype was within the coding region of *ADCY8*. Three of the four haplotypes contributed independently to Type III alcoholism and together generated a positive predictive value of 72% and a negative predictive value of 78% for distinguishing women with a Lesch Type III diagnosis versus women designated as Type I or II alcoholics.

Conclusions—Polymorphisms in *ADCY8* and *ADCY5* and within a lincRNA are associated with an AD phenotype in females, which is distinguished by co-morbid signs of depression. Each of these genetic locations can rationally contribute to the polygenic etiology of the alcoholism/depression phenotype and the use of these genetic markers may aid in choosing appropriate and beneficial treatment strategies.

Keywords

genetics; alcoholism; depression; Lesch typology; adenylyl cyclases

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INTRODUCTION

Many clinicians and researchers subscribe to the tenet that alcohol dependence (AD) encompasses a heterogeneous population of subjects. However, more effort needs to be made to assess genetic characteristics which may define more homogeneous subgroups of alcohol dependent subjects in order to direct the treatment specialist to more efficacious treatment strategies.

One of the characteristics that differentiate subtypes of alcoholics is the presence or absence of psychiatric co-morbidities (Lesch and Walter, 1996; Sintov et al., 2010), and one of the most prevalent co-morbidities in alcohol dependent subjects is major depressive disorder (MDD) (Grant et al., 2004). Several studies indicate that alcoholism and depression tend to occur together and to aggregate in the relatives of probands with both disorders (Merikangas and Gelernter, 1990; Merikangas et al., 1994). A recent analysis has confirmed that common genetic factors may contribute to the co-morbid occurrence of alcoholism and depression, even though both disorders also have independent determinants (Prescott et al., 2000).

One approach to the sub-classification of alcoholic subjects was introduced by Lesch and colleagues (1988 , 1996). Particularly relevant to the present study is the Lesch Type III category (Lesch et al., 2001), which segregates individuals who are classified as alcohol dependent and use alcohol to cope with their signs of depression (e.g., sleep disorders, where alcohol is used as a “self-medication”). This category is dominated by females (Walter et al., 2001).

A significant amount of evidence has appeared in the literature linking the cyclic AMP (cAMP) signaling cascades with both MDD and alcoholism (Desrivieres et al., 2011; Gass and Riva, 2007; Hines et al., 2006; Pandey et al., 2005). Although the greatest amount of interest has been devoted to PKA and CREB, the adenylyl cyclase (AC) family of enzymes is recently gaining more attention. ACs are a family of nine trans-membrane proteins (AC1–AC9) that display distinct responses to G protein-coupled receptors and other regulatory factors, leading to generation of cAMP from ATP. These enzymes play an essential role in learning and memory, synaptic plasticity and neurodegeneration. They are implicated in the development of addiction (DiRocco et al., 2009; Kim et al., 2006) and mood disorders. AC activity is increased by acute exposure to ethanol (Yoshimura et al., 2006), while chronic ethanol exposure often causes a decrease in cAMP production (Rabbani et al., 1999). Studies with knockout mice lacking certain ACs suggest a role in behavioral responses to ethanol in vivo, as well as in modulating complex behavioral outcomes such as depression and anxiety (Hines et al., 2006; Kim et al., 2008).

In the current work we present our analysis of association of polymorphisms in the genetic regions harboring the AC genes with the Lesch Type III category of alcohol-dependent subjects.

MATERIALS AND METHODS

Subjects

DNA extracted from blood samples was genotyped from a cohort of 333 alcohol dependent subjects assessed for AD and MDD, according to ICD-10 and DSM-IV, successfully classified into the Lesch typology using an electronic structured interview (http://www.lat-online.at), and not using illicit drugs at the time of enrollment (self-report and urine analysis). All subjects received out-patient treatment at the Medical University of Vienna, Austria, and all provided written informed consent. All the subjects were Vienna residents, of self-reported European ancestry.

Genotyping

Genomic DNA was genotyped using Affymetrix Genome-Wide Human SNP Array 6.0 according to manufacturer's protocols (Affymetrix, Santa Clara, CA). Prior to genotype calling, arrays were eliminated if their Contrast Quality Control metric, as calculated by the Affymetrix Genotyping Console 2.1, was less than 0.4 or when sample swaps were identified with Signature SNP genotypes using genotype calls from the Affymetrix software. Genotype calls and quality metrics were calculated using the second version of the Corrected Robust Linear Model with Maximum Likelihood Distance (CRLMM) method as implemented in the R package CRLMM (Carvalho et al., 2010). Calls were made separately for each batch of arrays processed (consisting of 36 to 87 subjects), with the exception of two consecutive batches that were combined due to low sample size.

Individual genotypes were recoded as missing if their CRLMM posterior probability was below 0.95. Entire SNPs were eliminated if their CRLMM quality metric was below 0.25 in any of the individual batches, if their minor allele frequency was less than 5%, if they were not in Hardy-Weinberg Equilibrium (HWE; unadjusted p-value<0.0001), if more than 20% of their genotypes were missing (i.e., posterior probability < 0.95), or if their concordance between sample duplicates (24 arrays representing 12 samples) was less than 90%. Arrays were eliminated if their CRLMM signal-to-noise ratio was below 5 or if more than 5% of their SNPs were missing genotype information. In addition, we compared the gender predicted by the CRLMM algorithm to the gender reported by the subject and eliminated arrays when they did not match. For statistical analysis, duplicate samples were consolidated to have one record per subject. If genotype calls differed for a SNP between replicate samples, the genotype call was treated as missing.

In the current study, we concentrated our analysis on 1,595 SNPs which occur in the genomic regions of the nine genes that code for the family of membrane bound ACs. Our full database can be accessed through http://phenogen.ucdenver.edu.

Case and Control

Our goal was to identify genetic factors that may distinguish Type III alcoholics (cases) from the Type I and Type II alcoholics (controls). This distinction between types is concordant with the prevalent co-morbidity of AD with depression for the Type III subjects and AD without concomitant depression for the Type I and Type II subjects (Lesch et al., 1988). Type IV subjects were excluded from the study because of the occurrence of early (childhood/adolescent) traumatic brain damage which would confound a genetic analysis.

Population Stratification

We examined the genome-wide genotype data for sample outliers and for population stratification that may confound associations with our case/control status using the principal component analysis (PCA) implemented in the smartpca program from the EIGENSTRAT package, EIGENSOFT 4.2 suite (Price et al., 2006). For calculating principal components, we only considered SNPs located on autosomal chromosomes and further pruned our data set using linkage disequilibrium. Pruning was done using the variance inflation factor in a sliding window as implemented in the PLINK software package (Purcell et al., 2007) (http://pngu.mgh.harvard.edu/purcell/plink/).

Statistical Analysis

Every SNP that passed quality control and was mapped to the genomic regions of interest was tested for association with Type III alcoholism using a genotype model with a Fisher's Exact test. We corrected for multiple testing by applying a permutation based false discovery rate (FDR, Xie et al., 2005).

Areas of interest from the SNP-wise associations were further explored for haplotype block structure. Haplotype block structure was examined with Haploview version 4.2 (Barrett et al., 2005) using the data collected from our subjects and the Centre d'Etude du Polymorphisme Humain collection of subjects of northern or western European descent living in Utah (CEU population) available through the HapMap project (Frazer et al., 2007). Haplotype blocks were defined using the method of Gabriel and colleagues (Gabriel et al., 2002) and a consensus was taken from the two populations.

When multiple suggestive SNPs were located within the same haplotype block, phased haplotypes and their associated posterior probabilities were calculated for each subject using the R function haplo.em from the haplo.stats package (Schaid et al., 2002). A global p value for the association of case/control status, as defined above (i.e., case = Type III alcoholism, control = Type I or Type II alcoholism), with each haplotype block, was calculated based on simulation methods (Schaid et al., 2002). For significant or suggestive (global p value < 0.10) haplotype blocks, we identified the specific haplotype with the strongest additive association with case/control status. These haplotypes were further investigated to identify the most appropriate genetic model using a weighted logistic regression. Weights were based on estimated posterior probabilities for each pair of phased haplotypes. Receiver operator curves (ROC) and associated statistics were calculated using the pROC library in R (Robin et al., 2011).

RESULTS

Of the 333 samples that were genotyped, 323 passed quality control standards (7 were eliminated prior to PCA and 3 were identified as outliers in the PCA). Further characteristics of subjects successfully genotyped are reported in Table 1. Of the 906,600 probe sets on the array, 620,594 probe sets remained after filtering for quality and retaining only informative SNPs. After filtering for autosomal chromosomes and pruning, 129,437 SNPs were included in the PCA. The population stratification analysis of the Vienna population generated a fixation index (F_{ST}) of zero showing that there is no genetic variation that would indicate stratification between cases and controls in this cohort.

We analyzed the genomic regions which contain the 9 membrane bound adenylyl cyclases for association with Type III alcoholism separately in males and females (Hines et al., 2006). We genotyped 1,595 SNPs in males and females and 1,152 SNPs met all quality control criteria and were analyzed individually. No single SNP in the vicinity of the 9 *ADCY* genes in either gender was significant (FDR < 0.05) after multiple testing correction. When adjusting for multiple comparisons in data generated from males, none of the FDR values for individual SNPs were less than 1. Therefore, we concentrated our analysis on the data obtained with female subjects. In females, an unadjusted SNP p value equal to 0.01 was equivalent to a permutation-based FDR of 0.33. We pursued further analysis of SNPs with an unadjusted p-value less than 0.01 with the caveat that conclusions reached are merely suggestive (i.e., hypothesis-generating) and that further studies are needed. To increase our confidence in positive results, we focused on areas of the genome with multiple "suggestive" markers (unadjusted p value <0.01) in close proximity to each other (i.e., haplotype blocks).

In females, there were 15 "suggestive" SNPs in the *ADCY8* region that were associated with the Type III phenotype (unadjusted p<0.01) (Table 2). We identified a haplotype block defined by nine SNPs (4 "suggestive" and 5 others; italicized SNPs are "suggestive"): *rs6998875,* rs7016933, rs12550604, *rs7829177*, rs7464362, *rs11781997*, rs13274422, *rs7843127* and rs12545028. This haplotype block spans two *ADCY8* exons (6 and 7) and

flanking introns (Chr 8: 131,902,911 to 131,922,027). The global p value for this haplotype block for association with the Type III phenotype was 0.18.

Another haplotype block was identified in the Chr 8: 131,987,325 to 132,002,814 area of the *ADCY8* gene in the female population and was defined by 11 SNPs (the "suggestive" SNPs are italicized): *rs6986982, rs4736465, rs11776982, rs7461448*, rs11779286, rs11776563, rs10956569, *rs17327852, rs17327900, rs13278912,* and *rs11991124* (Table 2, Figure 1A). This haplotype block encompasses exon 2 and flanking regions (Chr 8: 131,987,375 to 132,002,814). The global p value for this haplotype block indicated a significant association with case/control status (p value = 0.002). The 11 SNPs in this block defined three haplotypes with an estimated haplotype frequency greater than 5%. The ATCATCTTAAT haplotype was more prevalent in females with the diagnosis of Type I or II alcoholism (i.e., was protective against Type III alcoholism: OR = 0.13, p value = 0.002, dominant model; Table 3). Of women who were homozygous or heterozygous for this haplotype ("haplotype 1"), 0% and 19%, respectively, were classified as Type III alcoholics (Figure 2).

Seven SNPs associated with *ADCY5* were suggestive for association with Type III alcoholism (Table 2). Two haplotype blocks were identified in *ADCY5* that contained more than one of these suggestive SNPs. The first haplotype block included 6 SNPs (rs4677887, rs2877709, rs903572, *rs17295246*, rs9856983, *rs6764842*; "suggestive" SNPs are in italics). This haplotype block is within the intronic region between exon 1 and exon 2 (Chr 3: 123,100,223 to 123,106,287, Figure 1B). The global p value for this haplotype block indicated a significant association with case/control status (p value = 0.003). The 6 SNPs in this block defined three haplotypes, each with an estimated haplotype frequency greater than 5%. The GGCATA haplotype was more prevalent in females with the diagnosis of Type III alcoholism (i.e., predisposing: OR = 3.37, p value = 0.002, additive model).

The second haplotype block in *ADCY5* contained 5 SNPs including 4 associated with Type III alcoholism (*rs12496583*, rs6794936, *rs17295346*, *rs9840967*, *rs17295401*; "suggestive" SNPs are in italics). This haplotype block is adjacent to the other associated block and includes an alternative start site (Chr 3:123,121,286 to 123,128,413, Figure 1B). The global p-value for this haplotype block indicated a significant association with case/control status (p-value = 0.017) and 3 haplotypes had an estimated haplotype frequency >5%. The ATCCA haplotype ("haplotype 2") had the strongest association with Type III alcoholism and was more prevalent in females with the diagnosis of Type III alcoholism (i.e., predisposing: OR = 2.30, p-value = 0.008, additive model; Table 3). Of women who were homozygous or heterozygous for this haplotype, 53% and 100%, respectively, were classified as Type III alcoholics (Figure 2).

In the vicinity of *ADCY2* we found several "suggestive" SNPs associated with Type III alcoholism in females (Table 2). Three of the "suggestive" SNPs were near each other and, along with another SNP, rs4235569 ($p<0.09$), formed a haplotype block in this population that was also evident in the HapMap CEU population (Figure 1C and D). These SNPs (*rs4235567*, rs4235569, *rs12516822*, and *rs4541630*) ("suggestive" SNPs are italicized) cover an intergenic region between *ADCY2* and *PAPD7* (Chr.5: 7,177,338 – 7,178,622). More specifically, this haplotype block is located within the long intergenic non-coding RNA (lincRNA), referred to as the *RP11-122F24* gene (Figure 1C and D). These four SNPs were in tight linkage disequilibrium, and we were able to estimate phased haplotypes for each female subject with greater than 99% posterior probabilities. The global p-value for association between this haplotype block and case/control status was 0.011. For the four SNPs in this block, three haplotypes had an estimated haplotype frequency >5%. The haplotype of TGGC ("haplotype 3") was more prevalent in individuals with the Type III alcoholic diagnosis (i.e., predisposing: OR = 3.09, *p* value = 0.007, recessive model; Table

3). Of women who were homozygous or heterozygous for this haplotype, 64% and 40%, respectively, were classified as Type III alcoholics (Figure 2).

To investigate the combined effects of these haplotypes for distinguishing Type III alcoholics from non-Type III alcoholics, we modeled our case/control status using the four significant (global p-value<0.10) haplotypes: ATCATCTTAAT from *ADCY8,* GGCATA and ATCCA from *ADCY5*, and TGGC from *RP11-122F24*, simultaneously in a multivariate model. When using a backward-selection process, the GGCATA haplotype from *ADCY5* is the only haplotype eliminated from the full model. The final multivariate model containing one haplotype each from *ADCY8*, *ADCY5*, and *RP11-122F24* indicates that these potential genetic markers are independent of each other and when used together can distinguish Type III alcoholics from non-Type III alcoholics better than any haplotype alone. Using the three predictive haplotypes (Table 3) in a Receiver Operating Characteristic (ROC) analysis, the area under the ROC curve was 0.80, representing the fact that good discrimination can be achieved between women who are Type III vs non-Type III alcoholics by use of the identified genotypic markers. When we choose a diagnostic threshold that minimizes the error rate, the multivariate model has a positive predictive value of 72% and a negative predictive value of 78%.

DISCUSSION

The Type III alcoholics within the Lesch typology (Lesch et al., 1988) are distinguished by the co-occurrence of signs of depression and AD and are predominantly females. Epidemiologic studies show that such comorbidity in fact occurs at a higher rate in females compared to males (Helzer and Pryzbeck, 1988). In the current work we report three genomic regions of interest in females, within the *ADCY5* gene, the *ADCY8* gene and the *RP11-122F24* gene, which were found to be significantly associated with Type III alcoholism. The three haplotypes contribute independently to the prediction of Type III alcoholism and when used together provide good discrimination from Types I and II alcoholism (Lesch et al., 1988).

Twin studies have shown that a strong association exists between MDD and AD in females, but not in males (Cadoret et al., 1996). Thus, the markers we identified, together with some others, may be associated with a particular co-morbid phenotype which is evident primarily in women. Some genotypes that may be of interest to examine in combination with the genotypes we identified would be those reported by Philibert et al. (2003) and Wang et al. (2004), which were associated with alcohol dependence and comorbid MDD.

There is strong evidence to suggest a role for AC in behavioral responses to ethanol in vivo (Hines et al., 2006; Kim et al., 2011; Maas et al., 2005). The expression levels of AC7 are directly associated with depressive-like behavior in mice (Hines et al., 2006) as well as the level of adrenocorticotropin in the plasma under conditions of ethanol intoxication and/or stress (Pronko et al., 2010). Furthermore several genome-wide association studies have identified genes of the cAMP signaling pathway among the top candidates associated with psychiatric disorders: *ADCY3* in AD (Edenberg et al., 2010), *ADCY9* in MDD (Muglia et al., 2010), *ADCY8* in bipolar disorder (de Mooij-van Malsen et al., 2009) and *ADCY7* in alcoholism (Desrivieres et al., 2011).

Although the SNPs we identified cannot, at present, be linked directly to functional variants in the protein products of *ADCY5* or *ADCY8*, the *ADCY5* and *ADCY8* haplotypes may harbor other structural characteristics which are important for the regulation of the products of these genes. The genomic region of the *ADCY8* haplotype may contain genetic elements important for regulation of other genes. Computationally integrated ChIP-seq data

("Chromatin State Segmentation by HMM from ENCODE/Broad" UCSC Genome Browser track; http://genome.ucsc.edu) (Kent et al., 2002; Myers et al., 2011) identified regulatory segments (enhancers) in the region near haplotype 1. Enhancers may reside in introns or up- and downstream of the transcriptional unit they regulate, and generally harbor sites for tissue-specific DNA-binding proteins. Therefore regulatory elements within *ADCY8* could be affecting the transcription levels of *ADCY8* itself and/or other genes involved in alcoholism and depression.

The possible involvement of AC8 in psychiatric disorders has been well studied in animals. AC8 KO mice show hyperactivity, more risk taking behavior and do not show an increase in anxiety after repeated restraint stress (Schaefer et al., 2000). In contrast, female mice that displayed a higher level of avoidance behavior had an increased expression of AC8 in the brain (de Mooij-van Malsen et al., 2009). AC8 KO mice also showed decreased ethanol consumption compared to WT mice (Maas et al., 2005). Such studies suggest an involvement of AC8 in stress adaptation, alcohol consumption and mood disorders. In fact, recently a significant association of a marker (rs3914071) in *ADCY8* with bipolar disorder in humans was identified (de Mooij-van Malsen et al., 2009). Thus changes in the levels or activity of AC8 could have repercussions in the mood and addiction behavior of females.

The *ADCY5* gene is expressed primarily in regions of brain that receive dopaminergic input (Sanabra and Mengod, 2011) including nucleus accumbens and dorsal striatum. The type 5 adenylyl cyclase protein is the major transducer of signals generated by dopaminergic (D-1 and D-2) receptors and is also under substantial control of mu opioid receptors (Xie et al., 2012). Levels of AC5 protein have been linked to ethanol preference, levels of sedation produced by ethanol (Kim et al., 2011) and the anxiolytic actions of ethanol (Morales-Mulia et al., 2012). AC5 has also been noted to be elevated in its expression levels in subjects with current major depressive symptoms (Otsuki et al., 2010) and to be particularly important in procedural learning which plays an important role in both addiction and obsessive compulsive disorder (Kreitzer and Malenka, 2008). Given that the *ADCY5* haplotype includes an alternative start site, it is of interest to note that the N-terminus of AC5, which is coded by exons 1 and 2, can be alternatively spliced, and alterations in such events, as well as possible polymorphism-induced amino acid substitutions in the N-terminal regions, can alter the regulatory interactions between AC5 and other intracellular proteins (Wang et al., 2009).

The haplotype of interest in the vicinity of *ADCY2* is within the sequence of a long-intergenic non-coding RNA (lincRNA) gene [*RP11-122F24*; http://vega-previous.sanger.ac.uk (build VEGA45)], whose 678 nt long transcript has been shown to be expressed in the human amygdala (Kimura et al., 2006). LincRNAs have been implicated in risk for bipolar disorder, major depression (Chubb et al., 2008) and other disorders. One could speculate that the lincRNA identified in association with Type III alcoholism could be affecting the expression of multiple genes involved in mood and addiction behavior.

We have previously reported on a haplotype associated with MDD in women recruited in Montreal, Canada (Hines et al., 2006), consisting of 4 SNPs and a tetranucleotide repeat polymorphism that overlapped the *ADCY7* 3′ UTR. Further analysis of these data indicated that the odds ratio for distinguishing subjects diagnosed with depression from the non-depressed women is enhanced if we limit our analysis to women with a lifetime history of AD. In the current study, using subjects collected in Vienna, Austria, we did not find an association of polymorphisms in *ADCY7* and the Lesch Type III alcoholism versus the other subtypes of alcoholics. However, a number of significant differences distinguish the Montreal and Vienna subjects. First, from the information we had available we could not assign the Lesch phenotypes to the subjects in the Montreal population. Second, the genetic

population structure of the two populations was vastly divergent. The principal component analysis of 37 ancestral identification markers in the two populations combined, revealed a large genetic distance between the populations (F_{ST} = 0.025).

Dissecting the etiologic relationship of major depressive disorder and alcoholism is quite important in the realm of proper treatment and recovery of patients and reduction of cost to society. Pettinati and colleagues (2010) recently demonstrated the benefit of adding sertraline to naltrexone for treatment of alcohol dependent subjects with co-morbid MDD. It was clear that the combination of a serotonin uptake inhibitor with naltrexone promoted abstinence, delayed relapse and reduced signs of depression in subjects diagnosed with co-morbid alcoholism/depression (Pettinati et al., 2010). Our current data indicate that the three genetic haplotypes we described can generate a positive predictive value of 72% and a negative predictive value of 78% for distinguishing women with a Lesch Type III diagnosis (consisting of co-morbid alcoholism and depression) from subjects designated as Type I or II alcoholics. Our genetic information may assist in properly assigning individuals within a heterogeneous alcohol dependent subject population to effective treatment.

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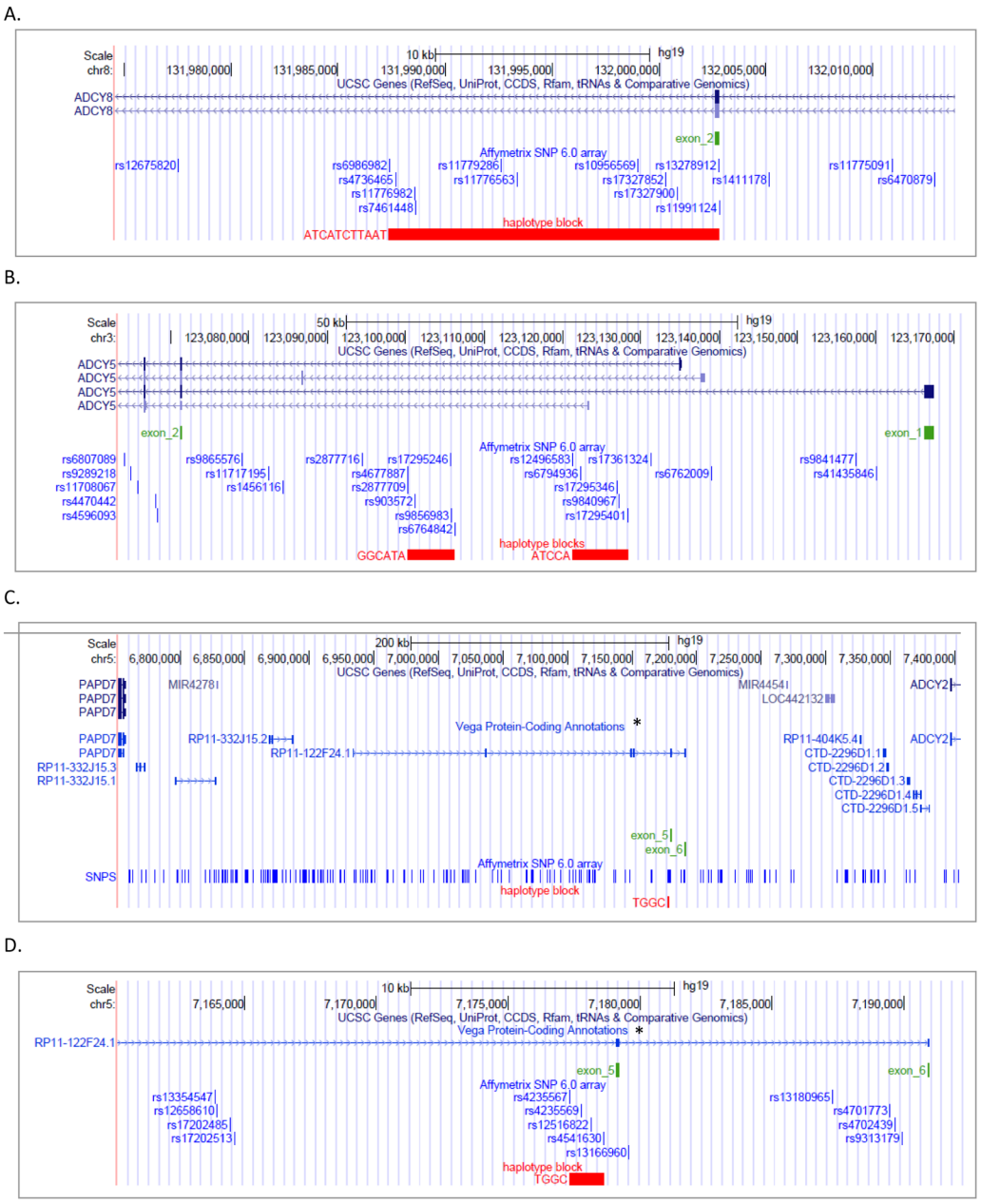

Figure 1. Location of haplotype blocks associated with Type III alcoholism in females

A) Haplotype block within the coding region of *ADCY8*. B) Haplotype blocks within *ADCY5*. C) Haplotype block within the lincRNA gene *RP11-122F24* located between *ADCY2* and *PAPD7*. D) Location of the same haplotype as in C showing dbSNP ID of the markers. * Please note that despite the UCSC track title, non-protein-coding transcripts are also included. Graphics created using the UCSC Genome Browser (Kent et al., 2002) (http://genome.ucsc.edu).

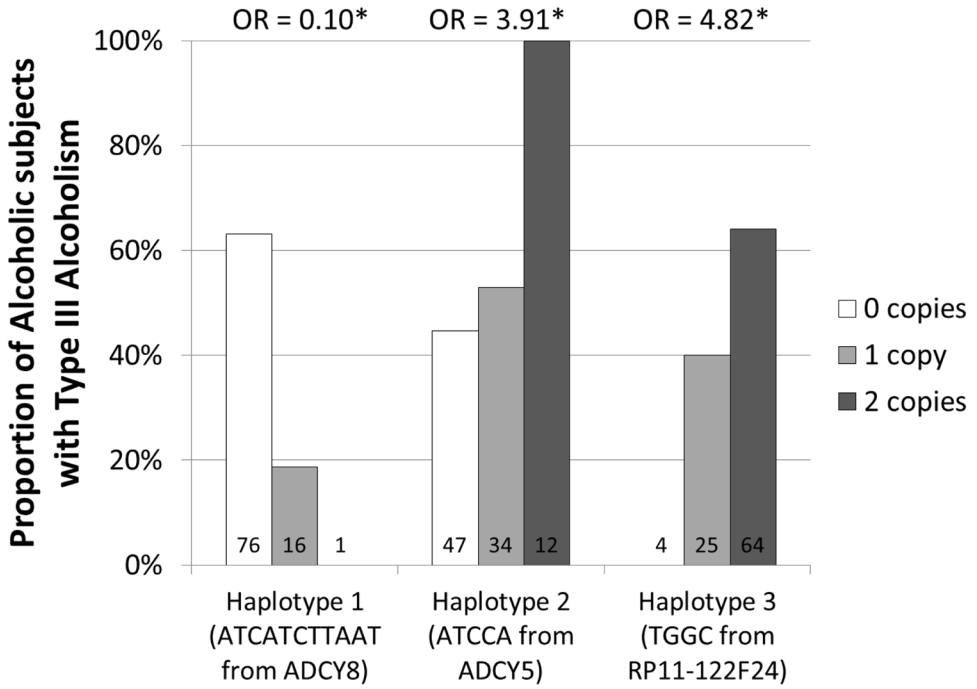

Figure 2. Proportion of female subjects with depression typology stratified by haplotype

The proportion of female subjects with Type III alcoholism according to the Lesch typology out of all alcohol-dependent female subjects is illustrated for individual haplotypes. The color of the bar represents the number of copies of the particular haplotype. The numbers at the bottom of each bar represent the total number of female subjects in each haplotype group. The odds ratio (OR) of the multivariate analysis is reported for each haplotype above the bars and represents a dominant model for haplotype 1, an additive model for haplotype 2, and a recessive model for haplotype 3. The *p* value for each haplotype is less than 0.01.

Table 1

Characteristics of Vienna Study Population

	Lesch Classification of Alcoholism			
	Type I	**Type II**	**Type III**	**Type IV**
FEMALE *(n = 116)*	11 (9%)[#]	31 (27%)	51 (44%)	23 (20%)
Endogenous depression[a] *(n = 44)*	0 (0%)[*]	0 (0%)	34 (67%)[d]	10 (43%)
Psychiatric disease in the family[b] *(n = 29)*	4 (36%)[*]	4 (13%)	15 (29%)	6 (26%)
Drinking > 60 g/day ethanol[c] *(n = 57)*	5 (45%)[*]	14 (45%)	24 (47%)	14 (61%)
MALE *(n = 207)*	44 (21%)[#]	52 (25%)	62 (30%)	49 (24%)
Endogenous depression[a] *(n = 48)*	0 (0%)[*]	0 (0%)	34 (55%)[d]	14 (29%)
Psychiatric disease in the family[b] *(n = 44)*	5 (11%)[*]	6 (12%)	20 (32%)	13 (27%)
Drinking > 60 g/day ethanol[c] *(n = 116)*	24 (55%)[*]	22 (42%)	42 (68%)	28 (57%)

[a] assessed by a psychiatrist using DSM-IV

[b] other than alcoholism

[c] patient's self-assessment of average daily alcohol consumption in 3 months prior to assessment (60 g/day is the established WHO criterion for hazardous/harmful alcohol use in males (Saunders and Lee, 2000)).

[d] Type III alcoholism is characterized by psychiatric symptoms which include major depressive disorder, severe suicidal ideations or attempts at suicide when not drinking, or severe sleep disorders.

[#] percentage of total subjects of the same gender

[*] percentage of subjects of the same subtype and gender

Table 2

Individual SNP Analyses in Female Subjects

Gene	Chr	Physical Location (bp)	dbSNP ID	Unadjusted *p* value
ADCY2	5	7164434	rs17202485	0.0051
	5	7177338	rs4235567 *	0.0081
	5	7178103	rs12516822 *	0.0081
	5	7178622	rs4541630 *	0.0093
	5	7424672	rs13175846	0.0017
	5	7433269	rs17227692	0.0016
	5	7627189	rs1541821	0.0087
ADCY5	3	122993710	rs4077111	0.0036
	3	123105721	rs17295246	0.0025
	3	123106287	rs6764842	0.0093
	3	123121286	rs12496583	0.0068
	3	123126972	rs17295346	0.0065
	3	123127106	rs9840967	0.0062
	3	123128413	rs17295401	0.0020
ADCY8	8	131892052	rs17248206	0.0019
	8	131894901	rs965815	0.0030
	8	131902911	rs6998875	0.0018
	8	131912052	rs7829177	0.0018
	8	131916318	rs11781997	0.0018
	8	131920536	rs7843127	0.0010
	8	131987375	rs6986982	0.0051
	8	131987659	rs4736465	0.0039
	8	131988562	rs11776982	0.0051
	8	131988609	rs7461448	0.0051
	8	132000301	rs17327852	0.0039
	8	132000828	rs17327900	0.0039
	8	132002770	rs13278912	0.0066
	8	132002814	rs11991124	0.0066
	8	132224892	rs4736472	0.0095

Individual SNPs from the genomic area of the 9 adenylyl cyclase genes that passed quality control standards were genotyped at varying probes per gene ratios: 137 for *ADCY1*, 365 for *ADCY2*, 28 for *ADCY3*, 5 for *ADCY4*, 35 for *ADCY5*, 7 for *ADCY6*, 8 for *ADCY7*, 478 for *ADCY8*, and 89 for *ADCY9*. The SNPs were investigated for their association with Type III alcoholism using a genotype model. Only SNPs with unadjusted p value < 0.01 for associations are shown.

* indicates SNPs within *RP11-122F24* gene.

Table 3

Multivariate Results Using Associated Adenylyl Cyclase Haplotypes

	Univariate Analysis		Multivariate Analysis (model p value < .0001)		ROC Analysis	
	OR (95% CL)	p value	OR (95% CL)	p value	univariate AUC (95% CL)	multivariate AUC (95% CL)
Haplotype 1 from ADCY8 (dominant model)	0.13 (0.03 – 0.48)	0.002	0.10 (0.02 – 0.47)	0.003	0.64 (0.56 – 0.72)	0.80 (0.71 – 0.89)
Haplotype 2 from ADCY5 (additive model)	2.30 (1.25 – 4.26)	0.008	3.91 (1.72 – 8.87)	0.001	0.65 (0.55 – 0.75)	
Haplotype 3 from RP11- 122F24 (recessive model)	3.09 (1.37 – 6.98)	0.007	4.82 (1.53 – 15.20)	0.007	0.63 (0.53 – 0.72)	

Haplotype 1 (ATCATCTTAAT) from *ADCY8*, haplotype 2 (ATCCA) from *ADCY5* and haplotype 3 (TGGC) from the lincRNA *RP11-122F24* were individually associated with Type III alcoholism in females. The multivariate analysis indicated that the predictive power of each haplotype was independent of the others. Using an ROC (receiver operating characteristic) analysis, combining the three *ADCY* haplotypes significantly improved the overall accuracy of predicting Type III alcoholism among alcohol dependent females. AUC, area under the curve.