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Sequence Variations of the Human *MPDZ* Gene and Association With Alcoholism in Subjects With European Ancestry

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Abstract

Background—*Mpdz* gene variations are known contributors of acute alcohol withdrawal severity and seizures in mice.

Methods—To investigate the relevance of these findings for human alcoholism, we resequenced 46 exons, exon–intron boundaries, and 2 kilobases in the 5' region of the human *MPDZ* gene in 61 subjects with a history of alcohol withdrawal seizures (AWS), 59 subjects with a history of alcohol withdrawal without AWS, and 64 Coriell samples from self-reported nonalcoholic subjects [all European American (EA) ancestry] and compared with the *Mpdz* sequences of 3 mouse strains with different propensity to AWS. To explore potential associations of the human *MPDZ* gene with alcoholism and AWS, single SNP and haplotype analyses were performed using 13 common variants.

Results—Sixty-seven new, mostly rare variants were discovered in the human *MPDZ* gene. Sequence comparison revealed that the human gene does not have variations identical to those comprising *Mpdz* gene haplotype associated with AWS in mice. We also found no significant association between *MPDZ* haplotypes and AWS in humans. However, a global test of haplotype association revealed a significant difference in haplotype frequencies between alcohol-dependent subjects without AWS and Coriell controls ($p = 0.015$), suggesting a potential role of *MPDZ* in alcoholism and or related phenotypes other than AWS. Haplotype-specific tests for the most common haplotypes (frequency > 0.05), revealed a specific high-risk haplotype ($p = 0.006$, maximum statistic $p = 0.051$), containing rs13297480G allele also found to be significantly more prevalent in alcoholics without AWS compared with nonalcoholic Coriell subjects ($p = 0.019$).

Conclusions—Sequencing of *MPDZ* gene in individuals with EA ancestry revealed no variations in the sites identical to those associated with AWS in mice. Exploratory haplotype and single SNP association analyses suggest a possible association between the *MPDZ* gene and alcohol dependence but not AWS. Further functional genomic analysis of *MPDZ* variants and investigation of their association with a broader array of alcoholism-related phenotypes could reveal additional genetic markers of alcoholism.

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Keywords

Genomics; Alcoholism; *MPDZ*; Withdrawal; Seizures

Twin And Linkage studies have revealed that alcohol dependence is highly heritable and allelic variants of several candidate genes have been found to be associated with altered risks for alcohol dependence and withdrawal (Higuchi et al., 2006; Liu et al., 2004; Prescott and Kendler, 1999; Schuckit et al., 1972; Tyndale, 2003). Using a combination of interval-specific congenic strains and recombinant progeny tests in mice (Buck et al., 1997; Fehr et al., 2002; Shirley et al., 2004), it was shown that the *Mpdz* gene and its variants could affect alcohol and barbiturate withdrawal liability measured by presence and severity of seizures. The human analog, *MPDZ*, is located on chromosome 9p24-p22 and encodes for the Multiple-PDZ-Domain protein, which is also known as MUPP1 and contains 13 PDZ domains (Ullmer et al., 1998). PDZ domains are protein-interaction domains and are often part of the multi-domain scaffolding proteins that assemble large molecular complexes, control synaptic protein composition and structure, and allow the dynamic trafficking of synaptic proteins in specific cell locations (Kim and Sheng, 2004). The *MPDZ* protein is known to be involved in learning and memory-related synaptic plasticity as well as seizures and epilepsy through its interaction with the serotonin 5-HT_{2C} receptor, glutamate NMDA receptors, GABA_B receptor, and dopamine D₂, D₃, and D₄ receptors (Bettler et al., 2004; Griffon et al., 2003; Krapivinsky et al., 2004; Parker et al., 2003).

Specific haplotypes associated with the production of the *Mpdz* protein variants II and III (NCBI# AAL37379.1 and AAL37390.1, respectively), differentiate strains of mice with severe withdrawal (including C57LJ and DBA/2J) from strains that have less severe withdrawal (including wild type C57BL/6J) and possess a haplotype that produces protein variant I (NCBI# AAL37377.2) (Fehr et al., 2002). Therefore, we hypothesized that genetic variability of the human *MPDZ* gene may also be associated with the risk of alcohol withdrawal seizures (AWS). This association may be due to variations in the coding sequence of the human *MPDZ* gene that are analogous to those described in mice (Fehr et al., 2002). Alternatively, variations in the regulatory regions could potentially affect protein production and contribute to the risk of alcohol withdrawal and AWS (Shirley et al., 2004).

To address these hypotheses, we performed direct comparisons between the human *MPDZ* gene and its mouse homolog *Mpdz* to identify potential variability in the human DNA sites that are homologous to the variability sites described as being part of the withdrawal seizure-related haplotype in mice (Fehr et al., 2002). The current version of the dbSNP (build 129) lists a total of 589 single nucleotide polymorphisms (SNPs) in the human *MPDZ* gene including 23 SNPs (12 nonsynonymous, 10 synonymous, and 1 frame shift) in coding regions (NCBI). In order to ensure the inclusion of all relevant variations, we performed resequencing of the *MPDZ* gene in a sample of alcohol-dependent subjects with European Ancestry and a sample of DNA from self reported nonalcoholic European Americans (EA) obtained from the Coriell Institute.

The results of this sequence comparison between mice and humans are reported as well as comparisons of sequence variation frequencies in groups of alcohol-dependent subjects with and without AWS and in the Coriell sample.

MATERIALS AND METHODS

Study Subjects

This study was conducted in compliance with the Code of Ethics of the World Medical Association (Declaration of Helsinki) and the standards established by the Institutional Review Board of the Mayo Clinic Rochester. Participants were recruited from subjects treated in the outpatient and residential addictions treatment programs or on the medical and surgical floors of the hospitals affiliated with the Mayo Clinic in Rochester, MN. All subjects provided informed consent. DNA samples were collected from 120 male and female EA subjects, 18 years or older, who met DSM-IVTR criteria for diagnosis of alcohol dependence (APA, 2000). Of these, 61 subjects had a history of alcohol withdrawal with seizures (AWS), including 15 subjects who also had a history of delirium tremens (DT). The remaining 59 subjects reported a history of alcohol withdrawal without seizures or DT. Potential study subjects were *excluded* if they had a history of: psychotic or bipolar I disorders; use of substances known to potentially cause seizures or delirium (e.g., barbiturates, benzodiazepines, opiates, hallucinogens, stimulants, cocaine, hallucinogens, PCP, anticholinergics) at the time of the first AWS; neurologic conditions with seizures and or EEG or imaging data indicating presence of the potentially epileptogenic focus prior to the first AWS; presence of somatic or metabolic conditions capable of provoking seizures and or delirium at the time of the first AWS (e.g., fever > 40°C, sodium < 120 or > 160 mEq/l, glucose < 60 mg%, magnesium < 1.0 mEq/l, calcium < 6.0 mEq/l, ammonia > 50 µgN/dl, BUN > 100 mg%, creatinine > 5.0 mg%, or osmolality 350 mOsm/l). A review of the medical records and diagnostic evaluation of each study participant by a Board Certified psychiatrist (VMK) was performed to ensure that DSM IV-TR criteria for alcohol dependence are met and none of the exclusion criteria are present. Genomic DNA from 64 self-reported EA nonalcoholic subjects obtained from the Coriell Institute was used as nonalcoholic control.

DNA Resequencing

Genomic DNA was extracted from peripheral blood lymphocytes using AutoPure LS (Gentra, Minneapolis, MN), according to the manufacturer's protocol. New pairs of primers were generated for all exons of the human *MPDZ* gene, including the exon-intron boundaries and 2 kilobases in the 5' region based on the chromosome 9p24-p22 genomic sequence (NT_008413). The coding sequence of the human *MPDZ* gene was compared with the Ensembl transcript OTTHUMT00000055488. All exons and introns, including 75 to 150 bp of the boundary regions of the respective exons, were amplified using polymerase chain reaction and sequenced using an ABI PRISM Big Dye Terminator, Cycle sequencing assay (Applied Biosystems, Foster City, CA), and an ABI 3730xl automated sequencer (Applied Biosystems; Genomics Technology Center, Mayo Clinic, Rochester, MN). After the sequencing, sequence variants were screened using the Polyphred program.

Data Analysis and Statistics

The ALIGN software (Pearson et al., 1997) and ClustalW program (<http://www.ebi.ac.uk/Tools/clustalw2/index.html>) were used to compare coding DNA (cDNA) sequence of the human *MPDZ* gene and protein with the sequences of *Mpdz* gene and proteins of 3 mouse strains. The Branchpoint (BP) Analysis program of EMBL-EBI web site (<http://www.ebi.ac.uk/asd-srv/wb.cgi?method=2>) was used to determine if variations were located in consensus areas potentially important for alternative splicing process. The SIGSCAN (<http://bimas.dcrt.nih.gov/molbio/signal/>) program was used to identify potential transcription enhancer/repressor consensus sequences in the putative promotor areas.

Haploview v3.2 software (Barrett et al., 2005) was used to determine linkage disequilibrium (LD) of *MPDZ* gene variants. Allele frequencies of all variants with a minor allele frequency

(MAF) ≥ 0.05 were compared in the 3 groups of subjects using a Fisher's exact test. Associations of these variants with AWS were explored by comparing allele frequencies between alcohol-dependent subjects with a history of AWS and alcohol-dependent subjects without AWS history. Associations with alcoholism were explored by comparing allele frequencies of alcohol-dependent subjects without AWS history and frequencies in the nonalcoholic Coriell subjects.

Haplotype association tests were performed on haplotypes composed of all SNPs with a MAF ≥ 0.05 , as well as tag-SNPs representative of these SNPs, using the HaploStats package (Schaid et al., 2002). First, a global test of association was performed. This test simultaneously considers all haplotypes (allelic combinations) of a specific set of tightly linked polymorphisms, uses a single statistic to test whether frequencies of any of these haplotypes differ between cases and controls and, thus, eliminates the need for correction for multiple testing. Subsequently, haplotype-specific tests for the most common haplotypes (frequency > 0.05) were performed. Each haplotype-specific test assesses whether the frequency of a particular haplotype differs between cases and controls. For the haplotype with the largest score statistic, demonstrating the strongest evidence of association with the phenotype of interest, a simulation-based p -value for the maximum score statistic was calculated. The maximum statistic simulated p -value is used to control for multiple testing in this case. It is expected that the global test may be more powerful when several haplotypes are associated with the trait, while the maximum score statistic is expected to be more powerful than the global test of association when the effect of a single haplotype is much larger than that of all the other haplotypes (see Schaid et al., 2002, for details).

RESULTS

Study Subjects

The group of alcohol-dependent subjects who had a history of AWS included 44 males (72%) and 17 females (28%) with a mean age of 50 ± 10 . The group of alcohol-dependent subjects without an AWS history included 45 males (76%) and 14 females (24%) who had a mean age of 52 ± 8 . The average age of the beginning of regular use of alcohol was 18 ± 4 in the AWS group and 18 ± 3 in the group of subjects without AWS history. Maximum drinking tolerance (defined as the maximum number of drinks ever consumed per 24 hour) was 29 ± 18 in the AWS group and 23 ± 10 in the group of alcohol-dependent subjects without AWS. This difference in maximum drinking tolerance was not statistically significant (Kruskal–Wallis test $p > 0.05$).

Resequencing of the Human *MPDZ* Gene

The 46 exons, exon–intron boundaries, and proximal promoter region of the *MPDZ* gene were resequenced and 92 variants were identified including 67 new variants. A detailed description of the identified variations is presented in Table 1 and their locations are illustrated in Fig. 1. Primer sequences are available in the Table S1. Of the 92 variants, 13 had MAF ≥ 0.05 in the combined set of all subjects (Table 1, Fig. 1). Figure 2 shows the LD pattern of these 13 SNPs with a MAF ≥ 0.05 .

Comparison of the Human *MPDZ* Gene With the Mouse Homolog

As allelic variation in specific gene locations was associated with differences in severity of alcohol withdrawal as defined by presence of seizures in inbred strains of mice (Fehr et al., 2002), sequence similarity between human and mouse variants was explored. The human gene sequence (Ensembl transcript OTTHUMT00000055488 updated with our resequencing data) was compared with the gene sequence of 3 strains of mice: the wild type C57BL/6J (NCBI# AF326531), the C57L/J (NCBI# AF326533), and the DBA/2J (NCBI# AF326544). The

alignment showed 76% global sequence identity between human *MPDZ* gene and its mouse homolog *Mpdz*. As shown in Table 2, at 12 of 18 cDNA positions forming withdrawal seizures-related haplotype in mice, the nucleotides in human sequence were identical with C57L/J strain and 10 of 18 of these haplotype-forming sites were the same in human and the DBA/2J strain. Both of these strains are prone to severe withdrawal and seizures. In contrast, only in 5 of 18 haplotype-forming sites nucleotides were identical in humans and the withdrawal seizure-resistant C57BL/6J strain. However, no genetic variability of these sites in human *MPDZ* gene was found in our sample of 240 chromosomes from alcoholics and 128 chromosomes from Coriell subjects (all with EA ancestry). This analysis had sufficient power (97.5%) to reveal rare polymorphisms and mutations (MAF = 0.01).

The amino acid sequence of the human *MPDZ* protein (NP_003820) was compared to those of the inbred lines, C57BL/6J (AAL37377), C57L/J (AAL37379), and DBA/2J (AAL37390). Alignment showed 83% global sequence identity between the human *MPDZ* protein and its mouse homolog. As shown in Table 2, of the 18 amino acids corresponding to the SNPs described as being part of the seizure-prone haplotype in mice, 6 were completely conserved between human and mice. Of the remaining 12 amino acids, 5 amino acids in C57L/J strain, 4 amino acids in DBA/2J strain, and 4 in C57BL/6J strain were identical to the human sequence (Table 2).

Comparison of the *MPDZ* Variations Frequency in the Alcoholic and Coriell Samples

To explore potential associations of the human *MPDZ* gene variations with AWS and with alcoholism, we studied 13 variants (Table 3) with $MAF \geq 0.05$. The synonymous rs13297480 G variant, in exon 7, was found to be significantly more prevalent in alcoholics without history of AWS compared to nonalcoholic Coriell subjects ($p = 0.019$), or to alcoholics with history of AWS ($p = 0.017$). Also, the intron 24 rs7024892 G variant was found to be less prevalent in AWS group compared to the group without AWS history. Given that these results are not corrected for the multiple comparisons performed, replication is necessary to confirm these findings.

The association between *MPDZ* haplotypes and alcoholism with and without AWS was further investigated by considering the haplotype that comprised all 13 SNPs with $MAF \geq 0.05$. The comparisons were made using the approach proposed by Schaid and colleagues (2002), as described in the Methods section. The seven 13-SNP haplotypes with frequencies > 0.05 used for these comparisons are presented in Table 4.

First, to test for association between the *MPDZ* gene and AWS, the frequencies of the 13-SNP haplotypes were compared between subjects with AWS history and alcohol-dependent subjects without AWS. No significant haplotype associations were identified (data not shown).

To test for association between *MPDZ* gene and alcoholism without AWS, we compared frequencies of the same 13-SNP haplotypes between the alcohol-dependent subjects without AWS and Coriell samples. The results of this comparison, including frequencies of the 7 most common haplotypes (frequency > 0.05) and score test simulation p -values are shown in Table 4. The global score test (Schaid et al., 2002) led to a simulation-based p -value of 0.015. As explained in the Methods section, only one global haplotype test was performed for this comparison, and the p -value does not require correction for multiple testing. Tests of individual haplotype effects shown in the last column of Table 4 indicate that haplotype #7 had the strongest association with alcoholism (uncorrected $p = 0.006$). The corresponding maximum statistic simulation p -value, which corrects for the multiple tests performed on all individual haplotypes shown in Table 4, was 0.051 suggesting that haplotype #7 confers a higher risk of alcoholism than the other haplotypes. This haplotype is one of only 2 common haplotypes that

carries the high risk rs13297480 G variant in the exon 7, indicating its potentially important role.

We also investigated association of the studied phenotypes with haplotypes composed of 9 tag-SNPs selected from the 13 common SNPs. The tag-SNPs are indicated in Table 4 with an asterisk (*) following the SNP rs number. Results were very similar to those described above for the 13-SNP haplotype: There was no association between the tag-SNP haplotypes and history of AWS; however, there was evidence of association of the tag-SNP haplotypes with alcoholism without AWS (global test simulation $p = 0.031$; uncorrected p -value for most strongly associated haplotype = 0.007; maximum statistic simulation p -value = 0.045).

DISCUSSION

Animal studies have demonstrated that genetic variations in coding sequence of *Mpdz* gene and changes in the expression of this gene might be associated with the severity of acute alcohol withdrawal and seizures (Fehr et al., 2002; Shirley et al., 2004). To investigate the significance of this finding in human subjects with alcoholism, sequence variations of the human *MPDZ* gene were examined in alcohol-dependent subjects (EA ancestry) with and without history of AWS and in the DNA samples from EA subjects available at the Coriell Institute (as representatives of the EA population). Sequencing of 240 chromosomes from the alcohol-dependent subjects and 128 chromosomes from the Coriell subjects provided sufficient power to reveal rare polymorphisms and mutations, with 97.5% power to detect variations with a MAF of 0.01 within specific ethnic group. We identified 67 new sequence variants but found no genetic variability in the human *MPDZ* gene sites homologous to the variability sites described as being part of a withdrawal seizure-related haplotype in mice. We also found no significant haplotype association of *MPDZ* gene with a history of AWS, although a larger study would be recommended to reliably rule out this possibility.

At the same time, a statistically significant haplotype association of *MPDZ* gene with alcoholism was demonstrated when subjects without AWS history were compared to Coriell controls. This association disappeared when haplotype association was tested in all 120 alcoholics (with and without AWS) versus Coriell controls (data not shown). Several important issues need to be considered in order to interpret this finding. First, different alcoholism-related phenotypes may have shared as well as independent genetic risk factors. For example, it is well known that AWS-resistant C57BL/6J mice consume significantly more alcohol than the AWS-prone DBA/2J mice (Belknap et al., 1993; Metten and Crabbe, 2005). Two (or more) different genetic variations may be responsible for the increased propensity to AWS and decreased preference of alcohol. Alternatively, the pleiotropic effects of a single genetic variation may contribute to both phenotypes, leading to inverse correlation between propensity to AWS and alcohol preference phenotypes in mice. Both of these possibilities are potentially applicable to humans and need to be investigated. Second, all alcohol-dependent subjects in our study had a history of alcohol withdrawal and the number of subjects with the history of AWS was increased compared to their frequency in general population or among treatment seeking alcoholics (Caetano et al., 1998). Consequently, it is possible that increased presence of the AWS phenotype may mask the association between *MPDZ* gene and other alcohol-related phenotypes, including alcoholism in general and alcohol withdrawal symptoms other than seizures. Comparison of alcohol-dependent subjects with and without history of withdrawal is necessary in order to investigate this possibility. In fact, in the model studies in mice, only one alcoholism-related phenotype, defined as “acute alcohol withdrawal,” has been studied for association with *Mpdz* gene variations and mRNA expression (Fehr et al., 2002; Shirley et al., 2004). The results of this study indicate that the role of *MPDZ* gene variability in a broader array of alcohol-related phenotypes needs to be considered. As a scaffolding protein, *MPDZ* is known to interact with serotonin, dopamine, glutamate, and GABA receptors in the brain

(Bettler et al., 2004; Griffon et al., 2003; Krapivinsky et al., 2004; Parker et al., 2003). Therefore, if particular variations of the *MPDZ* gene alter its expression or protein structure, these variants could be associated with changes in addictive behaviors (e.g., impulsivity, craving, level of response and/or withdrawal), which have been associated with serotonin, dopamine, glutamate, and GABA neurotransmission and are implicated in alcoholism-related phenotypes in humans and in animal models (Dahchour and De Witte, 2003; De Witte et al., 2003; Heinz et al., 2004; Kalivas and Volkow, 2005; Krystal et al., 2003; Nakamura et al., 1999).

The discrepancy between allelic variations in the coding sequence of the mouse *Mpdz* gene associated with severity of alcohol withdrawal and corresponding human sequence might explain the lack of genetic association of human *MPDZ* haplotypes and the history of withdrawal seizures. However, this conclusion should not be extended to an association between the abundance of the *Mpdz* mRNA in the different strains of mice and severity of alcohol withdrawal (Shirley et al., 2004). It is possible that noncoding variations may be responsible for the difference of *Mpdz* mRNA expression in different strains of mice and that these variations may potentially be conserved between species. Our findings also suggest that species-specific genetic variations may contribute to the susceptibility to the disease while the role of the gene remains similar between species. Although the pattern of results emerging in psychiatric genetics is generally consistent with the findings of behavioral genetics in animal models, for some behaviors, the pathway from genes to behavior differs meaningfully between species (Kendler and Greenspan, 2006). Further research in mice and human studies are necessary to address this issue.

Our analyses indicated that a common rs7024892 variant in intron 24 was less frequent in the AWS group when compared to alcoholics without AWS while the synonymous rs13297480 (c1074A/G, Thr358Thr) substitution in exon 7 showed association with alcoholism (although not after correction for multiple testing). The minor 1074G allele was twice as frequent in the alcoholic group without AWS when compared to either subjects with AWS or Coriell groups. The same 1074G allele also differentiated haplotype #7, which showed strongest association with alcoholism. This warrants further investigation of its functional importance. Although synonymous single-nucleotide polymorphisms do not produce altered coding sequences, the presence of a rare codon, marked by a synonymous polymorphism, may affect the timing of cotranslational folding and alter protein translation kinetics (Kimchi-Sarfaty et al., 2007). Thus, studies investigating effects of the rs13297480 SNP on *MPDZ* protein translation kinetics in the context of alcohol effects could be of interest. In addition, it was demonstrated that the expression of the Thr (TGT) tRNA is higher compared to the Thr (CGT) tRNA in most body tissues (Dittmar et al., 2006). These tRNA anticodons correspond to the ACA1074ACG variation of the Exon 7 SNP in the *MPDZ* gene. Thus, differences in the *MPDZ* expression in individuals with the c1074A and c1074G alleles may be tissue specific (e.g., different in the brain vs. liver). In this case, study of the tissue-specific response to alcohol and its withdrawal in subjects with different c1074 alleles would be of interest.

Although association tests were not performed for rare SNPs, we did note that 2 rare intronic variants (rs41265290 in Intron 15 and 7303GA in Intron 23) were more common in Coriell sample (3%) than in subjects with AWS history (0.8% in AWS group and 0.0% in alcohol-dependent subjects without AWS) (Table 1). Our sample does not provide adequate power to test for statistical significance of these differences and association with the group status. However, the last 2 variants are located near splice junctions and may influence alternative splicing patterns. Genomic variants located in or near consensus splice junctions at the exon–intron boundaries are important for specifying the splice sites, and estimated to affect more than 15% of human genetic diseases (Faustino and Cooper, 2003; Garcia-Blanco et al., 2004; Pagani and Baralle, 2004). Several transcripts of the *MPDZ* gene were reported in

humans. Thus, further investigation involving a sufficiently high number of study subjects should focus on these rare, but potentially important, variants and their contribution to the differential response to alcohol and its withdrawal in different subjects.

A limitation of this study is that only DNA from EA subjects was utilized, so it was not possible to assess variability in the human *MPDZ* gene in other ethnic groups. In addition, as the nonalcoholic control DNA was obtained from the Coriell Institute, rather than through random sampling from the same population as the alcoholic patients, potential effects of population stratification should be considered. As only EA subjects were enrolled in the study, we do not expect that population stratification was a serious problem. Another limitation is that there is no information about alcohol use by the subjects who provided DNA samples for the Coriell Institute. A consequence of using a control group for which detailed phenotyping is not available relates to the potential for mis-classification bias. Specifically, a proportion of the controls is likely to have the disease of interest or may develop it in the future. However, the resultant decrease in power should not be high. For example, if 5% of controls would meet the definition of cases, the loss of power is approximately the same as that due to a reduction of the sample size by 10% (Colhoun et al., 2003). As the prevalence of alcohol dependence is only 3.85% of white males and 2.37% in white females (Grant et al., 2004), the potential effect this has on the power of our study is modest. These considerations are in line with the approach used in several large case-control association studies [e.g., WTCCC Consortium (2007)]; however, a study design utilizing phenotypically screened controls would generally be desirable in future studies.

The association analyses between human *MPDZ* variation and alcoholism investigated here were an exploratory component of this study. A replication in an independent sample is necessary to confirm the obtained positive results. In addition, the possibility and nature of potential associations between the *MPDZ* gene variations, alcohol withdrawal, and/or other alcoholism-related phenotypes need to be further explored. If any of such associations were to be confirmed, it could be a useful genetic marker for increased risk of the development of alcoholism or related phenotypes.

In conclusion, our sequencing of the *MPDZ* gene in samples obtained from alcohol-dependent subjects and nonalcoholic control samples from Coriell Institute (all with EA ancestry) revealed 67 new, mostly rare variations. None of these variations was located in the sites identical to those associated with AWS sensitivity in mice. Exploratory haplotype and single SNP association analyses suggest possible association between the *MPDZ* gene and alcohol dependence and/or related phenotypes. Further investigation of *MPDZ* variations, including those that could alter *MPDZ* mRNA stability, translational efficiency, and/or alternative splicing (e.g., rs13297480 SNP in exon 7, rs41265290 in intron 15, and 7303G/A in intron 23) could provide important insights into the mechanism of this association and reveal valuable genetic markers of alcoholism and related phenotypes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Fig. 1.

Schematic representation of the known and newly discovered sequence variations in the human *MPDZ* gene. The arrows indicate approximate locations of the variant alleles. Pale arrows indicate variants with minor allele frequency (MAF) < 0.05 and dark arrows indicate variants with MAF > 0.05. The numbering of cSNPs corresponds to the start codon, ATG. Detailed information about each variant is presented in Table 1. Positions in the human *MPDZ* sequence homologous to 18 sites in mouse *Mpdz* sequence associated with variable propensity to alcohol-withdrawal seizures are indicated by *. Detailed information about each of these positions in the cDNA sequence is presented in Table 2.

Fig. 2.

LD and haplotype block analysis of 13 *MPDZ* variations with $\text{MAF} \geq 0.05$. LD plot prepared using Haploview (Barrett et al., 2005). Colors are used to display pairwise LD as follows: bright red, $\text{LOD} \geq 2$ and $D' = 1$; shades of pink/red, $\text{LOD} \geq 2$ and $D' < 1$; white, $\text{LOD} < 2$ and $D' < 1$. The numbers indicate pairwise r^2 values shown as a percentage. Blocks are defined based on the confidence interval method as described by Gabriel and colleagues (2002).

Table 1

Polymorphisms of *MPDZ* Gene of Alcoholic Subjects and Coriell Controls

Chr.	position	Gene position	dbSNP ID	Variation	Amino acid change	Location	Position	Minor allele frequency		
								Coriell <i>n</i> = 64	No AWS <i>n</i> = 60	AWS <i>n</i> = 60
1	13242041	-1727		G > C		Upstream-1727	Upstream-1727	0.0000	0.0085	0.0000
2	13241615	-1301		C > G		Upstream-1301	Upstream-1301	0.0078	0.0000	0.0000
3	13240778	-464		G > A		Upstream-464	Upstream-464	0.0000	0.0085	0.0000
4	13240750	-436		insT		Upstream-436	Upstream-436	0.2344	0.2458	0.1967
5	13240302	13		A > G	Ile5Val	Exon 1	c13	0.0000	0.0085	0.0082
6	13240274	41		C > T		Intron 1	IVS1 + 25	0.0078	0.0085	0.0164
7	13237636	2679		C > G	Gln61Glu	Exon 2	c181	0.0000	0.0085	0.0000
8	13237519	2796		A > G		Intron 2	IVS2 + 115	0.0000	0.0085	0.0082
9	13214491	25824	rs17273542	C > T	Ser92Leu	Exon 3	c275	0.0078	0.0169	0.0000
10	13214441	25874		G > T	Gly109Cys	Exon 3	c325	0.0000	0.0000	0.0082
11	13214410	25905		G > C	Cys119Ser	Exon 3	c356	0.0000	0.0000	0.0082
12	13214354	25961		A > C		Intron 3	IVS3 + 31	0.0156	0.0000	0.0000
13	13213592	26723		C > G	Gln171Glu	Exon 4	c511	0.0000	0.0000	0.0082
14	13213515	26800		G > A		Intron 4	IVS4 + 55	0.0000	0.0000	0.0082
15	13212200	28115		A > C		Intron 5	IVS5 + 32	0.0156	0.0085	0.0082
16	13211401	28914		A > G	Lys282Lys	Exon 6	c846	0.0078	0.0000	0.0000
17	13209839	30476		C > T		Intron 6	IVS6 + 1532	0.0078	0.0000	0.0000
18	13209797	30517-30518		delTA		Intron 6	IVS6 + 1573-74	0.0156	0.0000	0.0000
19	13209603	30712	rs34911705	G > C	Leu347Phe	Exon 7	c1041	0.0078	0.0000	0.0000
20	13209570	30745	rs13297480	A > G	Thr358Thr	Exon 7	c1074	0.0938	0.2034	0.0902
21	13209459	30856		A > G		Intron 7	IVS7 + 99	0.0000	0.0085	0.0000
22	13195789	44526		A > T		Intron 10	IVS10 + 126	0.0313	0.0000	0.0164
23	13195264	45051	rs2039333	T > C		Intron 10	IVS10 + 651	0.0313	0.0000	0.0164
24	13195020	45295		C > T		Intron 11	IVS11 + 15	0.0000	0.0085	0.0000
25	13186032	54283		T > C		Intron 12	IVS12 + 88	0.0078	0.0000	0.0082
26	13182360	57955		insA		Intron 13	IVS13 + 806	0.0156	0.0169	0.0164
27	13182320	57995	rs1331676	G > C		Intron 13	IVS13 + 846	0.0000	0.0000	0.0082

Chr. position	Gene position	dbSNP ID	Variation	Amino acid change	Location	Position	Minor allele frequency		
							Coriell n = 64	No AWS n = 60	AWS n = 60
28	13182201	58114	C > T	Arg633STOP	Exon 14	c1897	0.0000	0.0085	0.0000
29	13182048	58267	A > G		Intron 14	IVS14 + 82	0.0391	0.0169	0.0000
30	13182024	58291	C > T		Intron 14	IVS14 + 106	0.0234	0.0339	0.0164
31	13180292	60023	G > A	Val659Ile	Exon 15	c1975	0.0078	0.0085	0.0082
32	13180272	60043	C > T	Ile665Ile	Exon 15	c1995	0.0078	0.0000	0.0000
33	13180235	60080	A > T	Thr678Ser	Exon 15	c2032	0.0078	0.0000	0.0082
34	13180163	60152	G > A	Glu702Lys	Exon 15	c2104	0.0078	0.0000	0.0164
35	13180162	60153	A > T	Glu702Val	Exon 15	c2105	0.0078	0.0000	0.0164
36	13179093	61222	C > T		Intron 15	IVS15 + 1020	0.0000	0.0000	0.0082
37	13179017	61298	A > G		Intron 15	IVS15 + 1096	0.0313	0.0000	0.0082
38	13178754	61561	T > G		Intron 16	IVS16 + 29	0.0078	0.0000	0.0000
39	13178719	61596	T > C		Intron 16	IVS16 + 64	0.0000	0.0085	0.0000
40	13176355	63960	A > G	Lys799Glu	Exon 17	c2395	0.0078	0.0000	0.0082
41	13173701	66614	A > G		Intron 17	IVS17 + 2568	0.0000	0.0085	0.0000
42	13173486	66829	A > G	Leu860Leu	Exon 18	c2580	0.0156	0.0000	0.0082
43	13166312	74003	G > A	Ser918Ser	Exon 19	c2754	0.2734	0.3051	0.2049
44	13165987	74328	G > A		Intron 19	IVS19 + 148	0.2734	0.2966	0.2131
45	13165908	74407	C > T		Intron 19	IVS19 + 227	0.0391	0.0339	0.0164
46	13165719	74594-74596	delCTC		Intron 20	IVS20 + 30-32	0.3359	0.3390	0.2541
47	13165639	74676	G > T		Intron 20	IVS20 + 112	0.0000	0.0085	0.0000
48	13158588	81727	T > C		Intron 20	IVS20 + 7163	0.0000	0.0000	0.0082
49	13158251	82064	insT		Intron 21	IVS21 + 114	0.0000	0.0085	0.0000
50	13153010	87305	C > G		Intron 21	IVS21 + 5355	0.3359	0.3220	0.2295
51	13148261	92054	delC		Intron 22	IVS22 + 4429	0.0156	0.0085	0.0164
52	13148062	92253	G > A	Ser136Asn	Exon 23	c3407	0.0078	0.0085	0.0000
53	13140714	99601	G > A		Intron 23	IVS23 + 7303	0.0313	0.0000	0.0082
54	13140558	99757	T > G	Ser194Arg	Exon 24	c3582	0.0078	0.0085	0.0000
55	13140531	99784	A > G	Lys1203Lys	Exon 24	c3609	0.3359	0.3475	0.2377
56	13140506	99809	C > T		Intron 24	IVS24 + 4	0.0000	0.0000	0.0082
57	13137723	102592	A > G		Intron 24	IVS24 + 2787	0.3281	0.3644	0.2377

Chr. position	Gene position	dbSNP ID	Variation	Amino acid change	Location	Position	Minor allele frequency		
							Coriell <i>n</i> = 64	No AWS <i>n</i> = 60	AWS <i>n</i> = 60
58	13137602	102713	G > A	Arg1229Gln	Exon 25	c3686	0.0000	0.0085	0.0000
59	13137569	102746	A > T	Gln1240Leu	Exon 25	c3719	0.0000	0.0000	0.0082
60	13133591	106724	G > A		Intron 25	IVS25 + 3956	0.0000	0.0000	0.0082
61	13133470	106845	G > T	Asp1279Tyr	Exon 26	c3835	0.0000	0.0085	0.0000
62	13133254	107061	T > C		Intron 26	IVS26 + 211	0.0078	0.0000	0.0000
63	13130070	110245	G > A	Glu1307Lys	Exon 27	c3919	0.0000	0.0085	0.0000
64	13127982	112333	delT	Leu1392 ^a	Exon 28	c4174	0.0078	0.0000	0.0000
65	13127889	112426	A > G		Intron 28	IVS28 + 67	0.0000	0.0085	0.0082
66	13126160	114155	G > C	Gln1438His	Exon 30	c4314	0.0000	0.0000	0.0082
67	13123801	116514	A > G		Intron 31	IVS31 + 22	0.0000	0.0000	0.0082
68	13116836	123479	G > T		Intron 31	IVS31 + 6987	0.0078	0.0085	0.0000
69	13115415	124900	T > C		Intron 33	IVS33 + 1100	0.0078	0.0000	0.0000
70	13115201	125114	A > G		Intron 34	IVS34 + 14	0.3359	0.3390	0.2459
71	13113330	126985	T > C		Intron 34	IVS34 + 1885	0.0000	0.0000	0.0082
72	13109766	130549	A > G		Intron 37	IVS37 + 1972	0.0000	0.0085	0.0082
73	13105422	134893	A > C		Intron 38	IVS38 + 4079	0.3125	0.3644	0.2705
74	13105350	134965	insC		Intron 38	IVS38 + 4151	0.0078	0.0085	0.0082
75	13105349	134966	C > G		Intron 38	IVS38 + 4152	0.0313	0.0000	0.0082
76	13103159	137156	delA		Intron 40	IVS40 + 771	0.0313	0.0000	0.0082
77	13103006	137309	A > G		Intron 41	IVS41 + 4	0.0000	0.0085	0.0000
78	13102228	138087	C > G		Intron 41	IVS41 + 782	0.0078	0.0000	0.0000
79	13102024	138291	G > A	Arg1880Lys	Exon 42	c5639	0.0547	0.0339	0.0492
80	13102015	138300	G > A		Intron 42	IVS42 + 8	0.0000	0.0000	0.0082
81	13100138	140177	T > A		Intron 43	IVS43 + 497	0.0078	0.0000	0.0000
82	13100068	140247	C > T		Intron 43	IVS43 + 567	0.0078	0.0000	0.0000
83	13099969	140346	T > C	Ile1947Thr	Exon 44	c5840	0.0000	0.0085	0.0000
84	13099015	141300	G > T	Gly1968Cys	Exon 45	c5902	0.0000	0.0000	0.0082
85	13098915	141400	C > A		Intron 45	IVS45 + 20	0.0000	0.0000	0.0082
86	13098824	141491	G > A		Intron 45	IVS45 + 111	0.0078	0.0000	0.0000
87	13098794	141521	A > G		Intron 45	IVS45 + 141	0.4063	0.3729	0.4426

Minor allele frequency									
Chr. position	Gene position	dbSNP ID	Variation	Amino acid change	Location	Position	Cortell <i>n</i> = 64	No AWS <i>n</i> = 60	AWS <i>n</i> = 60
88 13097222	143093		C > T		Intron 45	IVS45 + 1713	0.0000	0.0000	0.0082
89 13097121	143194		A > G		Intron 45	IVS45 + 1814	0.0078	0.0085	0.0000
90 13096598	143717	rs3264	A > G		Exon 46	3'-UTR + 366	0.3359	0.4237	0.3689
91 13096469	143846		A > G		Intron 46	Intergenic + 109	0.0000	0.0000	0.0082
92 13096335	143980	rs722651	C > T		Intron 46	Intergenic + 243	0.3828	0.2627	0.3607

AWS, alcohol withdrawal seizure.

Variations included in the haplotype analysis are highlighted. AWS group included alcohol-dependent subjects with a history of withdrawal seizures. No AWS group included alcohol-dependent subjects without history of withdrawal seizures.

^a A frameshift.

Table 2

Comparison Between cDNA Sequence Positions of the Nucleotides Forming Withdrawal Seizures-Related Haplotype in Mouse *Mpdz* Gene and Nucleotides in the Homologues Positions in the Human *MPDZ* Gene

		1	2	3	4	5	6	7 ^a	8	9	10	11	12	13	14	15	16	17	18
Nucleotide change		C > T	A > G	A > G	G > T	A > G	A > G	G > A	G > C	T > C	C > T	A > G	T > C	G > A	T > A	C > T	T > C	C > A	A > G
Nucleotide	Mouse	261	669	1622	2072	2402	2575	2639	2742	2808	2930	2931	4002	4012	4298	4359	4365	5299	5505
location	Human	261	666	1643	2093	2423	2596	2659	2766	2832	2966	2967	4038	4048	4334	4395	4401	5344	5466
	Mouse	B6	C	A	A	G	A	A	G	C	C	A	T	G	T	C	T	C	A
	C57L/J	T	A	A	T	G	G	A	C	T	T	G	C	A	A	T	C	A	G
	D2	T	G	G	T	G	G	A	C	T	T	G	C	A	A	T	C	A	G
	Human	T	A	A	T	A	T	A	T	T	C	C	C	A	A	T	T	A	G
Amino Acid	Mouse	81	223	541	691	801	859	880	914	936	977	977	1334	1338	1433	1453	1455	1767	1835
location	Human	81	222	548	698	808	866	887-8	922	944	989	989	1346	1350	1445	1465	1667	1782	1822
	Mouse	B6	S	R	N	G	H	T	S	R	Y	A	G	V	I	C	T	H	K
	C57L/J	S	R	N	V	R	A	N	S	Y	V	V	G	M	N	C	T	N	K
	D2	S	R	S	V	R	A	N	S	Y	V	V	G	M	N	C	T	N	K
	Human	S	R	N	I	H	S	E/N	S	Y	A	A	G	M	N	T	T	N	K

^aX, association with the mild withdrawal/low propensity to seizures phenotype; ^aX, association with the severe withdrawal/high propensity to seizures phenotype; ^aX, nucleotide and amino acid residues in the human sequence, which are different from the mouse alleles; ^aX, amino acid residues identical in the all compared sequences.

^aSNP position corresponding to the "insertion" of the glutamate in the human protein sequence preceding asparagine/serine variants in mice.

Detailed information about the human and mouse sequences used for comparison is provided in the text. Color-coding is used to indicate the similarities and differences between the nucleotides and amino acids of the human and mice homologs.

Table 3
Polymorphic Variants of the *MPDZ* Gene Selected for LD and Haplotype Analysis

dbSNP ID	Variant	Location	Minor allele frequency			No AWS ^a versus Coriell ^b			AWS versus Alcoholics ^b		
			AWS	No AWS ^a	Coriell	p value	OR	95% CI	p value	OR	95% CI
N/A	insT	-436 upstream	0.197	0.246	0.234	0.882	1.064	0.567–1.994	0.437	0.752	0.388–1.450
rs13297480	A > G	Exon 7	0.090	0.203	0.094	0.019	2.459	1.113–5.701	0.017	0.390	0.163–0.878
rs2274856	G > A	Exon 19	0.205	0.305	0.273	0.673	0.858	0.475–1.546	0.078	1.700	0.909–3.218
rs1331674	G > A	Intron 19	0.213	0.297	0.273	0.777	0.893	0.494–1.614	0.142	1.554	0.832–2.930
rs3831219	delCTC	Intron 20	0.254	0.339	0.336	1.000	1.014	0.577–1.780	0.160	0.665	0.365–1.204
rs1331672	C > G	Intron 21	0.230	0.322	0.336	0.892	0.939	0.532–1.655	0.114	0.628	0.339–1.154
rs10756457	A > G	Exon 24	0.238	0.347	0.336	0.893	1.052	0.600–1.845	0.066	0.587	0.320–1.067
rs7024892	A > G	Intron 24	0.238	0.364	0.328	0.593	1.173	0.670–2.055	0.035	0.545	0.298–0.988
rs2297003	A > G	Intron 34	0.246	0.339	0.336	1.000	0.987	0.562–1.734	0.120	1.570	0.864–2.873
rs2274647	A > C	Intron 38	0.270	0.364	0.313	0.420	1.260	0.718–2.217	0.129	0.648	0.360–1.159
rs3765550	A > G	Intron 45	0.443	0.373	0.406	0.603	0.870	0.503–1.499	0.295	1.334	0.772–2.315
rs3264	A > G	Exon 46	0.369	0.424	0.336	0.188	1.451	0.838–2.522	0.429	0.796	0.458–1.379
rs722651	C > T	Intron 46	0.361	0.263	0.383	0.056	0.576	0.321–1.023	0.125	1.580	0.880–2.862

AWS, group of alcoholic subjects with a history of withdrawal seizure; OR, odd ratio; CI, confidence intervals.

^aNo AWS group included alcohol-dependent subjects without history of withdrawal seizure and/or delirium tremens.

^bStatistical analyses were performed using Fisher's exact test.

p values below 0.05 are presented in bold.

Table 4

Haplotypes With Alcoholism Without AWS

SNP															Max statistic simulated p-value
n	rs13297480*	rs2274856*	rs1331674	rs3831219*	rs1331672	rs10756457*	rs7024892	rs2297003	rs2274647*	rs3765550*	rs3264*	rs722651*	Haplotype frequency	Simulated p-value	
A		G	G		C	A	A	G	A	A	G	C	0.0621	0.087	
A		G	G		C	A	A	G	A	A	A	C	0.0725	0.164	
A		G	G		C	A	A	G	A	G	A	T	0.2421	0.162	
A		A	A	delCTC	G	G	G	A	C	A	A	C	0.0520	0.916	
A		G	G		C	A	A	G	C	A	A	C	0.0980	0.179	
A		G	G		C	A	A	G	A	G	G	C	0.0774	0.063	
G		A	A	delCTC	G	G	G	A	A	A	G	C	0.0746	0.006	
															0.051

re.

0, df = 7, Global simulation p-value = 0.015.

ed in bold.