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Novel MPDZ/MUPP1 transgenic and knockdown models confirm *Mpdz*'s role in ethanol withdrawal and support its role in voluntary ethanol consumption

Lauren C. Milner[1,3], **Renee L. Shirley**[1,3], **Laura B. Kozell**[1], **Nicole A. Walter**[1], **Lauren C. Kruse**[1], **Noboru H. Komiyama**[2], **Seth G.N. Grant**[2], and **Kari J. Buck**[1]

[1]Department of Behavioral Neuroscience, Department of Veterans Affairs Medical Center and Oregon Health & Science University, 3710 SW US Veterans Hospital Road, mail code RD40, Portland, OR 97239-3098, USA

[2]Edinburgh University, 49 Little France Crescent, Edinburgh EH16 4SB, UK

Abstract

Association studies implicate multiple PDZ domain protein (MPDZ/MUPP1) sequence and/or expression in risk for alcoholism in humans and ethanol withdrawal (EW) in mice, but confirmation has been hindered by the dearth of targeted genetic models. We report the creation of transgenic (MPDZ-TG) and knockout heterozygote (*Mpdz*$^{+/-}$) mice, with increased (2.9-fold) and decreased (47%) target expression, respectively. Both models differ in EW compared to wild type littermates ($p \leq 0.03$), providing compelling evidence for an inverse relationship between *Mpdz* expression and EW severity. Additionally, ethanol consumption is reduced up to 18% (p=0.006) in *Mpdz*$^{+/-}$, providing the first evidence implicating *Mpdz* in ethanol self-administration.

Keywords

MPDZ; MUPP1; ethanol; withdrawal; consumption; QTL

Alcohol (ethanol) is widely abused for its euphoric and sedative effects. Adoption and twin studies demonstrate that alcohol dependence (alcoholism) is 50–60% genetically determined (Ducci and Goldman, 2012). Although no animal model exactly duplicates alcoholism, models for specific factors, such as ethanol self-administration (ES) and withdrawal (EW), are useful to identify potential determinants of liability in humans. Using a robust behavioral model of ethanol physiological dependence, we identified a quantitative trait locus that accounts for ~14–26% of the genetic variance in EW convulsions in mice (Buck et al.,

Corresponding Author: Kari J. Buck, Professor, Behavioral Neuroscience, OHSU Research Scientist, VA Medical Center, Telephone: (503) 220-8262 x56659, Fax: 503-220-3411, buckk@ohsu.edu.
[3]Co-first authors.

COMPETING INTERESTS STATEMENT
The authors declare that they have no competing financial interests.

AUTHOR CONTRIBUTIONS
Concept and study design (KB). MPDZ-TG development (RS) and analyses (RS, LBK). *Mpdz*$^{+/-}$ development and backcrossing (NK, SG, LM), expression and additional molecular analyses (LCK, NW), and behavioral analyses (LM, LBK). KB, LM and LBK wrote the paper.

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1997). Positional cloning narrowed this locus to a 1.8 Mb interval syntenic with human chromosome 9p24-p22.3; with resident gene sequence and expression analyses identifying allelic variation in *Mpdz*, resulting in differential multiple PDZ domain protein (MPDZ/MUPP1) expression and/or sequence, as presumably causal (Fehr et al., 2002; Shirley et al., 2004).

MPDZ/MUPP1 was initially identified as a putative scaffolding protein, containing 13 PDZ (PSD-95, Dig, ZO-1) domains, with widespread expression in the brain (Sitek et al., 2003; Ullmer et al., 1998). PDZ proteins are scaffold proteins that impact the rate and/or fidelity of signal transduction mediated by the protein complexes they scaffold, including a number of G protein-coupled receptors that require an interacting partner for membrane expression (Romero et al., 2011). MUPP1 interacts with numerous partners, including serotonin-2C (5-HT_{2C}) and 5-HT_{2A} receptors (Becamel et al., 2001; Ullmer et al., 1998), with this interaction regulated by receptor phosphorylation (Parker et al., 2003) and crucial for 5-HT_{2A} receptor trafficking to the plasma membrane, with a key role in cortical dendritic spine morphology (Jones et al., 2009). Additionally, MUPP1-$GABA_B$ receptor interaction impacts receptor stability and function (Balasubramanian et al., 2007), and MUPP1-SynGAP-CaMKII complexes regulate synaptic NMDA receptor-dependent AMPA receptor potentiation (Krapivinsky et al., 2004).

Thus far, rigorous analyses that MPDZ/MUPP1 expression and/or structure impacts EW have been lacking due to the dearth of *Mpdz* targeted genetic models. This has also hindered the assessment of MPDZ's potential role in ES and additional behaviors genetically correlated with EW (Metten et al., 1998). Toward this end, we created MPDZ transgenic mice (MPDZ-TG) using an artificial bacterial artificial chromosome (BAC) construct containing the full *Mpdz* gene, but no other protein coding gene. Its injection into embryos resulted in a transgenic founder, with repeated backcrossing to DBA/2 (D2) strain mice producing the finished MPDZ-TG model [D2-Tg(RP23-119B7)^{1KB}; see Supporting Methods]. We also created *Mpdz* knockout heterozygote mice using an embryonic stem cell line with an insertional (null) mutation in *Mpdz* (XG734, Bay Genomics), with repeated backcrossing to C57BL/6 (B6) strain mice producing the finished *Mpdz*$^{+/-}$ model (B6-*Mpdz*$^{Gt(XG734)Byg(+/-)1KB}$; see Supporting Methods). QPCR confirmed that target expression in the brain of *Mpdz*$^{+/-}$ is reduced by 53±1% ($t_{1,19}$=7.1, p=0.0001), and in MPDZ-TG is increased 2.9±0.1 fold ($t_{1,13}$=21.4, p<0.001), compared to their respective wild type littermates (WT). In naïve animals, neither model showed overt anatomical or behavioral differences compared to WT (see Supporting Methods).

Detection and fine-mapping mapping of this EW locus used an established behavioral model, with EW assessed after administration of a single hypnotic dose of ethanol (4 g/kg, ip) using handling-induced convulsions (HIC) as a quantitative measure of EW severity. The present studies therefore assess EW severity using this acute model (see Supporting Methods). Neither *Mpdz* genetic model showed a main effect of sex or a sex x genotype interaction for baseline or EW enhanced HIC scores compared to WT (all p>0.25, NS), so data for both sexes were collapsed throughout. Baseline (pre-ethanol) HIC scores did not differ between MPDZ-TG and WT (Fig. 1a), or between *Mpdz*$^{+/-}$ and WT (Fig. 1b). As we predicted, MPDZ-TG demonstrated significantly less severe EW than WT (Fig. 1a).

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Furthermore, despite modest EW due to the B6 genetic background, EW scores were significantly higher in *Mpdz*$^{+/-}$ than WT (Fig. 1b). Blood ethanol concentrations (BEC) were assessed in parallel using separate animals. BEC values did not differ between *Mpdz*$^{+/-}$ and WT (Fig. S2A), indicating that the genetic differences are pharmacodynamic rather than pharmacokinetic. MPDZ-TG had slightly lower BECs than WT at some but not all time points (Fig. S2B), but this did not hasten the time course for EW in MPDZ-TG compared to WT (Fig 1a). No difference in pentylenetrazol (30 mg/kg, i.p.) enhanced HIC scores was detected between MPDZ-TG and WT (29.6±1.6 and 27.0±2.0, respectively, $F_{1,77}$=1.0, p=0.3, NS) or between *Mpdz*$^{+/-}$ and WT (27.3±1.3 and 24.2±1.6, respectively, p=0.2, NS), demonstrating that *Mpdz* expression does not affect seizure susceptibility in general. Taken together, these data confirm that varying *Mpdz* gene dosage regulates EW, with an inverse relationship between *Mpdz* expression and EW severity. The strengths of the BAC transgenic approach complement the limitations of the knockout approach, and *vice versa*, so the finding that both support a role for *Mpdz* expression in EW is compelling, and the first direct evidence that MPDZ impacts EW.

B6 background models may not be optimal for EW studies, but are preferred for analyses of voluntary ethanol consumption. In a meta-analysis, Metten *et al.* (1998) found that low voluntary ethanol consumption using a two bottle choice paradigm is significantly genetically correlated with severe EW (using both chronic and acute ethanol exposure models), and *vice versa*, even when tested independently in separate animals, suggesting that ES and EW may share specific (anonymous) genetic contributions. Development of *Mpdz*$^{+/-}$ animals allowed us to begin to test the hypothesis that *Mpdz* may be one of the shared genetic contributions. Here, using a two-bottle, free-choice protocol in which mice could choose either water or an ascending series of ethanol concentrations, *Mpdz*$^{+/-}$ consumed significantly less of the 6%, 10% and 20% ethanol solutions per kilogram body weight each day compared to WT littermates (Fig. 2a). Preference data indicated that both genotypes preferred 3%, 6% and 10% (preference ratios >0.5), but avoided 20% ethanol (preference ratio <0.5) (Fig. 2b). Both ethanol consumption and preference in WT mice were consistent with levels normally seen in the C57BL/6J background strain (Belknap et al., 1993; Melo et al., 1996). There was no volume compensation in water consumption (Fig. 2c), i.e., animals drinking less ethanol did not necessarily compensate by drinking more water. As a result, the total volume of fluid consumed is lower in *Mpdz*$^{+/-}$ than WT (Fig. 2f). One week after the ethanol drinking study, the same mice were tested for saccharin intake, selected for its sweet taste, lack of calories, and lack of known confounding pharmacological effects. Saccharin consumption and preference did not differ between *Mpdz*$^{+/-}$ and WT (Fig. 2d,e); nor did water consumption (not shown, p>0.1) or the total volume of fluid consumed (Fig. 2f). Thus, Mpdz+/− and WT do not consistently differ in total fluid consumption, with a difference found for some but not all studies (Fig 2f). In summary, these studies demonstate that Mpdz+/− consume significantly less ethanol than WT littermates, but do not differ from WT in free choice consumption of water or saccharin. These represent the first data implicating *Mpdz* in ES, as well as the first evidence implicating *Mpdz* in the genetic relationship between ES and EW in mice.

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Although limited to small populations thus far, recent human association studies find a promising association between the human homolog (*MPDZ*) and excessive ES (Tabakoff et al., 2009) as well as a possible association with diagnosis of alcoholism (Karpyak et al., 2009). Notably, if the relationship between high ES and low risk for EW in mice also exists in human populations, then one could predict an association with *MPDZ* (sequence and/or expression) in alcoholics with little or no history of EW, but not in alcoholics with a history of severe EW. This is in fact the case, with *MPDZ* haplotype status associated with the diagnosis of alcoholism in subjects with no history of severe EW, but not in alcoholics with a history of EW seizures and/or delirium tremens or heterogeneous populations (Karpyak et al., 2009).

In summary, the present studies use an integrated approach and state-of-the-art genetic models to confirm an inverse relationship between *Mpdz* expression and EW severity, and implicate *Mpdz* expression in ES, establishing MPDZ as a highly translational target. Importantly, our results may inform and help resolve an emerging dichotomy in human clinical studies suggesting *MPDZ* association with alcoholism depending on whether subjects with no history of severe EW are assessed independently. Although our results increase understanding of the genetic determination of EW and ES, there are limitations. The current ES studies are based solely on comparisons of $Mpdz^{+/-}$ and WT. Although potential limitations of conventional knockout models are mitigated in heterozygote models (Kalueff et al., 2007), future studies will be important to disentangle MPDZ actions from potential effects of embryonic stem cell derived genes and/or developmental compensation, e.g., using RNAi and/or conditional knockout models. Although the present studies do not directly assess how *Mpdz* expression translates through protein levels to behavior, we have demonstrated that lower *Mpdz* expression results in a corresponding decrease in MPDZ protein expression in brain (Shirley et al., 2004). Genetic variation in MPDZ sequence (structure) also exists in mice (Fehr et al., 2002), and may also contribute to MPDZ effects on EW. Currently, there is limited information available about how MPDZ relates to EW or its role in ES and other behaviors including response to additional drugs of abuse. Our MPDZ genetic models will be invaluable in this regard, and also to identify a *Mpdz* network, within which new biomarkers and “drugable” therapeutic targets (Hopkins, 2008) may exist, to improve prevention of alcohol abuse and/or treatment of dependent individuals. As more information becomes available on MPDZ-protein interactions and the relevant circuit, the mechanism by which MPDZ regulates EW and its contribution to ethanol use/abuse and relapse will become apparent.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

Balasubramanian S, Fam SR, Hall RA. $GABA_B$ receptor association with the PDZ scaffold Mupp1 alters receptor stability and function. J Biol Chem. 2007; 282:4162–4171. [PubMed: 17145756]

Becamel C, Figge A, Poliak S, Dumuis A, Peles E, Bockaert J, Lubbert H, Ullmer C. Interaction of serotonin 5-hydroxytryptamine type 2C receptors with PDZ10 of the multi-PDZ domain protein MUPP1. J Biol Chem. 2001; 276:12974–12982. [PubMed: 11150294]

Belknap JK, Crabbe JC, Young ER. Voluntary consumption of ethanol in 15 inbred mouse strains. Psychopharmacology (Berl). 1993; 112:503–510. [PubMed: 7871064]

Buck KJ, Metten P, Belknap JK, Crabbe JC. Quantitative trait loci involved in genetic predisposition to acute alcohol withdrawal in mice. J Neurosci. 1997; 17:3946–3955. [PubMed: 9133412]

Ducci F, Goldman D. The genetic basis of addictive disorders. Psychiatr Clin North Am. 2012; 35:495–519. [PubMed: 22640768]

Fehr C, Shirley RL, Belknap JK, Crabbe JC, Buck KJ. Congenic mapping of alcohol and pentobarbital withdrawal liability loci to a <1 centimorgan interval of murine chromosome 4: identification of *Mpdz* as a candidate gene. J Neurosci. 2002; 22:3730–3738. [PubMed: 11978849]

Hopkins AL. Network pharmacology: the next paradigm in drug discovery. Nat Chem Biol. 2008; 4:682–690. [PubMed: 18936753]

Jones KA, Srivastava DP, Allen JA, Strachan RT, Roth BL, Penzes P. Rapid modulation of spine morphology by the $5\text{-}HT_{2A}$ serotonin receptor through kalirin-7 signaling. Proc Natl Acad Sci U S A. 2009; 106:19575–19580. [PubMed: 19889983]

Kalueff AV, Ren-Patterson RF, Murphy DL. The developing use of heterozygous mutant mouse models in brain monoamine transporter research. Trends Pharmacol Sci. 2007; 28:122–127. [PubMed: 17275930]

Karpyak VM, Kim JH, Biernacka JM, Wieben ED, Mrazek DA, Black JL, Choi DS. Sequence variations of the human MPDZ gene and association with alcoholism in subjects with European ancestry. Alcohol Clin Exp Res. 2009; 33:712–721. [PubMed: 19175764]

Krapivinsky G, Medina I, Krapivinsky L, Gapon S, Clapham DE. SynGAP-MUPP1-CaMKII synaptic complexes regulate p38 MAP kinase activity and NMDA receptor-dependent synaptic AMPA receptor potentiation. Neuron. 2004; 43:563–574. [PubMed: 15312654]

Melo JA, Shendure J, Pociask K, Silver LM. Identification of sex-specific quantitative trait loci controlling alcohol preference in C57BL/6 mice. Nat Genet. 1996; 13:147–153. [PubMed: 8640219]

Metten P, Phillips TJ, Crabbe JC, Tarantino LM, McClearn GE, Plomin R, Erwin VG, Belknap JK. High genetic susceptibility to ethanol withdrawal predicts low ethanol consumption. Mamm Genome. 1998; 9:983–990. [PubMed: 9880664]

Parker LL, Backstrom JR, Sanders-Bush E, Shieh BH. Agonist-induced phosphorylation of the serotonin $5\text{-}HT_{2C}$ receptor regulates its interaction with multiple PDZ protein 1. J Biol Chem. 2003; 278:21576–21583. [PubMed: 12682077]

Romero G, von Zastrow M, Friedman PA. Role of PDZ proteins in regulating trafficking, signaling, and function of GPCRs: means, motif, and opportunity. Adv Pharmacol. 2011; 62:279–314. [PubMed: 21907913]

Shirley RL, Walter NA, Reilly MT, Fehr C, Buck KJ. Mpdz is a quantitative trait gene for drug withdrawal seizures. Nat Neurosci. 2004; 7:699–700. [PubMed: 15208631]

Sitek B, Poschmann G, Schmidtke K, Ullmer C, Maskri L, Andriske M, Stichel CC, Zhu XR, Luebbert H. Expression of MUPP1 protein in mouse brain. Brain Res. 2003; 970:178–187. [PubMed: 12706259]

Tabakoff B, Saba L, Printz M, Flodman P, Hodgkinson C, Goldman D, Koob G, Richardson HN, Kechris K, Bell RL, Hubner N, Heinig M, Pravenec M, Mangion J, Legault L, Dongier M, Conigrave KM, Whitfield JB, Saunders J, Grant B, Hoffman PL. Genetical genomic determinants of alcohol consumption in rats and humans. BMC Biol. 2009; 7:70. [PubMed: 19874574]

Ullmer C, Schmuck K, Figge A, Lubbert H. Cloning and characterization of MUPP1, a novel PDZ domain protein. FEBS Lett. 1998; 424:63–68. [PubMed: 9537516]

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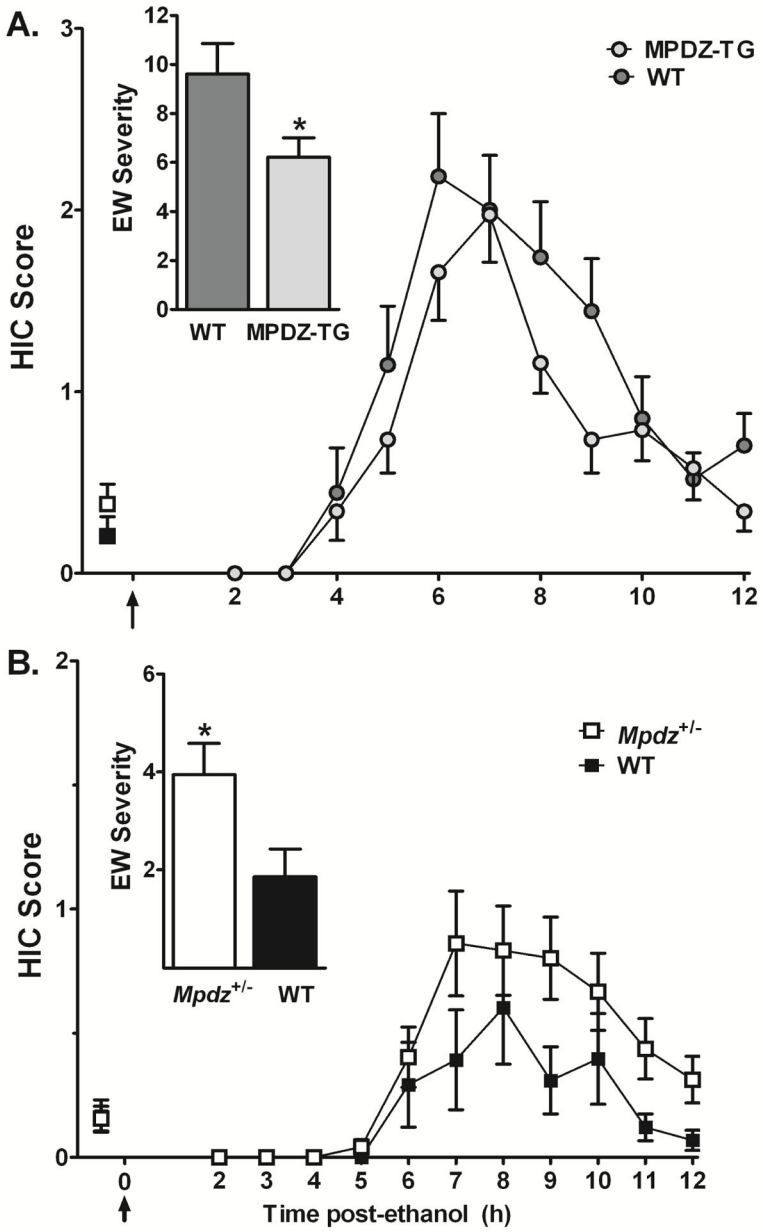

Figure 1.

Baseline and EW associated convulsion severity in MPDZ-TG and WT littermate mice. **(A)** MPDZ-TG (n=27) and WT (n=38) were scored twice for baseline HICs immediately before administration of 4 g/kg ethanol (the *arrow* marks ethanol administration at 0 h) and hourly between 2–12 h post-ethanol administration. Data represent mean HIC score ± SEM. Baseline HIC scores did not differ between MPDZ-TG and WT ($t_{1,63}$=1.1, p=0.27, NS). ***Inset:*** Data represent the EW score (corrected area under the curve, mean ± SEM), with EW significantly less severe in MPDZ-TG than WT ($t_{1,63}$=2.4, p=0.02). **(B)** EW severity in *Mpdz*$^{+/-}$ and WT littermates. *Mpdz*$^{+/-}$ (n=48) and WT (n=29) were tested as described above. Baseline HIC scores did not differ between *Mpdz*$^{+/-}$ and WT ($t_{1,73}$=-0.13, p>0.9,

NS). ***Inset:*** Data represent the EW score, with EW significantly more severe in *Mpdz*$^{+/-}$ than WT ($t_{1,75}$ =2.3, p=0.03). *p<0.05.

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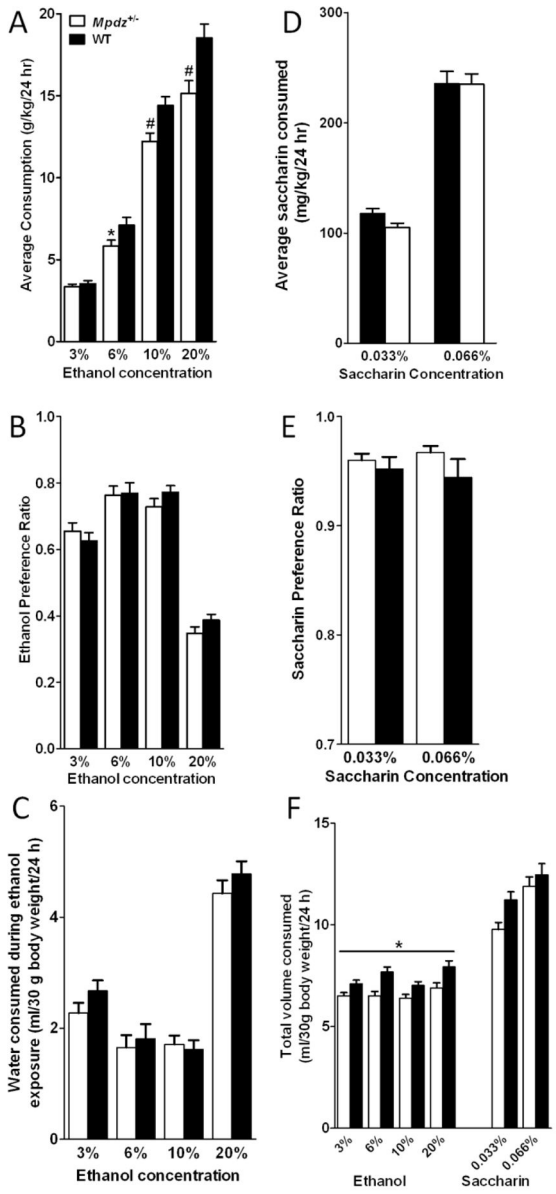

Figure 2.

Ethanol and saccharin consumption and preference in *Mpdz*$^{+/-}$ and WT littermate mice. **(A)** *Mpdz*$^{+/-}$ and WT were accessed for ethanol consumption using a two bottle free choice paradigm. Mean (± SEM) ethanol consumption (g/kg/24 h) was averaged across the second and fourth day of access for each concentration in *Mpdz*$^{+/-}$ (open bars) and WT (filled bars) littermates. Repeated measures ANOVA indicated a significant genotype and a genotype x ethanol concentration interaction ($F_{1,84}$=6.3, p=0.01 and $F_{3,82}$=3.1, p=0.03, respectively) and a main effect of sex ($F_{1,84}$=43, p=4×10^{-9}); but no sex x genotype interaction (p=0.6, NS), so the data shown are collapsed for both sexes. Post hoc analysis indicated that *Mpdz*$^{+/-}$ consumed less ethanol than WT for 6%, 10% and 20% ethanol (*p*=0.04, *p*=0.004, *p*=0.006, respectively), but not 3% ethanol. **(B)** Ethanol preference (volume of ethanol consumed divided by the total fluid volume consumed) was evident in both genotypes for

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3%, 6% and 10% ethanol, while both genotypes did did not prefer 20% ethanol (preference ratio < 0.5; $t_{1,19}$= 6.6, p=2.6×10^{-6}). No main effects of genotype or sex were detected by repeated measures ANOVA (both p>0.3). **(C)** There was no effect of genotype on water consumption ($F_{1,87}$=0.13, p=0.72, NS). There was a main effect of sex ($F_{1,87}$=7.2, p=0.008) with females drinking more than males but there was no genotype x sex interaction so data were collapsed across sexes.

(D) *Mpdz*$^{+/-}$ and WT were assessed for saccharin consumption using a two bottle free choice paradigm. Mean (± SEM) saccharin consumption (g/kg/24 h) was averaged across the second and fourth day of access for each concentration in *Mpdz*$^{+/-}$ (open bars) and WT (filled bars) littermates. A main effect of sex was detected by repeated measures ANOVA ($F_{1,86}$=48.7, p=5.9×10^{-10}), with females consuming more than males. No main effects of genotype or genotype x sex interactions were apparent (p>0.5, NS). **(E)** Saccharin preference (volume of ethanol consumed divided by the total fluid volume consumed) was evident in both genotypes for 0.033% and 0.066% saccharin solutions. No main effects of genotype or sex were detected by repeated measures ANOVA (both p>0.3).

(F) Total fluid volume consumption of ethanol solutions and water showed main effects of genotype ($F_{1,86}$=8.6, p=0.004) and sex ($F_{1,86}$=85, p=2×10^{-14}), but no interaction (all NS), so the results based on both sexes collapsed are given. Mpdz+/− mice consumed less volume than WT mice. Female mice consume a greater volume males. Total fluid volume consumption of saccharin solutions and water showed main effects of sex ($F_{1,86}$=102, p=1×10^{-15}), with females consuming a greater volume than males, but no effect of genotype ($F_{1,86}$=0.18, p=0.67), and no genotype x sex interactions ($F_{1,86}$=2.6, p=0.11).

*p<0.05, #p<0.01.