Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

HHS Public Access

Author manuscript

Alcohol. Author manuscript; available in PMC 2020 December 08.

Published in final edited form as:

Alcohol. 2020 December ; 89: 103–112. doi:10.1016/j.alcohol.2020.08.004.

Epigenetic changes on rat chromosome 4 contribute to disparate alcohol drinking behavior in alcohol-preferring and -nonpreferring rats

John Paul Spence[a], **Dongbing Lai**[b], **Jill L. Reiter**[b], **Sha Cao**[c], **Richard L. Bell**[d], **Kent E. Williams**[e], **Tiebing Liang**[e,*]

[a]Department of Pediatrics, Indiana University School of Medicine, Indianapolis, IN, 46202, United States

[b]Department of Medical and Molecular Genetics, Indiana University School of Medicine, Indianapolis, IN, 46202, United States

[c]Department of Biostatistics, Indiana University School of Medicine, Indianapolis, IN, 46202, United States

[d]Department of Psychiatry, Indiana University School of Medicine, Indianapolis, IN, 46202, United States

[e]Department of Medicine, Indiana University School of Medicine, Indianapolis, IN, 46202, United States

Abstract

Background: Paternal alcohol abuse is a well-recognized risk factor for the development of an alcohol use disorder (AUD). In addition to genetic and environmental risk factors, heritable epigenetic factors also have been proposed to play a key role in the development of AUD. However, it is not clear whether epigenetic factors contribute to the genetic inheritance in families affected by AUD. We used reciprocal crosses of the alcohol-preferring (P) and -nonpreferring (NP) rat lines to test whether epigenetic factors also impacted alcohol drinking in up to two generations of offspring.

Methods: F1 offspring derived by reciprocal breeding of P and NP rats were tested for differences in alcohol consumption using a free-choice protocol of 10% ethanol, 20% ethanol, and water that were available concurrently. In a separate experiment, an F2 population was tested for alcohol consumption not only due to genetic differences. These rats were generated from inbred P (iP) and iNP rat lines that were reciprocally bred to produce genetically identical F1 offspring that

*Corresponding author. Indiana Alcohol Research Center, Department of Medicine, Indiana University School of Medicine, Indianapolis, IN, 46202, United States. Tel.: +1 317 274 7813. fax: +1 317 274 2695., tliang@iu.edu (T. Liang).

Author credit statement

John Paul Spence: Conceptualization; Methodology; Writing – Original drafting, Reviewing and Editing. **Dongbing Lai:** Software; Formal analysis; Writing – Reviewing and Editing. **Jill L. Reiter:** Conceptualization; Writing – Reviewing and Editing. **Sha Cao:** Formal analysis. **Richard L. Bell:** Methodology; Formal analysis; Resources; Writing – Reviewing and Editing. **Kent E. Williams:** Formal analysis; Writing – Reviewing and Editing; Visualization. **Tiebing Liang:** Conceptualization; Supervision; Resources; Writing – Reviewing and Editing; Visualization.

Declaration of competing interest

The authors have no conflict of interest to declare.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

remained alcohol-naïve. Intercrosses of the F1 generation animals produced the F2 generation. Alcohol consumption was then assessed in the F2 generation using a standard two-bottle choice protocol, and was analyzed using genome-wide linkage analysis. Alcohol consumption measures were also analyzed for sex differences.

Results: Average alcohol consumption was higher in the F1 offspring of P vs. NP sires and in the F2 offspring of F0 iP vs. iNP grandsires. Linkage analyses showed the maximum LOD scores for alcohol consumption in both male and female offspring were on chromosome 4 (Chr 4). The LOD score for both sexes considered together was higher when the grandsire was iP vs. iNP (5.0 vs. 3.35, respectively). Furthermore, the F2 population displayed enhanced alcohol consumption when the P alleles from the F0 sire were present.

Conclusions: These results demonstrate that epigenetic and/or non-genetic factors mapping to rat chromosome 4 contribute to a transgenerational paternal effect on alcohol consumption in the P and NP rat model of AUD.

Keywords

alcohol-preferring and alcohol-nonpreferring rats; epigenetic; paternal alcohol exposure; transgenerational

Introduction

Alcohol use disorder (AUD) is a complex disease attributable to both genetic and environmental factors, with an estimated heritability of about 50% in twin studies (Verhulst, Neale, & Kendler, 2015). The lifetime odds for children of alcoholics to develop AUD are 2.5 times higher than that of individuals without an alcoholic parent (Yoon, Westermeyer, Kuskowski, & Nesheim, 2013). While previous studies provided evidence for a genetic contribution to AUD and AUD-related diseases (Enoch & Goldman, 2001; Reilly, Noronha, Goldman, & Koob, 2017; Schuckit, Goodwin, & Winokur, 1972; Slutske, Ellinsgon, Richmond-Rakerd, Zhu, & Martin, 2013), recent evidence suggests that epigenetic factors may also contribute to the inheritance of AUD (Gapp & Bohacek, 2018; Gapp et al., 2014). Results from recent genome-wide association studies (GWAS) indicate that the genetic heritability of AUD is significantly lower than previous epidemiological studies. Despite considerable effort and large sample sizes (>274,000 individuals), GWAS studies have yielded inconsistent results, with the exception of a consistent role of alcohol-metabolizing genes, such as *ADH1B* and *ALDH2* (Kranzler et al., 2019; Sanchez-Roige et al., 2019a,b) ; Walters et al., 2018). Therefore, the disparate results between twin studies and GWAS studies suggest that heritable factors other than genetic variants likely play an additional role in the inheritance of AUD (Chastain & Sarkar, 2017).

Paternal alcohol exposure has long been recognized as a risk factor in the development of AUD in offspring. Preclinically, several paternal-associated alcohol exposure-induced behavioral, epigenetic, and physiological changes have been identified in offspring. Two examples of this are attention deficit hyperactivity disorder (ADHD) and increased anxiety- and depression-like behavior (Kim et al., 2014; Liang et al., 2014). These psychiatric disorders are strongly correlated with the development of AUD (Walters et al., 2018) and

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

similarly have a strong genetic underpinning (Castillo-Carniglia, Keyes, Hasin, & Cerdá, 2019; Evangelou et al., 2019; Palmisano & Pandey, 2017). While previous studies strongly suggest a role for epigenetics in AUD, isolating specific epigenetic factors is a difficult task (Chastain & Sarkar, 2017).

Alcohol-preferring (P) and alcohol-nonpreferring (NP) rat lines were developed using bidirectional selective breeding for high and low alcohol preference and consumption. The selection criteria for P rats were an average intake greater than 5 g ethanol/kg body weight/day (g/kg/day) and a preference ratio greater than 2:1 (v/v) of 10% (v/v) ethanol vs. water consumed (Bell et al., 2012, 2016). For NP rats, the criteria were an intake of less than 1.5 g/kg/day intake and a preference ratio of less than 0.2:1 (Bell et al., 2012, 2016). Notably, P rats are considered a valid rodent model of AUD (Bell et al., 2012; McBride, Rodd, Bell, Lumeng, & Li, 2014). Following the successful divergence and plateau of alcohol consumption in each of the selected lines, brother-sister mating was initiated at the 30th generation of selection to develop the inbred strains (iP and iNP). After 20 generations of inbreeding, an F2 generation was created from reciprocal crosses of iP and iNP rats, which was used in our previous quantitative trait loci (QTL) study (Bice et al., 1998; Carr et al., 1998). A strong QTL for alcohol consumption was found on rat chromosome 4 (Chr 4, 95% confidence interval is located between 54.8 and 105 Mb) that included several genes that had been linked to AUD in humans, such as *NPY* and *SNCA*, among others (Bice et al., 1998; Li, Lumeng, & Doolittle, 1993). In addition, alcohol consumption has also been found to result in epigenetic changes in specific genes implicated in AUD, including the *dopamine transporter* (*DAT*) (Kim et al., 2014), as well as *Npy* and *Bdnf* (Lomazzo, König, Abassi, Jelinek, & Lutz, 2017; Palmisano & Pandey, 2017). Therefore, there is increasing evidence suggesting that epigenetic factors also contribute to the transmission of AUD across generations. We used the P and iP rats, and their nonpreferring counterparts, to test the hypothesis that epigenetic factors contribute to the heritability of alcohol consumption over subsequent generations.

In the present study, reciprocal crosses of selectively bred P and NP rats ($P_{male} \times NP_{female}$ and $NP_{male} \times P_{female}$) revealed that increased alcohol drinking levels in F1 rats were associated with the paternal P genotype. Next, in a separate experiment, we utilized linkage analysis to determine whether this paternal effect was transgenerational by comparing the effects of alcohol consumption between F2 rats bred from F0 iP and iNP progenitors. We then analyzed associations between genetic markers on rat Chr4 and alcohol consumption in the F2 rats. Our results demonstrate that both genetic and non-genetic factors contribute to alcohol drinking behavior of offspring in progeny of P and NP rats, likely due to alcohol-induced epigenetic changes.

Materials and methods

Animals

The alcohol-preferring P and alcohol-nonpreferring NP rat lines were developed by mass selection from a closed colony of Wistar [Wrm: WRC(WI)BR] rats at the Walter Reed Army Institute of Research, Washington, D.C., United States. Bidirectional selection has continued at the Indiana University School of Medicine. Because the P and NP rats were generated

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

from a closed colony through mass selection, evidence suggests that the frequency of genetic and non-genetic contributions related to alcohol consumption were fixed in early generations (Grahame, 2000; Hoffman et al., 2014). Selection of breeders in this study followed the standard protocol that has been used since the early selection of P and NP rat lines, which starts with a 4-day acclimation period of forced drinking of 10% ethanol followed by free choice of 10% ethanol and water (Li, Lumeng, McBride, Waller, & Murphy, 1986; Murphy et al., 1986). In this study, the protocol was modified following the 4-day forced drinking, with free choice of three-bottle access to water, 10% ethanol, and 20% ethanol, available concurrently. Standard rat chow was available *ad libitum*. The procedures performed herein were approved by the Indiana University School of Medicine Institutional Animal Care and Use Committee.

Alcohol consumption of F1 rats from reciprocal breeding

P and NP rats, at generation 72, were bred to generate F1 rats (Fig. 1A). Both male and female offspring from reciprocally bred $P_{male} \times NP_{female}$ (4 breeding pairs) and $NP_{male} \times P_{female}$ (3 breeding pairs) (n = 10 male and n = 10 female offspring per group) were used for this experiment. Following the acclimation period of 4 days (forced choice of 10% ethanol; i.e., the sole fluid), the F1 rats received continuous concurrent free-choice three-bottle access to 10% (v/v) ethanol, 20% (v/v) ethanol, and water for 8 weeks (Rodd et al., 2009). The water bottle was always presented on the left front side of the cage next to the food hopper. Due to the size of the water bottle and the cage configuration, it was not possible to switch the position of the water bottle with the ethanol drinking tubes. The positions of the two ethanol tubes on the right front side of the cage were randomly changed each day, and fluid intakes were recorded to the nearest 0.1 g by weighing the water and ethanol bottles before and after each 24-h period. Alcohol consumption was calculated as g of ethanol/kg body weight per day. Rat body weights were recorded twice weekly. Student's *t* tests were performed to test for differences in alcohol consumption between P- and NP-sired F1 rats. Alcohol consumption data and weekly measurements of total alcohol consumption were analyzed by repeated-measures ANOVA. A mixed-model ANOVA was used to analyze differences in weekly alcohol intake of 10% vs. 20% ethanol concentrations. Results were considered statistically significant when p was <0.05.

F2 animal breeding and alcohol consumption measurement

In previous work, reciprocal crosses of inbred iP and iNP rats were performed to generate genetically identical F1 rats. These F1 rats were crossed to generate F2 progeny for a genome-wide screen for QTLs influencing alcohol consumption and preference (Bice et al., 1998). Inbreeding of the P and NP lines started at the 30th generation of selection, and experiments occurred after 20 generations of inbreeding. Multiple breeding pairs of F0 $iP_{male} \times iNP_{female}$ and $iNP_{male} \times iP_{female}$ rats were used to generate F1 rats. F1 sister and brother mating occurred only within the same paternal genotype (i.e., intercross) group; that is, only F1 rats from an iP sire were mated to each other. Similarly, only F1 rats from an iNP sire were mated to obtain the F2 population. The F0 and F2 rats were tested for alcohol consumption using the same protocol described above, while the F1 rats remained alcohol-naïve (Li et al., 1993).

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Genome-wide linkage analysis

QTL Cartographer (v1.17) was used to conduct linkage analyses (Basten, Weir, & Zeng, 1994, 2002). This program uses a dynamic algorithm to map QTL on a linkage map using interval mapping methods. We included 137 previously identified microsatellite markers in our analyses (Bice et al., 1998). Three genome-wide linkage analyses were performed: i) all F2 animals (N = 381); ii) F2 offspring of iP grandsire only (N = 198); and iii) F2 offspring of iNP grandsire only (N = 183). Since the number of F2 offspring from iP and iNP grandsires differed, a permutation test was performed using the same number of F2 rats from an iP grandsire to rule out the possibility that the observed differences in LOD scores were due to different sample sizes. Linkage analysis was performed on 183 randomly selected F2 rats from an iP grandsire, and this procedure was repeated 5000 times to calculate the average maximum LOD scores and 95% confidence intervals.

Association of grandsire P allele with F2 alcohol consumption

Mean alcohol consumption scores were compared for each genetic marker using ANOVA. For each genetic marker, mean alcohol consumption scores were compared for the respective three genotype groups (P/P, P/NP, NP/NP) using ANOVA. For F2 rats, the independent variables examined included grandsire genotype as well as genetic markers on Chr4. Microsatellite markers included *D4Rat21, D4Mgh24, D4Rat29, D4Mit7-Npy, D4Rat33, D4Rat35, D4Rat51, D4Rat55, D4Mgh18, D4Mgh27, D4Mgh19, D4Mit27*, and SNPs including *Sspo* (Chr4: 78097074, G/A), *Snca* (Chr4; 90880297, G/A), and *Fam190a* (Chr4: 91374226, C/T). SNP marker information, genotyping data from exome sequencing, and data processing were described previously (Zhou et al., 2013). For each marker, a two-factor ANOVA was performed to determine main effects of grandsire and genotype as well as grandsire × genotype interactions. One-factor ANOVAs were then performed, followed by independent *t* tests using GraphPad Prism v8.

Results

Paternal genotype affects alcohol consumption in F1 progeny

We measured alcohol consumption over the course of 8 weeks in the F1 generation offspring from multiple families of reciprocal crosses of selectively bred P and NP rats (Fig. 1A; by convention, the male genotype is listed first in the cross). We found that the average daily alcohol consumption was higher in the offspring from P vs. NP sires (mean ± SEM: 6.94 ± 0.60 vs. 5.08 ± 0.25, $p = 0.008$; Fig. 1B). When the offspring were analyzed for sex differences, the results showed that female offspring consumed significantly more alcohol when the sire was P vs. NP (7.45 ± 0.93 vs. 4.82 ± 0.29 g ethanol/kg/day; $p = 0.039$). Male offspring of P sires also trended toward higher alcohol consumption compared to the offspring of NP sires, but the difference did not reach statistical significance (6.42 ± 0.78 vs. 5.31 ± 0.398 g ethanol/kg/day; $p = 0.672$) (Fig. 1C). Weekly measurements of total alcohol consumption also showed the same trend of higher alcohol drinking in male and female offspring of P vs. NP sires (Fig. 1D). Since both 10% and 20% ethanol were present, a mixed ANOVA of the ethanol concentration was performed. When comparing each group, we found an increased drinking preference related to ethanol concentration. The data revealed significant main effects of sire genotype ($p = 0.002$) and sex ($p = 0.042$) in the

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

consumption of 20% ethanol. In addition, we observed a trend for an interaction between sire genotype × sex ($p = 0.107$). No significant interactions were observed for either sire genotype or sex in the consumption of 10% ethanol. Together, these data indicate that non-genetic factors contribute to alcohol consumption in the F1 offspring, which is consistent with a paternal effect that was most pronounced in female offspring of P sires.

Grandsire genotype affects alcohol consumption in the F2 population

To determine whether this observed paternal effect was epigenetic in nature, we analyzed alcohol consumption data from a previous QTL study that included 384 F2 rats derived from reciprocal crosses of iP and iNP lines. These data were particularly useful in addressing this question because the F1 generation was not only genetically identical but was also alcohol-naïve. Fig. 2A depicts the breeding design that was utilized in generating the F2 rats. Reciprocal crosses of the iP and iNP lines produced F1 offspring that were heterozygous for P and NP alleles at all loci. Additionally, because intercrosses were performed at the F1 generation, the P alleles in the F2 rats could be traced back to the founder male or female in the F0 generation (Fig. 2A). Furthermore, because the F1 generation was alcohol-naïve, this breeding design controlled for the direct effects of alcohol on the male or female gamete. Therefore, this breeding design allowed us to test the hypothesis that heritable epigenetic factors play a role in alcohol consumption.

The F2 offspring of iP grandsires consumed more alcohol (3.56 ± 0.140 g/kg/day, mean ± SEM) than F2 offspring of iNP grandsires (2.94 ± 0.125) ($p = 0.0009$). This finding remained significant when the F2 animals were analyzed by sex; alcohol consumption was higher in both males (3.24 ± 0.159 vs. 2.55 ± 0.150) ($p = 0.002$) and females (3.90 ± 0.228 vs. 3.28 ± 0.188) ($p = 0.036$) when the grandsire was an iP compared to an iNP, respectively (Fig. 2B). Similar to previously published data on these F2 animals (Carr et al., 1998), our F2 data showed that alcohol consumption levels were not normally distributed when it was segregated by the F0 sire. The distribution was shifted, in that more rats drank lower levels of alcohol in their respective population whether the grandsire was iP or iNP (median levels were 3.46 vs. 2.47 g/kg/day, respectively) (Fig. 2C). This result is consistent with previous findings (Bell, Rodd, Engleman, Toalston, & McBride, 2014). We found that a higher percentage of both F2 males and females consumed ≥5 g/kg/day when the grandsire was iP vs. iNP (Fig. 2D). Due to the mating scheme, F2 rats would inherit the same P alleles whether they were descended from F0 iP males or females (i.e., iP grandsire group). These results indicate that inheriting the iP allele from the grandsire exerted a larger effect on ethanol consumption, than that observed when inheriting the iP allele from the granddam. Moreover, because the F1 generation did not consume alcohol, these results suggest that alcohol drinking in the F0 generation (which were tested for ethanol consumption to select the breeding pairs) likely altered epigenetic factors that were transgenerationally inherited by the F2 generation mainly through the male germline.

Genome-wide linkage analysis detects differences between F2 populations bred from iP and iNP grandsires

Having observed the F2 drinking differences between iP and iNP grandsire groups, we next screened for the linkage of a paternal effect on alcohol consumption between iP vs. iNP

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

grandsires using the previously published microsatellite markers within the rat Chr4 QTL region (Bice et al., 1998). This analysis included 381 F2 rats (198 from an iP grandsire and 183 from an iNP grandsire), and linkage was performed using alcohol consumption scores for the entire population and for each group separately. A locus on Chr4 showed strong linkage signals in all three analyses. The average maximum LOD score for rats with an iP grandsire was 5.07 (95% CI: 4.08–6.05), and the average maximum LOD score for rats with an iNP grandsire was 3.35 (Fig. 3). The chromosome location of QTL with LOD >2 in iP grandsire was from 0 to 27.86 centimorgan (cM), while the location in the iNP grandsire was from 5.76 to 29.87 cM; additionally, the peak LOD scores for each group were in different locations (Fig. 3). The magnitude of these scores indicated that the dissimilar sample sizes were not the driving force for the different LOD scores between rats with iP vs. iNP grandsires. Other suggestive QTLs for the observed effect included regions on Chr2 with LOD >0.5 (maximum LOD of iP grandsire = 0.7, iNP grandsire = 2.1), which was located between 39.17 and 73.62 cM for the iP grandsire group and between 57 and 125.44 cM for the iNP grandsire group, as well as regions on Chr18 with LOD >0.5 (maximum LOD for iP grandsire = 3.6, iNP grandsire = 0.6), which was located between 20 and 50.13 cM for the iP grandsire group and between 53.13 and 58 cM for iNP grandsire group. However, due to the low LOD scores, these regions were not analyzed further. Therefore, these results indicated that multiple genomic regions are likely linked to a paternal effect on offspring drinking in the F2 population. Additionally, this finding provides evidence that both genetic and transgenerational epigenetic factors contribute to the disparate alcohol drinking associated with the Chr4 QTL.

Effect of grandsire genotype on mean alcohol consumption scores for the alleles in the rat Chr4 QTL

Because linkage analysis detected the highest LOD scores for the paternal effect on rat chromosome 4, we asked whether genetic variants localized to the Chr4 region were associated with this effect on alcohol consumption. Importantly, the Chr4 region was also identified in the earlier QTL analysis of iP and iNP F2 animals to have the strongest linkage with alcohol consumption and alcohol preference (Bice et al., 1998). Fine mapping of rat Chr4 had been performed previously using microsatellite markers and other genetic variants identified from whole exome sequencing in the same F2 population (n = 384 rats) (Bice et al., 1998; Zhou et al., 2013). We used these same markers to calculate the relative contribution of the F2 Chr4 allelic genotype and the grandsire effect on alcohol consumption. The F2 population was categorized based on the F0 grandsire, and alcohol consumption was compared based on the F2 genotype for a given locus (i.e., P homozygotes, P/NP heterozygotes, and NP homozygotes). For the majority of markers used in this study, F2 rats showed higher average alcohol consumption when they were either homozygous or heterozygous for P alleles in the iP grandsire group, compared to F2 rats who inherited a P allele from an F0 female (i.e., $NP_{male} \times P_{female}$).

Table 1 depicts previously published microsatellite markers and more recently identified missense mutations within the rat Chr4 QTL region (Bice et al., 1998; Zhou et al., 2013). When analyzing the mean F2 alcohol consumption scores from the descendants of F0 iP vs. iNP grandsires, there were significant genotype effects ($p < 0.0001$) for eight of the Chr4

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

markers listed in Table 1. The magnitude of the genotype effect diminished toward more distal markers of the rat Chr4 QTL examined (i.e., *D4Rat55, D4Mgh19,* and *D4Mit27*). These data showed that the association between the grandsire's genotype and the offspring's allelic marker for the Chr4 QTL was consistent with a potential gene dosage effect(s). For instance, F2 animals homozygous for the P allele had the highest mean drinking scores, whereas F2 animals homozygous for the NP allele had the lowest mean drinking scores, with heterozygous rats having intermediate drinking scores (Table 1). Importantly, this effect was pronounced at the *Snca* marker, consistent with previous findings that showed *Snca* is differentially expressed in the hippocampus of the brain between iP and iNP rats (Liang et al., 2003).

Discussion

In the present study, we sought to determine whether epigenetic effects contribute to the disparate alcohol drinking observed between the alcohol-preferring (P) and alcohol-nonpreferring (NP) rats. First, using a reciprocal breeding design of P and NP rats, we found that the offspring (F1) of P × NP crosses (sire is indicated first) showed increased alcohol consumption compared to offspring of NP × P rats. A similar finding was also observed in the F2 generation of reciprocally bred inbred iP and iNP rats, where the F1 generation was genetically identical as well as alcohol-naïve. Next, separate linkage analyses for F2 offspring of P and NP grandsires showed that the LOD score of F2 rats for alcohol consumption on Chr4 from P grandsires was higher than that of F2 rats from an NP grandsire. Together, these findings demonstrate that epigenetic factors interact with genetic effects on Chr4 to increase alcohol consumption in P rats.

While other studies have demonstrated paternal effects following exposure to toxins, such as benzopyrene and dioxins (Viluksela & Pohjanvirta, 2019; Zhang, Yang, Lv, Li, & Qiang, 2019), our findings are the first to show that alcohol drinking leads to similar transgenerational effects in a well-established rodent model of AUD. Because the F1 rats were not exposed to alcohol, the differences observed in the F2 population are likely due to alcohol exposure in their respective grandsires. Alcohol consumption is known to alter epigenetic modifications that affect expression of genes important in AUD (Biermann et al., 2009; Bönsch, Lenz, Kornhuber, & Bleich, 2005; Downing et al., 2011; Zhang & Gelernter, 2017). Epigenetic effects could be linked to genetic markers by different mechanisms including changes in DNA methylation, expression of noncoding RNAs, or chromatin structure. These changes can be heritable when carried via the germline to the offspring, potentially through multiple generations, following exposure of the parent (F0 generation) to environmental factors, such as psychological stress, exposure to toxins, poor nutrition, drugs of abuse, and alcohol (Chastain & Sarkar, 2017; Levin et al., 2019; Lucas & Watkins, 2017). For example, a recent study found that pre-conception cigarette smoke exposure in male mice induced paternally mediated, heritable epigenetic changes that affected frontal cortical neurodevelopment in their offspring; these authors proposed that the mechanism involved altered DNA methylation of the sperm epigenome (Murphy et al., 2020). Similar findings in humans were also observed for the effects of pre-conception paternal smoke exposure (Jenkins et al., 2017). Furthermore, evidence also suggests that heritable epigenetic factors

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

in the sperm play a role in offspring alcohol consumption in animal models (Chastain & Sarkar, 2017; Finegersh, Rompala, Martin, & Homanics, 2015).

Heritable epigenetic factors in the sperm genome have been hypothesized to transmit acquired phenotypes to offspring via a phenomenon called epigenetic germline inheritance (Bohacek & Mansuy, 2015; Finegersh et al., 2015). Importantly, such epigenetic factors have been shown to modulate gene expression networks and modify brain plasticity (Godino, Jayanthi, & Cadet, 2015; Renthal & Nestler, 2008). Furthermore, previous studies observed alcohol-induced epigenetic changes in the sperm, and it was hypothesized that these changes contributed to the observed changes in alcohol sensitivity (Liang et al., 2014). Because the observed effect on alcohol consumption in our study was passed on from the F0 to the F2 generation along the male germline, the mode of inheritance would be consistent with a transgenerational effect of alcohol on the germline (Jirtle & Skinner, 2007; Skinner et al., 2018; Skinner, Manikkam, & Guerrero-Bosagna, 2010). Notably, recent findings suggest that alcohol induces changes in sperm small noncoding RNAs, which are currently thought to be likely mediators of intergenerational inheritance (Rompala et al., 2018).

We focused on the Chr4 region because linkage analysis detected the highest LOD scores, using alcohol consumption measurements, from F2 iP grandsire or iNP grandsire rats separately (Fig. 3). This region was previously fine-mapped using microsatellite markers and other genetic variants in the same F2 population (n = 384 rats). This fine-mapping study identified *Sspo* as a candidate gene in this region because of non-synonymous mutations (Zhou et al., 2013). Other genes within this linkage region have been reported to be regulated by epigenetic factors. For example, epigenetic alterations of *Cntnap2* and *Snca* have been associated with psychiatric disorders (Bönsch et al., 2005; Foroud et al., 2007; Mahurkar et al., 2016; Matsumoto et al., 2010; Schaafsma et al., 2017). Epigenetic changes that alter *neuropeptide Y* (*NPY*) expression have been suggested to contribute to the difference in alcohol consumption between iP and iNP rats (Lomazzo et al., 2017; Palmisano & Pandey, 2017). For example, alcohol exposure increased DNA methylation in the *NPY* promoter, leading to persistent downregulation of *NPY* expression (Palmisano & Pandey, 2017). Therefore, persistent downregulation of *NPY* expression due to the effects of alcohol exposure might lead to differences in alcohol drinking in both F1 and F2 rats.

Previously, grandparent-influenced alcohol preference had been mapped to mouse Chr2 using a congenic strain that had a QTL for alcohol consumption (Lesscher, Kas, van der Elst, van Lith, & Vanderschuren, 2009). These findings support the notion that genetic and epigenetic factors can affect alcohol consumption in the same genomic region, potentially acting through a common mechanism (e.g., gene dosage). In contrast to our findings of increased alcohol consumption in the offspring of high-alcohol drinking sires, other studies have found that alcohol exposure to sires resulted in a protective effect of reduced alcohol consumption in their offspring. Rompala and colleagues found a paternal effect on increased alcohol sensitivity and decreased consumption in the offspring in mice that were passively exposed to ethanol vapor (Rompala, Finegersh, Slater, & Homanics, 2017). However, ethanol vapor is also known to be a stressor that could have separate effects from alcohol exposure on the phenotype. The dual exposure of the sires to two stressors simultaneously may explain their finding of increased sensitivity of the offspring to alcohol. Beeler and

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

colleagues also reported a protective effect (i.e., reduced drinking in certain settings) in mice following paternal voluntary oral ethanol consumption (Beeler, Nobile, & Homanics, 2019). This study used inbred mice that were not selectively bred for alcohol consumption, and alcohol consumption was measured after a behavioral task, which may have complicated the interpretation of the data. Explanations for the observed differences in offspring alcohol consumption phenotypes following paternal alcohol exposure likely include species and strain differences as well as differences in experimental procedures. Nevertheless, all of these studies support the conclusion that paternal alcohol exposure affects offspring alcohol consumption.

Reciprocal breeding of the selectively bred P and NP rats, and the inbred iP and iNP rats, resulted in divergent drinking in their respective F1 and F2 progeny that was consistent with selection for an alcohol preference. For instance, the F1 generation arising from reciprocal breeding of F0 sires with the P genotype displayed greater alcohol consumption than those with a paternal NP genotype. In contrast, the potential effect of the dam on generational selection was inconsistent with selection, such that the F1 rats from dams of the NP genotype displayed increased alcohol consumption, while offspring from dams of the P genotype displayed decreased alcohol consumption (Fig. 1). Therefore, we propose that the effect on offspring alcohol consumption observed in our study is consistent with a paternal mode of inheritance. While the observation that offspring drinking behavior followed that predicted from the paternal genotype, this observation does not rule out a role for the maternal genotype. Epigenetic effects induced by the pre-, neo-, and post-natal environment undoubtedly play a role in determining the progeny's behavior (Brancato et al., 2018; Fleten et al., 2012; Nagamachi et al., 2018; Purcell et al., 2011), including ethanol intake. It would be interesting to dissect differences between P and NP rat maternal care, milk composition, and intrauterine environments.

In summary, we present evidence for a paternal effect on ethanol consumption that appears to be the result of ethanol-associated epigenetic changes in P and NP rats, which is a well-established model of AUD. Future studies using increased sample size and marker density may allow finer mapping resolution that could identify the genes involved in the observed paternal effect and their potential epigenetic regulation. Future studies will focus on how the potential transmission of epigenetic changes in the sperm from F0 P rats might alter genomic and/or biochemical changes in reward-related brain regions (e.g., nucleus accumbens, amygdala) of the offspring. Unlike genetic changes, epigenetic alterations can be reversible with positive environmental stimuli (Mitchell, Schneper, & Notterman, 2016; Yeshurun & Hannan, 2019), which can be as simple as increased paternal physical activity (Denham, O'Brien, Harvey, & Charchar, 2015; McPherson, Owens, Fullston, & Lane, 2015; Murashov et al., 2016; Short et al., 2017; Yeshurun & Hannan, 2019). Therefore, further study of these alcohol-induced changes could lead to the development of more effective preventive and therapeutic strategies.

Acknowledgments

We acknowledge Dr. Lucinda Carr and Dr. Tatiana Foroud for their previous design used to generate F2 rats, Dr. David Crabb and the late Dr. Lawrence Lumeng for their long-term support of genetic research using P and NP rats, as well as funding from the National Institute of Alcohol Abuse and Alcoholism (NIAAA, the United States of

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

America) R01 AA010707, U01 AA013522, and P60 AA007611. The authors also acknowledge the Indiana University Pervasive Technology Institute for providing high performance computing (Big Red II, Karst, Carbonate), visualization, database, storage, and consulting resources that have contributed to the research results reported within this paper. The opinions expressed in this manuscript do not necessarily represent those of the National Institutes of Health or NIAAA.

References

Basten CJ, Weir BS, & Zeng Z-B (1994). Guelph, Ontario: Canada.

Basten CJ, Weir BS, & Zeng Z-B (2002). QTL Cartographer, version 1.16. Raleigh, North Carolina: Department of Statistics, North Carolina State University.

Beeler E, Nobile ZL, & Homanics GE (2019). Paternal preconception every-other-day ethanol drinking alters behavior and ethanol consumption in offspring. Brain Sciences, 9(3), 56 10.3390/brainsci9030056

Bell RL, Hauser S, Rodd ZA, Liang T, Sari Y, McClintick J, Rahman S, & Engleman EA (2016). A genetic animal model of alcoholism for screening medications to treat addiction. International Review of Neurobiology, 126, 179–261. 10.1016/bs.irn.2016.02.017 [PubMed: 27055615]

Bell RL, Rodd ZA, Engleman EA, Toalston JE, & McBride WJ (2014). Scheduled access alcohol drinking by alcohol-preferring (P) and high-alcohol-drinking (HAD) rats: Modeling adolescent and adult binge-like drinking. Alcohol, 48(3), 225–234. 10.1016/j.alcohol.2013.10.004 [PubMed: 24290311]

Bell RL, Sable HJ, Colombo G, Hyytia P, Rodd ZA, & Lumeng L (2012). Animal models for medications development targeting alcohol abuse using selectively bred rat lines: Neurobiological and pharmacological validity. Pharmacology Biochemistry and Behavior, 103(1), 119–155. https://doi.org/101016/j.pbb.2012.07.007

Bice P, Foroud T, Bo R, Castelluccio P, Lumeng L, Li TK, & Carr LG (1998). Genomic screen for QTLs underlying alcohol consumption in the P and NP rat lines. Mammalian Genome, 9(12), 949–955. 10.1007/s003359900905 [PubMed: 9880658]

Biermann T, Reulbach U, Lenz B, Frieling H, Muschler M, Hillemacher T, Kornhuber J, & Bleich S (2009). N-methyl-D-aspartate 2b receptor subtype (NR2B) promoter methylation in patients during alcohol withdrawal. Journal of Neural Transmission, 116(5), 615–622. 10.1007/s00702-009-0212-2. (Vienna) [PubMed: 19350219]

Bohacek J, & Mansuy IM (2015). Molecular insights into transgenerational non-genetic inheritance of acquired behaviours. Nature Reviews Genetics, 16(11), 641–652. 10.1038/nrg3964

Bönsch D, Lenz B, Kornhuber J, & Bleich S (2005). DNA hypermethylation of the alpha synuclein promoter in patients with alcoholism. NeuroReport, 16(2), 167–170. 10.1097/00001756-200502080-00020 [PubMed: 15671870]

Brancato A, Castelli V, Cavallaro A, Lavanco G, Plescia F, & Cannizzaro C (2018). Pre-conceptional and peri-gestational maternal binge alcohol drinking produces inheritance of mood disturbances and alcohol vulnerability in the adolescent offspring. Frontiers in Psychiatry, 9, 150 10.3389/fpsyt.2018.00150 [PubMed: 29743872]

Carr LG, Foroud T, Bice P, Gobbett T, Ivashina J, Edenberg H, Lumeng L, & Li TK (1998). A quantitative trait locus for alcohol consumption in selectively bred rat lines. Alcoholism: Clinical and Experimental Research, 22(4), 884–887.

Castillo-Carniglia A, Keyes KM, Hasin DS, & Cerdá M (2019). Psychiatric comorbidities in alcohol use disorder. The Lancet. Psychiatry, 6(12), 1068–1080. 10.1016/s2215-0366(19)30222-6 [PubMed: 31630984]

Chastain LG, & Sarkar DK (2017). Alcohol effects on the epigenome in the germline: Role in the inheritance of alcohol-related pathology. Alcohol, 60, 53–66. 10.1016/j.alcohol.2016.12.007 [PubMed: 28431793]

Denham J, O'Brien BJ, Harvey JT, & Charchar FJ (2015). Genome-wide sperm DNA methylation changes after 3 months of exercise training in humans. Epigenomics, 7(5), 717–731. 10.2217/epi.15.29 [PubMed: 25864559]

Downing C, Johnson TE, Larson C, Leakey TI, Siegfried RN, Rafferty TM, & Cooney CA (2011). Subtle decreases in DNA methylation and gene expression at the mouse Igf2 locus following

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

prenatal alcohol exposure: Effects of a methyl-supplemented diet. Alcohol, 45(1), 65–71. https://doi.org/101016/j.alcohol.2010.07.006 [PubMed: 20705422]

Enoch MA, & Goldman D (2001). The genetics of alcoholism and alcohol abuse. Current Psychiatry Reports, 3(2), 144–151. 10.1007/s11920-001-0012-3 [PubMed: 11276410]

Evangelou E, Gao H, Chu C, Ntritsos G, Blakeley P, Butts AR, Pazoki R, Suzuki H, Koskeridis F, Yiorkas AM, Karaman I, Elliott J, Luo Q, Aeschbacher S, Bartz TM, Baumeister SE, Braund PS, Brown MR, Brody JA, & Elliott P (2019). New alcohol-related genes suggest shared genetic mechanisms with neuropsychiatric disorders. Nature Human Behaviour, 3(9), 950–961. 10.1038/s41562-019-0653-z

Finegersh A, Rompala GR, Martin DI, & Homanics GE (2015). Drinking beyond a lifetime: New and emerging insights into paternal alcohol exposure on subsequent generations. Alcohol, 49(5), 461–470. 10.1016/j.alcohol.2015.02.008 [PubMed: 25887183]

Fleten C, Nystad W, Stigum H, Skjaerven R, Lawlor DA, Davey Smith G, & Naess O (2012). Parent-offspring body mass index associations in the Norwegian mother and child cohort study: A family-based approach to studying the role of the intrauterine environment in childhood adiposity. American Journal of Epidemiology, 176(2), 83–92. 10.1093/aje/kws134 [PubMed: 22771730]

Foroud T, Wetherill LF, Liang T, Dick DM, Hesselbrock V, Kramer J, Nurnberger J, Schuckit M, Carr L, Porjesz B, Xuei X, & Edenberg HJ (2007). Association of alcohol craving with alpha-synuclein (SNCA). Alcoholism: Clinical and Experimental Research, 31(4), 537–545. 10.1111/j.1530-0277.2007.00337.x

Gapp K, & Bohacek J (2018). Epigenetic germline inheritance in mammals: Looking to the past to understand the future. Genes, Brain and Behavior, 17(3), Article e12407 10.1111/gbb.12407

Gapp K, Jawaid A, Sarkies P, Bohacek J, Pelczar P, Prados J, Farinelli L, Miska E, & Mansuy IM (2014). Implication of sperm RNAs in transgenerational inheritance of the effects of early trauma in mice. Nature Neuroscience, 17(5), 667–669. 10.1038/nn.3695 [PubMed: 24728267]

Godino A, Jayanthi S, & Cadet JL (2015). Epigenetic landscape of amphetamine and methamphetamine addiction in rodents. Epigenetics, 10(7), 574–580. 10.1080/15592294.2015.1055441 [PubMed: 26023847]

Grahame NJ (2000). Selected lines and inbred strains. Tools in the hunt for the genes involved in alcoholism. Alcohol Research & Health, 24(3), 159–163. [PubMed: 11199285]

Hoffman PL, Saba LM, Flink S, Grahame NJ, Kechris K, & Tabakoff B (2014). Genetics of gene expression characterizes response to selective breeding for alcohol preference. Genes, Brain and Behavior, 13(8), 743–757. 10.1111/gbb.12175

Jenkins TG, James ER, Alonso DF, Hoidal JR, Murphy PJ, Hotaling JM, Cairns BR, Carrell DT, & Aston KI (2017). Cigarette smoking significantly alters sperm DNA methylation patterns. Andrology, 5(6), 1089–1099. 10.1111/andr.12416 [PubMed: 28950428]

Jirtle RL, & Skinner MK (2007). Environmental epigenomics and disease susceptibility. Nature Reviews Genetics, 8(4), 253–262. 10.1038/nrg2045

Kim P, Choi CS, Park JH, Joo SH, Kim SY, Ko HM, Kim KC, Jeon SJ, Park SH, Han SH, Ryu JH, Cheong JH, Han JY, Ko KN, & Shin CY (2014). Chronic exposure to ethanol of male mice before mating produces attention deficit hyperactivity disorder-like phenotype along with epigenetic dysregulation of dopamine transporter expression in mouse offspring. Journal of Neuroscience Research, 92(5), 658–670. 10.1002/jnr.23275 [PubMed: 24510599]

Kranzler H, Zhou H, Kember R, Smith RV, Justice A, Damrauer S, Tsao PS, Klarin D, Rader DJ, Cheng Z, Tate JP, Becker WC, Concato J, Xu K, Polimanti R, Zhao H, & Gelernter J (2019). Genome-wide association study of alcohol consumption and use disorder in 274,424 individuals from multiple populations. Nature Communications, 10(1), 1499 10.1038/s41467-019-09480-8

Lesscher HM, Kas MJ, van der Elst S, van Lith HA, & Vanderschuren LJ (2009). A grandparent-influenced locus for alcohol preference on mouse chromosome 2. Pharmacogenetics and Genomics, 19(9), 719–729. 10.1097/FPC.0b013-3283311320 [PubMed: 19680168]

Levin ED, Hawkey AB, Hall BJ, Cauley M, Slade S, Yazdani E, Kenou B, White H, Wells C, Rezvani AH, & Murphy SK (2019). Paternal THC exposure in rats causes long-lasting neurobehavioral effects in the offspring. Neurotoxicology and Teratology, 74, 106806 https://doi.org/101016/j.ntt.2019.04.003 [PubMed: 31028824]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Li TK, Lumeng L, & Doolittle DP (1993). Selective breeding for alcohol preference and associated responses. Behavior Genetics, 23(2), 163–170. 10.1007/bf01067421 [PubMed: 8099788]

Li TK, Lumeng L, McBride WJ, Waller MB, & Murphy JM (1986). Studies on an animal model of alcoholism. NIDA Research Monograph, 66, 41–49. [PubMed: 3106817]

Liang F, Diao L, Liu J, Jiang N, Zhang J, Wang HJ, Zhou WH, Huang GY, & Ma D (2014). Paternal ethanol exposure and behavioral abnormities in offspring: Associated alterations in imprinted gene methylation. Neuropharmacology, 81, 126–133. 10.1016/j.neuropharm.2014.01.025 [PubMed: 24486713]

Liang T, Spence J, Liu L, Strother WN, Chang HW, Ellison JA, Lumeng L, Li TK, Foroud T, & Carr LG (2003). α-Synuclein maps to a quantitative trait locus for alcohol preference and is differentially expressed in alcohol-preferring and -nonpreferring rats. Proceedings of the National Academy of Sciences of the United States of America, 100(8), 4690–4695. 10.1073/pnas.0737182100 [PubMed: 12665621]

Lomazzo E, König F, Abassi L, Jelinek R, & Lutz B (2017). Chronic stress leads to epigenetic dysregulation in the neuropeptide-Y and cannabinoid CB1 receptor genes in the mouse cingulate cortex. Neuropharmacology, 113(Pt A), 301–313. 10.1016/j.neuropharm.2016.10.008 [PubMed: 27737789]

Lucas ES, & Watkins AJ (2017). The long-term effects of the periconceptional period on embryo epigenetic profile and phenotype; the paternal role and his contribution, and how males can affect offspring's phenotype/epigenetic profile. Advances in Experimental Medicine & Biology, 1014, 137–154. 10.1007/978-3-319-62414-3_8 [PubMed: 28864989]

Mahurkar S, Polytarchou C, Iliopoulos D, Pothoulakis C, Mayer EA, & Chang L (2016). Genome-wide DNA methylation profiling of peripheral blood mononuclear cells in irritable bowel syndrome. Neuro-Gastroenterology and Motility, 28(3), 410–422. 10.1111/nmo.12741

Matsumoto L, Takuma H, Tamaoka A, Kurisaki H, Date H, Tsuji S, & Iwata A (2010). CpG demethylation enhances alpha-synuclein expression and affects the pathogenesis of Parkinson's disease. PloS One, 5(11), Article e15522 https://doi.org/101371/journal.pone.0015522 [PubMed: 21124796]

McBride WJ, Rodd ZA, Bell RL, Lumeng L, & Li TK (2014). The alcohol-preferring (P) and high-alcohol-drinking (HAD) rats–animal models of alcoholism. Alcohol, 48(3), 209–215. 10.1016/j.alcohol.2013.09.044 [PubMed: 24268381]

McPherson NO, Owens JA, Fullston T, & Lane M (2015). Preconception diet or exercise intervention in obese fathers normalizes sperm microRNA profile and metabolic syndrome in female offspring. American Journal of Physiology. Endocrinology and Metabolism, 308(9), E805–E821. 10.1152/ajpendo.00013.2015 [PubMed: 25690453]

Mitchell C, Schneper LM, & Notterman DA (2016). DNA methylation, early life environment, and health outcomes. Pediatric Research, 79(1–2), 212–219. 10.1038/pr.2015.193 [PubMed: 26466079]

Murashov AK, Pak ES, Koury M, Ajmera A, Jeyakumar M, Parker M, Williams O, Ding J, Walters D, & Neufer PD (2016). Paternal long-term exercise programs offspring for low energy expenditure and increased risk for obesity in mice. Federation of American Societies for Experimental Biology Journal, 30(2), 775–784. 10.1096/fj.15-274274 [PubMed: 26506979]

Murphy JM, Gatto GJ, Waller MB, McBride WJ, Lumeng L, & Li TK (1986). Effects of scheduled access on ethanol intake by the alcohol-preferring (P) line of rats. Alcohol, 3(5), 331–336. 10.1016/0741-8329(86)90010-8 [PubMed: 3778650]

Murphy PJ, Guo J, Jenkins TG, James ER, Hoidal JR, Hueckstead T, Broberg DS, Hotaling JM, Alonso DF, Carrell DT, Cairns BR, & Aston KI (2020). NRF2 loss recapitulates heritable impacts of paternal ciga rette smoke exposure. PLoS Genetics, 16(6), Article e1008756 10.1371/journal.pgen.1008756 [PubMed: 32520939]

Nagamachi S, Nishigawa T, Takakura M, Ikeda H, Kodaira M, Yamaguchi T, Chowdhury VS, Yasuo S, & Furuse M (2018). Dietary L-serine modifies free amino acid composition of maternal milk and lowers the body weight of the offspring in mice. Journal of Veterinary Medical Science, 80(2), 235–241. 10.1292/jvms.17-0577 [PubMed: 29269705]

Palmisano M, & Pandey SC (2017). Epigenetic mechanisms of alcoholism and stress-related disorders. Alcohol, 60, 7–18. https://doi.org/101016/j.alcohol.2017.01.001 [PubMed: 28477725]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Purcell RH, Sun B, Pass LL, Power ML, Moran TH, & Tamashiro KL (2011). Maternal stress and high-fat diet effect on maternal behavior, milk composition, and pup ingestive behavior. Physiology & Behavior, 104(3), 474–479. 10.1016/j.physbeh.2011.05.012 [PubMed: 21605577]

Reilly MT, Noronha A, Goldman D, & Koob GF (2017). Genetic studies of alcohol dependence in the context of the addiction cycle. Neuropharmacology, 122, 3–21. 10.1016/j.neuropharm.2017.01.017 [PubMed: 28118990]

Renthal W, & Nestler EJ (2008). Epigenetic mechanisms in drug addiction. Trends in Molecular Medicine, 14(8), 341–350. https://doi.org/101016/j.molmed.2008.06.004 [PubMed: 18635399]

Rodd ZA, Bell RL, Kuc KA, Murphy JM, Lumeng L, & McBride WJ (2009). Effects of concurrent access to multiple ethanol concentrations and repeated deprivations on alcohol intake of high-alcohol-drinking (HAD) rats. Addiction Biology, 14(2), 152–164. 10.1111/j.1369-1600.2008.00140.x [PubMed: 19076927]

Rompala GR, Finegersh A, Slater M, & Homanics GE (2017). Paternal pre-conception alcohol exposure imparts intergenerational alcohol-related behaviors to male offspring on a pure C57BL/6J background. Alcohol, 60, 169–177. 10.1016/j.alcohol.2016.11.001 [PubMed: 27876231]

Rompala GR, Mounier A, Wolfe CM, Lin Q, Lefterov I, & Homanics GE (2018). Heavy chronic intermittent ethanol exposure alters small noncoding RNAs in mouse sperm and epididymosomes. Frontiers in Genetics, 9, 32 10.3389/fgene.2018.00032 [PubMed: 29472946]

Sanchez-Roige S, Fontanillas P, & Elson SL (2019a). Genome-wide association study of alcohol use disorder identification test (AUDIT) scores in 20 328 research participants of European ancestry. Addiction Biology, 24(1), 121–131. 10.1111/adb.12574 [PubMed: 29058377]

Sanchez-Roige S, Palmer AA, Fontanillas P, Elson SL, Adams MJ, Howard DM, Edenberg HJ, Davies G, Crist RC, Deary IJ, McIntosh AM, & Clarke TK (2019b). Genome-wide association study meta-analysis of the alcohol use disorders identification test (AUDIT) in two population-based cohorts. American Journal of Psychiatry, 176(2), 107–118. https://doi.org/101176/appi.ajp.2018.18040369 [PubMed: 30336701]

Schaafsma SM, Gagnidze K, Reyes A, Norstedt N, Mansson K, Francis K, & Pfaff DW (2017). Sex-specific gene-environment interactions underlying ASD-like behaviors. Proceedings of the National Academy of Sciences of the United States of America, 114(6), 1383–1388. 10.1073/pnas.1619312114 [PubMed: 28115688]

Schuckit MA, Goodwin DA, & Winokur G (1972). A study of alcoholism in half siblings. American Journal of Psychiatry, 128(9), 1132–1136. 10.1176/ajp.128.9.1132 [PubMed: 5060834]

Short AK, Yeshurun S, Powell R, Perreau VM, Fox A, Kim JH, Pang TY, & Hannan AJ (2017). Exercise alters mouse sperm small noncoding RNAs and induces a transgenerational modification of male offspring conditioned fear and anxiety. Translational Psychiatry, 7(5), e1114 10.1038/tp.2017.82 [PubMed: 28463242]

Skinner MK, Ben Maamar M, Sadler-Riggleman I, Beck D, Nilsson E, McBirney M, Klukovich R, Xie Y, Tang C, & Yan W (2018). Alterations in sperm DNA methylation, non-coding RNA and histone retention associate with DDT-induced epigenetic transgenerational inheritance of disease. Epigenetics & Chromatin, 11(1), 8 10.1186/s13072-018-0178-0 [PubMed: 29482626]

Skinner MK, Manikkam M, & Guerrero-Bosagna C (2010). Epigenetic transgenerational actions of environmental factors in disease etiology. Trends in Endocrinology and Metabolism, 21(4), 214–222. https://doi.org/101016/j.tem.2009.12.007 [PubMed: 20074974]

Slutske WS, Ellingson JM, Richmond-Rakerd LS, Zhu G, & Martin NG (2013). Shared genetic vulnerability for disordered gambling and alcohol use disorder in men and women: Evidence from a national community-based Australian twin study. Twin Research and Human Genetics, 16(2), 525–534. 10.1017/thg.2013.11 [PubMed: 23527679]

Verhulst B, Neale MC, & Kendler KS (2015). The heritability of alcohol use disorders: A meta-analysis of twin and adoption studies. Psychological Medicine, 45(5), 1061–1072. 10.1017/s0033291714002165 [PubMed: 25171596]

Viluksela M, & Pohjanvirta R (2019). Multigenerational and transgenerational effects of dioxins. International Journal of Molecular Sciences, 20(12), 2947 10.3390/ijms20122947

Walters RK, Polimanti R, Johnson EC, McClintick JN, Adams MJ, Adkins AE, Aliev F, Bacanu S-A, Batzler A, Bertelsen S, Biernacka JM, Bigdeli TB, Chen L-S, Clarke T-K, Chou Y-L, Degenhardt

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

F, Docherty AR, Edwards ACFontanillas P, … (2018). Transancestral GWAS of alcohol dependence reveals common genetic underpinnings with psychiatric disorders. Nature Neuroscience, 21(12), 1656–1669. https://doi.org/101038/s41593-018-0275-1 [PubMed: 30482948]

Yeshurun S, & Hannan AJ (2019). Transgenerational epigenetic influences of paternal environmental exposures on brain function and predisposition to psychiatric disorders. Molecular Psychiatry, 24(4), 536–548. 10.1038/s41380-018-0039-z [PubMed: 29520039]

Yoon G, Westermeyer J, Kuskowski MA, & Nesheim L (2013). Impact of the number of parents with alcohol use disorder on alcohol use disorder in offspring: A population-based study. Journal of Clinical Psychiatry, 74(8), 795–801. 10.4088/JCP.13m08350

Zhang H, & Gelernter J (2017). Review: DNA methylation and alcohol use disorders: Progress and challenges. American Journal on Addictions, 26(5), 502–515. 10.1111/ajad.12465 [PubMed: 27759945]

Zhang W, Yang J, Lv Y, Li S, & Qiang M (2019). Paternal benzo[a]pyrene exposure alters the sperm DNA methylation levels of imprinting genes in F0 generation mice and their unexposed F1–2 male offspring. Chemosphere, 228, 586–594. 10.1016/j.chemosphere.2019.04.092 [PubMed: 31059956]

Zhou Z, Karlsson C, Liang T, Xiong W, Kimura M, Tapocik JD, Yuan Q, Barbier E, Feng A, Flanigan M, Augier E, Enoch MA, Hodgkinson CA, Shen PH, Lovinger DM, Edenberg HJ, Heilig M, & Goldman D (2013). Loss of metabotropic glutamate receptor 2 escalates alcohol consumption. Proceedings of the National Academy of Sciences of the United States of America, 110(42), 16963–16968. 10.1073/pnas.1309839110 [PubMed: 24082084]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

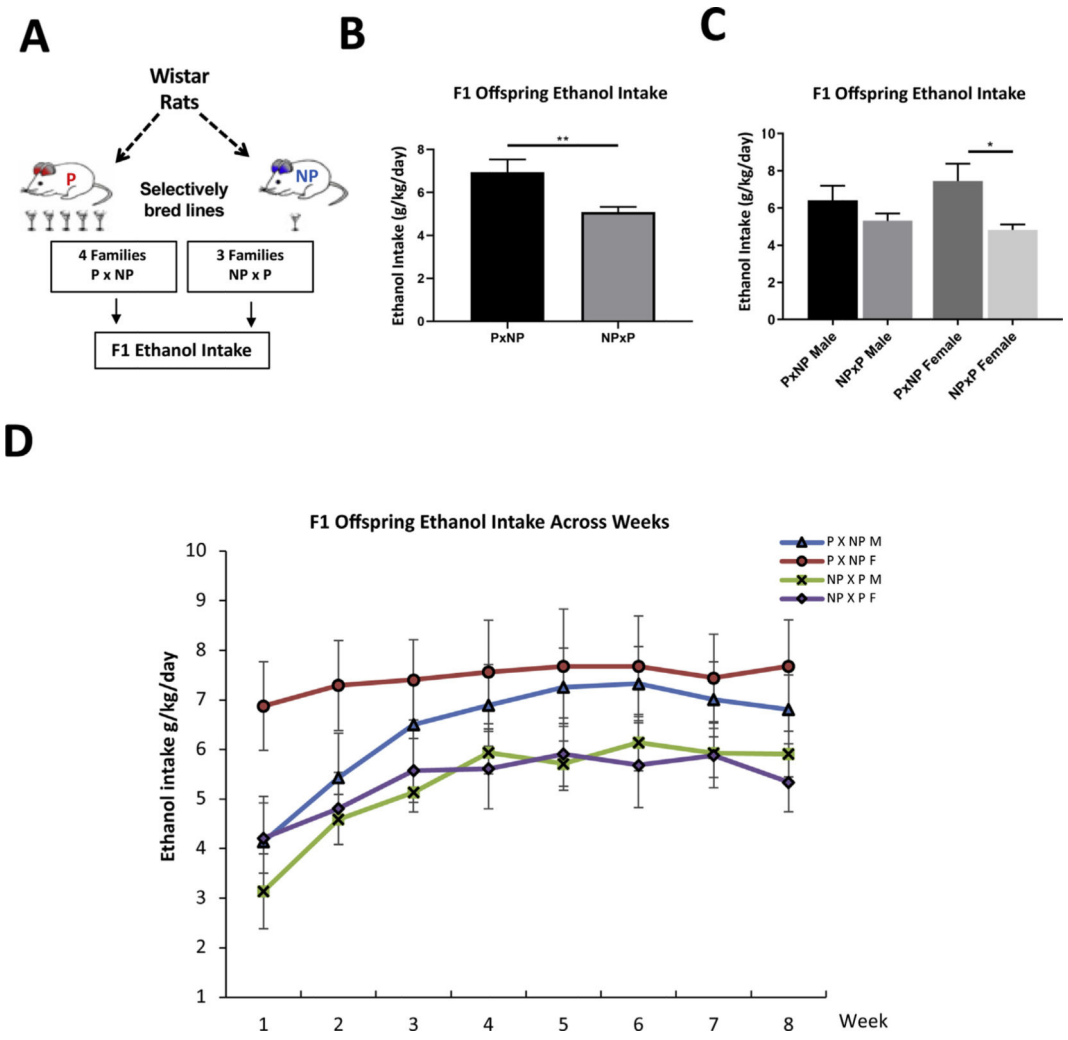

Fig. 1.

A) Representation of P and NP selective breeding paradigm and reciprocal breeding scheme for F1 ethanol consumption study. **B**) Average ethanol intake comparisons between F1 offspring of P × NP and NP × P reciprocal crosses; sire is indicated first (mean ± SEM). **C**) Average ethanol intake comparison between male and female F1 offspring of P × NP and NP × P reciprocal crosses (mean ± SEM). **D**) Weekly ethanol intake of male and female offspring of P × NP and NP × P reciprocal crosses (mean ± SEM). * and ** represent $p < 0.05$ and $p < 0.01$, respectively.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

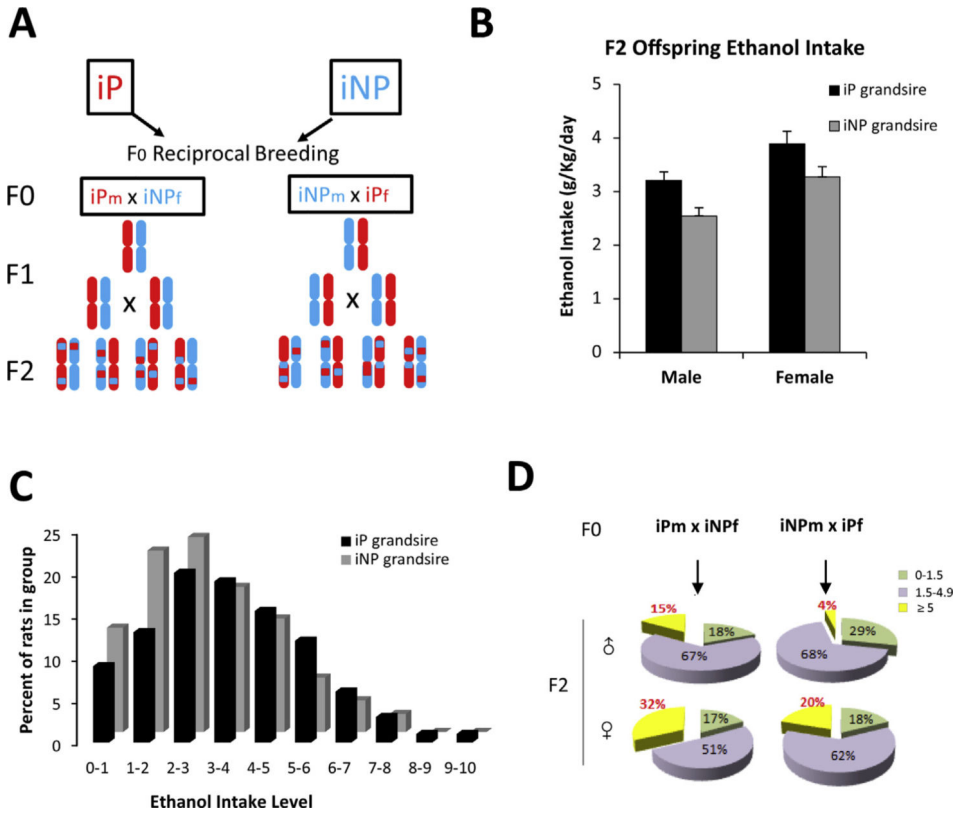

Fig. 2.

A) Representation of F_2 generation. Inbred P (iP) and iNP F0 reciprocal breeding paradigm used to generate F1 and the F1 × F1 breeding scheme used to produce the genetically diverse F_2 generation. **B**) Ethanol intake comparisons between male and female F_2 offspring derived from iP × iNP reciprocal crosses (mean ± SEM). **C**) Histogram depicting the percentages of F2 rats within iP- and iNP- grandsire-derived groups at distinct levels of average ethanol intake (g/kg/day). **D**) Pie charts depicting the percentages of rats within each group possessing high (≥5), moderate (1.5–4.9), and low (<1.5) average ethanol intake (g/kg/day). * and ** represent $p < 0.05$ and $p < 0.01$, respectively.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

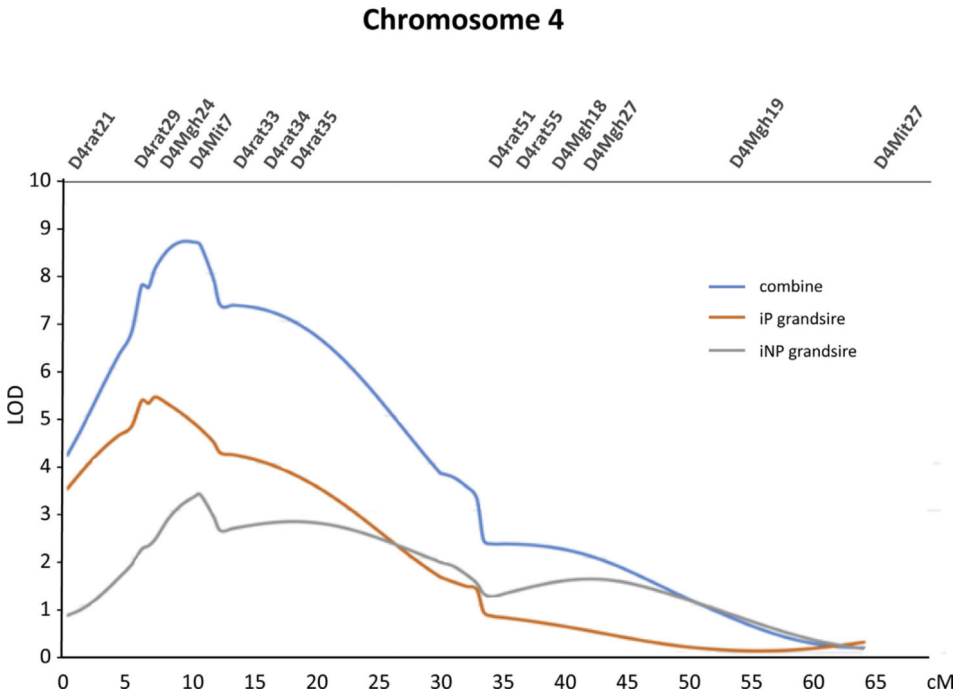

Fig. 3.

Multipoint LOD scores on rat Chr4 computed for alcohol consumption from iP-derived, iNP-derived, and combined F2 groups.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Table 1

Association between grandsire type and F2 allele genotype on mean alcohol consumption localized to rat chromosome 4.

Chr4	F2 genotype at marker	Homozygous P		Heterozygous P/NP		Homozygous NP		Variance explained by Genotype	Variance explained by Grandsire		
	Grandsire type	iP	iNP	iP	iNP	iP	iNP				
Marker	Position Start (Rn.6.0)	Mean Alcohol Consumption (±SD)						%	*p* value	%	*p* value
D4RAT21	61311474	4.32 (±2.13)	3.41 (±1.89)	3.51 (±1.95)	2.81 (±1.55)	2.75 (±1.41)	4.32 (±2.13)	7.5%	<0.0001	1.3%	0.0266
D4MGH24	75345304	4.32 (±2.15)	3.49 (±1.84)	3.66 (±1.92)	2.95 (±1.64)	2.52 (±1.37)	4.32 (±2.15)	7.3%	<0.0001	1.8%	0.0063
D4RAT29-Cntnap2	76673801	4.53 (±2.1)	3.6 (±1.82)	3.59 (±1.91)	2.92 (±1.64)	2.53 (±1.37)	4.53 (±2.1)	8.5%	<0.0001	1.9%	0.0044
Sspo	78097074	4.43 (±2.13)	3.64 (±1.83)	3.65 (±1.94)	2.86 (±1.65)	2.59 (±1.37)	4.43 (±2.13)	14.4%	<0.0001	1.5%	0.0056
D4MIT7-Npy	79575532	4.36 (±2.12)	3.63 (±1.83)	3.63 (±1.91)	3.03 (±1.59)	2.46 (±1.37)	4.36 (±2.12)	4.2%	0.0002	1.7%	0.0086
D4RAT33	81874073	4.39 (±2.09)	3.65 (±1.83)	3.67 (±1.9)	2.95 (±1.65)	2.54 (±1.36)	4.39 (±2.09)	8.5%	<0.0001	1.7%	0.0073
D4RAT34	86438317	4.23 (±2.14)	3.67 (±1.8)	3.63 (±1.92)	3.02 (±1.64)	2.43 (±1.29)	4.23 (±2.14)	9.3%	<0.0001	1.2%	0.0256
Snca	90880297	5.28 (±2.11)	4.5 (±3)	3.71 (±2.62)	3.12 (±3.17)	2.03 (±2.49)	5.28 (±2.11)	25.5%	0.0005	2.3%	0.2101
Fam190a	91374226	4.27 (±2.05)	3.62 (±1.76)	3.62 (±1.87)	3.03 (±1.7)	2.57 (±1.36)	4.27 (±2.05)	7.5%	<0.0001	1.3%	0.0266
D4RAT35	92490122	4.2 (±2.21)	3.62 (±1.76)	3.66 (±1.91)	2.99 (±1.7)	2.65 (±1.48)	4.2 (±2.21)	7.0%	<0.0001	1.6%	0.0109
D4RAT51	119538229	3.91 (±2.1)	3.58 (±1.98)	3.49 (±1.91)	2.96 (±1.61)	3.01 (±1.79)	3.91 (±2.1)	3.8%	0.0009	1.4%	0.0228
D4RAT55	125671525	4.02 (±2.06)	3.46 (±1.88)	3.48 (±1.84)	3.02 (±1.67)	3.26 (±1.97)	4.02 (±2.06)	2.4%	0.009	2.1%	0.0048
D4MGH18	125884464	3.92 (±1.95)	3.29 (±1.91)	3.52 (±1.94)	3.08 (±1.65)	3.23 (±2.04)	3.92 (±1.95)	2.0%	0.0214	2.2%	0.0033
D4MGH27	129308765	3.84 (±1.94)	3.34 (±1.9)	3.62 (±1.97)	3.04 (±1.65)	3.23 (±2.04)	3.84 (±1.94)	1.9%	0.0246	2.2%	0.0035
D4MGH19	147735145	3.63 (±1.95)	3.34 (±1.85)	3.66 (±1.86)	3.01 (±1.63)	3.36 (±2.25)	3.63 (±1.95)	1.0%	0.1458	2.0%	0.0062
D4MIT27	169215613	3.33 (±1.95)	2.84 (±1.57)	3.66 (±1.79)	2.92 (±1.65)	3.66 (±2.33)	3.33 (±1.95)	0.18%	0.7078	3.09%	0.0006