SWI/SNF chromatin remodeling regulates alcohol response behaviors in *Caenorhabditis elegans* and is associated with alcohol dependence in humans

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Alcohol abuse is a widespread and serious problem. Understanding the factors that influence the likelihood of abuse is important for the development of effective therapies. There are both genetic and environmental influences on the development of abuse, but it has been difficult to identify specific liability factors, in part because of both the complex genetic architecture of liability and the influences of environmental stimuli on the expression of that genetic liability. Epigenetic modification of gene expression can underlie both genetic and environmentally sensitive variation in expression, and epigenetic regulation has been implicated in the progression to addiction. Here, we identify a role for the switching defective/sucrose nonfermenting (SWI/SNF) chromatin-remodeling complex in regulating the behavioral response to alcohol in the nematode *Caenorhabditis elegans*. We found that SWI/SNF components are required in adults for the normal behavioral response to ethanol and that different SWI/SNF complexes regulate different aspects of the acute response to ethanol. We showed that the SWI/SNF subunits SWSN-9 and SWSN-7 are required in neurons and muscle for the development of acute functional tolerance to ethanol. Examination of the members of the SWI/SNF complex for association with a diagnosis of alcohol dependence in a human population identified allelic variation in a member of the SWI/SNF complex, suggesting that variation in the regulation of SWI/SNF targets may influence the propensity to develop abuse disorders. Together, these data strongly implicate the chromatin remodeling associated with SWI/SNF complex members in the behavioral responses to alcohol across phyla.

SWI/SNF | alcohol | *C. elegans* | chromatin remodeling | GWAS

Alcohol (ethanol) abuse has significant public health consequences. In the United States, the lifetime prevalence of alcohol abuse is nearly 13% (1). There are many factors affecting the liability to abuse alcohol, including both genetic and environmental components (2), although how these interact to influence the progression to abuse is not well understood. It has been difficult to identify the factors that modify liability, in part because of the complex genetic architecture of liability (3, 4) and in part because of the lack of understanding of how these factors modify phenotypes that are relevant to alcohol related behaviors.

The naive level of response (LR) is an individual's initial acute physiological reaction to alcohol, and it is a strong predictor of lifetime susceptibility to develop an alcohol use disorder. People with a low LR are more likely to become alcoholic than people with a high LR (5, 6), and the LR phenotype is heavily genetically influenced (7). Therefore, determining the factors that contribute to LR is a promising approach for the identification of abuse liability loci.

Epigenetic modification of gene expression lies at the intersection of genes and the environment. Recently, increasing attention has been paid to the roles of epigenetic mechanisms for regulating gene expression in response to alcohol (reviewed in ref. 8). Modification of gene expression is associated with the progression to addiction (9, 10). Epigenetic changes have been implicated in alcohol-dependent gene regulation (11), in the development of rapid tolerance to ethanol in a variety of model systems (12–14), and in alcohol-drinking behaviors in mammals (11, 15). Epigenetic factors also may modify the basal LR to alcohol.

One mechanism of epigenetic regulation is chromatin remodeling. One of the best studied chromatin-remodeling complexes is SWI/SNF, which is named for the yeast switching defective/sucrose nonfermenting genes. SWI/SNF chromatin-remodeling complexes are large, multiprotein complexes that use the energy of ATP hydrolysis to alter the interaction between nucleosomes and DNA. Moving nucleosomes can occlude or reveal transcriptional regulatory regions of the DNA, leading to changes in gene expression. Importantly, SWI/SNF complexes have been implicated in neuronal development and cognitive function (reviewed in ref. 16), and recently a mutation in an SWI/SNF component has been associated with a syndrome of autism and cognitive disability in humans (17), demonstrating the importance of SWI/SNF gene regulation in complex cognitive functions. Therefore, we examined the role of SWI/SNF in ethanol-response behaviors.

We use the neurobiological model organism *Caenorhabditis elegans* to study the molecular mechanisms that influence the acute behavioral response to ethanol. *C. elegans* are well suited to these studies because of the strong conservation in the machinery of nervous system function between worms and humans.

Significance

Alcohol abuse is a significant social problem for which the molecular etiology is poorly understood. One recognized component of the progression to abuse disorders is the development of alcohol tolerance, and recently epigenetic modification of gene expression has been implicated in tolerance. Here, we use *Caenorhabditis elegans* to show that the conserved switching defective/sucrose nonfermenting (SWI/SNF) chromatin-remodeling complex, which modifies the transcriptional status of target genes, is required in adults and neurons for the development of acute alcohol tolerance. We predicted that allelic variation in genes that modify tolerance phenotypes may modify abuse liability in humans, and we identified a significant association between variation in one member of the SWI/SNF complex and a diagnosis of alcohol dependence in a human genomewide association study.

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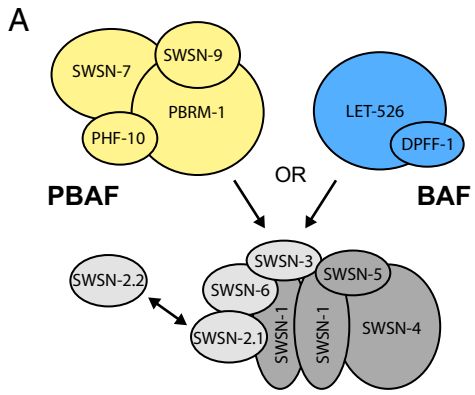
The molecular effects of ethanol on the nervous system are conserved functionally and molecularly in mammals and in worms (18–24).

We report here that SWI/SNF chromatin-remodeling complexes are required for the acute behavioral response to ethanol in *C. elegans*. Animals that lack SWI/SNF function have altered initial sensitivity and are unable to develop acute functional tolerance to ethanol, which in humans are two components of the LR phenotype (25). Further, we show that allelic variation in a member of the human SWI/SNF complex is associated with alcohol dependence (AD) in the Irish Affected Sib Pair Study of Alcohol Dependence (IASPSAD) genomewide association study (GWAS).

Results

Thirteen *C. elegans* SWI/SNF genes have been characterized to date, and they include homologs of all major human SWI/SNF subunits (26–31). Mammalian SWI/SNF complexes have been biochemically purified from different tissues and found to contain unique combinations of subunits depending upon cell type and developmental stage (32). Similarly, we predict that the *C. elegans* SWI/SNF subunits combine to form molecularly and functionally distinct complexes through the incorporation of different combinations of subunits (Fig. 1*A*). The complexes classically have been grouped into the Brg1-associated factors (BAF) and polybromo-associated BAF (PBAF) subfamilies based on the incorporation of complex-specific subunits (33, reviewed in 34). These distinctions are likely to extend to *C. elegans*, because mutations affecting BAF- and PBAF-specific subunits have distinct phenotypes in worms (26, 29).

SWI/SNF Genes Are Required for Acute Responses to Ethanol in *C. elegans*. To determine if SWI/SNF chromatin-remodeling complexes are important for acute behavioral responses to ethanol, we altered the function of each SWI/SNF subunit using genetic mutants or by RNAi and tested the affected animals for altered locomotion in response to acute ethanol exposure. Ethanol depresses the locomotion speed of wild-type worms, and after 30 min of exposure to ethanol the animals develop acute functional tolerance (AFT). AFT is not caused by increased metabolism or clearance of ethanol (35); instead it is likely to represent physiological compensation for the effects of ethanol in the affected tissues, which include neurons (22, 23). In this exposure paradigm, we observe the maximal reduction in speed at 10 min of exposure (35); we refer to this maximal reduction as the initial sensitivity to ethanol. AFT is observed as the recovery of speed between 10 and 30 min of exposure. We found that most of the 13 SWI/SNF subunits were required for the development of AFT, and some subunits were required for normal initial sensitivity to ethanol (Fig. 1*B*). The SWI/SNF genes fell into three phenotypic classes represented by *swsn-4*, *swsn-2.2*, and *let-526*. The *swsn-4(os13)* mutants have normal initial sensitivity but fail to develop AFT (Fig. 1*C*); *swsn-2.2(tm3395)* mutants initially are resistant to the depressive effects of ethanol on locomotion and do not develop AFT (Fig. 1*D*); and *let-526(RNAi)* animals initially are resistant to ethanol but develop AFT (Fig. 1*E*). These observations suggest that SWI/SNF complexes regulate at least two aspects of the acute ethanol response—initial sensitivity and AFT.



B

	C. elegans	Human	Allele/RNAi	Trials	Initial sensitivity	AFT
Core	*swsn-1*	*SMARCC1/C2*	RNAi	6	WT	No AFT
	swsn-4	*SMARCA2/A4*	*os13*	6	WT	No AFT
	swsn-5	*SMARCB1*	RNAi	7	WT	WT
Accessory	*swsn-2.1*	*SMARCD1-D3*	*tm3309*	6	WT	No AFT
	swsn-2.2	*SMARCD1-D3*	*tm3395*	6	Resistant	No AFT
	swsn-3	*SMARCE1*	*tm3647*	6	Sensitive	No AFT
	swsn-6	*ACT6A/6B*	RNAi	8	Resistant	No AFT
PBAF	*pbrm-1*	*PBRM1*	*ok847*	7	Resistant	WT
	swsn-7	*ARID2*	*gk1041*	6	WT	No AFT
	swsn-9	*BRD7/BRD9**	*ok1354*	7	WT	No AFT
	phf-10	*PHF10*	RNAi	6	WT	No AFT
BAF	*let-526*	*ARID1A/1B*	RNAi	6	Resistant	WT
	dpff-1	*DPF1 - DPF3*	*tm4287*	7	Resistant	WT

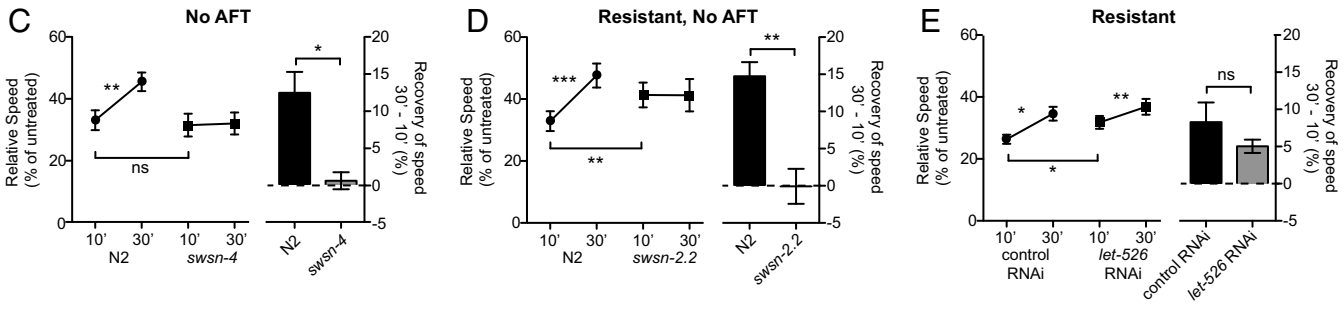


Fig. 1. SWI/SNF genes are required for acute alcohol responses in *C. elegans*. (*A*) Thirteen *C. elegans* SWI/SNF genes have been characterized genetically. By analogy with mammalian complexes, these subunits may combine to form functionally distinct complexes (indicated by arrows). Subunits shared by all SWI/SNF complexes include the enzymatic core (dark gray) and common accessory subunits (light gray). Two major subfamilies of SWI/SNF are BAF and PBAF, which are defined by their unique accessory subunits (PBAF, blue; BAF, yellow). (*B–E*) Locomotion speed was determined following 10- and 30-min exposure to 0 or 400 mM exogenous ethanol. For this and all subsequent figures, speeds are reported as a percentage of untreated controls (left *y* axes). AFT is quantified as the recovery of speed between 10 and 30 min of exposure (right *y* axes). All genotypes on the same graph were tested simultaneously on the same plates. Wild-type (N2) or control RNAi animals develop AFT. (*B*) SWI/SNF mutants and RNAi animals had altered acute responses to ethanol. Three phenotypic classes are shown. (*C*) *swsn-4(os13)* mutants have normal initial sensitivity to ethanol, but they fail to develop AFT ($n = 6$). (*D*) *swsn-2.2(tm3395)* mutants initially are resistant to ethanol and do not develop AFT ($n = 6$). (*E*) *let-526(RNAi)* animals initially are resistant to ethanol but still develop AFT ($n = 6$). Error bars indicate SEM. Paired two-tailed Student's *t* tests were used for statistical comparisons (ns, not significant; $*P \leq 0.05$; $**P \leq 0.001$; $***P \leq 0.0001$).

BAF and PBAF Complexes Function in Different Aspects of the Acute Behavioral Response to Ethanol. Our comprehensive analysis of SWI/SNF subunits allowed us to ask if BAF and PBAF have different functions in the acute response to ethanol. We found good correspondence between the mutant phenotype classes and the affected subunit type: (*i*) BAF subunits are required for normal initial sensitivity; (*ii*) PBAF subunits generally are required for AFT; and (*iii*) reduced functions of common subunits display a combination of phenotypes. This constellation of phenotypes suggests that distinct SWI/SNF complexes regulate different genes to control two aspects of the level of response to ethanol.

***swsn-9* Mutant Animals Have Normal Ethanol Entry and Metabolism.** *C. elegans* are resistant to tissue accumulation of exogenously applied ethanol and accumulate only ∼12% of the exogenous dose (35) because their cuticle is extremely resistant to passage by chemicals (36). One possible explanation for the failure of SWI/SNF mutants to develop AFT is that they have altered ethanol entry or metabolism, resulting in tissue ethanol concentrations different from those of wild-type animals. We determined internal ethanol concentrations after exposure to 400 mM ethanol and found that they were not different in *swsn-9(ok1354)* and wild-type (N2) worms at either the 10- or 30-min time points (Fig. S1). These data suggest that the SWI/SNF complex does not alter ethanol-response behaviors through the regulation of cuticle structures or metabolic enzymes.

***swsn-9* Is Expressed Broadly During Development and in Adulthood.** To determine the spatial requirements for the SWI/SNF complex in AFT, we used the *swsn-9(ok1354)* deletion mutant as the basis for transformation rescue experiments. This gene was selected for analysis because loss of *swsn-9* function affects only AFT, allowing us to focus on this aspect of the acute alcohol response. To begin, we determined the genomic sequences necessary for *swsn-9* function. We PCR amplified the genomic region encompassing the *swsn-9* coding and regulatory sequences, injected it into *swsn-9(ok1354)* mutants to generate extrachromosomal arrays, and then integrated one of the arrays into the genome to create stably transformed animals. The resulting integrated line rescued AFT in *swsn-9* mutants (Fig. 2*A*), suggesting that we have identified all *swsn-9* sequences that are required for AFT. Next, we generated a construct in which the *swsn-9* regulatory sequences drive expression of a SWSN-9::GFP fusion protein. This construct was injected into *swsn-9(ok1354)* mutants, integrated into the genome, and tested for rescue of ethanol-response behaviors. We found that *swsn-9::GFP* rescued AFT (Fig. 2*B*). We examined expression of SWSN-9::GFP in this line and found that it is expressed in many and perhaps all tissues, including neurons, somatic gonad, muscle, and intestine (Fig. S2). Of particular importance for this study, we found that SWSN-9::GFP is expressed in muscles and neurons throughout development and into adulthood (Fig. S2 *E*–*G*).

Expression of *swsn-9* in Neurons or in Body Muscle Is Sufficient for AFT. The *swsn-9* gene is expressed in many diverse cell types throughout the animal. To identify the tissues requiring *swsn-9* for the development of AFT, we used cell-type–specific promoters to drive the expression of functional *swsn-9* in specific tissues in an otherwise *swsn-9* mutant background. Because locomotion is a neuromuscular process, we focused on muscles and neurons. We used the *myo-3* promoter to drive expression in body wall muscle (Fig. 2*E*) (37) and the *H20* promoter to drive expression in neurons (Fig. 2*F*) (38). These constructs produce the same SWSN-9::GFP fusion protein that was used above (Fig. 2*B*). We found that SWSN-9::GFP is able to rescue AFT when expressed in either body muscle or neurons (Fig. 2 *C* and *D*). For each of the constructs, we examined four independent extrachromosomal arrays and found that in each case two of the four lines produced significant AFT (see Fig. S4). Although these promoters strongly and most consistently drive expression in the expected tissues, we found that there is some weak and variable

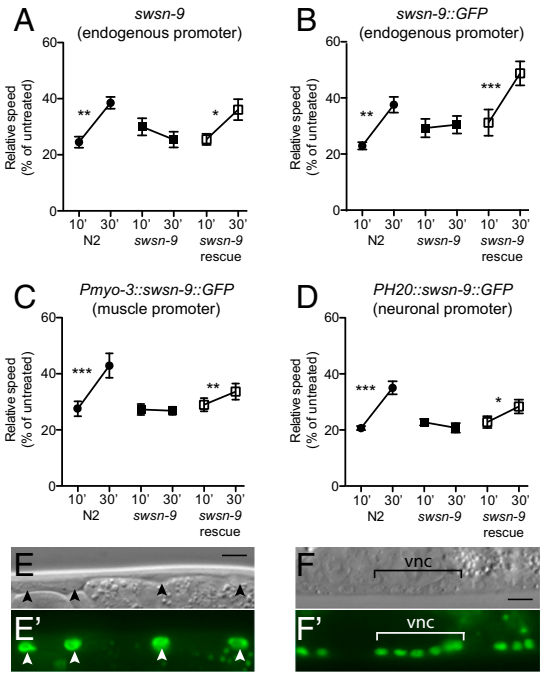


Fig. 2. *swsn-9* expression in neurons or body muscle is sufficient for the development of AFT. (*A*–*D*) Locomotion speed was determined following 10- and 30-min exposure to 0 or 400 mM exogenous ethanol. Wild-type (N2) animals develop AFT, but *swsn-9(ok1354)* mutants do not. (*A*) An integrated line containing *swsn-9* genomic sequence was able to fully rescue the AFT defect of *swsn-9(ok1354)* mutants ($n = 6$). (*B*) An integrated line containing *swsn-9* regulatory and coding sequences with a C-terminal GFP tag (*swsn-9::GFP*) also rescued AFT in *swsn-9(ok1354)* mutants ($n = 6$). (*C*) Expression of SWSN-9::GFP using the *myo-3* promoter was able to rescue AFT in *swsn-9(ok1354)* mutants. Four different extrachromosomal arrays were tested; two rescued AFT ($n = 8$). (*D*) Expression of SWSN-9::GFP using the *H20* promoter was able to rescue AFT in *swsn-9(ok1354)* mutants. Four different extrachromosomal arrays were tested; two rescued AFT ($n = 8$). Representative lines are shown for *C* and *D*; complete data are shown in Fig. S4. Error bars indicate SEM. Paired two-tailed Student's *t* tests were used for statistical comparisons. For indicated comparisons, ns, not significant; $*P \leq 0.05$; $**P \leq 0.001$; $***P \leq 0.0001$. (*E* and *F*) Corresponding differential interference contrast (DIC) (*E* and *F*) and fluorescent (*E′* and *F′*) images showing expression of SWSN-9::GFP. Images of the entire expression pattern appear in Fig. S3. (*E*) The *myo-3* promoter drives expression of SWSN-9::GFP in body wall muscle (arrowheads). (*F*) The *H20* promoter drives expression in head (Fig. S3) and ventral cord neurons (vnc). (Scale bar, 5 μm.)

GFP accumulation in additional tissue types, including intestine, pharynx, and hypodermis (Fig. S3). Expression of SWSN-9::GFP in intestine, pharynx, and hypodermis under the control of the *elt-2* promoter was unable to rescue the *swsn-9* AFT defect (Fig. S4), supporting the conclusion that SWSN-9::GFP expression in neurons or muscles is responsible for the rescue of the AFT defect. To confirm that PBAF has a role in muscles and neurons for AFT, we performed similar experiments with a second member of the complex, *swsn-7*. We found that *swsn-7* expression in muscles or neurons was able to rescue the AFT defect in *swsn-7*–null mutant animals (Fig. S5). We conclude that PBAF expression in either muscles or neurons is sufficient for the development of AFT.

***swsn-1* Is Required in Adult Tissues for the Development of AFT.** The SWI/SNF complex is important for embryonic and larval development (26, 28–31); therefore SWI/SNF may be required during development and/or in adulthood for AFT. To distinguish between these possibilities, we took advantage of a temperature-sensitive allele of *swsn-1* (28). This allele is viable and fertile at the permissive temperature (15 °C) and is embryonic lethal at the restrictive temperature (25 °C). First, we reared wild-type and

swsn-1(os22ts) animals at 15 °C continuously and performed locomotion assays in the presence and absence of ethanol. We found that both wild-type and *swsn-1(os22)* animals develop AFT to ethanol at 15 °C. We noticed that the recovery of speed at 15 °C in wild-type animals appears to be reduced compared with experiments conducted at 20 °C (Figs. 1 and 2 and Figs. S4 and S5), suggesting that temperature itself influences the AFT mechanism. Next, we reared wild-type and *swsn-1(os22ts)* worms at 15 °C through the fourth larval stage (L4) and then shifted them to 25 °C (Fig. 3*A*). The protein encoded by the *os22ts* allele is inactivated rapidly at the restrictive temperature (28); therefore this temperature-shift regimen is expected to produce functional SWSN-1 during development and inactive SWSN-1 in adults. We observed the behavioral response to ethanol 18 h after moving the animals to 25 °C and found that wild-type animals develop AFT, but *swsn-1(os22ts)* animals do not (Fig. 3*B*). This result indicates that *swsn-1* is required after the L4 stage for the development of AFT and suggests that the SWI/SNF complex has an ongoing role in differentiated tissues that is important for AFT to ethanol.

SWI/SNF Genes Are Associated with a Diagnosis of AD in a Human Population. Based on our finding that SWI/SNF mutants have profound defects in the behavioral response to ethanol in *C. elegans*, we reasoned that allelic variation in SWI/SNF genes might influence alcohol response phenotypes in human populations and therefore may be associated with alcohol use disorders. We queried the IASPSAD (39) using all 29 human SWI/SNF genes and found suggestive evidence of an association between polymorphisms in bromodomain containing 7 (*BRD7*) and SWI/SNF-related, matrix-associated, actin-dependent regulator of chromatin, subfamily A, member 2 (*SMARCA2*) and a diagnosis of AD in this population (Table S1). Our strongest single SNP signal is at rs116988464 (P = 6.94E-06, q = 0.24589), which is a polymorphism in *BRD7*, an accessory subunit found in PBAF complexes (40). The signal at *BRD7* (Fig. S6) is driven by a single imputed SNP of ~3% minor allele frequency (MAF). Robust associations usually display multiple correlated signals because of linkage disequilibrium (LD) with other SNPs (as seen for *SMARCA2*, below and Fig. 4). We investigated this SNP further to assess imputation quality and to determine whether its LD relationships with surrounding markers in the 1000 Genomes project database were consistent with the observed results. In this dataset, rs116988464 had Impute 2 certainty = 0.992 and a Modified Quasi-Likelihood Score (MQLS) R^2 = 0.776, both indicating high imputation accuracy. Additionally, rs116988464 has no r^2 >0.1 with any SNP in the 1000 Genomes data from individuals of European descent from the United Kingdom (browser.1000genomes.org/index.html), consistent with the lack of additional correlated signals we observe. Imputation accuracy depends on high-probability haplotype phasing and the specificity of alleles on phased haplotypes and is not affected by the lack of individual SNPs in LD with rs116988464. Together, these data provide strong support for the validity of the signal in *BRD7*.

Our next strongest signal is at rs79825622 (P = 7.29E-06, q = 0.25350), which is a polymorphism in *SMARCA2*, one of two ATPase subunits of human BAF and PBAF complexes; additional polymorphisms in *SMARCA2* give signals of similar magnitude (Fig. 4 and Table S1). Although these results do not achieve conventional, single-marker genomewide significance, the data from *C. elegans* alter the prior expectation that variation in these genes may influence risk of AD in our human sample.

We further assessed the evidence for a contribution from genes encoding elements of the SWI/SNF complex by performing gene-based analyses, which test for a departure from the expected distribution of P values across the genomic interval defined for the gene(s) in question, for all 29 human genes encoding known SWI/SNF subunits (Table S2). We observed enrichment of signals for *SMARCA2* (gene-based P = 0.00049, q = 0.01421). The signal in *SMARCA2* is dispersed broadly across multiple exons and introns, with the strongest signal (rs79825622) located in the ninth intron (Fig. 4). Taken together with our observation that SWI/SNF function is important in ethanol-response behaviors in worms, these data strongly support a role for SWI/SNF chromatin remodeling in the behavioral responses to alcohol across species.

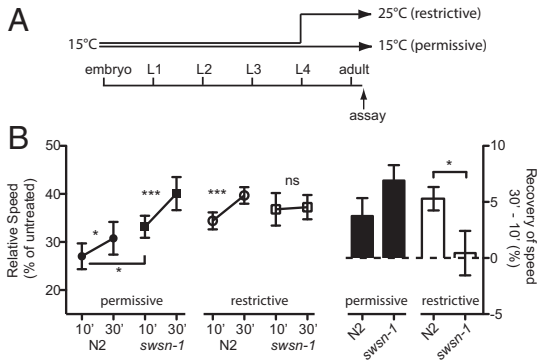


Fig. 3. *swsn-1* is required in adults for AFT. The *swsn-1(os22)* temperature-sensitive allele was used to determine when the SWI/SNF complex is required for AFT. This allele is viable at the permissive temperature (15 °C) and is lethal at the restrictive temperature (25 °C). (*A*) Wild-type (N2) and *swsn-1(os22)* animals either were maintained continuously at 15 °C (permissive) or were maintained at 15 °C until the L4 larval stage and then shifted to 25 °C (restrictive temperature). Animals were assayed on the first day of adulthood. (*B*) Locomotion speed was determined following 10- and 30-min exposure to 0 or 400 mM exogenous ethanol. A break in the *x* axis indicates animals that were tested on different plates. Wild-type (N2) and *swsn-1(os22)* animals develop AFT when maintained at 15 °C (n = 10). Wild-type (N2) animals also develop AFT when they are shifted to 25 °C as L4 larvae, but *swsn-1(os22)* animals do not develop AFT under this temperature-shift regimen (n = 10). Error bars indicate SEM. Paired two-tailed Student's t tests were used for statistical comparisons. For indicated comparisons, ns, not significant; *$P \leq 0.05$; ***$P \leq 0.0001$.

Discussion

Epigenetic modification of gene expression is likely to play an important role in the development of alcohol use disorders (8, 9). In this study, we examined the function of the SWI/SNF chromatin-remodeling complex in the acute behavioral response to ethanol. We found that the SWI/SNF complex functions in adults and in neurons and muscles for acute ethanol-response behaviors in *C. elegans* and that allelic variation in a member of the SWI/SNF complex is associated with AD in a human population. Together, these data demonstrate that SWI/SNF chromatin-remodeling complexes are important in a relevant physiological response to ethanol, the initial level of response, and suggest that members of the complex are good candidates for abuse liability loci in humans.

The phenotypes we observed in the acute response to alcohol correspond well with the two predicted *C. elegans* SWI/SNF complexes: Mutations affecting BAF subunits tend to cause resistance to ethanol but do not affect AFT, mutations affecting PBAF subunits typically do not alter initial sensitivity to ethanol but cause defects in AFT, and mutations affecting common subunits display a combination of these phenotypes. There are two exceptions to this general rule. First, *swsn-3(tm3647)* was the only SWI/SNF mutant that was hypersensitive to ethanol. This mutation is unusual in that it is predicted to produce a truncated SWSN-3 protein lacking the HMG domain (26) and therefore may produce a gain-of-function phenotype. Second, the *pbrm-1(ok843)* mutant was resistant to ethanol, and it developed AFT. Therefore, *pbrm-1(ok843)* appeared to be more similar to BAF than to PBAF subunits. There is evidence that mammalian PBRM-1 is present in additional high molecular weight complexes that are biochemically distinct from BAF complexes (41). Therefore, it is possible that *C. elegans* PBRM-1 functions in a different macromolecular complex. Alternatively, PBRM-1

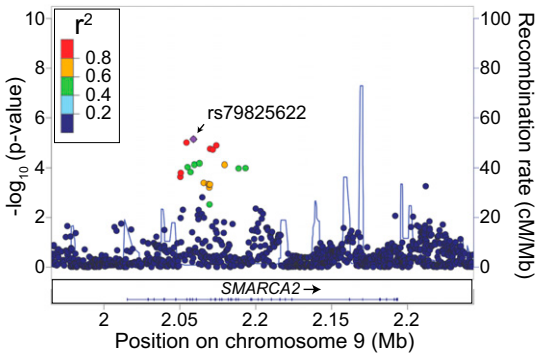


Fig. 4. SWI/SNF genes are associated with AD in the IASPSAD. A human GWAS identifies *SMARCA2* in association with a diagnosis of AD. Negative $\log_{10}$-transformed *P* values (left *y* axis) of SNPs within 50 kb of the *SMARCA2* locus (*x* axis) are depicted. Each dot represents an SNP tested in the GWAS. The arrow indicates SNP rs79825622, which had the lowest *P* value. LD is indicated as pairwise r^2 between this SNP and others in the region; the degree of LD is color-coded (legend). The right *y* axis represents recombination in the 1000 Genomes European-American reference panel. Note that the SNPs with the lowest *P* values lie between recombination peaks and are in LD with rs79825622. This figure was generated using LocusZoom (csg.sph.umich.edu/locuszoom/).

may act in a BAF-type complex to control initial sensitivity to ethanol. Biochemical purification of *C. elegans* SWI/SNF complexes will be required to distinguish between these possibilities.

We were surprised to find that expression of *swsn-9* or *swsn-7* in either neurons or muscles was able to rescue the AFT defect of the respective mutants. The finding that SWI/SNF function is not exclusive to neurons may suggest that the requirement is broad and that SWI/SNF acts in both tissues for AFT. None of these constructs completely restored AFT to the mutants, suggesting that AFT is the combined result of functions in both neurons and muscles. This result may suggest that ethanol acts on muscles as well as neurons to mediate its effects on locomotion.

An important aspect of epigenetic influences on the progression to addiction is dynamic gene regulation in the adult brain in response to chronic consumption of alcohol. SWI/SNF is important in the proper development of brain structures as well as in the function of differentiated neurons (42–45), so we asked if there is a role for SWI/SNF in adults for AFT. We identified a requirement for SWI/SNF function in adult cells using a temperature-sensitive mutant allele of *swsn-1*. The protein encoded by *swsn-1(os22)* is functional at 15 °C but is unstable and nonfunctional at 25 °C, so we were able to provide SWI/SNF function during development and remove it by shifting the animals to 25 °C at the L4 stage when most tissue development was complete. This experiment revealed a requirement for SWI/SNF function in differentiated cells in the acute behavioral response to ethanol. This result suggests that the SWI/SNF complex is functional and regulates genes involved in ethanol-response behaviors during the time at which plasticity in gene regulation is important in the development of addiction.

Nucleosome rearrangement influences the regulation of transcription. We predict that functional allelic variation in SWI/SNF complex components in humans may subtly alter their function and thereby influence the regulation of mediators of ethanol-response behaviors. In *C. elegans*, because AFT occurs quickly, within 30 min of the onset of exposure to ethanol, and because some SWI/SNF mutants demonstrate initial sensitivity defects within 10 min of exposure, we predict that the important SWI/SNF gene regulation for acute behavioral responses to ethanol occurs before the ethanol exposure and establishes the ability to respond acutely to ethanol. We speculate that in humans, extended or repeated exposure to ethanol may induce SWI/SNF-dependent epigenetic changes in the regulation of behaviorally relevant genes; these changes, in turn, may influence the progression to dependence.

Although we do not yet know the relevant targets of SWI/SNF regulation for ethanol-response behaviors, we have identified several genes that regulate initial sensitivity and AFT in worms that are excellent candidates for regulation by SWI/SNF, including the large conductance voltage and calcium sensitive potassium (BK) channel and genes that regulate lipid metabolism and utilization in the animal (23, 24, 46). Variation in SWI/SNF components may influence these and/or other as-yet-unidentified downstream mediators of the behavioral response to ethanol.

A major difficulty in understanding the genetics of alcoholism has been that very few strong candidate genes have come out of human GWASs, and even fewer have been supported strongly across different populations. One explanation for the failure to identify strong signals both within and across studies may be important natural variation in different components that affect the same biological process, so that different people may have allelic variations in different genes that work together to contribute to the same phenotypic outcome. Each signal, then, may identify only part of the actual variation in the important biological process underlying the susceptibility to addiction and therefore may not reach significance. By considering each gene in isolation for replication between studies rather than genes that act together, an underlying biologically relevant consensus that otherwise might be identified in a study may be missed. Therefore, we suggest that an appropriate unit of analysis for association with complex behavioral disorders may be a biological complex rather than an individual gene. Additionally, the strength of any weak individual signal in a study may be increased if it is combined with weak signals from other proteins that are known to interact with it; together these signals are likely to point to biological importance. Support for this hypothesis comes from our observation that two different components of the SWI/SNF complex, *SMARCA2* and *BRD7*, achieved suggestive association with AD in the IASPSAD GWAS. Together these components form an obligate protein complex that controls the same biological process, the regulation of chromatin structure. Variation in any one of the component proteins could produce the same phenotypic outcome, susceptibility to AD. Therefore it is not surprising that variation in different genes affecting the same protein complex would be found in human populations; the observation that both these genes demonstrate some association with AD enhances the significance of each of the individual results. This observation also suggests that it may be fruitful to query other human population datasets for signals in any SWI/SNF complex member rather than simply asking for replication of the particular SNPs identified here.

Materials and Methods

Strains. *C. elegans* strains were derived from the Bristol strain N2 and were maintained on Nematode Growth Medium at 20 °C unless otherwise specified (47). Strains used in this study are listed in *SI Materials and Methods*.

Locomotion Assays. Locomotion tracking and analysis was performed as described previously (22), with minor modifications (*SI Materials and Methods*). Briefly, 10 worms were placed in copper rings on plates containing no or 400 mM ethanol, and their locomotion was recorded for 2 min after 10 or 30 min of exposure to ethanol. The average speed was calculated for each group of worms ($n = 1$). At least six trials were performed for each genotype. Two-tailed paired Student's *t* tests were used for statistical comparisons. Only animals that were tested simultaneously on the same plates were compared with each other. All raw data are reported in Dataset S1.

RNAi. Feeding RNAi was performed as described in ref. 48, with some modifications (*SI Materials and Methods*). Briefly, synchronized populations of larval stage 1 (L1) animals were plated onto seeded RNAi plates and allowed to feed for 4 d. The resulting first-day adults were used in locomotion assays.

Rescue Constructs. All PCRs were performed with the iProof High Fidelity DNA Polymerase (Bio-Rad), and the resulting clones were sequenced. Rescue constructs were transformed into strains bearing the *swsn-9(ok1354)* or

swsn-7(gk1041) mutations, and lines with a high transmission frequency were selected for analysis (*SI Materials and Methods*).

GWAS. A sample of 816 related cases meeting criteria for a diagnosis of AD set by the *Diagnostic and Statistical Manual of Mental Disorders, version 4* (DSM-IV) (49) and 2,048 population controls were directly genotyped for ~1 million SNPs using the Affymetrix v6.0 microarray (Affymetrix). Measured genotypes for 890,920 autosomal SNPs were used as input for imputation to the April 2012 1000 Genomes reference panel of 36.5 million SNPs and 1.5 million structural variants derived from whole-genome sequencing of 1,092 individuals of multiple ethnicities (www.1000genomes.org).

After all quality control (QC) and data-cleaning steps, including post-imputation filtering of SNPs that (*i*) were monomorphic in our sample, (*ii*) had MAF <1%, or (*iii*) had information criterion values (an estimated correlation between imputed and true allele counts) ≤0.3, genotypes for 8,344,348 SNPs in 706 related cases and 1,755 controls were available for analysis using the Liang and Abecasis implementation of the MQLS (50); *P* values measure statistical significance, whereas *q* values estimate the proportion of results expected to be false discoveries at a specified *P* value threshold. We performed gene-based tests using KGG2.5 (51, 52), which accounts for LD based on the 1000 Genomes European reference sample. Gene-level *P* and *q* values are reported from these analyses. Detailed methods are included in *SI Materials and Methods*.

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1. Hasin DS, Stinson FS, Ogburn E, Grant BF (2007) Prevalence, correlates, disability, and comorbidity of DSM-IV alcohol abuse and dependence in the United States: Results from the National Epidemiologic Survey on Alcohol and Related Conditions. *Arch Gen Psychiatry* 64(7):830–842.
2. Prescott CA, Kendler KS (1999) Genetic and environmental contributions to alcohol abuse and dependence in a population-based sample of male twins. *Am J Psychiatry* 156(1):34–40.
3. Kendler KS, et al. (2012) Recent advances in the genetic epidemiology and molecular genetics of substance use disorders. *Nat Neurosci* 15(2):181–189.
4. Edenberg H, Foroud T (2014) Complex Genetics of Alcoholism. *Neurobiology of Alcohol Dependence*, eds Noronha A, Cui C, Harris RA, Crabbe JC (Academic, London), pp 539–546.
5. Schuckit MA (2000) Genetics of the risk for alcoholism. *Am J Addict* 9(2):103–112.
6. Schuckit MA (1994) Low level of response to alcohol as a predictor of future alcoholism. *Am J Psychiatry* 151(2):184–189.
7. Kalu N, et al. (2012) Heritability of level of response and association with recent drinking history in nonalcohol-dependent drinkers. *Alcohol Clin Exp Res* 36(6):1034–1041.
8. Marballi K, Ponomarev I, Mayfield RD, Harris RA (2014) Alcohol and the Brain: An Epigenetic Viewpoint. *Neurobiology of Alcohol Dependence*, eds Noronha A, Cui C, Harris RA, Crabbe JC (Academic, London), pp 349–358.
9. Starkman BG, Sakharkar AJ, Pandey SC (2012) Epigenetics-beyond the genome in alcoholism. *Alcohol Res* 34(3):293–305.
10. Hillemacher T (2011) Biological mechanisms in alcohol dependence—new perspectives. *Alcohol Alcohol* 46(3):224–230.
11. Pandey SC, Ugale R, Zhang H, Tang L, Prakash A (2008) Brain chromatin remodeling: A novel mechanism of alcoholism. *J Neurosci* 28(14):3729–3737.
12. Sakharkar AJ, Zhang H, Tang L, Shi G, Pandey SC (2012) Histone deacetylases (HDAC)-induced histone modifications in the amygdala: A role in rapid tolerance to the anxiolytic effects of ethanol. *Alcohol Clin Exp Res* 36(1):61–71.
13. Ghezzi A, Al-Hasan YM, Krishnan HR, Wang Y, Atkinson NS (2013) Functional mapping of the neuronal substrates for drug tolerance in Drosophila. *Behav Genet* 43(3):227–240.
14. Wang Y, Krishnan HR, Ghezzi A, Yin JC, Atkinson NS (2007) Drug-induced epigenetic changes produce drug tolerance. *PLoS Biol* 5(10):e265.
15. Warnault V, Darcq E, Levine A, Barak S, Ron D (2013) Chromatin remodeling—a novel strategy to control excessive alcohol drinking. *Transl Psychiatr* 3:e231.
16. Ronan JL, Wu W, Crabtree GR (2013) From neural development to cognition: Unexpected roles for chromatin. *Nat Rev Genet* 14(5):347–359.
17. Helsmoortel C, et al. (2014) A SWI/SNF-related autism syndrome caused by de novo mutations in ADNP. *Nat Genet* 46(4):380–384.
18. Kapfhamer D, et al. (2008) Loss of RAB-3/A in *Caenorhabditis elegans* and the mouse affects behavioral response to ethanol. *Genes Brain Behav* 7(6):669–676.
19. Bhandari P, et al. (2012) Chloride intracellular channels modulate acute ethanol behaviors in *Drosophila, Caenorhabditis elegans* and mice. *Genes Brain Behav* 11(4):387–397.
20. Treistman SN, Martin GE (2009) BK Channels: Mediators and models for alcohol tolerance. *Trends Neurosci* 32(12):629–637.
21. Thiele TE, Badia-Elder NE (2003) A role for neuropeptide Y in alcohol intake control: Evidence from human and animal research. *Physiol Behav* 79(1):95–101.
22. Davies AG, Bettinger JC, Thiele TR, Judy ME, McIntire SL (2004) Natural variation in the *npr-1* gene modifies ethanol responses of wild strains of *C. elegans*. *Neuron* 42(5):731–743.
23. Davies AG, et al. (2003) A central role of the BK potassium channel in behavioral responses to ethanol in *C. elegans*. *Cell* 115(6):655–666.
24. Bettinger JC, Leung K, Bolling MH, Goldsmith AD, Davies AG (2012) Lipid environment modulates the development of acute tolerance to ethanol in *Caenorhabditis elegans*. *PLoS ONE* 7(5):e35192.
25. Newlin DB, Thomson JB (1990) Alcohol challenge with sons of alcoholics: A critical review and analysis. *Psychol Bull* 108(3):383–402.
26. Large EE, Mathies LD (2014) *Caenorhabditis elegans* SWI/SNF subunits control sequential developmental stages in the somatic gonad. *G3 (Bethesda)* 4(3):471–483.
27. Hargreaves DC, Crabtree GR (2011) ATP-dependent chromatin remodeling: Genetics, genomics and mechanisms. *Cell Res* 21(3):396–420.
28. Sawa H, Kouike H, Okano H (2000) Components of the SWI/SNF complex are required for asymmetric cell division in *C. elegans*. *Mol Cell* 6(3):617–624.
29. Shibata Y, Uchida M, Takeshita H, Nishiwaki K, Sawa H (2012) Multiple functions of PBRM-1/Polybromo- and LET-526/Osa-containing chromatin remodeling complexes in *C. elegans* development. *Dev Biol* 361(2):349–357.
30. Cui M, Fay DS, Han M (2004) *lin-35/Rb* cooperates with the SWI/SNF complex to control *Caenorhabditis elegans* larval development. *Genetics* 167(3):1177–1185.
31. Weinberg P, Flames N, Sawa H, Garriga G, Hobert O (2013) The SWI/SNF chromatin remodeling complex selectively affects multiple aspects of serotonergic neuron differentiation. *Genetics* 194(1):189–198.
32. Wu JI, Lessard J, Crabtree GR (2009) Understanding the words of chromatin regulation. *Cell* 136(2):200–206.
33. Mohrmann L, Verrijzer CP (2005) Composition and functional specificity of SWI2/SNF2 class chromatin remodeling complexes. *Biochim Biophys Acta* 1681(2-3):59–73.
34. Kwon CS, Wagner D (2007) Unwinding chromatin for development and growth: A few genes at a time. *Trends Genet* 23(8):403–412.
35. Alaimo JT, et al. (2012) Ethanol metabolism and osmolarity modify behavioral responses to ethanol in *C. elegans*. *Alcohol Clin Exp Res* 36(11):1840–1850.
36. Burns AR, et al. (2010) A predictive model for drug bioaccumulation and bioactivity in *Caenorhabditis elegans*. *Nat Chem Biol* 6(7):549–557.
37. Okkema PG, Harrison SW, Plunger V, Aryana A, Fire A (1993) Sequence requirements for myosin gene expression and regulation in *Caenorhabditis elegans*. *Genetics* 135(2):385–404.
38. Shioi G, et al. (2001) Mutations affecting nerve attachment of Caenorhabditis elegans. *Genetics* 157(4):1611–1622.
39. Hack LM, et al. (2011) Limited associations of dopamine system genes with alcohol dependence and related traits in the Irish Affected Sib Pair Study of Alcohol Dependence (IASPSAD). *Alcohol Clin Exp Res* 35(2):376–385.
40. Middeljans E, et al. (2012) SS18 together with animal-specific factors defines human BAF-type SWI/SNF complexes. *PLoS ONE* 7(3):e33834.
41. Lessard J, et al. (2007) An essential switch in subunit composition of a chromatin remodeling complex during neural development. *Neuron* 55(2):201–215.
42. Bultman S, et al. (2000) A Brg1 null mutation in the mouse reveals functional differences among mammalian SWI/SNF complexes. *Mol Cell* 6(6):1287–1295.
43. Kim JK, et al. (2001) Srg3, a mouse homolog of yeast SWI3, is essential for early embryogenesis and involved in brain development. *Mol Cell Biol* 21(22):7787–7795.
44. Vogel-Ciernia A, et al. (2013) The neuron-specific chromatin regulatory subunit BAF53b is necessary for synaptic plasticity and memory. *Nat Neurosci* 16(5):552–561.
45. Wu JI, et al. (2007) Regulation of dendritic development by neuron-specific chromatin remodeling complexes. *Neuron* 56(1):94–108.
46. Raabe RC, Mathies LD, Davies AG, Bettinger JC (2014) The omega-3 fatty acid eicosapentaenoic acid is required for normal alcohol response behaviors in *C. elegans*. *PLoS ONE* 9(8):e105999.
47. Brenner S (1974) The genetics of *Caenorhabditis elegans*. *Genetics* 77(1):71–94.
48. Kamath RS, et al. (2003) Systematic functional analysis of the *Caenorhabditis elegans* genome using RNAi. *Nature* 421(6920):231–237.
49. American Psychiatric Association (1994) *Diagnostic and Statistical Manual of Mental Disorders* (American Psychiatric Association, Washington, DC), 4th Ed.
50. Thornton T, McPeek MS (2007) Case-control association testing with related individuals: A more powerful quasi-likelihood score test. *Am J Hum Genet* 81(2):321–337.
51. Li MX, Kwan JS, Sham PC (2012) HYST: A hybrid set-based test for genome-wide association studies, with application to protein-protein interaction-based association analysis. *Am J Hum Genet* 91(3):478–488.
52. Li MX, Sham PC, Cherny SS, Song YQ (2010) A knowledge-based weighting framework to boost the power of genome-wide association studies. *PLoS ONE* 5(12):e14480.