Genome **Biology**

The genetic basis of alcoholism: multiple phenotypes, many genes, complex networks

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Abstract

Alcoholism is a significant public health problem. A picture of the genetic architecture underlying alcohol-related phenotypes is emerging from genome-wide association studies and work on genetically tractable model organisms.

Keywords Addiction, alcohol sensitivity, disease susceptibility, genome-wide association studies, human genetics, model organisms.

Alcohol abuse and alcoholism are significant global public health problems. In the USA, alcoholism affects approximately 14 million people at a healthcare cost of $184 billion per year. Excessive alcohol consumption is associated with violent crime, aggression and increased risk of accidents, injury and death. The term 'alcoholism' was first coined by Magnus Huss to describe the persistence of drinking despite adverse health effects. The *Diagnostic and Statistical Manual of Mental Disorders* classifies alcoholism as an addictive disorder [1]. It is a complex disorder affected by genetic, epigenetic and environmental etiologic factors.

High levels of alcohol intake are associated with impairment of multiple organs, including brain, liver, pancreas and the immune system. The first stage of liver damage following chronic alcohol consumption is the development of fatty liver, which may be followed by inflammation, apoptosis, fibrosis and cirrhosis. Alcohol and its metabolite acetaldehyde are carcinogens, and excessive alcohol consumption is associated with increased risk for mouth and oropharyngeal cancer, breast cancer and liver cancer. The risk of upper gastrointestinal cancer is increased by a missense variant in the gene encoding aldehyde dehydrogenase (*ALDH*), which is found in some

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500 million East Asians [2]. Depression, epilepsy, hypertension and hemorrhagic stroke occur secondary to alcohol consumption [3]. Finally, alcohol consumption during pregnancy can result in birth defects that comprise fetal alcohol syndrome [4]. The diversity of pathologic effects of alcohol indicates that this drug exerts toxicity through multiple mechanisms, each of which can be modulated by different genetic variants.

Twin studies have demonstrated that the amount of alcohol one consumes has a genetic influence [5]. Age at first drink appears to be associated with alcohol-related problem behavior, but progression to alcoholism is under stronger genetic control than initiation, and the effect of early exposure to predict outcome is genetically mediated [6]. Alcohol-related phenotypes are typical quantitative traits, with population variation attributable to multiple segregating loci with effects that are sensitive to environmental exposures. Given that many loci are likely to affect alcohol drinking behavior and the development of dependence, we need to shift our focus from a 'one gene at a time' approach to genetic networks. This can be done by considering the effects of molecular polymorphisms on phenotypes mediated via complex networks of transcriptional, protein, metabolic and neurogenetic endophenotypes. Here, we review genetic risk factors and transcriptional correlates for alcohol consumption in humans, with insights from studies on model genetic organisms.
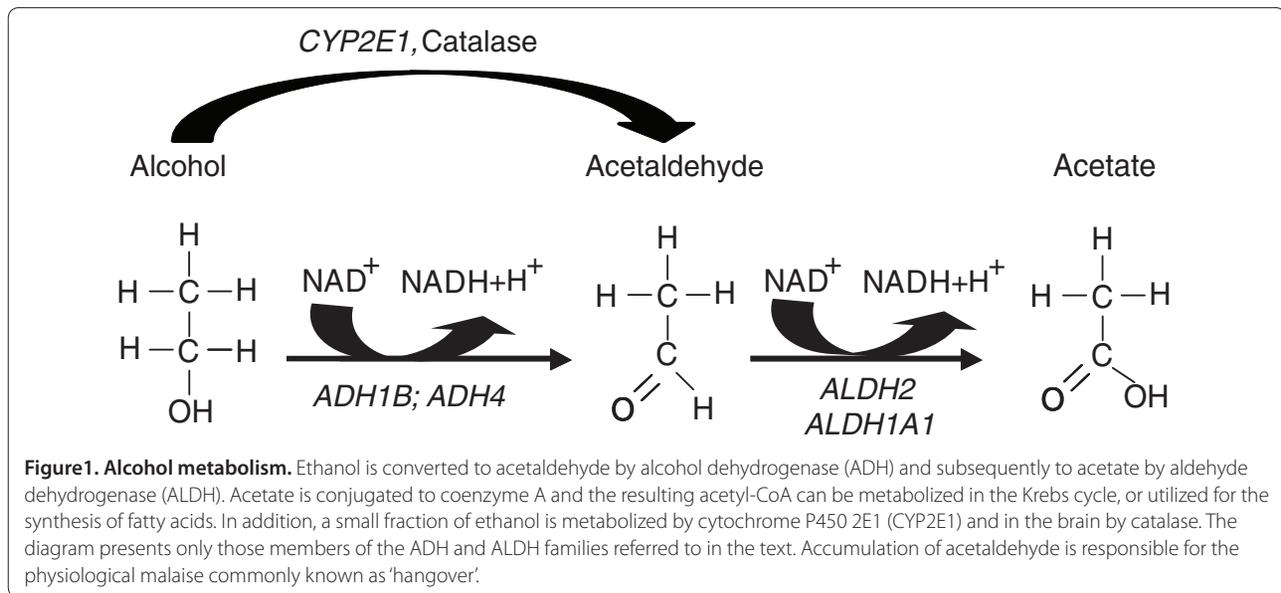
Human studies

Human genetic studies on alcohol-related phenotypes have used family-based linkage and population-based association analyses to identify quantitative trait loci (QTLs). Linkage studies are based on co-segregation between genetic markers and alcohol dependence in families with several affected members. By contrast, association studies evaluate the strength of association between genetic variants and alcohol phenotypes in samples of unrelated individuals; these can be attributable to a causal effect of the variant or linkage disequilibrium (LD) between the molecular variant and the true causal allele. Association analyses give more precise localization of QTLs than linkage studies; however,

Figure 1. Alcohol metabolism. Ethanol is converted to acetaldehyde by alcohol dehydrogenase (ADH) and subsequently to acetate by aldehyde dehydrogenase (ALDH). Acetate is conjugated to coenzyme A and the resulting acetyl-CoA can be metabolized in the Krebs cycle, or utilized for the synthesis of fatty acids. In addition, a small fraction of ethanol is metabolized by cytochrome P450 2E1 (CYP2E1) and in the brain by catalase. The diagram presents only those members of the ADH and ALDH families referred to in the text. Accumulation of acetaldehyde is responsible for the physiological malaise commonly known as 'hangover'.

false-positive associations can arise from population stratification of cases and controls, and by chance in small samples. In both designs, large numbers of individuals are required to detect QTLs with small effects. Early efforts to dissect the genetic basis of alcohol consumption and addiction in humans were based on candidate genes. The main pathway of ethanol metabolism involves its conversion to acetaldehyde by alcohol dehydrogenase (ADH; Figure 1). Acetaldehyde is oxidized to acetate by aldehyde dehydrogenase (ALDH). The activated form of acetate, acetyl-CoA, can be metabolized into ketone bodies, fatty acids, amino acids and steroids, in addition to oxidation in the Krebs cycle. Cytochrome P450s (for example, those encoded by the gene *CYP2E1*) and catalase also metabolize a small fraction of ingested ethanol. Multiple ADH and ALDH enzymes are encoded by different genes [7], and different *ADH* and *ALDH* alleles can differ in expression levels and in the rate at which their corresponding enzymes metabolize ethanol or acetaldehyde. The *ADH1B* His48Arg and *ALDH2* Lys487 polymorphisms have long been associated with risk of alcoholism, and directly and predictably lead to alcohol-induced flushing through molecular mechanisms that include accumulation of acetaldehyde and release of histamine [8]. *ADH1B*, *ALDH2* and *ADH4* influence alcohol consumption and have been implicated as risk factors for developing alcohol abuse or dependence [9-11].

The positive reinforcing effects of alcohol are mediated through the corticomesolimbic dopaminergic reward pathway, which extends from the ventral tegmental area to the nucleus accumbens and is modulated by a wide range of neurotransmitters. This pathway is indirectly activated by alcohol through the release of other neurotransmitters, including acetylcholine, dopamine, glutamate, gamma-aminobutyric acid (GABA), opioids and serotonin. Several candidate genes in neurotransmitter pathways associated with the ventral tegmental area and nucleus accumbens have been associated with alcohol dependence, including the genes encoding cholinergic receptor, muscarinic 2 (*CHRM2*) [12]; cholinergic receptor, nicotinic, alpha 5 (*CHRNA5*) [13]; catechol-*O*-methyltransferase (*COMT*) [9]; GABA A receptor, alpha 2 (*GABRA2*) [14]; glutamate receptor, metabotropic 8 (*GRM8*) [15]; solute carrier family 6 (neurotransmitter transporter, serotonin), member 4 (*5-HTT*) [16]; nuclear factor of kappa light polypeptide gene enhancer in B cells 1 (*NFKB1*) [17]; monoamine oxidase A (*MAOA*) [18]; neuropeptide Y receptor Y2 (*NPY2R*) [19]; opioid receptor, kappa 1 (*OPRK1*) [20]; opioid receptor, mu 1 (*OPRM1*) [21]; prodynorphin (*PDYN*) [20]; and tachykinin receptor 3 (*TACR3*) [22].

More recently, several genome-wide association studies (GWASs) using 500,000 to 1 million SNPs spanning the entire genome have provided unbiased screens for variants affecting alcohol-related behaviors [23-32] (Table 1; Additional file 1). Many of these studies have used samples from large consortia, such as the Collaborative Studies of Genetics of Alcoholism (COGA), the Study of Addiction: Genetics and Environment (SAGE) and the Australian Twin Registry. Consistent with GWAS for other traits [33], many novel loci have been implicated in alcohol dependence and alcohol consumption, but these loci have small effects and are thus difficult to detect with the available sample sizes, especially given the high significance threshold required to control for multiple tests. In one GWAS study, the gene encoding ACN9 homolog (*ACN9*), which is involved in gluconeogenesis and required for the assimilation of ethanol or

Table 1. Candidate genes for alcohol-related phenotypes replicated in several GWAS and transcriptional profiling studies

Protein (gene symbol)	Gene function[a]	Type of study	Phenotype(s) and reference(s)
Cadherin 13 (*CDH13*)	Calcium-dependent cell adhesion protein	GWAS	Alcohol dependence [27,28,31]; polysubstance abuse [29]
Rho GTPase activating protein 28 (*ARHGAP28*)	GTPase activator for the Rho-type GTPases	GWAS	Alcohol dependence [25,27]; alcohol consumption [23]
CUB and Sushi multiple domains 1 (*CSMD1*)	Potential suppressor of squamous cell carcinomas	GWAS	Alcohol dependence [25,27]; polysubstance abuse [29]
CUB and Sushi multiple domains 2 (*CSMD2*)	Potential suppressor of squamous cell carcinomas	GWAS	Alcohol dependence [25,27]; polysubstance abuse [29]
Catenin, delta 2 (*CTNND2*)	Transcriptional activator; may be involved in neuronal cell adhesion and tissue morphogenesis and integrity by regulating adhesion molecules	GWAS	Polysubstance abuse [29]; alcohol dependence [25]; alcohol consumption [23]
Kv channel interacting protein 1 (*KCNIP1*)	Modulates channel's density, inactivation kinetics, and rate of recovery from inactivation in a calcium-dependent and isoform-specific manner	GWAS	Alcohol dependence [25,26]; alcohol consumption [23]
Neuronal PAS domain protein 3 (*NPAS3*)	Encodes a member of the basic helix-loop-helix and PAS domain-containing family of transcription factors; may play a role in neurogenesis	GWAS	Polysubstance abuse [29]; alcohol dependence [25]; alcohol consumption [23]
Protein tyrosine phosphatase, receptor type, D (*PTPRD*)	The protein is a member of the protein tyrosine phosphatase family, members of which regulate a variety of cellular processes	GWAS	Polysubstance abuse [29]; alcohol dependence [25]; alcohol consumption [23]
Usher syndrome 2A (*USH2A*)	The protein contains laminin EGF motifs, a pentaxin domain, and many fibronectin type III motifs; involved in hearing and vision	GWAS	Alcohol dependence [26,27,32]
Angiotensinogen (*AGT*)	Essential component of the renin-angiotensin system, a potent regulator of blood pressure, body fluid, and electrolyte homeostasis	Microarray[b]	Frontal cortex [38,39]; prefrontal cortex [41]
Lysosomal-associated membrane protein 2 (*LAMP2*)	Member of a family of membrane glycoproteins; implicated in tumor cell metastasis	Microarray[b]	Temporal cortex [40]; frontal cortex and nucleus accumbens [36]; frontal cortex [38]
Peripheral myelin protein 22 (*PMP22*)	Integral membrane protein that is a major component of myelin in the peripheral nervous system; might be involved in growth regulation, and in myelinization	Microarray[b]	Prefrontal cortex [41]; frontal cortex and nucleus accumbens [36]
Selenoprotein P, plasma, 1 (*SEPP1*)	Might be responsible for some of the extracellular antioxidant defense properties of selenium or might be involved in the transport of selenium	Microarray[b]	Frontal cortex [39]; temporal cortex [40]; prefrontal cortex [41]
Transferrin (*TF*)	Responsible for the transport of iron from sites of absorption and heme degradation to those of storage and utilization	Microarray[b]	Frontal cortex [38,39]; frontal, motor cortices [37]

[a]Obtained from GeneCards® [103]; [b]Samples were obtained from postmortem human brains from alcoholics versus control.
GWAS, genome-wide association study.

acetate into carbohydrate [34], has been associated with susceptibility to alcohol dependence [35]. Two SNPs in the 3' flanking region of the gene encoding peroxisomal trans-2-enoyl-CoA reductase, which is a key enzyme for the peroxisomal fatty acid chain elongation pathway, achieved genome-wide significance for alcohol dependence in a study of German males [31]. A GWAS on pooled DNA samples from individuals with a lifetime history of alcohol dependence, nicotine dependence and co-morbid alcohol/nicotine dependence in an Australian population [28] identified three SNPs that reached genome-wide significance for co-morbid alcohol/nicotine dependence. The implicated genes were: (1) the gene encoding MAP/microtubule affinity-regulating kinase 1 (*MARK1*), which is a kinase involved in the phosphorylation of microtubule-associated proteins; (2) the gene encoding DEAD box polypeptide 6 (*DDX6*), which is a putative RNA helicase; and (3) *KIAA1409*, which encodes a component of the NALCN Na+ channel complex. A family-based association analysis for alcohol dependence that utilized both COGA and the Australian twin-family samples implicated the genes encoding endothelin receptor type B (*EDNRB*), Usher syndrome 2A (*USH2A*), TCDD-inducible poly(ADP-ribose) polymerase (*TIPARP*), monoamine oxidase A, Na+/K+ transporting ATPase interacting 2 (*NKAIN2*), and Down syndrome cell adhesion

molecule like 1 (*DSCAML1*) [32], with four SNPs in *DSCAML1* reaching genome-wide significance. A GWAS for alcohol consumption in Korean male drinkers [23] identified 12 SNPs in six genes (chromosome 12 ORF 51 (*C12orf51*), and the genes encoding coiled-coil domain containing 63 (*CCDC63*), myosin, light chain 2 (*MYL2*), 2'-5'-oligoadenylate synthetase 3 (*OAS3*), cut-like homeobox 2 (*CUX2*), and rabphilin 3A homolog (*RPH3A*)) on chromosome 12q24 associated with alcohol consumption at a genome-wide significance level. In contrast, two studies on alcohol dependence [25,26], including one of the largest GWASs to date, with over 10,000 individuals from the Australian Twin Registry, failed to identify any SNPs with genome-wide significance. A GWAS meta-analysis of approximately 2.5 million SNPs with alcohol consumption among 12 population-based samples of European ancestry, comprising more than 20,000 individuals [30], identified a single SNP, rs6943555, in the gene for autism susceptibility candidate 2 (*AUTS2*) associated with alcohol consumption at a genome-wide significance level.

Considering only SNPs in genes that achieve genome-wide significance reveals no overlap across the studies, with the exception of the large effects contributed by variation at *ADH1B* and *ALDH2* in Asian populations. Among all SNPs that were significant at a nominal *P*-value in the studies described above, the gene encoding cadherin 13 (*CDH13*) was replicated in four independent studies, and eight genes were common across any three studies (Table 1). In addition, five differentially expressed genes in different areas of postmortem human brains of alcoholics were replicated in any of three transcriptional profiling studies (Table 1) [36-41].

The lack of concordance across GWASs could be partially due to different measures of alcohol consumption used in different study populations or even across different samples from the same population. Common measures of alcohol consumption are frequency of drinking (weekly and annually), quantity by frequency, maximum drinks in a 24 hour period, frequency of heavy drinking and frequency of intoxication [5]; if these measures are not perfectly correlated, they will be associated with different SNPs. Association studies in humans are limited in resolution by the structure of LD; to the extent that LD varies among populations, different genes may be implicated in different studies. Moreover, rare alleles that contribute to variation in alcohol consumption are essentially blind to detection by association studies using common variants, and many SNPs with small effects may contribute to risk for alcohol dependence.

In summary, GWASs have been limited by difficulties in quantifying alcohol-related phenotypes and in obtaining large sample sizes, together with co-morbidity of alcoholism with other behavioral and neuropsychiatric disorders, gender effects and population admixture. Furthermore, the diversity of mechanisms of vulnerability and resilience to alcohol pose challenges for human genetic studies on alcoholism or alcohol consumption. It has become increasingly clear that, in addition to a few common alleles, many different rare alleles may contribute to vulnerability in different populations.

One strategy that circumvents the limitations of human GWASs relies on comparisons with genes associated with ethanol-related behaviors in genetically amenable model organisms.

Animal models

Given the evolutionary conservation of genes and pathways affecting key biological processes between vertebrates, invertebrates and humans, studies on model organisms (rats, mice, flies and nematodes) have played an important role in identifying potential candidate genes that contribute to alcohol intoxication. Invertebrate and vertebrate models show similar symptoms of alcohol intoxication, including loss of postural control, sedation, immobility and development of tolerance. After alcohol intoxication, mice and rats increase their alcohol consumption, develop tolerance and even alcohol dependence. *Drosophila* develops tolerance after a single exposure to ethanol [42]. In addition to rapid tolerance, flies develop chronic tolerance after prolonged exposure to a low concentration of ethanol [43]. *Caenorhabditis elegans* also exhibits tolerance after continuous ethanol exposure [44] and develops ethanol preference as a result of prolonged pre-exposure [45].

In addition to the behavioral similarities between invertebrate and mammalian models, invertebrates use similar neurotransmitter systems, neuropeptides, synaptic proteins, channels and signaling processes to mediate ethanol-induced behaviors [46]. These include genes encoding Ca^{2+}-sensitive adenylate cyclase and protein kinase A [47-49], BK channels [50-52], Homer [53,54], genes encoding proteins involved in GABA neurotransmission [55,56], the gene encoding protein kinase C [57,58], and genes encoding proteins involved in dopamine and serotonin signaling [45,59,60]. In vertebrates, neuropeptide Y (NPY) signaling plays a role in alcohol intake and dependence [61,62]. Invertebrates have an ortholog to NPY, neuropeptide F (NPF), and signaling via NPF also influences ethanol-related behaviors [44,63].

Rats meet all the criteria for animal models of alcoholism, including: the ability to orally self-administer ethanol; elevation of blood ethanol concentration after alcohol consumption; willingness to work for ethanol access; development of functional tolerance; and, after a deprivation period, relapse-like behavior [64]. A study on recombinant inbred rat strains identified several genomic regions on chromosomes 1, 6 and 12 that harbor

candidate genes for alcohol consumption, including the genes encoding actin filament associated protein, cholecystokinin 2 receptor, melanocortin 4 receptor; protein tyrosine phosphatase receptor type E and tubulin B6 [65]. Furthermore, several microarray studies have identified differential expression of genes between alcohol-preferring and alcohol-non-preferring rat strains, including the genes encoding alpha-adducin (*Add1*), retinal dehydrogenase 1 (*Aldh1a1*), adenylate cyclase type 3 (*Adcy3*), alpha-crystallin B chain (*Cryab*), glutamate decarboxylase 1 (*Gad1*) and NPY (*Npy*) [66,67]. However, most studies on the genetic underpinnings of alcohol-related phenotypes have focused on mice, because, in contrast to rats, they can be more easily genetically manipulated. Mice are amenable to complete elimination of the gene of interest, gene silencing by RNA interference (RNAi), overexpression and mutagenic technologies [68]. Numerous genetic models have been developed to investigate specific aspects of alcoholism in mice, including tolerance, withdrawal, motivational effects and high-dose sensitivity [68]. Over 90% of the mouse and human genomes can be partitioned into regions of synteny [69].

Among invertebrate models *Drosophila* is advantageous because large numbers of genetically identical individuals can be reared at relatively low cost and without regulatory restrictions, and many community resources are available for sophisticated genetic manipulations. *Drosophila* is also readily amenable to neuroanatomical studies.

C. elegans presents a useful model system for examining the effects of ethanol on development [70]. The lineage of each of its 302 neurons and their chemical synapses has been characterized. Nematodes have a short (approximately 3 days) reproductive cycle, enabling large-scale mutagenesis screens within a relatively short time, and they can be cryopreserved.

Mouse models

Studies on mice have identified more than 80 genes that affect alcohol preference drinking [59]. Pioneering work by Buck and colleagues identified three genomic regions on mouse chromosomes 1, 4 and 11 that influence acute alcohol withdrawal [71]. Through a succession of studies involving F_2 intercrosses, construction of recombinant inbred lines, and interval-specific congenic strains [71-73], the gene encoding multiple PDZ domain protein (*Mpdz*) was identified as a quantitative trait gene for alcohol withdrawal symptoms. In humans, MPDZ does not demonstrate an association with alcohol-induced withdrawal seizures, but haplotype and single-SNP association analyses suggest a possible association with alcohol dependence [74] and alcohol consumption [65]. Another QTL on chromosome 1 was mapped to a 0.44 Mb interval containing 15 candidate genes, including *Kcnj9*. *Kcnj9* encodes GIRK3, a subunit member of a family of G-protein-dependent inwardly rectifying K^+ channels that mediate postsynaptic inhibitory effects of $G_{i/o}$-coupled receptors [75]. *Kcnj9*-null mutant mice show reduced withdrawal from pentobarbital, zolpidem and ethanol [76].

QTLs for alcohol consumption and acute and chronic alcohol withdrawal on distal mouse chromosome 1 [71,73] are syntenic to a region on human chromosome 1q where several studies have identified QTLs for alcohol-related phenotypes [69,77,78]. The gene encoding 5-hydroxytryptamine receptor 1B (*Htr1b*) is located in this region, and mice in which this gene was knocked out were more aggressive and drank more alcohol, although the effects on alcohol consumption were influenced by unknown environmental factors in different laboratories [79]. In humans, *HTR1B* was subsequently associated with 'antisocial alcoholism (the dual diagnosis of alcoholism and antisocial personality disorder)' in two populations [80]. A QTL for severity of alcohol dependence and withdrawal on human chromosome 15 was identified in two human studies [77,81] and is syntenic with a region on mouse chromosome 9, where QTLs for alcohol preference have also been mapped [82,83]. Significant concordance between allelic variants of human GWASs and orthologous genes associated with alcohol-related phenotypes in mice [84,85] further demonstrate that mapping genes that underlie alcohol-related behaviors in mice is useful for identifying genes that govern alcohol-related phenotypes in people.

The *Drosophila* model: single gene mutations

Despite differences between the fly brain and the mammalian brain, *Drosophila* has been a valuable model system for studies on the genetics of alcohol sensitivity. When exposed to alcohol vapors, flies initially become hyperactive, but ultimately lose postural control; alcohol knockdown time provides a measure of sensitivity. Studies on flies have employed two complementary strategies: single mutant analyses and systems genetics approaches. A *P*-element mutagenesis screen for alcohol sensitivity revealed that a large fraction (approximately 30%) of the genome can contribute to alcohol sensitivity [86]. Indeed, most of the mutations that affect alcohol sensitivity in *Drosophila* have pleiotropic effects on other complex traits. The first mutants implicated the cyclic AMP signaling pathway, including: the *cheapdate* allele of *amnesiac* [49], which encodes a neuropeptide that activates the cyclic AMP signaling pathway [87]; the Ca^{2+}/calmodulin-dependent adenylate cyclase encoded by the *rutabaga* gene [49]; and *PkaR2*, which encodes a cyclic AMP-dependent protein kinase [47]. In addition, mutants affecting axonal migration, neural cell adhesion and neurotransmission have also been implicated in alcohol sensitivity, including the gene encoding the

axonal migration and cell adhesion receptor fasciclin II [88], the gene encoding GABA-B receptor 1 [56], and genes encoding NPF and its receptor [63]. Others include *slowpoke*, which encodes a large-conductance Ca^{2+}-activated K^+ channel, and *arouser*, which encodes a predicted adaptor protein homologous to the mammalian epidermal growth factor receptor substrate 8 (*Eps8*) family. Mutations in *slowpoke* prevent the development of tolerance [50]. Mutations in the gene encoding arouser (*aru*) result in increased ethanol sensitivity. The *aru* gene product interacts with the epidermal growth factor/ extracellular signal-regulated kinase and the phospoinositide 3-kinase/Akt pathways to regulate ethanol sensitivity [89].

Several transcription factors have been implicated in alcohol sensitivity and/or induction of tolerance in flies. The *hangover* gene encodes a transcription factor that contributes to the induction of alcohol tolerance [90]. Similarly, *dLmo/Beadex*, which encodes a transcriptional regulator, contributes to behavioral responses to ethanol [91]. The mouse ortholog gene encoding LIM domain only 3 (*Lmo3*) also affects alcohol sensitivity; reduced *Lmo3* expression correlates with increased sedation time and reduces voluntary consumption of ethanol [91].

The *Drosophila* model: genetic networks

While single gene approaches have advanced our understanding of how specific genes may influence responses to ethanol, it is becoming increasingly clear that a comprehensive understanding of the genetic architecture of alcohol sensitivity requires studies at the level of genetic networks. Such networks can be constructed based on covariance of transcript levels associated with alcohol sensitivity among different genotypes [92], or computational predictions based on genome-wide co-regulation of transcripts followed by experimental verification [86].

Morozova *et al.* [92] constructed modules of correlated transcripts associated with alcohol sensitivity and induction of tolerance; these models were validated by transposon-mediated disruption of focal genes. A second approach built computational networks of covariant transcripts around genes that affect sensitivity or resistance to alcohol exposure identified by *P*-element mutations [86]. Subsequent RNAi-mediated inhibition of genes connected to the focal genes in the networks confirmed their effects on alcohol-related phenotypes. Those genes could, in turn, serve as focal genes to grow the computational networks by iteration, allowing a stepwise expansion of the network with simultaneous functional validation.

From model organisms to human genetics

Evolutionary conservation of pathways offers opportunities for comparative cross-species analyses (Table 2).

For example, *AUTS2* was identified in human GWASs for alcohol consumption and verified by genotype-specific expression in human prefrontal cortex samples. Differences in expression of *Auts2* were also observed in whole-brain extracts of mice selected for differences in voluntary alcohol consumption, and downregulation of an *Auts2* homolog was causally associated with reduced alcohol sensitivity in *Drosophila*. Thus, evidence for the involvement of *AUTS2* in alcohol drinking or sensitivity is corroborated across three different species [30].

Because at least 60% of *Drosophila* genes have conserved human orthologs, the latter can be identified and superimposed on computationally predicted networks from *Drosophila*. This allows identification of candidate genes for subsequent human association studies based on a previous unbiased genome-wide approach in *Drosophila*. Not only can this strategy empower human association analysis by reducing the prohibitive multiple testing correction of a GWAS, but it provides also functional contexts to the candidate genes as they form part of defined networks.

To provide proof of principle for the potential of this translational approach, the human ortholog of the *Drosophila Men* gene, which encodes malic enzyme, was targeted as a candidate gene based on artificial selection, mutational and transcriptional profiling studies [86,93,94]. The gene encoding malic enzyme is also differentially expressed in mice upon acute alcohol treatment [95]. Malic enzyme represents a metabolic switch, converting malate into pyruvate while generating NADPH, an essential co-factor for fatty acid biosynthesis (Figure 2). Thus, the malic enzyme reaction enables the development of alcohol-induced fatty liver syndrome. Association studies on the Framingham Offspring cohort showed that intronic SNPs of the gene encoding malic enzyme 1 (*ME1*) were associated with amount of cocktail drinking, indicating that variation in expression of cytoplasmic malic enzyme contributes to variation in alcohol consumption. Thus, translational approaches from model organisms to humans can identify SNPs that are associated with drinking behavior, with an effect size that could not have been resolved with large-scale unbiased GWASs [92].
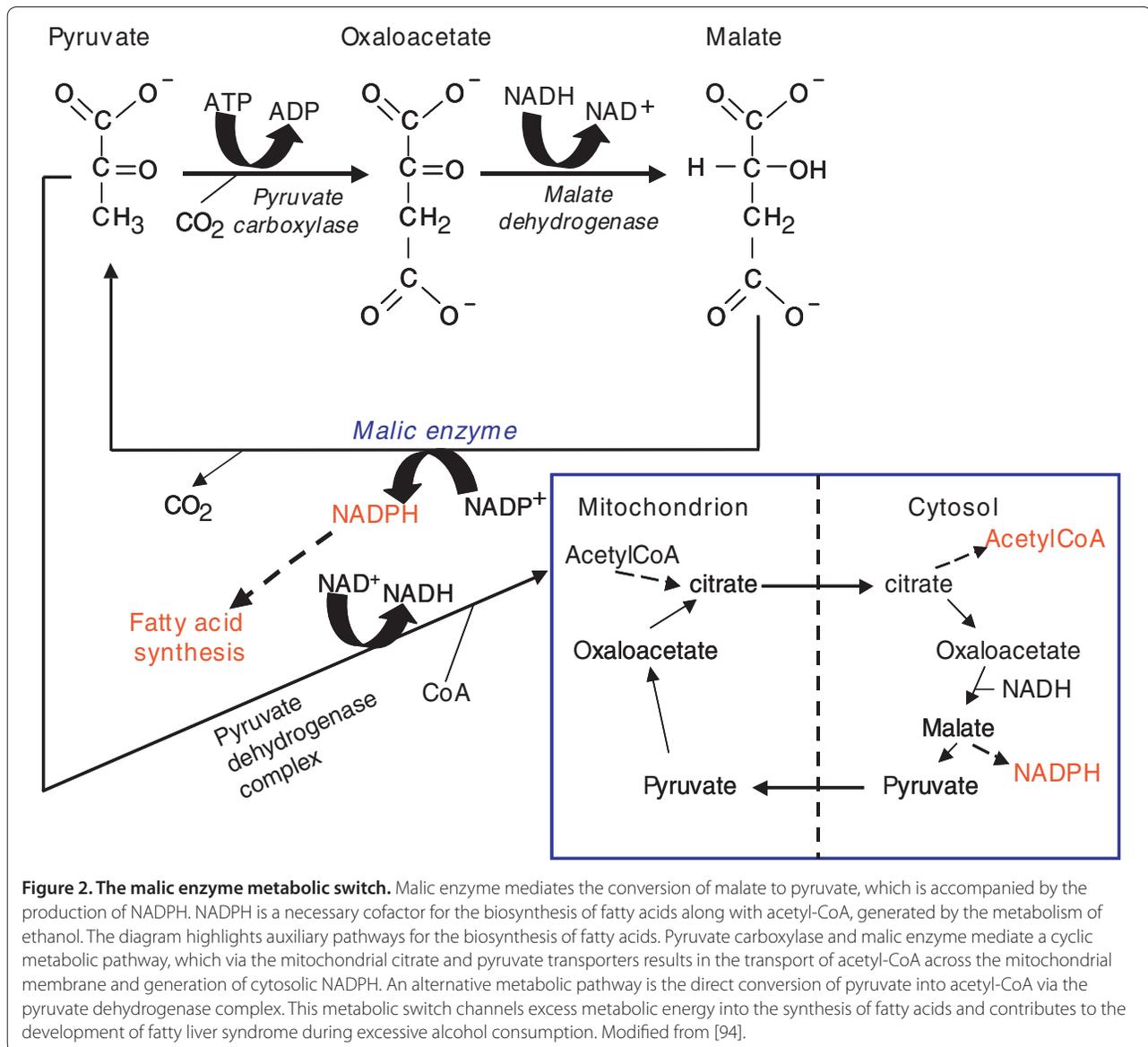
Concluding remarks

During the past decade a wealth of information on alcohol consumption has been obtained from human and model organism studies, but rarely have data from different studies been integrated to form a comprehensive blueprint of the genetic networks that contribute to alcohol drinking. In future, studies integrating data on alcohol-related phenotypes from GWASs and transcriptional profiling studies on both humans and model organisms will make it possible to construct biologically

Table 2. Orthologous candidate genes associated with human alcohol phenotypes, and their differential expression in three model organisms

Protein (gene symbol)	Human		Fly		Mouse		Rat	
	Phenotype and reference(s)	Gene function[a]	Gene symbol	Phenotype and reference(s)	Gene symbol	Phenotype and reference(s)	Gene symbol	Phenotype and reference(s)
Adducin 1 (ADD1)	Alcohol dependence [40]	Membrane–cytoskeleton-associated protein that promotes the assembly of the spectrin-actin network	hts	Ethanol knockdown [94]	Add1	Alcohol consumption [96]	Add1	Alcohol consumption [97]
Adenylate cyclase 3 (ADCY3)	Alcohol dependence [25,31]	Mediates odorant detection via modulation of intracellular cAMP concentration	Ac3	Ethanol knockdown [98]	Adcy3	Alcohol consumption [96]	Adcy3	Alcohol consumption [66]
Aldehyde dehydrogenase 1 family, member A1 (ALDH1A1)	Alcohol consumption [99]	Belongs to the aldehyde dehydrogenase family; binds free retinal and cellular retinol-binding protein-bound retinal	CG31075	Ethanol knockdown [60,93]	Aldh1a1	Alcohol consumption [100]	Aldh1a1	Alcohol consumption [97]
ATP-binding cassette, sub-family B, member 1 (ABCB1)	Alcohol dependence [25]	Member of the superfamily of ATP-binding cassette (ABC) transporters; energy-dependent efflux pump responsible for decreased drug accumulation in multidrug-resistant cells	Mdr50	Ethanol knockdown [93]	Abcb1b	Alcohol consumption [96]	Abcb1a	Alcohol consumption [66]
Crystallin, alpha B (CRYAB)	Alcohol dependence [41]	Member of the small heat-shock protein family; may contribute to the transparency and refractive index of the lens; expressed widely in many tissues and organs	l(2)efl	Ethanol knockdown [60,93,94]	Cryab	Alcohol consumption [96,101]	Cryab	Alcohol consumption [67]
Down syndrome cell adhesion molecule like 1 (DSCAML1)	Alcohol dependence [25]	Cell adhesion molecule that plays a role in neuronal self-avoidance	CG42256	Ethanol knockdown [93]	Dscaml1	Alcohol consumption [96]	Dscam	Alcohol consumption [66]
Glutamate decarboxylase 1 (GAD1)	Alcohol consumption [65]	The protein is one of several forms of glutamic acid decarboxylase, identified as a major autoantigen in insulin-dependent diabetes; catalyzes the production of GABA	Gad1	Ethanol knockdown [93]	Gad1	Alcohol consumption [96,101]	Gad1	Alcohol consumption [66,97]
Protein disulfide isomerase family A, member 3 (PDIA3)	Alcohol dependence [40]	A protein of the endoplasmic reticulum that interacts with lectin chaperones calreticulin and calnexin to modulate folding of newly synthesized glycoproteins	ERp60	Ethanol knockdown [93]	Pdia3	Acute ethanol injections [102]	Pdia3	Alcohol consumption [97]
Protein kinase C, alpha (PRKCA)	Alcohol dependence [25,31]	The protein is a calcium-activated, phospholipid-dependent, serine- and threonine-specific enzyme; plays a role in many different cellular processes	inaC	Ethanol knockdown [92,93]	Prkca	Alcohol consumption [96]	Prkca	Alcohol consumption [66]
Solute carrier family 2, member 14 (SLC2A14)	Alcohol dependence [25]	Member of the glucose transporter (GLUT) family that facilitates glucose transport	Glut1	Ethanol knockdown [93]	Slc2a3	Alcohol consumption [101]	Slc2a3	Alcohol consumption [66]
Tachykinin receptor 3 (TACR3)	Alcohol dependence [22]	Belongs to a family of proteins that function as receptors for tachykinins; associated with G proteins that activate a phosphatidylinositol-calcium second messenger system	Takr99D	Ethanol knockdown [93]	Tacr3	Alcohol consumption [96]	Tacr3	Alcohol consumption [66]

[a]Obtained from GeneCards® [103].
GABA, gamma-aminobutyric acid.

Figure 2. The malic enzyme metabolic switch. Malic enzyme mediates the conversion of malate to pyruvate, which is accompanied by the production of NADPH. NADPH is a necessary cofactor for the biosynthesis of fatty acids along with acetyl-CoA, generated by the metabolism of ethanol. The diagram highlights auxiliary pathways for the biosynthesis of fatty acids. Pyruvate carboxylase and malic enzyme mediate a cyclic metabolic pathway, which via the mitochondrial citrate and pyruvate transporters results in the transport of acetyl-CoA across the mitochondrial membrane and generation of cytosolic NADPH. An alternative metabolic pathway is the direct conversion of pyruvate into acetyl-CoA via the pyruvate dehydrogenase complex. This metabolic switch channels excess metabolic energy into the synthesis of fatty acids and contributes to the development of fatty liver syndrome during excessive alcohol consumption. Modified from [94].

meaningful networks of genes that contribute to alcohol consumption and dependence, and generate a deeper understanding of the genetic susceptibility for alcoholism.

Additional file

Additional file 1. Candidate genes at nominal *P*-value for alcohol dependence in human genome-wide association studies.

Abbreviations

ADH, alcohol dehydrogenase; ALDH, aldehyde dehydrogenase; COGA, Collaborative Studies of Genetics of Alcoholism; GABA, gamma-aminobutyric acid; GWAS, genome-wide association study; LD, linkage disequilibrium; Mb, megabase; NPF, neuropeptide F; NPY, neuropeptide Y; QTL, quantitative trait locus; RNAi, RNA interference; SNP, single nucleotide polymorphism.

Competing interests

The authors declare that they have no competing interests.

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