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Genetic approaches to addiction: genes and alcohol

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Abstract

Aims—Alcoholism is a chronic relapsing disorder with an enormous societal impact. Understanding the genetic basis of alcoholism is crucial to characterize individuals' risk and to develop efficacious prevention and treatment strategies.

Methods—We examined the available scientific literature to provide an overview of different approaches that are being integrated increasingly to advance our knowledge of the genetic bases of alcoholism. Examples of genes that have been shown to influence vulnerability to alcoholism and related phenotypes are also discussed.

Results—Genetic factors account for more than 50% of the variance in alcoholism liability. Susceptibility loci for alcoholism include both alcohol-specific genes acting either at the pharmacokinetic or pharmacodynamic levels, as well as loci moderating neuronal pathways such as reward, behavioral control and stress resiliency, that are involved in several psychiatric diseases. In recent years, major progress in gene identification has occurred using intermediate phenotypes such as task-related brain activation that confer the advantage of increased power and the opportunity of exploring the neuronal mechanisms through which genetic variation is translated into behavior. Fundamental to the detection of gene effects is also the understanding of the interplay between genes as well as genes/environment interactions. Whole Genome Association studies represent a unique opportunity to identify alcohol-related loci in hypothesis-free fashion. Finally, genome-wide analyses of transcripts and chromatin remodeling promise an increase in our understanding of the genome function and of the mechanisms through which gene and environment cause diseases.

Conclusions—Although the genetic bases of alcoholism remain largely unknown, there are reasons to think that more genes will be discovered in the future. Multiple and complementary approaches will be required to piece together the mosaic of causation.

Keywords

Alcoholism; GABA; HTT; intermediate phenotypes; MAOA; review; WGA

INTRODUCTION

Alcohol use disorders (AUD), including dependence and abuse, are common and etiologically complex diseases with a 12-month prevalence of 8.6% in the United States [1]. Alcohol is the most widely used addictive drug world-wide. According to the World Health Organization (WHO), there are 2 billion alcohol users (http://www.who.int/substance_abuse/facts/global_burden/en/). The impact on public health in terms of mortality and morbidity [2], and in terms of societal cost, is enormous (http://www.drugabuse.gov/about/welcome/aboutdrugabuse/magnitude). Understanding the genetic basic of alcoholism is a crucial step for the development of efficient prevention

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strategies and personalized treatments. For this purpose, it is important to identify genes predisposing individuals to alcoholism, genes moderating consequences of alcohol exposure, clinical course and treatment response, mechanisms through which genes exert their effects on behavior and interactions of genes with other genes and with environmental factors.

Although it is clearly known that genetic factors play a role in alcoholism, identification of the specific genes involved has proved challenging. Alcoholism is genetically complex, without a clear pattern of Mendelian inheritance. Major determinants of complexity are likely to include genetic heterogeneity (see Glossary at the end of this paper), heterogeneity at the level of neurobiological vulnerability, polygenicity, phenocopies, gene × environment interaction and incomplete penetrance. In recent years, the collection of large family-based and case–control samples informative for linkage and association, the more refined characterization of phenotypes, the use of statistical methods that take into account the multi-factorial etiology of alcoholism and advances in molecular genetic technologies have overcome many of the limits of early genetic studies. Consequently, several genetic loci that moderate vulnerability to alcoholism have been identified. At the phenotype level, major progress has been made through the use of intermediate phenotypes. In this regard, neuroimaging techniques, such as magnetic resonance, allow for a direct access to neuronal structure and activity in living humans and have been crucial to increase power to identify the effects of genetic variants in risk as well as to provide information on the neurobiological mechanisms through which genes exert their effects on behavior. As mentioned, alcoholism is a multi-factorial disease and several genes, each of small effect, as well as environmental variables, are likely to be involved. As will be discussed, in some instances common functional alleles of small effect have been identified, and in other cases uncommon alleles of strong effect are also known. From what is known, and from the large part of the genetic variance in risk that is still unexplained by genes identified so far, it is clear that methods are needed to identify additional loci whose individual influence is small at the population level. Our ability to detect gene effects is dependent upon the context in which their effects are measured, and it is becoming clear that we cannot ignore that genes act within a complex network that includes other genes, environmental variables and developmental timing. Finally, impressive advances have occurred in molecular genetic technologies. Although it is yet not technically feasible to re-sequence every base or genotype every known polymorphic site, technologies now available enable genome-wide searches for disease-causing genes with the linkage disequilibrium approach and genome-wide studies of gene expression and chromatin modifications that reflect the epigenetic response of the genome to environmental exposures, including the interactions of gene variations with those exposures.

This paper provides an overview of different approaches that are being integrated increasingly to advance our knowledge of the genetic bases of alcoholism. Examples of genes that alter risk for alcoholism and related phenotypes and treatment response are also discussed.

BEHAVIOUR GENETIC STUDIES

Heritability of alcoholism

Family, adoption and twin studies have clearly demonstrated that genetic factors are important in moderating vulnerability to alcoholism. Based on analyses of large, well-characterized cohorts of twins including nearly 10 000 twin pairs, alcoholism is a moderately to highly heritable psychiatric disease, with a heritability of more than 0.5 [3]. For comparison, heritability of generalized anxiety disorder has been estimated to be 0.32 [4] and that of autism has been estimated to be 0.9 [5]. Although early studies suggested that alcohol dependence was more heritable in men compared to women, more recent twin studies, based on larger sample sizes, have shown that genes contribute to vulnerability to alcohol dependence about equally in both sexes [6].

Shared versus unshared etiology

Alcoholism coexists frequently with other addictions, including illicit substance abuse and nicotine dependence more often than would be expected by chance [7]. Such comorbidity between disorders can indicate the existence of etiological factors that are shared (co-causation), but can also reflect inter-causation. Behavior genetic studies can establish the origins of comorbidity and evaluate the extent to which liability to different diseases is shared or unshared. This is conducted by looking at the cross-inheritance of different diseases within families and in twin pairs. Twin and family studies on alcoholism have revealed that genetic factors acting on alcoholism include both alcohol-specific genetic factors as well as genetic factors that are shared with other addictions (for review see Goldman & Bergen) [8]. For example, results from several twin studies [9-13] have detected consistently a considerable overlap in the genetic liability to alcoholism and nicotine dependence, particularly in individuals who drink or smoke heavily. Rates of smoking are declining; however, studies reported during the past 20 years have indicated that as many as 80% of alcohol-dependent individuals are heavy smokers [14,15]. Approximately 50% of the genetic vulnerability to nicotine dependence is shared with alcoholism, whereas 15% of the genetic vulnerability to alcoholism is shared with nicotine dependence [9].

Besides other addictions, alcoholism also coexists frequently with other psychiatric diseases, including both internalizing disorders (e.g. depression and anxiety) as well as externalizing disorders [e.g. antisocial personality disorder (ASPD), conduct disorder (CD) and attention deficit hyperactivity disorder (ADHD)] [1,7,16]. Twin studies reveal consistently the existence of shared genetic influences between alcoholism and externalizing disorders [17-20]. Longitudinal studies have shown that externalizing disorders of childhood such as CD and ADHD are important risk factors for the subsequent development of alcoholism [21]. Evidence from twin studies for shared genetic influences between alcoholism and internalizing disorders are more controversial [18,22,23]. However, longitudinal studies have shown that anxiety disorders such as panic disorder and social phobia predict subsequent alcohol problems in adolescents and young adults [24].

Identification of the specific etiological factors reflecting both the shared and unshared liability to alcoholism requires a redefinition of this disease and a process of simplification and deconstruction of etiology that may be achievable through the use of intermediate phenotypes.

INTERMEDIATE PHENOTYPES

Alcohol abuse and dependence as defined by the Diagnostic and Statistical Manual of Mental Disorders (DSM, American Psychiatric Association) are highly heterogeneous entities. DSM classification is based upon symptom clusters and as such is, to a large extent, an outcome-based classification, not reflecting multiple etiologies of vulnerability. One strategy to discover gene effects in etiologically complex diseases is through deconstruction of complexity into parts that are likely to be less etiologically heterogeneous. Intermediate phenotypes access mediating mechanisms of gene and environmental effects. Heritable intermediate phenotypes that are disease-associated have been termed endophenotypes, and these include biochemical, endocrinological, neuroanatomical, cognitive and neuropsychological parameters [25].

Several intermediate phenotypes have been linked specifically to alcoholism, perhaps reflecting alcohol-specific genetic liability. These include alcohol-induced flushing, which is a protective intermediate phenotype, and low response to the effects of alcohol, which is an endophenotype predictive of risk. The genetic origins of alcohol-induced flushing are discussed later. The level of response to alcohol is believed to reflect mainly pharmacodynamic variation in response [26]. A low response to alcohol predicts increased risk of developing alcohol use disorders [27-29] and has been associated with genetic variation in the serotonin transporter

gene and in the gene encoding the subunit α6 of the γ-aminobutyric acid receptor A (GABRA6) [30].

Other intermediate phenotypes predict diatheses that include alcoholism as well as other psychiatric diseases. Relevant phenotypes in this regard include electrophysiological, psychological, neuroendocrinological and, more recently, neuroimaging phenotypes. Neuroimaging provides access to the neuronal mechanisms underlying emotion, reward and craving and therefore represents an extraordinary tool to link genes to the neuronal pathways that produce behaviors (for review see Meyer-Lindenberg & Weinberger [31]; Xu *et al.* [32]). For example, amygdala activation after exposure to stressful stimuli predicts anxiety and captures inter-individual differences in emotional response and stress resiliency [33]. On the other hand, activation of the pre-frontal cortex during working memory performance is used to evaluate pre-frontal cognitive function which is impaired in several psychiatric diseases.

Several of the most relevant intermediate phenotypes for alcoholism are summarized in Table 1, which also includes the functional loci that have been linked consistently to such intermediate phenotypes.

FINDING GENES FOR ALCOHOLISM

Two main strategies have been used and are integrated increasingly to identify genetic variations influencing alcoholism vulnerability and other alcoholism-related phenomena: the candidate gene approach and the genome-wide linkage approach. In the former, genes known to influence processes involved in the pathogenesis or treatment of alcoholism are selected. In the latter the whole genome is interrogated simultaneously in a hypothesis-free fashion. A point of integration between the methods is the study of candidate genes located in chromosome regions implicated by genome-wide scans.

Candidate genes

Alcoholism is a disease of complex etiology. The process of addiction involves cellular molecular networks in which hundreds of genes are involved. As a result, many candidate genes for alcoholism have been postulated. So far, only a few functional loci moderating vulnerability to alcoholism have been identified. Some of these genes (e.g. catechol-*O*-methyltransferase: *COMT*) act on neurobiological mechanisms such as reward, behavioral control and stress resiliency that are involved in alcoholism as well as in other psychiatric diseases. In contrast, other genes (e.g. alcohol-metabolizing genes) are likely to reflect alcohol-specific liability. The use of intermediate phenotypes and studies taking into account the interaction between genes and the environment have been critical to identify and understand these loci, some of which are discussed below (see also Table 1).

COMT: warriors versus worriers—Different pathways can lead to the same outcome in different individuals. For this reason, alternative alleles at the same locus may increase vulnerability to alcoholism through different effects on brain function and behavior, and these risk effects may even be opposing. COMT is an enzyme that metabolizes dopamine (DA), norepinephrine (NE) and other catecholamines. COMT plays an important role in the regulation of dopamine levels in the pre-frontal cortex because of the paucity of dopamine transporter in this region [34,35]. *COMT* knock-out mice have increased levels of dopamine dramatically in the pre-frontal cortex [36,37]. Val158Met is a common functional polymorphism located in the coding sequence of the human *COMT* gene in which the amino acid at codon 158 can be either valine (Val) or methionine (Met). This missense variant alters enzyme activity by affecting enzyme stability [38,39]. The Val158 allele is three- to fourfold more active than Met158 [40] and the alleles act co-dominantly [41]. Therefore, Val158 is predicted to lower dopamine level in the frontal cortex. In line with this idea, the Val158 allele

has been linked to inefficient frontal lobe function evaluated with different methodologies [42-44]. On the other hand, the Met158 allele, although associated with better cognitive performance, is associated with decreased stress resiliency and increased anxiety. This allele has been associated with increased anxiety among women in two independent populations [45], with an increased pain–stress response and a lower pain threshold [46,47], and increased amygdala reactivity to unpleasant stimuli [48]. From an evolutionary perspective, balanced selection both against and for the Met and Val alleles has probably maintained these alleles across populations and generations. A heuristic for the phenomenon of maintenance on the basis of counterbalancing selection for stress resiliency (Val) and cognition (Met) is the 'warrior' (Val158) versus 'worrier' (Met158) model (Fig. 1) [49]. In certain addicted populations, for example polysubstance abusers [50], Val158 is associated with addiction, probably through the externalizing domain. On the other hand, in addicted populations with high frequencies of internalizing disorders such as late-onset alcoholics in Finland [51] and Finnish social drinkers [52], increased risk appears to be conferred by the Met158 allele.

Alcohol metabolizing enzymes: ALDH2 and ADH1B—In contrast to the complex and not completely understood effects of alcohol on the central nervous system, the hepatic metabolism of alcoholism is well understood and the most convincing examples of alcoholism-related genes are the alcohol dehydrogenase IB (*ADH1B*) and aldehyde dehydrogenase 2 (*ALDH2*) genes that encode for two enzymes catalyzing consecutive steps in alcohol degradation. ADH metabolizes ethanol to acetaldehyde, a toxic intermediate, which is then metabolized to acetate by ALDH. The most important functional loci at these genes are the His47Arg polymorphism in the *ADH1B* gene, where Arg47 is a superactive variant acting in co-dominant fashion, and the *ALDH2* Glu487Lys, in which the Lys487 allele inactivates ALDH2 dominantly. Either higher activity of ADH1B or lower activity of ALDH2 lead to accumulation of acetaldehyde following alcohol assumption which, in turn, causes an unpleasant and aversive response, termed the flushing reaction. The flushing reaction is characterized by facial flashing, headache, hypotension, palpitations, tachycardia, nausea and vomiting. Interestingly, the genotype-determined flushing reaction is equivalent to the effects of disulfiram (a drug used to prevent relapse), and certain antiprotozoal drugs, including metronidazole, that inhibit ALDH. In Chinese and Japanese populations where both His47 and Lys487 are highly abundant, and in Jewish populations where His47 is abundant, many individuals carry genotypes that are protective against the development of alcoholism. Both polymorphisms have been associated with alcohol dependence [53] as well as with risk of developing cancers of the oropharynx and esophagus [54]. The protective effect seems to vary across environments [55] and shows genotype–genotype additivity [56].

Predicting treatment response: OPRM1—Currently available medications for the treatment of alcoholism have limited efficacy. Therefore, the identification of genetic variation predicting treatment response would be useful for improving the clinical management of patients by personalizing treatment. Naltrexone, a μ-opioid receptor antagonist, is one of three pharmacotherapies currently approved for the maintenance of abstinence in alcoholism, the others being acamprosate and disulfiram. Results from clinical trials have shown moderate efficacy of naltrexone and efforts to identify genetic variants that moderate this effect have focused upon the gene encoding the μ-opioid receptor 1 (*OPRM1*). A functional polymorphism within the coding sequence of this gene (Asn40Asp) has been described [57]. In cell culture, μ-opioid receptors encoded by the Asp40 variant bind β-endorphin and activate G-protein-coupled protein potassium ion channels with three times greater potency than receptors encoded by the Asn40 variant [57].This polymorphism has been linked inconsistently to alcoholism and other addictions [57-61]. Recent evidence supports that this locus predicts response to naltrexone in clinical trials [62,63] with Asp40 being associated with better response, although another study was negative [64].

Gene × environment interaction: MAOA and HTT—Alcoholism occurs due to individual choice, environmental and genetic determinants and interactions between factors within these three domains of causation (Fig. 2). Environmental factors include alcohol availability, parental attitudes, peer pressure, underage drinking and childhood maltreatment. From this perspective the ability to detect gene effects is dependent upon context and timing [65]. Severe childhood stress and neglect increase vulnerability to alcoholism but also several alcoholism-related psychiatric diseases including ASPD, CD, anxiety and depression, with the risks of these common diseases being elevated several-fold in the stress-exposed [66,67]. However, not all subjects exposed to environmental stressors develop alcoholism or other psychiatric diseases, indicating that people differ widely in stress resiliency. Genetic variation is likely to account partially for much of the differential vulnerability. Gene × environment interaction (G × E) occurs when the effect of exposure to an environmental factor on a person's health is conditional upon his or her genotype (for review see Caspi & Moffitt [68]). The knowledge that differences in DNA sequence moderate individuals in their resiliency or vulnerability to environmental pathogens is well known for several complex diseases, including psychiatric diseases as well as cancer, diabetes and cardiovascular, infective and immune diseases, etc. G × E effects within the context of psychiatric diseases have been described for several genes including monoamine oxidase A (*MAOA*) [69], the serotonin transporter (*HTT*) [70], *COMT* [71], the corticotropin releasing hormones receptor 1 gene [72] and the dopamine transporter [73]. So far, results appear to be particularly robust because of validation from different perspectives for *MAOA* and *HTT*.

MAOA is an X-linked gene encoding monoamine oxidase A, a mitochondrial enzyme that metabolizes monoamine neurotransmitters including NE, DA and serotonin (5-HT). *MAOA* knock-out mice have higher levels of DA, 5-HT and NE, and manifest increased aggressive behavior and stress reactivity [74]. In the human, different *MAOA* genetic variants impair MAOA activity to different degrees and the reduction in enzyme activity appears to parallel the effect on behavior. In 1993, Brunner *et al*. [75] reported a Dutch family in which eight males were affected by a syndrome characterized by borderline mental retardation and impulsive behavior including impulsive aggression, arson, attempted rape, fighting and exhibitionism. The syndrome is due to a stop-codon variant in the eighth exon of *MAOA* leading to complete and selective deficiency of MAOA activity. More recently a common *MAOA* polymorphism influencing MAOA transcription was discovered [76]. This locus, termed the *MAOA*-linked polymorphic region (*MAOA–LPR*), is a variable number tandem repeat (VNTR) located approximately 1.2 kb upstream from the *MAOA* start codon and within the gene's transcriptional control region [76,77]. Alleles at this VNTR have a different number of copies of a 30-base pairs (bp) repeated sequence with the three and four repeats alleles being by far the most common. Alleles with four repeats are transcribed more efficiently than alleles with three copies of the repeat and are therefore associated with higher MAOA activity [76]. In a large longitudinal cohort of boys, Caspi *et al*. [69] found that *MAOA–LPR* moderates the effect of childhood maltreatment on vulnerability to develop antisocial behavior. In this study maltreated boys with the low activity genotype were more likely to develop antisocial problems later in life than boys with the high activity genotype. Several studies have tried to replicate this finding, and a recent meta-analysis revealed a significant pooled effect [78]. More recently it has been shown that the *MAOA* × environment interaction described for males is also valid for women. In a sample of Native American women with high rates of childhood sexual abuse (CSA), it was shown that the effect of CSA on risk of developing alcoholism and ASPD was contingent upon the *MAOA–LPR* genotype [79]. Sexually abused women who were homozygous for the low-activity *MAOA–LPR* allele had higher rates of alcoholism, particularly antisocial alcoholism, compared with women who were homozygous for the high-activity allele. Heterozygous women displayed an intermediate risk pattern. In contrast, there was no relationship between alcoholism/antisocial behavior and *MAOA–LPR* genotype in women who had not been sexually abused.

Genes can also influence the probability of stress exposure, a phenomenon known as G × E correlation. G × E correlation results in a contamination of genetic and environmental effects leading to difficulties in interpretating G × E interaction. In this regard, animal models are extremely useful because they offer the possibility of controlling environmental influences enhancing gene effects and eliminating confounds that might underlie correlations. Macaques with histories of stress exposure, such as maternal separation early after birth, show increased alcohol consumption, higher impulsive aggression and incompetent social behavior and serotonin dysfunction, and consume more alcohol and have increased behavioral and endocrine responsivity to stress compared to mother-reared animals (for review see Barr *et al.* [80]). Consistent with studies on humans, a polymorphism of *MAOA* in the rhesus macaque that is orthologous to the human *MAOA–LPR* has been associated with aggression, and this association depended on whether or not the monkey had been separated from the mother. The low-activity genotype was associated with higher aggression only in mother-reared male monkeys [81].

The effect of *MAOA* on the hippocampus, a brain region which is involved in the processing of emotional experience, may underlie the interaction between *MAOA* and childhood trauma. Carriers of the low activity variant of *MAOA–LPR* display hyperactivation of the hippocampus and amygdala during the retrieval of negatively valenced emotional material but not during the retrieval of neutral material [82]. Therefore, the increased sensitivity to adverse experiences of carriers of the low activity *MAOA* genotype might be due to their impaired ability to extinguish adverse memories and conditioned fears. Other important mechanisms that are likely to underlie G × E interactions include hormonal effects. Steroid hormones including estrogens, progesterones, androgens and glucocorticoids modulate *MAOA* expression [83,84]. Both glucorticoids and androgens increase *MAOA* expression through response elements that are located within the *MAOA* promoter [83]. Recently, in a sample of criminal alcoholics, it has been shown that the effect of testosterone on aggression and alcoholism is contingent upon *MAOA–LPR* genotype [85]. Indeed, a positive correlation between testosterone level and antisocial behavior was found only among carriers of the low-activity allele, suggesting that this VNTR might influence the effect of testosterone on the *MAOA* promoter.

HTT is responsible for serotonin re-uptake and is a key regulator of serotonin availability in the synaptic cleft. In two unrelated families, an uncommon coding region mutation, Ile425Val was found in subjects with treatment-resistant obsessive compulsive disorder and carriers also had other diseases including alcoholism, Asperger's syndrome, social phobia, anorexia nervosa and tic disorder [86]. Additional rare HTT missense variants have been described in recently autism [87]. A common polymorphism of the *HTT* promoter region (5-*HTTLPR*) affects expression, with the major alleles involving 16 (L) or 14 (S) copies of a 20–23 bp imperfect repeated sequence [87]. Recently, it has been shown that 5-*HTTLPR* is actually a functionally tri-allelic locus due to a relatively common, functional A > G substitution within the L allele [88]. The low transcribing s allele has been associated inconsistently with anxiety and alcoholism. However, the effect of this allele on behavior appears stronger if stress exposure is taken into account. *5-HTTLPR* moderates the impact of stressful life events on risk of depression and suicide [70,89,90]. Carriers of the low transcribing s allele exhibit more depression and suicidality following stressful life events compared to individuals with two copies of the L allele [70]. Furthermore, 5-*HTTLPR* has been shown to moderate the functions of brain regions, such as the amygdala, that are critical in emotional regulation and response to environmental changes. Carriers of the low-activity allele display increased amygdala reactivity to fearful stimuli [33], reduced amygdala volume [91] and enhanced functional coupling between the amygdala and the ventromedial pre-frontal cortex [92]. Closer to the function of the gene, an effect of 5-*HTTLPR* genotype on transporter expression in brain *in vivo* has been reported in some studies [93] but not in others [94]. Consistent with findings in humans, the macaque orthologous *rs-5HTTLPR* polymorphism was shown to influence alcohol

consumption and stress response, depending on rearing conditions. Carriers of the low expressed genotype who were separated from their mother at an early age displayed higher stress reactivity and ethanol preference [95]. The combined effect of *rh-HTTLPR* and environment on stress reactivity suggests that the influence of *HTTLPR* on behavior might be traced to altered regulation of the hypothalamic–pituitary–adrenal (HPA) axis.

Genome-wide scans

Genome-wide scans, including whole genome linkage and whole genome association (WGA), allow the hypothesis-free mapping of disease-causing loci within the genome. Chromosome regions and genes implicated by these studies, as well as potential strengths and limitations of WGA methodologies, are discussed below.

Whole genome linkage—In whole genome linkage studies a panel of polymorphisms is tested for meiotic linkage to a disease (marker-disease coupling or repulsion) in family-based samples, identifying chromosome regions that are shared more often among phenotypically concordant relatives compared to phenotypically discordant family members. The implicated chromosomal regions are usually broad, for example greater than 10 megabases. Therefore, a more refined search for candidate genes within the region of interest is subsequently conducted. To perform whole-genome linkage analysis for alcoholism, several large family-based data sets have been collected. These include the Collaborative Study on the Genetics of Alcoholism (COGA) [96], the Roscommon study of Irish families [97]; a sample of multiplex families collected in the Pittsburg area [98]; and samples collected from relatively isolated populations including Native Americans [99-100] and Finns [101, Enoch *et al*. unpublished]. Such isolated populations, and large families within them, are likely to confer the advantage of reduced genetic heterogeneity. A non-exhaustive list of convergent findings across studies includes a region on chromosome 4q, that contains the alcohol dehydrogenase (ADH) gene cluster [96, 97,99,100], and a chromosome 4p region near the centromere containing a γ-aminobutyric acid receptor ($GABA_A$) gene cluster [96,99]. In the COGA sample there was also evidence for linkage to chromosomes 1 and 7, and to chromosome 2 at the location of an opioid receptor gene [96]. A region on chromosome 1 was linked to alcoholism and affective disorder in the COGA data set [102], supporting further the existence of a genetic overlap between alcoholism and internalizing disorders. Linkage analyses have also been conducted with intermediate phenotypes for alcoholism including low response to alcohol [29], neurophysiological endophenotypes such as P300 [103] and reduced alpha power (Enoch *et al*., unpublished), and chromosome regions identified by these studies overlap partially with those reported for alcoholism.

The $GABA_A$ receptor: γ-aminobutyric acid (GABA) is the primary inhibitory neurotransmitter in the central nervous system. $GABA_A$ receptor-mediated chloride currents into neurons are facilitated by various drugs including ethanol, benzodiazepines and barbiturates. Several lines of evidence suggest that GABA is involved in many effects of alcohol including tolerance, dependence and cross-tolerance to benzodiazepines and barbiturates. A series of mouse ethanol-related behaviors, including preference, withdrawal severity and sedation sensitivity, map quantitative trait loci *(QTL)* regions where $GABA_A$ receptor-gene clusters are located [104,105]. In the rat, a Arg100Gln missense variant located in the $GABA_A$ α6 subunit gene (*GABRA6*) was associated with variation in ethanol and benzodiazepine sensitivity [103]. In humans, AUD has been linked to both the chromosome 4 [99,106] and chromosome 5 [107] GABA clusters. Linkage signals appear to derive from *GABRA6* on chromosome 5 [107] and the $GABA_A$ α2 (*GABRA2*) [106,108] on chromosome 4. A human variant of *GABRA6* (Pro385Ser), which is located in the chromosome 5 cluster, was associated with sensitivity to alcohol [30] and benzodiazepine [109].

Whole-genome association—Large-scale genotyping techniques have recently been made available and genome-wide analyses for several complex diseases including amyotrophic lateral sclerosis [110], Type 2 diabetes [111], neuroticism [112], obesity [113] and human immunodeficiency virus 1 (HIV-1) [114] have been run within large data sets of unrelated individuals using dense panels including more than 500 000 polymorphisms. These WGA studies have the advantage of increased power for detecting effects of relatively common alleles (>0.05) and more refined localization of signals to smaller chromosome regions compared to family-based linkage analyses, which have a reciprocal advantage of being powerful for detecting effects of rare and uncommon alleles that are present in only a small proportion of probands and their families.

A WGA scan has been run recently in a sample of unrelated alcohol-dependent (n = 120) and control (n = 160) individuals sampled from the COGA pedigrees [115]. This study identified several candidate genes that might moderate vulnerability to alcoholism and whose products are involved in cellular signaling, gene regulation, development, cell adhesion and Mendelian disorders. However, these findings are weakened by the small sample size and the consequent lack of power to identify exhaustively common alcoholism-causing alleles. Indeed, the typical effect size of genetic variations acting on complex diseases is small [116] and the median sample size required for a WGA study to have 90% power even at an alpha level of 0.05 has been estimated to be as high as 15 000 participants [117]. A WGA study searching for genes influencing seven complex disorders, including bipolar disorder, have been completed recently in a sample of 14 000 cases (2000 cases per disease) and 3000 controls and can be considered as illustrative of the general potential of WGA (Welcome Trust study [118]). For the seven diseases studied in the Wellcome Trust study, a total of 24 genome-wide significant signals were found. The maximum genotype-specific odds ratios for significant disease loci ranged from 1.5 to 18.5, with a median of 1.9 (see Fig. 3). Furthermore, the disease loci found accounted for only a small portion of the genetic variance of these diseases (consistent with the common allele/moderate effect model). For example, the 16p12 locus found for bipolar disorder with an odds ratio of approximately 2.1 accounts, perhaps, for 4% of the genetic risk for bipolar disorder at the general population level, although its impact might be higher within certain families. WGA in large case–controls data sets has not yet been reported for alcoholism. However, this approach, even when large case–control samples will be available for alcoholism, will not enable identification of the complete list of genetic loci involved in alcoholism. Another important strategy that can be used to increase power of WGA studies is through the use of intermediate phenotypes. Indeed, effect sizes of genetic variations acting on intermediate phenotypes more proximal to gene action and including measures such as task-related brain activation appear to be larger than effects on complex disease phenotypes [116]. In Fig. 3 effect sizes reported for well-characterized functional loci including *MAOA–LPR* [82], *5-HTTLPR* [33] and *COMTVal158Met* [42] are compared to odds ratios reported for the complex diseases studied by the Welcome Trust and to the odds ratio reported by a large meta-analysis [119] evaluating the effect of *5-HTTLPR* on harm avoidance, which can also be considered a complex phenotype (Fig. 3, see also Goldman & Ducci [116]). A whole-genome scan on intermediate phenotypes relevant for alcoholism is an alternative and potentially very powerful approach in the search for the alcoholism-causing genes.

As well as power and the false-negative issue, another important concern of WGA analyses is false positive findings due to the extremely high number of markers evaluated and statistical tests performed. In this regard, particular attention has been placed on the threshold used to defined statistical significance and on replication in independent populations. Furthermore, current marker panels used in WGA are composed of single nucleotide polymorphisms (SNPs) and do not include other types of genetic variation such as copy number variations (CNVs), whose importance in disease susceptibility is being recognized increasingly. In this regard, recent whole-genome analysis has revealed that both CNVs and SNPs can alter patterns of

mRNA expression, but the SNP variation does not track most of the effects of CNVs, indicating that both types of polymorphisms should be interrogated to perform a comprehensive genome-wide evaluation of the effects of genomic variation on disease vulnerability [120]. Finally, the ultimate validation of WGA results will be represented by the identification of the specific causing variants within the disease-associated genomic regions.

UNDERSTANDING GENE FUNCTION

Our understanding of the protein-coding function of the genome is still limited, and we have even less understanding of non-protein coding transcripts and genome elements regulating gene expression. However, new technologies including gene expression profiling using microarrays and methods to evaluate chromatin remodeling are available, enabling a detailed exploration of the genome function and promising an increase of our knowledge of mechanisms through which genetic variation influence protein level/function and environment interacts with the genome.

DNA microarray

DNA microarrays are used for the simultaneous measurement of the expression of thousands of genes and represent a tool that can be used to identify genes that are expressed differentially within QTL linked previously to specific alcohol-related phenotypes using inbred or selected lines of animals. Indeed, several strains of animals have been generated that display differences in alcohol-related behaviors, such as animals with high or low sensitivity to various effect of alcohol or with high or low preference for alcohol [104]. Kerns *et al.* [121] studied differences in basal or ethanol-responsive gene expression in the brains of two strains of mice that differ markedly in a number of behavioral responses to ethanol. Several genes that were expressed differentially in the two strains as well as several ethanol-regulated genes were found within brain regions that are involved in reward, including the nucleus accumbens, pre-frontal cortex and ventral tegmental area. Multiplex evaluation of gene expression by microrrays enables the exploration of gene networks. Tabakoff *et al.* [122] compared the mRNA expression profiles of mouse strains displaying marked differences in acute tolerance to alcohol and results from this study indicate the importance of a signal transduction cascade that involves the glutamatergic pathway.

Epigenetics—Complex epigenetic mechanisms that regulate gene activity without altering DNA code have been shown to produce long-lasting changes in gene expression essential to development and cellular differentiation and to adaptation to environmental changes. These mechanisms, that include DNA methylation, post-translational covalent modifications of histones, nucleosome sliding and nucleosome and histone substitution, cause modifications of chromatin conformation which, in turn, regulate gene expression (for review see Tsankova *et al.* [123]). For example, the amount of DNA methylation in promoter regions correlates with gene inactivation. Chromatin remodeling can be studied at the single locus level; however, methods such as the chromatin immunoprecipitation in combination with DNA sequencing (ChIP-seq) allow for high-resolution genome-wide analysis. Alcohol exposure has been shown to cause changes in chromatin structure in rat brain [124,125]. Alpha synuclein (*SNCA*) is a gene that maps to a QTL for alcohol preference, and expression of alpha synuclein is increased in different brain areas in rats displaying alcohol preference [126]. Recently, an increase in alpha synuclein promoter DNA methylation has been found in patients with alcoholism [127], and genetic variation within the human *SNCA* gene has been linked to alcohol craving [128]. DNA methylation also appears to regulate expression of alcohol dehydrogenase [129] and *HTT* [130] genes.

CONCLUSION

Although most of the genetic determinants of alcoholism remain to be discovered there are reasons for optimism. In recent years a technological revolution has occurred producing a shift from single-locus studies to genome-wide searches. The genomes trascriptome, epigenome and, to some extent, proteome, can now be assessed at a level of detail that was previously inconceivable. Innovations are required at the analytical level to integrate and validate the massive amounts of data produced by these new technologies and different approaches. However, these tools promise to increase our understanding of the mechanisms by which genetic variation alters molecular function and predisposes individuals to alcoholism and other diseases.

GLOSSARY

Genetic heterogeneity, a model of genetic determinism in which different alleles lead to the same phenotype in different individuals, but an individual allele can suffice to produce the phenotype.

Polygenicity, a model of genetic determinism in which many alleles function in combination to produce a phenotype.

Phenocopies, a phenotype of environmental origin that mimics a phenotype of genetic origin.

Penetrance, the probability of expressing a phenotype that is determined by a genotype.

Intermediate phenotype, mechanism-related manifestation of a more etiologically complex phenotype.

Allele, alternative form of a genetic locus.

Epigenetics, biological mechanisms that regulate gene activity without altering DNA sequence, and that can result in long-lasting changes in gene expression.

Genetic locus, a particular location on a chromosome.

Microarray, a genetic analysis tool containing a large number of features such that many different DNAs, RNAs or proteins can be measured simultaneously.

Inbred lines, strains by repeated matings of genetically related individuals such that most or all genetic variation has been eliminated.

Chromatin remodeling, dynamic changes in DNA/protein structure altering the packaging and function of DNA.

Quantitative trait locus (QTL), region of a chromosome that contains one or more genetic loci that contribute to a phenotypic difference.

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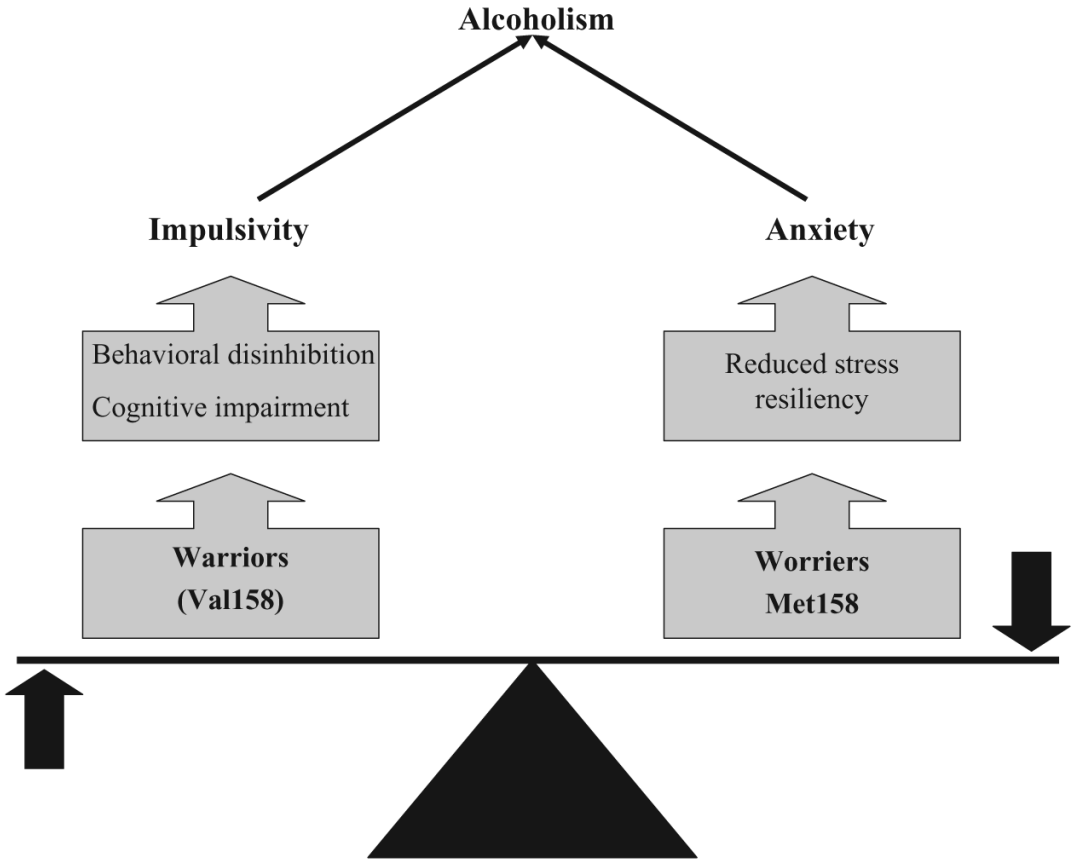

Figure 1.
Catechol-*O*-methyltransferase (*COMT*): the warrior (Val158) versus worrier (Met158) model

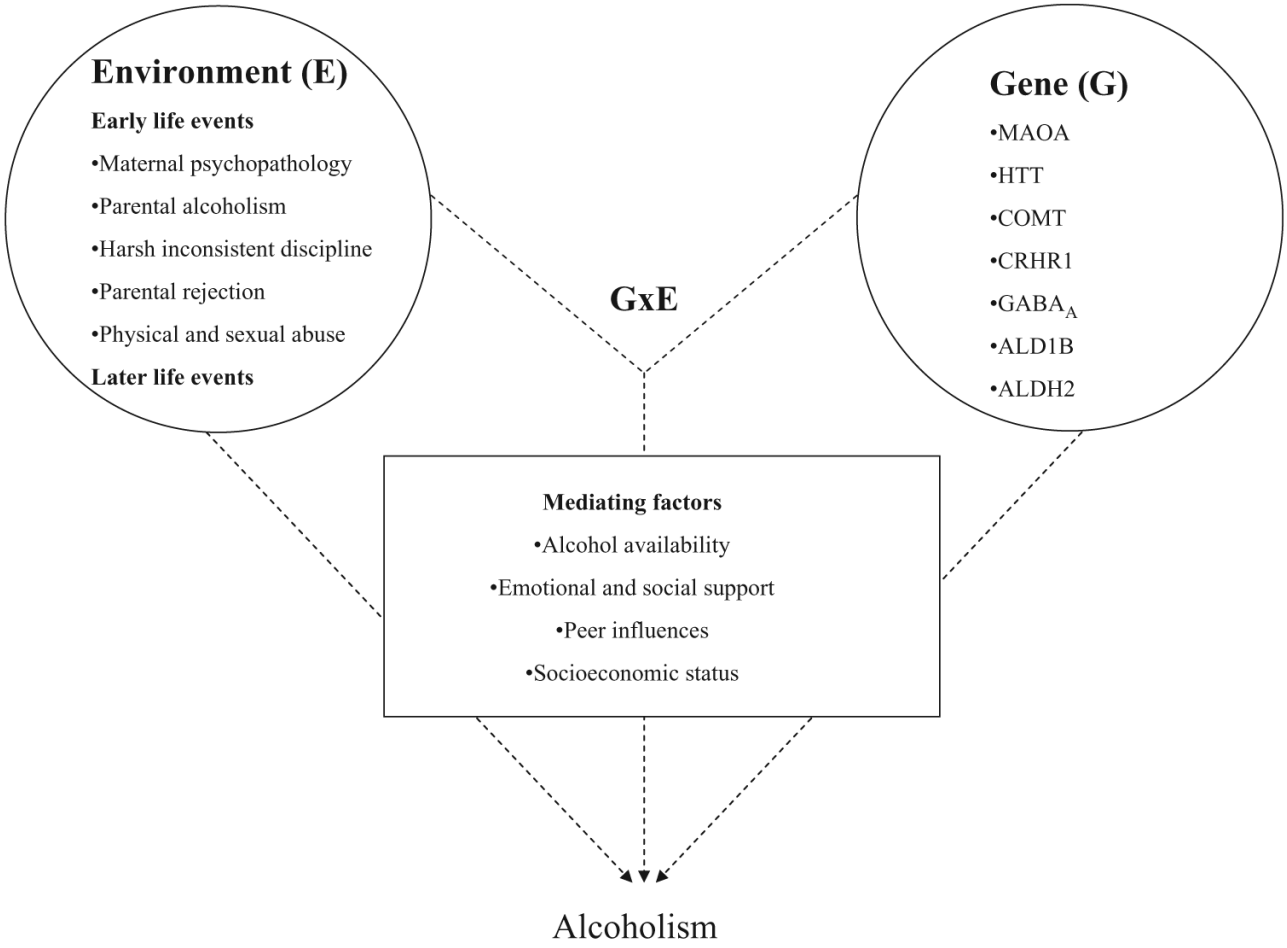

Figure 2.
Main and interactive effects of genetic and environmental risk factors for alcoholism

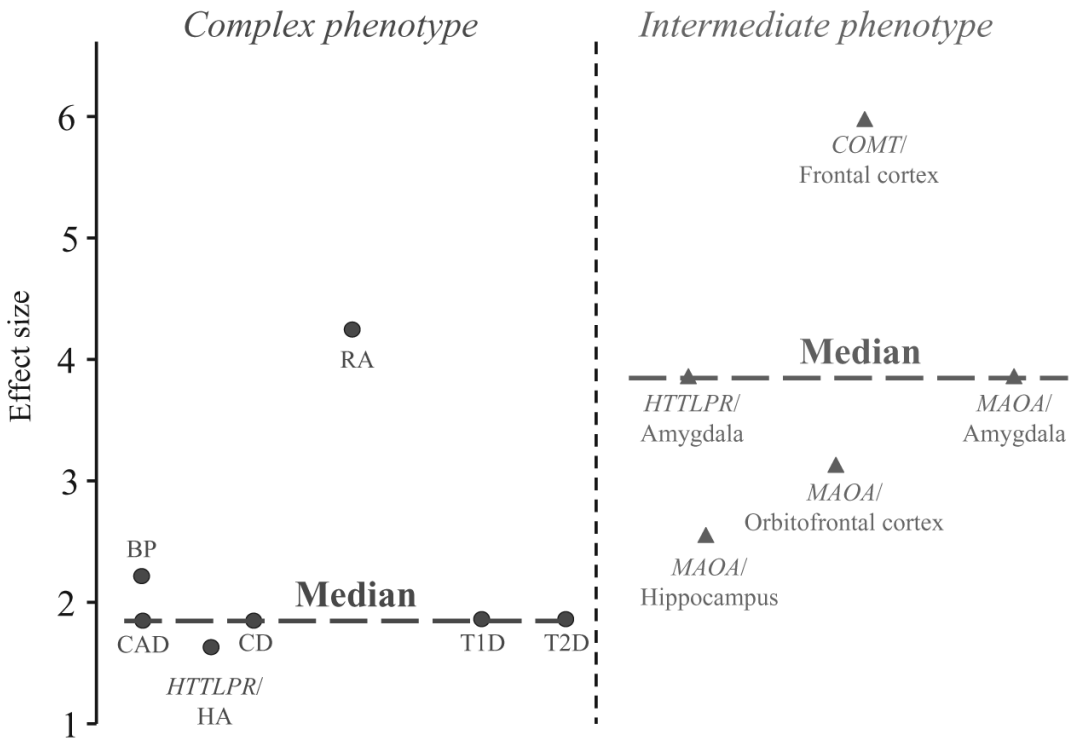

Figure 3.

Genotype-specific effect size (odds ratios) for complex versus intermediate phenotypes. CAD = coronary artery disease; T1D = Type 1 diabetes; T2D = Type 2 diabetes; CD = Crohn's disease; BP = bipolar disorder; RA = rheumatoid arthritis; HA = harm avoidance; *MAOA* = monoamine oxidase A; *HTT* = serotonin transporter; *LPR* = *linked polymorphic region*; *COMT* = catechol-O-methyltransferase

Table 1

Gene effects on intermediate phenotypes for alcoholism.

Domain	***Mode of assessment***	***Locus***
Attention/dyscontrol	Low amplitude P300	*COMT Val158Met*
	Frontal cognitive tasks	*MAOA–LPR*
	Task-specific MRI	
	Questionnaires	
Pharmacokinetics	Flushing questionnaires	*ALDH2 Glu487Lys*
	Metabolite levels	*ADH1B His47Arg*
Level of response	Alcohol challenge/clamp	
	Questionnaire	
Treatment response	Response in clinical trials	*OPRM1 Asn40Asp*
Anxiety/dyscontrol	α, β resting EEG power	
Mesolimbic reward	Task-specific MRI, PET	
Stress/resiliency	Task-related MRI, PET	*HTTLPR (La, S, Lg)*
	Endocrine responses	*COMT Val158Met*
	Questionnaires	*NPY*
Brain volume/structure	Structural MRI	*BDNF Met66Val*
		MAOA–LPR
		HTTLPR
		COMT Val158Met