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CONSENSUS PAPER

Consensus paper of the WFSBP task force on biological markers: Biological markers for alcoholism

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Abstract

Objectives. This article presents an overview of the current literature on biological markers for alcoholism, including markers associated with the pharmacological effects of alcohol and markers related to the clinical course and treatment of alcohol-related problems. Many of these studies are well known, while other studies cited are new and still being evaluated. **Methods.** In this paper we first describe known biomarkers of alcohol-related disorders, review their features and the problems involved in their use. We then consider future developments on biomarkers and their possible impact on the field. **Results.** More recent findings cited include the work on type 7 adenylylase (AC) polymorphism and its lower expression levels in female alcoholics. Neuroimaging studies involving biomarkers have also reported brain volume reductions of gray and white matter, including amygdala and subcortical regions in alcoholic patients, while a high association between the copy number variations (CNVs) in 6q14.1/5q13.2 and alcohol dependence has more recently been identified in genetic studies. **Conclusions.** In addition to their possible importance for diagnosis, biomarkers may have utility for predicting prognosis, progression of the disorder, the development of new treatments, and monitoring treatment effects. Although such findings should be verified in independent studies, the search for new biomarkers is continuing. Several potential candidate biomarkers have been found recently in blood, imaging, and genetic studies with encouraging results.

Key words: alcohol, biochemical markers, abuse, alcohol dependence, alcohol use disorder

Introduction

Alcohol (ethanol) is one of the most widely misused drugs in the world. Humans respond to low doses of ethanol with euphoria, but with disinhibition, incoordination and lethargy at high doses. Alcohol abuse and alcohol dependence signify alcohol use disorders characterized by chronic heavy drinking, culminating in serious adverse outcomes and loss of control. Alcohol dependence is characterized by an unhealthy drive to drink alcohol that leads to an inability to control intake on any given occasion and an increasing tolerance to alcohol's

effects. The identification of biochemical substances in the body suggesting the repeated use of heavy doses of alcohol as possibly part of an alcohol use disorder and the identification of genetic susceptibility factors for alcoholism (i.e. “biomarkers for alcoholics”) will provide important tools for future investigation.

The goal of this paper is to provide a guide to the optimal application of biomarkers for heavy drinking and alcohol use disorders, and to facilitate objective and quantitative data gathering in both clinical and research settings.

Pharmacological effects of alcohol

Many neurochemical systems have been implicated in the biology of alcohol intoxication, but two systems are the most relevant: (1) gamma-aminobutyric acid (GABA) and its receptors; (2) glutamate and the *N*-methyl-D-aspartic acid (NMDA) receptor (α glutamate receptors) and the opioid systems (Addison and Kurtz 1986). GABA receptors include ion-selective and ligand-gated ion channels, while GABA itself is the major inhibitory neurotransmitter in the brain that interacts with a family of receptors that recognize anxiolytic and sedative drugs. For example, benzodiazepines, which share bio-behavioural properties with alcohol, enhance Cl⁻ transport through GABA_A receptors. Drugs that simulate the effect of GABA enhance and prolong the behavioural effects of alcohol, whereas drugs opposing the effect of GABA antagonize alcohol's effects. In animal studies, benzodiazepine receptor antagonists have been shown to block many alcohol-induced cognitive, behavioural and neurophysiological effects of ethanol.

Several lines of evidence implicate opioid peptides, such as beta-endorphin (an endogenous opioid receptor ligand), in both the perception of the rewarding effects of ethanol and in the risk for developing alcoholism. Alcohol is believed to activate the brain reward system, especially in the mesolimbic dopamine system, in part by increasing beta-endorphin release. The direct mechanism of alcohol's action on dopamine release in the ventral tegmental area (VTA) is intriguing and has been widely investigated. Recent patch clamp studies suggest that alcohol excites VTA dopamine neurons, partly by increasing ongoing opioid-mediated suppression of local GABAergic inhibition.

Glutamate, the major excitatory neurotransmitter in the brain, is also believed to be involved in alcohol-induced intoxication and behaviour changes. Electrophysiological studies in animals show that antagonizing NMDA receptors produces similar behavioural effects to alcohol.

Tolerance to both the sedative and intoxicating effects of alcohol is partly due to a compensatory decrease in GABA-mediated inhibition in the brain. Alcohol-induced alterations in the function of the GABA_A receptor Cl⁻ channels that remain after cessation of drinking are believed to contribute to the clinical features associated with ethanol withdrawal. Decreased GABA-mediated inhibition of the neuronal functions and increased activity of NMDA receptors may help explain important characteristics of alcohol withdrawal. Hypersensitive, NMDA-induced calcium flux occurs following chronic alcohol exposure and may contribute to the hyperexcitability and seizures that can be seen during

ethanol withdrawal. Chronic alcohol consumption may also augment the sensitivity to NMDA by increasing the density of NMDA receptors, thereby increasing biosynthesis or release of glutamate. Thus, the effects of ethanol on calcium homeostasis can have an important impact on the clinical features of alcoholism.

Therapeutic studies in alcoholics

The opioid receptor antagonists naltrexone and nalmefene have been shown to be effective in reducing alcohol consumption in both animal and human studies. Naltrexone's propensity to reduce alcohol intake may be negatively correlated with baseline beta-endorphin levels as mu-opioid receptor antagonists attenuate alcohol consumption. Nalmefene also works as an opioid receptor antagonist and is helpful in the treatment of alcohol dependence. Nalmefene has a longer half-life than naltrexone, better bioavailability, and a more favourable tolerance profile in achieving its benefit. Because alcohol is believed to activate the brain reward system by increasing the release of beta-endorphin, an endogenous opioid receptor ligand, it is possible that some of the rewarding and pleasant effects of alcohol related to this physiological effect promote drinking behaviour. Although the precise mechanisms underlying the actions of nalmefene remain to be elucidated, endogenous opioid peptides as well as metabolic by-products of ethanol (e.g., tetrahydroisoquinolines and beta-carbolines) may mediate these effects and may also involve interactions between cannabinoids and the opioid receptor system. These rewarding and pleasant effects are potentially associated with both internal and external drinking cues. Analogous to the concept of negative reinforcement or relief craving (euphoria and disinhibition), the latter type of craving could theoretically be classified as positive craving. In the first case alcohol acts as a positive reinforcer, in the second instance it serves as a negative reinforce (Mann et al. 2009).

A second medication that has been shown to decrease alcohol use and alcohol problems in alcoholics is acamprosate. This compound works as an excitatory amine antagonist, mainly through glutamate but it is also a GABA stimulant. It is believed that this action reduces alcohol craving and promotes abstinence. While the precise beneficial mechanisms of action for treating alcoholism are not completely understood, the relationship of the glutamatergic system and the mechanism of action of acamprosate serves as one example of how our understanding of the pharmacological effects of alcohol may offer important new leads for developing or modifying alcoholism treatments.

Comorbidity of alcoholism and psychiatric disorders

As with all complex diseases, alcoholism can be regarded as a clinical syndrome resulting from a combination of multiple risk factors; consequently individuals can present with diverse sets of symptoms and severity of disease (Hines et al. 2005). For example, the prevalence of psychiatric symptoms is higher in individuals who drink more heavily and more regularly, i.e. patients with alcohol dependence, compared to those with a diagnosis of alcohol abuse. In the National Comorbidity Study, 29.2% of respondents with alcohol dependence experienced either an independent or a substance-induced mood disorder within the past 12 months of their assessment, a rate that was 3.9 times higher than those who were not alcohol dependent. Bipolar disorder over the previous year was seen in 1.9% of the respondents with alcohol dependence, a rate 6.3 times greater than non-alcoholics (Regier et al. 1990; Feinman and Dunner 1996; Cornelius et al. 2003). Furthermore, among people with alcohol dependence, 36.9% met the criteria for an anxiety disorder during the previous year, including 11.6% with generalized anxiety disorder (GAD), 3.9% with panic disorder, and 7.7% with PTSD, all representing higher rates than those seen in the general population. Further, alcohol- and substance-use disorders are very common in patients with schizophrenia. The Epidemiologic Catchment Area (ECA) study (Regier et al. 1990) reported that 47% of patients with schizophrenia had a lifetime history of a substance-use disorder and that 34% of these patients have a lifetime diagnosis of an alcohol-use disorder (Le Fauve et al. 2004).

Genetic influences in alcoholism

Like other complex genetic disorders, alcohol use disorders are heterogeneous in their clinical presentation and their course. Combined, genetic factors explain an estimated 60% of the variance, interacting with environmental factors that contribute to the remaining 40% (Schuckit 1999; Kendler et al. 2003). Genetic factors that affect susceptibility to alcoholism may be related to certain components of alcoholism, such as alcohol metabolism, personality, cognitive function, and neurophysiology. A classical approach for identifying alcohol susceptibility genes is to focus on particular features of alcoholism dependence, i.e. intermediate phenotypes that likely influence susceptibility to alcohol dependence, also known as endophenotypes (Hines et al. 2005). These studies have often been able to identify genes that impact the alcoholism risk, including alcohol

metabolizing enzymes, the sensitivity to alcohol and impulsivity and related personality characteristics, as discussed further below in the section on trait markers of risk.

Significance of biomarkers for studying alcoholism

Biomarkers that relate to recent heavy drinking (state markers) have several possible applications, namely as (1) diagnostic tools; (2) screening tools; or (3) for use in early or pre-symptomatic identification. Any biological characteristic that can be objectively measured and reliably indicates a predisposition to a specific condition, or the presence or progress of a pathological state can be regarded as a biomarker (Atkinson et al. 2001). Biochemical state markers for alcoholism can provide clinicians with objective measures of patient's recent drinking patterns, whether heavy drinking or a more modest intake. The availability of state markers for alcoholism may also facilitate optimal treatment(s). Clinicians would be greatly assisted by biological markers that accurately reflect both the degree of problematic drinking and the presence of a genetic predisposition to alcoholism. Not surprisingly, considerable recent effort has been expended on developing objective, biologically based, and easily measured biomarkers for alcoholism.

Currently, there are no biomarkers that can directly identify alcoholism. Much of state marker research in studies of alcoholics has been focused on finding clinically useful alcohol consumption biomarkers or markers that can detect the timing and intensity of an individual's alcohol use. The identification of additional state markers to assess the effectiveness of treatments would be of considerable value.

On the other hand, trait markers are biochemical markers that reveal some possible genetic links between the inherited risk for heavy drinking and the consequent alcohol problems. Trait markers must be clinically validated by testing "at risk" individuals before the onset of alcoholism. Marker-positive patients would become prime candidates for prevention programs since early warning may make it possible to avoid alcoholism. Individuals with a family history of alcoholism are 3–5 times more likely to develop alcoholism than are individuals with no such family history. One important feature of trait biomarkers of alcoholism is to provide information regarding a person's inherited risk of alcoholism. A good biomarker, whether state or trait, should be sensitive (i.e. accurate for most if not all drinkers) and specific (i.e. linked to alcohol use but not to other psychiatric conditions). The biomarker tests

should be noninvasive, easy-to-perform, inexpensive, rapid, have stable values, and especially should allow reproducibility in laboratories worldwide. Indeed, numerous biomarkers of alcohol use have been identified that can measure patterns of previous alcohol use from hours to days and weeks, but with variable accuracy (Table I).

State markers for alcoholism

State markers can be used for diagnosis or screening, prognosis, determining disorder stage, or for monitoring the effectiveness of an intervention. Unfortunately, state markers are currently limited to measuring patterns of alcohol consumption rather than directly measuring the entire spectrum of alcoholism including dependence symptoms and evidence of harmful use. Despite this limitation, alcohol

consumption patterns provide essential information regarding the extent of a patient's alcohol use, the risk of having or developing alcoholism, and related alcohol-related adverse effects (Peterson 2004–2005). More specifically, biomarkers that can estimate the amount of alcohol consumed over various periods of time could assist clinicians in verifying vital information such as the time of the last drink and the current pattern of alcohol use (harmful, hazardous, or non-hazardous).

Alcohol consumption biomarkers

Current biomarkers for alcohol consumption include carbohydrate-deficient transferrin (CDT), gamma-glutamyl transferase (GGT), aspartate aminotransferase (AST), alanine aminotransferase (ALT), mean corpuscular volume (MCV), and direct measures of

Table I. Possible state and trait markers for alcoholics.

Biomarker	Remarks	Sensitivity	Specificity	Possible or current use
<i>State markers (recent drinking activity)</i>				
GGT (gamma-glutamyltransferase)	Early indicator of chronic heavy drinkers, liver disease	61	n/a	Chronic alcohol abuse
ALT (alanine aminotransferase)	More useful for liver disease; AST/ALT ratio: heavy alcohol consumption	n/a	n/a	Chronic alcohol abuse
AST (aspartate aminotransferase)		56	n/a	Chronic alcohol abuse
MCV (mean corpuscular volume)	Less useful, but high level is maintained for several months after stop drinking	47	n/a	Heavy alcohol use
Beta-Hex (<i>N</i> -acetyl-b-hexosaminidase)	Elevated in heavy drinkers; difficult to assay	94	91	Heavy alcohol use
CDT (carbohydrate-deficient transferrin)	Higher amounts of CDT in heavy drinkers; highly specific to alcohol consumption; difficult to measure	26–83	92	Heavy alcohol use
SIJ (plasma sialic acid index of apoJ)	Sialylated ApoJ decrease after alcohol consumption	n/a	n/a	
TSA (total serum sialic acid)	Elevated in alcoholics; long-term elevation even during abstinent	n/a	n/a	
5-HTOL (5-hydroxytryptophol)	24-h biomarker; useful in forensic toxicology	n/a	n/a	Monitoring sobriety
FAEE (fatty acid ethyl esters)	24-h biomarker; distinguishable social drinkers from heavy drinker or alcoholics	100	90	Recent heavy alcohol use
EtG (ethyl glucuronide)	24-h (blood) or 36-hour (urine) biomarker; detectable in other body fluids tissue or hair	n/a	n/a	Monitoring sobriety; forensics
WBAA (whole blood-associated acetaldehyde)	Alcohol specific biomarker; Hb-bound acetaldehyde accumulate in RBC over 120 days	100	95	Recent alcohol consumption at all levels; monitoring abstinence
Salsolinol	Better marker for chronic alcohol consumption (blood); no difference between alcoholics and nonalcoholics (brain)	n/a	n/a	Chronic alcohol consumption
CPK (creatine phosphokinase)	Elevated in alcoholics(hallucination, delirium)	n/a	n/a	
Fisher ratio (BCAA/AAA)	Low level in alcohol dependence	n/a	n/a	
MAO-B (monoamine oxidase B)	Low level in hazardous/harmful alcohol use	n/a	n/a	Recent alcohol consumption; monitoring success of treatment

(Continued)

Table I. (Continued)

Biomarker	Remarks	Sensitivity	Specificity
<i>Trait markers (genetic predisposition)</i>			
AC (adenyl cyclase)	Not specific to alcoholic (c.f. marijuana and other drug use)	n/a	n/a
GABA (gamma-aminobutyric acid)	Low level in alcohol dependence	n/a	n/a
Dopamine	Low level even after abstinent for 7 years	n/a	n/a
b-endorphin	Low level in alcohol dependence	n/a	n/a
Serotonin	Higher serotonin transporter activity than non- alcoholics	n/a	n/a

Refs: Peterson K. *Alcohol Res Health* 2004/2005;28:30–37; Saito et al. *Alcohol Alcoholism* 194;29(Suppl 1):133–135; Snell et al. *Alcoholism: Clin Exp Res* 2012;36:322–341.

ethanol in blood and breath, and ethanol's metabolites. Of these tests, CDT appears to be the most sensitive and specific single test for detecting more recent moderate to heavy alcohol intake (~7–10 drinks a day). The CDT test and assay are highly standardized, automated and inexpensive; consequently, it is commonly used in clinical practice. When used alone, these tests are only moderately sensitive, but their combined use greatly improves sensitivity without a marked decrease in specificity (Hietala et al. 2006). A wide range of medications affect GGT, particularly those that induce the microsomal enzymes (Table I). A variety of hepatic or biliary conditions can affect GGT, including hepatic congestion in cardiac failure. Disorders of other body sites where GGT is found can also affect GGT levels (e.g., diabetes and pancreatitis) (Conigrave et al. 2003).

AST/ALT and MCV are often used in clinical practice to detect chronic heavy drinking, but are much less sensitive and specific for detecting heavy alcohol use. Aminotransferases are less sensitive than GGT in the detection of excessive alcohol consumption. The AST/ALT ratio appears to be a useful index for distinguishing non-alcoholic steatohepatitis (NASH) from alcoholic liver disease (a ratio of <1 suggests NASH and values >2 are strongly suggestive of alcoholic liver disease (Sorbi et al. 1999). However, the AST/ALT ratio is thought to be indicative advanced alcoholic liver disease rather than heavy alcohol consumption (Nyblom et al. 2004). Since the life-span of a red blood cell is about 120 days, it may take several months for changes in drinking to be reflected in MCV levels (Hasselblatt et al. 2001). Sustained and regular excessive drinking is important for elevating MCV levels (Meerkerk et al. 1999) and the levels may continue to rise upon cessation of drinking in alcohol dependence (Monteiro et al. 1986). The half-lives of plasma GGT, MCV and CDT are, respectively, 4 weeks, 2–3 months and 14–16 days.

Direct measurement of ethanol is a widely used measure of acute intake, but its relatively short half-life (a few hours) limits its usefulness. Ethyl glucuronide (EtG) and ethyl sulfate (EtS), direct metabolites of ethanol, can indicate even a minor intake of alcohol up to 80 h after alcohol has been eliminated from the

body (Wurst et al. 2003). Fatty acid ethyl esters (FAEE) are known to be a direct alcohol intake marker and are often investigated in hair and skin surface samples. The highest FAEE/sebum are detected 7–9 days after the days of high alcohol consumption (González-Illán et al. 2011). Phosphatidylethanol (PEth) is a glycerophospholipid homologue containing an amino-alcohol by phospholipase D. Since the formation of PEth is specifically dependent on ethanol, the diagnostic specificity of PEth as an alcohol biomarker is theoretically 100% and its half-life in blood is approximately 4 days. The amount of alcohol consumed highly correlates with the blood concentration of PEth; thus, PEth appears to be a more sensitive indicator of alcohol consumption than traditional alcohol markers, such as CDT, GGT, and MCV (Isaksson et al. 2011). Recently, novel serum markers have been identified as possible candidate markers for alcoholism or at least heavy alcohol consumption. Pigment epithelial-derived factor (PEDF) has been found in moderate to heavy habitual drinkers, but not in healthy subjects with no drinking history (Sogawa et al. 2011). Elevation of N-terminal pro-BNP (NtBNP), a circulating neurohormone which is also a marker of cardiac dysfunction, has been found in alcoholic patients. An elevated level of NtBNP was reversed and significantly decreased after therapy for withdrawal symptoms (Hoefer et al. 2011).

Use of alcohol consumption biomarkers in clinical practice

In current clinical practice, the diagnosis of alcohol-related disorders usually depends on patient self-reporting. While the veracity of self-reporting is a relatively accurate measure of alcohol use (Babor et al. 1989), combining biological markers with performance of the alcohol use disorders identification test (AUDIT) questionnaire can be helpful in clinical settings. A positive predictive value (PPV) of 17.3% for an AUDIT score 8 or more in the detection of withdrawal, increased to 47.1% when used in combination with at least two other abnormal biological markers including MCV, AST, ALT, and GGT (Dolman et al. 2005).

Recovery programs

Although biomarkers are not widely used in specialized alcohol treatment programs, they may be particularly useful in detecting relapse. CDT is significantly more sensitive than GGT in detecting relapses. Chen et al. reported that CDT combined with GGT was successful in monitoring relapse in inpatient alcoholics (Chen et al. 2003). Due to their ability to detect small amounts of alcohol, urinary EtG/EtS can potentially monitor relapse in patients in active treatment programs. However, PEth measurement in whole blood is a more sensitive biomarker than serum CDT for the detection of relapse drinking because the PEth test can detect lower consumption levels. The half-lives for total PEth and for CDT (the relative disialotransferin) are estimated to be 3.5–9.0 days (mean 6.1) and 8.5–15 days (mean 12.6), respectively (Helander et al. 2012).

Primary care

Because heavy alcohol use can cause or aggravate numerous common medical conditions, biomarkers for heavy alcohol use could yield vital information to primary care providers. CDT levels have been found to be useful in detecting and/or confirming high-risk alcohol use in patients treated for type 2-diabetes and hypertension in a primary care setting (Fleming et al. 2004). The CDT test, in addition, to patient self-report may provide an economic healthcare benefit by identifying a larger number of heavy drinkers in the primary care population. The positive net benefit (cost savings) can be attributed to improved detection and intervention in cases of heavy drinking and the ability of intervention(s) to reduce the occurrence of expensive medical and legal events (Dillie et al. 2005).

Hospital settings

Alcoholism is often associated with medical complications in the clinical course of patients with trauma or who are undergoing surgery (Miller et al. 2006; Fleming et al. 2009). CDT has been found to be an accurate marker for detecting patients at-risk for alcohol-related surgical complications, alcohol withdrawal, an increased risk of complications, and a prolonged ICU stay after severe trauma (Spies et al. 1998). Recently, fatty acid ethyl esters (FAEEs) in meconium, particularly ethyl linoleate and ethyl AA, emerged as reliable, direct biological markers for establishing gestational ethanol exposure among recently delivered babies. Among the minor non-oxidative products of ethanol metabolism, ethyl glucuronide (EtG) and ethyl sulfate (EtS) may also be measured (Lamy and Thibaut 2011). Although

none of these markers singularly has adequate sensitivity and specificity for screening, their diagnostic utility increases when measured as part of a panel of markers. These latter markers are also durably deposited in hair (fifth month of pregnancy in total) and may also be measured in mothers or newborns' hair. Indeed, the retrospective detection of alcohol consumption during pregnancy is an important part of the diagnosis of foetal alcohol syndrome and foetal alcohol spectrum disorders. Ethanol metabolites, in conjunction with the AUDIT, were most likely to detect drinking in 2nd-trimester pregnant women in a hospital setting (Wurst et al. 2008).

Workplace and criminal justice settings

A survey of aftercare programs in the US for health care professionals with substance abuse problems found that a range of alcohol biomarkers are being used to monitor abstinence (Hayes et al. 1989). Additionally, there is some evidence that CDT is a complementary test to the AUDIT in screening for alcohol abuse disorders among transportation workers (Hermansson et al. 2000). Similarly, there is a considerable need for better alcohol use disorders diagnostic tools in criminal justice settings. One study found that under-reporting was common in "driving while impaired" (DWI) offenders (Lapham et al. 2004).

Others

It was reported that ethanol induces elevation of Fas/Apo-1 mRNA and activated caspase 3 (Saito et al. 1999; Cheema et al. 2000); on the other hand, in neuronal cells, a biphasic response to the Ca²⁺-channel (NMDA receptor) depends on the duration of ethanol ingestion (Kumari and Ticku 2000). Short-term exposure attenuates the channel activity; however, long-term exposure up regulates the number of channels, which in turn increases the intracellular Ca²⁺-concentration. Using neuronal and glial cell cultures, increased expression of annexin IV, a calcium and phospholipid binding protein, has been found in relation to heavy ethanol exposure (Ohkawa et al. 2002) and annexin IV may be a specific marker for the effects of ethanol. The augmented expression of annexin IV in specimens from alcoholics suggests that it may be involved in the recovery from the damage caused by alcohol.

Trait markers for alcoholism

Trait markers for alcoholism could potentially help identify those individuals genetically predisposed to develop alcoholism. Trait markers should be

heritable, associated with alcoholism in the general public and be present before alcoholism develops (Schuckit 1986). Ideally, a trait biomarker should share many of the same characteristics required of an ideal state biomarker: specific to the illness, easy to measure, reproducible, not be harmful to the individual, and be cost effective.

Clinical significance of trait biomarkers

Trait biomarkers could be useful in estimating the risk of developing alcoholism and, potentially, for predicting the course of the illness. Initially, knowledge of alcoholism susceptibility might facilitate early diagnosis (McCaul et al. 1991). Furthermore this information might have a significant influence on preventing alcoholism. For example, if the individual is an adolescent, identifying a trait related to the potential development of later alcohol problems might encourage the initiation of parental or school preventive actions, both of which have demonstrated some effectiveness in preventing the start of substance use (McCaul et al. 1990). Knowledge of trait biomarkers could also affect selection of treatment strategies, particularly the intensity of treatment and its timing, by providing information about the severity or prognosis of the illness. They also may assist in identifying/subtyping different classes of alcoholism leading to personalized treatment options. One example of the benefits of using trait markers comes from the PREDICT Study being conducted in Germany (Mann et al. 2009). Here, biomarkers (fMRI, PET, genetic analysis) are used to divide alcoholics into two types: relief craving/drinkers and reward craving/drinkers. These two subtypes are then used in the evaluation of the treatment efficacy of naltrexone and acamprosate (Mann et al. unpublished observations). One arm of the study examines whether variation in the mu-opioid receptor gene *OPRM1*, which is associated with enhanced alcohol consumption, predicts naltrexone efficacy (Oslin et al. 2003). *OPRM1* may be a trait biomarker useful for both subtyping alcoholics and predicting the efficacy of anti-relapse agents. Similarly, a *GATA4* gene polymorphism may be useful in helping to predict the response to acamprosate (Kiefer et al. 2010).

Approaches to trait biomarker discovery

There are no trait biomarkers for alcoholism in routine clinical use today, mainly because of the complexity of the illness. Beginning with Jellinek (1960) various subtype classification systems of alcoholics have been proposed, reflecting clinical recognition of the heterogeneous nature of the disease (Jellinek

1960; Dick et al. 2006a). While there may be genes that directly or indirectly impact the susceptibility for developing alcoholism, it is more likely that there are a larger number of genes that affect intermediate characteristics, or endophenotypes, that then affect alcoholism "risk". Because genetically influenced characteristics (endophenotypes) are presumed to be closer to the genotype than the features of the disease syndrome itself, endophenotypes represent a potentially powerful tool in psychiatric diagnosis, as well as a strategy to explore the genetic basis of complex illnesses such as alcoholism, although this view has been contested (Flint and Munafò 2007).

Numerous endophenotypes have been proposed for alcoholism. These include a person's low level of response (or low sensitivity) to alcohol (i.e. a low LR) and personality features such as impulsivity, novelty-seeking and disinhibition. Also included are other major psychiatric disorders (primarily schizophrenia and bipolar disorder), alcohol craving, the opioid peptide system, and characteristics of at-risk populations derived from electrophysiological and neuroimaging assessments (Winokur et al. 1996; D'Souza et al. 2006; Barr et al. 2007). Endophenotypes potentially possess features desired in trait biomarkers, including specificity and state-independence; however, highly reproducible and easily measurable endophenotypic tests are currently lacking. Furthermore, several proposed alcoholism endophenotypes may also be confounded by the diagnosis of other co-occurring psychiatric disorders, rendering them more complicated diagnostically than alcoholism itself. Despite these obstacles, the endophenotypic strategy has been used to uncover potential trait biomarkers as well as candidate genes involved in the aetiology of alcoholism. Other approaches to this problem include neurochemistry (primarily second messengers and neurotransmitters), neuroimaging, and electrophysiological analysis of at-risk populations for alcoholism, such as the children of alcoholics (COA).

Biochemical measures

Using the definition of an endophenotype, a trait marker must be: (1) heritable (co-segregates with the disease within families and represents the genetic liability among non-affected relatives of subjects); (2) associated with the disease in the general population; (3) state independent; (4) measurable; (5) associated with the causal pathway of the disease; and (6) expected to be genetically less complex. Ratsma's group examined five neurotransmitters as potential markers for alcoholism vulnerability (Ratsma et al. 2002). Two markers were identified: namely, increased basal activity of the serotonin transporter

in platelets and increased responsiveness of the pituitary beta-endorphin system to alcohol challenges that met all of the criteria. The exploration of the serotonin transporter and endorphin systems includes the GABA and adenylyl cyclase (AC) systems. Numerous reports implicate cAMP-dependent protein kinases (PKA) and cAMP responsive element binding proteins (CREB) in alcohol dependence and tolerance. The attenuation of CsF⁻ or forskolin-stimulated platelet AC activity (Menninger et al. 2000) and a quantitative decrease of type 1 AC mRNA (Sohma et al. 1999) were also reported in alcoholic patients. These factors are also considered as markers for lifetime prevalence of alcohol dependence. Recently, a higher alcohol preference has been found in female type 7 AC knockout mice, which is consistent with the findings of type 7 AC polymorphism and its lower expression levels in female alcoholic patients (Desrivieres et al. 2011).

Neuroimaging studies

Imaging studies have shown that chronic alcohol intake is accompanied by volume reductions of grey and white matter, as well as microstructural disruption of various white matter tracts (Bühler and Mann 2011). Distinctively impaired brain functions are associated with volume loss in several key regions such as the hippocampus, in which the visuospatial and learning/memory functions are localized (Pfefferbaum et al. 1995; Sullivan et al. 1995; Agartz et al. 1999). Furthermore, alcohol abuse was associated with a functionally disordered brain reward system including subcortical striatopallidal and extended amygdala. Craving for alcohol leads to functional activation of the amygdala in recently studied abstinent alcoholic patients (Schneider et al. 2001). The volume of the amygdala has been reported to be smaller in chronic alcoholics than in controls (Wrase et al. 2008; Fein et al. 2009). Subcortical volume was found to be less in patients with a comorbid psychiatric diagnosis than controls (Sameti et al. 2011). Behavioural problems, including externalizing disorders, anxiety disorders and mood disorders followed by substance abuse can be related to structural abnormalities of the amygdala, hippocampus, nucleus accumbens, putamen and thalamus (Sullivan et al. 2005; Benegal et al. 2007; Makris et al. 2008). In addition, most neurocognitive studies have focused on testing for specific characteristics in adolescents and young adults (e.g., impulsivity, risk-taking, and novelty-seeking) that might be impaired in groups at-risk for alcoholism, particularly adolescents with a family history of alcoholism (Schweinsburg et al. 2004). From a cognitive neuroscience perspective, adolescence is a

period in which developing “top down” cognitive control processes compete with earlier “bottom up” motivational processes (Casey and Jones 2010). The “top down” processes important for alcoholism vulnerability include resisting temptation and delaying immediate gratification for more long-term goals; “bottom up” processes involve motivational incentives in the environment that might be more responsible for risk-taking and novelty-seeking behaviour (Finn 2002).

Electrophysiology

Numerous EEG studies of at-risk for alcoholism populations have described potential electrophysiological trait biomarkers for alcoholism (Porjesz et al. 1998b). Most notably, electrophysiological endophenotypes have been explored to identify genes involved in alcoholism predisposition (Dick et al. 2006b, among others). Based on the observation that resting EEG beta power is highly heritable and is increased in alcoholics and the offspring of male alcoholics, linkage and linkage disequilibrium analyses were conducted. A strong association between the resting beta frequency, and GABRA2, a gamma-aminobutyric acid (GABA_A) receptor gene on chromosome 4, in alcoholism was subsequently discovered (Dick et al. 2004; Edenberg et al. 2004). Increased resting beta power has been hypothesized to represent an overall CNS disinhibition/hyperexcitability, which might lead to increased alcohol use for its normalizing effect (Begleiter and Porjesz 1999; Rodriguez Holguin et al. 1999).

One of the most consistent electrophysiological findings in alcoholics and their offspring is a lower amplitude P300 (P3) waveform of the event-related potential (ERP) (Porjesz et al. 1998a). This phenomenon is also observed in individuals with other disinhibitory conditions such as conduct disorder, anti-social personality disorder and attention deficit hyperactivity disorder (Cappadocia et al. 2009; Szuromi et al. 2011).

Low response to alcohol

A low alcohol response (LR) is a frequently observed phenomenon in which affected and many “at risk” individuals require more than the usual amounts of alcohol in order to experience the desired alcohol effects (Enoch et al. 2003). Native Americans and Koreans, two groups with high rates of alcohol use disorders, appear to be more likely to have a low LR to alcohol early in life and prior to developing alcoholism (Ehlers et al. 1999; Wall et al. 1999). LR is genetically influenced as shown by twin studies that

indicate that genetics accounts for 60% of the variance of risk for this characteristic (Heath et al. 1999; Viken et al. 2003). Many (~40%) offspring of alcoholics also have a low response to alcohol before they engage in heavy drinking; a low LR early in life is a good predictor of later heavy drinking and alcohol problems (Pollock 1992; Schuckit et al. 1996, 2000). Currently, the best assessment of LR is through subjective testing following alcohol use. LR as measured by a retrospective questionnaire that records the number of drinks typically needed for a range of effects is also both genetically influenced and a good predictor of future alcohol problems. The search for the genes underlying LR is underway. Candidate genes related to the serotonin transporter, *GABA_A* receptor, adenylyl cyclase, and potassium channels are being considered. Recently, polymorphisms in *CYP2E1*, a gene involved in alcohol metabolism, and *GABRG1*, which encodes the *GABA_A* receptor γ -1 subunit, have been linked to the response to alcohol (Ray and Hutchison 2009; Webb et al. 2011).

Others

The ratio of the lengths of the second and fourth finger of the right hand (smaller 2D:4D ratio) have been investigated in alcohol-dependent patients (Casey and Jones 2010). The variation of 2D:4D is reported to be related to the (CAG)*n* tri-nucleotide repeat found within the coding region of the androgen receptors (Manning et al. 2003). Low 2D:4D is known to be associated with psychological traits such as physical aggression, novelty seeking and higher dominance – features frequently reported as possible predictors of substance abuse (Addison and Kurtz 1986; Wills et al. 1994; Williams et al. 2003). A significant association is also reported between the (CAG)*n* tri-nucleotide repeat and craving for alcohol in male patients during withdrawal (Lenz et al. 2009).

Genetics and alcohol use behaviours

A significant genetic influence exists in most psychiatric disorders and accounts for the high frequency of a positive family history seen in these patients. Recent advances in genetic technologies are rapidly expanding the understanding of the mechanisms by which genes influence the onset and course of alcohol use behaviour, including dependence. As more genetic information becomes available, its clinical application will expand and more health care providers will be able to use the information in their clinical practice.

Disease aetiology: ancestry and geography

Genetic research has contributed considerably to the understanding of the aetiology and natural history of all health problems and diseases and their worldwide distribution partly reflects both ancestral and geographic variations. In the case of alcohol use behaviours, the gene coding for aldehyde dehydrogenase, *ALDH2*2*, is known to protect against heavy drinking and alcohol dependence that characterizes some Asian populations. Variations in disease prevalence are due to differences in population composition by race and ethnicity, as well as differences in geographic location. Environmental factors, including health care access, family and neighbourhood environments, social relationships, and other factors also influence both health and disease prevalence.

Genetic traits

Genetic traits in humans are either simple or complex. Simple genetic traits depend on variations in a single gene, cf. Huntington's disease and cystic fibrosis, in which a single gene mutation alters or destroys a particular biological function. However, single gene disorders are quite rare in the human population and are often observed in less than one in five thousand individuals. Complex genetic traits are influenced by both genetic and environmental factors. Most commonly, these traits have multiple gene influences where variations in one gene influence the risk for developing a disease by interacting with other genes and/or the environment. Complex genetic traits are common in the human population, for example, heart disease, Alzheimer's disease, and diabetes.

Psychiatric disorders, including alcohol use disorders, are also considered to be complex traits, with many pathways leading to their development. The prevalence of alcohol use disorders among biological family members is high, with more than 80% of patients having at least one first- or second-degree affected relatives. Genetic and other psychological and environmental factors (which can also be passed between generations) are likely to be involved in the development of a mental disorder in an affected individual. While genetic influences are clearly important in determining susceptibility for alcohol use disorders, genetic and other biological factors alone cannot fully explain a person's vulnerability for developing an alcohol use disorder or alcohol-related problems.

Genetic studies of alcohol use disorders

Several sources, including basic laboratory research, family studies, and molecular genetic studies provide evidence for the genetic basis of alcohol use disorders

(Ducci et al. 2008; Enoch 2012). Studies of the genetic causes of both medical and behavioural disorders begin with establishing whether genetic influences are involved. Evidence for this depends on studies of the pedigrees of large and multigenerational families, fraternal and identical twins, or of persons who were adopted at an early age and raised apart from their biological parents in a different family environment. Each of these methods has strongly suggested a role for genetic influences in susceptibility to alcohol use disorders. Once the familial/genetic nature of the trait is established through these studies, how the disorder is transmitted across generations and the strength of the genetic influence can then be investigated. Each participant (affected individual and family members) is interviewed, including a standardized diagnostic assessment for phenotyping purposes, and a tissue sample (blood or saliva) is obtained. Linkage studies are then conducted to identify chromosomal regions, followed by association studies to identify specific genes. The function of each identified gene (functional genomics) can then be studied to better determine the possible genetic or biological mechanisms that link the gene to the trait of interest (Nurnberger and Bierut 2007).

The Collaborative Study of the Genetics of Alcoholism (COGA)

One of the best examples of a genetic study of alcohol use behaviours, including alcohol dependence, the Collaborative Study of the Genetics of Alcoholism (COGA), is funded by the US National Institutes of Health (NIH). Beginning in 1989, it is a multi-site national study, currently involving 11 sites. COGA uses an extended family pedigree design and has collected data from over 12,000 adults and 4,500 children and adolescents representing over 1,900 families. The primary goal of the COGA project is to characterize the familial distribution of alcohol dependence within families and to identify vulnerability genes for alcohol dependence and related conditions using genetic linkage and association methods. To date, more than 25 different genes have been identified that are associated with alcohol dependence and related conditions (Table II).

These genes are known to have roles in the different neurotransmitter systems, alcohol metabolism, sensitivity to the effects of alcohol, or taste preference. Most of these genes are associated with an increased risk for alcohol dependence, but some are protective. From both the literature and Table II, it has become clear that few genes predispose to only a single condition. Few genes are trait specific, but more often genes contribute to the predisposition to

various psychiatric conditions and related traits. For example, the *GABRA2* gene is associated with conduct disorder, antisocial personality disorder, alcohol dependence and other drug dependencies (Dick et al. 2007). These results validate the model proposed by Kendler and colleagues that many complex traits/psychiatric disorders are influenced by a cluster of genes that are common to several related psychiatric conditions (Kendler et al. 2003). As part of COGA, Edenberg et al. (2006) found that some genes are associated with more severe alcoholism, including an early age of onset of drinking and the development of alcohol dependence and more severe symptoms, and also to conduct problems.

Copy number variations (CNVs) have also been examined for their role in alcohol dependence susceptibility. A recent report by the Study of Addiction: Genetics and Environment (SAGE) investigators (2011) has identified CNVs in 6q14.1 ($P = 1.04 \times 10^{-6}$) and 5q13.2 ($P = 3.37 \times 10^{-4}$) as being highly significantly associated with alcohol dependence after adjusting for multiple testing. On chromosome 5q13.2, there were multiple candidate genes previously associated with various neurological disorders. This same region on chromosome 6q14.1 has also been associated with mental retardation and language delay.

To date, the use of genome-wide association studies (GWAS) of alcohol dependence and related problems has had limited scientific impact because extremely large samples are required to satisfy the statistical power requirements of such studies. A successful study typically requires pooling multiple data sets from different investigators and often, different populations. Consequently, insufficient phenotypic information is available to test complex traits related to a diagnosis. Rather, only simple phenotypes, such as average alcohol consumption, can be tested. A report by Schumann et al. (2011) using a combined sample of approximately 26316 subjects of European descent drawn from 12 different studies, identified a single nucleotide polymorphism (SNP) in the autism susceptibility gene *AUST2* as being associated with alcohol consumption.

Gene–environment interplay

There is a rapidly growing literature from twin studies and other investigations documenting how specific environmental factors may moderate the impact of genetic effects on alcohol use behaviours and alcohol dependence. An important example of gene–environment interaction by Heath et al. (1989) demonstrated that genetic influences on alcohol use were greater among unmarried women, whereas having a marriage-like relationship reduced the impact of

Table II. Vulnerability genes for alcohol dependence and related conditions using genetic linkage and association methods in COGA studies.

Genetic location	Encoded protein function	Linked to other traits gene effect
<i>ADH4</i> Chromosome 4	Alcohol dehydrogenase; alcohol metabolizing enzyme	None Increased risk
<i>ALDH2</i> Chromosome 12	Aldehyde dehydrogenase; aldehyde metabolizing enzyme	None Protective
<i>CHRM2</i> Chromosome 7	Muscarinic acetylcholine receptor M2; regulates neural signalling	Major depression; drugs Increased risk
<i>DRD2/ANKK1</i> Chromosome 11	Dopamine D2 receptor; regulates reward reinforcement	Habitual smoking Increased risk
<i>GABRG3</i> Chromosome 15	GABAa receptor g3 subunit; regulates neural signalling	Drug dependence, CD Increased risk
<i>GABRA2</i> Chromosome 4	GABAa receptor a2 subunit; regulates neural signalling	Drugs; CD, ASPD Increased Risk
<i>GABRA1</i> Chromosome 5	GABAa receptor a1 subunit; regulates neural signalling	Drinking patterns
<i>HTAS2R16</i> Chromosome 4	hTAS2R16 receptor; contributes to bitter taste sensitivity	Increased drinking Increased risk
<i>HTAS38R</i> Chromosome 4	hTAS2R16 receptor; contributes to bitter taste sensitivity	Heavy consumption Increased risk
<i>CHRNA5</i> Chromosome 15	Nicotinic acetylcholine receptor; nACHr modulated by ethanol	Alcohol, Tobacco Dependence Increased risk
<i>CHRNA3</i> Chromosome 15	Nicotinic acetylcholine receptor; nACHr modulated by ethanol	Nicotine Dependence Increased risk
<i>ADH1A/ADH1B</i> Chromosome 4	Alcohol dehydrogenase; alcohol metabolizing enzyme	None Increased Risk
<i>CNR1</i> ; Chromosome 6	Cannabinoid receptor 1: regulates dopamine reward system	Cannabis dependence Increased risk
<i>OPRK1</i> ; Chromosome 8	Kappa opiod receptor 1; regulates neural signalling	None Increased risk
<i>PDYN</i> Chromosome 20	Kappa opiod receptor 1; regulates neural signalling	Alcohol Dependence Increased risk
<i>POMC</i> Chromosome 2	Adrenocorticotrophic hormone	Opioid dependence Increased risk
<i>PENK</i> Chromosome 8	Proenkephalin	Opioid dependence Increased risk
<i>OPRL1</i> Chromosome 20	Opiate receptor-like	Opioid dependence
<i>NPY2R/NPY5R</i> ; Chromosome 4	Neuropeptide Y receptors; anxiolytic regulation	Alcohol Dep &Withdrawal Increased risk
<i>NFKB1</i> Chromosome 4	Transcription factor NF- B-1; regulates neural signalling	Alcohol Dependence Increased risk
<i>CRHR1</i> Chromosome 17	Corticotropin releasing hormone receptor	VP3 amplitude; alcohol dependence Increased risk
<i>TACR3</i> Chromosome 4	Tachykinin receptor 3	Alcohol dependence; cocaine dependence Increased risk
<i>GRM8</i> Chromosome 7	Glutamate receptor, metabotropic	Alcohol dependence; ERO Increased risk
<i>ACN9</i> Chromosome 17	ACN9 homologue (<i>S. cerevisiae</i>)	Alcohol dependence; ERO Increased risk
<i>SNCA</i> Chromosome 4	Synuclein, alpha	Alcohol craving Increased risk
<i>SLC6A4</i> Chromosome 17	Solute carrier family 6 (serotonin transporter)	Depression Increased risk

genetic influences on drinking. Dick et al. (2006b) have reported that both *GABRA2* and marital status contribute independently to the development of alcohol dependence. The high-risk genotype at *GABRA2* was also related to a decreased likelihood of marrying and an increased likelihood of divorce,

which appeared to be mediated in part by personality characteristics. There was also a differential risk for alcohol dependence associated with the *GABRA2* genotype according to marital status. A similar interaction has been shown with social support, in general, and the *GABRA2* genotype (Pescosolido et al.

2008). Religious beliefs have also been shown to moderate genetic influences on alcohol use among females, with genetic factors playing a larger role among individuals without a religious upbringing (Koopmans et al. 1999).

The importance of genetic influences on alcohol use can also vary as a function of neighbourhood and socio-regional factors such as urban/rural residency, neighbourhood stability, and regional alcohol sales (Dick et al. 2001; Rose et al. 2001). Genetic influences on adolescent substance abuse appear enhanced in environments with less parental monitoring (Dick et al. 2007b) and in the presence of substance-using friends (Dick et al. 2007c). Genetic and environmental risks for substance use disorders typically do not only add together, but also interact with each other, during development (Kendler 2012). In summary, there are a number of environmental factors, across a variety of different domains that moderate the importance of genetic influences on patterns of alcohol use. With respect to alcohol use, it appears that environments permitting greater opportunity to express genetic predispositions, as exemplified by environments with low parental monitoring and high(er) peer alcohol use, are important moderators of genetic effects.

Using genetic information in clinical practice

Genetic information can be used in many ways to improve clinical practice, although genetic testing and the implications of sharing genetic information raise several issues and concerns. In the future, using information from a patient's genome, clinicians will be able to predict whether a client is likely to develop a disease and, if he does, to make an early diagnosis. Further, patients will be able to receive personalized treatment and prevention strategies based on his/her genetic profile.

Genetic screening is presently available for several diseases including breast cancer, Huntington's disease, and Alzheimer's disease; however, to date, no specific screening is available for psychiatric disorders. Any screening test needs to be accurate (i.e. have both sensitivity and specificity) to be useful, but the nature of complex gene disorders limits the application of a reliable screening test. Recently, pharmacogenetic analyses of treatments for alcohol dependence have attempted to predict treatment response and side-effect risk for specific medications. Variation in the *DRD4* gene, which encodes the dopamine D4 receptor, is suggested to predict better response to naltrexone and olanzapine. A polymorphism in the serotonin transporter gene *SLC6A4* promoter region has been related to differential treatment response to sertraline, depending on the

subject's age of onset of alcoholism (Arias et al. 2012).

Whenever sharing genetic information, the clinician must ensure that the patient understands that for complex trait disorders, genetic susceptibility is not absolute and that much of the heritable component of chronic disease remains to be discovered. Common questions from patients include: How much risk do I actually have? What treatments might work best for me? What is my long-term prognosis? It is important for patients to understand that even complete knowledge of genetic susceptibility factors will not totally determine the risk for developing a disorder, as many cases are due to non-genetic factors and are labelled "sporadic cases". A patient's own responsibility and accountability are vital factors in managing the patient's risks and symptoms.

The prospects of biomarkers for alcoholics in the future

Studies of state markers are best conducted in animal or other model organisms, such as inbred mice, because genetic and environmental factors that influence alcohol-related traits can better be manipulated under controlled environmental and genetic conditions. If animal models are available, improved studies of specific alcohol-related endophenotypes (e.g., alcohol preference, sensitivity, tolerance, and dependence) will advance scientific progress. Endophenotypes can help improve our understanding of the aetiology of the endophenotype and provide a means for identifying which genetic factors might be most fruitful to study in humans. Several inbred mice lines have been widely used in mapping quantitative trait loci (QTL) for certain endophenotypes. These selected lines differ with respect to various alcohol-related traits and the genes that contribute to differences in the alcohol response.

Alcoholic consumption varies depending on mouse strains; some strains have a tendency to readily consume alcohol and demonstrate alcohol-related physical symptoms. Animal models of addiction can be organized within the stages of the addiction cycle, including: binge/intoxication, withdrawal/negative affect (anxiety-like responses, conditioned place aversion, elevated reward thresholds, withdrawal-induced increases in drug self-administration), and preoccupation/anticipation (drug-induced reinstatement, cue-induced reinstatement, stress-induced reinstatement) (Koob 2012). These models have the possibility to provide insights into the neurobiological mechanisms of addiction. However, it is possible that the mouse model does not accurately reflect the human condition. Further, the diverse

human genetic background due to the admixture of different populations complicates genetic studies. However, human population studies are essential to elucidate the pathophysiology of human disease.

Since blood sampling is routinely done for annual health check-ups, plasma biomarkers would be ideal for identifying alcohol-related conditions. To identify plasma biomarkers, proper procedures are essential to eliminate unnecessary blood components (e.g. albumin) and to isolate some fractions in which necessary component(s) are enriched. However, the great differences in concentrations and the vast number of plasma protein constituents make it almost impossible to directly identify plasma biomarkers for alcoholism, even with high throughput techniques.

Conclusions

The search for optimal biomarkers of alcohol consumption (state) and for the genetic predisposition toward alcohol dependence (trait) continues. Although currently used state markers are of some value, their limitations and weaknesses justify the continued search for more sensitive and specific markers.

The importance of a marker's precision, accuracy, sensitivity, and specificity cannot be overstated. Although it is unlikely that researchers will find a single marker to satisfy all clinical needs, they may eventually develop combinations of markers for specific clinical purposes, from unselective screening (i.e. drinking versus not drinking) to confirming a suspicion of alcohol abuse or dependence.

Like most human behaviours, alcohol consumption patterns are aetiologically and phenotypically complex. Clinicians often need to detect patterns of drinking other than the chronic, heavy drinking patterns revealed by GGT, AST, ALT, and CDT. For example, clinicians may need to know whether a person has been drinking recently or the type of drinking that has occurred (e.g. heavy or social drinking). Therefore, finding new biomarkers that measure the many different aspects of alcohol consumption will vastly improve the clinician's ability to manage alcohol-related problems.

In addition, the ability to study in-depth the multiple factors that contribute to the development of "alcoholism" will depend on creating more homogeneous subgroups by use of endophenotypes or other complex phenotypic models. This can be achieved through the development of new classification schemes based on genetic/biological, physiological, and behavioural factors, including alcohol-metabolizing enzymes, neurophysiological waveforms, a low level of response to alcohol, externalizing or disinhibited

behaviours, and possibly other psychiatric conditions. Efforts are underway to identify genes that contribute to each of these and other intermediate phenotypes. Additionally, it will be important to understand how the phenotypes/endophenotypes and multiple related genes correlate and interact with environmental and cultural forces to enhance or diminish the risk for alcoholism.

An ultimate (and implicit) goal of the work described in this article is to develop more effective prevention techniques. Increased understanding of the biological mechanisms and genetic impact associated with a specific type of increased vulnerability to alcoholism could enhance prevention efforts in several ways. For instance, children at high risk for developing alcohol-related problems, including children of alcoholics, could be screened to determine if their risk is likely to operate through LR, externalizing behaviours, or other phenomena, such as cognitive/neurophysiological processes. This information may then be used to suggest which specific environmental or cultural factors enhance the risk for a specific mechanism and, more importantly, identify those factors that might diminish the risk. Armed with these data, more focused and effective preventative trials can be developed.

An equally important goal is to expand our understanding of data which might enhance the evaluation of existing treatments and the development of new therapeutic approaches for alcohol use disorders. The more that is known about the specific neurochemical systems that contribute to alcoholism, the better our ability to develop new and more effective pharmacologic and behavioural approaches to help alcoholics recover.

Finally, researchers should further develop the markers described here and seek new biomarkers. These findings will contribute to a stronger basis for clinical care and a more objective assessment of alcohol consumption and possibly the genetic predisposition to alcohol use disorders.

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