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Genetically selected alcohol preferring rats to model human alcoholism

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Abstract

Animal models have been successfully developed to mimic and study alcoholism. These models have the unique feature of allowing the researcher to control for the genetic characteristics of the animal, alcohol exposure and environment. Moreover, these animal models allow pharmacological, neurochemical and behavioural manipulations otherwise impossible. Unquestionably, one of the major contributions to the understanding of the neurobiological basis of alcoholism comes from data that have been obtained from the study of genetically selected alcohol-preferring rat lines and from the consequences that alcohol drinking and environmental manipulations (/i.e., protracted alcohol drinking, intoxication, exposure to stress etc) have on them. In fact, if on the one hand genetic factors may account for about 50–60% of the risk of developing alcohol dependence, on the other hand protracted alcohol exposure is a necessary precondition to actually develop the disease, while environmental vulnerability factors may be crucial for disease progression. The present article will offer an overview of the different genetically selected alcohol preferring rat lines developed and used to study alcoholism. The predictive, face and construct validity of these animal models and the translational significance of findings achieved through their use will be critically discussed.

Keywords

alcohol preferring rat; genetic selection; animal models; alcohol drinking; relapse; self-administration

Introduction

Alcohol dependence develops gradually, occurs over the course of years, and requires prolonged and repeated exposure of the brain to significant blood-alcohol levels. Both preclinical and clinical research have clearly demonstrated that the presence of genetic traits provides an important contribution to the development of this pathological condition (Cloninger et al. 1981; Sigvardsson et al. 1996), and recent twin studies estimate the contribution of genetic susceptibility factors to 48–58% (Kendler et al. 1997; Prescott and Kendler 1999). However, whether genetically encoded vulnerability is present or not, the process of actually developing dependence is influenced by a number of other factors, such

as drug availability, environmental conditions and stress (Ciccocioppo et al. 2001; Katner et al. 1999; Le et al. 1998; Martin-Fardon et al. 2000; Martin-Fardon et al. 2010; Monti et al. 1999; Rohsenow et al. 2000). The pathological traits of alcoholism are complex and over the years various theoretical frameworks have been proposed to explain it. A common consensus has been reached, however, on the concept that alcoholism is polygenic in nature, that are different typology of patients and that pharmacotherapies should be optimized according to the patient subgroup treated (Goldman et al. 2005; Heilig and Egli 2006; Heilig et al. 2011). Translated into preclinical research, all these levels of complexities are such that they cannot be mimicked by univocal experimental protocols or laboratory animal models. Nevertheless, while it is recognized that animal models of alcoholism may not be entirely congruent with the human condition, it should be agreed that there are minimal criteria that must be met for an animal model to be considered valid. Therefore, as discussed for other psychiatric disorders (McKinney and Bunney 1969; Newport et al. 2002; Willner 1984) an animal model must resemble the human condition in several respects: 1) should be sensitive to amelioration or attenuation of the symptoms by treatments effective in humans, and conversely insensitive to those treatments that are inactive in attenuating the human disorder (*predictive validity*); 2) should mimic the fundamental behavioral characteristics of human alcoholism and should be characterized by the same symptoms profile (*face validity*); 3) the pathology should be triggered by events thought to be important in eliciting the human disorder and should involve similar neurochemical, neurobiological and psychobiological mechanisms (*construct validity*).

Over the years intense work from several research groups has allowed the development of a number of new preclinical procedures and new animal models to integrate the study of the genetics of alcoholism and the role played by the environment in disease progression. The use of rat lines genetically selected for high ethanol preference or excessive alcohol drinking represented one of the first and most important preclinical approaches to the study of alcohol addiction. Another important contribution to the field has come from the development of well validated experimental paradigms to induce excessive alcohol drinking or chronic alcohol intoxication leading to dependence. In fact if the genetics is a predisposing factor, exposure to repeated intoxicating alcohol experiences is a necessary condition to facilitate the progression from alcohol use to abuse and dependence.

The present article has the objectives of: 1) providing an update on the different rat lines that have been genetically selected for high alcohol preference; b) to discuss the contribution of these models in advancing our understanding of the genetics the neurobiology and the physiopathology of alcoholism; c) to provide a critical discussion on the validity of these animal models and the translational significance of findings achieved through their use.

History

Historically, the rat is one of the laboratory animals most frequently and widely used to study the physiopathology and the pharmacology of alcoholism. However this animal, especially under continuous exposure does not drink alcohol spontaneously or if it does it consumes low amounts. About 60 years ago, to overcome this problem, alcohol researchers initiated, at that time, very ambitious programs to obtain new rat lines genetically selected

for high alcohol drinking and preference. This objective was achieved by selective mating of animals with a higher spontaneous preference for alcohol. After repeated mating across generations the genetic traits sub-serving excessive alcohol drinking have been permanently segregated into these rat lines. Simultaneously, control nonpreferring lines were selected for extremely low alcohol preference. As a result of this work we have now available at least 6 different genetically selected alcohol preferring and nonpreferring rat lines around the world (Table 1). The first selective breeding program for rats differing in alcohol consumption began in 1950 at Universidad de Chile (UCH) with the development of high alcohol-drinking (UChB) and low alcohol-drinking (UChA) lines (Mardones and Segovia-Riquelme 1984; Mardones et al. 1953; Quintanilla et al. 2006). About ten years later another selection program was started at Alko Research Laboratories in Finland where through bidirectional selection two rat lines, AA and ANA, were selected for high (Alko; Alcohol) and low (Alko, Non-Alcohol) alcohol preference, respectively (Eriksson 1968). Following Mardones' (1953) and Eriksson's (1968) pioneering work two similar programs were initiated in USA (Indianapolis, IN) and in Italy (Cagliari, Sardinia). These programs resulted in the development of the alcohol preferring (P) and non preferring (NP) rats and of the sardinian preferring (sP) and sardinian nonpreferring (sNP) rats, respectively (Colombo et al. 2006; Li et al. 1979). A few years later, at the University of Indianapolis the same research group initiated a new breeding program for a bidirectional separation of another line of alcohol preferring and nonpreferring rats. This led to the generation of a replicate line named High Alcohol Drinking (HAD) and Low Alcohol drinking (LAD) respectively (Murphy et al. 2002). Over the years in a few cases these rat lines have been transferred in laboratories other than those where they were original developed. In the new laboratories these rat lines were re-derived. For example, 1988 a few pairs of sP rats were donated by Prof Gessa (University of Cagliari, Italy) to colleagues at the University of Camerino (Italy). In 1998, after 20 generations of selective breeding, at the Dept. of Experimental Medicine and Public Health of the University of Camerino, these animals have been re-named msP (for details see; Ciccocioppo et al. 1999a). This distinction was made for several reasons: first, when the genetic selection from sP started in Camerino the high alcohol drinking phenotype of the original sP line was only partial. In addition, the two breeding programs were carried out under different husbandry conditions and used slightly different selection criteria. Hence, the genotypic and phenotypic characteristics of sP and msP rats might have been different. About 15 years ago another colony derived from sP rats was established at The Scripps Research Institute (La Jolla, CA). For this line the original name sP was maintained and the first scientific article ever published with animals belonging to this colony appeared about five years ago (Sabino et al. 2006).

In the recent years also the Indiana P line was re-derived to obtain an inbred strain (iP) that is now maintained also at the Howard Florey Institute (University of Melbourne). This inbred strain has retained the high alcohol drinking phenotype of the parental line and has been extensively use for pharmacological and for genetic studies (Carr et al. 2007; Cowen et al. 2005; Hargreaves et al. ; Kimpel et al. 2007; Rodd et al. 2007).

The first paper on genetically selected alcohol preferring rats by Mardones et al. appeared in 1953 but since than the number of publications on these rat lines has grown constantly and now we can count a few hundred research papers already published. The work conducted

with these animals has provided a unique contribution for the understanding of the genetics, the neurobiology and the physiopharmacology of alcoholism

Genetically selected alcohol preferring rats: Predictive Validity

In recent years one of the most exciting developments in the field of alcoholism is the introduction of effective medications such as naltrexone and acamprosate (Sass et al. 1996; Volpicelli et al. 1992). These agents proved the feasibility of pharmacological treatment of alcoholism. More recently, other drugs have been tested in the clinic for their ability to reduce ethanol drinking and relapse. The results of these initial studies showed, for example, that ondansetron, an antagonist of the serotonin 5HT₃-receptor, exerts marked beneficial effects, but did so exclusively in early-onset patients (Johnson et al. 2000a; Johnson et al. 2000b). Other drugs of interests are those compounds that modulate central GABAergic transmission or reduce neuronal excitability; such as topiramate, gabapentin and pregabalin, as well as the GABA B receptor agonist baclofen (Addolorato et al. 2000; Johnson 2005; Martinotti et al. 2008; Mason et al. 2009; Stopponi et al.). A large body of evidence indicates that genetically selected alcohol preferring rats are highly sensitive to inhibition of alcohol consumption by treatments that have shown efficacy also in humans which may support the predictive validity of these animal models. For example naltrexone, a drug extensively used in the clinical practice and that reduces alcohol consumption and cue reactivity in humans lowers alcohol intake also in several lines of alcohol preferring rats. The efficacy of naltrexone was documented in the P (Dhaher et al.) the HAD (Krishnan-Sarin et al. 1998), the sP (Sabino et al. 2006), the AA (Koistinen et al. 2001) and the msP lines (Ciccocioppo et al. 2007; Perfumi et al. 2005). Another drug of proven efficacy in alcoholics is acamprosate (Mann et al. 2008). Studies in iP and in AA alcohol preferring rats confirmed the efficacy of this drug on alcohol drinking thus offering additional evidence for the predictive validity of these animal models (Cowen et al. 2005).

Another example supporting the predictive validity of alcohol preferring rats is 5-HT₃ antagonism. Relatively recent studies, in fact, revealed promising therapeutic effects with the 5-HT₃ selective antagonist ondansetron that appeared to be particularly effective in early onset alcoholics (Johnson et al. 2000a; Johnson et al. 2000b). Notably, ondansetron was able to reduce alcohol intake also in the P rat. While MDL72222, another selective 5-HT₃ receptor antagonist resulted effective in P and in sP rats (Fadda et al. 1991; McKinzie et al. 2000; Rodd-Henricks et al. 2000)

According to the definition of predictive validity, if a medication is inactive in humans alcoholics it should also be inactive in alcohol drinking animals. An interesting example is offered by the selective serotonin 5-HT₂ receptor antagonist ritanserin that was shown to be unable to reduce ethanol drinking in patients (Johnson et al. 1996) and also in msP (at that time named sP) rats (Ciccocioppo et al. 1995). In this case, the predictive value of msP rats appeared to be higher than that of AA and P rats in which blockade of 5-HT₂ receptors reduced ethanol drinking (Overstreet et al. 1997; Roberts et al. 1998). Of interest is the unusual case of the Selective Serotonin Reuptake Inhibitors (SSRI). In preclinical research, these drugs showed efficacy in almost all experimental animal models used to investigate their effect on alcohol drinking, including genetically selected alcohol preferring rats

(Ciccocioppo et al. 1997; Maurel et al. 1999; Murphy et al. 1985; Rezvani et al. 2000). In addition, reinstatement studies (ie., resumption of extinguished drug-paired lever responding following extinction or after an imposed period of abstinence) demonstrated that fluoxetine reduces also stress-induced relapse in rodents (Le et al. 1998). Contrary to what animal research predicted, treatment with this class of compounds showed very little, if any, efficacy in humans (Garbutt et al. 1999; Nunes and Levin 2004). If we consider that SSRIs markedly inhibit ingestive behavior in general, one could explain this false positive by hypothesizing that the reduction of ethanol drinking in laboratory animals is an epiphenomenon associated to the anorectic effects of these agents. This could be particularly true for genetically selected alcohol-preferring rats because due to their high ethanol consumption (6–8 g/kg day) they retain a considerable amount of calories from alcohol. Hence, their drinking behavior could be highly sensitive to pharmacological manipulation of feeding related mechanisms. Some clinical evidence suggest, however, that SSRIs may have some beneficial effects on ethanol drinking and on other ethanol related behaviors in patients with a diagnosis of comorbid depression (Nunes and Levin 2004). In a forced swimming test study it was shown that msP and sP rat have a particularly high level of depressive-like behaviors. Those were reversed by repeated intragastric ethanol administrations or by treatment with the anti-depressant drug desipramine (Ciccocioppo et al. 1999a). These data may suggest that in these rat lines ethanol has an antidepressant-like action that may contribute to their high motivation to drink ethanol for self-medication purposes. This may provide an alternative explanation for which treatment with fluoxetine (Ciccocioppo et al. 1997) or desipramine, removing the depressive-like negative state typical of these animals, may significantly lower their spontaneous ethanol drinking.

Genetically selected alcohol preferring rats: Face Validity

In the Diagnostic and Statistical Manual of Mental Disorders (DSM IV), alcohol dependence is defined as a maladaptive pattern of drug use leading to clinically relevant impairment and distress associated with specific phenomena such as drug intoxication, development of tolerance, occurrence of withdrawal, uncontrollable drug seeking and continuous use of the drug despite knowledge of its negative effects. To have face validity, an animal model of alcoholism has to mimic the fundamental behavioral characteristics of human alcoholism and should be characterized by the same symptoms profile. Some of these characteristics are intrinsically expressed in all genetically selected alcohol preferring rat lines. For example, all of them are characterized by consumption of pharmacologically relevant daily doses (6–8 g/kg even 10 g/kg) of ethanol. In addition, several reports showed that alcohol consumption in these animals is largely concentrated during the active phase (night) of the light dark cycle when intake is organized in bouts of several millilitres of ethanol (up to 10–15 ml of 10% ethanol) leading to blood alcohol levels (BAL) above 50 mg/dl, thus indicating that intake is largely driven by the pharmacological properties of alcohol. (Ciccocioppo et al. 2006). Few studies also evaluated the taste reactive response of these rat lines to alcohol. Results showed, for instance, that compared to ANA controls, naïve AA rats make significantly higher levels of ingestive responses to ethanol. A further increase is observed in ethanol experienced AA rats. These two rat lines emit identical aversive responses to alcohol but following a period of acclimation they are reduced in the AA but not in the ANA line

(Badia-Elder and Kiefer 1999). A similar finding was reported in a taste reactivity study in msP rats in which it was shown that these animals do not show aversive reactions to ethanol following its passive infusion into the mouth (Polidori et al. 1998). This can, at least in part, explain why msP rats voluntarily drink large amount of alcohol from the very first presentation (Ciccocioppo et al. 2006). Lastly, studies in UChB and in P rats, showed that when compared to their nonpreferring counterparts they have less sensitivity to the aversive effects of alcohol (Froehlich et al. 1988; Quintanilla et al. 2001). Notably, UChB rats appear to be insensitive to the aversive effects of acetaldehyde, the main alcohol metabolites, that in UChA rats produces, instead, a marked conditioned taste aversion (Quintanilla et al. 2002).

Overall these data demonstrate that genetically selected alcohol preferring rats seek ethanol and shape their behavior to obtain pharmacological effects from its intake. This concept is further supported by self-administration experiments showing, for example, that P rats can lever press to infuse alcohol directly into the stomach or the ventral tegmental area of the brain (Gatto et al. 1994; Waller et al. 1984). Consistent findings were obtained also in msP rats in which place conditioning studies revealed that in this rat line intragastric administration of 0.7–1.5 g/kg of alcohol elicited a marked conditioned place preference (Ciccocioppo et al. 1999b). Conversely, in nonselected Wistar rats, intragastric alcohol administration leads to conditioned aversive responses (Fidler et al. 2004).

Another key feature in alcohol addiction is that subjects voluntarily drink intoxicating doses of alcohol that, after abrupt discontinuation of intake, terminates into a withdrawal syndrome. Drinking to intoxication is very difficult to observe in laboratory animals. However, it was shown that P rats can develop physical dependence upon protracted exposure to 10% alcohol under free choice (water vs alcohol) condition (Kampov-Polevoy et al. 2000). Such evidence was not confirmed in msP rats, neither to our knowledge, in other preferring lines. However, this should not be surprising, considering that in msP rats the BALs reached following voluntary ethanol intake generally remain below 100 mg/dl (Ciccocioppo et al. 2006). In fact, as reported in many studies, physical symptoms of alcohol withdrawal appears following intoxication paradigms aimed at reaching BALs of at least 150 mg/dl (Braconi et al. 2009; Hermann et al. ; Majchrowicz 1975; Penland et al. 2001; Rimondini et al. 2002) In humans, alcohol withdrawal is also characterized by a number of psychological symptoms that includes agitation, anxiety, depression and dysphoria. Some of these symptoms (i.e., anxiety- and depressive-like signs) can be detected also in laboratory animals, in which they appear after intoxicating doses of alcohol leading to lower BALs compared to those needed to observe physical withdrawal. In one study examining the behavior of msP rats in the forced swimming test it was shown that naïve animals exhibit a longer period of immobility compared to alcohol experienced msP rats allowed to voluntarily drink ethanol for 10 days before the forced swimming test. After 10 days of voluntary 10% ethanol drinking, if alcohol is removed from the home-cage for 10 days, immobility score increases again to values similar to those of naïve rats. Voluntary ethanol consumption or intragastric administration of appropriate doses of alcohol (6.3 g/kg of ethanol given in 9 boluses of 0.7 g/kg of ethanol) administered during the 24 hours preceding the swimming test reduced the immobility time (Ciccocioppo et al. 1999a). Overall these data show that while ethanol exerts an antidepressant-like action at doses that alcohol-preferring rats voluntarily take, an imposed abstinence in alcohol experienced animals exacerbate depressive-

like symptoms (as expected in human abstinent alcoholics). Of note, in the P rat withdrawal from alcohol is followed by increase in anxiety-like behaviors, another affective sign of abstinence (Kampov-Polevoy et al. 2000). Consistently sP rats, compared to their nonpreferring sNP counterpart, showed higher anxiety-like behaviours that was, however, attenuated following alcohol consumption (Colombo et al. 1995). A similar phenotype was described also in the msP line in which heightened anxiety was linked to a genetic mutation occurring at corticotrophin releasing hormone receptor-1 gene (Hansson et al. 2006).

Another interesting phenomenon occurring in alcohol preferring rats and that is associated with alcohol abstinence is the occurrence of a robust alcohol deprivation effect (ADE). If ethanol experienced alcohol preferring rats are withdrawn from ethanol for a few days or weeks, when alcohol access is returned they show a clear shift toward a higher level of drinking. This phenomenon was observed in P, and though to a lower extent in sP and msP rats (Agabio et al. 2000; Perfumi et al. 2005; Rodd et al. 2003). Contrasting findings were described in HAD rats while no ADE was reported in the AA line (Rodd et al. 2009; Vengeliene et al. 2003). The ADE in alcohol preferring rats should be interpreted as the intense motivation of these animals to resume ethanol use following an abstinence period. During disease progression alcohol deprivation experiences are recurrent also in alcoholics. Like in animals, following abstinence episodes, these individuals often report an increasing urge to drink that normally terminates with an uncontrollable severe alcohol intoxication episode. With respect to these characteristics the P and the sP lines appear to more closely mimic human behavior compared to AA and HAD rats.

Clinical studies reveal that conditioning factors and stress may play a major role in maintaining addictive behaviors and in facilitating relapse (Koob and Le Moal 1997; O'Brien et al. 1998). Conditioning hypotheses are based on observations that relapse is often associated with exposure to ethanol-related environmental stimuli. According to this view, environmental stimuli that have become associated with the subjective actions of ethanol by means of classical conditioning throughout an individual's history of ethanol abuse elicits subjective states that can trigger resumption of drug use. Stress may, instead, result in mood dysregulation, disruption of neuroendocrine homeostasis and somatic symptoms such as insomnia that may motivate alcoholic patients to resume drinking to alleviate negative affective states. Alcohol preferring rats represent an excellent model to reproduce these complex behavioral traits described in the human literature. For example, it has been shown that msP rats trained to operantly self-administer 10% ethanol or water in 30-min daily session in the presence of discriminative stimuli associated with the availability of ethanol vs. water, following an extinction period resume their lever pressing for ethanol, but not for water associated cues. Similar behavior was observed also in nonselected Wistars; however, remarkable line differences in the magnitude and persistence of the response-reinstating effect of ethanol-associated stimuli was observed between the two rat lines (Ciccocioppo et al. 2006). Using slightly different conditioning/reinstatement models identical results have been described also in sP and P rat lines (Ciccocioppo et al. 2001; Ciccocioppo et al. 2006; Maccioni et al. 2007). Another important finding was that compared to nonpreferring controls or heterogenous Wistars in the preferring lines cue exposure resulted in a more persistent ability to trigger relapse to alcohol seeking also after a protracted period of abstinence (Ciccocioppo et al. 2001; Ciccocioppo et al. 2006).

These findings not only confirm that the reinforcing properties of ethanol are increased in rats with a genetic predisposition toward heightened ethanol intake but provide evidence that genetically-determined alcohol preference extends to greater responsiveness to the motivating effects of ethanol-associated stimuli. In a recent self-administration study it was also shown that in an extinction-reinstatement paradigm exposure to intermittent foot-shock stress reinstates lever pressing for ethanol in both msP and Wistar rats. However, msP rats showed the highest reinstatement levels following administration of 0.3 mA foot-hock current intensity whereas the maximal responses in Wistars were observed after exposure to 1.0 mA electric current. At 1.0 mA the locomotor behavior of msP rats was impaired because freezing behavior occurred (Hansson et al. 2006). Altogether these data suggest that msP rats are characterized by a heightened sensitivity to stress which may contribute to shape their high ethanol drinking phenotype (Hansson et al. 2006). Of note, in a study where Wistars, AA, HAD and P rats were tested for the alcohol deprivation effect (also a model of relapse) following exposure to foot-shock stress it was found that shock increased alcohol consumption in all rats lines, but the most pronounced effects were observed in the HAD and in the P lines (Vengeliene et al. 2003). Overall these data suggest that genetically selected preferring lines and heterogeneous nonselected rats both show relapse-like behaviors after exposure to stressful stimuli but in the preferring lines the sensitivity appears to be higher. This reflects the results of several clinical studies showing that a large population of alcoholics have lower ability to engage into stress-coping strategies and that resumption of alcohol abuse is often a mechanism to ameliorate the negative affective state in which they precipitate following exposure to anxiogenic stimuli or stress, especially during protracted withdrawal (Bartlett and Heilig 2011; Sinha 2011).

Genetically selected alcohol preferring rats: Construct Validity

An animal model of alcoholism should rely on similar neurochemical, neurobiological, and physiological mechanisms and should be sensitive to the same events thought to be important in eliciting the human disorder in order to have construct validity. Many years of clinical and experimental research have demonstrated that alcoholism is a multifactorial disorder where genetic predisposition associated to environmental factors can contribute to the final level of abuse vulnerability. The fact that genetic selection has lead to obtain animal lines expressing high ethanol drinking phenotype is *per se* an important element of construct validity because it shows that, like in humans, vulnerability to abuse ethanol can be inherited. An additional level of validity comes from co-segregation of the excessive drinking phenotype with high anxiety- and depressive-like affective traits as observed in P, sP and msP lines, reflecting pathological conditions described in large alcohol addict subpopulations (Ciccocioppo et al. 1999a; Colombo et al. 1995; Stewart et al. 1993). On the other hand, in the HAD and in the AA alcohol preference has been associated with impulsive traits suggesting that these rat lines may resemble a different population of alcoholic patients like those characterized by early disease onset, high impulsive behavior and antisocial personality (Enoch 2003; Moller et al. 1997; Sommer et al. 2006; Wilhelm and Mitchell 2008).

An ideal genetic animal model of alcoholism should carry the same genetic traits that are linked to alcoholism in humans. In recent years a wealth of work has been carried out to

understand the genetic basis of alcoholism and a lot of information has been collected. It is now clear that alcoholism is a multigenic disorder and various genetic polymorphisms have been associated to alcohol abuse vulnerability. The most compelling evidence is that polymorphism in the genes encoding different alcohol and acetaldehyde dehydrogenase isoforms can dramatically affect an individual's risk to develop alcoholism. For instance, the slow isoform acetaldehyde dehydrogenase2 (ALDH2) and the fast isoform alcohol dehydrogenase (ADH) are protective against alcoholism (Higuchi 1994; Thomasson et al. 1991; Tu and Israel 1995). Consistent with this finding in humans it was shown that the low alcohol drinking line UChA carries a slow form of ALDH that is not present in the alcohol preferring UChB, thus allowing these latter to consume higher doses of alcohol without experiencing aversive reactions (Quintanilla et al. 2005a; Quintanilla et al. 2005b). Other genes linked to increased vulnerability to develop alcoholism are those encoding for specific variants of GABA_A receptor (*GABRA2* and *GABRG3*), muscarinic cholinergic (*CHRM2*) receptors, opioid receptors (OPRK1, PDYN, OPRL1), alpha-synuclein protein (SNCA), neuropeptide Y (NPY) etc (Dick et al. 2008; Dick et al. 2004; Dick et al. 2006; Foroud et al. 2007; Lappalainen et al. 2002; Xuei et al. 2006; Xuei et al. 2008; Xuei et al. 2007). Polymorphisms at dopamine D2, μ -opioid receptor, and serotonin transporter genes have also been associated with increased vulnerability to develop alcoholism and with a different response to pharmacological interventions see for review (Heilig et al. 2011). The significance of these genes in alcohol preferring rats have not been systematically investigated yet. However, in quantitative trait loci (QTL) mapping studies it was found that in inbred P (iP) rats chromosome 4 is associated with alcohol preference. Compared to the nonpreferring inbred counterpart (iNP) approximately 11% of the phenotypic variability appears to be linked to this QTL. Noteworthy, several candidate genes identified in the human studies (ie. SNCA, NPY, CHRM2, TAS2R16 and ACN9) have homologous located on this rat chromosome (Carr et al. 1998; Carr et al. 2007; Liang et al.).

In a relatively recent study an extensive gene mapping study has been undertaken in msP rats. The most striking evidence obtained in these animals is that they carry with high correlation two single nucleotide polymorphisms on the promoter region of the gene encoding for the CRF₁ receptor. Combining this finding with the observation that msP rats have a higher expression of CRF₁ receptor mRNA and CRF₁ receptor protein density in various brain regions one may speculate that the gene variant identified in msP rats may be functionally relevant (Hansson et al. 2007; Hansson et al. 2006). Interestingly, in a recent investigation it has been reported that also in humans, polymorphisms at the level of the promoter region for the CRF₁ receptor gene are linked to alcohol use disorder. For example, in an adolescent at risk population it was found a significant correlation between two SNPs (Reference SNP IDs-number; rs242938 and rs1876831), binge drinking and lifetime prevalence of drunkenness (Treutlein et al. 2006). The same association was found in an independent sample of adult alcohol dependent patients in which rs1876831 polymorphism was linked to higher levels of alcohol drinking (Treutlein et al. 2006)(Blomeyer et al. 2008; Chen et al. ; Treutlein et al. 2006).

Altogether these findings suggest that alcohol preferring rats and humans, at least in part, share common genetic predisposing factors to alcoholism.

Conclusions and remarks

Genetically selected alcohol preferring rat lines were developed several decades ago, when other genetic tools such as engineered mice and sophisticated high-throughput gene expression and gene sequences analysis for human studies were not available yet. Hence for several years these rat lines have offered a unique opportunity to investigate the genetics of alcoholism. Often observations in genetically selected alcohol preferring rats have inspired hypothesis driven genetic studies in humans or have stimulated further *ad hoc* genetic studies using engineered animals. These rat lines have also offered a unique help for advancing our knowledge of the neurobiology of alcoholism and has allowed the possibility to carry out pharmacological studies to evaluate drug effects on alcohol drinking. In fact, non selected rodents and laboratory animals in general do not readily drink alcohol and in most of the cases their consumption is too low to pharmacologically manipulate alcohol drinking or to evaluate the neurobiological consequences of alcohol exposure. These limitations have been now, partially overcome with the more recent development of nongenetic animal models of excessive drinking (see chapter by Becker).

A wealth of data collected over the years of research suggests that the alcohol preferring rat may indeed represent an animal model of alcohol abuse endowed with good predictive, face and construct validity. Nevertheless, the clinical translational value of results collected in alcohol preferring rats remains less clear. In fact, on the one hand some genetic traits linked to alcohol abuse vulnerability seems to be shared by humans and alcohol preferring rats. On the other hand, the breeding and selection programs of these rat lines were merely based on alcohol preference criteria. It is unlikely, therefore, that human alcoholism which is characterized by complex phenotypic traits such as drinking to intoxication despite the negative consequences associated to it and rodent alcohol preference are subserved by the exact same genetics. In this regard, among the different alcohol preferring rat lines only the P seems, under certain experimental condition, to spontaneously drink enough alcohol to achieve intoxication and dependence (Kampov-Polevoy et al. 2000).

Another consideration is that alcoholism is a heterogeneous disorder to which several genetic factors may contribute. Indeed, several phenotyping and genotyping criteria have been proposed to group alcoholic patients into more homogeneous subpopulations (Cloninger 1987; Heilig et al. 2011). Similarly, also alcohol preferring rat lines appear to differ in their phenotypes. For example the P, the sP and the msP lines are characterized by high anxiety-like traits; the AA and the LAD not. sP and msP rats appear also to show depressive-like symptoms that are medicated by alcohol intake (Ciccocioppo et al. 1999a). While in the UChB/UChA lines the pharmacokinetics of alcohol seems to play a critical role (Quintanilla et al. 2006). Hence, it is likely that the different lines of genetically selected alcohol preferring rats may resemble subpopulation of alcoholics; generalization of the findings in these animals may be of questionable value.

At present only two drugs have been FDA approved for the treatment of alcoholism and relapse prevention; namely naltrexone and acamprosate. These two agents have been extensively tested in animal models of alcohol drinking including genetically selected rats. In most of these experiments results correctly predicted drug efficacy in humans (Cowen et

al. 2005; Dhaher et al. ; Koistinen et al. 2001; Krishnan-Sarin et al. 1998; Perfumi et al. 2005; Sabino et al. 2006). These findings support the translational value of pharmacological findings in alcohol preferring rats. However, as described in previous paragraphs, there are other examples (i.e., the case of 5-HT₂ receptor antagonists or the case of SSRIs) in which preclinical data in genetically selected alcohol preferring rat lines was unable to clearly predict actual clinical outcomes (Ciccocioppo et al. 1997; Ciccocioppo et al. 1995; Johnson et al. 1996; Maurel et al. 1999; Murphy et al. 1985; Overstreet et al. 1997; Rezvani et al. 2000; Roberts et al. 1998).

In conclusion, there should be no doubt that genetically selected alcohol preferring rat lines represent a very useful model to study alcoholism. Human alcoholics, on the other hand, consist of a heterogeneous population of individuals with alcohol abuse as a common problem. These individuals are characterized by genetic variability, life history, drug exposure (i.e., time of exposure and amounts), environment etc. All these factors are important and they all contribute to shaping the trajectory of disease progression. Considering these levels of complexities it appears unlikely that a single animal model of alcoholism or a single line of alcohol preferring rats may mimic the human condition in a satisfactory way. Rather, it is reasonable to believe that any different alcohol preferring rat line, or animal model, may catch some aspects of the human disorder but not all. To maximize the translational power of preclinical research it is important, therefore, to collect evidence from as many different animal models as possible. The different lines of alcohol preferring rats may be viewed as important tools to achieve this objective.

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Abbreviation

UChB	Universidad de Chile “Bebidores” high alcohol-drinking line
UChA	low alcohol-drinking line
AA	Alko Alcohol preferring
ANA	Alko, NonAlcohol preferring
P	alcohol Preferring
NP	alcohol NonPreferring
sP	sardinian alcohol Preferring
sNP	sardinian alcohol NonPreferring
msP	marchigian sardinian alcohol Preferring
HAD	High Alcohol Drinking
LAD	Low Alcohol Drinking

ADE Alcohol Deprivation Effect

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Table 1

List of the different genetically selected alcohol-preferring and nonpreferring lines available

Rat line	Predictive	Face	Construct	Notes
UChB/UChA	NA	+	+++	Not enough information to evaluate its predictive value.
AA/ANA	++	++	+	Little data are available to evaluate the construct validity of this rat line
P/NP	++	+++	+++	The only line shown to voluntarily drink alcohol to intoxication
sP/sNP	++	++	++	Alcohol preference was co-segregated with heightened anxiety and depressive-like traits
HAD/LAD	++	++	++	Best genetic criteria for line selection
msP	+++	++	+++	Nonpreferring line is not available. The msP showed the highest predictive validity

Not available (NA), acceptable (+), good (++); very good (+++)