Network: Systematic Gene Identification and Functional Analyses in Common CNS Disorders

Project: Identification of Quantitative Trait Loci Involved in Alcohol Drinking, Stress-induced Alcohol Drinking and Relapse

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Introduction

Studies in animals and humans have shown that most of the common allelic variations contribute small quantitative effects to addiction-related phenotypes (1,2). Attempts to map these allelic variations in humans have not been successful in the past (3). This is probably due, at least in part, to the many environmental and socioeconomic factors that also contribute to the development of drug addiction (4, 5). These environmental factors, including exposure to the drug and stress, can be controlled in animals and thus all variations in the phenotype of genetically diverse animal models can be attributed to genetic differences. The core of this research effort is therefore the identification of quantitative trait loci (QTL) associated with alcohol preference in mice.

The validity of this approach has been demonstrated over the last decade by the identification of QTLs and, in some instances candidate genes, that are associated with physical dependence to alcohol sensitivity, alcohol metabolism and physiological responses to acute ethanol exposure (2, 6-9). The focus of this project will be on the identification of QTLs that modulate the effects of stress on alcohol consumption and other behaviors. This strategy is based on our findings in knockout mice, which suggests that stress-induced drug responses and other behaviors associated with stress may be controlled by similar sets of genes (10-14). Indeed, there is also evidence from human studies for a genetic relationship between alcoholism and depression (15).

The major aim of this project is the identification of QTL involved in alcohol consumption, stress-induced alcohol drinking, and somatic symptoms of ethanol withdrawal. A minor aim of this project is to determine if anxiety-related and stress-induced behavioural traits are associated with a preference for drugs of abuse under normal and stressful conditions.

Project Status

C57BL/6J and C3H/HeJ mice respond differently in many stress-related paradigms and they differ in behaviours related to addiction to alcohol and other drugs of abuse. The C57BL/6J strain shows higher anxiety levels and an increased preference for ethanol when compared to the C3H/HeJ strain. To identify genomic loci that contribute to these behavioural traits, we have analyzed mice from the F2 generation of an intercross between these two strains. We tested the acute and chronic effect of ethanol, determined the development of addiction and tolerance, and the preference to the alcohol. To this end, we have obtained behavioural phenotyping data from 150 F2 mice. The ethanol naïve animals showed dose dependent decrease in body temperature after the intraperitoneal injection of different doses of alcohol. After two weeks of forced ethanol drinking (a 16% ethanol solution was the only source of liquid available), mice from the two parental strains became tolerant to the hypothermic effects of alcohol and therefore showed a significantly lower temperature change after intraperitoneal ethanol administration. In contrast, animals from the F2 generation did not show such a tolerance effect, due to the large individual variation.

Ethanol dependence was evaluated after 4 weeks of forced alcohol drinking. We assessed somatic withdrawal symptoms such as handling-induced tremors and convulsions, as well as behaviours associated with anxiety and hyperactivity in zero-maze and in open field test. The parent strains showed elevated anxiety levels three days after ethanol withdrawal. In contrast, mice of F2 generation only showed a significantly increased anxiety in zero maze, but not in the open field test. Again, the individual responses in the F2 generation were highly variable.

To examine ethanol preference, the animals had free access to an ethanol solution or water. Ethanol preferring animals consumed more ethanol than water. As described in the literature, we found a higher ethanol preference in C57BL/6J mice than in C3H/HeJ animals. The preference ratio of the F2 mice was between those of the parental strains.

A correlation analysis of the seven phenotype parameters evaluated in the F2 generation of mice showed different degrees of dependence and independence of the behavioural responses, thus suggesting different degrees of genetic co-regulation. Based on these behavioural data, we have begun to select mice that are most informative for subsequent QTL analysis. We are aiming to analyze a total of 1500-2000 mice.

Fig 1: Two bottle choice procedure. The animals had free access to 8% ethanol solution and water.

Outlook

As the next step, we will perform a whole genome scan for the identification of genetic loci that contribute to the quantitative behavioral traits. For this purpose, we have isolated DNA from all F2 animals. The analysis wil be done using 314 fluorescently-labeled microsatellite markers, spaced at a distance about 5 cM. These microsatellite markers were selected from the Whitehead MIT mouse genome database and are polymorph between C57BL/6J and C3H/HeJ mice. Interesting candidate loci will be further characterized by fine mapping strategies, and eventually validated using transgenic and/or knockout animals. We have also archived brain from these behaviourally and genetically well-characterized and diverse animals. These brains will be an important resource for the downstream analysis of candidate genes for drug addiction.

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