GENETIC AND ENVIRONMENTAL FACTORS FOR THE ALCOHOLISM RISK

Schuckit M (USA)

Great strides have been made in the last decade regarding the importance of both genetic and environmental contributors to the risk for heavy drinking, alcohol problems, and alcohol use disorders. This lecture reviews recent research findings regarding one potentially important endophenotype contributing to the risk, namely a low level of response (sensitivity) to alcohol.

The genetic findings will highlight several genes that appear to contribute to the level of response to alcohol in both animals and humans (including the l allele of the serotonin transporter and a polymorphism of a potassium channel gene), review ongoing steps to identify additional genes in several large population studies, present the approach being used to extend data to additional relatives for relatively rare polymorphisms contributing to the sensitivity to alcohol, while also presenting information on the search for environmental factors that contribute to the risk. The latter includes the results of structural equation models and growth curve analyses in evaluating models of how an endophenotype (e.g., the low response to alcohol) appears of both genetic and environmental contributors to the risk for heavy drinking and alcohol-related life problems using results of structural equation models and growth curve analyses in evaluating models of how an endophenotype (e.g., the low response to alcohol) appears of both genetic and environmental contributors to the risk for heavy drinking and alcohol-related life problems using data from a 25-year prospective study across two generations of San Diego families.

WHY MICE, RATS AND SOME HUMANS DRINK


The revolution in high throughput assessment of genetic, genomic and proteomic has produced novel opportunities to search for genetic contributors to complex phenotypes. Alcohol consumption by animals, including humans, is a quantitative trait which has both genetic and environmental determinants. Much work has been expended to identify the regions of the genome and the actual genes that contribute to the large variation evident within and across species in alcohol consumption. During the past several years, we have developed experimental approaches, generated transcriptome and genetic data, and analyzed data using a process which we call MAGIC-B, to identify genes which differ in their levels of transcript and are involved in modulating alcohol consumption. Whole brain transcriptome information was generated for 30 strains of the BXD RI mouse panel, for 27 strains of the HXB RI rats and for 20 strains of inbred mice. Genetic marker information was generated or obtained from public sources for all strains of animals. Behavioral data on the phenotype of voluntary alcohol consumption, using a two-bottle choice procedure, was generated for all strains of mice and rats. Using this large collection of data, we determined quantitative trait loci for the behavioral phenotype (b-QTLs) and the QTLs for gene expression in brains of mice and rats (e-QTLs). We performed correlation analysis between brain gene expression and voluntary alcohol consumption across the mouse and rat panels of animals. We used the premises of MAGIC-B to filter the correlated genes through the e-QTL and arrive at lists of genes whose expression levels correlated with alcohol consumption and whose transcriptional regulatory control was located in the same area of the genome as the b-QTL for alcohol consumption in mice and rats. We then ascertained the location of the ‘candidate’ genes in the human genome and ascertained whether the ‘candidate’ genes were located in QTLs which were determined for alcohol consumption in humans. Overall, this analysis produced the indication for the involvement of the following genes in the quantitative trait loci: Prat, Magt3, Prpf, Myoc, Galm1, Nck2, Pcdhg3 and PZrx4. The biochemical pathways and physiologic and behavioral functions determined by these genes will be discussed. Support by NIAAA and the Banbury Fund.

ALCOHOL, LIVER FIBROSIS AND CIRRHOSIS: FROM PATHOGENESIS TO ANTIFIBROTIC THERAPY

Schuppan D (USA)

Cirrhosis is a major determinant of morbidity and mortality in patients with alcohol abuse. Even low grade alcoholic hepatitis frequently progresses to cirrhosis. Progression depends to a large extent on additional environmental factors (second hits) and genetic predisposition. After elimination of alcohol and second hits, such as obesity and hepatitis C, advanced fibrosis and even cirrhosis are (partly) reversible. Novel drugs are aimed at speeding up or inducing reversal of advanced fibrosis/cirrhosis. Numerous agents that affect mitotic stimuli in liver fibrosis and that improve survival in patients with cirrhosis is difficult due to the slow progression from normal to cirrhosis (10–40 years) and the unreliability of liver biopsy to quantify fibrosis (biopsy sampling error: 30 and 60% for 1 out of 4 fibrosis...
stages). Current serum fibrosis markers are ill validated and too insensitive to confirm antifibrotic drug effects. Transient elastography adds a new dimension of fibrosis assessment, but is again too insensitive to monitor fibrosis progression or regression. Therefore, much effort is currently invested to develop quantitative imaging of liver fibrosis and fibrogenesis by use of molecular probes that are targeted at collagen or at the fibrogenic cells, and to identify sensitive serum markers of progression and regression by use of advanced transcriptomics and proteomics. Apart from liver transplantation which is only available to a minority of patients with end stage alcoholic liver disease, future reversal therapies are anticipated that use combinations of anti-fibrotic agents, likely in concert with hepatocyte regenerative approaches, such as stem or progenitor cell transplantation.

SYMPOSIUM 1–36
SYMPOSIUM I, SUNDAY SEPT. 23RD 2 PM–3.30 PM; ROOM: LECTURE HALL 1
Gene expression and genetic variability in alcohol preferring
trat lines
Chairpersons: Hyytia P (Finland), Sommer W (USA)
Presentation S1-1
VARIATION AT CANDIDATE GENE LOCI AND THEIR
FUNCTIONAL IMPORTANCE IN RODENT MODELS OF
ETOHOL DEPENDENCE
Bjork K (USA)
Aims. The overall aim of this study is to identify candidate genes in key
brain areas for ethanol dependence in genetically selected alcohol-preferring
rats and in addition, to search for genetic variation associated with these
genes.
Methods. Microarrays and in situ hybridization was used to screen for genes that
differentially expressed in brain areas of genetically selected alcohol-
preferring rats (AA and msP lines) compared to their non-preferring coun-
terparts. Expression differences were confirmed using quantitative reverse
transcription Real-Time PCR, in situ hybridization or Western blot. To identify
cis-regulatory elements driving expression differences screening of the
candidate gene promoters for genetic variation was also performed.
Results. Several genes were identified as differentially expressed in alcohol-
preferring rats compared to non-preferring rats in the initial microarray
screening. Genes with robust differences in several brain regions and involved
in biological processes relevant to ethanol dependence were chosen for further
analysis. β-arrestin 2 (Arrb2), corticotrophin releasing hormone receptor
subtype 1 (Crh1) and Glutathione S-transferases a 4 (Gst4) fulfilled both
of these criteria. Differential expression of these genes was confirmed using
alternative methods. Furthermore, sequence analysis revealed allelic variants of
two genes that correlate with altered expression levels.
Conclusions. The combined use of high-throughput methods and rats
genetically selected for high ethanol consumption provide a powerful tool
to identify candidate genes for ethanol dependence. All of the genes that we
could confirm displayed genetic variation at the candidate gene loci which
suggests that these genes are mainly cis-regulated. In addition to revealing
novel candidate genes for ethanol dependence the approach described above
may also provide insight into how these genes are regulated.

Presentation S1-2
ROLE OF BRAIN CRH SYSTEMS IN ETHANOL DEPENDENCE
Hansson AC, Rimondini R, Cippitelli A, Terasmaa A, Thorsell A,
Ciccioppo R, Sommer WH, Heilig M, Conrod P, Castellanos N (USA)
Aims. Alcoholism is a chronic, relapsing disorder. It develops through a
long history of repeated cycles of intoxication and withdrawal. Over the
course of this process, a major shift occurs from positively reinforced,
‘reward drinking’, to negatively reinforced ‘relief drinking’. This in turn has
been postulated to result from neuroadaptations that lead to a recruitment of
anti-reward systems, i.e. negative reinforcement through stress and fear systems
involving extra-hypothalamic corticostriphin-releasing hormone
(CRH) systems. We demonstrated the value of this theoretical framework for
the design and selection of model phenotypes with good predictive validity
for medication development.

Methods. Two animal models of high voluntary ethanol consumption were used:
1) prolonged exposure to repeated cycles of alcohol intoxication
and withdrawal in Wistar rats, and 2) the genetically selected Marchigian-
Sardinian alcohol-preferring (msP) rat line. Behavioral sensitivity to stress
was examined using fear suppression of behavior in the punished drink-
ing (Vogel) conflict test, forced swim stress on voluntary alcohol intake,
conditioned emotional response and stress-induced reinstatement of ethanol
seeking. The expression of CRH, CRH-R1 and CRH-R2 transcripts within the
amygdala complex and hypothalamus was analyzed by in situ hybridization
histochemistry. Elevated levels of CRH-R1 transcripts were pharmacologi-
cally validated by use of two selective CRH-R1 antagonists (antalarmin
and MTIP).

Results. Prolonged exposure to repeated cycles of alcohol intoxication and
withdrawal induces a ‘post-dependent’ state, a stable behavioral syndrome
characterized by excessive ethanol consumption and behavioral sensitivity to
stress. We found that both the elevated self-administration of alcohol and the
increased behavioral sensitivity to stress in the post-dependent state is in large
part mediated by an up-regulation of the CRH-R1 subtype of CRH receptors
in the amygdala. This converges with findings of a stress-sensitive/anxious
behavioral phenotype in the msP rat, accompanied by an innate up-regulation
of CRH-R1 receptors in the amygdala and several other brain regions in
this line. Using the selective CRH-R1 antagonists antalarmin and MTIP we
demonstrate that this accounts for the elevated self-administration of alcohol
and the anxious phenotype of both rat models.

Conclusions. Similarly to human alcoholics, the post-dependent state in rats
is associated with an enhanced behavioural sensitivity to stress. The increased
response to external stressors translates into elevated motivation to consume
alcohol in post-dependent animals. High alcohol preference together with
increased behavioral sensitivity to stress makes the msP rat a phenocopy of
the post-dependent state. A recruitment of intra-amygdala, but not
hypothalamic CRH systems seems to be driving both the post-dependent and
msP phenotype. In summary, our data support the hypothesis of recruitment
of anti-reward systems during the descent into ethanol dependence and their
likely importance for maintaining the dependent state. Our findings provide
a solid validation of the CRH-R1 receptor as a treatment target.

Presentation S1-3
THE USE OF ALCOHOL PREFERRIND RAT LINES FOR GENE
EXPRESSIO PROFILING IN ALCOHOL RELATED DISORDERS
Spanagel R (Germany)
Aims. Our study aimed to identify new candidate genes, which might be
involved in (i) alcohol craving and relapse and (ii) in the induction of alcohol-
induced organ damage.
Methods. In order to find changes in gene expression following long-term
alcohol consumption, we studied gene expression profiles (i) in the striatal
dopamine system and (ii) in the pancreas by using DNA microarrays of three
different alcohol-preferring rat lines (AA, HAD and P).

Results. Our data revealed an up-regulation of the dopamine D3 receptor
(D3R) following one year of voluntary alcohol consumption in the striatum
of alcohol preferring rats that was confirmed by qRT-PCR. In the pancreas,
among several differentially regulated genes, the regenerating genes Reg1 and
Reg3a showed a consistent down-regulation in all 3 lines following long-term
alcohol consumption, which was confirmed by qRT-PCR and on the protein
level by immunohistochemistry and Western Blot.

Conclusions. We conclude that long-term alcohol consumption leads to
an up-regulation of the dopamine D3R that may contribute to alcohol-
seeking and relapse. We therefore suggest that selective antagonists of
this pharmacological target provide a specific treatment approach to reduce
alcohol craving and relapse behavior. Reg genes seem to be critically
involved in alcohol-induced pancreatitis and can be used as diagnostic
markers as well.

Presentation S1-4
EXPRESSION PROFILING IN THE PREFRONTAL CORTEX:
IMPLICATION FOR ETHANOL CONSUMPTION IN ANIMAL
MODELS OF DEPENDENCE
Sommer WH (USA)
Aims. The AA (Alko, Alcohol) and ANA (Alko, Non-Alcohol) rat lines
were among the earliest rodent lines produced by bi-directional selection for
ethanol preference. AA rats display high ethanol consumption together with