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

Project: Validation in Humans of Genes Involved in Alcohol Drinking, Stress-induced Alcohol Drinking and Relapse

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Introduction

The aim of the project is the validation and functional characterization in humans of candidate genes derived from animal models, which are involved in alcohol drinking, stress-induced alcohol drinking and relapse. In order to attain this goal, we intend to apply a combination of three fundamental strategies:

1. To detect small to medium gene effects, large samples of alcohol dependent patients, which have been specifically characterized for personality factors related to anxiety and depression will be analyzed. In order to control for false positive results we investigate several populations with the same phenotypes from the same homogeneous population background in parallel.
2. To identify genes specific for alcohol dependence (vs. abuse vs. recreational use), population-based samples with different amounts of drug use without fulfilling clinical criteria for dependence will be analyzed and compared to patient samples; again several populations will be investigated in parallel.
3. To characterize the genotype-phenotype relationship in human patient and population samples for those genes and their variants identified in animal experiments which influence drinking behaviour such as stress-reactivity.

Rationale:

We assume that stress related drinking might induce harmful drinking and alcohol dependence in vulnerable subjects.

Samples:

Therefore, we explore the impact of variance of candidate genes in:

a) human samples with information on drinking patterns as well as on stress responsivity (e. g. through appropriate questionnaires or experimental settings) and
b) general population samples with drinking related phenotypes (abstinent, non-harmful and harmful drinking) as well as patient samples with alcohol dependence (in comparison to controls).

On this behalf a collaboration of all German Addiction Research Networks (GARN) funded by the BMBF join together with individual researchers in offering suitable cohorts of patients and controls.

Specific biological phenotypes of stress reactivity will be assessed in two samples: Firstly, analysis of the genetic influence on stress-related alcohol intake patterns in a non-addicted adolescent population (MARC-study) of 360 subjects aged 18-20 years of whom about 30% were already heavy consumers at age 16. These adolescents have been assessed for environmental stress by identifying stressful life events as well as chronic social difficulties. Experimental stress induction measures include spectral analysis of EEG-recorded white noise at 105 db and the d2 stress test. In addition, we propose to perform a test for psychosocial stress, the Trier Social Stress Test (TSST), which produces autonomic activation and stimulation of stress hormones, norepinephrine and epinephrine (Schommer et al. 2003). The influence of candidate gene variations on outcome parameters of the TSST and alcohol use status will be analyzed. Secondly, investigation of the genetic contribution to psychosocial stress reactivity and relapse behaviour in 200 alcohol dependent patients and 100 controls. We will perform the Trier Social Stress Test (TSST) after a minimum of six weeks of documented abstinence in the patients. Relapse will be assessed 3 and 6 months after application of the TSST. The influence of candidate gene variations on outcome parameters of the TSST and remission status will be analyzed.

Genes identified in animal models will be prioritized according to positional data (see 8.4.1, 8.4.2) as well as functional relations of the differentially expressed genes with each other or with genes known to be relevant for the specific phenotype. In addition, genes relevant for the CRH/CRHR1 and the GR pathway will be analyzed (see 8.4.4, 8.4.5). Human homologues of 20 genes with the highest priority identified in animal models will be systematically analysed for genetic variations in exons, introns and the 3' and 5' regions of the gene These studies will provide important information on frequency and linkage disequilibrium and help in the selection of SNPs for genotyping, and will lead to the identification of a number of structural or functional variants of interest for further biochemical investigations in collaboration with the Molecular Genome Analysis laboratory at the DKFZ (Wiemann), with whom a collaboration was established during NGFN1.

Results

The Trier Social Stress Test (TSST) as a tool to phenotype populations with a defined risk for alcoholism

The method of the TSST was successfully established at our new laboratory facilities, including approval of the study by the institutional review board. In a first step we began to study the adolescent participants of the Mannheim risk children study (MARS) who are now 18-19 years old. Up to now, we tried to contact 190 subjects. 7 were lost to follow-up, 8 refused to participate because they objected to blood draws. The remaining 175 (=92%) consented to participate. 33 probands were scheduled for the experiment, 30 (91%) of whom actually showed up and were investigated. There were no dropouts during the experiment and the tests were well tolerated in all instances. Endocrine data are complemented by self-ratings of subjective stress, the temperament and character inventory (TCI) and questionnaires on recent alcohol and cigarette consumption. Projecting our current recruitment rate, we expect to be able to study 282 of the available 338 MARS participants, which is even more than was expected before starting.

While recruitment of the adolescent sample analysed with TSST is ongoing, we have performed a systematic analysis of

Genes of the hypothalamo-pituitary-adrenocortial (HPA) axis.

Environmental stressors increase the predisposition of an individual to self-administer drugs: The stress reaction is mediated via the hypothalamo-pituitary-adrenocortical (HPA) system. We presently analyse associations of all relevant genes of the HPA-axis with alcohol dependence in a cohort of 3000 individuals (cases + controls). Functional SNPs and tagging SNPs derived from the HAPMAP project were selected. The combination of the two approaches (see table) will detect genes and polymorphisms of the HPA-axis that are relevant for phenotypic traits of alcohol dependence.

We have recently completed a first study to assess the role of CRH-receptor 1 genotypes in alcohol drinking phenotypes:

HPA-axis gen	Polymorphisms under analysis
Glucocorticoid receptor [NR3C1]	Functional SNPs: Bcl1-, TthIII-, 363-polymorphisms Haplotype tagging SNPs: rs190488, rs10482672, rs4986593, rs33389
Glucocorticoid modulatory binding protein 1 [GMEB1]	Haplotype tagging SNPs: rs12042034
Glucocorticoid modulatory binding protein 2 [GMEB2]	Functional SNPs: rs310670, rs414897 Haplotype tagging SNPs: rs1151621, rs149462, rs2872810
Arginin-Vasopressin receptor 1b [AVPR1b]	Functional SNPs: AVPR1b-SNPS3
FK506-BINDING PROTEIN 5 [FKBP 5]	Functinal SNPs: rs3800373,rs 473916, rs1360780 Haplotype tagging SNPs: rs7757037, rs755658, rs3798346, rs737054
Corticotropin releasing hormone receptor 1 [CRHR1]	Haplotype tagging SNPs: rs1876831, rs242938

Genetic variation of the human corticotropin releasing hormone receptor (hCRHR1) is involved in alcohol intake patterns and binge drinking

To analyse the role of the corticotrophin-releasing-hormon (CRH)-receptor 1 in human alcohol drinking patterns and its possible contribution to alcohol dependence, we successively analysed two relevant samples for genotype-phenotype association of haplotype tagging (ht)SNPs and specific patterns of alcohol drinking: First, an adolescent sample, which consisted of individuals from the "Mannheim Study of Risk Children" (MARC), who had little previous exposure to alcohol, and secondly, a sample of alcohol dependent adults, which met DSM-IV criteria of alcohol dependence. Following determination of allelic frequencies of polymorphisms of the hCRHR1 gene, these two independent samples were genotyped and systematically analysed for association with two htSNPs of hCRHR1. Significant group differences in genotypes were observed in lifetime prevalence of alcohol intake, lifetime prevalence of drunkenness, maximal amount of alcohol intake per occasion, binge drinking, but not in the frequency of drinking or age. The sample of adult, alcohol dependent patients showed association with high amount of drinking as well as high depression score in the personality testing schedule. This is the first time an association of hCRHR1 with specific patterns of alcohol dependence has been reported.

We have recently shown that the circadian rhythm gene Per2 is associated with high alcohol intake in adult patients with alcohol dependence (Nature Med (2005) 11: 35-42). We were now interested to analyse the role of circadian rhythm genes Per1 and Per2 in stress-related alcohol drinking in different populations:

Circadian rhythm genes Per1 and Per2 regulate gene-environment interactions and are associated with alcohol drinking behaviour in humans

We have now found evidence for the regulation of alcohol intake by Per1 and report a role for Per2 in the regulation of alcohol intake in juveniles.

We performed a SNP-discovery and frequency analysis of the human Per1 gene. 18 genetic variations of the *Per1* gene were identified in the regulatory domains, exons and exon-intron boundaries. htSNPs were identified and genotyped in a sample of 206 adult patients, which were assessed for alcohol intake. We found a significant association of high vs. low alcohol intake with one htSNP (p=0.0041, OR 2.12) localised in the promoter region of the gene. In the adolescent sample we analysed frequency of alcohol intake and maximum alcohol intake per occasion (a measure for binge drinking) for an association with Per1 htSNPs. We found an association of one htSNP with frequency (p=0.032) as well as maximum amount of alcohol intake/occasion (p=0.021). Interestingly, these associations were only present in adolescents with a high amount of psychosocial stress.

Additionally, the adolescent sample was assessed for a possible association of maximum alcohol intake per occasion with Per2 htSNPs. We found an association of a haplotype with high vs. low maximum alcohol intake per occasion, which was mainly driven by the same SNP, which accounted for the association with high alcohol intake in adult patients with alcohol dependence (p=0.0046; OR 2.85). Thus, our findings suggest a role of the Per1gene in gene-environment regulation of alcohol drinking behaviour in humans and extend our previous results regarding the role of Per2 in the regulation of alcohol intake.

Since aversive stimuli are at the core of stressfull, negative life events and may lead to development or relapse of addictive behaviour, we performed a functional gen x neuroimaging analysis of emotional reactivity to aversive stimuli:

Genetic variants of the serotonin transporter and Catechol *O*-methyltransferase additively affect brain processing of unpleasant stimuli

Emotional reactivity and regulation are fundamental to human behavior. Since inter-individual behavioral variation is affected by a multitude of different genes, there is intense interest to investigate gene-gene effects. Functional sequence variation at two genes has been associated with response and resiliency to emotionally unpleasant stimuli. These genes are the catechol-O-methyltransferase gene (*COMT Val^{158}Met*) and the regulatory region of the serotonin transporter gene (*5-HTTLPR*). Using functional magnetic resonance imaging (fMRI) we observed an additive effect of both *COMT* and *5-HTTLPR,* accounting for 29% of the inter-individual variance in fMRI activity of amygdala, hippocampal and limbic cortical regions elicited by unpleasant stimuli.

Outlook

We will further study the observed gene-environment effect mediated by Per1 and continue to systematically analyse HPA-axis phenotypes in various samples, characterised clinically, neuroendocrinologically and by neuroimaging analysis. We have established samples and techniques necessary for the analysis of novel genes, as they will emerge from the animal studies associated with his project.

*Lit.: **1.** Ramoz N, et al. (2005) Pharmacogenetic aspects of the treatment of alcohol-dependence, Current Pharmacogenetics (in press). **2.** Hinckers, A et al. (2005) Low level of response to alcohol as associated with serotonin transporter genotype and high alcohol intake in adolescents (Biological Psychiatry, in press). **3.** Smolka M et al. Genetic variants of the serotonin transporter and Catechol O-methyltransferase additively affect brain processing of unpleasant stimuli (Nature NS, in review). **4.** Spanagel R et al. (2005) The clock gene Period2 influences the glutamatergic system and thereby modulates alcohol consumption. Nature Medicine 11: 35-42. **5.** Spanagel R et a. (2005) Alcohol consumption and the body`s biological clock. Alc Clin Exp Res (in press). **6.** Treutlein J et al. Genetic variation of the human corticotropin releasing hormone receptor (hCRHR1) is involved in alcohol intake patterns and binge drinking. (submitted). **7.** Wrase, J et al. (2005) CB1/Cnr1 Association to Amygdala Volume, Craving and Alcohol Relapse (Neuron, in revision).*