of some polymorphisms, such as DAT1 VNTR and 5HTT LL ins/del. No relationship was found between a preference for high concentrations of.

Conclusion. There are no direct links between probands belonging to specific subtypes of alcoholism inherited in the family (according to Lesch’s and Cloninger typology) and a preference for high concentrations of saccharose (sweet liking). (The study was supported by MNISW grant no NN402 18935.)

S23.2

INFLUENCE OF CANDIDATE GENE VARIANTS ON EARLY ONSET OF DRINKING AND ALCOHOL DEPENDENCE: PERTAINING TO ALCOHOL DEPENDENCE FAMILY HISTORY

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Introduction and Aim. Several lines of evidence support the hypothesis that a positive family history (FHP) of alcohol dependence (AD) in first-degree relatives is a significant risk factor for an individual to develop AD during their lifetime. However, little is known about the role of specific candidate genes variants which may transmit the risk. The aim of this analyses of the CIGAR (Collaborative initiative on Genetics of alcoholism in Central Europe) is to investigate the influence of known candidate gene variants (ADH4, GABRA2 and NR2A) posing a risk for AD on age of first drinking and age of onset in FHP vs. FHN (Family history negative) individuals.

Patients and Methods. A total of 1351 inpatient subjects with DSM-IV AD from three addiction treatment centres were included. Characteristics of AD and related phenotypes first ages of drinking (FD) and alcohol dependence onset (A0O) were obtained using standardized structured interviews. All subjects were genotyped for ADH4, GABRA2 and NR2A polymorphisms.

Results. FHP vs. FHN individuals had significantly more severe characteristics of alcohol dependence and more comorbidity. FD before age 15 was associated with higher rates of A0O before 30 in FHP. Variants of NR2A were associated with both early FD and A0O in male FHP individuals while an ADH4 variant was associated with FHP.

Conclusions. This study confirms the significant role of FHP in development of alcohol dependence. The results support the relationship of ADH4 and NR2A variants with FD and related traits in the development of AD.

S23.3

IMPULSIVE BEHAVIOR IN ALCOHOL DEPENDENCE: IS IT GENETICALLY DETERMINED?

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It has been established that high levels of impulsivity may increase the vulnerability to develop alcohol dependence. Impulsiveness is also considered to be a predictor of poor addiction treatment outcomes and is regarded as a candidate for an endophenotype in alcohol dependence. The heritability of impulsivity has been confirmed in animal as well as human subjects, accounting for about 45% of the variance. A subtype of alcohol dependence, characterized by both a family history and an early onset of the disorder, as well as increased impulsiveness, further suggests a genetic component. To date, only a few studies have investigated the associations between the levels of impulsivity and polymorphisms of candidate genes. However, most of the results did not employ measures of impulsiveness per se, but rather analyses of aggressive or suicidal behaviors, which are often not always associated with high levels of impulsivity. Findings suggest that functional polymorphisms in genes associated with serotonin and dopamine activity may be a key to understanding the mechanisms underlying impulsivity. Current studies indicate that the serotonin system may exert its effects on impulsivity through dopamine and glutamate as well as GABA neurotransmission. In particular, polymorphisms in the TP12, HTR2A and SLCO2A4 genes may be important in the process of impulse control. Other studies pertaining to a relationship between gene polymorphisms and impulsivity in alcohol-dependent individuals revealed a possible influence of the MAOA gene as well as DRD2 gene. Recent studies performed in Polish population of alcohol-dependent patients provide a new interesting perspective, suggesting that the level of behavioral impulsiveness is determined primarily by the genetic component, whereas cognitive impulsivity is associated mainly with demographic and psychosocial factors.

S23.4

IN A SEARCH OF ALCOHOLISM PHENOTYPES: TYPOLOGIES

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Endophenotype is a psychiatric concept and biomarker of mental disorders (Gottesman and Goldin 2003). Currently, advances in genetics and neurobiology are much better developed than clinical knowledge about psycho-pathological traits or symptoms closely related to genetic polymorphisms. One of the first research in molecular genetics has shown that ‘severe alcoholism’ could be related with DRD2 gene polymorphism (Blum et al., 1993). Although that findings were not satisfactorily confirmed, that was an inspiration to investigate the relation between genotype and more precisely defined types of an alcoholism. On a beginning, alcoholism types were promising subjects of research due to close relations between Cloninger’s subtypes and characteristics of an activity of neurotransmission systems, which are mostly genetically determined (Cloninger et al., 1987). In a light of current knowledge, typologies seems to be insufficient to name them endophenotypes but during research on them, many traits and symptoms were identified as a promising subjects for future research (Lee et al., 2008; Pomo and Lesch, 2008; Ersche et al., 2010).

S23.5

NEUROBIOLOGY OF LEARNING DYSFUNCTION IN ALCOHOLISM

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The disposition and maintenance of alcohol dependence has been associated with dysfunctional learning, particularly with increased salience attribution to alcohol-associated stimuli and Pavlovian-to-instrumental transfer, which establishes an effect of alcohol-associated cues on operant alcohol seeking and consumption. Previous imaging studies showed that dopamine dysfunction in the ventral striatum, the core region of the so-called reward system, is associated with increased brain activation elicited by alcohol-associated cues in brain areas associated with attention. Furthermore, brain activation elicited by non-alcohol (e.g. monetary) reward was decreased in detoxified alcoholics. Here we present findings of functional magnetic resonance imaging (fMRI) studies showing that in detoxified alcohol-dependent subjects, reward-dependent reversal learning is impaired compared to healthy controls. This impairment correlates with reduced functional connectivity between the ventral striatum and the dorsolateral prefrontal cortex.

S24

5-ADENOSYLMETHIONINE IN THE PATHOGENESIS AND TREATMENT OF ALCOHOLIC LIVER DISEASE

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S24.1

THE ROLE OF ALTERED SAME METABOLISM IN ALCOHOL-INDUCED ER STRESS AND LIVER INJURY

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ER stress is a condition under which unfolded or malfolded proteins accumulate in the endoplasmic reticulum. ER stress triggers the unfolded protein response (UPR) which can either result in the recovery of ER homeostasis or activate a cascade of events which result in inflammation and steatosis and ultimately cell death. Alcohol-induced alterations of molecular markers of the UPR were first revealed by microarray analysis in the liver of mice with intrahepatic alcohol infusion (French-Tsukamoto model) in 2001. The role of ER stress in alcohol-induced liver disease (ALD) has since been investigated using genomic and proteomic profiling as well as a series of transgenic or gene-knockout cell and animal models. Robust support for a contributing as opposed to a secondary role for ER stress response is seen in ALD. Potential mechanisms that trigger alcohol-induced ER stress response include alcohol-induced hyperhomocysteinemia (HHcy) causing protein
homocysteinylation, alcohol metabolite acetaldehyde forming protein adducts and alcohol-induced reduction in the ratio of liver S-adenosylmethionine (SAM) to S-adenosylhomocysteine (SAH) increasing hepatic exposure to homocysteine as well as causing epigenetic regulations of the UPR signaling pathway. Mice with liver-specific deletion of the major chaperone GRP78/BiP was recently created. The GRP78 loss exacerbated ER stress and liver injury in the knockout mice fed orally low doses of alcohol. The role of S-adenosylhomocysteine (SAH) in the hepatic ER stress of fatty liver injury were induced in wild-type mice. The evidence support the existence of a complex interplay between impaired SAMe/Hcy metabolism, continuous high alcohol consumption that promotes it and ER stress responses that result from it. Such a vicious cycle may be the key pathophysiologic concept in ALD. Therapeutic approaches aimed at interrupting the cycle may dampen the stress response and the ensuing injury. (This study was supported by US NIH/NIAAA grants R01AA018846 and R01AA018612.)

S24.2 RESCUE OF ALTERED METHYLATION REACTIONS BY BETAINA: A THERAPEUTIC APPROACH FOR THE TREATMENT OF ALCOHOLIC LIVER INJURY

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Alcoholic liver disease is one of the most serious medical consequences of chronic alcohol use in the USA and worldwide. In our on-going investigation to understand the mechanism of alcohol-induced liver injury, we have shown that ethanol causes alterations in many specific steps involved in methionine metabolism. Ethanol consumption predominantly inhibits the activity of a vital cellular enzyme, methionine synthase, involved in remethylation homocysteine. By way of compensation in some species, ethanol increases the activity of the enzyme, betaine homocysteine methyltransferase. This enzyme catalyzes an alternate pathway in methionine metabolism and utilizes hepatic betaine to remethylate homocysteine to form methionine and maintain levels of S-adenosylmethionine (SAM), the key-methylating agent. Under extended periods of ethanol feeding, however, this alternate pathway cannot be maintained resulting in a decrease in the hepatocyte level of SAM and increases in two toxic metabolites, S-adenosylhomocysteine (SAH) and homocysteine ultimately causing a reduction in the hepatocellular SAM:SAH ratio. This ethanol-induced reduction in the SAM:SAH ratio is a profound metabolic stressor and affects many crucial methylation reactions catalyzed by diverse methyltransferases in the liver. The reduced activities of the methyltransferases results in the generation of many hallmark features of alcoholic liver injury, including increased triglyceride accumulation, increased apoptosis, enhanced accumulation of damaged proteins, decreased creatine synthesis, impaired proteasome activity and altered protein–protein interactions. We have further shown that the impaired methylation also alters cytoplasmic lipid droplet dynamics disrupting normal VLDL assembly and secretion contributing to the development of alcoholic steatosis. Treatment with betaine by virtue of its ability to remethylate homocysteine increases hepatic SAM levels and lowers SAH preserving normal SAM:SAH ratios to maintain crucial methylation reactions. Overall, betaine supplementation offers an exciting therapeutic option to prevent and protect against the development of alcoholic liver injury. (This study was supported by Department of Veterans Affairs National Merit Review grant and NIH/NIAAA grant R21AA017296.)

S24.3 ROLE OF SAME IN HEPATOCELLULAR CARCINOMA

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HCC is a heterogeneous pathology with a variety of risk factors. Steatosis and NASH are hallmarks of non-alcoholic fatty liver disease (NAFLD). The molecular mechanisms of NASH to HCC progression remain unknown. Human patients with liver cirrhosis at a high risk of HCC have impaired methionine metabolism and abnormal S-adenosylmethionine (SAMe) levels. SAMe, the main methyl donor in cells, plays a critical role in cell proliferation, differentiation and apoptosis. The two main enzymes involved in SAMe synthesis and catabolism are methioninedesulfoxtransferase (MAT) and glycine N-methyltransferase (GNMT), respectively. MAT1A and GNMT deficiency have been reported in human HCC. Our laboratory generated MAT1A-KO and GNMT-KO mice, with decreased and increased hepatic SAMe content, respectively. Both models develop HCC spontaneously. GNMT-KO mice develop steatosis that progresses to fibrosis and HCC, being DNA hypermethylation the main mechanism involved. We isolated a cell line derived from a hepatic tumour of GNMT-KO mice, OKER cells. Our data indicate that hyper-phosphorylation of LKB1-Ser428 plays a critical role in proliferation of the tumour. SAMe excess promotes hyperactivation of Ras due to promoter methylation and subsequent silencing of RASSF1 (Ras inhibitor). Wild-type Ras pathway is responsible for the LKB1-hyperphosphorylation (Ser428) in an ERK/P90Rsk-dependent fashion, leading to LKB1-AMPK misconnection. Treatment with demethylating agent, 5-azacytidine, abolished Ras pathway activation, induced AMPK activation and promoted apoptosis. Ablation of LKB1 triggered apoptosis. Finally, in a xenograft model in nude mice, LKB1 suppression by IP injections of siRNA revealed a decrease in tumour growth, increase in necrotic regions and AMPK activity emphasizing the proliferative role of LKB1 in OKER cells. Critically, our results open a novel therapeutic strategy in HCC treatment and highlight the importance of LKB1 activity so far considered as a tumor suppressor.

S24.4 THE EFFECTS OF SAME ON METHIONINE METABOLISM AND TREATMENT OF ALD

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S-adenosyl-L-methionine (SAM) is the methyl donor for all methylation reactions, including those for DNA and histones, and regulates the synthesis of glutathione (GSH), the main cellular antioxidant. Previous experimental and clinical studies suggested that SAM may benefit patients with established alcoholic liver diseases (ALD). A multicenter 2-year European trial in 123 ALD patients found that SAM at 1.2 g/day reduced mortality or liver transplant incidence from 30% in the placebo group to 16% in the SAM group, but was significant only when patients with more advanced clinical disease were excluded from the analysis. We conducted a double-blind, placebo-controlled clinical trial on the effects of 24 weeks of treatment with SAM for ALD. The primary endpoints were changes in serum aminotransferase levels and liver histopathology scores, and the secondary endpoint was changes in serum levels of methionine metabolites. We randomized 37 patients with ALD to receive 1.2 g of SAM by mouth or placebo daily. Subjects were required to remain abstinent from alcohol drinking. A baseline liver biopsy was performed in 24 subjects and a posttreatment liver biopsy was performed in 14 subjects. Fifty-eight percent of subjects presented bridging fibrosis or cirrhosis at baseline. Comparisons at baseline with 26 alcoholics without liver disease and 28 healthy subjects showed decreased vitamin B6 levels in ALD that is associated with alterations in the transsulfuration pathway of homocysteine metabolism. Fasting serum SAM levels were increased over time intervals in the SAM treatment group. The entire cohort showed an overall improvement of AST, ALT and bilirubin levels after 24 weeks of treatment, but there were no differences between the treatment groups in any clinical or biochemical parameters nor any intra- or intergroup differences or changes in liver histopathology scores for steatosis, inflammmatory, fibrosis or Mallory-Denk hyaline bodies. In conclusion, whereas abstinence improved liver function, 24 weeks of therapy with SAM was no more effective than placebo in the treatment of ALD. The possible reasons for the observed lack of efficacy include the high rate of advanced stage of fibrosis in the studied group, reduced retention of SAM by injured hepatocytes and concomitant vitamin B6 deficiency.