World Health Organization/International Society for Biomedical Research on Alcoholism Study on State and Trait Markers of Alcohol Use and Dependence: Back to the Future

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This article summarizes content proceedings of a symposium held at the 2004 International Society for Biomedical Research on Alcoholism Congress in Mannheim, Germany. The chairs were Boris Tabakoff and Friedrich M. Wurst. The presentations were (1) Genetic associations with alcoholism and affective disorders, by Paula Hoffman; (2) Proteomic analysis of blood constituents in alcoholism, by Boris Tabakoff; (3) Contrasts between the responses of GGT and CDT to high alcohol intake, and a test of their combined use, by John Whitfield; (4) Direct ethanol metabolites such as ethyl glucuronide, fatty acid ethyl esters, phosphatidylethanol and ethyl sulfate: a new line of sensitive and specific biomarkers, by Friedrich Martin Wurst; and (5) Genetic studies of alcoholism subtypes in a Han Taiwanese population, by Ru-Band Lu.

Key Words: Alcoholism, Biomarkers, Direct ethanol metabolites, CDT, GGT, WHO/ISBRA study, Proteomics, Affective disorders, Genetics, Subtypes.

THE GLOBAL BURDEN of disease from alcohol exceeds that of tobacco and is on a par with the burden attributable to unsafe sex worldwide (WHO, 1999). The

World Health Organization/International Society for Biomedical Research on Alcoholism (WHO/ISBRA) Study on State and Trait Markers of Alcohol Use and Dependence was initiated in 1988 and involved an international network of clinical and biochemical assay centers that participated in a study focused on the characterization of alcohol consumption and alcohol dependence in different countries. The identification of individual characteristics, both biochemical and genetic, that might serve as state or trait markers for assessing the current level of alcohol consumption by an individual, and/or the individual's predisposition to alcohol dependence, respectively, was an important goal of the study. The rationale for the study was that the development of state markers of heavy alcohol consumption might assist physicians in the early detection of harmful alcohol consumption, and promote prevention and intervention efforts. The generation of candidate trait markers could provide information on the biological etiology of alcohol dependence as well as generate novel means of identifying alcohol dependence subtypes and approaches for matching patients to treatment programs. The study used a verbal assessment instrument developed in concert with the Alcohol Use Disorders and Associated Disabilities Interview Schedule (AUDADIS) to assess alcohol consumption, dependence, and various other psychiatric, medical, and demographic characteristics. A total of 1,863 subjects were recruited from five clinical centers in Helsinki,

Finland; Sydney, Australia; Sao Paulo, Brazil; Montreal, Canada; and Sapporo, Japan. These individuals completed the verbal assessment and provided blood and/or urine samples, using the informed consent procedures of each of the participating countries. The demographic characteristics of the study population, as well as results of biochemical assays of state markers and possible trait markers, have been published (Conigrave et al., 2002; Figlie et al., 2002; Glanz et al., 2002; Helander et al., 2002; Hoffman et al., 2002; Kovac et al., 2002; Snell et al., 2002; Martinez et al., 2002; Wurst et al., 2002). Currently, a second phase of the WHO/ISBRA study is being organized, and this study will focus on the use of cutting-edge proteomic, genomic, and metabolomic techniques, as well as haplotype and single nucleotide polymorphisms (SNP) analyses, to further assess state and trait markers of alcohol use and dependence. This symposium was designed to present the results of genomic, proteomic, and biochemical analyses that have been carried out to date with the material obtained in Phase I of the WHO/ISBRA study as well as to introduce the work of some of the proposed participants in the Phase II study.

GENETIC ASSOCIATIONS WITH ALCOHOLISM AND AFFECTIVE DISORDERS

P. L. Hoffman, A. Kaiser, L. Hines, and B. Tabakoff

A goal of the WHO/ISBRA project is to identify novel trait markers for a genetic predisposition to alcoholism and other complex psychiatric disorders. This presentation focused on the identification of candidate genes that may contribute to alcohol dependence or related affective disorders, since alcoholics are frequently vulnerable to comorbid disorders such as anxiety and depression (Grant et al., 2004). We have previously found that platelet adenylyl cyclase activity is lower in alcoholics who have abstained from alcohol for a period of time and that activity is also lower in individuals with major depression (Hoffman et al., 2002; Menninger and Tabakoff, 1997; Tabakoff et al., 1988). We have identified an isoform of adenylyl cyclase, AC7, which is highly expressed in platelets (Hellevuo et al., 1993). The *AC7* gene is localized on human chromosome 16 (Hellevuo et al., 1995) and has a tetranucleotide repeat polymorphism in the 3'-UTR (Hellevuo et al., 1997). This polymorphism was used to carry out association studies in the WHO/ISBRA population (white subjects from Montreal, Canada). A significant association was found between alleles containing the seven-repeat polymorphism in the *AC7* gene and familial depression in women. Haplotype analysis has now been carried out in the region of the *AC7* gene on chromosome 16. A total of 10 SNPs were selected, with varying distances from the *AC7* gene repeat polymorphism. A haplotype block containing the polymorphic 3'-UTR repeat region was identified within the *AC7* gene (Haploview, v. 2.05) (Barrett et al., 2004). These SNPs demonstrated suggestive associations with familial depression. Haplotypes were predicted for each individual (Phase,

v. 2) (Stephens and Donnelly, 2003; Stephens et al., 2001), and a high-risk haplotype was identified that was associated with a significantly increased risk for familial depression (particularly in women). Such associations between haplotypes and the phenotype examined are expected to increase our understanding of the role of AC7 in depression and other populations are being subjected to similar analyses.

PROTEOMIC ANALYSIS OF BLOOD CONSTITUENTS IN ALCOHOLISM

B. Tabakoff, L. Snell, C. Wu, and P. Hoffman

Clear evidence exists that chronic consumption of ethanol, in quantities deemed hazardous or harmful to health (Saunders and Lee, 2000), can alter the levels of certain proteins circulating in the blood. Changes in these proteins can reflect organ pathology and such proteins are secreted into the circulation when cells are damaged (e.g., GGT and ASAT). Other proteins in the circulation are altered through ethanol, through its metabolites (acetaldehyde), through interference with pathways responsible for post-translational modification of proteins (e.g., carbohydrate deficient transferrin, CDT), or they undergo the covalent binding of the ethanol metabolite acetaldehyde to modify proteins through formation of Shiff bases or other chemical reactions.

Measures of proteins, whose levels and/or structure are modified by ethanol/acetaldehyde, have been used extensively as "state markers" of the levels of ethanol intake and as surrogates for the verbal diagnostic tests of alcohol dependence (Allen and Litten, 2001). It has to be emphasized that current psychiatric diagnostic criteria for alcohol dependence (i.e., DSM-IV or ICD-10) do not consider measures of ethanol intake as part of their diagnostic scheme for alcohol dependence. The diagnostic criteria concentrate primarily on behavioral and medical (ICD-10) aberrations occurring as the result of alcohol consumption. Additionally, all currently available protein markers of ethanol intake have several important limitations to their use: 1) no currently available marker protein is altered in a manner that is directly proportional to levels of alcohol intake, 2) each of the currently available markers has confounding influence of a number of environmental/physiological situations unrelated strictly to quantities of alcohol intake (e.g., the effect of sex on the utility of the use of CDT (Fleming et al. 2004), and 3) the most popular proteins for measures of alcohol intake (i.e., GGT and ASAT) are actually measures of organ damage and are influenced significantly by individual differences in sensitivity to organ damage by ethanol.

The advent of technology to simultaneously identify and quantify large numbers of proteins has allowed for global searches for novel proteins that might better reflect the quantitative aspects of alcohol use. Current proteomic techniques use one of two methods of separation of proteins or the peptides derived from the digestion of proteins

before analysis by mass spectroscopic techniques. It is these preparatory methods that in most cases determines which proteins will be amenable to identification and/or quantitation. The use of two-dimensional gel electrophoresis followed by in-spot digestion usually is best suited for soluble proteins, whereas methods that use digestion methods as an initial step and follow this step with column chromatographic separation of resultant peptides can produce an enrichment of the preparation with fragments from membrane-bound proteins. In either case, one needs to concern oneself with maintaining a good dynamic range in the measurement technology and not overwhelm the system with highly represented proteins in the assayed sample (e.g., albumin in plasma).

We have used both gel and column separation techniques to examine samples of plasma and platelets derived from individuals who have a history of consuming alcohol in the range of 0 to over 300g absolute ethanol per day (on average) over the last month. The two-dimensional gel chromatography/electrophoresis of plasma proteins, in our hands, produced results in which only 50 to 70 proteins could be identified even after preabsorption of albumin and certain immunoglobulins. Of the protein spots consistently visualized and identified, we found only one protein that was well correlated with the average daily intake of ethanol. The levels of α2-macroglobulin were well correlated with alcohol intake in the ranges of 0 to 275 g/d ($r = 0.642, p < 0.005$). On much more careful analysis of variables that could confound our results, we discovered that the age of our subjects was inversely correlated with the amount of alcohol they drank, and the results we obtained were severely compromised by a strong relation between age and α2-macroglobulin levels in plasma.

On the other hand, when we used multidimensional protein identification techniques (Mud PIT) as described by Wu and Yates (Wu and Yates, 2003) to analyze platelet membrane proteins of individuals who consumed alcohol at various levels, we were more successful in simultaneously sampling large numbers of proteins. Hydrolysis of the membrane-bound proteins, followed by separation of the peptide fragments on micro-liquid chromatography columns consisting of hyrophilic/hydrophobic and ion exchange resins and then identifying the fragments by MS/MS spectroscopic techniques, produced information on 500 to 600 proteins. Several of these proteins were found to vary between individuals with low or high alcohol intake. We have prepared antibodies to two of these proteins and are screening close to 500 subjects to assess the utility of these previously unquantified proteins to distinguish between nonhazardous and hazardous/harmful alcohol use. Our results should produce a prototype process for screening and then quantifying proteins that may provide a mirror for reflecting alcohol use. Such markers used alone or in conjunction with other markers will be a significant aid to monitor abstinence or screen for alcohol use among individuals who may put themselves or others at risk because of their high ethanol intake.

CONTRASTS BETWEEN THE RESPONSES OF GGT AND CDT TO HIGH ALCOHOL INTAKE AND A TEST OF THEIR COMBINED USE

John Whitfield

Many laboratory test results vary with alcohol intake, but only a few show sufficient response to serve as biological markers and discriminate between acceptable and probably safe and excessive and probably harmful alcohol use. The best tests for assessing average alcohol intake over the previous two to four weeks are plasma GGT and CDT. Both are far from perfect, but two lines of inquiry may lead to improvements. First, we need to understand the factors (other than alcohol intake) that affect them; second, we should evaluate combinations of test results, with or without other information, to improve performance in the diagnostic, screening or monitoring roles.

Factors such as age, sex, and body mass index (BMI) can affect GGT or CDT independent of alcohol intake. If the size of such effects is constant across alcohol intake categories, the test sensitivity and specificity for a constant cutoff value will change but the overall receiver operating characteristic (ROC) curve will have the same shape and position across, for example, age and sex categories. However, if such factors change the slope of the alcohol/test dose-response curve, the groups with a flatter dose-response curve will show poor diagnostic performance for this test regardless of the cutoff point chosen. Therefore, the shape of the ROC curve will change, and it may be necessary to specify that the test has good performance in (for example) obese people or in men but is not useful in people of normal BMI or in women. Although restrictive, this could be a useful strategy for test utilization.

A number of studies have shown that GGT and CDT values are affected by sex and also by BMI and aspects of the metabolic syndrome (Fagerberg et al., 1994a, 1994b; Whitfield et al., 1998). There are also known associations with iron status (De Feo et al., 1999; Stauber et al., 1996; Whitfield et al., 2001) and cardiovascular risk factors (Nikkari et al., 2001). Some of these studies suggest that such factors may also affect the alcohol/GGT or alcohol/CDT dose-response curves (Fagerberg et al., 1994a; Whitfield et al., 1998; Whitfield et al., 2001). In nearly all cases, the effects on test results or on the responsiveness of test results to alcohol intake are in opposite directions for GGT and CDT. These reports need further confirmation, and the data from the WHO-ISBRA study provide an opportunity for this.

The WHO-ISBRA data also allow investigation of the performance of some combination of GGT and CDT results and confirmation or modification of the formula previously proposed (Sillanaukee and Olsson, 2001). Because the effects of extraneous factors on GGT and CDT are in

opposite directions, inclusion of both variables may cancel out these distortions and provide a significant improvement in test performance.

The results presented are based on data on 1,863 participants in the WHO-ISBRA Study and are drawn from two published reports (Chen et al., 2003; Conigrave et al., 2002). Approximately two thirds of participants were men and one third were women. Overall, the correlation between alcohol intake and GGT was 0.38 for men and 0.37 for women, and the values for CDT were 0.46 and 0.27, respectively. These values are consistent with previous estimates.

The relations between self-reported recent alcohol intake (average daily intake over the previous month) and plasma GGT and CDT results were first examined by age group. Participants aged between 18 and 20 years showed no increase in mean GGT with increasing alcohol intake, and a similar lack of response was found for CDT. Although previous studies have found that GGT is less affected by alcohol in younger than in older people, the lack of CDT response to drinking in young people was unexpected. The effects of BMI on the response of GGT or CDT to alcohol were broadly consistent with expectations; people in the lowest quintile for BMI showed the greatest increase in CDT and people in the highest BMI quintile the least, with intermediate results for the second, third and fourth quintiles. GGT was also affected by BMI, but the main difference was between people in the top BMI quintile and all other participants. Obese people showed higher GGT than others for any level of alcohol use and showed a greater increase in mean GGT with increasing alcohol intake (Conigrave et al., 2002).

Although it was not possible to examine other characteristics of the study participants such as iron status and metabolic syndrome, which have been reported to affect the marker response to alcohol, these results reinforce the view that the test results are not simply passive markers of alcohol intake. They interact with and reflect the dynamic metabolic state of the individual and particularly with the cluster of variables that are affected by insulin resistance and the metabolic syndrome.

The second question about GGT and CDT that can be addressed by using the WHO-ISBRA study data are whether a combination of these test results provides better information than either singly. Results on this question have recently been published (Chen et al., 2003), and the main conclusions will be summarized. First, independent derivation of an equation combining GGT and CDT results ($y = 0.9 \times \ln \text{GGT} + 1.7 \times \ln \text{CDT}$) gave a result very close to that from Sillanuakee's group ($y = 0.8 \times \ln \text{GGT} + 1.3 \times \ln \text{CDT}$). The ROC areas under the curve for these two equations, applied to the WHO-ISBRA data set, were 0.811 and 0.810. Second, the combination of GGT and CDT in this manner gave better results than either test alone in men, but for women, GGT was best and addition of CDT results did not show any benefit.

The conclusions from this work are that GGT and CDT have in many ways opposite properties, although both of them increase with increasing alcohol intake. These opposite properties may make them complementary, and there is some support for use of a combination of test results as a marker of alcohol intake. However, CDT is a comparatively expensive test and may (depending on the subjects tested) provide no benefit when applied to women. The more general conclusion is that sample and data collections such as the WHO-ISBRA Study can be an ongoing resource for testing of hypotheses and for evaluation of new tests or combinations of existing tests before clinical application. Such evidence of effectiveness will be increasingly required before the introduction of new tests.

DIRECT ETHANOL METABOLITES SUCH AS ETHYL GLUCURONIDE (ETG), ETHYL SULFATE (ETS), FATTY ACID ETHYL ESTERS (FAEES), AND PHOSPHATIDYL ETHANOL (PETH): A NEW LINE OF SENSITIVE AND SPECIFIC BIOMARKERS

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Among the most important criteria for alcohol biomarkers are the time spectrum of detection they reflect and the influences of age, of sex, of other substances, and of non–alcohol-associated diseases on them (Laposata, 1999). During the past decade, nonoxidative direct ethanol metabolites have been developed and appear to meet the need for sensitive and specific biomarkers of ethanol intake. These markers include ethyl glucuronide (EtG) (Wurst et al., 2003b), phosphatidyl ethanol (PEth) (Varga et al., 2001; Wurst et al., 2003a, 2004a), fatty acid ethyl esters (FAEE) (Diczfalusy et al., 2001), and ethyl sulfate (EtS) (Dresen et al., 2004; Helander and Beck, 2004). Each provides a unique time spectrum for detection of ethanol consumption. For example, FAEEs remain detectable in serum up to 24 hours after cessation of ethanol intake, EtG and EtS in urine up to five days, and PEth in whole blood for more than two weeks. Additionally, EtG and FAEE can be found in hair for months (Wurst et al., 2003b, 2004a).

Wurst and colleagues reported on basic characterization and clinical dimensions of EtG, EtS, FAEEs, and PEth, such as excretion characteristics, distribution in various body fluids, tissues, and hair. Furthermore, they described the use of the direct ethanol metabolites in monitoring of psychiatric patients and physicians recovering from addiction, evaluation of recent alcohol use although blood alcohol was zero in an emergency unit, and giving motivational feedback to rehabilitating alcohol-dependent patients.

The studies on EtG completed so far, which cumulatively consist of more than 5,000 urine and serum samples of more than 1,900 individuals, include participation in the WHO/ISBRA study (Wurst et al., 2004b) and the very first comparisons of this new line of markers with each other. These new markers have also been compared with traditional markers and self-reports (EtG, FAEE, PEth, GGT, MCV, CDT; EtG, HTOL/HIAA ratio, GTOL; breath and urinary ethanol).

A variety of techniques have been developed for measuring EtG, including gas chromatography/mass spectrometry (GC/MS), liquid chromatography-tandem MS (LC/MS-MS)[very recently with two ion transitions monitored, according go forensic standards], using deuterium labeled EtG as internal standard [a gold standard], and so forth (Weinmann et al., 2004).

Ethyl sulfate, another direct ethanol metabolite formed by sulfotransferases (Vestermark and Boström, 1959) has recently also gained attention as a biomarker for ethanol intake (Dresen et al., 2004; Helander and Beck, 2004). EtS showed similar but not identical excretion characteristics as EtG (Dresen et al., 2004) and was detectable for up to one and a half days after intake of moderate amounts of ethanol. Furthermore, Wurst and colleagues reported on a controlled drinking experiment with ten healthy volunteers: Of the 77 urine samples, 14 were positive for urine alcohol concentration (UAC), 59 for EtG, and 61 for EtS. Four samples were found to be positive for EtG only and six for EtS only. Ethyl sulfate, standardized to a creatinine level of 100 (EtS 100) reached its maximum about two hours (median) after start of the experiment. When monitoring abstinence in alcoholics after withdrawal (during a rehabilitation program), of the 98 urine samples tested, seven were positive for EtS but not for EtG, and in 20 samples both ethanol metabolites were detectable. Our preliminary data suggest that the percentage of ethanol excreted as EtS is comparable to that of EtG (<0.1%). In samples of patients and volunteers, the Spearman rank correlation between EtG and EtS and between EtG 100 and EtS 100 was high and significant. In some subjects we found EtS were positive for drinking, whereas EtG values were below the limit of quantitation and in others the opposite. This could be explained by having different pathways of formation of EtG and EtS. This might turn out to be an advantage offering the opportunity of complementary use of these markers as well as one marker serving as confirmation for the other.

For FAEEs in serum, Wurst et al. (2003b) found that the usefulness might be restricted by the undulating time course in which FAEEs in serum intermittently may return to zero. EtG in contrast appears to be more reliable and accurate in serum. In contrast, FAEEs in hair appear to be promising: ROC curve analysis of FAEE in hair was conducted distinguishing between teetotalers/social drinkers and heavy drinkers/alcoholics. The area under the curve was 0.982, indicating high sensitivity and specificity for the sum of four major FAEEs in hair (Wurst et al., 2004a).

For PEth, which becomes positive after intake of 40 to 60 g ethanol/day for about two weeks and remains positive after cessation of alcohol intake for about two to three weeks, there were no false-negatives in alcohol withdrawal patients and no false-positives in sober patients (Wurst et al., 2004a, 2003a). In conducting the ROC curve analysis of PEth for the drinkers versus sober patients, the resulting AUC was 1.0 ($p < 0.0001$; CI, 1.0 to 1.0). At a cutoff close to the limit of quantification, sensitivity was 97.6% and specificity 100%. These data suggest that PEth is an excellent candidate for a sensitive and specific marker, which in previous studies has reflected longer-lasting intake of higher amounts of alcohol. However, to avoid in vitro formation of PEth in blood, samples containing ethanol should be kept frozen at $-80°C$ or at $+4°C$ (Aradottir et al., 2004).

The complementary use of direct ethanol metabolites together with other biological state markers and self-reports is expected to lead to significant improvement in treatment outcome, therapy effectiveness, and health, social, and socioeconomic benefits. The direct markers, including EtS, may be expected, in fact, to have important applications in fields of medicine, including psychiatry, as well as public health and public safety as a more objective method for documenting ethanol exposure.

GENETIC STUDIES OF ALCOHOLISM SUBTYPES IN A HAN TAIWANESE POPULATION

Ru-Band Lu, Huei-Chen Ko, San-Yuan Huang, Tso-Jen Wang, and Yi-Syuan Wu

Family, twin, and adoption studies have suggested that there is a strong hereditary component in alcoholism. The enzymes involved in the metabolism of alcohol were considered to be one of the major biological factors influencing drinking behavior and the development of alcoholism. In vitro enzymatic studies have demonstrated that ADH1B*2/*2-encoded enzymes exhibit 30 to 40 times the V_{max} for ethanol oxidation exhibited by ADH1B*1/*1-encoded enzymes. ADH1C*1/*1-encoded enzymes oxidize ethanol twice as fast as ADH1C*2/*2-encoded enzymes. The ALDH2*1/*1-encoded enzyme is an active form, whereas the enzyme encoded by ALDH2*1/*2 or ALDH2*2/*2 is an inactive form. A subject with ADH1B*2, ADH1C*1, and ALDH2*1 is thus more at risk than a subject with ADH1B*1, ADH1C*2, and ALDH2*2 because of the faster alcohol and aldehyde metabolite rate; this risk increases with the amount of alcohol consumed.

However, Thomasson et al. (1991) found that alcoholics among Han Chinese men in Taiwan had significantly lower frequencies of ADH1B*2, ADH1C*1, and ALDH2*2 alleles than did the nonalcoholics. With ALDH deficiency resulting from ALDH2*2, which slows the elimination of acetaldehyde, the more active isozymes produced by ADH1B*2 and ADH1C*1 could generate higher acetaldehyde levels and thus deter heavy drinking. That is, alcohol-

metabolizing genes might have protective effects against alcoholism through slower removal or faster production of acetaldehyde.

Many later studies supported the hypothesis of Thomasson et al.: 1) The alleles ADH1B*2, ADH1C*1, and ALDH2*2 occur significantly less frequently in alcoholics than in the general population of East Asians. The alcoholism rate is only 1.5 to 1.8% among Han Chinese in Taiwan, and ADH1B*2 and ADH1C*1 are the predominant alleles among that population. ALDH2*2 only appears in East Asian populations. 2) ALDH2*2 homozygosity alone, regardless of the functional polymorphisms at ADH1B and ADH1C, appeared to be completely protective against alcoholism, and ALDH2*1/*2 heterozygosity displays partial protection against alcoholism, being found in only 10 to 18% of Han Chinese alcoholics versus 40% of normal control subjects. 3) The ADH1B*2 and ADH1C*1 alleles are found at higher frequencies in normal control subjects than in alcoholics.

A series of recent studies, however, has shown evidence that contradicts the hypothesis of Thomasson et al. (1991): Chen et al. (1999) and Osier et al. (1999) found the effect of ADH1C polymorphism on propensity to alcoholism to be neutral or very small. Because of the similar activity and close vicinity of the ADH1B and ADH1C loci, which are only ~15 kb apart, Osier et al. (1999) investigated their functional variants as haplotypes and reported that linkage disequilibrium occurred at the ADH1B and ADH1C loci. Chen et al. (1999) considered ADH1C singly and found no statistically significant causative relation between ADH1C and the risk of alcoholism. The ADH1B*2-ADH1C*1 haplotype is higher in nonalcoholics, and the ADH1B*1-ADH1C*1 haplotype is higher in alcoholics. These results suggest that the ADH1B and ADH1C genes behave in transmission as a single allele. ADH1C might have no effect on alcoholism (Osier et al., 1999).

In our study, we increased the study sample sizes to prove the hypothesis of Chen et al. (1999) and Osier et al. (1999), collecting 340 alcoholics and 545 nonalcoholics. After multiple logistic regression, we confirmed the findings of Chen et al. (1999) that only variant ADH1B genotypes influence drinking behavior.

Genetic studies of the population show alcoholism rates of about 15% in Han Chinese, 20.7% in Ami aborigines, 17.1% in Atayal aborigines, and 15% in whites. ADH1B*1 is found in most people worldwide. ADH1B*2 is found in 70% of East Asians, and ADH1B*2 is found in about 80% of such Taiwanese aborigines as the Ami and the Atayal. One protective factor in Taiwanese aborigines and limited protective factors in whites have been found. However, the alcoholism rate is higher among Taiwanese aborigines than among Han or whites. Our study corroborated these findings, showing the ADH1B*2 allele present equally in alcoholics and control subjects. All the above study results have shown that ADH1B*2 might not serve as a protective factor in alcoholism.

Additionally, in the alcohol challenge test, we gave 0.3 g/kg alcohol to normal subjects with ALDH2*1/*1 and ALDH2*1/*2 genotypes. We found in normal volunteers no influence of blood ethanol in variant ADH1B genotypes only at 120 minutes after alcohol consumption. Moreover, we found with Peng et al. (1999) no accumulation of blood acetaldehyde in the variant ADH1B genotype in subjects with ALDH2*1/*1. All of these results argue against Thomasson's suggestion that elimination of acetaldehyde, higher acetaldehyde levels generated by the more active ADH isoenzymes should deter heavy drinking.

On the other hand, dopamine is metabolized either through oxidative deamination by monoamine oxidase (MAO) or O-methylation by catecho-O-methyltransferase (COMT). About 90% of dopamine is metabolized to 3,4-dihydroxyphenylacetaldehyde (DOPAL) by MAO in the rat corpus striatum. DOPAL is then either oxidized to 3,4-dihydroxyphenylacetic acid (DOPAC) by ALDH or reduced to 3,4-dihydroxyphenylethanol (DOPET) by aldehyde or aldose reductase (ARs). Inhibition of ALDH and ARs would both increase the level of DOPAL and decrease the formation of DOPAC and DOPET. Thus, ALDH and ADH not only play an important role in ethanol catabolism but also in dopamine catabolism.

Furthermore, one recent study also found that Chinese herbs containing ALDH2 inhibitors might suppress alcohol intake through inhibition of dopamine metabolism (Keung and Vallee, 1998; Rooke et al., 2000). From the above, we hypothesize that ADH1B and ALDH2 genotypes might interact with the dopamine D2 receptor gene (DRD2) to influence drinking behavior. In our recent study, we found that DRD2 is not associated with pure alcoholism or anxiety-depression disorder but is associated with anxiety-depression alcoholism and is only under the control of the ALDH2*1/*1, ADH1B*1/*1, and ADH1B*1/*2 genotypes. Therefore, we suggest that anxiety-depression alcoholism is a specific subtype of alcoholism, and DRD2 might interact with ADH1B or ALDH2 in the Han Taiwanese population (Huang et al., 2004).

We concluded first that there should be more studies of the role of ADH genotypes in ethanol metabolism in different ethnic groups. Second, population genetic studies showed the increased statistical power for a disease of 1% prevalence compared with a disease of 10% prevalence, for a fixed heritability and a fixed number of trait loci. Third, study of Han Chinese populations with specific variant ADH1B and ALDH2 genes might contribute greatly to the understanding of the relation between molecular genetics and alcoholism. Last, the study of alcoholism may play an important role in the study of substance use disorders and addictive behavior in general.

CONCLUSIONS

This symposium links Phase I and the proposed Phase II of the WHO/ISBRA Study on State and Trait Markers of

Alcohol Use and Dependence. Results presented in the fields of genomic, proteomic, and biochemical analyses have been carried out with material obtained in Phase I of the WHO/ISBRA study. Furthermore, the work of some of the proposed participants in the Phase II study has been introduced.

For the characterization of the phenotype "alcoholism," biological state and trait markers and marker combinations capable of monitoring alcohol consumption with a high sensitivity and specificity over a broad time spectrum and indicating individual susceptibility are needed. Recent advances in high-throughput technologies in genomics, proteomics, and metabolomics provide unique possibilities to identify novel biomarkers. This type of research is not only valuable for discovering new state and trait markers but also for identifying biomarkers of alcohol use disorders such as alcoholic liver disease and other tissue damage and biomarkers that allow a subtyping of alcoholism and co-morbid psychiatric disorders.

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