Research Highlights

Highlights from the latest articles in pharmacogenomics

A polymorphism in the *SOD2* gene and its association with esophageal cancer

Evaluation of: Sun L, König IR, Homann N: Manganese superoxide dismutase (*MnSOD*) polymorphism, alcohol, cigarette smoking and risk of oesophageal cancer. *Alcohol Alcoholism* 44(4), 353–357 (2009).

As alcohol consumption, smoking and Barrett's syndrome can result in gastro-esophageal reflux, they are important risk factors for esophageal cancer. In addition, Zhang *et al.* have shown that a genetic component may be implicated in the pathogenesis of esophageal squamous cell carcinoma and adenocarcinoma [1]. With regard to the molecular mechanism involved in oesophageal carcinogenesis, oxidative stress is likely to play a role and is mediated by the production of reactive oxidative species (ROS). The manganese superoxide dismutase (*MnSOD*) enzyme, which is found in mitochondria, is part of the antioxidant defense system that counteracts the possible damaging effects of ROS. Polymorphisms in the *SOD2* gene encoding *MnSOD*, which cause an altered level of gene expression, may subsequently influence the cell's ability to counteract ROS-induced damage. A polymorphism in *SOD2*, consisting of a T to C substitution and resulting in an amino acid change from valine (Val) to alanine (Ala) has been associated with several types of cancer [2], but studies on the link with esophageal cancer have not been reported.

Sun, König and Homann examined three Caucasian cohorts for the *MnSOD* allele frequencies; an esophageal cancer cohort (adenocarcinoma: *n* = 61; squamous cell carcinoma: *n* = 109), a population-based healthy cohort (*n* = 400) and a cohort of heavy drinkers (*n* = 160) [3]. Although the Ala/Ala genotype was

more frequent in cancer patients (27.7%) than in healthy blood donors (12.5%) and heavy drinkers (23.1%), these differences were not statistically significant when the researchers controlled for age, alcohol and tobacco use. Similarly, non-significant differences in genotype frequencies were obtained when the cancer cohort was subdivided according to cancer type and compared with the healthy controls or heavy drinkers. Interestingly, in an additional, exploratory analysis, it was found that *SOD2* Ala/Ala genotype carriers among the three cohorts did consume significantly more alcohol (uncorrected *p* < 0.0001) and cigarettes (uncorrected *p* = 0.041) than those carrying Val/Ala or Val/Val genotypes.

The Sun *et al.* study was the first to examine the association between a polymorphism in the *SOD2* gene and esophageal cancer [3]. However, whether the *SOD2* gene and environmental risk factors interact in increasing the risk for esophageal carcinoma remains a topic for future research. Esophageal cancer is a complex disease that is most likely affected by multiple genes [4], environmental factors and by the interaction between these genes and environment. Hence, the relationship between *SOD2* genotype and esophageal cancer might be dependent on levels of alcohol use.

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Relationships between the *ADH1C* gene, alcohol use and risk factors for cardiovascular disease

Evaluation of: Latella MC, Di Castelnuovo A, De Lorgeril M *et al.*: Genetic variation of alcohol dehydrogenase type 1C (*ADH1C*), alcohol consumption, and metabolic cardiovascular risk factors: Results from the IMMIDIET study. *Atherosclerosis* (2009) (Epub ahead of print).

Epidemiological studies showed that light-to-moderate alcohol consumption protects against cardiovascular diseases (e.g., [1]). The mechanism involved in the cardioprotection through alcohol might involve its enhancing effect on high-density lipoprotein (HDL) cholesterol levels. In addition, interindividual variability in alcohol metabolism may help understand the cardioprotective effects of alcohol [2]. In the human body, alcohol is converted by alcohol dehydrogenase (ADH) into acetaldehyde, which is subsequently oxidized into acetate by acetaldehyde dehydrogenase. The $\gamma 2$ low-activity allele of a polymorphism in the *ADH1C* gene (rs689) has been associated with higher levels of HDL cholesterol and a lower risk at myocardial infarction, in individuals consuming at least one alcoholic drink per day [3]. These results, however, have not been replicated in subsequent studies.

Latella *et al.* examined 1604 subjects (from 974 male–female pairs participating in the IMMIDIET study) from Italy, Belgium and England for relationships between *ADH1C* genotype, alcohol consumption and various metabolic cardiovascular risk factors in a cross-sectional design [2]. In general, no direct associations between *ADH1C* genotype and cardiovascular risk factors or alcohol use were found. Independent of *ADH1C* genotype, alcohol intake was significantly and positively related to several cardiovascular risk factors, including HDL-cholesterol levels. An interaction effect in which *ADH1C* $\gamma 2$ allele carriers who consumed over 48 g of pure ethanol per day showed higher levels of BMI and waist circumference than males who did not carry this allele, but this was not observed in females. This effect of *ADH1C* genotype did not seem to be mediated via the levels of either HDL cholesterol or other metabolic factors investigated. In fact, *ADH1C* $\gamma 2$ allele carriers showed even lower HDL-cholesterol levels at high-alcohol consumption levels than subjects homozygous for the $\gamma 1$ allele.

The attempt to clarify the complex relationships between alcohol, specific genes and cardiovascular risk factors in the cross-sectional study by Latella and

coworkers is admirable, as is their attempt to use multivariate analyses to approach this subject [2]. However, a prospective study, in which the participants are regularly examined for cardiovascular disease, would potentially provide a more ideal setting for such a study. Although the researchers used a multivariate design, ample different analyses were still carried out, which may have created a multiple testing problem. Since p-values were not corrected, and effect sizes were relatively low, the results should be replicated before firm conclusions can be drawn from this study.

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Polymorphisms in the *CYP2E1*, *GSTM1* and *GABRG2* genes and risk for alcoholic liver cirrhosis

Evaluation of: Khan AJ, Ruwali M, Choudhuri G *et al.*: Polymorphisms in cytochrome P450 2E1 and interaction with other genetic risk factors and susceptibility to alcoholic liver cirrhosis. *Mutat. Res.* 664, 55–63 (2009).

Although heavy alcohol consumption is a major risk factor for liver diseases, only 10–20% of heavy drinkers develop liver cirrhosis. Cirrhosis is marked by the replacement of normal liver tissue by collagen scar tissue, which disrupts the blood flow through the liver and results in loss of liver function [1]. Genetic factors,

particularly those that influence alcohol metabolism or risk for alcoholism, may be involved in the risk for cirrhosis [1,2]. Since the cytochrome P450 2E1 (*CYP2E1*) enzyme is a key player in alcohol oxidization, differences in *CYP2E1* activity caused by polymorphisms in the *CYP2E1* gene may help explain individual

variability in susceptibility for liver cirrhosis. Several polymorphisms have been identified in the *CYP2E1* gene, of which the *CYP2E1*5B* (*RsaI*) SNP (C>T) leads to an increased transcription and enzyme activity. In previous studies, some of the *CYP2E1* polymorphisms have been related to liver diseases, though with inconsistent results.

Kahn *et al.* examined associations between three polymorphisms in the *CYP1E1* gene (*CYP2E1*5B*, *CYP2E1*6* and *MspI*) and alcohol liver cirrhosis in an Indian sample, consisting of 160 alcoholic cirrhotic patients, 125 nonalcoholic cirrhotic patients, 250 nonalcohol dependent controls and 100 subjects who were alcohol dependent but showed no signs of liver disease [3]. In addition, two polymorphisms in genes involved in the detoxification of ROS and in alcohol consumption (glutathione *S*-transferases M1 [*GSTM1*] and γ -aminobutyric acid receptor γ 2 [*GABRG2*], respectively) were also included in the analyses. Allele frequency of the rare *CYP2E1*5B* variant, but not of the more common *CYP2E1*6* and *MspI* variants, was significantly higher in alcoholic cirrhotic patients compared with all other groups ($0.002 \leq p \leq 0.034$). The haplotype carrying the variant allele of *CYP2E1*5B* and the wild-types of the *CYP2E1*6* and *MspI* was significantly more frequent in alcoholic cirrhotic

patients than in nonalcoholic controls (odds ratio: 5.3; 95% CI: 1.46–19.60; $p = 0.005$). Also, a *GSTM1* gene deletion polymorphism and a functional *GABRG2* variant (intron 8, 84435G>A) separately showed a significant association with alcohol-related cirrhosis. Two combined gene effects were also found: carriers of both the *CYP2E1*5B* variant allele and the *GSTM1* null allele showed a significantly higher risk for alcoholic liver cirrhosis (odds ratio: 7.7; 95% CI: 1.60–37.16; $p = 0.003$), as did those with both the *CYP2E1*5B* variant allele and the *GABRG2* A allele (odds ratio: 6.4; 95% CI: 1.92–21.50; $p < 0.001$).

That epistasis (i.e., the interaction between genes) is highly likely to affect complex human traits such as liver diseases is nicely shown by the results of the paper by Kahn and coworkers [3]. Testing for epistasis may be especially important since the effect of one locus can be masked by the effects of a second locus [4]. Epistasis effects may therefore explain considerably more phenotypic variance than two single variants, helping to explain the relative paucity of significant findings so far identified in genome-wide association studies on complex disorders [5]. There are presumably more than three loci involved in the development of alcoholic liver diseases and testing for multiway genetic interactions would further

increase our knowledge of the etiology of liver diseases. However, a concern with the investigation presented in the current paper is the fact that the frequency of the risk alleles (especially the *CYP2E1*5B* allele) is low, reducing generalizability of the findings and making replications in larger samples necessary before firm conclusions can be drawn.

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Interactions between the *GPX1* Pro198Leu polymorphism and substance use and susceptibility to colorectal cancer

Evaluation of: Hansen RD, Krath BN, Frederiksen K *et al.*: *GPX1* Pro198Leu polymorphism, erythrocyte GPX activity, interaction with alcohol consumption and smoking, and risk of colorectal cancer. *Mutat. Res.* 664, 13–19 (2009).

There is evidence that oxidative stress plays a role in the pathogenesis of colorectal cancer, since increased levels of ROS and lipid peroxidation have been found in colorectal cancer tissue. The antioxidant GPX1 and the DNA-repairing OGG1

enzymes are known to counteract oxidative DNA damage. Therefore, genetic polymorphisms in *GPX1* and *OGG1*, such as the *GPX1* rs1050450 (Pro¹⁹⁸Leu) and the *OGG1* rs1052134 (Ser³²⁶Cys) variants, that result in lower enzyme expression levels, might play a role in colorectal carcinogenesis. In addition, fruit, vegetable and alcohol intake, tobacco smoking and selenium supplementation may influence GPX1 enzyme activity. Although the *GPX1* and *OGG1* variants have not consistently been associated with colorectal cancer in previous studies [1], Kim and colleagues did report interactions between

the *OGG1* ³²⁶Cys allele and meat consumption, and between the *OGG1* ³²⁶Cys allele and smoking, which both increased the risk for colorectal cancer [2].

Hansen *et al.* examined whether 375 patients with colorectal cancer and 779 control subjects selected from a large, prospective cohort study differed in *GPX1* ¹⁹⁸Leu or *OGG1* ³²⁶Cys allele frequencies and/or various lifestyle factors [3]. *GPX1* Pro¹⁹⁸Leu and *OGG1* Ser³²⁶Cys genotypes were not directly related to colorectal cancer. Direct differences in lifestyle factors between patients with colorectal cancer and controls were also not observed.

However, significant interaction effects were found; alcohol consumption and smoking augmented risk of colorectal cancer among homozygous *GPX1* ¹⁹⁸Leu allele carriers ($p = 0.02$, uncorrected), while intake of vitamin C from diet reduced risk among homozygous carriers of the *GPX1* ¹⁹⁸Pro allele ($p = 0.05$). In contrast to the findings by Kim *et al.*, the *OGG1* Ser³²⁶Cys polymorphism showed no significant interaction with lifestyle factors in the current study. Hansen *et al.* also measured erythrocyte GPX activity in prediagnostic blood samples. In univariate analyses GPX activity proved to be lower in women, *GPX1* ¹⁹⁸Leu allele carriers, smokers and subjects who consumed less fruit and vegetables. In the multivariate

analysis, however, these findings did not remain significant, except for fruit and vegetable intake, which explained approximately 5% of variance. Selenium supplementation and alcohol use were not related to erythrocyte GPX enzyme activity.

The setup of this study, a large ($n = 57,053$) prospective follow-up design, is ideally suited for the analyses of interest. Multivariate analyses like these, including both environmental and genetic factors, such as the one described by Hansen and coworkers [3], are likely to provide a better insight into the etiology of colorectal cancer and other complex disorders than the analysis of single factors or domains. Nevertheless, the number of cases that could be investigated is still limited, and findings from this study did not reach significance when a Bonferroni correction for multiple testing was applied.

This study clearly needs replication, but is an important starting point that future studies on colorectal carcinogenesis may elaborate on.

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