Proceedings of the Nutrition Society (2004), **63**, 73–79 DOI:10.1079/PNS2003321

Genetic modification of the effect of alcohol consumption on CHD

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The deleterious health effects of high alcohol consumption are numerous and well recognized; however, the effect of moderate alcohol consumption on overall health continues to be a debated issue. Among the more prevalent diseases in Westernized countries, epidemiological research suggests that alcohol in moderation substantially reduces the risk of CHD, while it modestly increases the risk for certain cancers, such as breast and colon cancer. Despite the overwhelming data supporting the beneficial effect of moderate alcohol consumption on the cardiovascular system, some researchers are not convinced. Sceptics argue that the reduction in risk is attributed to a favourable lifestyle factor associated with moderate alcohol consumption, or that it may be attributed to constituents of alcoholic beverages other than ethanol, such as the antioxidants in the grapes. In order to promote overall health for the general public, it is necessary to elucidate these issues. One approach is to study population differences in alcohol metabolic efficiency, which is likely to contribute to an individual's susceptibility to alcohol-associated diseases. Among the population there is substantial variability in the efficiency to metabolize alcohol. Genetic variation among the alcohol-metabolizing genes is known to produce isoenzymes with distinct kinetic properties. Studying genetic differences that potentially influence disease susceptibility among populations may provide insight into the mechanism(s) for the relationship between risk factor and disease, such as alcohol and CHD.

Alcohol consumption: Alcohol metabolism: CHD

Epidemiological data on the relationship between alcohol consumption and CHD

Epidemiological data strongly support an inverse association between moderate alcohol consumption and risk of CHD and/or CHD mortality (Grobbee *et al*. 1999; Sesso & Gaziano, 1999; Rimm & Stampfer, 2000; Gall, 2001). This relationship has been observed in diverse populations that differ in relation to incidence of CHD and preference of alcoholic beverage, such as populations from Yugoslavia, UK, Denmark, China, France and Japan (Kozararevic *et al*. 1980; Doll *et al*. 1994; Gronbaek *et al*. 1995; Yuan *et al*. 1997; Kitamura *et al*. 1998; Renaud *et al*. 1998). The reproducibility of this finding refutes previous ecological data suggesting that only wine is beneficial (Rimm *et al*. 1996). Taking into account other known CHD risk factors, an overall estimate from prospective studies is a 30–40% reduction in risk of CHD for two drinks per d among men and one drink per d among women, with minimal additional benefit for higher consumption levels. Depending on the study and the beverage type, a typical drink has been defined as containing 10–15 g alcohol. Evidence suggests a 'U'-shaped relationship between the level of alcohol consumption and CHD, with estimates of the bottom of the curve ranging from two to six drinks/d (Grobbee *et al*. 1999; Rimm & Stampfer, 2000). Recent epidemiological studies have shown that alcohol consumption on a regular basis (≥3 or 4 d/week) is more beneficial than consuming the entire week's allocation in a few days (Kannel, 1988; Kauhanen *et al*. 1997; Mukamal *et al*. 2003). There is no clear evidence to suggest that there is a difference in risk of CHD between alcohol consumption with meals and alcohol consumption without meals (Mukamal *et al*. 2003).

Although the epidemiological evidence is overwhelming, the specific mechanism(s) for the inverse association has not been completely elucidated. Over eighty experimental studies on human subjects have examined cardiovascular effects of alcohol on lipids, coagulation factors and other cardiovascular markers (Rimm *et al*. 1999).

Abbreviations: ADH, alcohol dehydrogenase; ALDH, acetaldehyde dehydrogenase.

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The majority of experimental studies utilized a cross-over study design, in which biological markers were measured among participants while consuming a fixed amount of alcohol after a washout period and during abstinence. These typically short-term studies (<3 months) are useful in the investigation of the effects of alcohol on biological predictors of CHD, but not necessarily on clinical events.

Based on a meta-analysis of these experimental studies, which was conducted by Rimm *et al*. (1999), the effect of alcohol consumption on risk of CHD is attributed to HDL (60%), fibrinogen (20–30%), insulin (5–10%) and haemostatic factors (0–5%). Observational data, simultaneously estimating the relationships between alcohol, HDL level and risk of CHD have also demonstrated that at least half the beneficial effect is attributed to HDL (Criqui *et al*. 1987; Langer *et al*. 1992). Although the protective effect of HDL is understood, the mechanism(s) by which alcohol raises HDL levels is not clear. Some investigators have speculated that alcohol may have a direct effect on the liver; it may increase hepatic production and secretion of apo and lipoprotein particles, increase triacylglycerol lipases and decrease removal of circulating HDL (Dreon & Krauss, 1996).

The observed increase in HDL level occurs after 1–2 weeks of daily alcohol consumption and remains unless the alcohol consumption level is decreased (Rimm & Stampfer, 2000). Early observations suggested that the effect of alcohol was only on HDL-3 particles, not HDL-2 particles, but recent work has found that it increases both types of particle (Gaziano *et al*. 1993; Clevidence *et al*. 1995). Although epidemiological evidence suggests a 'U'-shaped association between alcohol consumption and CHD risk, the relationship between the amount of alcohol consumed and HDL level is linear and extends beyond the range of moderate consumption (Rimm *et al*. 1999). Rimm *et al*. (1999) estimated that 30 g alcohol/d (slightly more than two drinks/d) would raise HDL by 0·1 mmol/l (40 mg/l), which translates to a 17% reduction in risk of CHD. Gender, beverage choice or duration of study did not noticeably influence this estimate. Alcohol consumption has been shown to have a greater impact on HDL levels among subjects with baseline levels below 1·0 mmol/l (400 mg/l; Rimm & Stampfer, 2000).

Gender differences

Although lifetime mortality from CHD is similar for men and women, the disease primarily affects middle-aged men and older women. Experimental studies have shown a cardio-protective effect of ovarian hormones, such as increasing HDL levels, decreasing LDL levels and other vasculo-protective effects (Farhat *et al*. 1996). There is evidence to suggest that moderate alcohol consumption affects oestrogen levels, which could contribute to the beneficial effect of alcohol consumption on the risk of CHD (Purohit, 1998). However, recent clinical trial data do not support previous epidemiological data of a protective effect for exogenous oestrogens, specifically post-menopausal hormone therapy (Rossouw *et al*. 2002). Since the incidence of CHD in premenopausal women is very low, it is difficult to assess the association between alcohol consumption and CHD risk in this population. Epidemiological studies support a protective effect of alcohol consumption on CHD risk in post-menopausal women, and this benefit appears to be associated with levels of alcohol consumption that are lower than those observed for men (one drink/d *v*. two drinks/d; Grobbee *et al*. 1999). The gender difference has been attributed to three main factors: (1) body size; (2) alcohol solubility; (3) efficiency of alcohol metabolism (Meister *et al*. 2000). Women generally have a smaller body size and a higher proportion of body fat. Since alcohol is more soluble in body water than in body fat, the physiological effects of alcohol occur at a lower dose among women (Goist & Sutker, 1985). Furthermore, experimental data indicate that men and women differ in relation to their efficiency in alcohol metabolism (Frezza *et al*. 1990). This difference could be attributed to factors such as hormones, which affect regulation of the alcohol metabolic pathway (Teschke & Heymann, 1982; Teschke *et al*. 1986; Qulali *et al*. 1991).

Genetic variation of metabolic genes and disease: a possible link to a 'causal' interpretation

The majority of associations obtained from epidemiological studies are based on observational data. Since these studies are conducted in a non-randomized setting, they are susceptible to confounding variables. Some investigators argue that the observed inverse association between moderate alcohol consumption and CHD is attributed to other favourable lifestyle factors (Hart *et al*. 1999). As a result of the observed increase in risk for certain cancers, the questionable causality of the association with CHD and the fear of promoting alcohol abuse among susceptible individuals, the use of moderate alcohol consumption for overall health remains controversial. A randomized clinical trial would be ideal for validating observational data; however, this approach may not be feasible because of the potential detrimental effects of moderate alcohol consumption. An alternative approach is to study the effect of genetic variation among genes that contribute to metabolism or transduction of the suspected factor associated with a given disease. An observed modifying effect of genetic differences (e.g. a specific genotype) among alcohol-metabolizing genes on the relationship between alcohol consumption and CHD adds support to a causal interpretation of the observed association for two main reasons. First, it is unlikely that an individual's genetic composition is associated with any of the potentially confounding variables, such as smoking or exercise. Furthermore, functional genetic differences in metabolic genes will improve the possibility of identifying the specific disease-causing factors. Unlike animal experimental models, human subjects are exposed to a complex mixture of many compounds. Identifying the key factor that predisposes an individual to a given disease is difficult. For example, some sceptics argue that the reduction in risk of CHD is attributed to constituents of alcoholic beverages other than ethanol, such as the antioxidants in the grapes (Frankel *et al*. 1993; Whitehead *et al*. 1995; Bell *et al*. 2000). Since the predominant function of alcohol-metabolizing genes is to metabolize ethanol rather than other constituents in

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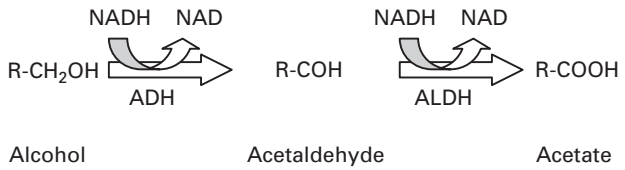

Fig. 1. Alcohol metabolic pathway. ADH, alcohol dehydrogenase; ALDH, acetaldehyde dehydrogenase.

alcoholic beverages, an observed modifying effect of genetic differences in alcohol-metabolizing genes on the relationship between alcohol consumption and CHD would suggest that ethanol is responsible for the association between alcohol consumption and alcohol-related diseases.

The Human Genome Project has demonstrated that many genes contain variants, several of which are likely to have an impact on function. Traditional epidemiological methods have been successful in identifying several lifestyle and environmental factors that contribute to disease susceptibility. However, lifestyle and environmental factors alone are not the only contributors to disease susceptibility. The ability to incorporate genetic differences will provide another perspective to the understanding of disease aetiology.

The genetics of alcohol metabolism

The pharmaco-kinetics of alcohol metabolism have been well studied. The initial steps of the predominant pathway for metabolizing alcohol are illustrated in Fig. 1. The rate-limiting step in this pathway is believed to be the oxidation of alcohol to acetaldehyde by the alcohol dehydrogenase (ADH) enzyme, a dimeric protein that consists of two 40 kDa subunits (Bosron *et al*. 1988). In man there are at least seven *ADH* genes that convert alcohol to acetaldehydes (Osier *et al*. 2002). Located in tandem on chromosome 4, the *ADH* are divided into five different classes depending on their preferential substrate. The class I enzymes, encoded by *ADH1A*, *ADH1B* and *ADH1C* (also known as *ADH1*, *ADH2* and *ADH3*), predominantly metabolize short-chain alcohols such as ethanol. The class I ADH isoenzymes share approximately 93% homology, but differ in their substrate specificity and tissue expression (Niederhut *et al*. 2001).

Among the class I genes, both *ADH1B* and *ADH1C* have polymorphisms that have been well characterized (Bosron *et al*. 1988). At the *ADH1B* locus, the β_1 allele differs from the other alleles by one amino acid: position 47 (arginine to histidine) for the β_2 allele; position 369 (arginine to cysteine) for the β_3 allele. At the *ADH1C* locus, the γ_1 allele differs from the γ_2 allele by two amino acids at positions 271 (arginine to glycine) and 349 (isoleucine to valine). *In vitro* studies have shown that these polymorphisms produce enzymes with distinct kinetic properties (Table 1). At the *ADH1B* locus, the β_2 homodimer is approximately forty times faster than the β_1 homodimer. The difference in kinetic activity at the *ADH1C* locus is not as dramatic; the γ_1 homodimer is approximately 2·5 times faster than the γ_2 homodimer. This difference is thought to affect the rate of oxidation of blood ethanol

Table 1. The kinetic characteristics of the alcohol dehydrogenase (ADH) subunits ADH1B and ADH1C (Bosron *et al.* 1988)

	ADH1B			ADH1C	
Homodimer	$\beta_1\beta_1$	$\beta_2\beta_2$	$\beta_3\beta_3$	$\gamma_1\gamma_1$	$\gamma_2\gamma_2$
Maximum velocity (/min)	9·2	400	270	87	35

Table 2. Allele frequencies (%) of the alcohol dehydrogenase (*ADH*) polymorphisms *ADH1B* and *ADH1C* by ethnicity (Bosron *et al.* 1988; McCarver *et al.* 1997)

Population	*ADH1B*1* (β_1)	*ADH1B*2* (β_2)	*ADH1B*3* (β_3)	*ADH1C*1* (γ_1)	*ADH1C*2* (γ_2)
Caucasian	90–95	<5	<5	60	40
Asian	35	65	<5	95	5
African-American	75	<5	25	85	15

(Bosron *et al*. 1988), although the *ADH1C* polymorphism had no apparent effect on blood alcohol levels in a short-term study of high-dose alcohol consumption in human subjects (Whitfield, 1994).

The allele frequencies of the class I *ADH* polymorphisms vary depending on the ethnic background of the population (Table 2). Among Caucasian populations, the predominant allele at the *ADH1B* locus encodes for β_1, the slowest-oxidizing subunit; the β_2 and β_3 alleles are quite rare (<5%). At the *ADH1C* locus, there is a relatively even distribution of the γ_1 and γ_2 alleles, with the γ_1 allele being slightly more common.

Multiple molecular forms of acetaldehyde dehydrogenase (ALDH1A1 and ALDH2), the next step in the alcohol metabolic pathway, have also been identified. A variant form of *ALDH2 (ALDH2*2)*, predominantly observed in Asian populations, has low acetaldehyde-oxidizing activity, which results in a facial flushing response associated with the toxicity of acetaldehyde (Takeshita *et al*. 1994). This variant has been shown to be protective against the development of alcoholism (Thomasson *et al*. 1991; Higuchi *et al*. 1995; Chen *et al*. 1996).

In addition to genetic determinants, the efficiency of alcohol metabolism is also affected by factors that can regulate the activity of the genes involved in alcohol metabolism. Glucocorticoids, growth hormone and retinoids have been shown to induce ADH activity (Dong *et al*. 1988; Potter *et al*. 1989; Duester *et al*. 1991). Furthermore, other systems such as the microsomal ethanol-oxidizing system, also contribute to alcohol metabolism (Lieber, 1999). To what extent these factors contribute to population variability in alcohol metabolism needs to be determined.

Alcohol dehydrogenase-1C gene, alcohol consumption, HDL levels and risk of myocardial infarction

Genetic determinants of alcohol metabolic capacity could play a role in the susceptibility to alcohol-related diseases,

https://doi.org/10.1079/PNS2003321 Published online by Cambridge University Press

such as CHD. Although the difference in kinetic activity among the β subunits is considerably greater than that among the γ subunits, the homogeneity at the *ADH1B* locus among Caucasian populations suggests that the *ADH1C* polymorphism may contribute more to variability in metabolic capacity at the population level. The variant *ADH1C* allele is common and functional, making it an ideal candidate for molecular epidemiology studies among Caucasian populations.

The relationship between the *ADH1C* polymorphism, alcohol consumption and risk of myocardial infarction was investigated in a case–control study nested within the prospective Physicians' Health Study cohort, a predominantly Caucasian population (Hines *et al*. 2001). It was hypothesized that if ethanol is responsible for the observed beneficial effect on risk of CHD, a slower rate of clearance of ethanol may enhance the beneficial effect of moderate alcohol consumption on the risk of CHD. In addition, the relationship between alcohol intake and *ADH1C* genotype on plasma HDL levels was examined among this study population and in a similar cohort of women.

The Physicians' Health Study commenced in 1982 as a randomized double-blinded placebo-controlled trial of aspirin and β-carotene (Steering Committee of the Physician's Health Study Research Group, 1989). It consisted of 22 071 US male physicians between the ages of 40 and 84 years without a previous diagnosis of myocardial infarction or stroke. Before randomization, a blood sample was requested from all the participants. Specimens were received from 68% of the physicians who form the baseline cohort for this study (Ma *et al*. 1996).

Information on cardiovascular risk factors and disease status was obtained through biannual mailings and medical records. Follow-up for fatal and non-fatal outcomes was 99%. By 1994, 396 eligible incident myocardial infarction cases were identified. When possible, each case was matched to two randomly-selected control subjects based on age, smoking status and time from randomization, yielding a total of 1166 individuals (396 cases and 770 controls).

A strong interaction between the *ADH1C* genotype and the level of alcohol consumption in relation to the HDL level and the risk of myocardial infarction was observed (Hines *et al*. 2001). Homozygosity for the slow-oxidizing allele (γ_2) was associated with a reduced risk of myocardial infarction (relative risk, 0·65; 95% CI, 0·43, 0·99). Moderate alcohol consumption was associated with decreased risk for all genotypes; however, *ADH1C* genotype modified this association ($P = 0{\cdot}01$). Among men who were homozygous for the fast-oxidizing allele (γ_1), daily drinkers had a relative risk of 0·62 (95% CI, 0·34, 1·13) compared with men consuming less than one drink per week. Daily drinkers who were homozygous for the slow-oxidizing allele (γ_2) had a larger and highly significant 86% reduced risk (relative risk, 0·14; 95% CI, 0·04, 0·45; Fig. 2). Furthermore, slow oxidizers who consumed alcohol daily had higher plasma HDL levels ($P = 0{\cdot}05$).

The interaction between *ADH1C* genotype, alcohol consumption and plasma levels of HDL was confirmed in an independent study of 325 post-menopausal women not taking hormone-replacement therapy ($P = 0{\cdot}02$; Hines *et al*. 2001). These women were participants in a nested case–control study of breast cancer among the 33 826 Nurses' Health Study participants who donated blood in 1989–90, as described elsewhere (Haiman *et al*. 1999).

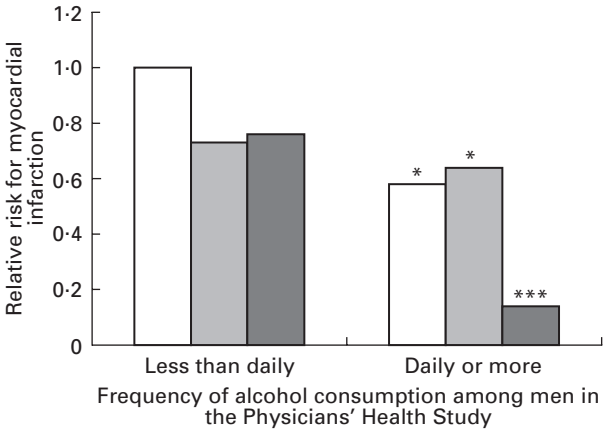

Fig. 2. Multivariate relative risks for myocardial infarction *v.* aldehyde dehydrogenase-3 genotype and daily alcohol consumption ($P = 0{\cdot}02$ for interaction). (□), Fast oxidisers, individuals homozygous for the γ_1 allele; (▨), intermediate oxidisers, individuals heterozygous for the γ_1 allele; (■), slow oxidisers, individuals homozygous for the γ_2 allele. In addition to the matching factors of age, smoking use and follow-up time, adjustment was made for BMI, vigorous physical activity, family history of myocardial infarction, assignment to aspirin use and history of hypertension, diabetes or angina at enrollment. Mean values were significantly different from those for fast oxidizers who consumed less than one drink daily: $^*P = 0{\cdot}04$, $^{***}P < 0{\cdot}001$; relative risk was 0·14 (95% CI 0·04, 0·42).

The data of Hines *et al*. (2001) suggest that moderate drinkers who are homozygous for the slow-oxidizing *ADH1C* allele (γ_2) have higher HDL levels and a substantially decreased risk of myocardial infarction. Since the predominant function of ADH1C is to metabolize ethanol, this study strengthens the evidence that the reduction in the risk of heart disease is attributed to the ethanol in alcoholic beverages.

Additional studies

Other studies have also assessed the relationship between genetic differences in alcohol metabolism, alcohol consumption and cholesterol levels. Nakamura *et al*. (2002) studied the variation at the *ALDH2* locus among a Japanese population. They did not observe any modifying effect of the *ALDH2* genotype on the relationship between alcohol consumption and HDL levels. However, because of the strong toxicity of acetaldehyde, individuals with the variant *ALDH2* allele consumed virtually no alcohol. Among the sixty-eight men and ninety-six women who were homozygous for the variant *ALDH2* allele, only six men and two women consumed more than half a drink per week. The immediate physiological effects observed among individuals with the variant *ALDH2* allele is a strong influential factor on the amount of alcohol consumed, as reflected by its protective effect on alcoholism (Thomasson *et al*. 1991; Higuchi *et al*. 1995; Chen *et al*. 1996). Thus,

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this population may not be ideal for investigating the relationship between alcohol metabolism and cholesterol levels.

Hashimoto *et al*. (2002) studied the relationship between variation at the *ADH1B* and *ALDH2* loci, alcohol consumption and serum lipids among a Japanese population who consumed high amounts of alcohol. The study population consisted of 133 male hospital employees who reportedly consumed >300 g/week, which is equivalent to more than twenty-three drinks per week. To what extent the ADH system contributes to alcohol metabolism at high levels of alcohol consumption is unclear. Previous evidence suggests that other alcohol-metabolizing systems, such as the microsomal ethanol-oxidizing system, play a more substantial role in alcohol metabolism at high levels of alcohol consumption (Lieber, 1999). Surprisingly, the authors did not observe a marked difference in alcohol consumption level based on *ALDH2* genotype among this population. This finding could be partially attributed to the fact that the heterozygotes and the homozygotes for the *ALDH2*2* allele were combined into one category, although previous data suggest that heterozygotes also experience facial flushing (Takeshita *et al*. 1994). Individuals with the variant *ALDH2*2* had noticeably higher HDL levels than those who did not (820 mg/l (2·12 mmol/l) *v*. 650 mg/l (1·68 mmol/l)). At the *ADH1B* locus, higher HDL levels were observed among individuals homozygous for the slow-oxidizing allele (β_1) compared with either heterozygotes or homozygotes for the fast-oxidizing allele (β_2; 708 mg/l (1·83 mmol/l), 634 mg/l (1·64 mmol/l) and 634 mg/l (1·64 mmol/l) respectively). These observations were not statistically significant; however, the sample size of this study was small. Although previous evidence suggests that other alcohol-metabolizing systems play a greater role in alcohol metabolism at high levels of alcohol consumption, these data do support a role of ADH in the effect of alcohol consumption on HDL levels.

Whitfield *et al*. (2003) investigated the individual effects of both the *ADH1B* and *ADH1C* polymorphisms on the relationship between alcohol consumption and HDL levels among an Australian population that was originally selected for a twin study. The authors observed no modifying effect for either of the variant genes. This inconsistency could be attributed to differences in the study populations. Whitfield *et al*. (2003) studied a population that consisted of both men and women, with the age for the women ranging from 29 to 92 years. Data were only presented for men and women, pre- and post-menopausal, combined. In the Hines *et al*. (2001) study, this interaction was observed only among men and post-menopausal women who were not taking post-menopausal hormones. Premenopausal women and post-menopausal women who use post-menopausal hormones are known to have substantially higher HDL levels compared with men and post-menopausal women who do not use post-menopausal hormones (Wenger, 1996). Among post-menopausal hormone users, the composition, dosage and period of usage are also likely to influence HDL levels. The effect of metabolic capacity on HDL levels and CHD risk among individuals who have oestrogen-elevated HDL levels has not been elucidated. The lack of effect modification by *ADH1C* genotype could suggest a threshold for HDL level. Thus, individuals with oestrogen-elevated HDL levels may receive minimal additional increases in the HDL level from alcohol consumption, which can be supported by experimental data suggesting no effect of alcohol consumption on HDL levels among runners who have exercise-elevated HDL levels (Hartung *et al*. 1983, 1993). Alternatively, the specificity of the observed *ADH1C*–alcohol interaction could be attributed to the regulatory effect of oestradiol on ADH. Animal studies have demonstrated that oestradiol administration results in an induction of *ADH* expression and an increase in ADH activity, which is likely to affect the rate of alcohol metabolism (Teschke & Heymann, 1982; Teschke *et al*. 1986; Qulali *et al*. 1991).

Conclusion

There is substantial epidemiological and experimental evidence to support a strong inverse relationship between moderate alcohol consumption and CHD; however, the basis for this relationship is not completely understood. The physiological effects of alcohol and the mechanisms for alcohol metabolism have been well studied, but the means by which alcohol affects the cardiovascular system in relation to CHD risk are complex and unclear. In addition to the intricacies of alcohol metabolism, a number of *in vitro* studies have shown that ethanol influences gene expression in a variety of genes that may ultimately contribute to the development of heart disease, such as *PPARα* (Galli *et al*. 2001), cytochrome P450 2E1 (Ingelman-Sundberg *et al*. 1994), *c-myc* (Paice *et al*. 1996), tissue-type plasminogen activator (Grenett *et al*. 1998), urokinase-type plasminogen activator (Grenett *et al*. 1998) and plasminogen activator inhibitor-1 (Grenett *et al*. 2000). It is now well recognized that many diseases cannot be attributed to a single cause, but rather a combination of several contributing factors. Furthermore, these factors will not necessarily be the same for every individual. Thus, it is important to assess both genetic and lifestyle factors in order to improve our understanding of the pathogenesis of disease. Advances in genomic and proteomic research will provide a more comprehensive approach to elucidating disease development at a sophisticated level of molecular detail. A more complete understanding of these elaborate mechanisms will assist in making recommendations at the individual level, which would be the most effective means for promoting overall health. Furthermore, with any medical advice where there is risk and benefit; caution should be exercised in framing guidelines for alcohol and they should be kept in the larger context of other favourable lifestyle factors, such as exercise and diet.

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