Alcohol Drinking, Apolipoprotein Polymorphisms and the Risk of Cardiovascular Diseases

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Abstract: Lipoprotein disorders are a major risk factor for atherosclerotic neuro-cardiovascular disease (ACVD) and are heavily influenced by lifestyle, including alcohol drinking. Moderate drinkers have a lower ACVD risk than abstainers due to their higher levels of high-density lipoprotein (HDL) cholesterol, an important protective factor against ACVD. On the contrary, heavy drinking increases ACVD risk. According to an extensive literature body, ethanol intoxication modifies lipid serum profile and induces endothelial dysfunction. Single nucleotide polymorphisms may influence the relationship between alcohol drinking, HDL cholesterol level, and atherosclerotic risk. The risk of ACVD in heavy drinkers seems enhanced in patients with apolipoprotein E4 allele, interleukin-6-174 polymorphism, and cholesteryl ester transfer protein TaqB polymorphism. Apolipoprotein E4 is a known risk factor for ACVD, while apolipoprotein E2 has mixed effects. Therefore, even if a “protective role” may be attributed to moderate drinking, this effect cannot be extended to everyone.

Keywords: Alcoholism, genetic polymorphism, atherosclerotic cardiovascular disease, apolipoprotein E, adiponectin.

1. INTRODUCTION

Lipoprotein's primary function is to transport cholesterol and triglycerides. Insoluble in water, from the liver and intestine to peripheral tissue, it return lipids and cholesterol to the liver for clearance and recycling [1]. Lipoproteins are complex particles composed of a central hydrophobic core of non-polar lipids (triglycerides and cholesterol esters), surrounded by a hydrophilic membrane and consisting of phospholipids, free cholesterol, and apolipoproteins (Apo) (Fig. 1) [2].

The Apo amphipathic properties allow them to surround the lipids, creating a water-soluble particle. Lipoproteins are classified according to their increasing density in chylomicrons, very-low-density lipoprotein (VLDL), intermediate-density lipoprotein (IDL), low-density lipoprotein (LDL), and high-density lipoprotein (HDL) [3, 4].

The main functions of Apo are stabilizing lipoprotein structure and solubilizing lipid fraction, acting as a ligand for lipoprotein receptors and serving as activators or inhibitors of enzymes involved in the metabolism of lipoproteins [1, 5, 6]. There are various classes of Apo and several subclasses. Apolipoprotein E (ApoE) is a component of chylomicrons, chylomicron remnants, VLDL, IDL, and a subgroup of HDL, which promotes the hepatic clearance of triglyceride-rich lipoproteins owing to the binding to the low-density lipoprotein receptor (LDLR) [7, 8]. ApoE was initially described as a lipid transport protein and major ligand for LDL receptors (mediated via clathrin-coated vesicles) [9]. There are three genetic variants of ApoE: ApoE2, ApoE3, and ApoE4 [10]. Patients who are homozygote for APO E2 gene display the worst affinity for LDLR and higher plasma cholesterol levels, leading to type III hyperlipidemia in humans [11]. Whereas, ApoE4 is associated with an increased risk of Alzheimer’s disease and an increased risk of atherosclerosis [12]. Taken together, this may open new therapeutic and nutraceutical, including moderate alcohol drinking, approaches to reduce apoE4 pathology in both and atherosclerotic cardiovascular diseases (ACVD), neurovascular disorders, and Alzheimer’s disease.

ACVD is a large group of atherosclerosis-related diseases, including coronary heart disease, myocardial infarction, infarction, an ischemic or hemorrhagic stroke of the brain or the spinal cord, and peripheral arterial disease (carotid and
legs atherosclerosis) [13-17]. Lipoprotein disorders are one of the main risk factors for ACVD [18-20]. High levels of LDL and triglycerides and low levels of HDL are the most important players in the process of atherogenesis [21-24]. Furthermore, an incorrect diet and lifestyle may enhance the risk: high cholesterol and high glucose diets, as well as smoking and heavy alcohol drinking may contribute to increased ACVD risk [25-28]. Several case-control and cohort studies have described a J- or U-shaped relationship between alcohol intake and ACVD: abstainers and heavy drinkers show greater ACVD risk than moderate drinkers [29-34]. The limit between moderate and heavy drinking is not established. According to the indications of the National Institute of Alcohol Abuse and Alcoholism (NIAAA) and the British Society of Cardiology, we considered “at-risk” people drinking up to 4 drinks per day or 14 per week for men (in Italy 1 drink = 12 g of alcohol), more than 3 drinks per day or 7 drinks per week for women [35-38]. NIAAA defines heavy drinking as 5 or more standard drinks in a day for a man and 4 or more standard drinks for a woman. However, women should avoid alcohol consumption during gestation and breastfeeding [39-43]. Furthermore, it is difficult to define a drink since alcoholic beverages can significantly differ in their alcohol content even within the same type of drink (e.g., beer, wine, or distilled spirits) [37]. Drinking patterns may be a confounding factor as well [44-46]. Binge drinking increases cardiovascular risk, while many studies suggest that alcohol consumed in moderation is beneficial for the cardiovascular system [47-49]. Also, red wine presents protective effects due to its antioxidant effect and the combination of ethanol with better nutrition. Phenolic compounds as resveratrol in the red wine are thought to be responsible for the protective effects as shown in humans and animal models [50-64]. Considering all these observations, it is not surprising that several studies regarding alcohol consumption and ACVD did not report consistent results.

2. POLYMORPHISMS AND LIPID METABOLISM IN ALCOHOL DRINKERS

2.1. Positive Effects

The S447X polymorphism results in the premature truncation of lipoprotein lipase (LPL), a fundamental enzyme of lipid metabolism [65]. In Asia, the S447X allele of the LPL gene is frequent, and S447X carriers have a low level of triglycerides in the plasma and a high level of HDL cholesterol. In this population, the S447X allele seems to be associated with a less atherogenic lipid profile [66]. In Korea, the effect of the S447X allele was higher in men and women who consumed moderate levels of alcohol [67].

2.2. Negative Effects

Patients with ApoE4, an isoform of the APOE gene in humans, display increased plasma LDL cholesterol levels and lower HDL cholesterol plasma levels. Therefore, ACVD risk seems to be enhanced [68-72]. As for the Interleukin-6 (IL-6) genotype, drinkers and smokers with CC IL-6-174 polymorphism or the CG IL-6-174 polymorphism have a higher risk of coronary artery diseases [73, 74].

2.3. Mixed Effects

Alcohol-dehydrogenase enzymes (ADH) encoded by the ADH1A, ADH1B, and ADH1C genes are mainly responsible for oxidizing ethanol to acetaldehyde [75, 76]. Moderate drinkers who are homozygote for the slow-oxidizing ADH1C allele have higher HDL levels and a lowered risk of myocardial infarction. However, conflicting results are reported by others [77-79].

![Fig. (1). Scheme of one molecule LDL, a particle with a diameter of 22 nm surrounded by a single lipid layer composed of around 800 phospholipids and 500 unesterified cholesterol molecules accompanied by one apolipoprotein B100 (ApoB-100) molecule and by minor apolipoproteins like apolipoprotein E (ApoE). In the core of the LDL particle are stored 1500 molecules of esterified cholesterol and about 170 triglycerides. LDL particles are a high-risk factor for developing cardiovascular disease. Modified from ref. [3].](image-url)

ApoE is a circulating glycoprotein with a central role in lipid metabolism, promoting the clearance of residues of triglyceride-rich lipoproteins from the circulation into the liver [80]. Regarding ApoE polymorphisms, APOE ε2 heterozygotes are associated with increased ApoE levels, lower levels of cholesterol, lower LDL cholesterol levels, and lower triglycerides levels, when compared with APOE ε3 homozygotes [81, 82]. Whereas, ApoE4 heterozygotes are correlated with higher levels of cholesterol, LDL cholesterol, and triglycerides [82, 83]. Of these two, the ε4 isoforms are associated with higher coronary and carotid atherosclerosis risk and higher cardiovascular risk in diabetes mellitus [68, 84, 85].

Considering TaqIB polymorphism at cholesteryl ester transfer protein (CETP) locus, it has been shown that the B2 allele has increased HDL cholesterol level in moderate drinkers and higher levels in heavy drinkers with decreased atherogenic risk [86, 87]. On the contrary, the B1B1 genotype seems to be a genetic risk factor for ACVD [88].
3. ALCOHOL DRINKING AND ACVD RISK

3.1. Mechanisms

Alcohol can be beneficial or harmful to the cardiovascular system, depending on the amount consumed, the characteristics of the consumer, and the quality of the alcohol. Most of the beneficial effect of moderate alcohol drinking against the atherosclerosis risk seems due to the increased HDL cholesterol, reduced triglycerides, total cholesterol, and LDL cholesterol [89-91]. Moderate alcohol consumption may enhance the cardioprotective function of HDL by upregulating the capacity of removing cholesterol, esterification of cholesterol, and the transfer of cholesteryl ester from HDL to the liver [92, 93]. Also, the elevation in HDL cholesterol levels may be due to increased hepatic production or increasing transport rate of apoA-I and apoA-II [91, 94, 95]. Lipoprotein lipase (LPL) is a fundamental enzyme responsible for the hydrolysis and transport of triglycerides [96]. Alcohol could also protect the cardiovascular system by increasing LPL activity which in turn decreases the triglyceride concentration in those subjects who consume a low quantity of alcohol [90].

Of note, the plasma level of adipokine adiponectin, a vasogenic protein produced by the adipocytes [25], increased in moderate drinkers [97, 98]. Adiponectin may exert anti-inflammatory and anti-atherogenic effects; it, therefore, plays a protective role against neuro-cardiovascular injuries [99, 100].

The relation between alcohol consumption and HDL cholesterol is mediated by the effect of alcohol on cholesteryl ester transfer protein (CETP) activity. The transfer of cholesteryl ester from HDLs to triglyceride-rich lipoproteins in exchange for triglycerides is driven by CETP: the increase in CETP activity reduces HDL cholesterol level and promotes atherosclerosis [101, 102]. On the contrary, alcohol drinking tends to deplete CETP activity, enhancing HDL cholesterol levels [103, 104].

While moderate drinking seems to have a protective role, heavy drinking is associated with endothelial and adipose dysfunction, increased atherosclerosis progression. Besides, a wide cluster of hemodynamic and vascular abnormalities indicate an unfavorable lipid profile and atherogenic risk even in former alcoholics who are disease-free [105-109]. Several studies show that heavy alcohol drinking alters lipid blood profile, causing hypertriglyceridemia, a risk factor for the development of atherosclerosis [110]. This alteration is due to increased levels of chylomicron and VLDL due to types of lipoproteins rich in triglycerides [111, 112].

Another mechanism by which alcohol abuse induces endothelial dysfunction and atherosclerosis is the alteration of oxidative stress and antioxidant defense [113]. Oxidative stress is defined as an imbalance between the oxidant and antioxidant system, causing an increase of reactive oxygen species (ROS) [114, 115]. Acetaldehyde, a product of alcohol metabolism, modifies the structure of the mitochondrial causing high production of ROS and lower synthesis of ATP [116]. Oxidative stress is a well-studied component of atherosclerosis pathogenesis, occurring in parallel with inflammation [114]. The excessive production of ROS is responsible for LDL oxidation [115]. Due to such modifications, LDL becomes atherogenic and accumulates in specific areas of the vascular wall. The subsequent internalization of LDL oxidized into macrophages gives rise to the foam cells, which is a hallmark of the early atherosclerotic lesion (Fig. 2) [117, 118]. Recent findings showed that resveratrol and
polyphenols extracted from the olives could counteract the oxidative stress induced by alcohol abuse throughout also a modulation of neurotrophins [50, 52-54].

4. GENETIC POLYMORPHISMS, ALCOHOL DRINKING, AND ACVD RISK

4.1. APOE Polymorphisms

ApoE lipoproteins rich in triglycerides are recycled in the liver from which HDL enriched in ApoE are secreted [119, 120]. The main APOE alleles are E2, E3, and E4. These three alleles lead to different metabolic properties and different atherosclerosis risks [121, 122]. The distribution of these alleles is not the same between different populations, even though E3 is the most widespread, followed by E2 and E4 [123, 124].

The allele E4 is the ancestral form in humans and presents two arginines at positions 112 and 158 of the ApoE amino acid sequence [80, 121, 125]. The affinity of these lipoproteins for the hepatic LDLR varies with the ApoE isoform [68, 126]. The recycling of ApoE4 lipoproteins by the liver is reduced, and causes intracellular cholesterol accumulation, lower HDL-receptor expression and HDL cholesterol plasma level, reduced expression of LDLRs, and increasing plasma LDL cholesterol level [68, 69, 126]. All together, these alterations may increase atherosclerosis risk [70]. Furthermore, in ApoE4 non-obese postmenopausal women, alcohol drinking is associated with hypertension, which is a significant risk factor for the development of atherosclerosis [127, 128]. Moreover, ApoE4 is the major genetic risk factor for Alzheimer’s disease as this carrier is less efficient in transporting lipids from astrocytes to neurons than other isoforms [129, 130].

The E2 allele is the second most widespread allele and presents a cysteine and an arginine at positions 112 and 158 of the ApoE amino acid sequence [80, 121, 125]. Subjects with this polymorphism have reduced affinity for LDLRs, leading to low LDL cholesterol and high HDL cholesterol levels, accumulating triglycerid-rich lipoprotein containing ApoE2 in plasma, and elevation of triglycerides level [131]. Moreover, a combination of E2 with the mutation E1 Arg142Ser seems to be associated with severe type III hyperlipoproteinemia in patients with familial hypocholesterolemia [132]. Regarding Alzheimer’s disease, ApoE2 protects the brain by accumulating less amyloid β in the brain than other isoforms [133, 134].

Different studies report that alcohol may exert a positive action, but heavy drinking is responsible for atherosclerotic risk having a negative action on the release of nitric oxide by the endothelium [135, 136]. This leads to vascular oxidative stress and reduces nitric oxide production, which are key events in the development of atherosclerosis [137, 138]. The different ApoE isoforms can exert different antioxidant effects resulting in more or less atherogenic [139, 140]. In heavy drinkers, the oxidative stress and the HDL levels are increased: so the differences in atherogenic effect due to the various ApoE isoforms may be further enhanced [137, 141, 142].

4.2. IL-6-174 Polymorphism

Heavy alcohol drinkers with CC IL-6-174 polymorphism have higher levels of IL-6 and a higher risk of developing coronary artery diseases [73, 143]. On the contrary, moderate alcohol drinking is correlated with lower levels of IL-6, lower levels of C-reactive protein, and reduced carotid intima-media thickness progression [144-146]. These results strongly suggest that inflammation is one of the mechanisms by which alcohol intoxication triggers the phenomenon of atherosclerosis, as also suggested by the close relationship between atherosclerosis and IL-6, the upstream inflammatory cytokine [147, 148].

4.3. TaqIB Polymorphism and Cholesterylester Transfer Protein

Transfer of cholesteryl ester from HDLs to triglyceride-rich lipoproteins in exchange for triglycerides is promoted by CETP: this transfer is increased in ACVD patients causing lower levels of HDL cholesterol [149, 150]. Instead, CETP deficiencies are associated with high HDL cholesterol serum levels and marked variations in the size and lipid composition [151, 152]. TaqIB, a CETP polymorphism, is associated with high plasma HDL cholesterol levels, and the TaqIB B2B2 genotype shows the highest levels of HDL cholesterol [153, 154]. The use of CETP inhibitors, alone or in combination with a statin, could be a valid option for patients with atherosclerotic disease as these drugs showed promising results in increasing HDL cholesterol levels [155, 156].

4.4. Gender Effects

The association between plasma lipids and alcohol intake depends on a context defined by gender, age, body mass index, and ApoE genotype [157-160]. As far as we know, the incidence of ACVDs in pre-menopausal women is virtually non-existent: so it is hypothesized a cardio-protective role of ovarian hormones achieved through a more favorable lipid profile [161, 162]. In postmenopausal women, it appears that a beneficial effect of moderate alcohol consumption can be obtained on cardiovascular risk but with lower alcohol intake than men as women metabolize alcohol differently [163, 164]. Alcohol catabolism is impaired in women due to smaller body size, larger body adipose tissue mass (alcohol is less soluble in body fat than in body water), and lower alcohol-dehydrogenase activity with decreased first-pass metabolism of alcohol in the stomach before arriving in the systemic circulation [165-168].

In a Chinese population, men with small artery occlusion who consume alcohol show the worst outcome, greater risk factors, and high LDL cholesterol plasma levels concerning women [169]. In APOE E4 carriers, plasma LDL cholesterol level, and ACVD risk were significantly higher in drinking than in non-drinking men, whereas in women, no differences were found [170]. Compared to women, men are more exposed to atherosclerotic risks [171]. The anti-atherogenic property of estrogens is mediated via at least two mechanisms: firstly by affecting plasma lipoprotein profiles promoting high levels of HDL and low levels of LDL; se-
condary by downregulating the expression of adhesion molecules of the vessel wall after the exposure to atherogenic stimuli [172-174].

4.5. Age Effects

Among elderly postmenopausal women (over 65 years), ApoE4 carriers were found a negative effect of alcohol drinking concerning abstainers [175]. In non-obese postmenopausal women, alcohol consumption is associated with early systolic and diastolic hypertension in APOE genotype ε3/3 compared to ε4 carriers [122]. Furthermore, ApoE4 aged patients abusing alcohol display lower cognitive abilities [176, 177].

Similar results were obtained for ischemic stroke risk in elderly subjects: in the ApoE4 negative subjects, moderate drinking was associated with a lower risk than in abstainers, whereas in ApoE4 patients, the risk among moderate drinkers was higher than abstainers. In this study of older adults, the association of alcohol use and risk of ischemic stroke was U-shaped, with a modestly lower risk among consumers up to 6 drinks per week. However, ApoE genotype may modify this association, and even moderate alcohol intake may be associated with an increased risk of ischemic stroke among ApoE4-positive older adults [178].

Despite the substantial scientific evidence linking moderate alcohol use to lower risk of coronary heart diseases, the shape of the dose-response relationship between alcohol intake and coronary risk remains less consistent. No relationship was found between ApoE genotype and risk of myocardial infarction or coronary death in subjects over 65 years [179].

CONCLUSION

Many studies indicate that the ACVD risk in heavy drinkers is increased by genetic factors. An important goal is to provide genetic information for improving our ability to identify individuals with increased atherosclerosis risk, thus increasing our skill in the development of prevention programs.

ACVD risk in drinkers may be modulated by different polymorphisms well documented as ApoE, IL-6-174, S447X, TaqIB, and ADH1C. However, we focused on ApoE SNPs due to the relationship between alcohol drinking, ApoE single nucleotide polymorphism, and ACVD. In the primary prevention of alcoholism, people should be advised that heavy drinking may increase the atherosclerosis risk and that this risk may be further enhanced by specific genetic personal traits. At the present time, there is little awareness of these problems, and it could be quite useful to spread such information mainly among family doctors and other health and social professionals providing primary care.

The results relating to the risk of neuro-cardiovascular diseases in ApoE4 patients are worrisome since, in Caucasians, this isoform is present in approximately 13-25% of the general population. As a clinical strategy, the ApoE SNP should be routinely assessed, and the data should be considered for the treatment: in fact, the ACVD risk is enhanced for drinking ApoE4 people. This strategy would raise the cost of prevention since skillful operators and expensive equipment are needed. However, the relationship between cost/benefit may be favorable. As the prevalence of ApoE4 carriers is high in Caucasians and cardiac ischemic disease and ischemic stroke, that are the most worrisome ACVD outcomes heavily affect the individuals and society in terms of human suffering, loss of productivity, and health costs.

When a heavy drinker results in ApoE4-positive, a more careful clinical evaluation is needed to detect clinical symptoms and signs (if any) of ACVD. Furthermore, the patients should be advised that their risk of ACVD is increased due to both drinking habits and genetic patterns, and they should quit drinking. In alcohol-dependent subjects, abstinence should be supported by psychological and/or pharmacological treatments in specialized units.

Since most of the literature evidence that moderate alcohol decreases ACVD risk and that ACVD is the main cause of death in Western countries, moderate use of alcohol could be hypothetically recommended to decrease mortality in these countries. However, clinicians should not recommend moderate drinking to prevent coronary heart disease based on this evidence alone as current NIAAA guidelines suggest a limit of one drink per day in adults [95, 180].

When we consider the age effect, it should be noted that in older people (over 65 years), the relationship between alcohol-ischemic stroke and alcohol-carotid thickness is modified by the ApoE genotype. Some results show that the risk among moderate drinkers is lower than abstainers ApoE4-negative subjects. However, the risk among moderate drinkers is higher than abstainers among ApoE4-positive subjects. These data are worrisome and, if confirmed by further research in more extensive series and different countries, they can make alcohol abstinence mandatory for all elderly ApoE4 carriers.

The identification of ApoE genotypes may be important for pharmacological treatment. ApoE genotypes have been associated with the response of plasma lipids to lipid-lowering drugs, as statins are known to decrease LDL cholesterol levels and ACVD risk. Although the effect of Apo E gene polymorphism on the response to treatment with statins has been studied, the results are conflicting. Age and gender were found to influence low-density lipoprotein cholesterol response to a similar extent as the most pronounced genetic effects. Among SNP tested with an allele frequency of at least 5%, only those in ApoE significantly influence statin response [181-183]. Beneficial effects of statin treatment were found in ApoE4 carriers by most authors [183-186]. Mixed or negative effects were found in a few studies [187, 188]. ApoE genotype had no significant effect on the response to treatment with atorvastatin in patients with heterozygous familial hypercholesterolemia [188].

In the future, gene therapy could be the best approach for ACVD prevention and cure. Severe hypercholesterolemia and atherosclerosis were successfully treated in ApoE deficient mice (ApoE-/-) and transgenic animals expressing an ApoE defective gene [189-191]. Promising results were also obtained using a gene therapy based on a viral vector expressing apolipoprotein A-I, which reduced atherosclerotic lesion growth in mice fed with a high-fat diet [192]. Furthermore, gene editing using the CRISP/Cas9 tech-
nique showed substantial improvements in the atherosclerosis condition in a mouse model of hypercholesterolemia [193].

LIST OF ABBREVIATIONS

ACVD = Atherosclerotic Neuro-Cardiovascular Diseases
ADH = Alcohol-Dehydrogenase Enzymes
ApoE = Apolipoprotein E
CETP = Cholesterol Ester Transfer Protein
HDL = High-Density Lipoprotein
IDL = Intermediate-Density Lipoprotein
IL-6 = Interleukin-6
LDL = Low-Density Lipoprotein
LDLR = Low-Density Lipoprotein Receptor
LPL = Lipoprotein Lipase
VLDL = Very-Low-Density Lipoprotein

CONSENT FOR PUBLICATION

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CONFLICT OF INTEREST

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Alcohol Drinking, Apolipoprotein Polymorphisms and ACVD

Current Neurovascular Research, 2021, Vol. 18, No. 1 157

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