Valine-Alanine Manganese Superoxide Dismutase Polymorphism Is Not Associated With Alcohol-Induced Oxidative Stress or Liver Fibrosis

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The role of genetic factors in the pathogenesis of alcohol-induced liver disease (ALD) is receiving increasing attention. Recently, it has been reported that homozygosity for a valine to alanine substitution in the mitochondrial targeting sequence of manganese superoxide dismutase (Mn-SOD) represents a risk factor for severe ALD. Because this mutation is postulated to modify enzyme transport into mitochondria, we have sought confirmatory evidence of this association in a larger group of patients and investigated whether this polymorphism might influence alcohol-induced oxidative stress. Genotyping for the valine-alanine (Val-Ala) polymorphism of the Mn-SOD gene in 281 patients with advanced ALD (cirrhosis/fibrosis) and 218 drinkers without liver disease showed no differences in either the heterozygote (55% vs. 50%) or the homozygote (19% vs. 23%) frequency for the alanine allele. By measuring the titers of circulating antibodies against oxidized cardiolipin (OX-CL) and malondialdehyde (MDA) or hydroxy-ethyl radical (HER) adducts as markers of oxidative stress, we found a significant increase in ALD patients compared with healthy controls. However, the carriers of the alanine Mn-SOD allele had titers of anti-MDA, anti-HER, and anti-OX-CL IgG comparable with heterozygotes and patients homozygous for the valine allele. Similarly, the frequency of subjects with antibody titers above the 95th percentile of controls was not increased among homozygotes for the alanine Mn-SOD allele. In conclusion, in our population Val-Ala polymorphism in Mn-SOD influences neither susceptibility to alcohol-induced liver fibrosis nor alcohol-induced oxidative stress. (HEPATOLOGY **2002;36: 1355-1360.**)

Only a small proportion of heavy drinkers develop advanced alcohol-induced liver disease (ALD). Because of the complexity of ALD pathogenesis, there are many candidate genes that could potentially influence susceptibility. Functional polymorphisms in genes encoding proteins involved in ethanol metabolism, oxidative stress, fatty liver, and inflammatory/immune responses may all play a role. IL-10,[1] TNF-α,[2] CD14 promoter polymorphisms,[3] and a polymorphism in exon 1 of the gene encoding the T-cell surface molecule CTLA-4 have all been associated with ALD, implicating the response to endotoxin and immune mechanisms in disease pathogenesis. Although it is well accepted that oxidative stress generated during ethanol metabolism promotes hepatocyte injury and collagen deposition,[4] there is less evidence that genetic variability in the enzymes involved in the production or control of oxidative stress dictates ALD susceptibility. Recently, however, a polymorphism in the mitochondrial targeting sequence of manganese superoxide dismutase (Mn-SOD or SOD2), an enzyme involved in detoxifying reactive oxygen species (ROS), has been associated with severe ALD.[5]

Animal models of alcohol-induced liver injury have shown that ethanol can induce oxidative stress in hepatocyte mitochondria by several mechanisms, including depletion of mitochondrial glutathione (GSH) and TNF-α-stimulated oxygen radical formation.[6] The main source of

Abbreviations: ALD, alcohol-induced liver disease; Mn-SOD, manganese superoxide dismutase; ROS, reactive oxygen species; OX-CL, oxidized cardiolipin; HER, hydroxyethyl radical; MDA, malondialdehyde; PCR, polymerase chain reaction; HSA, human serum albumin; PBS, phosphate-buffered saline.

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this TNF-α is Kupffer cells, which are putatively activated by gut-derived endotoxin. Removing endotoxin,[7] inactivating Kupffer cells[8] in rats, and "knocking out" the TNF-α gene in mice[9] all ameliorate alcohol-induced liver injury. ROS generated within mitochondria are reduced by Mn-SOD to hydrogen peroxide, which is further reduced by GSH peroxidase to water. The importance of superoxide dismutase is highlighted by the fact that over expression of Mn-SOD in the mitochondria of rats fed an ethanol-rich diet leads to a significant reduction in liver steatosis, inflammation, necrosis, and the production of free radicals.[10]

A thymine to cytosine substitution at position −1,183 in the human Mn-SOD gene results in an alanine for valine substitution in the leader amino acid sequence that is responsible for the mitochondrial localization of the enzyme.[11] Structural analysis predicts that the presence of an alanine would modify the amphiphilic helical structure of this sequence, thus favoring Mn-SOD transport into the mitochondrial matrix. Degoul et al.[5] demonstrated an increased frequency of Mn-SOD alanine homozygotes in heavy drinkers with microvesicular steatosis (12/28; 43%) and in ALD patients with alcoholic hepatitis (7/12; 58%) or cirrhosis (9/13; 69%) as compared with controls (19/79; 24%). In view of the small numbers of this study, resulting in inadequate statistical power, and the fact that this polymorphism would intuitively lead to more mitochondrial Mn-SOD and, subsequently, reduced mitochondrial oxidative stress and liver damage, we sought to investigate the frequency of this polymorphism in a larger cohort of well-characterized heavy drinkers with and without ALD.

Ethanol metabolism results in the production of reactive oxygen species, such as the hydroxyethyl radical (HER),[12] and lipid peroxidation products, such as oxidized cardiolipin (OX-CL) and malondialdehyde (MDA). Lipid peroxidation products can be directly immunogenic (OX-CL), whereas the aldehyde end products of lipid peroxidation (MDA, HER) and reactive oxygen species both modify host proteins forming immunogenic adducts.[13,14] We have previously reported that patients with ALD have increased titers of antibodies directed towards these antigens than controls.[15] These antibody markers of oxidative damage are also more prominent in patients with more advanced liver lesions, providing further evidence for the role of oxidative stress in the pathogenesis of ALD. To evaluate the influence of the Mn-SOD genotype on the development of alcohol-induced oxidative stress, circulating IgG against OX-CL and MDA and HER adducts were also measured and correlated with genotype.

Materials and Methods

Study Population. Informed consent was obtained before 5-mL blood samples were collected after an overnight fast. Serum was used for the ELISA tests, and an EDTA sample collected for the preparation of leukocyte DNA. Patients from both the drinking and the control groups were white and originated in the northeastern part of the United Kingdom as did their parents and grandparents. All patients from the drinking group had been consuming more than 80 grams ethanol/d for at least 10 years at the time of presentation. Severity of disease was assessed initially with liver function tests. If the alanine transaminase, alkaline phosphatase, or bilirubin were more than twice normal on 2 separate occasions within a 6-month period, an ultrasound scan was also arranged, and a liver biopsy was performed in patients without biliary dilatation. Patients with advanced ALD, defined as the presence of extensive fibrosis or cirrhosis with or without alcohol-induced hepatitis, were then placed in one study group (AALD), and patients with no liver disease, or steatosis only, were placed in another (NALD). Patients in the NALD group had either normal liver function tests on 2 separate occasions (excluding γ glutamyl transferase) or, for those with abnormal liver function tests, a liver biopsy that revealed either a normal liver or simple steatosis only, with no evidence of steatohepatitis or fibrosis. Exclusion criteria were serologic evidence of previous hepatitis B virus or hepatitis C virus infection, autoimmune liver disease, histologic evidence of other liver disease, excessive liver iron staining, or homozygosity for the C282Y mutation in the HFE gene. Healthy controls all drank within the WHO guidelines for sensible limits (21 units per week for men and 15 units per week for women). The study was planned according to the guidelines of the local ethical committee.

Determination of Mn-SOD Genotype. The polymerase chain reaction (PCR) was used to amplify a 172-bp fragment around the first exon of the Mn-SOD gene, containing the C1183T base exchange, using the previously described primers 5′-CAGCCCAGCCTGCGTAGACGG-3′ and 5′-GCGTTGATGTGAGGTTCCAG-3′.[11] Alleles were distinguished on the basis of their digestion patterns with *Bsa*WI because the C1183T substitution results in the creation of a site for this enzyme. Amplification was in a 50 μL reaction mix containing the following: KCl reaction buffer (Bioline, United Kingdom), 100 μmol/L dNTP, 0.125 μmol/L of each primer, 0.5 to 0.8 μg of genomic DNA, and 2 U of *Taq* polymerase (Bioline) and involved 35 cycles at 93°C for 1 minute, 59°C for 1 minute, and 72°C for 1 minute. Subsequently, 10 μL of the amplified DNA was digested

with 2 U of *BsaW1* at 50°C and the 3 Mn-SOD genotypes distinguished by electrophoresis on 1.5% agarose gels with ethidium bromide staining. AA, AV, and VV controls were included in each batch, and 5 cases from each genotype were spot-checked by sequencing of the PCR fragments. To purify PCR products for DNA sequencing, approximately 300 μL of PCR product was mixed with 700 μL binding solution (0.1% [wt/vol] diatomaceous earth in 8 mol/L guanidine HCl). The suspension was transferred to a minispin filter in a 1.5 mL microcentrifuge tube and centrifuged at 3,000*g* for 2 minutes. The filtrate at the bottom of the tube was discarded and the filter replaced in the same tube. Six hundred microliters of wash buffer [200 mmol/L NaCl, 10 mmol/L EDTA, 50 mmol/L Tris-HCl, pH 7.4, in 50% (vol/vol) ethanol] was added, and the pellet was washed by centrifugation at 5,000*g* for 2 minutes. This wash step was repeated, followed by a final centrifugation for 2 minutes at 5,000*g* to remove all traces of wash. Finally, the filter was transferred to a 1.5-mL collection tube, and the bound DNA was eluted in 35 μL of water by centrifugation at 10,000*g* for 5 minutes. The DNA concentration was estimated by analysis on an agarose gel and approximately 100 to 500 ng sequenced using fluorescently labelled dye terminators (Fig. 1) (MWG Biotech., Milton Keynes, United Kingdom).

Antigen Preparation. Adducts between human serum albumin (HSA) and MDA were prepared by reacting 1 mg/mL HSA with 50 mmol/L MDA, obtained by the acid hydrolysis of malonildialdehyde-bis-dimethylacetal, for 2 hours at 37°C. Unbound aldehyde was removed by overnight dialysis at 4°C against phosphate-buffered saline (PBS), pH 7.4. The presence of MDA adducts was evaluated by measuring the fluorescence intensity at 399/471 nm exc/em wavelength pairs according to Cominacini et al.[16] Cardiolipin (88 μg/mL suspension in PBS) was oxidized by incubation with 1 mmol/L 2,2′-azo-bis-(2-amidinopropane) hydrochloride (Polyscience Inc., Warrington, PA) at 37°C. The kinetics of phospholipid oxidation were monitored spectrophotometrically by measuring conjugated diene absorbance at 234 nm. The reaction was stopped by the addition of 0.1 mmol/L diphenylphenylendiamine (DPPD) when the relative difference of absorbance from the solution at time 0 was 0.5 OD. OX-CL was extracted in 5 mL of chloroform, dried under nitrogen, and resuspended in ethanol (100 μg/mL final concentration). Hydroxyethyl radical adducts with HSA were prepared by reacting HSA (1 mg/mL in PBS, pH 7.4) for 30 minutes at 25°C with 1 mg of freshly prepared 1,1′-dihydroxyazoethane crystals, synthesized as described by Stoyanovsky et al.[17]

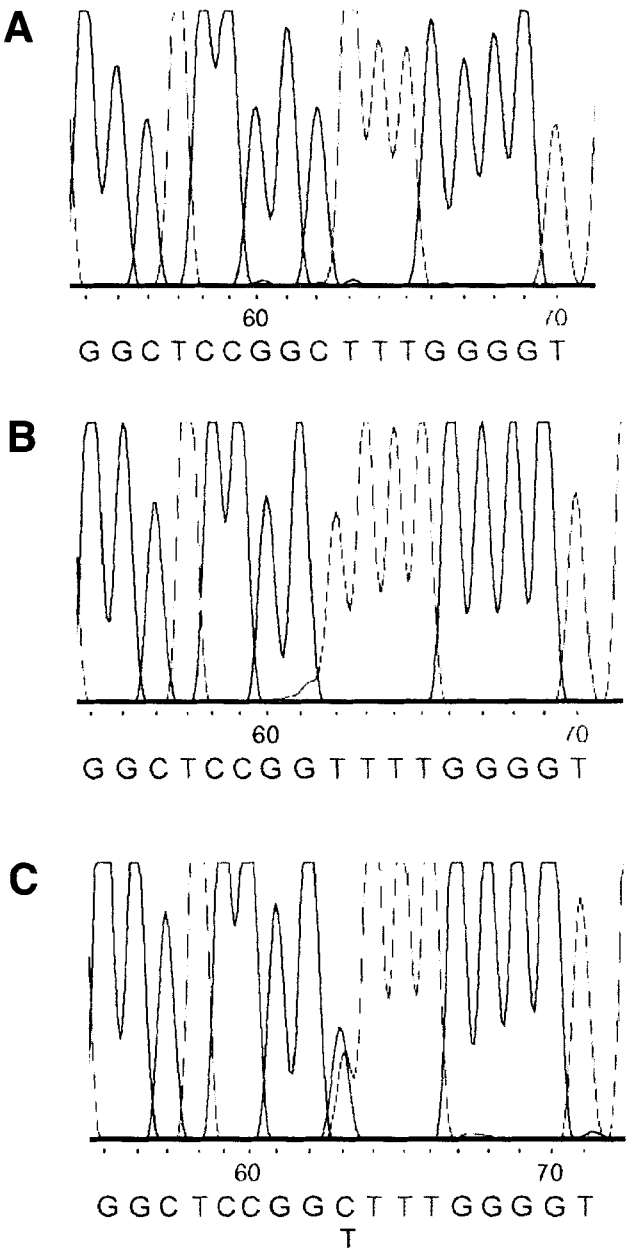

Fig. 1. Sequencing analysis confirmed genotypes determined by PCR-RFLP. (A) Homozygous AA have only a cytosine, (B) homozygous VV have only a thymine, and (C) heterozygous AV have both cytosine and thymine at position 1,183 of the MnSOD gene. The BsaWI restriction site TCCGGT is only present when thymine is at position 1,183 of the Mn-SOD gene.

Determination of the Immune Reactivity Toward Lipid Peroxidation Products. Polystyrene microwell plates for ELISA (Immunolon IV, Nunc; Fisher Scientific St. Louis, MO) were coated for 4 hours at 37°C with 0.05 mg/mL of MDA-conjugated HSA or unmodified HSA solubilized in 0.1 mol/L bicarbonate buffer, pH 9.6. After incubation, solutions were removed and replaced by 0.3 mL of coating buffer containing 3% bovine serum albumin (BSA) in PBS, pH 7.4. The plates were further incubated for 1 hour at 37°C to block nonspecific binding

sites. The coated wells were washed 3 times with PBS containing 0.25% Triton X-100. Patients' sera was diluted 1:50 with the coating buffer, added in duplicate as aliquots of 0.20 mL to the appropriate wells, and incubated 1 hour at 37°C. After washing 3 times with PBS 0.25% Triton X-100, peroxidase-linked goat anti-human IgG (dilution 1:6,000; Dako S.P.A., Milano, Italy) was added and incubated for 60 minutes at 37°C. The antibody binding was revealed by the addition of 0.15 mL of a reaction mixture containing 0.4 mg/mL of 1-phenylendiamine, 0.4 μL/mL hydrogen peroxide (30%), 5.1 mg/mL citric acid, and 6.1 mg/mL anhydrous Na_2HPO_4 at pH 5.0. The reaction was stopped after 15 minutes by adding 50 μL 2 N H_2SO_4, and absorbances were measured at 490 nm using a Bio-Rad microplate reader (Bio-Rad Laboratories Inc., Hercules, CA).

For the determination of the immune reactivity toward OX-CL, ELISA plates were coated by adding 30 μL of OX-CL ethanol solution to each well and then evaporating the solvent under vacuum. The same amount of ethanol was added to reference wells. After 2 washes with 0.3 mL PBS, non-specific binding sites were blocked by 1-hour incubation at 37°C with a coating buffer containing 1% (vol/vol) polyethylenglycol in PBS, pH 7.4. The coated wells were washed 3 times with PBS, and 0.2 mL aliquots of the sera (1:50 dilution in PBS) from patients and controls were added in duplicate to the appropriate wells and incubated for 1 hour at 37°C. IgG binding was revealed by peroxidase-linked goat anti-human IgG as described above. The results were expressed by subtracting the background reactivity of the sera in the wells containing unmodified HSA or treated with ethanol.

Data Analysis and Statistical Calculations. Statistical analysis was performed by Instat-3 statistical software (GraphPad Software Inc, San Diego, CA) using 1-way ANOVA test. Distribution normality of the groups considered was preliminarily evaluated by Kolmogorov and Smirnov test. Relative risk and association analysis were performed by odds ratio and χ^2 tests. Significance was taken at the 5% level.

Results

The frequency of the mutation resulting in a Val-Ala substitution in the mitochondrial targeting sequence of Mn-SOD was determined in 281 patients with biopsy specimen-proven cirrhosis/fibrosis (AALD), in 218 heavy drinkers without liver damage or with fatty liver only (NALD), and in 244 healthy controls (Table 1).

The distribution of the genotypes in patients and controls was in Hardy Weinberg equilibrium. There was no significant difference in the distribution of the genotype frequencies either between heavy drinkers with or without advanced ALD or between either of the heavy drinking groups and healthy subjects. Furthermore, alanine-Mn-SOD homozygosity was not associated with a younger age at presentation (AA 47.7 ± 9.3, AV 49.1 ± 9.1, VV 47.9 ± 10.5) or an increased frequency of alcohol-induced hepatitis features on liver biopsy (AA 40%, AV 42%, VV 42%).

Table 1. Distribution of Polymorphism in the Mn-Superoxide Dismutase Gene in Healthy Controls and in Heavy Drinkers With (AALD) and Without (NALD) Advanced ALD

	VV	AV	AA
AALD, n = 281 (%)	72 (25.5)	156 (55.5)	53 (19)
Controls, n = 244 (%)	64 (26)	125 (51)	55 (22.5)
NALD, n = 218 (%)	58 (27)	109 (50)	51 (23)

Sera were available for antibody studies on 87 of the 281 AALD patients. The samples were collected prospectively from patients still attending the clinic. As we have reported previously,[15] the antibody markers of oxidative stress were significantly increased in ALD patients as compared with healthy controls (Fig. 2).

However, within the AALD group, the titers of anti-MDA, anti-HER, and anti-OX-CL IgG in the alanine-Mn-SOD homozygotes (AA) were similar to heterozygotes (AV) and valine-Mn-SOD homozygotes (VV) (Fig. 2). There was also no significant difference in the frequency of subjects with antibody titers above the 95th percentile of control population among homozygotes for alanine-Mn-SOD and heterozygotes or homozygotes for valine-Mn-SOD (Table 2).

Discussion

The Val-Ala polymorphism in the mitochondrial-targeting sequence of Mn-SOD varies in frequency between ethnic groups with the reported frequency for the alanine allele ranging from 12% in Japanese to 40% to 50% in whites.[18] In this latter population, the alanine variant of Mn-SOD has been associated with an increased risk of breast cancer in premenopausal women,[19,20] sporadic motor neurone disease,[21] diabetic nephropathy,[22] and ALD,[5] whereas the association with Parkinson's disease, first observed in the Japanese,[11] has not been confirmed in a subsequent study performed in Germany.[23] In contrast to the French study, we found no association between the alanine-Mn-SOD allele and advanced ALD in our cohort of 499 patients. The alanine polymorphism alters the secondary structure of the Mn-SOD mitochondrial-targeting sequence and probably results in increased mitochondrial levels of the enzyme, a situation that would be intuitively protective against oxidative stress. Degoul et al.[5] explain the disease association by suggesting that in-

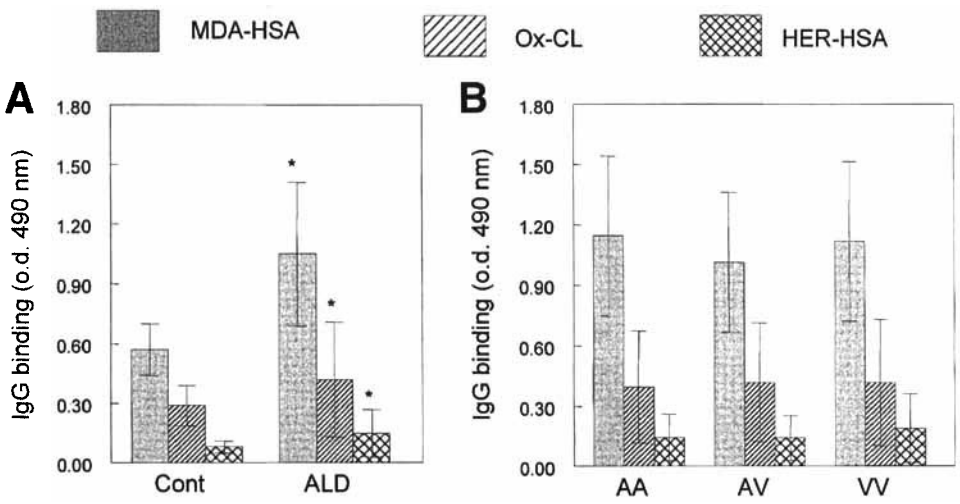

Fig. 2. (A) Titers of antibodies against lipid peroxidation- and hydroxyethyl free radical-derived adducts in controls and patients with advanced ALD and (B) the effect of the Mn-SOD Val-Ala polymorphism on these titers in patients with advanced ALD. The IgG titers were measured in the sera (1:50 dilution) of 87 patients with advanced ALD and 55 healthy controls using microplate ELISA plates coated with either oxidized cardiolipin (OX-CL) or human serum albumin adducted with malonildialdehyde (MDA-HSA) or hydroxyethyl free radical (HER-HSA) and peroxidase-linked goat anti-human IgG antiserum. The results are expressed as absorbance at 490 nm after subtracting the background reactivity. Statistical significance: $*P < .0001$.

creased mitochondrial expression of Mn-SOD will lead to overproduction of hydrogen peroxide and more oxidative stress. However, we found no evidence that the Val-Ala Mn-SOD polymorphism affected the degree of oxidative stress in patients with ALD. The magnitude of oxidative stress was determined indirectly by measuring antibody titers to oxidation products in the sera. We have previously shown that antibodies to products of lipid peroxidation and ROS adducted to host proteins are present in the sera of patients with ALD and that titers are related to disease severity.[15] Among the 87 patients from whom serum samples were available, there was no appreciable difference in antibody titers to MDA or HER adducts or OX-CL between AA homozygotes, AV heterozygotes, and VV homozygotes. There was also no significant difference in the frequency of antibody titers above the 95% confidence interval among the 3 genotypes (Table 2).

There are several potential explanations for the discrepancy between our results and those of the previous study, with accordingly different implications. The first and most obvious explanation is that the previous results obtained in only 79 patients and 71 controls were spurious, attributable to a lack of statistical power, and that there is no association between the Val-Ala Mn-SOD polymorphism and either ALD or the degree of oxidative stress. The implications of this negative result are either that the polymorphism has no functional effect on Mn-SOD activity or that it has a functional effect, but factors other than Mn-SOD are more important in determining the degree of oxidative stress and, therefore, disease risk in heavy drinkers. These factors seem likely to include those involved in the generation of free radicals, such as ethanol-metabolizing enzymes and TNF-α, as well as antioxidant defense systems including mitochondrial glutathione peroxidase, normally responsible for the detoxification of hydrogen peroxide arising from Mn-SOD-catalyzed reduction of superoxide.

Table 2. Influence of the MnSOD Polymorphism on the Antibody Responses Against MDA and HER-Derived Antigens and Oxidized Cardilipin (Ox-CL) in Patients With Advanced ALD

	VV	AV	AA
Anti-MDA (%)	10/16 (63)	40/57 (70)	10/14 (71)
Anti-Ox-CL (%)	4/14 (29)	16/51 (31)	4/11 (36)
Anti-HER (%)	8/16 (50)	21/57 (37)	5/14 (36)

NOTE. Table displays the percentage of patients who have IgG titers above the 95th percentile of the control population.

The second explanation for the discrepancy between the 2 studies is that the association is real but is confined to certain populations. It may be that the association is indirect and attributable to a linked polymorphism in the Mn-SOD gene or a neighboring gene that varies in frequency between populations. Alternatively, a population-specific effect of a functional Mn-SOD polymorphism may be explained by differences in drinking behavior or dietary factors. The French pattern of chronic, daily, moderate alcohol consumption may mimic the situation observed in chronic ethanol feeding of rats, in which up-regulation of Mn-SOD and a decrease in glutathione peroxidase activity has been reported.[24,25] This would be expected to magnify the effect of any functional Mn-SOD polymorphism. In contrast, the binge pattern of drinking seen in the United Kingdom might not be expected to exert such profound effects on the activities of these enzymes, minimizing the effect of any functional Mn-SOD polymorphism on ethanol-related oxidative stress.

There seems little doubt that oxidative stress plays a role in the pathogenesis of alcohol-induced liver disease. Although ALD susceptibility polymorphisms have been found in cytokine and other immunoregulatory genes, none have yet been found in this important pathway. The

Mn-SOD gene is a good candidate for ALD susceptibility. Mn-SOD plays a crucial role in detoxifying superoxide radicals and, if over-expressed in mitochondria, attenuates experimental alcohol-induced liver injury in the rat.[10] The polymorphism studied, however, does not appear to affect the level of oxidative stress in ALD in a United Kingdom population and is not a susceptibility factor in our cohort. A further, recent report from Australia in a population of 57 ALD cirrhotic patients found no association between the A allele and disease.[26] It remains possible that other as yet unidentified polymorphisms in coding or regulatory sequences of Mn-SOD may affect risk of ALD development and/or that this polymorphism is important in other populations.

References

1. Grove J, Daly AK, Bassendine MF, Gilvarry E, Day CP. Interleukin 10 promoter region polymorphisms and susceptibility to advanced alcoholic liver disease [see comments]. Gut 2000;46:540-545.
2. Grove J, Daly AK, Bassendine MF, Day CP. Association of a tumor necrosis factor promoter polymorphism with susceptibility to alcoholic steatohepatitis. HEPATOLOGY 1997;26:143-146.
3. Jarvelainen HA, Orpana A, Perola M, Savolainen VT, Karhunen PJ, Lindros KO. Promoter polymorphism of the CD14 endotoxin receptor gene as a risk factor for alcoholic liver disease. HEPATOLOGY 2001;33:1148-1153.
4. Albano E. Free radical mechanisms of ethanol toxicity. In: Rhodes CJ, ed. Toxicology of the Human Environment: The Critical Role of Free Radicals. London: Taylor & Francis, 2000:235-264.
5. Degoul F, Sutton A, Mansouri A, Cepanec C, Degott C, Fromenty B, Beaugrand M, et al. Homozygosity for alanine in the mitochondrial targeting sequence of superoxide dismutase and risk for severe alcoholic liver disease. Gastroenterology 2001;120:1468-1474.
6. Fernandez-Checa JC, Kaplowitz N, Garcia-Ruiz C, Colell A, Miranda M, Mari M, Ardite E, et al. GSH transport in mitochondria: defense against TNF-induced oxidative stress and alcohol-induced defect. [Review]. Am J Physiol 1997;273:G7-G17.
7. Adachi Y, Moore LE, Bradford BU, Gao W, Thurman RG. Antibiotics prevent liver injury in rats following long-term exposure to ethanol. Gastroenterology 1995;108:218-224.
8. Adachi Y, Bradford BU, Gao W, Bojes HK, Thurman RG. Inactivation of Kupffer cells prevents early alcohol-induced liver injury. HEPATOLOGY 1994;20:453-460.
9. Yin M, Wheeler MD, Kono H, Bradford BU, Gallucci RM, Luster MI, Thurman RG. Essential role of tumor necrosis factor α in alcohol-induced liver injury in mice. Gastroenterology 1999;117:942-952.
10. Wheeler MD, Nakagami M, Bradford BU, Uesugi T, Mason RP, Connor HD, Dikalova A, et al. Overexpression of manganese superoxide dismutase prevents alcohol-induced liver injury in the rat. J Biol Chem 2001;276:36664-36672.
11. Shimoda-Matsubayashi S, Matsumine H, Kobayashi T, Nakagawa-Hattori Y, Shimizu Y, Mizuno Y. Structural dimorphism in the mitochondrial targeting sequence in the human manganese superoxide dismutase gene. A predictive evidence for conformational change to influence mitochondrial transport and a study of allelic association in Parkinson's disease. Biochem Biophys Res Comm 1996;226:561-565.
12. Albano E, Tomasi A, Goria-Gatti L, Dianzani MU. Spin trapping of free radical species produced during the microsomal metabolism of ethanol. Chem Biol Interact 1988;65:223-234.
13. Moncada C, Torres V, Varghese G, Albano E, Israel Y. Ethanol-derived immunoreactive species formed by free radical mechanisms. Mol Pharmacol 1994;46:786-791.
14. Palinski W, Witztum JL. Immune responses to oxidative neoepitopes on LDL and phospholipids modulate the development of atherosclerosis. J Intern Med 2000;247:371-380.
15. Mottaran E, Stewart S, Rolla R, Vay D, Cipriani V, Moretti M, Vidali M, et al. Lipid peroxidation contributes to immune reactions associated with alcoholic liver disease. Free Radic Biol Med 2002;32:38-45.
16. Cominacini L, Garbin U, Davoli A, Micciolo R, Bosello O, Gaviraghi G, Scuro LA, et al. A simple test for predisposition to LDL oxidation based on the fluorescence development during copper-catalyzed oxidative modification. J Lipid Res 1991;32:349-358.
17. Stoyanovsky DA, Wu D, Cederbaum AI. Interaction of 1-hydroxyethyl radical with glutathione, ascorbic acid and α-tocopherol. Free Radic Biol Med 1998;24:132-138.
18. Van Landeghem GF, Tabatabaie P, Kucinskas V, Saha N, Beckman G. Ethnic variation in the mitochondrial targeting sequence polymorphism of MnSOD. Hum Hered 1999;49:190-193.
19. Mitrunen K, Sillanpaa P, Kataja V, Eskelinen M, Kosma VM, Benhamou S, Uusitupa M, et al. Association between manganese superoxide dismutase (MnSOD) gene polymorphism and breast cancer risk. Carcinogenesis 2001;22:827-829.
20. Ambrosone CB, Freudenheim JL, Thompson PA, Bowman E, Vena JE, Marshall JR, Graham S, et al. Manganese superoxide dismutase (MnSOD) genetic polymorphisms, dietary antioxidants, and risk of breast cancer. Cancer Res 1999;59:602-606.
21. Van Landeghem GF, Tabatabaie P, Beckman G, Beckman L, Andersen PM. Manganese-containing superoxide dismutase signal sequence polymorphism associated with sporadic motor neuron disease. Eur J Neurol 1999;6:639-644.
22. Chistyakov DA, Savost'anov KV, Zotova EV, Nosikov VV. Polymorphisms in the Mn-SOD and EC-SOD genes and their relationship to diabetic neuropathy in type 1 diabetes mellitus. BMC Med Genet 2001;2:4-8.
23. Grasbon-Frodl EM, Kosel S, Riess O, Muller U, Mehraein P, Graeber MB. Analysis of mitochondrial targeting sequence and coding region polymorphisms of the manganese superoxide dismutase gene in German Parkinson disease patients. Biochem Biophys Res Comm 1999;255:749-752.
24. Koch OR, De Leo ME, Borrello S, Palombini G, Galeotti T. Ethanol treatment up-regulates the expression of mitochondrial maganese superoxide dismutase in rat liver. Biochem Biophys Res Commun 1994;201:1356-1365.
25. Husain K, Scott BR, Reddy SK, Somani SM. Chronic ethanol and nicotine interaction on rat tissue antioxidant defense system. Alcohol 2001;25:89-97.
26. Frenzer A, Butler WJ, Norton ID, Wilson JS, Apte MV, Pirola RC, Ryan P, et al. Polymorphism in alcohol-metabolising enzymes, glutathione S-transferases and apolipoprotein E and susceptibility to alcohol-induced cirrhosis and chronic pancreatitis. J Gastroenterol Hepatol 2002;17:177-182.