

Leading article

Genetic predisposition to alcoholic liver disease

Most people who abuse alcohol will develop hepatic steatosis but only a few alcoholics develop more serious forms of liver disease – hepatitis, fibrosis, and cirrhosis. Despite extensive research, the mechanisms underlying between-individual variation in susceptibility to alcohol induced liver damage remain elusive. There is some evidence to support a dose-response relationship between the total amount of alcohol consumed and the risk of developing cirrhosis¹ but within drinking categories there is considerable variability in the presence and severity of liver damage.² Recent prospective studies have been unable to confirm a linear relationship between alcohol intake and the development of cirrhosis,^{3,4} and even for steatosis, the severity has been found to be unrelated to the amount, duration, or type of alcohol consumed.⁵ Perhaps most important is the observation that up to a third of confirmed alcoholics have completely normal liver histology.^{6,7} Clearly, therefore, factors other than the cumulative amount of alcohol consumed are involved in determining which patients develop liver disease. These factors are likely to include both other exogenous (environmental) and endogenous (genetic) influences.

Environmental factors that have received most attention include nutritional status and past or present hepatitis B virus (HBV) infection. The specific contribution of malnutrition to the pathogenesis of alcoholic liver disease has been extensively reviewed and remains highly controversial.^{8,9} There is no doubt that some patients with alcoholic liver disease have evidence of malnutrition, but it is not a universal finding.¹⁰ Furthermore, fibrosis and cirrhosis have been produced in the baboon model of alcoholic liver disease in animals fed diets purportedly high in nutritional value,¹¹ although this has been disputed.^{9,12} The extent to which variation in nutritional status contributes to tissue injury remains unclear but it seems to offer only part of the explanation. With regard to HBV, markers of past or current infection have been found more commonly in patients with alcoholic liver disease than in the general population in some studies^{13,14} but not others.¹⁵ In view of these discrepant results it seems unlikely that coexisting HBV infection plays a significant role in the aetiopathogenesis of alcohol related liver damage, at least in areas of low HBV prevalence such as Britain.

With regard to endogenous factors, there is no doubt that gender differences play a role, with women more susceptible to alcohol induced liver injury than men. This is probably the result of both the lower volume of distribution and the lower first-pass gastric metabolism of alcohol in women compared with men.¹⁶ Clearly, however, gender differences do not explain differences in susceptibility to the hepatic effects of alcohol among members of the same sex.

Evidence for a genetic predisposition to alcoholic liver disease

The absence of any clear cut environmental influences on the variable hepatic response to alcohol has recently diverted attention towards a search for genetic factors that might offer an explanation. Evidence supporting a genetic component to predisposition comes mainly from a large study of 15 924 male twin pairs.¹⁷ The concordance rate for alcoholic cirrhosis was 14.6% in monozygotic twins and 5.4% in

dizygotic twins. Importantly, the difference in concordance rates could not be accounted for by the different concordance rates for alcoholism alone. This remarkable study has provided the stimulus for a number of investigations employing the 'candidate gene' strategy, to search for genes involved in predisposition to alcohol induced liver injury. In this approach, a gene (or nearby DNA sequence) suspected of involvement in predisposition to disease, is used specifically as a probe in an association or linkage study. However, a number of problems confront investigators applying this technique to studies of alcoholic liver disease. There is convincing evidence that a predisposition to alcoholism itself has a significant genetic component¹⁷⁻¹⁹ and therefore to examine the role of any genetic factors in predisposition to end-organ damage distinct from predisposition to alcoholism, any study should ideally include two groups of alcoholics, well matched for alcohol intake, with and without end-organ damage. In the case of alcoholic liver disease, this may be logistically impossible, since even if biopsy specimens could be obtained from alcoholics with normal liver blood tests to ensure the absence of occult disease, there is no way of predicting whether these patients will develop disease at a later date. In addition, since it is probable that alcohol produces liver damage by a number of different mechanisms, any explanation for a genetic predisposition to cirrhosis is likely to involve several genes, rather than variation at a single locus. Potential investigators must therefore also cope with problems inherent in investigating a 'polygenic disease'. An appreciation of these difficulties is important when interpreting the results of studies which have examined factors potentially involved in genetic susceptibility to alcoholic liver disease.

'Candidate genes' in predisposition to alcoholic liver disease (Table)

GENES ENCODING ENZYMES INVOLVED IN ETHANOL METABOLISM

It seems likely that at least some of the pathological effects of alcohol are related to its tissue concentration or the concentration of its metabolites, in particular acetaldehyde.²⁰ The vast majority of alcohol metabolism occurs in the liver. At least three hepatic enzyme systems are capable of catalysing the oxidation of alcohol to acetaldehyde (see Figure), although under normal conditions only alcohol dehydrogenase (ADH) is important. Aldehyde dehydrogenase (ALDH) is the only enzyme that catalyses the oxidation of acetaldehyde to acetate. The recent identification, therefore, of polymorphisms at the gene loci encoding alcohol dehydrogenases and aldehyde dehydrogenases,²¹ had led to speculation concerning their potential role in predisposition to alcoholic liver disease. ADH subunits are encoded at five different gene loci on chromosome 4 and polymorphisms have been observed in two: *ADH2* (producing three different β subunits) and *ADH3* (producing two different γ subunits). The variant homodimeric and heterodimeric isoenzymes arising from these subunits exhibit widely different kinetic properties with correspondingly different rates of alcohol oxidation in vitro.^{22,23} Human ALDHs are encoded at four

Candidate genes in alcohol-related liver injury

Candidate gene	Chromosomal location	Polymorphism described*
Alcohol dehydrogenase genes:		
<i>ADH1</i> (α -ADH)	4q21-q25	No
<i>ADH2</i> (β -ADH)	4q21-q25	Yes ³⁸
<i>ADH3</i> (γ -ADH)	4q21-q25	Yes ³⁸
<i>ADH4</i> (π -ADH)	-	No
<i>ADH5</i> (χ -ADH)	4q21-q25	No
σ -ADH \ddagger	-	No
Mitochondrial aldehyde dehydrogenase (<i>ALDH2</i>)	12	Yes ^{33 37}
Ethanol inducible cytochrome P-450: IIE1	10	Yes
Fatty acid ethyl ester synthase	-	No
Type I collagen genes:		
<i>COL1A1</i>	17q21-qter	Yes ^{48 49}
<i>COL1A2</i>	7q22	Yes ^{48 49}
Class I HLA-A and B	6p21	§Yes ⁵⁰⁻⁵⁶
Class II HLA	6p21	§Yes ^{59 52 56 59}
Alpha-1-antitrypsin	14q31-q32.3	Yes ⁷²

*References given where polymorphisms have been screened at the genotyping level for genetic linkage to alcohol related liver damage. §References refer to phenotyping studies for those genes where no genotyping studies have yet been reported. ¶The gene encoding the recently identified gastric alcohol dehydrogenase; σ -ADH, has not yet been designated a number.

independent loci on four different chromosomes.²⁴ *ALDH2* on chromosome 12^{25 26} encodes the major mitochondrial enzyme, which has a low K_m for acetaldehyde and is responsible for the majority of acetaldehyde oxidation. The *ALDH2* gene exists in at least two allelic forms, *ALDH2*1* and *ALDH2*2*.^{27 28} Isoenzymes present in individuals homozygous for the *ALDH2*2* allele have little or no catalytic activity, while those present in heterozygotes have measurable, although reduced, activity compared with the isoenzymes present in *ALDH2*1* homozygotes.^{29 30}

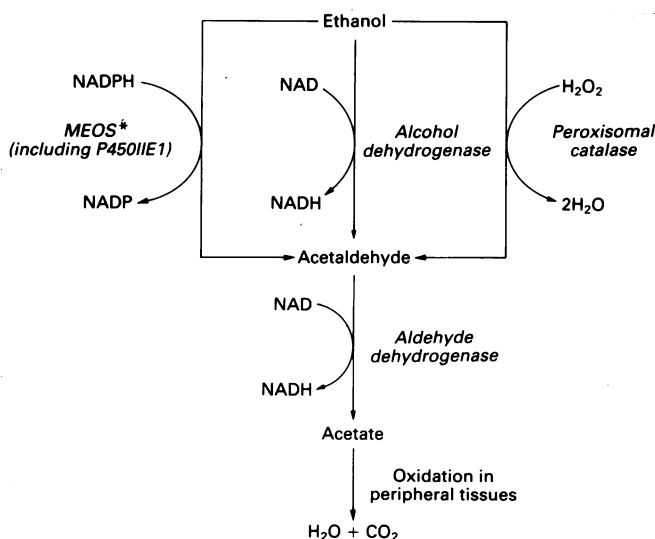
Investigation of the role of these polymorphisms in a predisposition to alcoholic cirrhosis has, until recently, been hampered by the need to use liver biopsy specimens to determine the isoenzyme phenotype. However, advances in molecular biology have overcome this problem, allowing direct genotyping of ADH³¹ and ALDH²⁹ loci using allele specific oligonucleotides to probe amplified leukocyte DNA. It has been known for some time that the inactive form of *ALDH2* is present in about 50% of Japanese and Chinese^{22 32 33} and is associated with high acetaldehyde concentrations, an exaggerated flush response to alcohol, and seems to protect against alcoholism.^{34 35} It is now evident that possession of the most active forms of *ADH2* and *ADH3* (*ADH2*2* and *ADH3*1*) is also 'protective' against alcoholism, presumably because of faster rates of acetaldehyde accumulation and lower alcohol tolerance.³⁶ Perhaps

not surprisingly, in view of its negative association with alcoholism, patients with alcoholic liver disease have a much lower incidence of the deficient *ALDH2*2* allele compared with controls, with no *ALDH2*2* homozygotes and only occasional heterozygotes in the disease populations studied.^{33 37} However, the most recent of these studies has also shown that habitual drinkers who are heterozygotes for the normal and mutant *ALDH2* genes develop alcoholic liver disease at lower levels of alcohol intake than alcoholics who are homozygotes for the normal gene.³⁷ This is consistent with the recent finding of an increased frequency of the more active *ADH3* allele, *ADH3*1*, in patients with fibrotic alcoholic liver disease compared with locally matched healthy controls.³⁸ Together, these studies suggest that although individuals with the less active form of ALDH and more active forms of ADH are less likely to become alcoholic, they have a greater risk of developing alcoholic liver disease if they do drink than people with the normal form of ALDH and less active forms of ADH. These results strongly implicate acetaldehyde as one of the important pathogenetic factors in the development of alcoholic liver disease.

The role of genes encoding other alcohol metabolising enzymes in a genetic predisposition to alcoholic liver damage has yet to be explored. The gastric oxidation of alcohol has been implicated in the enhanced vulnerability of women to complications of alcoholism.¹⁶ The gastric mucosa was originally thought to contain only the *ADH3* and *ADH5* encoded isoenzymes (γ -ADH and χ -ADH respectively), but recently a 'new' alcohol dehydrogenase isoenzyme type (designated σ -ADH) has been identified in the stomach of both rats³⁹ and humans.⁴⁰ The human enzyme has now been purified and characterised and its properties suggest that, at the high ethanol concentrations in the stomach after drinking, σ -ADH is probably the ADH form with the largest contribution to gastric alcohol metabolism.⁴⁰ Identification of the gene encoding σ -ADH and the eventual search for polymorphisms are awaited with interest. Also of potential importance concerning genetically determined individual variation in alcohol metabolism, is the gene encoding the ethanol inducible form of cytochrome P-450, P450IIE1; this enzyme probably plays an important role in alcohol metabolism in chronic alcoholics following enzyme induction.⁴¹ The gene has been cloned and sequenced,⁴² and a restriction fragment length polymorphism (RFLP) has been described,⁴³ no studies on these genetic variants in alcoholic liver disease have yet been reported. Recently, a non-oxidative pathway of alcohol metabolism that leads to the production of fatty acid ethyl esters (FAEEs) has been described, catalysed by FAEE synthase.⁴⁴ This pathway was originally thought to be important only in those tissues lacking ADH, such as the heart, but a recent study has shown in vivo formation of FAEEs in the liver and pancreas during ethanol exposure.⁴⁵ In view of the suggestion that FAEEs may have a pathophysiological role via their effect on lipid metabolism and their incorporation into cell membranes,⁴⁶ the gene encoding FAEE synthase, when it is identified, would seem to be another 'candidate gene' worthy of study in relation to alcoholic liver disease.

GENES INVOLVED IN FIBROGENESIS

Since the final common pathway in the pathogenesis of cirrhosis must be either excess production or deficient degradation of collagen by the liver, genes involved in these mechanisms are an obvious second group of potential candidates for consideration as a basis for predisposition to alcoholic fibrosis. Type I collagen is the predominant collagen in cirrhotic livers, and RFLPs have been observed at both of the loci encoding its two different constituent polypeptide chains; *COL1A1* on chromosome 17, encoding



Schematic representation of ethanol metabolism in the liver. *MEOS = microsomal ethanol oxidising system.

the $\alpha 1(1)$ chain and *COL1A2* on chromosome 7, encoding the $\alpha 2(1)$ chain.⁴⁷ The role of these polymorphisms in a predisposition to alcoholic liver disease has now been examined in two studies. The earliest study reported a significant association of a particular RFLP haplotype of the *COL1A2* locus and alcoholic cirrhosis.⁴⁸ This suggested that *COL1A2* might be a susceptibility locus for alcoholic cirrhosis with one, or very few, predisposing mutations possible. However, a more recent study, with larger numbers of patients and controls, drawn from an identical 'genetic pool' has not confirmed the initial report; no particular haplotype of either type I collagen gene was found to be associated with cirrhosis.⁴⁹ Polymorphisms of the genes encoding enzymes involved in the metabolism of collagen, such as the collagenases and their inhibitors, or genes controlling the transcription of collagen may ultimately prove to be more important than polymorphisms of the collagen genes themselves in determining individual susceptibility to the fibrotic effect of alcohol.

GENES ENCODING HISTOCOMPATIBILITY ANTIGENS

There is some evidence to support an immunological component in the pathogenesis of alcoholic liver disease.⁵⁰ In view of this, numerous groups have investigated the role of the highly polymorphic HLA antigen system (which determines immune responsiveness) in susceptibility to alcohol related liver damage. Several studies, in diverse populations, have reported isolated and conflicting significant associations of the class I HLA-A and B antigens with alcoholic liver disease including A1,⁵¹ A2,⁵² A9,⁵¹ A28,⁵¹ B5,⁵³ B8,^{54,55} B13,⁵⁶ B15,⁵⁷ Bw35,⁵¹ and B40.^{58,59} Other studies have failed to show any relationship between these histocompatibility antigens and susceptibility.^{60,61} Several groups have also examined HLA class II antigen frequency in alcoholic liver disease and reported associations with DR2,⁶² DR3,^{52,55} and DRw9.⁵⁹ Many of these reports should be interpreted with caution in the light of several factors that can account for misleading results. Only one investigation took into account the patient's alcohol history; interestingly this showed a shorter duration of intake in cirrhotic patients with HLA-B8/DR3 compared with those without this haplotype.⁵⁵ Many of the studies screened large numbers of HLA antigens in large population samples which, of course, can lead to the occurrence of significant associations by chance. If the results are corrected for the number of alleles screened, the significance of the reported associations disappears.⁶³ A large number of alleles in the human major histocompatibility complex on chromosome 6p21 have now been cloned, generating a considerable number of informative RFLPs. In other 'polygenic' diseases, such as insulin dependent diabetes mellitus, the HLA system has now been extensively studied at the molecular level and alleles identified which determine both disease susceptibility and resistance.^{64,65} However, given the inconsistency of the tissue type associations with alcoholic liver disease, it is perhaps not surprising that no studies have yet been reported using molecular techniques to examine the role of HLA class I and II genes in susceptibility to alcohol related end-organ damage.

OTHER 'CANDIDATE GENES'

Several distinct pathogenetic mechanisms are currently considered to play a role in the hepatotoxicity of alcohol, with investigators disagreeing as to their relative importance.⁶⁶ These include induction of a hypermetabolic state, alteration of cell membrane structure and function, lipid peroxidation, induction of cytochrome P-450 enzymes increasing the hepatotoxicity of environmental toxins, acetaldehyde toxicity, immunologically mediated mechanisms, and endotoxin mediated cytokine release. Each of these mechanisms

may involve proteins encoded by polymorphic genes which would therefore be potential 'candidate genes' explaining individual susceptibility to alcoholic liver injury. Further genetic studies must await more precise information on the biochemical pathways underlying these different mechanisms.

Alpha-1 antitrypsin (α_1 AT) gene, whose structure and protein biochemistry have been extensively studied is worthy of consideration as a potential 'candidate gene' in predisposition to alcoholic cirrhosis. Alpha-1 antitrypsin is the major serum protease inhibitor, the glycoprotein is encoded by a single locus on chromosome 14 and is principally produced by hepatocytes.⁶⁷ Over 30 phenotypes of α_1 AT can be differentiated by isoelectric focusing. Some phenotypes are associated with serum deficiency (PiZ, PiS, and PiM_{malton}) and intrahepatic α_1 AT inclusion granules (PiZ, PiM_{malton}). Individuals homozygous for PiZ are at increased risk of adult chronic liver disease: chronic active hepatitis, cirrhosis, and hepatocellular carcinoma.⁶⁸ It is thus possible that the α_1 AT gene is a susceptibility locus for alcohol induced liver damage. This hypothesis has been examined in several studies looking at the PiZ phenotype and periportal α_1 AT granules in patients and controls, and no evidence to support involvement of α_1 AT in genetic predisposition has been found.^{69,70} However, more heterogeneity is seen at the gene level and it is now possible to examine the α_1 AT genotype using techniques including polymerase chain reaction and hybridisation to ASO probes.⁷¹ A preliminary study using these molecular techniques has also failed to show a significant increased risk of alcoholic liver disease in individuals who are homozygous or heterozygous for both the PiZ and PiS mutant alleles.⁷²

Conclusions and perspectives

In summary, why only a few alcoholics develop significant liver disease remains largely a mystery. Cumulative alcohol intake certainly plays a role and other environmental factors, in particular nutrition, may also be important. There is now little doubt that women are more susceptible to the hepatic complications of alcohol excess than men. Neither environmental influences or gender differences, however, offer the whole explanation and evidence from twin concordance studies suggests at least a contributory role for genetic factors in determining individual susceptibility to alcohol related liver disease. This susceptibility is not caused by a single gene defect but is thought to result from the cumulative interaction of a number of genes. Analysis of such a complex 'polygenic' disorder is not easy but 'candidate genes' can be identified and recombinant DNA techniques used to investigate whether any particular genotype or haplotype is associated with increased risk of disease. This approach is already suggesting that genetically determined variation in the oxidative metabolism of alcohol may play a role in individual susceptibility to alcohol related liver damage implicating acetaldehyde as an important factor in disease pathogenesis. Thus, the molecular genetic approach has the potential to contribute to our understanding of the underlying biochemical mechanisms of alcoholic liver disease and may ultimately enable 'high risk' individuals to be identified in whom preventive measures such as counselling can be undertaken.

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