Who gets alcoholic liver disease: nature or nurture?

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ABSTRACT – the factors determining why fewer than 10% of drinkers develop advanced alcoholic liver disease remain largely unknown. There is a weak relationship between disease risk and the dose and pattern of alcohol consumed. Obesity increases the risk of all stages of alcoholic liver disease, probably reflecting the role of steatosis in the pathogenesis of more advanced disease. Women develop disease at a lower intake than men due, in part, to their lower volume of distribution for alcohol, but also potentially to increased gut permeability to endotoxin. Recent studies suggest a non-gender-linked genetic component to disease susceptibility and recent case-control studies have suggested that polymorphisms of genes encoding cytokines and other immunoregulatory molecules may exert a significant effect. The pattern of polymorphisms associated with risk suggests that antibody-mediated mechanisms play a role in disease pathogenesis. This has implications for treatment and for identifying high risk individuals at an early stage.

Alcoholic liver disease (ALD) represents a considerable burden to the practising gastroenterologist and hepatologist. In 1998 alone, almost 500 in- and out-patients with ALD were seen in the regional liver unit in Newcastle upon Tyne. More than half had established cirrhosis and 70% of these had evidence of hepatic decompensation. A related audit of all ‘liver-related’ admissions to the three Newcastle teaching hospitals demonstrated that the largest proportion (38%; 297/787) was due to ALD. In 1997 ALD was the second commonest indication for orthotopic liver transplantation in Europe, accounting for 3,335 (27%) of all transplants performed. Unfortunately, this burden is increasing in the United Kingdom. Death rates from cirrhosis (all aetiologies) almost doubled between 1987 and 1997, and the observation that 38% of liver-related admissions were due to ALD in our 1998 audit compares with only 19% in a similar study from the Royal Free Hospital reported in 1977. This increase almost certainly reflects the continuing increase in per capita alcohol consumption, which is in marked contrast to many other countries in the developed world where per capita intake and cirrhosis death rate are falling.

Despite this burden, as we enter the new millennium surprisingly little consensus exists in the field of ALD, perhaps the only exceptions being that excessive alcohol intake is associated with an increased risk of liver disease and that some drinkers never develop disease. However, there is no consensus on the precise dose-response relationship between alcohol intake and risk of ALD, on disease pathogenesis and, as a result, on the factors that determine an individual’s disease susceptibility. Figure 1 depicts the relationships between the various stages of ALD and the frequencies of these lesions in histological screening studies of heavy drinkers. Only between 1 in 4 and 1 in 12 heavy drinkers ever progress to cirrhosis, with fewer than 1 in 3 developing hepatitis. In most studies up to a third of heavy drinkers have completely normal liver biopsies under light microscopy. These studies have led to the obvious question – what determines whether or not a heavy drinker develops advanced ALD?

Dose and pattern of alcohol consumption and risk of ALD

The most obvious explanation for susceptibility to ALD is that it depends on the dose and pattern of alcohol consumed. The effect of dose has been best demonstrated by a remarkable study that surveyed the dietary and alcohol habits of the entire population of two towns in Northern Italy¹. All subjects underwent biochemical liver function testing, those with abnormal tests underwent ultrasound, and those with abnormalities underwent a liver biopsy. The study showed a linear correlation between the number of alcohol units consumed per day and the risk of liver disease and cirrhosis. However, only 6% of individuals drinking more than 12 drinks per day had cirrhosis, and in our own studies we have found no difference in cumulative lifetime alcohol intake between drinkers with cirrhosis, fibrosis and fatty liver alone (steatosis)². The Northern Italian study, along with a more recent Italian study, has shown that the risk of ALD may also depend on the pattern of intake independently of the absolute levels of consumption. Disease risk appears to be increased by drinking alcohol at other than meal times, drinking several rather than a single type of alcoholic beverage, and drinking every day versus weekend drinking. While these studies have provided evidence that dose and pattern of alcohol intake play a role in determining ALD risk, they have clearly demonstrated that other factors are likely to be equally if not more important. Before considering the other

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exogenous (environmental) and endogenous (genetic) factors that have been suggested to play a role in determining disease risk, it is necessary to review what are currently believed to be the mechanisms of hepatocyte injury and fibrosis in alcohol-related liver disease.

Mechanisms of hepatocyte injury and fibrosis in ALD

Hypothetical mechanisms of alcohol-related hepatocyte injury are depicted in Figure 2. The left-hand side of the scheme represents the hypothesis that hepatocyte injury is due primarily to increased gut permeability to endotoxin (lipopolysaccharide [LPS]) – a component of the cell wall of Gram-negative bacteria. LPS stimulates the release of pro- and anti-inflammatory cytokines and reactive oxygen species (ROS) by hepatic Kupffer cells. If the balance between these cytokines favours necroinflammation and/or apoptosis then hepatocyte injury occurs. The right-hand side of the scheme represents the hypothesis that hepatocyte injury is related primarily to the metabolism of alcohol. This can occur either via the cytosolic alcohol dehydrogenase (ADH) system, or the ethanol-inducible cytochrome P450, CYP2E1 system. Metabolism of alcohol via ADH generates acetaldehyde, which can be further metabolised by enzymes generating ROS, or can bind covalently to protein lysine residues to form acetaldehyde adducts. This irreversible acetaldehyde binding interferes with protein function and generates neo-antigens capable of eliciting an immune response, which can lead to immunologically mediated hepatocyte injury. Metabolism by CYP2E1, in conjunction with free iron in the liver, generates reactive free radicals that can cause oxidative stress and, like acetaldehyde, the formation of adducts. Oxidative stress leads to peroxidation of the phospholipid constituents of plasma and intracellular membranes, resulting in necrosis and/or apoptosis. The aldehyde end-products of lipid peroxidation (malondialdehyde [MDA] and 4-hydroxynonenal [4-HNE]) can also form adducts capable of eliciting an immune response. The fibrosis that follows hepatocyte injury is due principally to activation of hepatic stellate cells from their quiescent, vitamin A-storing phenotype to an activated phenotype secreting excessive amounts of extracellular matrix components, in particular type 1 collagen. This activation is induced by cytokines and growth factors released during necroinflammation and may also be induced directly by oxidative stress, MDA, 4-HNE and acetaldehyde. The environmental and genetic factors considered to play a role in determining the risk of ALD can be understood in terms of their effects on these putative disease mechanisms.

Dietary factors and risk of ALD

The environmental factor that has received most attention as a potential determinant of ALD risk is the diet. Studies in the Tsukamoto-French rodent model of ALD, which involves continuous intragastric infusion of ethanol, have shown that diets high in polyunsaturated fat and iron and low in carbohydrate increase the severity of hepatic inflammation. Free iron favours the production of ROS in the liver during ethanol metabolism, a diet high in polyunsaturated fat and low in carbohydrate induces CYP2E1, and polyunsaturated fats in membrane phospholipids are the substrate for lipid peroxidation. Data from humans are relatively sparse, although one case-control study from France demonstrated that the risk of cirrhosis was increased by diets high in fat and alcohol and low in carbohydrate. We have recently compared the micronutrient intake of heavy drinkers with cirrhosis with that of drinkers with steatosis or normal livers, and healthy controls. We found no evidence of an increased intake of polyunsaturated fat or iron in the cirrhotic patients. They did, however, consume significantly less copper, zinc and selenium, essential cofactors for the anti-oxidant enzymes that form the body's principal defences against oxidative stress. These studies require confirmation in prospective studies as it is difficult
to know whether the dietary differences were a cause or an effect of the liver disease in these drinkers. A more obvious role for diet in ALD risk has been suggested by a recent study showing that obesity increases the incidence of all stages of ALD in heavy drinkers. This observation was most striking for steatosis, leading to the hypothesis that the association between obesity and cirrhosis risk was most likely to be indirect, and attributable to the role of steatosis in the pathogenesis of more advanced ALD.

The role of steatosis in the pathogenesis of advanced ALD

Previously established dogma that steatosis is an entirely ‘benign’ lesion has recently been challenged by several studies from our group. In addition to continuing alcohol intake, the severity and pattern of steatosis at index biopsy predicts the subsequent risk of fibrosis/cirrhosis over an 11 year follow-up. In that study, 30% of patients with ≥ grade 2 (out of 3) steatosis, progressed to cirrhosis over follow-up compared with fewer than 5% of patients with grade 1. Patients with a ‘mixed’ pattern of micro- and macrovesicular steatosis were also more likely to progress than those with macrovesicular steatosis only. We have also demonstrated that the degree of hepatic stellate cell activation correlates with the severity of fat in the liver biopsies of heavy drinkers. These observations have now been explained by studies in animal models demonstrating that, compared to normal livers, fatty livers are more sensitive to the effects of oxidative stress and endotoxin – the main triggers of hepatocyte injury in ALD. Fatty livers generate more lipid peroxidation products during oxidative stress, and genetically obese mice with fatty liver develop florid alcohol-like hepatitis in response to single intraperitoneal dose of endotoxin whereas their non-obese litter mates are unaffected. This latter observation may be explained by defective Kupffer cell function in fatty livers. These human and animal studies have led us to develop the concept of ALD as a disease of ‘two hits’, with steatosis as the first ‘hit’ amplifying the deleterious effects of the various second ‘hits’ or ‘triggers’. This hypothesis is supported by recent observations that the steatosis observed in other conditions can also progress to inflammation and fibrosis – so called non-alcoholic steatohepatitis or ‘NASH’ – probably due to the same second ‘hit’ – oxidative stress and endotoxin. Recognition of the role played by steatosis in the pathogenesis of more advanced liver disease suggests that factors determining its severity may play a key role in determining the risk of cirrhosis. Clearly genetic and environmental factors determining the degree of obesity would fall into this category, as would functional polymorphisms of genes encoding enzymes involved in hepatic lipid metabolism. Of interest in this respect is the observation that the activity of the rate-controlling enzyme in hepatic triglyceride synthesis – phosphatidate phosphohydrolase (PAP) – correlates with the severity of steatosis in

Key Points

| Advanced alcoholic liver disease develops in less than 10% of heavy drinkers. |
| The risk of disease increases with total alcohol intake but also with daily versus weekend drinking and drinking away from meal times. |
| Obesity significantly increases the risk of all stages of alcoholic liver disease. |
| Steatosis (fatty liver) plays a role in the pathogenesis of more advanced liver disease, probably by increasing the sensitivity of the liver to endotoxin and oxidative stress. |
| Genetic factors play a role in susceptibility and results to date suggest that polymorphisms of cytokines and other immunoregulatory genes may be most closely associated with disease risk. |

Fig 2. Mechanisms of alcohol-related hepatocyte injury.

- ADH = alcohol dehydrogenase;
- CYP2E1 = cytochrome P450 2E1;
- Fe = iron;
- IL-10 = interleukin 10;
- RES = reticuloendothelial system;
- ROS = reactive oxygen species;
- TNFα = tumor necrosis factor α.
heavy drinkers. Clearly, the molecular basis for this increased activity is worthy of further study as a potential determinant of ALD risk.

**Gender and risk of ALD**

The most obvious 'genetic' factor determining ALD risk is female gender. It has long been appreciated that women develop ALD at a lower intake of alcohol than men. The traditional explanation has been that women develop higher blood alcohol concentrations per unit of alcohol consumed due to their lower volume of distribution for alcohol. This, in turn, is attributed to their lower body mass index and to fat constituting a higher percentage of their body mass than in men. More recent evidence has, however, suggested an explanation based on disease mechanisms. Thurman and colleagues have demonstrated in the rat model that oestrogen increases gut permeability to endotoxin and accordingly up-regulates endotoxin receptors on Kupffer cells leading to an increased production of tumour necrosis factor in response to endotoxin. These exciting data suggest several new directions for research into human gender-specific susceptibility to ALD.

**Non-gender linked genetic factors and risk of ALD**

Evidence for non-gender linked genetic susceptibility to ALD comes principally from a twin study showing that the concordance rate for alcoholic cirrhosis was three times higher in monozygotic than in dizygotic twin pairs. This difference in concordance rates was not entirely explained by the difference in concordance rates for alcoholism per se. This seminal publication has paved the way for several studies investigating the potential genetic basis for ALD risk. The difficulty in identifying 'informative' families (who must drink excessively!) has thus far largely restricted studies investigating potential candidate genes to case-control studies in large groups of well-characterised drinkers with or without advanced ALD, matched for alcohol intake and ethnic origin. As with other 'polygenic' diseases, such as diabetes and coronary artery disease, identification of genes involved in predisposition is hampered by the likelihood that susceptibility is determined by additive small effects of multiple genes.

**Genes influencing oxidative stress and risk of ALD**

The mechanisms outlined in Figure 2 identify several categories of 'candidate' genes worthy of study in ALD. These include genes influencing oxidative stress, genes encoding cytokines, genes influencing the immune response and genes influencing the severity of obesity and/or steatosis. With respect to oxidative stress, the recent identification of mutations of the HFE gene strongly associated with genetic haemochromatosis suggested an obvious candidate gene for ALD, since liver iron promotes oxidative stress and iron deposition is common in ALD.

Unfortunately, in a case-control study of over 400 patients and controls, we found no evidence of an association between ALD and either of the HFE mutations associated with haemochromatosis. This lack of association was explained by the observation that hepatic iron content did not differ between patients with and without the mutations. The principal class of genes that influences oxidative stress in heavy drinkers is that which encodes enzymes involved in alcohol metabolism. Polymorphisms have been identified in two of the seven genes encoding alcohol dehydrogenases, in the promoter region of the CYP2E1 gene and in the coding region of the gene encoding the mitochondrial form of aldehyde dehydrogenase (ALDH2). However, in Caucasian populations the ALDH2 polymorphism and one of the two ADH polymorphisms (ADH2) are extremely rare. The other ADH polymorphism – in the ADH3 gene – is observed in this population; however, several studies have found no evidence of an association between this polymorphism and the risk of either alcoholism per se or ALD. Several studies have looked for an association between the C2 promoter polymorphism of the CYP2E1 gene and ALD in Caucasians. *In vitro* studies have shown that this polymorphism has ten times the transcriptional activity of the C1 allele, leading to the hypothesis that individuals with this polymorphism will have higher CYP2E1 activity and, as a result, generate more oxidative stress during alcohol metabolism. We recently 'pooled' the data from our own and other Caucasian studies, and showed that the C2 allele is more common in ALD patients than controls, with an odds ratio of 2.4 [1.3–4.3]. Other evidence supporting a role for this allele in disease risk was provided by our own study showing that the cumulative lifetime alcohol intake of patients with ALD heterozygous for the C2 allele was almost half that of patients with ALD homozygous for the C1 allele. We also observed that the combination of the slow ADH3 allele (ADH3*2) and the C2 allele was more common in ALD patients than controls, and suggested that patients with this combination will metabolise more alcohol through CYP2E1 and generate more oxidative stress and so increase the risk of ALD. The rarity of the C2 allele in Caucasians, however, implies that other factors are likely to be important in determining genetic susceptibility to ALD.

**Cytokine genes and risk of ALD**

Evidence supporting a role for cytokines in the pathogenesis of ALD, together with the identification of promoter polymorphisms in several of their genes, has recently suggested an alternative set of 'candidates' to explain genetic susceptibility to ALD. Interleukin-10 (IL-10) is the classical anti-inflammatory cytokine which inhibits:

- the activation of CD4+ T-helper cells
- the function of cytotoxic CD8+ T-cells and macrophages
- class II HLA/B7 expression on antigen-presenting cells
- hepatic stellate cell collagen synthesis.
A variant C→A substitution at position -627 in the IL-10 promoter has been associated with decreased reporter gene transcription, decreased IL-10 secretion by peripheral blood monocytes and an increased response to α-interferon in patients with chronic hepatitis C – all consistent with the polymorphism being associated with lower IL-10 production. We have recently reported a strong association between this polymorphism and ALD, with the A allele being more common in drinkers with advanced fibrosis/cirrhosis than in drinkers with normal/fatty liver or healthy controls. This is consistent with low IL-10 favouring inflammatory and immune-mediated mechanisms of disease as well as hepatic stellate cell collagen production. We have also studied polymorphisms in the promoter region of the TNF-α gene. We found the mutant G→A allele at position -238 was strongly associated with alcoholic hepatitis. This polymorphism has also been associated with hepatitis B virus persistence, increased insulin resistance in patients with non-insulin dependent diabetes mellitus, and decreased in vitro expression. All of these observations suggest that, as with the IL-10 mutation, the TNFα promoter mutation is associated with decreased TNFα transcription. Initially, this observation was thought to be somewhat paradoxical for a pro-inflammatory cytokine, and led us further to consider the immunoregulatory role of TNF-α and IL-10.

Immune response genes and risk of ALD

When an antigen is presented to a CD4+ Th helper cell by an antigen-presenting cell, the T-cell differentiates into either a Th1 helper cell which favours a cell mediated immune response, or a Th2 cell, which favours a humoral, antibody-mediated immune response (Fig 3). The fate of the naive Th cell is determined by the cytokine milieu during antigen presentation. High levels of TNF-α, IL-12 and γ-interferon favour a Th1 response, while IL-4 favours a predominantly Th2 response. IL-10, meanwhile, acts principally to down-regulate antigen presentation by antigen-presenting cells and subsequent naive Th-cell activation. We considered, therefore, that the association between ALD and ‘low’ TNF-α and IL-10 genotypes was compatible with a role for Th2 antibody-mediated immune mechanisms in the pathogenesis of ALD. Several lines of accumulating evidence support this hypothesis. These include:

- demonstration of ethanol-derived adducts in the blood and liver of heavy drinkers
- the presence of adducts on the surface of hepatocytes from alcohol-fed rodents
- the presence of anti-adduct antibodies in the serum of heavy drinkers
- the demonstration that these antibodies are capable of mediating antibody-dependent cellular cytotoxicity (ADCC)

Perhaps the most persuasive evidence of a role for immunological mechanisms in disease pathogenesis comes from a study showing that immunising guinea pigs with acetaldehyde-albumin adducts leads to a severe form of ‘alcohol-like’ hepatitis following the consumption of negligible amounts of alcohol.

Prompted by these observations and our results with the IL-10 and TNF-α promoter polymorphisms, we proceeded to look for an association between ALD and a recently described polymorphism in the T-cell surface molecule cytotoxic T lymphocyte antigen-4 (CTLA-4). This molecule competes with CD28 on the CD4+ Th cell’s surface for the
antigen-presenting cell co-stimulatory molecule B7. When CTLA-4, rather than CD28, engages B7 a negative signal is delivered to the T-cell leading to its apoptosis. CTLA-4 knockout mice develop lethal autoreactive lymphoproliferative disease and an A→G polymorphism leading to a Thr→Ala substitution has recently been associated with autoimmune liver diseases, insulin-dependent diabetes and autoimmune thyroid disease. We have found this polymorphism to be strongly associated with advanced ALD, with an odds ratio of 2.2 [1.4–3.3], p<0.005, thus offering further support for the immune hypothesis of ALD²⁰. As discussed, the principal cytokine favouring a Th2-mediated antibody response to antigen presentation is IL-4. Recently, a QwR polymorphism has been identified in the gene encoding the IL-4 receptor. This polymorphism has been linked to increased IgE levels in patients with atopy, and in vitro functional studies have confirmed that it results in an increase in the effects of IL-4. We have recently observed a strong association between this polymorphism and advanced ALD. This result supports a role for the antibody response to alcohol-derived neoantigens in disease pathogenesis, although a recent study showing that IL-4 induces CYP2E1 transcription offers a further explanation for the association.

Conclusions

Environmental and genetic factors determine which particular heavy drinker develops ALD. The risk of ALD can be decreased by:

- reducing daily alcohol intake (with women consuming less than men)
- consuming alcohol with food and sticking to one type of beverage
- limiting dietary excess to avoid obesity
- consuming a diet high in carbohydrate, protein and anti-oxidant trace elements and low in fat.

As regards genetic factors, polymorphisms of ‘metabolic’ genes play only a minor role in Caucasians, while associations with polymorphisms of the gene encoding CTLA-4, TNFRα, IL-10 and the IL-4 receptor support a role for antibody-mediated immune responses in disease pathogenesis. In addition, the associations with the IL-4 receptor, CYP2E1 and IL-10 polymorphism will favour oxidative stress and fibrosis respectively. ALD appears to be a polygenic disease with the risk of disease increasing with the number of ‘at risk’ alleles a drinker possesses. Most of the recently reported associations require confirmation in other populations; however, these genetic studies are beginning to provide clues on disease pathogenesis with potential implications for the development of novel treatment strategies. Of equal importance, the reported associations could enable the identification of ‘high risk’ individuals before they begin to drink.

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