THE ROLE OF GENETIC POLYMORPHISMS IN ALCOHOLIC LIVER DISEASE

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Abstract — Chronic alcohol consumption is a major cause of liver cirrhosis which, however, develops in only a minority of heavy drinkers. Evidence from twin studies indicates that genetic factors account for at least 50% of individual susceptibility. The contribution of genetic factors to the development of diseases may be investigated either by means of animal experiments, through linkage studies in families of affected patients, or population based case–control studies. With regard to the latter, single nucleotide polymorphisms of genes involved in the degradation of alcohol, antioxidant defense, necroinflammation, and formation and degradation of extracellular matrix are attractive candidates for studying genotype–phenotype associations. However, many associations in early studies were found to be spurious and could not be confirmed in stringently designed investigations. Therefore, future genotype–phenotype studies in alcoholic liver disease should meet certain requirements in order to avoid pure chance observations due to a lack of power, false functional interpretation, and insufficient statistical evaluation.

INTRODUCTION

Alcoholic liver disease (ALD) accounts for >50% of all chronic liver diseases in industrialized countries and is responsible for >50,000 annual deaths due to cirrhosis and associated complications (Corrao et al., 1997; Kim et al., 2002; John and Hanke 2002).

ALD comprises various degrees of liver injury due to direct and indirect effects of continuous exposure towards toxic amounts of alcohol, including alcoholic fatty liver, alcoholic steatohepatitis, as well as alcohol-induced hepatic fibrosis and cirrhosis, either with or without inflammation (Ishak et al., 1991). Moreover, chronic alcohol consumption is an established risk factor for the development of hepatocellular carcinoma in patients with liver cirrhosis (Stickel et al., 2002; Morgan et al., 2004). While nearly all heavy drinkers reveal fatty liver, 10–35% of alcoholics are diagnosed with alcoholic hepatitis, and 10–20% develop cirrhosis (Teli et al., 1995). Hence, only a minority of heavy drinkers develop severe liver disease. The cause for this variable susceptibility towards alcohol is yet unclear.

As with other chronic liver diseases, an alcoholic individual’s risk to develop ALD is governed by a complex interplay of numerous genes with several known or unknown environmental factors. For example, it is well established that the amount of alcohol consumed is highly correlated with evolution of cirrhosis (Bellentani et al., 1997). In addition, alcoholics with chronic hepatitis C virus infection or obesity display more severe liver damage (Wiley et al., 1998; Raynard et al., 2002). However, these factors are not sufficient to explain the wide diversity of hepatic damage suggesting a role of certain host factors. Accordingly, female gender and the presence of the hemochromatosis gene mutation were identified to increase the likelihood of alcohol-related hepatic damage. Evidence has accumulated supporting the concept that genetic factors unrelated to gender contribute to the emergence of ALD (Hrubec and Omenn, 1981). For example, twin studies have shown that the concordance for alcoholic cirrhosis is significantly greater in monozygotic than in dizygotic twins, leading to the conclusion that ~50% of the phenotypic variation of alcoholism can be attributed to genetic modifiers (Reed et al., 1996). The identification of these factors would improve our understanding of the pathophysiology of ALD and greatly help managing affected patients. Identification of genetic factors indicative of rapid disease progression would augment preventive strategies and the timing of therapeutic interventions including liver transplantation. Identified genetic risk factors may also play a role in the progression to cirrhosis in other chronic liver diseases as it represents a common end point of many chronic liver diseases.

METHODOLOGY AND ANALYSIS OF GENETIC STUDIES

While some diseases, such as cystic fibrosis, alpha-1 antitrypsin deficiency, and phenylketonuria, are related to mutations of single gene loci inherited according to Mendelian rules, ALD and the majority of other liver disease are polygenic and represent ‘complex traits’. Considerable efforts are required to determine the role of a single or a group of genes, since their effects on disease manifestation are usually smaller than crucial gene defects in monogenic disorders.

Genetically modified animals may be useful in the analysis of disease-specific gene loci either through using knockout mice deficient for the gene of interest, or conversely, transgenic mice which overexpress genes that are relevant for the manifestation or progression of certain diseases (Hillebrandt et al., 2003). Recently, an elegant technique termed ‘quantitative trait loci’ (QTL) analysis has been introduced which combines molecular biology tools with classical approaches of genetics to search for genetic determinants which so far had not been linked to a disease’s pathogenesis (Darvasi, 1998; Korstanje et al., 2002). In QTL
analysis, all crossbred animals in an experiment are both phenotyped with regard to a certain quantitative marker and genotyped through a ‘genome-wide scan’ for markers with known localization within the genome. Using this technique, Hillebrandt et al. (2002) have described a susceptibility locus on chromosome 15 that significantly affects the stage of fibrosis in fibrosis-susceptible BALB/c mice. Excitingly, the same group later confirmed their findings by showing that the corresponding gene in humans which codes for complement factor 5, is in fact a quantitative trait gene that modifies liver fibrosis in patients with chronic hepatitis C (Hillebrandt et al., 2005).

The detection of disease-modulating genes in humans is more difficult and challenging. Currently, three different approaches have been applied: family-based linkage analyses, candidate gene association studies, and genome-wide polymorphism studies. It is beyond the scope of this review to describe family-based linkage and genome-wide scanning analyses and to dissect their advantages and disadvantages in detail, and therefore, readers are referred to concise review articles focused on this issue (Tabor et al., 2002; Day, 2003). Here, emphasis will be exclusively put on genotype–phenotype associations studies.

In humans, candidate gene case–control studies investigating associations between single nucleotide polymorphisms (SNPs) and certain disease end-points have been the most extensively applied method (Hirschhorn et al., 2002). In this kind of study, the frequency of one or several polymorphisms are compared between a group of patients displaying the phenotype of interest and another group of unaffected controls exposed to the same insult, e.g. alcohol or viral hepatitis (Gambaro et al., 2000; Day, 2003). An association with the disease is assumed should there be a significantly higher frequency of one allele or genotype in either of the groups. Genotype–phenotype studies most commonly focus on the role of SNPs. In an SNP, a single base is substituted for another one thereby leading to an altered base triplet that potentially could code for a different amino acid when located in the coding sequence. In effect, this could result in an altered function of the generated protein. Also, some SNPs result in altered quantitative transcription of the respective gene if located in the promoter sequence. However, most polymorphisms are situated in non-coding regions of genes and, therefore, have little or no impact. This has to be taken into account, when choosing polymorphisms for association studies. SNPs are the most common type of allelic variation and can be found throughout the human genome at a frequency of 1 every 1000–2000 bp (http://www.ncbi.nlm.nih.gov/SNP/). So far nearly three million of them are described. Genotyping of SNPs is possible through electrophoresis-based techniques, such as restriction fragment length polymorphism (RFLP) analysis in which a restriction enzyme cuts a PCR product at the site of the SNP thereby generating DNA fragments of different sizes which are then made visible by agarose gel electrophoresis. Since only a proportion of SNPs can be detected through RFLP, PCR techniques using sequence-specific primer pairs that solely amplify the corresponding wild-type or mutated DNA template have been established. Other experimental tools are more complex and include oligonucleotide ligation assays, single-strand conformational polymorphism analysis, or DNA sequencing. While all these techniques are appropriate for small-scale studies on single or a limited number of SNPs, high-throughput genotyping platforms are required to study larger cohorts and multiple SNPs such as in silico mapping (Wang and Rannala, 2005), fluorescent dye-based genotyping (Wilson et al., 1990), DNA microarrays (Lau et al., 2005), and mass spectrometry technologies (Younossi et al., 2005).

The selection of a candidate gene for an association case–control study is usually based on biological plausibility in chosen genes that play a putative role in the pathogenesis of the studied disease (Day, 2003). With regard to ALD, it is important to separate genes that are related to alcoholism from those that affect progression of liver disease. In addition, some genes are known to be mutated in a hereditary type of the disease and, therefore, become an interesting candidate for a case–control study that investigates the sporadic type of the disorder. As mentioned above, genes that were identified through research with knockout or transgenic animals are ideal candidates to be further studied in human cohorts. Novel candidate genes can also be identified through DNA microarrays, and then screened for in larger cohorts of affected individuals. After a gene has been selected to serve as a candidate, it should be clarified whether SNPs with a functional implication reside in the corresponding gene. Only genetic variants that result in altered transcription, RNA stability, or protein function are likely to modulate disease progression (Daly, 2003). So, the functional implications of a SNP or a haplotype (a cluster of gene loci that co-segregate) should be characterized through in vitro and in vivo evidence prior to their testing in association studies.

An important task in genotype–phenotype association studies in ALD is the selection of appropriate cases and controls matched for potential modifiers of liver damage. Therefore, a suitable recruitment strategy should control for potential confounders of an association such as age and gender, extent of alcohol exposure, co-morbidities and co-medication, and ethnicity. These attempts should be made in order to avoid a confounding effect by population stratification due to a marked variation of the allele frequency of certain genes among subgroups with a different baseline risk for the disease (Cardon and Palmer, 2003). Both cases and controls need to be well characterized, and subjects with a similar alcohol consumption and a near normal histology on liver biopsy represent ideal controls. Obviously, this is a difficult task in many instances, so alternatively, controls should at least have normal liver-specific laboratory results and a normal appearance of some type of liver imaging, e.g. ultrasound (Day, 2003).

Statistical issues have become increasingly complex in genetic studies and, particularly, statistical power is important since it reflects the probability that a statistically significant effect is demonstrated when it really exists. Currently, a power of 0.8 is generally accepted for genetic association studies which translates into a 80% chance of detecting a true association (Bataller et al., 2003). In ‘underpowered’ studies, both a type I and a type II error may occur. Type I errors refer to false-positive associations frequently seen in studies with a low sample size, whereas type II errors represent false-negative findings that may result from insufficient patient characterization or population stratification. Recently, a sample size of ≥150 has been defined as a critical threshold for the replication validity of genetic association studies
(Ioannidis et al., 2001). So, a power calculation prior to patient recruitment based on the known allele frequency should become an integral part of the planning of any candidate gene association study.

For data analysis, adequate statistical means have to be applied that adjust for all potential co-factors of the disease. In studies with small sample sizes, a Fisher’s exact test is appropriate, whereas larger sets of data require the application of a $\chi^2$-test. Most well-analyzed genetic case–control studies performed a multivariate logistic regression analysis which allows for correction of quantitative and qualitative covariates as predictors of the disease outcome. If all these prerequisites are taken into account, large numbers of patients and controls are usually necessary to give a study a sufficient power to detect a significant effect (Cardon and Bell, 2001). This highlights the need for networking in the scientific community and calls for cooperation among different research centers.

**PATHOGENESIS OF ALCOHOLIC LIVER DISEASE: POSSIBLE CANDIDATE GENES**

Alcohol is hepatotoxic through a variety of mechanisms which lead to acute and chronic tissue injury, and possibly, to cirrhosis. With regard to the liver, alcohol-induced tissue damage is primarily based on the toxicity of its first metabolite acetaldehyde. In addition, the increased formation of reactive oxygen species (ROS), such as hydrogen peroxide (H$_2$O$_2$) and superoxide anions (O$_2^-$) have been implicated as a cause of liver injury in various forms of chronic liver diseases including ALD (Parola and Robino, 2001). Increased ROS formation is mainly caused by the induction of CYP2E1. The elevated generation of ROS produces cytotoxic oxidative stress and increased lipid peroxidation through the formation of 4-hydroxy 2,3-nonenal (HNE), 4-hydroxy-2,3-alkenals (HAKs) and malondialdehyde (MDA). The capacity of CYP2E1 to oxidize ethanol is increased up to 10-fold in heavy consumers which consecutively increases the pro-oxidative burden (Kessova and Cederbaum, 2003). Defense mechanisms such as antioxidant mitochondrial and cytosolic enzymes can offset some of the toxicity derived from oxidative stress but may become saturated as it persists, or downregulated as liver damage progresses. Furthermore, excessive alcohol consumption can lead to an increased portosystemic uptake of gut-derived endotoxins from gastrointestinal bacteria which contribute to necroinflammation in alcoholic hepatitis by activating Kupffer cells through the CD14/toll-like receptor-4 complex to produce ROS via NADPH oxidase (Parlesak et al., 2000; Wheeler et al., 2001).

The uniform morphologic response of liver tissue to repeated injury from various sources, including alcohol, is fibrosis which closely resembles the process of scar forming (Bataller and Brenner, 2005). As a response to triggers such as acetaldehyde, ROS, lipid peroxides, and endotoxins, liver macrophages (Kupffer cells) and other inflammatory cells become activated to produce a battery of growth factors and cytokines including the powerful mitogen platelet-derived growth factor (PDGF) and the most important profibrogenic cytokine transforming growth factor $\beta_1$ (TGF$\beta_1$) which stimulate hepatic stellate cells (HSC) and portal fibroblasts (Fig. 1). The transformation of quiescent HSC rich in vitamin A into an activated myofibroblast (MFB)-like phenotype rich in $\alpha$-smooth muscle actin and devoid of vitamin A is considered the central event in the pathophysiology of liver fibrosis (Bataller and Brenner, 2001). Activated HSCs/MFBs markedly increase the production of extracellular matrix.
(ECM) molecules which comprise collagens, non-collagenous glycoproteins, proteoglycans, and glycosaminoglycans. However, the accumulation of fibrous material does not simply result from increased production of ECM, but also from its impaired degradation which plays an equally important role (Schuppan et al., 2001). In normal liver tissue, a subtle balance is maintained between matrix synthesis and degradation. The latter is exerted by a group of matrix metalloproteinases (MMPs) released by various liver cells. MMPs process a wide range of ECM substrates (Benyon and Arthur, 2001) and their activity is regulated by a group of specific inhibitors produced by activated HSCs/MFBs termed tissue inhibitors of matrix-metalloproteinases (TIMPs). Upregulation of TIMP-1 and TIMP-2 is responsible for the loss of MMP expression during fibrogenesis in the liver (Herbst et al., 1997).

All phases in the development of ALD are regulated by a number of genes that either alone or in combination may represent genetic risk constellations that influence the biological reaction towards alcohol. Figure 2 depicts some of the possible genetic candidates that are involved in the pathogenesis of alcohol-related liver injury.

**FIG. 2.** Genetic susceptibility for the onset or progression of alcoholic liver disease could be related to genetic variation at any step of alcoholic liver damage (e.g., alcohol metabolism, inflammation, and fibrogenesis).

**CASE–CONTROL STUDIES ON CANDIDATE GENES IN ALD**

In the following, case–control association studies aiming at the identification of genetic risk markers for ALD will be described and weighed regarding their strengths and weaknesses. The description of the studies is divided into sections to pay tribute to the functional role of the tested genes in ALD.

**Polymorphisms of genes involved in alcohol metabolism**

After absorption, alcohol is degraded in the liver and other tissues by alcohol dehydrogenase (ADH) in the cytosol and cytochrome P450 2E1 in microsomes. Acetaldehyde is further converted by aldehyde dehydrogenases (ALDH) to acetate which, after release from the liver, is metabolized by heart and skeletal muscle tissue.

Different classes of ADH isoenzymes are known and a new nomenclature has been introduced recently (Duester et al., 1999). As most studies have applied the old ADH classification, it also will be used in this review. All ADHs are dimeric zinc-containing enzymes classified according to their metabolic properties and sequence similarities. Class I ADH comprises isoenzymes with α, β, and γ subunits coded by corresponding gene loci termed ADH1, ADH2, and ADH3 (Bosron et al., 1993). Among the human ADH gene loci, two class I ADH genes are polymorphic with three alleles existing for either ADH2 and ADH3 which reveal substantially different enzymatic characteristics. ADH2 alleles are ADH2*1 found in Caucasians and ADH2*2 detectable in Asians which encode the low activity β1 and the high activity β2 subunits, respectively. The resulting dimeric isoenzymes have markedly different $k_{cat}$ values of 9.2 min$^{-1}$ for β1β1 and 400 min$^{-1}$ for β2β2, respectively. The ADH3*1 and ADH3*2 alleles produce the γ1 and γ2 subunits, and the γ1γ1 isoenzyme is twice as active as the γ2γ2 isoenzyme ($k_{cat}$ 87 min$^{-1}$ vs 35 min$^{-1}$). A recently described ADH3*3 allele has not been enzymatically characterized (Osier et al., 2002).

According to the differences in the capacity to metabolize alcohol to acetaldehyde, it has been speculated that individuals with the more active ADH2*2 and ADH3*1 alleles are at increased risk of developing alcohol-related organ damage due to a higher acetaldehyde exposure (Couzigou et al., 1994). The details of population-based case–control studies that have tested a possible association of ADH variants with ALD are summarized in Table 1. Studies were carried out in Asian and Caucasian populations, but due to the fact that the ADH2*2 allele is rare in Caucasians, most data regarding this variant stem from investigations in Asians. However, results are inconsistent. Earlier studies identified the
Table 1. Case–control studies on the association between polymorphisms of alcohol-metabolizing enzymes and ALD

<table>
<thead>
<tr>
<th>Authors</th>
<th>Tested gene(s)</th>
<th>Sample size</th>
<th>Definition of cases/controls</th>
<th>Functional data</th>
<th>Principle finding</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Couzigou et al., 1990)</td>
<td>ADH2, ADH3</td>
<td>46 alcoholic cirrhosis 39 controls</td>
<td>Alcoholic cirrhosis: 100 g alcohol/day for &gt;5 years Signs of portal hypertension</td>
<td>None</td>
<td>No association between either ADH and ALD</td>
</tr>
<tr>
<td>(Day et al., 1991)</td>
<td>ADH2, ADH3, ALDH2</td>
<td>59 alcoholic cirrhosis 13 alcoholic pancreatitis 79 healthy Subjects</td>
<td>Alcoholic cirrhotics: 80 g alcohol/day for &gt;10 years Liver biopsy in all cirrhotics</td>
<td>None</td>
<td>ADH3*1 more frequent in cirrhotics than in controls (62.7 vs 55.1%, P &lt; 0.05)</td>
</tr>
<tr>
<td>(Sherman et al., 1993)</td>
<td>ADH2</td>
<td>38 ALD 23 healthy controls</td>
<td>Alcoholics: 80 g alcohol/day for &gt;2 years Liver biopsy in all ALD</td>
<td>None</td>
<td>ADH2*B allele associated with ALD</td>
</tr>
<tr>
<td>(Chao et al., 1994)</td>
<td>ADH2, ADH3, ALDH2</td>
<td>27 alcoholic cirrhosis 23 viral cirrhosis 30 gastroduodenal ulcers</td>
<td>None</td>
<td>None</td>
<td>ADH2<em>2, ADH3</em>1 and ALDH2*2 alleles significantly less frequent in alcoholic cirrhotics than in controls</td>
</tr>
<tr>
<td>(Yamauchi et al., 1995a)</td>
<td>ADH2, ALDH2, CYP2E1</td>
<td>27 alcoholic fatty liver 7 non-specific changes 46 alcoholic cirrhosis 60 healthy controls</td>
<td>Alcoholics: 120g alcohol/day for &gt;10 years Liver histology in all patients Controls: random</td>
<td>None</td>
<td>ADH2*2/2 genotype associated with alcoholic cirrhosis (OR 4.6; no CI)</td>
</tr>
<tr>
<td>(Yamauchi et al., 1995b)</td>
<td>ADH2</td>
<td>42 alcoholic cirrhosis 34 noncirrhotic alcoholics</td>
<td>Alcoholics: 120g alcohol/day for &gt;10 years Liver histology in all patients Controls: random</td>
<td>None</td>
<td>ADH2*2/2 genotype associated with alcoholic cirrhosis</td>
</tr>
<tr>
<td>(Tanaka et al., 1996)</td>
<td>ADH2, ALDH2</td>
<td>31 ALD 90 alcohol addicts 66 healthy controls</td>
<td>Alcoholics: 80g alcohol/day for &gt;10 years Liver histology in all alcoholics Controls: random</td>
<td>None</td>
<td>ADH2<em>1/1 and ALDH2</em>1/1 more frequent in alcoholics/ALD than in controls</td>
</tr>
<tr>
<td>(Tanaka et al., 1997)</td>
<td>ADH2, ALDH2, CYP2E1</td>
<td>26 ALD 189 controls</td>
<td>Alcoholics: 80g alcohol/day for &gt;10 years Liver histology in all alcoholics Controls: random</td>
<td>None</td>
<td>ADH2<em>1/1, ALDH2</em>1/1 and CYP2E1 c2 more frequent in ALD than in controls</td>
</tr>
<tr>
<td>(Ceni et al., 1997)</td>
<td>ADH2, ADH3, ALDH2, CYP2E1</td>
<td>100 alcoholics (26 fatty liver, 29 steatofibrosis, 19 cirrhosis, 26 nonspecific changes)</td>
<td>Alcoholics: 150g alcohol/day or &gt;10 years Liver histology in alcoholics</td>
<td>None</td>
<td>No association with severity between ALD and any of the tested polymorphisms</td>
</tr>
<tr>
<td>(Chao et al., 1997)</td>
<td>ADH2, ADH3, ALDH2, CYP2E1</td>
<td>75 alcoholic cirrhosis 48 acute pancreatitis 19 heavy drinkers 235 controls</td>
<td>Alcoholics: 60g alcohol/day for &gt;7 years Cirrhosis: CHILD B and C, esophageal varices</td>
<td>None</td>
<td>ADH2<em>1 and ALDH2</em>1 more frequent in alcoholic cirrhosis than in controls plus pancreatitis No difference for ADH2 and CYP2E1</td>
</tr>
<tr>
<td>(Grove et al., 1998)</td>
<td>ADH3, CYP2E1</td>
<td>264 ALD 121 random controls</td>
<td>Alcoholics: 80g alcohol/day for &gt;10 years Liver biopsy 228/264 patients</td>
<td>None</td>
<td>No significant association between ADH3 and CYP2E1, and ALD</td>
</tr>
<tr>
<td>(Borras et al., 2000)</td>
<td>ADH2, ADH3</td>
<td>180 alcoholic cirrhosis 231 alcoholics 199 viral cirrhosis 224 healthy controls</td>
<td>Alcoholics: 100g (70g) alcohol/day for &gt;10 years Non-alcoholics: &lt;40g (20g)/day</td>
<td>None</td>
<td>Linkage between ADH2<em>2 and ADH3</em>1 ADH2*2 allele more frequent in non-alcoholics but overall frequency very low (0–4.8%)</td>
</tr>
<tr>
<td>(Monzoni et al., 2001)</td>
<td>ADH2, ADH3, CYP2E1, TNFα</td>
<td>158 alcoholics</td>
<td>Alcoholics: 120g (60g) alcohol/day (questionnaire)</td>
<td>None</td>
<td>ADH3*2 and CYP2E1 c2 allele associated with severity of ALD</td>
</tr>
<tr>
<td>Authors</td>
<td>Tested gene(s)</td>
<td>Sample size</td>
<td>Definition of cases/controls</td>
<td>Functional data</td>
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<tr>
<td>(Lee et al., 2001)</td>
<td>ADH2, ADH3, ALDH2, CYP2E1</td>
<td>56, 52, 64</td>
<td>Alcoholics: 80g alcohol/day for &gt;10 years Cirrhosis: ascites, esophageal varices, encephalopathy</td>
<td>None</td>
<td>No association with severity between alcoholic cirrhosis and any of the tested Genes</td>
</tr>
<tr>
<td>(Frenzer et al., 2002)</td>
<td>ADH2, ADH3, ALDH2, CYP2E1, ApoE, GSTM1/T1</td>
<td>57, 71, 57, 200</td>
<td>Alcoholic cirrhosis: biopsy-proven or clinical evidence</td>
<td>None</td>
<td>ADH3<em>2/2 genotype more frequent in cirrhotics than in blood donors All cirrhotics had ADH2</em>1/1</td>
</tr>
<tr>
<td>(Vidal et al., 2004)</td>
<td>ADH2, ADH3, CYP2E1</td>
<td>99, 118, 47</td>
<td>Alcoholics: 100 g alcohol/day for &gt;10 years Liver histology in alcoholics Non-alcoholics: &lt;20 g/day</td>
<td>None</td>
<td>No association with severity between ALD and any of the tested Polymorphisms</td>
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<tr>
<td>(Tsutsumi et al., 1994)</td>
<td>CYP2E1 (RsaI + PstI)</td>
<td>50, 10, 34</td>
<td>Alcoholics: 80g alcohol/day for &gt;5 years Liver biopsy in all ALD No characterization of controls</td>
<td>Acetaminophen elimination test showed higher activity in c2 homozygotes</td>
<td>Alcoholic cirrhotics had higher rate of mutant c2 allele and c2 homozygosity</td>
</tr>
<tr>
<td>(Maewawa et al., 1994)</td>
<td>CYP2E1 (RsaI + PstI)</td>
<td>20, 62, 66</td>
<td>Alcoholics: 120 g alcohol/day for &gt;10 years Liver biopsy in all ALD</td>
<td>None</td>
<td>Genotype CYP2E1 c1c1 more frequent in advanced ALD</td>
</tr>
<tr>
<td>(Chao et al., 1995)</td>
<td>CYP2E1 (RsaI + PstI)</td>
<td>54, 23, 33</td>
<td>Alcoholics: 80g alcohol/day for &gt;8 years All cirrhotics had decompensated liver disease</td>
<td>None</td>
<td>No association of CYP2E1 alleles with alcoholic cirrhosis marked ethnic differences between Asians and Caucasians/African Americans</td>
</tr>
<tr>
<td>(Pirmohamed et al., 1995)</td>
<td>CYP2E1 (RsaI + PstI)</td>
<td>95, 58, 47</td>
<td>Alcoholics: 190g alcohol/day (median) &gt; 10 years 33% of ALD with biopsy</td>
<td>None</td>
<td>c2 allele more frequent in ALD (OR 4.5; CI 1.9-10.9)</td>
</tr>
<tr>
<td>(Carr et al., 1995)</td>
<td>CYP2E1 (RsaI + PstI)</td>
<td>53, 21, 18</td>
<td>ALD: average intake 257g alcohol for 27 years Alcoholism: DSM II criteria</td>
<td>None</td>
<td>No association of CYP2E1 alleles with alcoholic cirrhosis</td>
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<tr>
<td>(Ball et al., 1995)</td>
<td>CYP2E1 (RsaI + PstI)</td>
<td>37, 24, 39</td>
<td>Alcoholics: 80g alcohol/day for &gt;3 years Liver biopsy in all ALD</td>
<td>None</td>
<td>No association of CYP2E1 alleles with alcoholic cirrhosis</td>
</tr>
<tr>
<td>(Carr et al., 1996)</td>
<td>CYP2E1 (RsaI + PstI)</td>
<td>46, 108</td>
<td>Alcoholics: 80g alcohol/day Alcoholism: DSM II criteria</td>
<td>None</td>
<td>No association of CYP2E1 alleles with alcoholic cirrhosis and alcoholism</td>
</tr>
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</table>
### Table 1. Continued

<table>
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<tr>
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<th>Principle finding</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Lucas et al., 1996)</td>
<td>CYP2E1 (RsI, DraI), CYP1A1 (MspI)</td>
<td>202 alcoholics without ALD; 110 alcoholic cirrhosis</td>
<td>Alcoholic cirrhosis confirmed through biopsy</td>
<td>None</td>
<td>No association of CYP2E1 alleles with alcoholic cirrhosis DraI SNP more frequent among alcoholics</td>
</tr>
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<td>(Agundez et al., 1996)</td>
<td>CYP2E1 (RsI)</td>
<td>58 alcoholic cirrhosis; 137 normal controls</td>
<td>Alcoholics: 100g alcohol/day for &gt;10 years</td>
<td>None</td>
<td>No association of CYP2E1 RsI SNP with alcoholic cirrhosis</td>
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<tr>
<td>(Savolainen et al., 1997)</td>
<td>CYP2E1 (RsI, PstI, DraI, MspI)</td>
<td>Male autopsy cases; 243 alcoholics; 43 moderate drinkers; 33 controls</td>
<td>Alcoholics: &gt;80 g alcohol/day Moderate drinkers: 10–80 g Controls: 0–10 g</td>
<td>None</td>
<td>No association of CYP2E1 alleles with ALD RsI + PstI very infrequent</td>
</tr>
<tr>
<td>(Rodrigo et al., 1998)</td>
<td>CYP2E1 (RsI + PstI)</td>
<td>174 alcoholics; 89 controls</td>
<td>Alcoholics: Cloninger criteria Liver histology in all cirrhotics</td>
<td>None</td>
<td>No association of CYP2E1 alleles with alcoholic cirrhosis or alcoholism</td>
</tr>
<tr>
<td>(Itoga et al., 1999)</td>
<td>9 exons and exon-intron junctions of CYP2E1</td>
<td>44 ALD; 96 alcoholics without ALD; 115 healthy controls</td>
<td>ALD: liver biopsy in all ALD patients Alcoholism: DSM III-R Controls: non-drinking, light consumption (22–44g/day &gt; 5 years), moderate consumption (&gt;66g/day &gt; 5 years)</td>
<td>None</td>
<td>No association between any nucleotide replacement and ALD</td>
</tr>
<tr>
<td>(Wong et al., 2000)</td>
<td>CYP2E1 (RsI, PstI, TaqI)</td>
<td>61 ALD; 46 HCC; 375 healthy controls</td>
<td>Alcoholics: 60g alcohol/day for &gt;10 years Liver biopsy in all ALD subjects</td>
<td>None</td>
<td>No association between CYP2E1 RsI and PstI SNPs and alcoholic cirrhosis TaqI SNP protective towards alcoholic cirrhosis</td>
</tr>
<tr>
<td>(Pastorelli et al., 2001)</td>
<td>CYP2E1 + (PstI), DRD2 and SLC6A4</td>
<td>60 alcoholics; 18 cirrhosis; 64 healthy control</td>
<td>Alcohol quantification via interview</td>
<td>None</td>
<td>No association with severity between ALD and any of the tested polymorphisms</td>
</tr>
<tr>
<td>(Plee-Gautier et al., 2001)</td>
<td>CYP2E1 (1C + 1D alleles)</td>
<td>98 alcoholic cirrhosis; 148 alcoholics without ALD; 103 random controls</td>
<td>Alcoholics: 80g alcohol/day for more than 'several' years Chlorzoxazone test</td>
<td>None</td>
<td>No association between CYP2E1 1C and 1D alleles and alcoholic cirrhosis</td>
</tr>
</tbody>
</table>

ADH*2 allele and genotype ADH*2/2 as a risk factor for ALD (Sherman et al., 1993; Chao et al., 1994; Yamauchi et al., 1995a, 1995b), whereas subsequent investigations rather found the allele ADH2*1 and genotype ADH2*1/1 to be associated with ALD (Tanaka et al., 1996, Tanaka et al., 1997; Chao et al., 1997; Frenzer et al., 2002). Among the former it is noteworthy to mention, that Yamauchi et al. (Yamauchi et al., 1995a, 1995b) were seemingly able to publish the same data twice. While several investigators reported associations between the ADH3*2 allele or the ADH3*2/2 genotype, respectively, and ALD (Monzoni et al., 2001; Frenzer et al., 2002), others suggested a role of the high turnover allele ADH3*1 to confer risk to the development of ALD (Day et al., 1991). However, a number of studies found no association at all (Table 1). The true role of ADH genotypes in the risk of ALD is difficult to define since many of the published studies reveal important limitations as regards the interpretability of the data. For example, most studies reveal a very low sample size and, therefore, a lack of statistical power likely hampers the significance of the key finding. 'Underpowered' investigations are highly susceptible towards chance observations which may explain the discrepancies between many of the studies. Also, relatively simple tests were used for statistical analyses, such as mere counting of alleles and genotype frequencies, Student’s t-tests and Fisher’s exact tests which are inappropriate for determining the influence of certain factors in complex settings. Only one study has applied a multiple logistic
regression analysis that takes numerous modifiers of disease into account (Yamauchi et al., 1995b). Moreover, no study presented in vitro or in vivo data supporting the functional significance of the association and the hypothesis, such as the testing for differences in acetaldehyde levels in individuals with different genotypes. Overall, the available data does not provide clear evidence that demonstrates a contribution of ADH genotypes to the development of ALD.

A similar situation prevails with regard to genetic variants of CYP2E1. In contrast to ADH and ALDH, CYP2E1 is an inducible enzyme and its activity can increase up to 20-fold following continuous alcohol consumption. Therefore, functional polymorphisms are just one possible factor in the individual variation of CYP2E1 activity. There are several polymorphic loci within the human CYP2E1 gene of which four are identifiable via RFLP (Hayashi et al., 1991). Two mutations were found to be in linkage disequilibrium, giving rise to the c1 and c2 allele (Watanabe et al., 1990). The CYP2E1 c2 (mutated) allele is associated with an up to 10-fold higher gene transcription, protein level, and enzyme activity than the c1 allele and could result in a higher exposure of the liver towards acetaldehyde and ROS (Watanabe et al., 1994). Therefore, CYP2E1 is an interesting candidate gene in case–control association studies and published reports are listed in Table 1. However, many of the limitations outlined for ADH and ALDH genotype association studies also hold true for studies investigating CYP2E1 SNPs including lack of statistical power, insufficient characterization of cases and controls, inappropriate statistical analysis, and lack of functional data. Altogether, a significant contribution of CYP2E1 variants to the emergence of ALD is unlikely.

While both ADH3 and CYP2E1 genotypes do not seem to be a strong risk factor of progression of ALD, their significant contribution to the development of HCC was suggested by two reports which showed that individuals homozygous for the alleles CYP2E1 c1 (Yu et al., 1995) and ADH3*1/ADH1C*1 (Homann et al., 2006) are at an increased risk to develop hepatoma. In the latter study, 818 alcoholics either with or without alcohol-related disorders including liver cirrhosis, chronic pancreatitis, alcohol-related carcinoma of the esophagus, head and neck, and liver (n = 86) were genotyped for ADH3/ADH1C variants. The odds ratio for genotype ADH3*1/1 regarding the development of HCC was 3.36 (CI 1.33–9.53) and multivariate analysis identified the ADH3*1 allele and its homozygosity as independent risk factors for HCC in heavy drinkers with preexisting cirrhosis.

Genetic polymorphisms of antioxidant enzymes

Numerous lines of evidence point to an important role of oxidative stress in the pathogenesis of ALD. During alcohol metabolism, ROS are generated as a result of the production of NADH from the conversion of ethanol to acetaldehyde by ADHs, and of NADPH from the metabolism of ethanol by CYP2E1. ROS are highly reactive and can damage lipids, proteins, and DNA (Arteel, 2003). Several enzymes exist which counteract oxidative stress generated in ALD including glutathione-S-transferases (GSTs) and superoxide dismutase. GSTs are expressed in the liver and other organs and comprise several gene subfamilies encoding sulphur-containing enzymes which inactivate ROS and many toxic and carcinogenic xenobiotics through conjugation with glutathione (GSH) (Armstrong, 1997). GST isoenzymes reveal profound differences in their structure and substrate specificity and >30 polymorphic variants have been identified (Hayes et al., 2005). Among these, polymorphisms of the α (GSTA), μ (GSTM), τ (GSTT), and π (GSTP) classes have been studied in patients with ALD and the major characteristics of the published reports are outlined in Table 2.

Most case–control studies investigated the GSTM1 and GSTT1 genes due to the existence of ‘null’ allelic variants resulting from a partial deletion in either gene locus with absence of enzyme activity. Consequently, a loss or deficiency in GSTM1 and GSTT1 enzyme activity could potentially increase the levels of toxic intermediates generated along with chronic alcohol consumption. However, only few studies have found an association between ‘null’ genotypes of GSTM1 and GSTT1, and ALD (Groppi et al., 1995; Groppi et al., 1996), while several reports showed no relation (Savolainen et al., 1996; Rodrigo et al., 1999; Frenzer et al., 2002; Burim et al., 2004; Hayes et al., 2005). However, in the largest study, a significantly increased frequency of GSTM1 ‘null’ genotype in heavy drinkers with advanced ALD has been demonstrated (Savolainen et al., 1996). Moreover, a recent study from Spain showed an increased risk for ALD in individuals with combined carriage of GSTM1 and GSTT1 ‘null’ genotype (Ladero et al., 2005). Although attractive, genetic polymorphisms of GST isoenzymes cannot be considered as firmly established since findings are still conflicting or have been generated in studies with an insufficient design. Our own findings in a larger case–control series indicate no risk of GSTP1 polymorphisms (Eurich, D., Friess, H., Hellerbrand, C., Homann, N., Kolb, A., Österreicher, C. H., Patsenker, E. et al., unpublished data). However, we have recently found genotype GSTP1 Val/Val to be associated with the development of cirrhosis in patients with hereditary hemochromatosis (Stickel et al., 2005). As in ALD, oxidative stress plays an important role in the pathogenesis of hemochromatosis.

Hepatocyte mitochondria are prime targets of oxidative stress generated in chronic alcoholism and alterations of mitochondrial function and structure, such as the breakdown of mitochondrial membrane potential, have been recognized as key events in the onset of alcohol-driven apoptosis (Adachi and Ishii, 2002). The ability to resist oxidative pressure largely depends on the mitochondrial GSH content and antioxidant mitochondrial enzymes. Mitochondria-derived ROS are detoxified to hydrogen peroxide and water by the successive action of manganese superoxide dismutase (MnSOD) and GSH peroxidase, respectively (Wallace, 1999). MnSOD is synthesized with a cleavable N-terminal mitochondrial target sequence that enables its transport into mitochondria (Shimoda-Matsubayashi et al., 1996). A SNP within codon 16 of the precursor protein leads to either alanine (Ala) or valine (Val) at amino acid position –9 of the target sequence resulting in enhanced translocation into mitochondria and higher concentration of active MnSOD in case of the Ala-sequence (Sutton et al., 2003). Table 2 summarizes case–control studies that investigated MnSOD variants.

Degoul et al. (2001) have genotyped 71 patients with ALD stratified according to the degree of liver damage and have found that the Ala/Ala genotype occurs more frequently in
<table>
<thead>
<tr>
<th>Authors</th>
<th>Tested gene(s)</th>
<th>Sample size</th>
<th>Definition of cases/controls</th>
<th>Functional data</th>
<th>Principle finding</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Groppi et al., 1991)</td>
<td>GSTM1</td>
<td>45 alcoholics</td>
<td>Cirrhotics: &gt;100 g alcohol/day for &gt;5 years Presence of ascites, varices</td>
<td>None</td>
<td>No relationship between alcoholic cirrhosis and GSTM1 genotypes</td>
</tr>
<tr>
<td>(Savolainen et al., 1996)</td>
<td>GSTM1</td>
<td>313 alcoholics</td>
<td>Alcohols: &gt;80 g alcohol/day Moderate drinkers: 10–80 g Controls: 0–10 g</td>
<td>None</td>
<td>GSTM1 'null' genotype more frequent among ALD with advanced fibrosis (OR 2.3; CI 1.11–4.76)</td>
</tr>
<tr>
<td>(Rodrigo et al., 1999)</td>
<td>GSTM1, CYP2E1 (PstI), ADH2, NAT2</td>
<td>120 alcoholic cirrhosis 30 alcoholics without ALD 200 healthy controls</td>
<td>Alcoholics: 200 g alcohol/day for &gt;20 years</td>
<td>None</td>
<td>GSTM1 polymorphism not related to cirrhosis</td>
</tr>
<tr>
<td>(Frenzer et al., 2002)</td>
<td>GSTM1/T1, ADH2, ADH3, ALDH2, CYP2E1, ApoE</td>
<td>57 alcoholic cirrhosis 71 alcoholic pancreatitis 57 alcoholics 200 blood donors</td>
<td>Alcoholic cirrhosis: biopsy-proven or clinical evidence</td>
<td>None</td>
<td>No association between alcohol-related disorders and GST genotypes</td>
</tr>
<tr>
<td>(Burim et al., 2004)</td>
<td>GSTM1, T1, P1, CYP2E1, CYP1A1</td>
<td>65 alcoholic cirrhosis 14 alcoholic pancreatitis 41 alcoholics 221 non-alcoholic controls</td>
<td>Alcoholics: &gt;40g alcohol/day obtained from case records Mixed ethnicity</td>
<td>None</td>
<td>GSTs not associated with any of the endpoints CYP1A1 associated with cirrhosis (OR 5.33; CI 1.23–23.14)</td>
</tr>
<tr>
<td>(Brind et al., 2004)</td>
<td>GSTM1, M3, P1, T1, A1</td>
<td>Unclear number of healthy controls, alcoholics without ALD, and ALD patients</td>
<td>Alcoholics: different definitions in three centers</td>
<td>None</td>
<td>No association between ALD and any of the tested SNPs</td>
</tr>
<tr>
<td>(Ladero et al., 2005)</td>
<td>GSTM1, T1</td>
<td>153 ALD 241 random controls</td>
<td>Alcoholics: 150 g alcohol/day (median) for &gt;10 years Cirrhosis: clinical and imaging evidence of portal hypertension</td>
<td>None</td>
<td>GSTT1 'null' genotype associated with ALD (OR 1.67; CI 1.03–2.71) Carriers of GSTT1 and M1 'null' genotypes are at increased risk for ALD (OR 4.3; CI 1.89–9.97)</td>
</tr>
<tr>
<td>(Degoul et al., 2001)</td>
<td>MnSOD</td>
<td>71 ALD 79 blood donors</td>
<td>Average alcohol intake 151 g (±95)/day for 18 ± 6 years Liver biopsy in all ALD patients</td>
<td>None</td>
<td>Rate of MnSOD Ala homozygosity increased significantly with severity of ALD (RR of cirrhosis for Ala/Ala 9.6; CI 2.6–35.4)</td>
</tr>
<tr>
<td>(Stewart et al., 2002)</td>
<td>MnSOD</td>
<td>281 advanced ALD 218 alcoholics without ALD 244 healthy controls</td>
<td>Alcoholics: 80g alcohol/day for &gt;10 years ALD: clinical and biochemical evidence, imaging Controls: alcohol &lt;21 U (men), &lt;15 U (women)</td>
<td>Circulating antibodies raised against markers of oxidative stress</td>
<td>No difference in MnSOD genotype and allele frequencies between groups, Serum markers of oxidative stress similar in different genotypes</td>
</tr>
<tr>
<td>(Brind et al., 2003)</td>
<td>MnSOD</td>
<td>357 ALD 93 alcoholics without ALD 474 non-drinking controls</td>
<td>Alcoholics: different definitions in three centers</td>
<td>None</td>
<td>No difference in MnSOD genotype and allele frequencies between groups</td>
</tr>
<tr>
<td>(Nahon et al., 2005)</td>
<td>MnSOD</td>
<td>Longitudinal study in 264 alcohol cirrhotics</td>
<td>Alcoholic cirrhosis: liver biopsy, for &gt;80 g alcohol/day, alphafetoprotein &lt;50 ng/ml</td>
<td>None</td>
<td>RR for HCC with 1 Ala allele 4.59 (CI 1.61–13.06) RR for death due to cirrhosis with 1 Ala allele 2.49 (CI 1.36–4.57)</td>
</tr>
</tbody>
</table>

Table 2. Case–control studies on the association between polymorphisms of antioxidant enzymes and ALD
patients with severe ALD. They assessed an OR of 9.6 (CI 2.6–35.4) for genotype MnSOD Ala/Ala for the presence of cirrhosis. However, the study included only 13 cirrhotic patients. Although the authors did not provide a power calculation, there is an obvious lack of statistical power due to a small sample size. The same group later presented data from a longitudinal study suggesting that the presence of at least one Ala MnSOD allele increases the risk for developing cirrhosis in French subjects and, furthermore, with the development of HCC and death due to cirrhosis (Nahon et al., 2005). However, case–control studies from other researchers with larger numbers of patients and controls could not confirm these findings (Stewart et al., 2002; Brind et al., 2003).

Polymorphic genes coding for cytokines involved in ALD

Compelling evidence points to an important role of the immune system in mediating alcoholic liver injury. The innate immune response acts as the first line of nonspecific defense against exogenous pathogens such as ROS, lipid peroxides, and endotoxins/lipopolysaccharides derived from the outer cell membrane wall of gram negative bacteria in the intestine which activate immune effector cells. Particularly, Kupffer cells play an important role and experimental inhibition of Kupffer cells prevents an array of hepatic reactions in response to ethanol including the elevation of serum transaminases, steatosis, inflammation, and necrosis (Hines and Wheeler, 2004). Numerous experimental studies found that Kupffer cells are stimulated by various triggers to produce a number of cytokines including tumor necrosis factor α (TNFα), a proinflammatory cytokine known to potentially cause hepatocyte death. Apart from TNFα, several other cytokines including interleukins, interferons, chemokines, and certain growth factors regulate hepatic inflammation, apoptotic and necrotic cell death, cholestasis, and fibrosis (Tilg and Diehl, 2000). For several genes that code for proteins involved in these processes, polymorphisms with functional implications have been detected that render these variants interesting candidates for case–control association studies.

Excess TNF-α production is a typical feature of ALD. The biological response is dependent on the level of TNF-α the liver is exposed to and may result in increased hepatocyte proliferation, activation of cell survival factors such as upregulation of MnSOD and bcl-xL, or cell death through initiation of apoptosis or necrosis. Therefore, polymorphic variation of the TNF-α gene leading to variable TNF-α levels may influence TNF-α-dependent inflammation following alcohol intake. In fact, two SNPs at position –308 (G→A) and –238 (G→A), respectively, of the TNF-α gene are associated with increased TNF-α expression thereby possibly influencing the progression of ALD (Wilson et al., 1993; D’Alfonso and Richiardi, 1994). Grove and co-workers (Grove et al., 1997) were the first to study these two TNF-α polymorphisms in a cohort of patients with ALD. While the distribution of the polymorphism at position –308 was not different between the cases and controls, an excess of the rare TNFA-A allele at position –238 was found in patients with ALD. The OR for this variant vs non-diseased patients was 3.5 (0.4–28) with regard to cirrhosis and 4 (1.2–14) for alcoholic steatohepatitis. All patients had biopsy-proven ALD and the distribution of genotypes was in Hardy–Weinberg equilibrium in all groups. However, the authors did not provide evidence that the tested polymorphisms resulted in differences in TNF-α serum levels, neither did they show SNP-dependent variation in hepatic TNF-α expression. Two subsequent cohort studies also tested TNF-α variants but found no association (Bathgate et al., 2000; Ladero et al., 2002). However, one study testing the TNF-α –308 SNP investigated only 25 patients with ALD and, therefore, could have missed a relationship due to low sample size (Bathgate et al., 2000). The other study included a larger number of ALD patients but was carried out in Spain, so ethnic differences may have contributed to the discrepancy (Ladero et al., 2002). The latter two studies also included several polymorphisms in the interleukin-10 (IL-10) gene which alter gene transcription and IL-10 serum concentrations. IL-10 has emerged as an important inhibitor of inflammatory responses such as the downregulation of proinflammatory cytokines including IL-1, TNF-α, IL-6, IL-8, and IL-12. Moreover, IL-10 was shown to upregulate the expression of the IL-1R antagonist, to inhibit collagen gene transcription, and to increase collagenase expression in HSC (Wang et al., 1998). However, no association was found in contrast to a previous report in 287 patients with biopsy-proven ALD in which the possession of the A allele in the IL-10 promoter was associated with an increased risk of advanced ALD (Grove et al., 2000).

Two reports are available on the association of a polymorphism in the interleukin-1 receptor antagonist (IL-1Ra) gene with ALD (Takamatsu et al., 1998; Pastor et al., 2000). IL-1Ra is a potent antiinflammatory cytokine that can inhibit immune-mediated inflammatory reactions. IL-1Ra relates to IL1 which binds to its corresponding receptor IL-1 (IL-1R). A variable nucleotide tandem repeat polymorphism resulting in different allele sizes due to variable numbers of repeats (A1: 4 repeats; A2: 2 repeats; A3: 5 repeats; A4: 3 repeats) was shown to alter IL-1Ra expression in monocytes in vitro (Danis et al., 1995). One study included Spanish alcoholics with alcohol addiction, significant alcohol abuse, and alcoholic cirrhosis, respectively (Pastor et al., 2000). While the presence of the A1 allele increased the risk for alcoholism, no relationship with ALD was detected. In the Japanese study, genotype and allele distributions differed from that in Caucasians and heterozygosity for the A1 allele was more frequent in alcoholics with fibrosis than in those without (Takamatsu et al., 1998). However, this difference did not reach statistical significance. Owing to the low number of cases, the findings of the latter study are highly suspicious of being chance observations which particularly occur in genotypes with low frequencies (Day, 2003).

In addition, one study each was performed to investigate a possible relationship between polymorphisms of interleukin-1β (IL-1β), a promoter polymorphism of the CD14 endotoxin receptor, and the cytotoxic T-lymphocyte antigen-4 gene (CTLA-4), and ALD. The details of studies on polymorphic variation of cytokine genes in the development of alcoholic hepatitis and cirrhosis are outlined in Table 3. All three reports found associations between certain genotypes and the development of alcoholic cirrhosis; however, no other study has so far repeated, let alone confirmed these findings.
Genetic variants of genes relevant for fibrogenesis and fibrolysis

Genes that govern the production and degradation of fibrous tissue are interesting with regard to many etiologies of chronic liver disease since the formation of fibrosis in different disease entities share many similarities (Friedman, 2000). These genes could represent genetic markers of progression rather than of the susceptibility towards a certain disease trigger since fibrosis develops with a long latency that is unlikely to influence behavioral aspects of alcoholism. Consequently, genes that were identified to confer risk to advanced liver damage in one form of chronic liver disease is likely to be involved in another etiology as well. Accordingly, several candidate genes that are involved in connective tissue turnover have

<table>
<thead>
<tr>
<th>Authors</th>
<th>Tested gene(s)</th>
<th>Sample size</th>
<th>Definition of cases/controls</th>
<th>Functional data</th>
<th>Principle finding</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Grove et al., 1997)</td>
<td>TNFα</td>
<td>150 ALD (cirrhosis, ASH)</td>
<td>Alcoholics: 80 g alcohol/day &gt;10 years Liver biopsy in all ALD</td>
<td>None</td>
<td>−238 TNF-α G/A genotype associated with cirrhosis (OR 3.5; CI 0.4–28) and ASH (OR 4; CI 1.2–14)</td>
</tr>
<tr>
<td>(Takamatsu et al., 1998)</td>
<td>IL-1R antagonist</td>
<td>46 alcoholic cirrhosis</td>
<td>Alcoholics: 120 g alcohol/day &gt;10 years Liver biopsy in all ALD</td>
<td>None</td>
<td>IL-1Ra A1 heterozygotes more frequent in fibrosis/cirrhosis Cumulative alcohol intake lower in A1 carriers with alcoholic fibrosis</td>
</tr>
<tr>
<td>(Grove et al., 2000)</td>
<td>IL-10</td>
<td>287 advanced ALD</td>
<td>Alcoholics: 80 g alcohol/day &gt;10 years ALD: clinical and biochemical evidence, imaging, biopsy</td>
<td>None</td>
<td>Carriage of A allele (~627) of IL-10 promoter associated with advanced ALD (OR 2.04; CI 1.42–2.92)</td>
</tr>
<tr>
<td>(Bathgate et al., 2000)</td>
<td>TNFα, IL-10, TGFβ1</td>
<td>25 ALD</td>
<td>All patients transplant recipients Alcohol consumption not detailed</td>
<td>None</td>
<td>None of the tested genotypes associated with ALD</td>
</tr>
<tr>
<td>(Ladero et al., 2002)</td>
<td>TNFα, IL-10</td>
<td>147 advanced ALD (steatofibrosis, cirrhosis, alcoholic hepatitis)</td>
<td>Alcoholics: 120 g alcohol/day for &gt;10 years Liver biopsy in all ALD</td>
<td>None</td>
<td>Single SNPs not related to ALD Excess of G11-GCC haplotype in ALD(OR 2.08; CI 1.31-3.31)</td>
</tr>
<tr>
<td>(Takamatsu et al., 2000)</td>
<td>IL-1β</td>
<td>142 ALD</td>
<td>Alcoholics: 120 g alcohol/day (mean) &gt;10 years ALD: clinical evidence, imaging results, endoscopy, biopsy (N = 12)</td>
<td>None</td>
<td>Carriers of –511 IL-1β allele 2 more frequent among alcoholic cirrhotics (OR 2.3; CI 1.1–4.8) Haplotype IL-1β –511 allele 2/4395 allele 1 associated with alcoholic cirrhosis</td>
</tr>
<tr>
<td>(Jarvelainen et al., 2001)</td>
<td>CD14-Endotoxin receptor</td>
<td>48 alcoholic cirrhosis</td>
<td>Alcoholics: 40g alcohol/day for 24.9 (±10.8) years ALD: autopsy</td>
<td>None</td>
<td>T allele of CD14-Endotoxin receptor associated with alcoholic hepatitis (OR 2.48; CI 1.17–5.24) and alcoholic cirrhosis (OR 3.45; CI 1.49–7.99)</td>
</tr>
<tr>
<td>(Valenti et al., 2004)</td>
<td>CTLA-4</td>
<td>183 ALD (cirrhosis, fibrosis, steatosis)</td>
<td>Alcoholics: 60 g (men) / 40 g (women) alcohol/day for &gt;5 years Alcohol cirrhosis assessed with biopsy in 128/183</td>
<td>None</td>
<td>Genotype CTLA-4 G/G associated with ALD (OR 3.5; CI 1.1–11)</td>
</tr>
<tr>
<td>(Pastor et al., 2000)</td>
<td>IL-1R antagonist</td>
<td>30 alcoholic cirrhosis</td>
<td>Alcoholics: 100g alcohol/day for &gt;5 years Alcohol cirrhosis: biopsy (n = 24), biochemical evidence (n = 6)</td>
<td>None</td>
<td>Presence of the A1 IL-1R antagonist allele associated with alcoholism but not with alcoholic cirrhosis</td>
</tr>
<tr>
<td>(Oliver et al., 2005)</td>
<td>TGFβ1</td>
<td>165 advanced ALD</td>
<td>Alcoholics: 167 g alcohol/day (mean) &gt;10 years ALD: clinical evidence, imaging results, endoscopy, biopsy (n = 16)</td>
<td>None</td>
<td>No association between tested SNP and ALD</td>
</tr>
</tbody>
</table>
been tested in other diseases, such as TGF-β1 in chronic hepatitis C (Powell et al., 2000) and hemochromatosis (Österreicher et al., 2003; Lose et al., 2005). MMPs in primary sclerosing cholangitis (Satsangi et al., 2005), TIMPs in the development of asthma and arterial aneurysms (Krex et al., 2003; Lose et al., 2005).

With regard to ALD, only data from studies on the role of TGF-β1 polymorphisms are available so far (Table 3). Two studies have analyzed several TGF-β1 polymorphisms which lead to elevated TGF-β1 expression in the setting of ALD, neither found an association with alcoholic cirrhosis (Bathgate et al., 2000; Oliver et al., 2005). Our own data on a polymorphism at codon 25 of the signal sequence (Arg→Pro) within the TGFβ1 gene resulting in higher TGFβ1 levels showed no difference in a cohort of 153 alcoholic cirrhosis compared with a cohort of 118 matched heavy drinkers without liver damage (Eurich, D., Friess, H., Hellerbrand, C., Homann, N., Kolb, A., Österreicher, C. H., Patsenker, E. et al., unpublished data).

With regard to the progression of fibrosis, MMPs qualify as ideal candidates since their function is closely linked to the accumulation of ECM. Among several MMPs that are expressed in the liver, MMP-3 (stromelysin) is crucial because of its capacity to degrade a broad spectrum of ECM molecules and to activate other MMPs. The presence of 5 adenosines (5A) instead of 6 adenosines (6A) at bp –1171 results in increased MMP-3 activity (Ye et al., 1996). As mentioned above, a recent study found an association between genotype MMP-3 5A/5A and the progression of liver damage in patients with primary sclerosing cholangitis (Satsangi et al., 2001), but its role in ALD is unknown. In a recent study from our own group, MMP-3 5A/5A genotype was found more frequently in alcoholic cirrhotics than in those without (30.1 vs 15.3%, \[P = 0.017\]), and multivariate analysis identified age and genotype MMP-3 5A/5A as independent risk factors for alcoholic cirrhosis (Stickel et al., manuscript in revision).

The adjusted odds ratio of genotype MMP-3 5A/5A for the development of cirrhosis was 1.52 (95% CI 1.108–2.086, \[P = 0.010\]). Notably, RT–PCR revealed significantly higher MMP-3 transcription in individuals with MMP-3 5A/5A genotype compared with the MMP-3 5A/6A (6A/6A) variant. However, whether the MMP-3 5A/5A genotype represents the first fibrosis-associated genetic risk factor for the progression of ALD remains to be confirmed in an independent cohort of alcoholic cirrhotics.

**DESIGN OF GENETIC ASSOCIATION STUDIES**

Many published genotype–phenotype association studies were carried out based on exciting hypotheses but collected questionable genetic data since important requirements to design and statistical interpretation were not met. Consequently, not only results were published that could not be reproduced due to lack of a true association with the studied disease, but also some investigations may have even missed an association because of an insufficient approach. Frequently encountered problems of previous case–control association studies in many diseases include the lack of statistical power due to small sample size (Ioannidis et al., 2003), population stratification (Cardon and Bell, 2001), ethnic heterogeneity (Deng, 2001), deviation from Hardy–Weinberg equilibrium due to errors in patient and control selection or genotyping, and lack of control for confounding factors (Colhoun et al., 2003). Consequently, replication validity of genetic association studies has been unsatisfactory because results of the first study rarely correlated with subsequent findings. This discrepancy is more likely to occur as more studies are performed on a potential association and when the sample size of the first study has been low (Ioannidis et al., 2001).

Notably, ‘index’ studies on a genotype–phenotype association are often published in prestigious journals that yield high impact factors, whereas subsequent research with often better design face problems to get accepted at all, or appear in journals with lower average impact factors. The same accounts for the calculated odds ratios for a given association: while the first study indicated impressive estimates of predisposition against or protection from a certain disease, subsequent data tended to show much less pronounced relationships or even the opposite (Ioannidis et al., 2001).

In order to avoid these mistakes and to prevent much energy and financial resources from being wasted on time-consuming and, lastly, unproductive efforts, some recommendations are proposed for the design of genetic case–control association studies (Table 4).

**CONCLUSION**

Agreement exists that the manifestation of ALD is partly determined by genetic factors and substantial efforts have

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**Table 4. Recommendations for design and data analysis of genetic case–control association studies**

<table>
<thead>
<tr>
<th>Recommendation</th>
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<tbody>
<tr>
<td>Candidate gene</td>
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<tr>
<td>Genetic variant</td>
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<tr>
<td>Selection of cases and controls</td>
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<td>Data analysis</td>
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been made to identify such genetic modifiers, mostly by means of genetic case–control association studies. SNPs of genes that code for proteins that play a role in the pathogenesis of ALD were tested. So far, results are conflicting and initial euphoria over seemingly identified genetic markers has faded since many results could not be reproduced. Therefore, future studies have to adopt certain criteria that assure statistical power via large-scale multicenter cooperations, functional relevance of the tested genes/haplotypes, and consider functional proteomics and genomics. Bearing this in mind, research efforts will contribute to better disease management and patients’ benefit.

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