ants can best be explained by either increased rates of formation (high activity ADH) or decreased rates of clearance of acetaldehyde (ALDH2 deficiency), which can cause aversive reactions to drinking.

Two new polymorphisms in *ADH* and *ALDH2* genes have recently been reported. An A/C substitution at −75 bp in the promoter of *ADH4* (encoding π ADH) was found to affect the expression of transfected reporter plasmids.[5] A mutation in the promoter of the *ALDH2* promoter was simultaneously reported by Harada *et al.*[6] and the author's laboratory.[7] This A/G variant occurs at about −360 bp from the start site and is adjacent to a site bound by transcription factors belonging to the steroid receptor family. The A allele is less active than the G allele in reporter gene transfection assays. Of great interest, Harada's group showed that the A allele was also less common in a group of alcoholics with active ALDH2. These variants were found in all ethnic groups examined. It will be very interesting to see if the observations on the association of the A allele with protection from alcoholism can be extended to Caucasians and Africans.

The National Institute on Alcohol Abuse and Alcoholism has initiated a genomewide search for other genes underlying the observations that alcoholism is both familial and heritable. In the first 10 years of funding of the Consortium on Genetics of Alcoholism, screening and diagnostic tools were created and validated, families with multi-generational alcoholism were identified, and the patients' phenotypes were established. DNA has been banked for a subset of families and the first pass of screening for microsatellite repeats has been completed for over 250 families. A number of potential loci associated with alcoholism have been identified which are currently being studied with more closely spaced markers.[8]

As far as genetic risk of alcoholic liver disease, the largest existing study is the U.S. VA Twin Panel Study. This study reported in 1981 that there was a substantially higher concordance for cirrhosis in monozygotic twins than in dizygotic twins, indicating the presence of a genetic component to the risk.[9] This database was re-analyzed in 1994. The analysis again supported the notion that concordance for cirrhosis was higher in the monozygotic twins, but found that most of the genetic liability for cirrhosis was the result of shared risk for alcoholism.[10] Thus, the idea that there is a genetic risk of alcoholic liver injury has yet to find support in large population studies. However, case control studies have indicated the possibility of increased genetic risk for alcoholic liver disease through inheritance of *ALDH2*2*, and polymorphisms of the tumor necrosis factor-α (TNF-α) and interleukin-10 (IL-10) promoter, and glutathione S-transferase.

Genesis of Fatty Liver

Fatty liver has long been recognized as the earliest response of the liver to chronic alcohol consumption. This was attributed to the effect of increased levels of cytosolic and mitochondrial NADH, which inhibits oxidation of fatty acids and stimulates their synthesis. Although this hypothesis has been dominant in the field, it was challenged as early as the mid 1960's by studies in which anti-oxidants prevented development of fatty liver in animals receiving large single doses of alcohol or chronic feeding of alcohol in the diet. The mechanisms by which alcohol imposes oxidative stress are discussed in the next section. We recently addressed this question by generating cell lines expressing rat class I ADH. These cells lack other forms of ADH and ALDH, as well as cytochrome P4502E1. The cell lines express high levels of ADH and metabolize alcohol present in the culture medium. Moreover, the rate of NADH production in these cells is sufficient to increase the lactate/pyruvate ratio in the medium from about 9 to 45. This degree of redox stress is at least as great as that occurring in the liver during alcohol metabolism. Coincident with this, the HeLa cells accumulate large amounts of triglyceride and free fatty acids. This was associated with inhibition of fatty acid oxidation and an increased rate of fatty acid synthesis.[11]

To determine the mechanism underlying this effect, various inhibitors were tested. As expected, the ADH inhibitor 4-methylpyrazole blocked the accumulation of fatty acids and triacylglycerols. Addition of tocopherol to the medium did not prevent fat accumulation, arguing that oxidative stress was not involved. On the other hand, methylene blue, which non-enzymatically accepts electrons from NADH, reduced the lactate/pyruvate ratio and attenuated the accumulation of fat in the cells. These results argue that a high $NADH/NAD^+$ ratio alone is sufficient to initiate the accumulation of fat and the development of fatty liver.

An interesting development in our understanding of lipid metabolism may be relevant to the development of alcoholic fatty liver. The peroxisome proliferator activated receptor (PPAR) is now recognized to be a fatty acid receptor.[12] This receptor forms heterodimers with retinoid X receptor, binds consensus response elements (PuGGTCAnPuGGTCA), and activates gene transcription. Many genes involved in fatty acid binding (fatty acid binding protein), fatty acid oxidation (peroxisomal fatty acyl CoA oxidase, mitochondrial medium chain fatty acyl CoA dehydrogenase, microsomal lauryl hydroxylase), and lipoprotein synthesis (apo CIII and AI) appear to be regulated by PPAR.[13] It would be expected that high levels of fatty acids in the livers of individuals who drink alcohol would activate these genes, but some of them (e.g., fatty acyl-CoA oxidase)