Polymorphisms in interleukin-1 gene cluster are associated with increased risk of alcoholic liver cirrhosis

Petrasek J, Reznakova S, Hubacek JA, Sperl J, Trunecka P, Spicak J, Jirsa M

Institute for Clinical and Experimental Medicine, Prague, the Czech Republic

Aim of the study

The objective of the study was to test whether the *IL-1B* polymorphism -31C/T, known to increase transcription of *IL-1B,* and penta-allelic 86-bp tandem repeat in intron 2 of IL-1 receptor antagonist *IL-1RN,* known to increase secretion of IL-1β, are associated with alcoholic liver cirrhosis.

Background

Apart from the direct effect of ethanol on hepatocyte metabolism, inflammatory response elicited by gut endotoxins (lipopolysaccharide, LPS), is involved in the pathogenesis of alcoholic liver cirrhosis (ALC). Circulating LPS activates hepatic Kupffer cells that produce the tumor necrosis factor α (TNF-α) and the interleukin 1 β (IL-1β). These proinflammatory cytokines induce hepatocyte death and inflammatory infiltrate of the portal areas (figure 1). The role of polymorphisms in TNF-α is controversial. The *IL-1* gene cluster on chromosome 2q contains the *IL-1B* and *IL1-RN* genes, which encode IL-1β and its receptor antagonist IL-1ra, respectively. The promoter polymorphism *IL-1B* -31C/T results in a fivefold increase in *IL-1B* transcription activity (ref. 1). The *IL-1RN* gene contains a penta-allelic 86-bp tandem repeat in intron 2, of which the less common IL-1RN*2 allele enhances the IL-1β concentration *in vitro*. Both polymorphisms were associated with the risk of gastritis and gastric cancer (ref. 1). Haplotype *IL-1RN**2*2/IL-1B* -31T+ further increased the disease risk, indicating additive effect of both polymorphisms on IL-1β secretion (ref. 1). To our best knowledge, the role of this haplotype in the pathogenesis of ALC has not been studied.

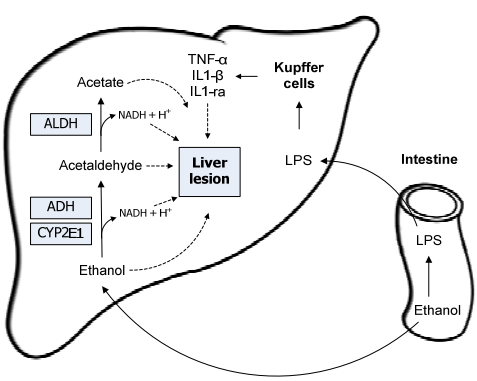

Figure 1. Metabolic and inflammatory pathway of alcoholic liver disease. ADH, alcohol dehydrogenase; ALDH, aldehyde dehydrogenase; CYP2E1, cytochrome P4502E1; LPS, lipopolysaccharide; TNF-α, tumor necrosis factor alpha; IL-1β, interleukin 1 beta; IL-1ra, interleukin 1 receptor antagonist.

References

1. El-Omar et al., Nature. 2000;404:398-402.
2. Multinational Monitoring of Trends and Determinants in Cardiovascular Diseases: "MONICA Project." Manual of operations, WHO/MNC 82.2.

Conclusions

The *IL-1RN**2*2 and *IL-1B* -31T+ haplotype increases the risk of ALC 8.3 times and represents 7.8% of the genetic predisposition to ALC. Our observation is in line with the previous studies indicating that synergistic action of the *IL-1B* -31T and *IL-1RN**2 alleles is responsible for a IL-1β high secretory phenotype. Such phenotype promotes liver necroinflammation and progression of fibrosis and explains the molecular background of the increased risk of progression of ALC in predisposed individuals.

Results

Allelic association was found for the carriage of neither *IL-1B* -31T nor *IL1-RN**2 allele. Similarly, no associaton was found for the homozygotes. However, simultaneous presence of homozygous state for the *IL-1RN**2 allele and either hetero- or homozygous state for *IL-1B* -31T allele (i.e. *IL-1RN**2*2 and *IL-1B* -31T+ haplotype) increased the risk of ALC 8.3 times (table 1).

Distribution of genotypes						
		IL-1RN				
Patients (N=100)		**1/1**	**1/2**	**2/2**	**1/3,4,5**	**2/3,4,5**
	C/C	1	12	1	0	0
***IL-1B* -31**	**C/T**	16	23	6	0	0
	T/T	25	12	3	1	0
Controls (N=180)		**1/1**	**1/2**	**2/2**	**1/3,4,5**	**2/3,4,5**
	C/C	3	11	9	0	0
***IL-1B* -31**	**C/T**	33	43	1	3	2
	T/T	46	26	1	2	0

Haplotype association with ALC			
Haplotype	***P***	**OR (CI 95%)**	**Population attributable risk**
*IL-1RN**2*2 and *IL-1B* -31T+	0.009	8.3 (1.7 – 40.7)	7.8% (CI 95%, 2.1 – 13.5)

Table 1. *IL-1B* and *IL-1RN* haplotype frequencies in patients with alcoholic cirrhosis (ALC) and controls. Alleles of *IL-1RN* were coded as follows: allele 1 = 4 repeats, allele 2 = 2 repeats, allele 3 = 5 repeats, allele 4 = 3 repeats, allele 5 = 6 repeats. The rare alleles 3, 4 and 5 were grouped in the statistical analysis.

Patients and methods

Subject characteristics

We included 100 Caucasian patients with alcoholic liver cirrhosis (ALC) who consulted the liver unit of our institute from March 2004 to June 2006. The diagnosis of ALC was based on laboratory signs of liver synthetic dysfunction and on signs of portal hypertension on physical examination, liver ultrasonography and gastroscopy (ascites, jaundice, encephalopathy, esophageal varices or portal gastropathy). Patients with a positive serology of hepatitis B or C, detectable antinuclear or antimitochondrial antibodies and with liver tumor were not included. The median age of patients with ALC was 52.6 (range, 26-72) years, 72 patients were male. The median alcohol consumption was 100 grams per day (interquartile range, 75-174).
As controls, 225 non-abstaining volunteers - participants of the MONICA project (ref. 2) - without history of liver disease were enrolled. Genotyping of both loci was successful in 180 of 225 control subjects. The median age of controls was 47.9 (range, 24-73) years, 142 were male. The median alcohol consumption was 21.5 grams per day (interquartile range, 14-40). Except for the liver disease, there were no clinically meaningful differences between the groups. The study was approved by the institutional review board. Written informed consent was obtained from all subjects.

Gene polymorphisms

Genotyping of the *IL-1B* -31C/T polymorphism was performed by the PCR-restriction fragment length polymorphism analysis. Genotyping of the *IL-1RN* polymorphism was performed by the PCR-fragment length polymorphism analysis. The PCR products were separated by polyacrylamide electrophoresis.

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