KEY POINTS

- Mutations in K8 and K18 genes may constitute risk factors for liver disease of multiple aetiologies.
- Other data support a hepatoprotective role for human K18 in transgenic mice, but K8 has not so far been tested similarly.
- The present mutation analysis showed none of these mutations in 256 patients with liver disorder, but did show a novel polymorphism in the K8 gene.
- Further work with animal models and European liver patients is needed.

MATERIALS AND METHODS

Patients

Genomic DNA from peripheral leucocytes or liver biopsies was prepared using standard procedures. Of the 256 patients recruited for this study, 21 were infected with hepatitis B virus (HBV); 126 with hepatitis C virus; 11 had non-alcoholic steatohepatitis; 32 had primary biliary cirrhosis; 18 had primary sclerosing cholangitis; 22 had autoimmune hepatitis; five were in fulminant hepatitic failure; 10 were suffering from cryptogenic cirrhosis; and 11 had liver disorder of unknown aetiology. These patients were chosen because alterations in the keratin cytoskeleton, such as reorganization and formation of aggregates, have been described in these disorders, and because certain viral proteases cleave keratins 8 and 18. As a reference group, 100 white blood donors from the Bonn University transfusion centre were selected randomly.

PCR amplification

PCR was performed using 1U Platinum Taq (Invitrogen, Karlsruhe, Germany), 200 μM dNTPs, 1.5–2.0 mM MgCl₂ (see table), 5% DMSO, and 0.5 μM of the corresponding primer (table 1), in a total volume of 25 μl. Conditions were

**ELECTRONIC LETTER**

A frequent keratin 8 p.L227L polymorphism, but no point mutations in keratin 8 and 18 genes, in patients with various liver disorders

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as follows: five minutes at 94°C (25 seconds at 94°C, 25 seconds at 61.3–65.6°C (see table), and one minute at 72°C) × 35 cycles. Of the reaction, 10 μl was checked for the expected product on an agarose gel. PCR products from genomic DNA of one blood donor were fully sequenced and used as controls. Equal amounts of control product were mixed with the patient samples, denatured for 10 minutes at 94°C, and allowed to cool slowly to room temperature for heteroduplex formation.

### RESULTS

Genomic DNA from peripheral blood samples was used for PCR amplification with primers specific for all exons of K8 and K18. After analysis of fragments on a DHPLC high throughput system, all detected mutations were identified by sequencing. In none of 256 patients suffering from a variety of liver diseases could mutations altering amino acids be detected. However, a g.740A>G sequence variation p.L227L was found very frequently in all patients (fig 1A, B). Of the 256 patients, 54% were heterozygous, 26% homozygous at this CTG, and 20% homozygous for CTA. This polymorphism was observed in the analysed controls as well: 46% were heterozygous, 24% homozygous at this CTG, and 30% homozygous for CTA. Another polymorphism in the K18 gene was detectable in one patient suffering from HBV at the end of intron 1 (fig 1C, D). This g.418-4C>G variation as well as from the control RNA (fig 2). This clearly demonstrates that the g.418-4C>G variation does not affect the splicing of the K18 cDNA.

### DISCUSSION

Data from transgenic mice have suggested a correlation between mutations in keratin 8 and 18 and liver diseases in humans. These mice carried a point mutation in keratin 18, in analogy to a hot spot mutation in epidermolysis bullosa simplex, and as a consequence suffered from chronic hepatitis. The mutations found in patients with cryptogenic liver disease are listed in table 1.

### Table 1

<table>
<thead>
<tr>
<th>Exon</th>
<th>Sequence</th>
<th>MgCl2</th>
<th>Anoeing temp</th>
<th>Product size</th>
<th>Temp gradient</th>
</tr>
</thead>
<tbody>
<tr>
<td>K8 Ex1 5'</td>
<td>5'-AAAGACAGTCCTCCGCTC-3'</td>
<td>2.0 mM</td>
<td>64.1°C</td>
<td>452</td>
<td>62.5–65.5°C</td>
</tr>
<tr>
<td>K8 Ex2 5'</td>
<td>5'-GAAGACATCCGATCCCTCC-3'</td>
<td>1.5 mM</td>
<td>64.1°C</td>
<td>322</td>
<td>60.0–62.0°C</td>
</tr>
<tr>
<td>K8 Ex3 5'</td>
<td>5'-GGCTCCCCTCTCCTCATC-3'</td>
<td>2.0 mM</td>
<td>64.1°C</td>
<td>148</td>
<td>59.5–60.5°C</td>
</tr>
<tr>
<td>K8 Ex4 5'</td>
<td>5'-TCTCTGCGACCAGGAAC-3'</td>
<td>2.0 mM</td>
<td>64.1°C</td>
<td>142</td>
<td>60.0–61.0°C</td>
</tr>
<tr>
<td>K8 Ex5 5'</td>
<td>5'-ACCACCTGACCCTTCC-3'</td>
<td>2.0 mM</td>
<td>65.6°C</td>
<td>458</td>
<td>60.0–62.0°C</td>
</tr>
<tr>
<td>K8 Ex6 5'</td>
<td>5'-AGCATGATCTGCTCAGCC-3'</td>
<td>1.5 mM</td>
<td>65.6°C</td>
<td>373</td>
<td>63.5–65.5°C</td>
</tr>
<tr>
<td>K8 Ex7 5'</td>
<td>5'-GAAGGCTTGTGCTTCC-3'</td>
<td>1.5 mM</td>
<td>65.6°C</td>
<td>188</td>
<td>61.0–66.0°C</td>
</tr>
<tr>
<td>K8 Ex8 5'</td>
<td>5'-AGGGGATTCGACGGG-3'</td>
<td>1.5 mM</td>
<td>65.6°C</td>
<td>246</td>
<td>64.0–66.0°C</td>
</tr>
<tr>
<td>K8 Ex9 5'</td>
<td>5'-CTGCCCTTGTCCCTCTCC-3'</td>
<td>1.5 mM</td>
<td>61.3°C</td>
<td>461</td>
<td>62.0–67.0°C</td>
</tr>
<tr>
<td>K8 Ex10 5'</td>
<td>5'-GCTGGCTACCTCTCTCC-3'</td>
<td>1.5 mM</td>
<td>62.0°C</td>
<td>241</td>
<td>60.0–61.0°C</td>
</tr>
<tr>
<td>K8 Ex11 5'</td>
<td>5'-GGTAGATCGAAAGG-3'</td>
<td>1.5 mM</td>
<td>62.0°C</td>
<td>248</td>
<td>60.0–63.0°C</td>
</tr>
<tr>
<td>K8 Ex11 5'</td>
<td>5'-TCTCTGCGACCCTC-3'</td>
<td>1.5 mM</td>
<td>62.0°C</td>
<td>248</td>
<td>60.0–63.0°C</td>
</tr>
<tr>
<td>K8 Ex12 5'</td>
<td>5'-GCAGTGGATCGGGC-3'</td>
<td>1.5 mM</td>
<td>62.0°C</td>
<td>276</td>
<td>60.5–63.5°C</td>
</tr>
<tr>
<td>K8 Ex13 5'</td>
<td>5'-TTCAGACGAGTGC-3'</td>
<td>1.5 mM</td>
<td>62.0°C</td>
<td>245</td>
<td>61.5–63.5°C</td>
</tr>
</tbody>
</table>

**Temp. temperature.**
cirrhosis were predominantly and most frequently p.Y54H and p.G62C in the head domain of K8. Cells transiently transfected with keratin 8 carrying those mutations showed a normal keratin cytoskeleton under standard culture conditions, but displayed alterations in the filaments after applying pharmacological stress, including okadaic acid, hydrogen peroxide, or heat. However, there is no evidence yet from mouse models that the p.Y54H and p.G62C-mutations in K18 predispose to liver disease or cause an increased susceptibility to stress factors. Mutations used so far for liver studies in transgenic mice were p.R90C, p.S34A, p.S53A in K18, K8 null, and K18null. Such mutations have not been detected in humans so far. It would be useful to develop mouse models carrying p.Y54H- and p.G62C-mutations in K8 in order to investigate conditions under which these mutations affect disease progression. The contrary result of our study to the mutations reported so far suggests that allele frequencies might possibly differ between European and North American populations. It might be also possible that additional risk factors coincide with K8 and K18 mutations in Northern American but not European liver patients. The question arises as to whether patients suffering from liver diseases are the most likely disease group to carry mutations in K8 and K18. Evidence from animal models strongly suggests an essential role for K8, and during embryonic development. Mice deficient for K8 in the C57BL/6 strain and with a compound deficiency for K18/19 display embryonic lethality during mid gestation. It seems likely that mutations in analogy to hot spot mutations in human skin fragility syndromes at the ends of the rod domain are able to cause early embryonic lethality in humans.

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REFERENCES


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