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

M Hesse, T Berg, B Wiedenmann, U Spengler, R P Woitas, T M Magin


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.

Keratins (K) 8 and 18 are the first intermediate filament proteins present in the early embryo, and form the backbone of the intermediate filament cytoskeleton in all simple internal epithelia. Mice with a combined deficiency in K8/K19 and K18/K19 suffer from midgestational lethality with full penetrance, which strongly supports an essential keratin function in extraembryonic epithelia. Moreover, mutations in K8 and K18 have been reported to present risk factors in liver disease of multiple aetiologies. This mutation analysis of the complete K8 and K18 genes, using DHPLC technology, involved screening blood samples from 256 patients diagnosed positive for various liver disorders, and from 100 individuals serving as controls. None of the previously reported mutations in K8 and K18 was found in any of the samples, nor was a positive correlation observed between K8 and K18 mutations to cryptogenic cirrhosis or to chronic liver disease of other origin. However, a novel polymorphism was detected in exon 4 of the K8 gene, leaving L227 unaltered, in both patient and control samples.

Type I and II keratins belong to the large gene family coding for intermediate filament proteins. Among the 27 type I and 27 type II keratins known to exist in the human genome, type I K18 and type II K8 form copolymers and are coexpressed in all embryonic and internal epithelia. DNA linkage analysis and transgenic animal experiments have established that dominant acting point mutations in epidermal keratin genes lead to a large number of skin fragility syndromes, including epidermolysis bullosa simplex. The majority of these mutations are highly conserved with regard to their position. The molecular mechanism leading to cell fragility is not well understood, but it is clear that an intact cytoskeleton is required for epithelial homeostasis. A more detailed biochemical analysis has demonstrated a strong genotype-phenotype correlation, in the sense that mutations located in conserved protein coding motifs produce more severe pathology than mutations elsewhere.

With respect to internal and embryonic epithelia, studies of keratin gene knockout mice so far have underlined an essential function of these proteins in extraembryonic tissues, but have failed to support a vital role in adult internal epithelia. Expression of a mutated human K18 gene in transgenic mice, on the other hand, has revealed fragile hepatocytes and supports a hepatoprotective role for this keratin. There is evidence that such a function includes modulation of apoptotic pathways by interaction of keratins with effectors of TNF and Fas pathways. Mutant K8 has so far not been tested in transgenic mice. Collectively, these and additional data support the hypothesis that K8 and K18 have a non-structural function in digestive epithelia and that corresponding mutations might predispose individuals to liver or gastrointestinal disease. In fact, several reports have now identified mutations in either K8 or K18 genes as risk factors for liver disease of multiple aetiologies. Of these mutations, six of each were found in K8 and K18 genes, respectively, in non-conserved locations with regard to those observed in epidermal keratin genes. All of these represent heterozygous single base exchanges. The total frequency of K8 and K18 mutations amounted to 0.036, compared with 0.0057 in control groups.

In order to assess the role of K8 and K18 mutations as potential risk factors for patients with various chronic liver disorders in the European Caucasian population, samples from more than 250 subjects suffering from a range of liver disorders, and from a control group of 100 unaffected individuals, were analysed and compared.

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

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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) x 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.

**DHPLC analysis and sequencing**

Mixed amplicons were analysed by DHPLC using the Transgenomic WAVE system (Transgenomic, Omaha, Neb., USA). For validation, positive control samples with the p.Y54H and p.G62C variations in K8 and the p.R90C variation in K18 were used (gift from B Omary). These variations could be detected, using exactly the same conditions as for the screening protocol: 5 µl of the mixed amplicons were loaded on a DNasep column and eluted on a linear acetonitril gradient in a 0.1 M triethylamine acetate buffer (pH 7) with a constant flow rate of 1.5 ml/minute. All samples were injected at different temperatures, to minimise the risk of possibly affecting the nearby splicing site and lead to an alternative spliced product. To test this, RT-PCR with primers specific for the 5’- and 3’-end of the human K18 cDNA was performed on total liver RNA. Only one product with the expected size of 1.3 kb was obtained from the RNA of the patient carrying the 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.

**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 could possibly affect the nearby splicing site and lead to an alternative spliced product. The mutations found in patients with cryptogenic

**DISCUSSION**

Data from transgenic mice have suggested a correlation between mutations in keratin 8 and 18 and liver diseases in humans.15 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

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**Table 1** Primer sequences, PCR conditions, temperatures, and temperature gradients used for DHPLC mutation screening of genes K8 and K18

<table>
<thead>
<tr>
<th>Exon</th>
<th>Sequence</th>
<th>MgCl₂</th>
<th>Anealing temp</th>
<th>Product size</th>
<th>Temp gradient</th>
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<tr>
<td>K8 Ex1 5’</td>
<td>5’-AGAAGCAGCTCCTGCCTC-3’</td>
<td>2.0 mM</td>
<td>64.1°C</td>
<td>452</td>
<td>62.5–65.5°C</td>
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<tr>
<td>K8 Ex1 3’</td>
<td>5’-GGACTACAGGAGGAAAAGGG-3’</td>
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<td>64.1°C</td>
<td>322</td>
<td>60.0–62.0°C</td>
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<td>K8 Ex2 5’</td>
<td>5’-AAAGAGCTCCTGTTCCTCCTC-3’</td>
<td>1.5 mM</td>
<td>64.1°C</td>
<td>148</td>
<td>59.5–60.5°C</td>
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<tr>
<td>K8 Ex2 3’</td>
<td>5’-AGGAGCTCAGTGGTGTTGCC-3’</td>
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<td>64.1°C</td>
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<td>K8 Ex4 5’</td>
<td>5’-GGCCCTCTTCTCCATGAC-3’</td>
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<td>65.6°C</td>
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<tr>
<td>K8 Ex4 3’</td>
<td>5’-TCCTCTCAGACCATGAA-3’</td>
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<td>60.0–62.0°C</td>
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<td>K8 Ex5 5’</td>
<td>5’-ACCCATGTCCCTTCCGCC-3’</td>
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<td>65.6°C</td>
<td>373</td>
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<td>65.6°C</td>
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<td>5’-GACAGGACCACTACACCACAC-3’</td>
<td>1.5 mM</td>
<td>65.6°C</td>
<td>246</td>
<td>64.0–66.0°C</td>
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<tr>
<td>K8 Ex6 3’</td>
<td>5’-CCGACCCGGCCGAGCAACC-3’</td>
<td>1.5 mM</td>
<td>65.6°C</td>
<td>461</td>
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<tr>
<td>K8 Ex7 5’</td>
<td>5’-TGAGGGCAGCATGCCGCAC-3’</td>
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<td>65.6°C</td>
<td>241</td>
<td>60.0–61.0°C</td>
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<td>K8 Ex9 5’</td>
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<td>5’-GGCCCTCTTCTCCATGAC-3’</td>
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<td>65.6°C</td>
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<td>K8 Ex11 5’</td>
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<td>65.6°C</td>
<td>241</td>
<td>60.0–61.0°C</td>
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<tr>
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<td>5’-GGCCCTCTTCTCCATGAC-3’</td>
<td>1.5 mM</td>
<td>65.6°C</td>
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<td>K8 Ex12 5’</td>
<td>5’-AGAGGCAGCTCCTGCCTC-3’</td>
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<td>K8 Ex12 3’</td>
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<td>1.5 mM</td>
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<td>5’-TGAGGGCAGCATGCCGCAC-3’</td>
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<td>1.5 mM</td>
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<td>K8 Ex15 5’</td>
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<td>65.6°C</td>
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<td>1.5 mM</td>
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<td>60.0–61.0°C</td>
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<td>K8 Ex16 5’</td>
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<td>1.5 mM</td>
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<td>1.5 mM</td>
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<td>65.6°C</td>
<td>241</td>
<td>60.0–61.0°C</td>
</tr>
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</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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