Keratin 8 Y54H and G62C mutations are not associated with liver disease


METHODS

Study subjects

We recruited 1668 patients with various liver disorders in our hospital between 1998 and 2003. According to the study protocol, which was approved by the Charité Ethics Committee, blood samples were obtained at the time of routine clinic attendance from patients who had either undergone liver transplantation or who suffer from chronic liver disease. The respective aetiologies of liver disease in our population are shown in table 1. The majority of our patients (1617 out of 1668) were Caucasians with 1400 patients suffering from distinct liver diseases and no association with cryptogenic cirrhosis or with any of the other investigated liver diseases was found. Genotype/phenotype analysis revealed no particular findings in patients carrying one mutant allele.

In contrast to previous reports, KRT8 mutations neither predispose to cryptogenic cirrhosis nor to chronic liver disease of other aetiologies.

Key points

- Keratin 8 is one of the major intermediate filament proteins expressed in single-layered epithelia of the gastrointestinal tract. Animal models provided evidence that keratin 8 (KRT8) has an important protective role against mechanical and toxic stress in hepatocytes. Recently, KRT8 mutations were identified and linked to cryptogenic and non cryptogenic liver disease.

- In order to investigate the relevance of two hitherto described KRT8 mutations, Y54H and G62C, we genotyped 1668 patients with liver disorders of different aetiologies (viral, autoimmune, cryptogenic, alcoholic, and others) as well as 679 healthy controls for Y54H and G62C. Samples were analysed by PCR amplification and subsequent melting curve analysis with fluorescence resonance energy transfer (FRET) probes.

- The genotype distributions of both mutations were similar in patients and controls. The G62C variation was detected in 27 out of 1668 patients (1.6%) compared to 12 out of 679 controls (1.8%). The Y54H alteration was found in two patients (0.1%) and one control (0.1%). Patients carrying a mutation suffered from distinct liver diseases and no association with cryptogenic cirrhosis or with any of the other investigated liver diseases was found. Genotype/phenotype analysis revealed no particular findings in patients carrying one mutant allele.

- In contrast to previous reports, KRT8 mutations neither predispose to cryptogenic cirrhosis nor to chronic liver disease of other aetiologies.

Abbreviations: BASE, Berlin Aging Study; FRET, fluorescence resonance energy transfer
(n = 168) and parents of healthy newborns (n = 347) as well as healthy volunteers of the Berlin Aging Study (BASE) (n = 164). Information about the ethnic background of the control subjects was available in 547/679 subjects (81%). All parents of healthy newborns and all individuals of the Berlin Aging Study were of German ancestry. Among the medical students and medical staff ethnicity was known in 36 individuals (34 German, one Asian, and one African-German).

**Genetic analysis**

DNA was extracted from peripheral blood leukocytes using spin columns (Qiagen, Hilden, Germany). We amplified exon 1 of *KRT8* using 5'-CGGTCCTTCTAGGATCTCG-3' as forward primer and 5'-GGCACAGTCAGCCAGCGAG-3' as reverse primer. We designed primers and FRET probes according to the published *KRT8* sequence (GenBank #M34482). We performed PCR using 0.75 U AmpliTaq Gold (Applied Biosystems, Weiterstadt, Germany), 400 μM dNTPs, 1.5 mM MgCl₂, and 0.1 μM of each primer in a final volume of 25 μL. The reaction mix was denatured at 95°C for 12 min followed by 48 cycles of denaturation at 95°C for 20 s, annealing at 64°C for 40 s, elongation at 72°C for 90 s, and a final extension step for 2 min at 72°C in an automated thermocycler (Biometra, Göttingen, Germany).

The detection of the mutant alleles was carried out by melting curve analysis with fluorescence resonance energy transfer (FRET) probes in a LightCycler (Roche Diagnostics, Mannheim, Germany). The probes were designed complementary to the mutant allele of both codons. For detection of Y54H variation the sequence of the sensor fluoresceine labelled probe was 5'-CCCCACCATGGCCGCC-FL and that of the anchor probe 5'-TCAGCAGGCTCTGGT GACGCTAACTGC-FL were used. All FRET probes were designed and synthesised by TIB MOLBIOL, Berlin, Germany.

We numbered the mutations according to the recommendations of the Nomenclature Working Group for human gene mutations. Thus, we apply the correct numbering Y54H and G62C for the *KRT8* alterations as previously described by Lee et al.¹⁴

**RESULTS**

The frequency of *KRT8* Y54H and G62C variations in a large cohort of 1668 patients suffering from various liver diseases is shown in table 1. The G62C mutation was detected in 27 patients (1.6%) and 12 healthy controls (1.8%), p = 0.8. The frequency of G62C among the three control populations did not differ significantly. Two patients (0.1%) and one control (0.1%) were heterozygous carriers of the Y54H variation (p = 0.9). We did not detect any homozygous or compound heterozygous carriers of these two mutations. Overall, there were no significant differences in genotype distribution of both mutations between the various groups of liver diseases investigated. Genotype/phenotype analysis of patients carrying *KRT8* mutations revealed no specific characteristics. In particular, the mean age of patients with wild type and mutant *KRT8* was similar (51 years; range 7–87 v 51 years, range 20–67). Moreover, we observed no differences concerning disease severity or request for liver transplantation between these two groups of patients. In 794 patients liver transplantation was required for advanced liver disease with 14 (1.8%) of these patients being carriers of a mutant *KRT8* allele compared to 15 (1.7%) out of 874 patients with no need for a liver graft (p = 0.9).

Among those patients carrying a G62C mutation were 26 patients of German ancestry and one Turkish patient. Both patients with an Y54H variation originated from Africa. Eleven control subjects with G62C were of German origin. Information about ethnicity was unavailable in one control with G62C and in the control carrying Y54H.

**DISCUSSION**

Animal models clearly showed that *KRT8*18 transgenic and knock out mice were predisposed to liver damage. However, these animal studies suggested a complex inheritance pattern for *KRT8*18 mutations. The importance of keratins in the pathogenesis of liver damage was further supported by their modulation of Fas-mediated apoptosis.¹⁵ ¹⁶

The relevance of the *KRT8* mutations Y54H and G62C was studied in a large population of 1668 patients with liver disease and 679 healthy controls. In contrast to the results of Ku et al.,¹¹ ¹² the hitherto described human *KRT8* mutations were associated neither with cryptogenic nor with non cryptogenic chronic liver disease in our population. An allele frequency of 0.8% and 0.9% was observed for the G62C alteration in patients and healthy controls, respectively. For the Y54H mutation the respective allele frequencies were 0.06% and 0.08%. The discrepancies between our study and the previous reports are readily explained by the differences in the number of patients and controls investigated in both groups. To investigate whether keratin mutations have any impact on liver disease severity, we studied patients with advanced liver disease requiring liver transplantation as well as patients who suffered from chronic liver disease including all stages of fibrosis. No differences in *KRT8* mutations were found in relation to fibrosis stage indicating that patients carrying *KRT8* mutations are not more prone to develop severe liver disease and cirrhosis. Among our patients were 1617 (97%) Caucasians. Therefore, another possible explanation for the difference between our results and those

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**Table 1**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Number of individuals studied</th>
<th>G62C</th>
<th>Y54H</th>
</tr>
</thead>
<tbody>
<tr>
<td>All patients</td>
<td>1668</td>
<td>27 (1.6%)</td>
<td>2 (0.1%)</td>
</tr>
<tr>
<td>Chronic HCV infection</td>
<td>672</td>
<td>13 (1.9%)</td>
<td>1 (0.1%)</td>
</tr>
<tr>
<td>Alcoholic liver disease</td>
<td>215</td>
<td>4 (1.9%)</td>
<td>–</td>
</tr>
<tr>
<td>Chronic HBV infection</td>
<td>200</td>
<td>1 (0.5%)</td>
<td>1 (0.5%)</td>
</tr>
<tr>
<td>Primary biliary cirrhosis</td>
<td>162</td>
<td>1 (0.6%)</td>
<td>–</td>
</tr>
<tr>
<td>Autoimmune hepatitis</td>
<td>104</td>
<td>4 (3.8%)</td>
<td>–</td>
</tr>
<tr>
<td>Cryptogenic cirrhosis</td>
<td>61</td>
<td>1 (1.6%)</td>
<td>–</td>
</tr>
<tr>
<td>Primary sclerosing cholangitis</td>
<td>54</td>
<td>2 (3.7%)</td>
<td>–</td>
</tr>
<tr>
<td>Non alcoholic fatty liver</td>
<td>52</td>
<td>1 (1.9%)</td>
<td>–</td>
</tr>
<tr>
<td>disease</td>
<td>118</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Controls</td>
<td>679</td>
<td>12 (1.8%)</td>
<td>1 (0.1%)</td>
</tr>
</tbody>
</table>

*The group of patients with miscellaneous liver disorders included 21 patients with hepatocellular carcinoma, six patients with cholangiocellular carcinoma, two patients with liver metastases of neuroendocrine tumours, 32 patients with acute liver failure, 14 patients with Budd–Chiari’s syndrome, one patient with pregnancy induced cholestasis, 54 patients with metabolic/genetic liver disorders, nine patients with drug-induced liver disease, six patients with biliary cirrhosis, two patients with extrahepatic biliary atresia, and one patient with sarcoidosis. HBV, hepatitis B virus; HCV, hepatitis C virus.
of Ku et al might be the greater ethnic homogeneity of our patient and control population. It is important to note that both patients with Y54H were of African origin. In the population studied by Ku et al three out of five patients and the control subject carrying the Y54H alteration were also African-Americans. These observations suggest that the Y54H variation occurs more frequently among individuals of African origin.

Evidence is increasing that cryptogenic cirrhosis is a heterogeneous disorder. Patients presenting no evidence that Y54H occurs mainly among people of African origin. These rare sequence variations may therefore rather diseases. Furthermore, there is conclusive as suffering from cryptogenic cirrhosis though this diagnosis stills depends on how carefully other causes are excluded. We thoroughly re-evaluated all patients with an initial diagnosis of cryptogenic cirrhosis as described previously. Differentiation of cryptogenic liver disease from an underlying autoimmune process is particularly challenging. Ku et al reported that two of their five patients with KRT8 mutations and cryptogenic cirrhosis had some autoimmune features which were not particularly defined. Interestingly, we found the highest frequency of KRT8 mutations in patients suffering from autoimmune hepatitis and primary sclerosing cholangitis. Therefore, the association of KRT8 mutations and cryptogenic cirrhosis documented in previous reports might be in part due to an underlying autoimmune liver disease in these patients. Keeping these facts in mind the exact role of KRT8 in hepatic autoimmune processes remains to be elucidated.

In conclusion, our study clearly demonstrates that carriers of the two KRT8 mutations, Y54H and G62C, are not at increased risk for developing cryptogenic or non cryptogenic chronic liver diseases. Furthermore, there is conclusive evidence that Y54H occurs mainly among people of African origin. These rare sequence variations may therefore rather represent non pathogenic genetic alterations than disease-causing mutations.

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