Hepatic Manifestation Is Associated with ALK1 in Hereditary Hemorrhagic Telangiectasia: Identification of Five Novel ALK1 and One Novel ENG Mutations

Heidi K. A. Kuehl†1, Martin Caselitz‡2,3, Sandra Hasenkamp1, Siegfried Wagner2,3, El-Harith A. El-Harith†1, Michael P. Manns2, and Manfred Stuhrmann†1

1Institut fuer Humangenetik, 2Abteilung fuer Gastroenterologie, Hepatologie und Endokrinologie, Medizinische Hochschule Hannover, D-30625 Hannover, Germany; 3Medizinische Klinik, Klinikum Deggendorf, D-94469 Deggendorf, Germany

*Correspondence to Prof. Dr. Manfred Stuhrmann, Institut fuer Humangenetik, Medizinische Hochschule Hannover, Carl-Neuberg-Str. 1, D-30625 Hannover, Germany; Fax: (49) 511 532 5865; E-mail: stuhrmann.manfred@mh-hannover.de

†These authors contributed equally to this study.

Grant sponsor: Alexander von Humboldt Foundation (Germany); Fellowship grant to E. A. El-Harith

Communicated by Claude Ferec

Hereditary hemorrhagic telangiectasia (HHT), or Osler-Rendu-Weber syndrome, is a heterogeneous inherited disorder characterized by multi-systemic vascular dysplasia and wide variation in its phenotypic expression. Hepatic manifestation is seen in about 8 to 30% of the patients. The molecular basis for liver involvement is unknown. We screened the two known HHT disease loci, the ALK1 (ACVRL1) and ENG genes, for mutations in a clinically well-characterized group of HHT patients with or without liver involvement. Mutations in the ALK1 gene were detected in eight out of 10 HHT patients with hepatic manifestation. Among nine HHT patients without liver involvement, four had mutations in the ALK1, and three in the ENG genes, respectively. In one patient with hepatic manifestation a mutation was detected in both the ALK1 and ENG genes. No mutation could be detected in two patients with liver involvement and, likewise, in two patients without hepatic manifestation. In this study, we have identified five novel ALK1 and one ENG disease-causing mutations. We conclude that hepatic manifestation in HHT patients is associated with mutations in the ALK1 gene, but rarely with ENG mutations. © 2005 Wiley-Liss, Inc.

KEY WORDS: HHT; Osler-Rendu-Weber syndrome; ALK1; ACVRL1; ENG

INTRODUCTION

Osler-Rendu-Weber syndrome or hereditary hemorrhagic telangiectasia (HHT, MIM# 187300 and 600376) is an autosomal dominant disease with age-dependent penetrance and variable expression of the clinical manifestation. The estimated prevalence is in the order of 1 out of 10,000 (Guttmacher et al., 1995). According to the Curacao

Received 16 July 2004; accepted revised manuscript 30 November 2004.
criteria (Shovlin et al., 2000), the clinical diagnosis of HHT requires that at least three out of four conditions, i.e. epistaxis, telangiectasia, visceral lesions, and a family history with HHT, should be present in order to ensure the diagnosis. While the cutaneous and mucocutaneous manifestations have mostly a relative good prognosis, the involvement of the visceral organs, if untreated, can trigger mortality (Kjeldsen et al., 1999; Shovlin and Letarte, 1999).

Hepatic manifestation of HHT is estimated to affect about 8 to 30% of the patients (Reilly and Nostrant, 1984; Bauer et al., 1995; Kjeldsen et al., 1999). The hepatic vascular malformation can be diagnosed by ultrasound (Caselitz et al., 2003) and is mostly associated with fibrosis and/or atypical cirrhosis (Reilly and Nostrant, 1984). In severe cases, the reduced liver function associated with HHT may lead to progressive hepatic failure (Weik and Greiner 1999).

Little is known about the genetic basis of the observed clinical heterogeneity of HHT. Even within the same family there could be great variations with respect to manifestation and severity of the disease. (Shovlin, 1997). Disease-causing mutations had been identified in both the endoglin (ENG, MIM# 131195) gene (McAllister et al., 1994) on chromosome 9 (HHT type 1) and in the activin receptor-like kinase (ALK1, also designated ACVRL1, MIM# 601284) gene (Johnson et al., 1996) on chromosome 12 (HHT type 2). The presence of two disease loci provided the rationale for genotype/phenotype studies of the disease. Recently, a large questionnaire based study led Berg and colleagues to conclude that the HHT1 phenotype is distinct from, and more severe than the HHT2 phenotype (Berg et al., 2003). In that study, an earlier onset of epistaxis and telangiectasia was present in patients with HHT1. In addition, pulmonary arteriovenous malformations (PAVM) were only seen in HHT1 (Berg et al., 2003), which confirmed earlier observations (Berg et al., 1996). However, PAVMs can also be encountered in some patients with HHT2 (McDonald et al., 2000; Kjeldsen et al., 2001). Furthermore, ALK1 mutations were also identified in families with pulmonary hypertension and HHT (Trembath et al., 2001; Harrison et al., 2002).

With respect to liver involvement in HHT, Olivieri and colleagues hypothesized from their observations on the presence of intrahepatic AV shunts in six of 10 patients with HHT2, that mutations in the ALK1 gene may be associated with higher risk of liver AV malformations (Olivieri et al., 2002). A significantly higher liver involvement had also been reported previously for two large HHT2 families (McDonald et al., 2000; Lin et al., 2001). The main objective of the present study is to screen systematically for mutations in the ENG and ALK1 genes of patients with HHT and liver involvement as compared to patients with HHT and no liver disease. This approach should enable a confirmation of the above mentioned hypothesis. In addition, we reviewed the latest literature in search for the genotype-phenotype relationships in HHT with respect to liver disease.

**PATIENTS AND METHODS**

**Patients**

Nineteen non-related patients [9 male, 10 female, mean age 56 (35-72) years] with HHT were investigated at the Hannover Medical School (MHH). Liver involvement of HHT was confirmed in 10 patients [7 female, 3 male, mean age 56 (35-72) years]. All patients fulfilled at least three of the four diagnostic criteria for HHT (Shovlin et al., 2000). Hepatic involvement of HHT was assessed by one of us (M.C.) by ultrasound as described previously (Caselitz et al., 2003).

The study was conducted with approval of the local ethical committee (IRB protocol No. 2785). Individuals were clinically assessed before the genotype information was available. Peripheral blood was obtained with informed consent.

**Healthy controls**

Control DNA was extracted from 50 healthy German individuals, who gave written consent that their DNA may be investigated anonymously in research projects. These samples were used to confirm absence of the novel identified mutations in the general population, in order to prove that these mutations are disease-causing.

**Methods**

DNA was extracted using a standard salting-out method (Miller et al., 1988). Polymerase chain reaction (PCR) for the nine coding exons and the flanking intronic sequences of the ALK1 gene was performed as described elsewhere (Berg et al., 1997). PCR primers for the entire coding sequence and flanking intronic sequences of the
ENG gene were derived from McAllister et al. (1994) (ENG exons 2, 4 -7 and 10 – 14) and Gallione et al. (1998) (ENG exons 1, 3, 8, 9A, 9B). PCR products were sequenced using the BigDye Terminator Cycle Sequencing Kit (Applied Biosystems, Darmstadt, Germany) according to the manufacturer’s instruction. Sequencing products were analyzed by capillary electrophoresis using the ABI PRISM 310 Genetic Analyser (Applied Biosystems, Darmstadt, Germany). Mutations were named according to international recommendations (http://www.hgvs.org/mutnomen/). The term “mutation” refers to a sequence variant with predicted phenotype-modifying effect, i.e. presumably causative of HHT. Analysis was performed using the following reference sequences: ENG genomic sequence: 3513290 (AH006911.1), ALK1 genomic sequence: 2228561 (AH005451.1), ENG cDNA: 33871100 (BC014271.2), ALK1 cDNA: 4557242 (NM_000020.1); cDNA numbering begins with +1 as the A of the ATG initiation codon.

Literature and database search

A Medline literature search (http://www.ncbi.nlm.nih.gov/PubMed/) was performed on 14th July, 2004 using the following key words: ENG, endoglin, ALK1, ACVRL1, each in combination with mutation, Osler, HHT, hereditary haemorrhagic telangiectasia. All articles found were screened for genotypic and phenotypic information. If necessary, phenotypic information was derived from references mentioned in these articles. In addition, ENG and ALK1 mutations were searched for in the Human Gene Mutation Database (HGMD; http://www.uwcm.ac.uk/uwcm/mg/hgmd).

Statistical analysis

Differences in the ENG and ALK1 mutation frequencies among HHT patients with and without liver involvement were assessed by Fisher’s exact test.

RESULTS

Eight of 10 patients with liver involvement carried a mutation in the ALK1 gene. One of these patients additionally carried a mutation in the ENG gene (table 1). We were not able to identify a mutation in the remaining two HHT patients with liver disease. Of the 9 patients without liver involvement, 4 carried a mutation in the ALK1 gene, 3 in the ENG gene, and no mutation was identified in two of these. There is a trend towards a higher rate of ALK1 and a lack of ENG mutations in HHT patients with liver involvement, although the differences in the distribution of ALK1 and ENG mutations among HHT patients with and without liver involvement do not reach statistical significance (p = 0.076, Fisher’s exact test).

Five of the ALK1 mutations and one of the ENG mutations detected in this study were novel. None of the missense mutations were present in any of the 50 healthy controls. Two novel ALK1 mutations were located in the extracellular domain: first, a nonsense mutation c.149G>A, changing codon 50 from tryptophan to stop (p.Trp50X), and second, a deletion of nucleotide 246 (C), leading to a frameshift from codon 82 (Thr) onwards to a premature stop 39 codons further downstream (p.Thr82fsX121). The three remaining novel ALK1 mutations that occurred in the intracellular kinase domain were as follows: a missense mutation that affects nucleotide 1273, and thus causes a change from phenylalanine to valine (p.Phe425Val), a TCT deletion from nucleotides 1274 to 1276 that leads to the deletion of codon 425 (p.Phe425del), and a potential splicing mutation that affects the first nucleotide of intron 9 (c.1377+1G>A). The latter mutation decreases the Shapiro-Senepathy consensus value (CV) (Shapiro and Senapathy, 1987) from 0.84 (G-allele) to 0.66 (A-allele). Six of the ALK1 mutations detected in this study had been previously described (Berg et al., 1997; Abdalla et al. 2000; Trembath et al., 2001; Olivieri et al., 2002; Lesca et al., 2004).

In the extracellular domain of the ENG gene, one novel ENG mutation occurred at nucleotide 1844 (C>T) changing codon 615 in the transmembrane domain from serine to leucine. This latter missense mutation occurred in patient P3 who additionally carried mutation p.Glu379Lys in the ALK1 gene. The other three ENG mutations detected in our patients had been reported by other workers (Gallione et al., 1998; Pece-Barbara et al., 1999; Lesca et al., 2004).

According to our literature and database survey, up to the present time (14th July, 2004) 116 different mutations have been identified in the ENG gene. Fifty seven mutations were listed in the recent review by van den Driesche and colleagues (van der Driesche et al., 2003) while 59 further novel mutations have been published subsequently as follows: 31 mutations (Lesca et al., 2004), 17 (Cymerman et al., 2003), 4 (Lastella et al., 2003), 4 (Berg et al,
Together with the one novel ENG mutation detected in this study, the total number of ENG mutations is now 117.

Table 1. Alk-1 and ENG Mutations in Morbus Osler Patients With or Without Hepatic Manifestation

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Sex</th>
<th>Liver disease</th>
<th>Gene</th>
<th>Exon</th>
<th>Nucleotide-change</th>
<th>Mutation effect</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>P35</td>
<td>35</td>
<td>f</td>
<td>yes</td>
<td>ALK1</td>
<td>3</td>
<td>c.149G&gt;A</td>
<td>p.Trp50X</td>
<td></td>
</tr>
<tr>
<td>P4</td>
<td>59</td>
<td>f</td>
<td>yes</td>
<td>ALK1</td>
<td>3</td>
<td>c.199C&gt;T</td>
<td>p.Arg67Trp</td>
<td>Olivieri et al. 2002</td>
</tr>
<tr>
<td>P13</td>
<td>38</td>
<td>m</td>
<td>no</td>
<td>ALK1</td>
<td>3</td>
<td>c.199C&gt;T</td>
<td>p.Arg67Trp</td>
<td>Olivieri et al. 2002</td>
</tr>
<tr>
<td>P20</td>
<td>53</td>
<td>f</td>
<td>yes</td>
<td>ALK1</td>
<td>3</td>
<td>c.246delC</td>
<td>novel</td>
<td></td>
</tr>
<tr>
<td>P27</td>
<td>70</td>
<td>f</td>
<td>no</td>
<td>ALK1</td>
<td>8</td>
<td>c.1120C&gt;T</td>
<td>p.Arg347Trp</td>
<td>Berg et al., 1997</td>
</tr>
<tr>
<td>P3*</td>
<td>53</td>
<td>f</td>
<td>yes</td>
<td>ALK1</td>
<td>8</td>
<td>c.1135G&gt;A</td>
<td>p.Glu379Lys</td>
<td>Lesca et al., 2004</td>
</tr>
<tr>
<td>P6+</td>
<td>69</td>
<td>f</td>
<td>yes</td>
<td>ALK1</td>
<td>8</td>
<td>c.1221G&gt;T</td>
<td>p.Glu407Asp</td>
<td>Abdalla et al., 2000</td>
</tr>
<tr>
<td>P9</td>
<td>49</td>
<td>f</td>
<td>yes</td>
<td>ALK1</td>
<td>8</td>
<td>c.1221G&gt;T</td>
<td>p.Glu407Asp</td>
<td>Abdalla et al., 2000</td>
</tr>
<tr>
<td>P32</td>
<td>72</td>
<td>m</td>
<td>yes</td>
<td>ALK1</td>
<td>8</td>
<td>c.1231C&gt;T</td>
<td>p.Arg411Trp</td>
<td>Trembath et al., 2001</td>
</tr>
<tr>
<td>P26</td>
<td>63</td>
<td>f</td>
<td>no</td>
<td>ALK1</td>
<td>9</td>
<td>c.1273T&gt;G</td>
<td>p.Phe425Val</td>
<td>novel</td>
</tr>
<tr>
<td>P5</td>
<td>45</td>
<td>m</td>
<td>no</td>
<td>ALK1</td>
<td>9</td>
<td>c.1274_1276delTCT</td>
<td>p.Phe425del</td>
<td>novel</td>
</tr>
<tr>
<td>P22</td>
<td>44</td>
<td>m</td>
<td>yes</td>
<td>ALK1</td>
<td>9</td>
<td>c.1377+1G&gt;A</td>
<td>splicing</td>
<td>novel</td>
</tr>
<tr>
<td>P2</td>
<td>61</td>
<td>f</td>
<td>yes</td>
<td>no mutation identified</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P8</td>
<td>64</td>
<td>m</td>
<td>yes</td>
<td>no mutation identified</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P1</td>
<td>69</td>
<td>m</td>
<td>no</td>
<td>no mutation identified</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P7</td>
<td>63</td>
<td>m</td>
<td>no</td>
<td>no mutation identified</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P25</td>
<td>39</td>
<td>m</td>
<td>no</td>
<td>ENG</td>
<td>5</td>
<td>c.662T&gt;C</td>
<td>p.Leu221Pro</td>
<td>Pece-Barbara et al., 1999</td>
</tr>
<tr>
<td>P23</td>
<td>68</td>
<td>m</td>
<td>no</td>
<td>ENG</td>
<td>6</td>
<td>c.787_789delATC</td>
<td>p.Ile263del</td>
<td>Lesca et al., 2004</td>
</tr>
<tr>
<td>P28</td>
<td>48</td>
<td>f</td>
<td>no</td>
<td>ENG</td>
<td>8</td>
<td>c.1278_1281delCAG</td>
<td>p.Gln359fsX366</td>
<td>Gallione et al., 1998</td>
</tr>
<tr>
<td>P3*</td>
<td>53</td>
<td>f</td>
<td>yes</td>
<td>ENG</td>
<td>13</td>
<td>c.1844C&gt;T</td>
<td>p.Ser615Leu</td>
<td>novel</td>
</tr>
</tbody>
</table>

*P3 carries mutations in both genes. †P6 is affected with pulmonary hypertension. Liver disease was determined by ultrasound as described previously (Caselitz et al., 2003). ‡f: female, m: male. Novel mutations are given in bold. DNA numbering is based on cDNA sequences (GenBank: BC014271.2 and NM_000020.1) and cDNA numbering begins with +1 as the A of the ATG initiation codon. The mutation effect on the corresponding proteins is predicted but was not experimentally assessed. No novel neutral/silent variants were identified by systematic sequencing of both genes.

In the ALK1 gene, 81 mutations have so far been described; 50 in the review mentioned above (van der Driesche et al., 2003), 27 (Lesca et al., 2004), and 4 (Harrison et al., 2003). The detection of five novel ALK1 mutations in this study rises the total number of ALK1 mutations to 86.

In most of these studies (including the original studies listed in the mentioned review (van der Driesche et al., 2003), liver involvement was not routinely assessed. Among all published patients with known mutation and hepatic disease, only five were reported to carry a mutation in the ENG gene (Shovlin et al., 1997; Berg et al., 2003; Cymerman et al., 2003; Harrison et al., 2003). ALK1 mutations and liver involvement was reported for single patients (Johnson et al., 1996; Berg et al., 1997; Trembath et al., 2001), for 2 patients each (Abdalla et al., 2003a; Berg et al., 2003), for 6 unrelated patients (Olivieri et al., 2002), and for multiple familial cases (Piantanida et al., 1996; McDonald et al., 2000; Lin et al., 2001; Abdalla et al. 2003b).
DISCUSSION

The scarcity of genotype/phenotype relationships in HHT, particularly with respect to liver complications in HHT, has initiated us to screen for mutations causing HHT in a group of German patients who are clinically well characterized, and who presented with or without hepatic manifestation.

Altogether, we have identified 14 different mutations (table 1). None of the novel missense mutations were present in 50 healthy controls. The other novel mutations introduce premature stop codons or affect splicing, as deduced from the altered Shapiro-Senepathy CV in the latter case. Hence, we assume that we have detected the disease causing mutations in 15 of 19 patients with HHT.

Our data clearly indicate that the liver involvement in patients with HHT is predominantly associated with mutations in the \textit{ALK1} gene, which were present in eight of ten patients (80%). Only one mutation was detected in the \textit{ENG} gene in HHT patients with liver involvement. This patient (P3, table 1), however, also carried a mutation in the \textit{ALK1} gene. To the best of our knowledge, this is the first case of a HHT patient with mutations in both the \textit{ALK1} and the \textit{ENG} genes. It is likely that the \textit{ALK1} mutation p.Glu379Lys (which has recently been identified independently by Lesca et al., 2004) is the predominant disease causing mutation. This substitution of an acidic amino acid by a basic amino acid in the functional important kinase domain of the ALK1 protein is more likely to lead to important functional consequences than the \textit{ENG} mutation p.Ser615Leu, a substitution of a neutral polar by a neutral nonpolar amino acid in the transmembrane domain. Unfortunately, no further family members were available to study the segregation of these mutations, and the disease, in the family of this patient.

The predominance of \textit{ALK1} mutations in HHT with hepatic manifestation does not reach statistical significance due to the small sample size. A follow up study with more HHT patients with liver involvement is needed to finally prove the association between \textit{ALK1} mutations and this particular HHT phenotype. Our results are well in accordance with previous publications on hepatic manifestations in patients with HHT. Although liver involvement was reported for a few patients with \textit{ENG} mutations, (Berg et al., 2003; Harrison et al., 2003; Cymerman et al., 2003; Shovlin et al., 1997) it was much more often reported for patients carrying mutations in the \textit{ALK1} gene (Johnson et al., 1996; Piantanida et al., 1996; Berg et al., 1997; McDonald et al., 2000; Lin et al., 2001; Trembath et al., 2001; Olivieri et al., 2002; Abdalla et al., 2003a & b ; Berg et al., 2003). Liver involvement was not routinely assessed in most of these studies, since the emphasis was mainly on the genotype and not the phenotype, or the reports were on multiple family members and not on unrelated individuals. Therefore, it did not seem appropriate to statistically compare the occurrence rates of \textit{ALK1} versus \textit{ENG} mutations among HHT patients with hepatic manifestation from previous reports.

Additionally, further family studies are needed to determine the risk for hepatic disease in HHT patients with \textit{ALK1} mutations. It is noteworthy that, even in families with high rates of liver involvement, only a subset of the mutation carriers finally develops hepatic disease (Piantanida et al., 1996; McDonald et al., 2000; Lin et al., 2001; Abdalla et al., 2003b). The phenotypic variability of HHT type 2 is also evident from our study: the same \textit{ALK1} mutation (p.Arg67Trp) was present in two unrelated HHT patients, one with (P4), and one without (P13) hepatic manifestation. The age difference of about 20 years between patients P4 and P13 may argue in favor of an age dependent liver phenotype, whereas the lack of liver involvement in older HHT patients in the families mentioned above points towards a reduced penetrance of liver disease in HHT patients.

A similar high predominance of \textit{ALK1} mutations has recently been described for HHT patients with primary pulmonary hypertension.(Trembath et al., 2001; Harrison et al., 2003). Familial primary pulmonary hypertension is mostly due to mutations in the gene bone morphogenetic receptor type 2 (BMPR2) (International PPH Consortium, 2000), which codes for another member (like endoglin and ALK1) of the membrane bound receptors of the transforming growth factor \(\beta\) (TGF\(\beta\)) signalling superfamily (Shi et al., 2003). Pulmonary hypertension was present in patient P6 (table 1), who carries the \textit{ALK1} mutation p.Glu407Asp. However, neither patient P9, who was also shown to carry p.Glu407Asp, nor any other of our patients with or without \textit{ALK1} or \textit{ENG} mutations was affected with pulmonary hypertension. We assume that the pulmonary hypertension (mean pulmonary artery pressure of 37 mm Hg) in patient P6 may be a secondary consequence of increased cardiac output (8 l/min) due to hepatic vascular malformations. However a primary form of pulmonary hypertension can not be ruled out in this patient as well (Trembath et al., 2001).

Whereas the mutations in our HHT2 patients with liver involvement were evenly distributed in exons 3, 8 and 9 of the \textit{ALK1} gene, in Italian HHT2 patients the causative mutations were detected in exons 3, 7 and 8. It seems possible that specific nucleotide changes in those four exons 3, 7, 8 and 9 of the \textit{ALK1} gene could confer susceptibility to liver involvement in HHT. However, due to the lack of significantly high number of observations,
it is not possible to date to answer the question whether or not certain types or positions of mutations in the ALK1 gene are associated with a higher risk for liver involvement (or pulmonary hypertension) in HHT patients.

In two of our ten patients with liver disease and two of the nine patients without liver disease, we were not able to identify a mutation in either the ALK1 or the ENG genes. However, one has to take into account that the overall mutation detection rate in our study was 78%. In a recently published extensive study a detection rate of 68% has been reported in patients with a confirmed diagnosis of Morbus-Osler according to the Curaçao criteria (Lesca et al., 2004). Although we have been sequencing the whole coding sequences of both genes, we can not rule out that we missed mutations like large deletions or insertions, or mutations involving non-coding regions of these genes. Hence, it is possible that one or two of the HHT-patients with hepatic manifestations and no ALK1 mutations might carry a non-identified ENG mutation. Even if this was the case, we would still have identified ALK1 mutations as the predominant cause for liver involvement in HHT. Alternatively, it is also possible that HHT may – at least in some cases - be caused by mutations in a third locus, other than the ALK1 or ENG genes. Evidence for existence of a third HHT locus had been mentioned previously (Piantanida et al., 1996; Wallace and Shovlin 2000). However, in one of these cases (Piantanida et al. 1996) an ALK1 mutation was finally detected, after reevaluation, in a large Italian HHT family with liver involvement (Olivieri et al., 2002).

The age and sex distributions among HHT patients in our study confirm former reports on a high proportion of females (Selmaier et al., 1993; Shovlin and Letarte, 1999) with higher age (Plauchu et al., 1989) among HHT patients with liver involvement. The same trend was seen in HHT patients with pulmonary hypertension (Harrison et al, 2003), pulmonary vessel malformations (Shovlin et al., 1995), and cerebral involvement (Shovlin and Letarte, 1999). Estrogen responsive elements had been identified in the endoglin promoter (Rius et al., 1998), linking HHT expression to female hormones. However, to the best of our knowledge, there is no final proof for a direct influence of hormones on the development of certain HHT symptoms.

Visceral manifestations were reported to occur at least in 30% of patients with HHT2 (Abdalla et al., 2003b). Intrahepatic shunts were found only in 6% of 93 HHT2 patients from nine families (Abdalla et al., 2003b). It is very likely that these figures represent underestimates, since the screening for visceral manifestations is highly variable and sometimes incomplete in different clinical centers. The identification of disease causing ALK1 mutations in patients with liver disease enables us to investigate their families in a future study, which will help to come to a better estimation of the risk for liver involvement in HHT. We conclude from our study and from our literature survey that the risk for liver disease is higher in HHT2 than in HHT1. If confirmed by further studies, this will have important consequences for disease management in HHT patients with known mutations as well as for mutation screening in newly diagnosed HHT patients. The latter should be performed primarily at the ALK1 gene, if liver involvement is known in the patient or family members.

ACKNOWLEDGMENT

We thank Prof. Jörg Schmidtke, Hannover, for his continuous support. The financial support of this study (scholarship to E.A. El-Harith) by the Alexander von Humboldt Foundation (Germany) is hereby gratefully acknowledged.

REFERENCES


