A newborn with hereditary haemorrhagic telangiectasia and an unusually severe phenotype

Loukas Argyriou[a, d], Johannes Wirbelauer[b], Arvind Dev[a], Irakli Panchulidze[a], Moneef Shoukier[a], Ute Teske[a], Karim Nayernia[c]

[a] Georg-August University Göttingen, Institute of Human Genetics, Göttingen, Germany
[b] University of Würzburg, University Children's Hospital, Würzburg, Germany
[c] Institute of Human Genetics, University of Newcastle upon Tyne, Newcastle, UK
[d] University of Schleswig-Holstein, Campus Lübeck, Institute of Human Genetics, Lübeck, Germany

Summary

Hereditary haemorrhagic telangiectasia (HHT), associated with arteriovenous malformations, is a genetic disease of the vascular system with a frequency of approx. 1:10,000. Genetic diagnosis serves to identify individuals at risk of developing the disease and is a useful tool for genetic counselling purposes.

Questions under study: Here we report on a child presenting severe arteriovenous malformations leading to heart failure. Her mother and grandmother present fewer symptoms of hereditary haemorrhagic telangiectasia. In this study we identify the cause of HHT in the family.

Methods: Clinical examination, PCR, DNA sequencing, quantitative PCR, Southern blot, x-ray, ultrasound, cardiac catheterisation and angiocardiography.

Results: Initially the sequence variant in c.392C>T in the endoglin gene was detected in the grandmother, but not in other affected family members. Further analyses revealed a deletion of exon 1 of endoglin, segregating with the phenotype.

Conclusions: This report points out the need for careful evaluation of molecular genetic findings, particularly in diseases with highly variable phenotype.

Key words: Osler-Weber-Rendu; mutation; gene polymorphisms; heart hypertrophy; epistaxis; telangiectasia

Introduction

Hereditary haemorrhagic telangiectasia (HHT) is an autosomal dominant disorder manifested in about 1/10,000 individuals [1]. The leading criteria for the clinical diagnosis in patients with HHT are recurrent nosebleeds, cutaneous telangiectases, internal arteriovenous malformations (AVMs) and positive family history. At least 3 out of these 4 criteria are necessary to establish a definite clinical diagnosis. However, age of onset and severity of symptoms are highly variable even in affected members of the same family. Individuals may remain undiagnosed until a life-threatening complication occurs. AVMs may occur in the lungs, liver, central nervous system or, rarely, in other organs, leading to stroke, various haemorrhages and severe anaemia. Mutations in at least three genes are causally related to HHT [2, 3, 5]. Mutations in the *endoglin* (*ENG*; OMIM 187300) gene are associated with a high prevalence of pulmonary AVMs [4], whereas mutations in the *activin receptor-like kinase-1* gene (*ACVRL1*; ALK-1, OMIM 600376) are linked to a lower frequency of pulmonary and cerebral AVMs but to a higher incidence of liver involvement [4]. Additionally, mutations in an unidentified third gene linked to chromosome 5, as well as mutations in *SMAD4* (also: *MADH4*, OMIM 600993) are likely to account for some HHT cases [5]. (Mutations in SMAD4 are also known to cause a combined syndrome of HHT and juvenile polyposis [6]). The large number of truncating mutations in HHT1, together with experimental evidence [7, 8], point to haploinsufficiency as the main causative mechanism for HHT mutations.

List of abbreviations (not explained in text)

OMIM	Online Mendelian inheritance in man
PCR	Polymerase chain reaction
NCBI	National Centre for Biotechnology Information

Figure 1

Pedigree of the family with the novel endoglin deletion. Circles symbolise females, squares males, shadow symbolises clinical symptoms of HHT. Numbers out of brackets below symbols symbolise age. Arrows indicate individuals who were tested for mutations. The mutations found are represented next to each person on pedigree. WT: wildtype E: spontaneous and recurrent epistaxis, T: telangiectases, AVM: arteriovenous malformation, P: pulmonary, AB: intraabdominal, SP: spinal, GI: gastrointestinal. III-(1) is not shadowed, because initially she was not diagnosed with HHT.
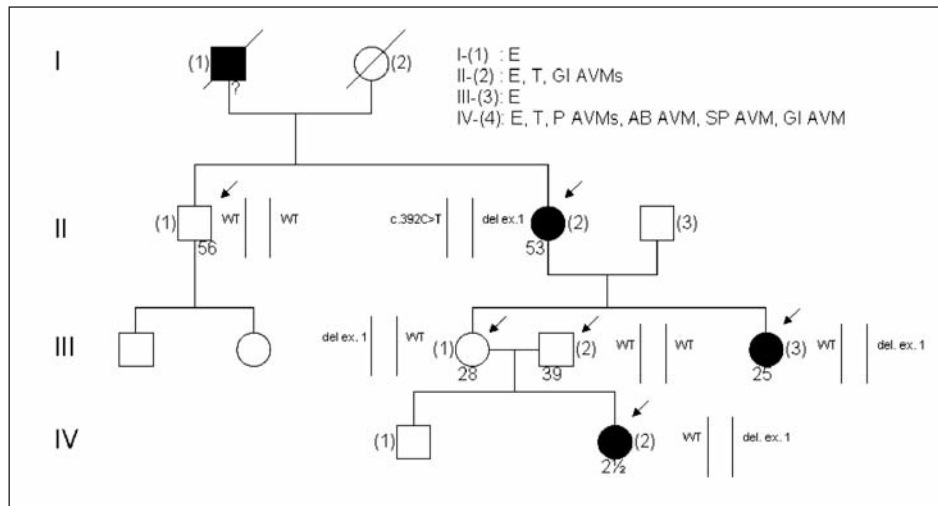
Patients, material and methods

Blood samples from an affected family underwent molecular genetic analyses in the Institute of Human Genetics, Göttingen. A pedigree is given in Fig. 1. The molecular diagnosis for every patient analysed was conducted after the referring physician had received consent. Written consent for anonymous publication of the study was obtained from all family members analysed. Consent for the newborn child was obtained from the parents.

Genomic DNA was isolated from peripheral blood leucocytes. The coding regions of both endoglin and ACVRL1 were amplified on a PTC-100™ thermal cycler (MJ Research, Waltham, USA) (primer sequences are available on request). Polymerase chain reaction products were purified with Millipore Microcon PCR Filter tubes (Milian SA, Geneva, Switzerland) and were directly sequenced in both directions on an Amersham MEGABACE® DNA Sequencer. Sequence variations were tested in 50 unrelated control individuals from the same ethnic background. The detection of deletions was performed in both endoglin and ACVRL1 by quantitative PCR [9]. The analysis was conducted on the ABI Prism 7900 Sequence Detection System (PE Applied Biosystems and 384-well plates from AB gene, Hamburg, Germany). The results were analysed using the Sequence Detection System software (SDS version 2.0, PE Applied Biosystems). We used the same reaction reagents, concentrations and quantitative PCR conditions as described by Boehm et al. [9]. Primer sequences are available on request. For detection of the exact deletion length a three-step approach was selected: as a first step, 3 amplicons for ACVRL1 and 5 amplicons for endoglin were designed, spanning the whole genomic sequence of the genes. Once a deletion could be detected, 6 further amplicons were designed in a second step, 3 in the 5' region of the initially deleted amplicon and 3 in its 3' region. After narrowing of the 5' and 3' breakpoints, several primers were designed in the putative breakpoint regions. As the third step, non-quantitative PCRs were conducted with various primer combinations from the putative breakpoint regions. The conditions for these PCRs were: denaturation at 95 ºC for 15 min, denaturation at 95 ºC for 30 seconds, annealing at 60 ºC for 1 min, elongation at 72 ºC for 2 min, the latter 3 repeated for 35 cycles. A longer elongation time in comparison with standard PCR conditions was selected to allow amplification for longer amplicons also (up to about 2kb). Due to the great distance between the F and R primer on undeleted wildtype genomic DNA, only DNA from the deleted allele could be amplified during this last step. The product of this "deletion-specific PCR" was then sequenced as mentioned above. A comparison of the provided sequence with the sequence published on the NCBI webpage was performed to provide the 5' and 3' breakpoints.

The advantage of the approach described is that it provides relatively rapid identification of breakpoints without the need to clone genomic DNA to separate the wildtype from the deleted allele. To confirm the deletion by an independent method we performed a Southern blot analysis. For this purpose, *SacI* digested DNA was electrophoresed on a 0.7% agarose gel at 25 V for 24 h. Gels were blotted onto Hybond-XL membranes in 20×SSC overnight. Filters were baked for 1 h at 80 °C. For hybridisation a 700 bp fragment of intron 1 of endoglin gene was amplified by PCR. The PCR fragment was cloned in the pGEM T-Easy vector (Promega) and then extracted with *EcoRI* restriction enzyme. The gel-extracted fragment of the endoglin gene was labeled with 32P-dCTP using the Rediprime II system (Amersham Pharmacia). Filters were hybridised with the probe in Rapid-hyb buffer (Amersham Pharmacia) with 100 µg/mL denatured salmon sperm DNA at 65 °C for 16 h. Filters were washed at room temperature in 2× SSC, 0.2% SDS for 15 min followed by 15 min in 0.5× SSC, 0.2% SDS at 65 °C, and were then exposed with x-ray film for 24h.

Results

As the second child of a now 28-year-old healthy mother (III-1) the affected girl (IV-2) was born without complications at 39 weeks gestational age (see fig. 1 for pedigree). The birth weight was 2680 g (5th perc.) and Apgar score was 10/10 after 5 and 10 minutes respectively. The girl

Figure 2

A + B: Southern blot analysis of genomic DNA isolated from blood. Upon digestion with Sac1, the wildtype allele gives rise to a 6.9kb fragment, while the DNA of IV-(2) generates the 6.9kb and 5.4 fragments, detected with a 3′probe located in intron1 (Wt: wildtype). C: Deletion-specific sequencing of the affected allele depicts the regions 5′ and 3′ from the deletion (10064 bases including exon 1 with ATG codon are deleted) and heterozygous c.392C>T mutation in exon 4 of the endoglin gene.
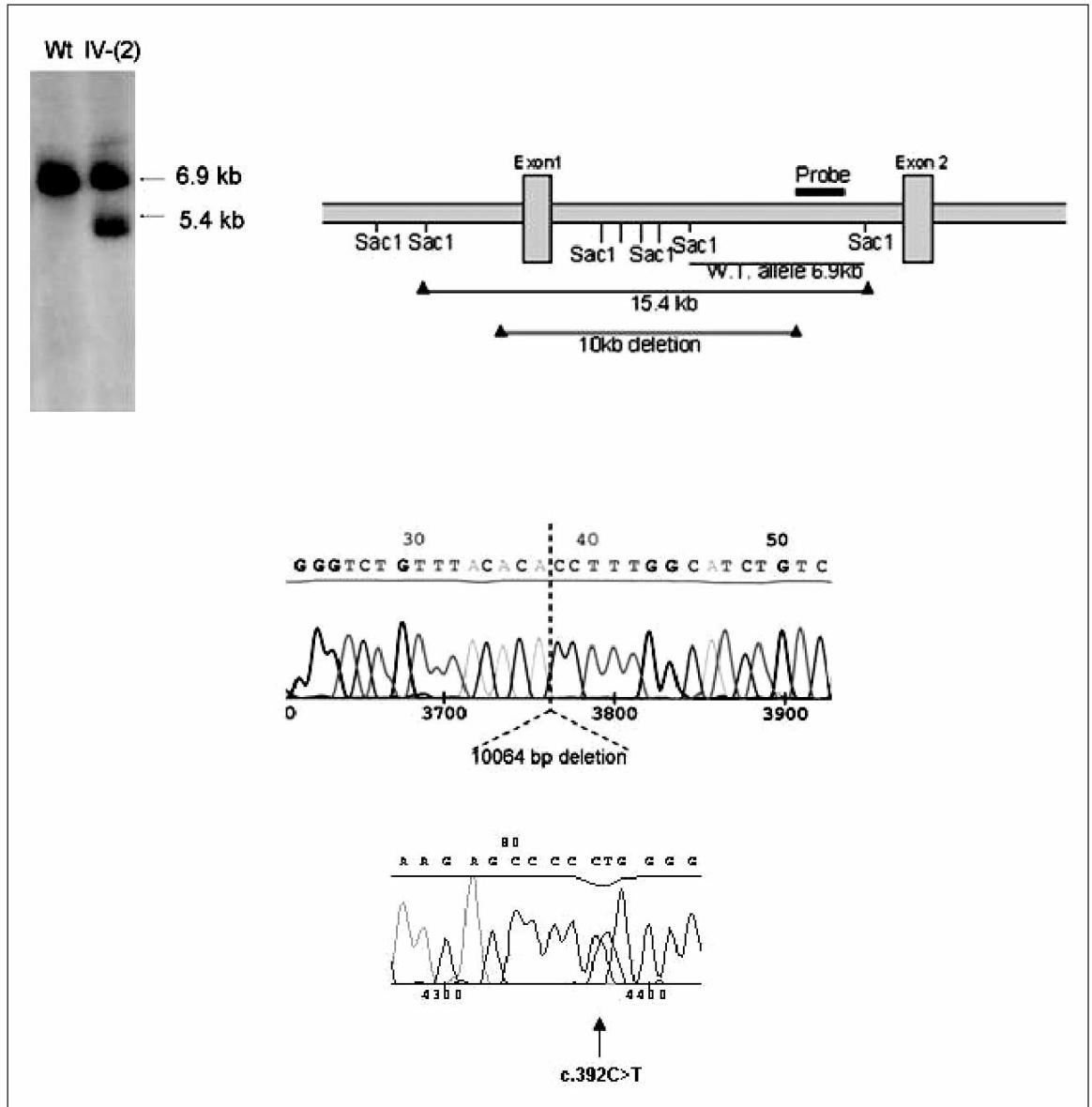

presented epistaxis for the first time at the age of three weeks. A paediatrician was consulted after another episode of epistaxis at the age of 14 weeks. At that time the girl was cyanotic and oxygen saturation was measured at 75% in room air. Cyanotic heart failure was ruled out by ultrasound. Tachypnoea and elevated density in chest x-ray prompted an initial diagnosis of pneumonia. Further examination with ultrasound of the abdomen revealed increased blood flow in the portal vein. This proved to be due to an arteriovenous anastomosis between splenic artery and portal vein. Cardiac catheterisation and angiocardiography revealed multiple arteriovenous fistulas of the pulmonary vessels. Implantation of 21 coil devices to block the blood flow into these AVMs (fig. 3) improved saturation in the systemic arteries to 90%. At the age of 7 months laser coagulation had to be administered to the nasal mucous membrane. Due to gastrointestinal hemorrhages an

Figure 3

On the left, chest x-ray at the age of 14 weeks depicting cardiac hypertrophy; in the middle, chest x-ray at the age of 20 months, after multiple coil implantation; on the right, T2 MRT of the spinal cord (at the age of 2 years) depicting a large arteriovenous malformation at the level of the 6th thoracic spinal body.
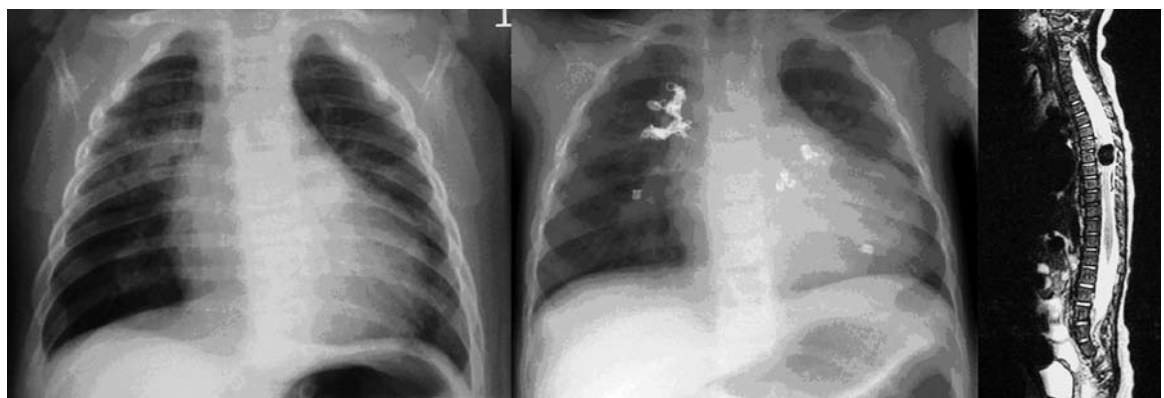
endoscopic intervention was necessary at the age of ten months. The source was telangiectasia in the ascending colon. Cerebral bleeding due to a cerebral AVM was the reason for a prolonged half-side seizure at the age of 21 months. Before the age of 21 months, the underlying cerebral AVM had not evidenced itself, which is why no brain CT or arterial angiography was performed earlier. After detection of the cerebral AVM, MRT of the spine was performed to detect or exclude further AVMs of the central nervous system. A large arteriovenous malformation at the level of the 6th thoracic spinal body (fig. 3) was detected, which, interestingly enough, creates no neurological problems.

The grandmother (II-2) of the affected girl had been diagnosed with HHT. Due to this previous history of HHT in the family, HHT was suggested as the most probable diagnosis for the girl, although her mother (III-1) seemed to be unaffected initially (25 years old at birth of the child). At 26 years, however, spontaneous and recurrent epistaxes presented for the first time. Even after clinical examination by a dermatologist no mucosal or cutaneous telangiectases were detected. Further ultrasound and MRT examinations and central nervous system arteriographies are now planned to detect possible AVMs preclinically. Initially the disease causing mutation in the family was thought to be the c.392C>T in the endoglin gene, which is also cited in published reports disease causing [10]. Because it was not found in the girl's genomic DNA, the girl was expected to have another mutation in endoglin or ACVRL1 or another disease with symptoms resembling HHT (e.g. OMIM entities 108010, 608354). Since direct sequencing does not reveal large rearrangements, we decided to perform a deletion analysis in both known HHT genes, as described above. Southern blot analysis revealed a deletion of approximately 10kb (fig. 2). The further analysis proved that the deletion spans exactly 10064 base pairs on the endoglin gene including exon 1 (fig. 2).

Discussion

The 10 kb deletion segregates with the disease in the family (Fig. 1), whereas the initially detected mutation does not. This prompts the conclusion that: (1) the two mutations are probably biallelic in the grandmother, since they do not cosegregate (crossing over is very unlikely within a region of some 22kb, which is the distance between the deletion and the point mutation) and (2) the mutation c.392C>T is probably not causative of disease. This finding is concordant with the HHT Mutation Database (http://137.195.14.43/cgibin/WebObjects/hht.woa/2/wo/qaLwSLQJJO2gIUSR3HWEIg/8.3.17.8.18), which declares the variation c.392C>T as a rare polymorphism, a finding which is also supported by Abdalla et al. [1]. Moreover, family member II-2, who has both the 10 kb deletion and the point mutation, does not present more severe HHT symptoms than IV-2. Thus, the intrafamilial variability does not depend on the presence of c.392C>T in the endoglin gene.

Our analysis identified a large deletion affecting exon 1 (including the ATG codon) of the endoglin gene. A similar deletion, also affecting exon 1, has already been described as causative of disease [11]. Since there is no alternative ATG codon we predict that no protein is built from the allele with the 10kb deletion. The fact that members of this family present so different degrees of HHT symptoms (the grandmother has had telangiectases since the age of 35 and an AVM of the gastrointestinal tract diagnosed at the age of 40, the mother remained without symptoms until the age of 26, and the girl has been severely affected since birth) proves that no prediction based on molecular analysis can be made even in members of the same family. Moreover, c.392C>T in the endoglin gene is probably not connected to HHT in this family. Also, it cannot be assumed that patients with large deletions are more severely affected than patients with missense mutations. Since the affected girl is the only member in the family with symptoms since childhood we assume that she could have inherited a modifying polymorphism from her father. Hence we also obtained a blood probe from him, in order to check for sequence variants on genomic DNA level. Sequence analysis and deletion analysis for both endoglin and ACVRL1 did not reveal any amino-acid alteration, nonsense mutation or large rearrangement. A reason for the severe form of HHT in IV-2 could thus be an additional inherited sequence variant from the father in the promoter region of the gene (not tested in our analysis) or a mutation in another modifier gene, which leads to a severe phenotype only when it is combined with a mutation in endoglin or ACVRL1. This hypothesis, in our case postulated for HHT, has been proven for other diseases [12, 13].

In conclusion, we report on a family with a highly variable HHT phenotype, including an unusual phenotype in a child. Several cases of children with HHT have been described in the literature [14]. Our case, involving this extreme intrafamilial variability in phenotype, adds to identical descriptions in the literature [e.g. 15] and points to the fact that severe HHT cases may

occur even in families with otherwise mildly affected individuals. Although the reason for this phenomenon could not be explained by our analyses, we would like to point out that the molecular diagnosis of HHT should include analysis of both endoglin and ACVRL1. Even when a mutation is found, sequencing should be completed for every exon of these genes and every positive result should be proved on further affected and non-affected members of the family. If this procedure is not followed it is possible that a mutation will be falsely declared as causative of disease in a family, resulting in wrong predictive diagnostics for at-risk members.

Correspondence:
Dr. med. Loukas Argyriou
Institute of Human Genetics
University of Lübeck
Ratzeburger Allee 160
23538 Lübeck
Germany
E-Mail: argi13@yahoo.com

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