Am. J. Hum. Genet. 61:60–67, 1997

The Activin Receptor–Like Kinase 1 Gene: Genomic Structure and Mutations in Hereditary Hemorrhagic Telangiectasia Type 2

Jonathan N. Berg,[1,3] Carol J. Gallione,[1] Timothy T. Stenzel,[1,2] David W. Johnson,[1] William P. Allen,[4] Charles E. Schwartz,[4] Charles E. Jackson,[5] Mary E. M. Porteous,[3] and Douglas A. Marchuk[1]

Departments of[1]Genetics and [2]Pathology, Duke University Medical Center, Durham; [3]Department of Human Genetics, University of Edinburgh, Edinburgh; [4]Greenwood Genetics Center, Greenwood, SC; and [5]Department of Medicine, Division of Clinical and Molecular Genetics, Henry Ford Hospital, Detroit

Summary

The activin receptor–like kinase 1 gene (ALK-1) is the second locus for the autosomal dominant vascular disease hereditary hemorrhagic telangiectasia (HHT). In this paper we present the genomic structure of the ALK-1 gene, a type I serine-threonine kinase receptor expressed predominantly in endothelial cells. The coding region is contained within nine exons, spanning <15 kb of genomic DNA. All introns follow the GT-AG rule, except for intron 6, which has a TAG|gcaag 5′ splice junction. The positions of introns in the intracellular domain are almost identical to those of the mouse serine-threonine kinase receptor TSK-7L. By sequencing ALK-1 from genomic DNA, mutations were found in six of six families with HHT either shown to link to chromosome 12q13 or in which linkage of HHT to chromosome 9q33 had been excluded. Mutations were also found in three of six patients from families in which available linkage data were insufficient to allow certainty with regard to the locus involved. The high rate of detection of mutations by genomic sequencing of ALK-1 suggests that this will be a useful diagnostic test for HHT2, particularly where preliminary linkage to chromosome 12q13 can be established. In two cases in which premature termination codons were found in genomic DNA, the mutant mRNA was either not present or present at barely detectable levels. These data suggest that mutations in ALK-1 are functionally null alleles.

Introduction

Hereditary hemorrhagic telangiectasia (HHT) is an autosomal dominant disease of blood vessels that is characterized by severe recurrent nosebleeds, mucocutaneous telangiectases, gastrointestinal hemorrhage, and a high incidence of vascular malformations in the lung and brain (Guttmacher et al. 1995). Most of the published families with HHT fall into two linkage groups: HHT1, mapping to chromosome 9q33 (McDonald et al. 1994; Shovlin et al. 1994); and HHT2, mapping to chromosome 12q13 (Johnson et al. 1995; Vincent et al. 1995). Patients with HHT linked to 9q33 may have a higher incidence of symptomatic pulmonary arteriovenous malformations than is seen in those with HHT linked to 12q13 (Heutink et al. 1994; McAllister et al. 1994*b;* Porteous et al. 1994; Berg et al. 1996), suggesting that, in the pulmonary vasculature, the HHT1 gene has an additional role not shared by HHT2. A third rare variant of HHT has been reported in one large family with hepatic involvement as the major manifestation (Piantanida et al. 1996), with exclusion of linkage to both chromosome 9 and chromosome 12.

The genes involved at the two major loci have been identified. HHT1 is the endoglin gene on chromosome 9 (McAllister et al. 1994*a*); HHT2 is the ALK-1 gene on chromosome 12 (Johnson et al. 1996). Both of these genes are members of the TGF-β receptor superfamily (Cheifetz et al. 1992; Attisano et al. 1993; ten Dijke et al. 1993). The ALK-1 protein has the properties of a type I serine-threonine kinase receptor (Attisano et al. 1993; ten Dijke et al. 1993). It has been shown to bind either activin or TGF-β in the presence of their respective type II receptors but does not bind ligand alone (Attisano et al. 1993). The mechanism for downstream signaling of ALK-1 has yet to be elucidated, but it seems probable that it involves pathways similar to those implicated in the signaling of other serine-threonine kinase receptors (Niehrs 1996; Zhang et al. 1996). Expression studies of cell lines by reverse-transcriptase–PCR (RT-PCR) have shown that significant transcription of ALK-1 occurs only in endothelial cell lines (Attisano et al. 1993), although we have demonstrated that it is possible to amplify ALK-1 message from peripheral blood leukocyte mRNA. This has permitted identification of three

Received November 20, 1996; accepted for publication April 25, 1997.

Address for correspondence and reprints: Dr. Douglas A. Marchuk, Department of Genetics, Duke University Medical Center, Box 3175, Durham, NC 27710. E-mail: march004@mc.duke.edu

0002-9297/97/6101-0011$02.00

mutations in ALK-1 in unrelated individuals affected with HHT2 (Johnson et al. 1996).

In this paper we have identified the size and position of introns in the coding portion of the ALK-1 gene. We have developed PCR assays to amplify each exon of the coding region of ALK-1, from genomic DNA. The ALK-1 gene was sequenced in a panel of 12 unrelated patients with HHT, 6 of whom have either evidence of HHT linkage to markers on 12q13 or exclusion of linkage of HHT to 9q34; for the remaining 6 insufficient linkage data were available. The position and type of each mutation found are discussed in relationship to both the function of the protein and the pathobiology of the disease.

Subjects, Material, and Methods

Human Subjects

Individuals with HHT had been assessed in, and DNA samples had obtained from, families 1–5, 17, and 92, as described elsewhere (McAllister et al. 1994*b;* Porteous et al. 1994; Johnson et al. 1995; Berg et al. 1996), after informed consent had been obtained. Single samples had also been collected from four unrelated individuals by use of the same methods.

Identification of a Genomic P1 Artificial Chromosome (PAC) Clone Containing ALK-1

DNA from pools of a two-dimensional arrayed PAC library (Ioannou et al. 1994) was screened by use of the primers 5′-CGCGTGTCACACTTCATGGCTC-3′ and 5′-ATCAGAAGGCCTTTCCTGGGGG-3′, designed to the 5′ UTR of ALK-1 published by Attisano et al. (1993. The positive clone from a single positive 384-well plate (plate 265) was identified at position C14 by hybridization using standard protocols (Sambrook et al. 1989). This clone was designated "265C14." Subclones were generated by use of *Bgl*II-digested PAC cloned into *Bam*HI-digested pBluescript phagemid (Stratagene).

Localization of Introns

With a range of primers designed from the published cDNA sequence, PCR reactions were performed to amplify segments of genomic DNA containing the ALK-1 gene. Whenever a PCR fragment generated was larger than that predicted from the cDNA sequence, it was assumed to contain an intron and was sequenced. Where sequencing was unclear, further sequence was generated from the plasmid subclones by use of different primers designed for the ALK-1 cDNA sequence. Intron size was estimated by size of PCR product, or, for three of the introns, sequence was generated for the entire intron.

PCR Amplification of Individual Exons

With the intronic sequence generated, primer pairs were designed to amplify each exon of the coding part of the ALK-1 gene. Primer sequence and conditions are given in table 1. PCR was carried out for 35 cycles: 95°C for 45 s, annealing temperature for 45 s, and 72°C for 1 min. One hundred nanograms of each primer, 1 × buffer, 50 ng of genomic DNA, 0.2 mM dNTPs, and 1 unit of Gibco-BRL *Taq* polymerase were used in each 25-μl reaction. The buffer used was either that supplied by Boehringer Mannheim Biochemicals (BMB) or that originally used by Weissenbach (W; Gyapay et al. 1994); in both cases the magnesium ion concentration was 1.5 mM.

Mutation Analysis

For each patient in the study, each exon was amplified and sequenced. Sequencing was performed by use of the Amersham Thermosequenase cycle sequencing kit, which uses P^{33}-labeled dideoxy terminators to reduce artifactual bands. Samples of each nucleotide-specific termination reaction were loaded in adjacent lanes, making a mutation in any single lane very easy to identify. In one instance, for family 92, it was necessary to clone the exon into PCR Script by using the Invitrogen TA cloning kit, prior to sequencing. Conservation of the region affected by the mutation was determined by use of the BLAST algorithm, to match the ALK-1 region containing the mutation against the Genbank nonredundant protein database.

RT-PCR

For RT-PCR, peripheral blood leukocyte mRNA was reverse transcribed by use of a mixture of oligo dT and random hexamers. A 250-bp segment of ALK-1 exon VII was amplified by use of primers 5′-TGTGGCACG-GTGAGAGTGTGGCC-3′ and 5′-GTTGCTCTTGAC-CAGCACAT-3′. A minus-reverse-transcriptase control was included.

Polymorphism Screen

For each mutation, a panel of 100–120 normal individuals was screened to ensure that the mutation did not arise as a population polymorphism. The screening was performed by altered restriction-enzyme site, where possible. In one case in which no enzyme site was altered, the mutant PCR product gave a heteroduplex band that could be resolved by gel electrophoresis as described elsewhere (McAllister et al. 1995). For two mutations it was necessary to screen the population samples by sequencing the PCR product. In these instances, only a single termination reaction was run for each individual screened.

Results

Genomic Structure of ALK-1

A single PAC clone containing the ALK-1 gene was isolated from a human genomic PAC library (Ioannou

Table 1

Primers for Amplification of ALK-1 Gene Exons

Exon	5′ Assay Primer	3′ Assay Primer	Annealing Temperature (°C)	Buffer	Size (bp)
II	CTCTGTGATTTCCTCTGGGCA	TACATTCTCCCCAGCTTCTCAA	62[a]	BMB	266
III	AGCTGGGACCACAGTGGCTGA	GGAGGCAGGGGCCAAGAAGAT	64	W	345
IV	AGCTGACCTAGTGGAAGCTGA	CTGATTCTGCAGTTCCTATCTG	60	W	318
V	AGGAGCTTGCAGTGACCCAGCA	ATGAGAGCCCTTGGTCCTCATCCA	68[a]	BMB	242
VI	AGGCAGCGCAGCATCAAGAT	AAACTTGAGCCCTGAGTGCAG	60	BMB	294
VII	TGACGACTCCAGCCTCCCTTAG	CAAGCTCCGCCCACCTGTGAA	65[a]	W	388
VIII	AGGTTTGGGAGAGGGGCAGGAGT	GGCTCCACAGGCTGATTCCCCTT	65	BMB	293
IX	TCCTCTGGGTGGTATTGGGCCTC	CAGAAATCCCAGCCGTGAGCCAC	68[a]	BMB	256
X	TCTCCTCTGCACCTCTCTCCCAA	CTGCAGGCAGAAAGGAATCAGGTGCT	65[a]	BMB	197

[a] Hot start improves amplification.

et al. 1994). This PAC clone was designated "265C14." In Southern blot analysis, cDNA from the entire coding region of ALK-1 hybridized to genomic fragments totaling 15 kb. The position of each intron and the size of each exon, as determined by PCR amplification from the PAC clone, are shown in figure 1. Exon I contains the 5′ untranslated sequence published by ten Dijke et al. (1993) and was shown, by sequencing of a subclone, to be present in 265C14.

The 5′ untranslated sequence of ALK-1 differs in the two published descriptions of this gene (Attisano et al. 1993; ten Dijke et al. 1993). Genomic sequencing shows that the 5′ untranslated sequence published by Attisano is part of exon II. ten Dijke et al.'s version is a splice variant arising from splicing of exon I to a consensus splice junction present 7 bp upstream of the start codon in exon II, where identity with Attisano et al.'s version of this sequence begins. This splice is marked with an arrow in figure 1. A consensus splice is present at the 3′ side of exon I. Both splice variants can be amplified by RT-PCR of mRNA extracted either from endothelial cell lines or from peripheral blood leukocytes.

The coding region of ALK-1 is contained within nine exons. All except one intron follow the GT-AG rule. Intron 6, between exons VI and VII, has a nonconsensus sequence at the 5′ junction. The most likely position for the splice, however, gives the splice-junction sequence TAG|gcaag, which is the sequence most commonly reported for nonimmunoglobulin eukaryotic nonconforming 5′ splice sites (Senapathy et al. 1990). The 3′ splice of this intron would then have the usual consensus sequence, cag|G. Sequence data from all the intron-exon borders of the coding region are available as Genbank accession numbers U77707–U77713.

Comparison with a Murine Type I Kinase

Another type I serine-threonine kinase receptor for which the genomic structure has been published is the mouse receptor TSK-7L (Ebner et al. 1993; Schmitt et al. 1995), a homologue of human ALK-2 and activin type I receptor. The amino acid sequence of the kinase domain is 79% identical to human ALK-1, but the extracellular domain is only 23% identical. Comparison of the intron-exon positions of ALK-1 with those of TSK-

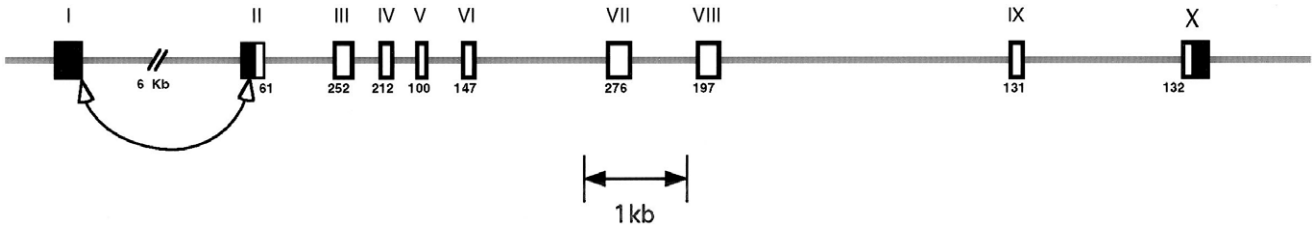

Figure 1 Diagrammatic representation of the ALK-1 gene, approximately to scale. The exons are marked by boxes—unblackened for the coding regions and black for the noncoding regions. The exon number referred to in the text is given as a roman numeral above the exon. Exact exon size (in bp) is given below the exon, in arabic numerals. The curved arrow marks the putative alternative splice, in the 5′ UTR, that would lead to the 5′ sequence published by ten Dijke et al. (1993). For the 5′ Attisano et al. (1993) sequence, transcription would start farther 5′ in exon II.

7L shows almost exact conservation of the position of introns within the kinase domain. It is notable that four of the intron-exon splices occur between the first and second bases of a codon in the human sequence. Wherever this happens, the amino acid involved is uncharged. This is also the case with the murine receptor. Although the location of intron 6 is exactly conserved between mouse and human, the mouse intron follows the GT-AG rule, whereas the human intron 6 has a nonconsensus sequence, as outlined above.

Novel Mutations Detected in the ALK-1 Gene

The entire coding portion and intron-exon borders of the ALK-1 gene were sequenced in 12 unrelated individuals affected with HHT. Mutations were found in ALK-1 in nine of the patients. Of these, six were from families showing either strong evidence of HHT linkage to chromosome 12 (either a LOD score >3.0, with closely linked markers, or a LOD score close to the maximum predicted [in the previously published families 2, 3, and 17] or exclusion of chromosome 9 linkage with inconclusive evidence of linkage to chromosome 12 [in previously published families 4 and 5 and new family 92]). Mutations were also found in three of six patients with HHT who were from small families for which there were inclusive linkage data. For each mutation, 100–120 unrelated individuals were analyzed, to show that the sequence change was a true mutation and not a population polymorphism. When other family members were available, the mutation was shown to be present in the other affected individuals from the same kindred. The mutations and their positions in the gene are described in table 2. Figure 2 shows sequence data and cosegregation of the ALK-1 mutation with disease status in a part of the previously unpublished family 92.

For two of the three individuals for whom no mutation was found, a genomic Southern blot of DNA digested with *Hin*dIII, *Eco*RI, or *Bgl*II and probed with ALK-1 cDNA showed no evidence of a gross genomic rearrangement. DNA available from the third individual was insufficient for Southern blot analysis.

All 12 mutations seen so far (9 described here and 3 described elsewhere) leave the putative signal peptide and the transmembrane domain of ALK-1 intact. Two mutations lead to the change of a conserved amino acid in the extracellular domain. Two mutations would lead to premature truncation of the ALK-1 protein just after the transmembrane domain, if translated. The remaining eight mutations either change residues conserved in the serine-threonine kinase family or lead to a frameshift and premature stop in the kinase domain.

In order to determine whether a mutant protein is required for the pathogenesis of the disease, we investigated the presence of mutant transcripts in patient mRNA. In two cases in which a premature termination codon is created in exon VII by either a frameshift or a nonsense mutation, mRNA was isolated from peripheral blood leukocytes from an affected family member and was amplified by RT-PCR, by use of a single round of PCR. In both cases, only the wild-type allele was represented at any appreciable level in the product. These procedures were replicated, with the same result. Figure 3 shows this for a frameshift mutation in exon VII, where no mutant transcript is visible.

Discussion

The position of introns within the kinase domain is very highly conserved between mouse Tsk7L and human ALK-1. In both genes, four of the splice sites fall between the first and second bases of a codon that encodes a neutral amino acid. It has been hypothesized that this allows alternative splicing of the kinase domain, which are still in frame but have variations in kinase activity (Schmitt et al. 1995). Intron 6 of the ALK-1 gene has a variant splice sequence at the 5′ side—AG/gcaag, instead of the usual AG/gt. This has been observed in other genes (Senapathy et al. 1990). The 3′ splice junction is the usual consensus, cag/G. The function of this variant splice sequence is unknown, but it may represent a means for controlling the amount of ALK-1 mRNA that is spliced. This splice-sequence variation is not seen in the mouse, but the splice location occurring between the first and second bases of a glycine codon is the same.

Mutations were found in ALK-1 in all three of the families in which the disease is known to be linked to the ALK-1 region, as well as in all three families in which linkage to HHT1 on chromosome 9 had been excluded. When no linkage data were available, mutations were found in three of the six individuals analyzed. The three patients in whom no mutation was found may come from families in which the disease is linked to other loci, or they may have a form of mutation not easily detected by PCR and sequencing of the coding exons.

Genomic sequencing may prove a more reliable diagnostic test than RT-PCR from peripheral blood leukocyte mRNA. A low level of the ALK-1 transcript in leukocytes in addition to unstable mutant mRNA of some mutations may lead to difficulties in detection of the mutation by RT-PCR. Our comparatively high rate of mutation detection and the small size of the ALK-1 gene make genomic sequencing a viable diagnostic test for HHT2, particularly if a family has previously shown linkage of the disease to chromosome 12q13. Since a clinical diagnosis of HHT in young adults is often uncertain, presymptomatic diagnosis of HHT will allow screening for the serious complications to be targeted only to those at risk.

Table 2

Summary of ALK-1 Mutations in HHT2

Exon	Protein Region	Mutation Found (Position)	Effect on Protein
II	Start	None	
	Signal peptide		
III	Extracellular domain	G→T (150)	Trp (conserved)→Cys
		G→A (200)	Arg (conserved)→Gln
IV	Extracellular domain	G→A (423)	Premature STOP (intracellular)
	Transmembrane domain	G→T (475)	Premature STOP (intracellular)
	Start of intracellular domain		
V	Glycine-Serine domain	None	
VI	ATP binding site	3-bp deletion (694)[a]	Deletion Ser in frame
	Start of kinase domain		
VII	Kinase domain	Ins T (865)	Frame shift and premature STOP
		C→A (924)	Premature STOP
		G→T (998)	Ser (conserved)→Ile
VIII	Kinase domain	C→T (1120)	Arg (conserved)→Trp
		T→G (1126)[a]	Met (conserved)→Arg
		G→A (1232)[a]	Arg (conserved)→Gln
IX	Kinase domain	C→A (1270)	Pro (conserved)→Thr
X	End of kinase domain	None	

[a] Previously published by Johnson et al. (1996).

All 12 mutations identified in ALK-1 leave the signal peptide and transmembrane domain intact. Two extracellular mutations cause changes of conserved amino acids, and there are two premature stops before the glycine-serine domain. The remaining mutations disrupt the kinase domain either by amino acid change or by small insertion/deletion. Such mutations may function in one of several ways, including a dominant-negative effect of the mutant ALK-1 receptor, haploinsufficiency of ALK-1 protein, or complete loss of functional ALK-1 because of a second somatic mutation in the normal allele.

The hypothesis of a dominant-negative effect requires that an altered protein be transcribed and translated from the mutant allele—and that this protein interfere with the function of the heteromeric receptor signaling complex. Support for this mechanism includes the conservation of signal peptide and transmembrane regions in all mutations found to date. If translated, the abnormal ALK-1 protein may retain the ability to bind a type II receptor in the presence of ligand; but it may not be able to transduce signal, either because of disruption of the kinase domain or because of failure to bind ligand because of alteration of the extracellular domain. Similar mutations in another type I receptor show a dominant-negative effect. Wild-type Mv1Lu cells, which express the type I TGF-β receptor, show reduced TGF-β signaling after transfection with an inactivated TGF-β type I receptor (Feng et al. 1995; Yamamoto et al. 1996), created either by deletion of the cytoplasmic domain or by point mutation in the kinase domain. This dominant-negative effect is even more pronounced with truncated forms of the type II receptor.

However, RT-PCR of two premature protein-truncating mutations observed in exon VII exhibit very low to undetectable levels of the mutant transcript from cDNA of peripheral blood leukocytes, suggesting that some mutant transcripts are unstable. Vascular endothelial cells from patients were unavailable for this expression study. Nonetheless, these data suggest that a mutant protein product may not be present for at least some ALK-1 mutations and that a dominant-negative effect is not required for the pathogenesis of HHT.

The vascular lesions observed in HHT may develop in response to reduced levels of ALK-1 that are due to haploinsufficiency. The mutations identified to date are consistent with the hypothesis that they are all functionally null alleles. The inheritance of a single mutant copy would then predispose the individual to develop the vascular lesions observed in HHT. Lesion formation itself could be due to local environmental or mechanical effects at the lumen of the vessel or to additional genetic alterations.

Finally, it is possible that the development of a vascular lesion initiates with a complete loss of ALK-1 signaling in an endothelial cell. This could be due to somatic mutation of the normal ALK-1 allele. Some support for the possibility of a "second hit" is given by the apparently random distribution of discrete lesions, the number of which increases with age. Mucocutaneous telangiec-

tases appear abruptly and are progressive (Plauchu et al. 1989). However, one study has shown that the classical telangiectases arise as a result of dilatation of blood vessels (Braverman et al. 1990) and do not appear to be proliferative lesions. Also, studies on the development and growth of pulmonary arteriovenous malformations have shown gradual size increases with time, requiring as long as a decade or more to double in diameter (Vase et al. 1985; Masakazu et al. 1988). These studies suggest that, if ALK-1 requires a somatic second mutation for the formation of a vascular lesion, it is not acting as a tumor-suppressor gene. Since TGF-β signaling in endothelial cells modulates vascular remodeling by inducing changes in the extracellular matrix (Madri et al. 1989; Merwin et al. 1990), complete loss of ALK-1 signaling may induce remodeling of the vascular bed, rather than directly affect the rate of endothelial cell proliferation. The proof of this mechanism would require detection of either loss of heterozygosity or somatic mutation of the normal ALK-1 allele in the single layer of endothelium removed from an HHT-associated vascular lesion. In a similar analysis of the renal cysts seen in autosomal dominant polycystic kidney disease type 1, loss of heterozygosity and somatic mutation of the normal PKD1 allele were observed only when methods were developed to isolate the single layer of epithelium lining the renal cysts (Qian et al. 1996).

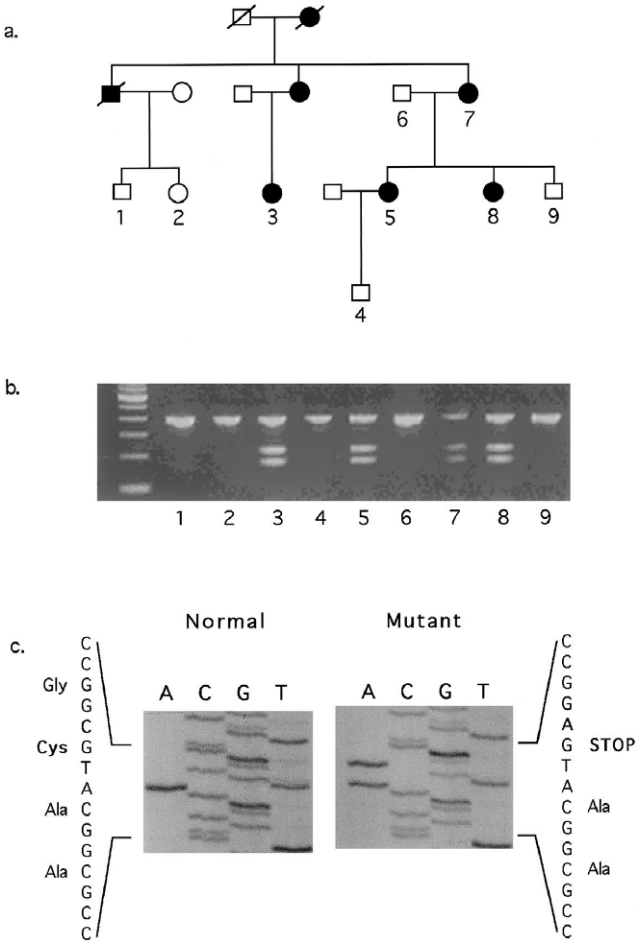

Figure 2 Molecular analysis of the ALK-1 mutation in family 92. *a,* Portion showing affected family members. The disease status cosegregates with presence of a *Stu*I site in the exon VII product shown in panel *b*. *b, Stu*I digest of the exon VII PCR product from family 92. The mutation creates a site that leads to generation of fragments of ~170 and ~210 bp, as well as to an undigested fragment of 380 bp. In unaffected individuals, only the undigested fragment is seen. A 100-bp ladder is used as the size standard. *c,* Sequence from an affected member of family 92. Exon VII product was subcloned as described and was sequenced. Sequences from mutant and wild-type clones are shown. In the mutant clone a C→A base change causes a premature stop. This base change creates a *Stu*I site in the mutant exon VII product.

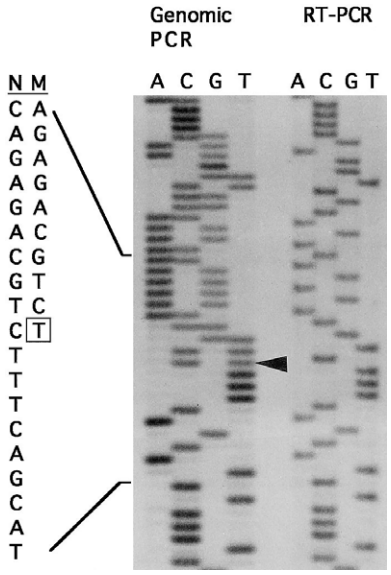

Figure 3 Unstable transcript of the mutant ALK-1 allele in family 17. DNA and mRNA from peripheral blood leukocytes of an affected individual (family 17) were used in genomic PCR and RT-PCR, respectively. Both PCR products were then sequenced by use of an identical primer within the exon. The sequence of the normal (N) allele is apparent in both samples, whereas that of the mutant (M) allele is seen only in the genomic PCR product. Insertion of a T (boxed in the sequence at the left, indicated by an arrow to the right of the band) produces, beyond the inserted nucleotide, superimposed sequences of the normal and mutant alleles, only in the genomic product.

Acknowledgments

We are grateful to Dr. P. de Jong for a copy of the PAC library, to Mary Robyn Tudor for assistance in preparing the manuscript, and to Laura Giovanelli and Yan Xue for technical assistance. We thank Robert I. White, Jr., for discussions of the rate of growth of pulmonary arteriovenous malformations.

Above all, we thank the family members with HHT, the Telangiectasia Self-Help Group (UK), and the HHT Foundation, International, without whom this work could not take place. This work is supported by NIH grant HL49171 to D.A.M., who is also an Established Investigator of the American Heart Association. J.N.B. is supported by a Mrs. Jean V. Baxter Medical Research Fellowship awarded by the Scottish Hospital Endowment Research Trust. T.T.S is supported by NIH fellowship 1F32HL09394, and D.W.J. is supported by NIH fellowship 1F32HL09349.

References

Attisano L, Carcamo J, Ventura F, Weis FMB, Massague J, Wrana JL (1993) Identification of human activin and TGF-β type I receptors that form heteromeric kinase complexes with type II receptors. Cell 75:671–680

Berg JN, Guttmacher AE, Marchuk DA, Porteous MEM (1996) Clinical heterogeneity in hereditary hemorrhagic telangiectasia: are pulmonary arteriovenous malformations more common in families linked to endoglin? J Med Genet 33:256–257

Braverman IM, Keh A, Jacobson BS (1990) Ultrastructure and three-dimensional organization of the telangiectases of hereditary hemorrhagic telangiectasia. J Invest Dermatol 95: 422–427

Cheifetz S, Bellon T, Cales C, Vera S, Bernabeu C, Massague J, Letarte M (1992) Endoglin is a component of the transforming growth factor-β receptor system in human endothelial cells. J Biol Chem 267:19027–19030

Ebner R, Chen RH, Shum L, Lawler S, Zioncheck TF, Lee A, Lopez AR, et al (1993) Cloning of a type I TGF-β receptor and its effect on TGF-β binding to the type II receptor. Science 260:344–348

Feng XH, Filvaroff EH, Derynck R (1995) Transforming growth factor-β (TGF-β)-induced down-regulation of cyclin A expression requires a functional TGF-β receptor complex. J Biol Chem 270:24237–24245

Guttmacher AE, Marchuk DA, White RI (1995) Current concepts: hereditary hemorrhagic telangiectasia. N Engl J Med 333:918–924

Gyapay G, Morissette J, Vignal A, Dib C, Fizames C, Millasseau P, Marc S, et al (1994) The 1993–94 Généthon human genetic linkage map. Nat Genet 7:246–339

Heutink P, Haitjema T, Breedveld GJ, Janssen B, Sandkuijl LA, Bontekoe CJM, Westerman CJJ, et al (1994) Linkage of hereditary hemorrhagic telangiectasia to chromosome 9q34 and evidence for locus heterogeneity. J Med Genet 31:933–936

Ioannou PA, Amemiya CT, Garnes J, Kroisel PM, Hiroaki S, Chen C, Batzer MA, et al (1994) A new bacteriophage P1-derived vector for the propagation of large human DNA fragments. Nat Genet 6:84–89

Johnson DW, Berg JN, Baldwin MA, Gallione CJ, Marondel I, Yoon SJ, Stenzel TT, et al (1996) Mutations in the activin receptor-like kinase 1 gene in hereditary haemorrhagic telangiectasia type 2. Nat Genet 13:189–195

Johnson DW, Berg JN, Gallione CJ, McAllister KA, Warner JP, Helmbold EA, Markel DS, et al (1995) A second locus for Hereditary Hemorrhagic Telangiectasia maps to chromosome 12. Genome Res 5:21–28

Madri JA, Kocher O, Merwin JR, Bell L, Tucker A, Basson CT (1989) Interactions of vascular cells with transforming growth factor beta. Ann NY Acad Sci 593:243–258

Masakazu T, Akioka K, Yasuda M, Ikuno Y, Oku H, Takeuchi K, Takeda T (1988) Case report: hereditary hemorrhagic telangiectasia with growing pulmonary arteriovenous fistulas followed for 24 years. Am J Med Sci 295:545–547

McAllister KA, Baldwin MA, Thukkani AK, Gallione CJ, Berg JN, Porteous ME, Guttmacher AE, et al (1995) Six novel mutations in the endoglin gene in hereditary hemorrhagic telangiectasia type 1 suggest a dominant-negative effect of receptor function. Hum Mol Genet 4:1983–1985

McAllister KA, Grogg KM, Johnson DW, Gallione CJ, Baldwin MA, Jackson CE, Helmbold EA, et al (1994*a*) Endoglin, a TGF-β receptor of endothelial cells, is the gene for hereditary hemorrhagic telangiectasia type 1 (OWR1) Nat Genet 8:345–351

McAllister KA, Lennon F, Bowles-Biesecker B, McKinnon WC, Helmbold EA, Markel DS, Jackson CE, et al (1994b) Genetic heterogeneity in hereditary haemorrhagic telangiectasia: possible correlation with clinical phenotype. J Med Genet 31:927–932

McDonald MT, Papenberg KA, Ghosh S, Glatfelter AA, Biesecker BB, Helmbold EA, Markel DS, et al (1994) A disease locus for hereditary haemorrhagic telangiectasia maps to chromosome 9q33-34. Nat Genet 6:197–204

Merwin JR, Anderson J, Kocher O, van Itallie C, Madri JA (1990) Transforming growth factor β1 modulates extracellular matrix organization and cell-cell junctional complex formation during in vitro angiogenesis. J Cell Physiol 142: 117–128

Niehrs C (1996) Growth factors: mad connection to the nucleus. Nature 381:561–562

Piantanida M, Buscarini E, Dellavecchia C, Minelli A, Rossi A, Buscarini L, Danesino C (1996) Hereditary haemorrhagic telangiectasia with extensive liver involvement is not caused by either HHT1 or HHT2. J Med Genet 33:441–443

Plauchu H, de Chadarevian J-P, Bideau A, Robert J-M (1989) Age related clinical profile of hereditary hemorrhagic telangiectasia in an epidemiologically recruited population. Am J Med Genet 32:291–297

Porteous MEM, Curtis A, Williams O, Marchuk D, Bhattacharya SS, Burn J (1994) Genetic heterogeneity in hereditary haemorrhagic telangeiectasia. J Med Genet 31:925–926

Qian F, Watnick TJ, Onuchic LF, Germino GG (1996) The molecular basis of focal cyst formation in human autosomal dominant polycystic kidney disease type 1. Cell 87:979–987

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY

Schmitt J, Mielke R, Schrewe H (1995) Genomic organization of a mouse type 1 activin receptor. Biochem Biophys Res Commun 213:211–217

Senapathy P, Shapiro MB, Harris NL (1990) Splice junctions, branch point sites, and exons: sequence statistics, identifica-

tion, and applications to genome project. Methods Enzymol 183:252–278

Shovlin C, Hughes JMB, Tuddenham EGD, Temperley I, Perembelon YFN, Scott J, Seidman CE, et al (1994) A gene for hereditary hemorrhagic telangiectasia maps to chromosome 9q3. Nat Genet 6:205–209

ten Dijke P, Ichijo H, Franzen P, Schulz P, Saras J, Toyoshima H, Heldin CH, et al (1993) Activin receptor-like kinases: a novel subclass of cell-surface receptors with predicted serine/threonine kinase activity. Oncogene 8:2879–2887

Vase P, Holm M, Arendrup H (1985) Pulmonary arteriovenous fistulas in hereditary hemorrhagic telangiectasia. Acta Med Scand 218:105–109

Vincent P, Plauchu H, Hazan J, Faure S, Weissenbach J, Godet J (1995) A third locus for hereditary haemorrhagic telangiectasia maps to chromosome 12q. Hum Mol Genet 4:945–949

Yamamoto H, Ueno H, Ooshima A, Takeshita A (1996) Adenovirus-mediated transfer of a truncated transforming growth factor-β (TGF-β) type II receptor completely and specifically abolishes diverse signaling by TGF-β in vascular wall cells in primary culture. J Biol Chem 271:16253–16259

Zhang Y, Feng XH, Wu RY, Derynck, R (1996) Receptor-associated Mad homologues synergize as effectors of the TGF-β response. Nature 383:168–172