Autosomal dominant hereditary benign telangiectasias maps to the CMC1 locus for capillary malformation on chromosome 5q14

F Brancati, E M Valente, G Tadini, V Caputo, A Di Benedetto, C Gelmetti, B Dallapiccola

LETTER TO JMG

Telangiectases are characterised by an abnormal permanent dilatation of end vessels—mainly venules but occasionally also capillaries and arterioles—in the sub-papillary plexus in the upper part of the dermis.1 Hereditary benign telangiectasia (HBT; OMIM 187260) is a rare genetic skin disorder classified among the primary or idiopathic telangiectases.1 Affected individuals present with widespread benign telangiectasia (HBT; OMIM 187260) is a rare genetic disorder.2 3 5–10 However, owing to the small size of these families, no locus has been identified so far.12

Capillary malformation (CM or “port wine stain”; OMIM 163000) is a common vascular anomaly occurring in 0.3% of newborns and can be inherited as an autosomal dominant trait with incomplete penetrance and variable expression.13–15 CM usually presents as a single flat lesion located in the head and neck, typically changing in colour from pink to purple with age.11 In published reports, HBT and CM have often been considered distinct disorders, based on the clinical presentation of cutaneous lesions, but overlapping phenotypes have been described.6 12

The family was first tested for linkage to the genetic regions containing Endoglin and ALK1, the two known genes responsible for hereditary haemorrhagic telangiectasia, on chromosomes 9q (ORW1 – OMIM 187300) and 12q (ORW2 – OMIM 600376), respectively.12

Before the reported linkage of CMC1 to chromosome 5q,12 we analysed 380 microsatellite markers covering the whole genome, with an average distance between two adjacent markers of 10 cm (ABI PRISM linkage mapping set, version 2). Markers were run on a 3100 automated DNA sequencer (ABI PRISM) and analysed using Genescan and Genotyper software. Two-point LOD scores were generated with the FASTLINK version of the MLINK program,16 assuming equal male–female recombination rate, autosomal dominant inheritance, a gene frequency of 0.0001, equal allele frequencies for each marker, and reduced penetrance (0.50 to 0.95). Haplotypes were manually constructed and phase was assigned based on the smallest number of recombinants. Genetic distances between markers were taken from the Marshfield Centre for Medical Genetics (www.marshfieldclinic.org/research/genetics/), while physical distances were taken from the University of California Santa Cruz draft of the human genome, release “November 2002” (www.genome.ucsc.edu) (table 1).

To refine the upper and lower limit of the linked region, three novel polymorphic markers (arbitrarily named RST2, RST3, and RIT3) were selected from the human genome.

Key points

- The opportunity was taken to study a large Italian kindred with 13 individuals in three consecutive generations with autosomal dominant hereditary benign telangiectasia (HBT; OMIM 187260), the benign variant of hereditary haemorrhagic telangiectasia (HHT or Rendu-Osler-Weber disease; OMIM 187300 and 600736).4 Several familial cases showing autosomal dominant inheritance have been described.5–10 However, owing to the small size of these families, no locus has been identified so far.

- A genome-wide search in this family allowed mapping the disease to a 7 Mb (about 11 cM) interval on chromosome 5q14. A locus for familial capillary malformation (CM or “port wine stain”; OMIM 163000), named CMC1, was recently assigned to the same chromosomal interval, although CMC1 spans a larger region of 19 Mb (23 cM).

- HBT and CM have usually been considered distinct disorders, based on the different clinical presentation of cutaneous lesions. However, overlapping phenotypes have been described in some families, suggesting that both conditions are part of the wide phenotypic spectrum of the same clinical entity. Thus the narrowing of the CMC1 locus represents an important step towards the identification of the disease causing gene.

METHODS

We ascertained a family with HBT from northern Italy. After obtaining informed consent, 20 family members and four spouses underwent a detailed dermatological examination and a blood sample was taken. Three affected individuals had a skin biopsy. Skin samples were stained with haematoxylin-eosin and examined by light microscopy.

Genomic DNA was extracted from blood samples following standard procedures.

The family was first tested for linkage to the genetic regions containing Endoglin and ALK1, the two known genes responsible for hereditary haemorrhagic telangiectasia, on chromosomes 9q (ORW1 – OMIM 187300) and 12q (ORW2 – OMIM 600376), respectively.

Abbreviations: CM, capillary malformation; HBT, hereditary benign telangiectasia; HHT, hereditary haemorrhagic telangiectasia

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These markers were amplified using the following pairs of primers: RST2-F: 5'-CCCTGCTGCTGTTTATTTTT-3' and RST2-R: 5'-AGGAGCCATAGCCTCTCTTT-3'; RST3-F: 5'-ACTTCCTAGCAAACTCCCACC-3' and RST3-R: 5'-GCGACCTAATGCGGGTTTCTT-3'; RIT3-F: 5'-GCCAACCTAGGAAACC TTTAAC-3' and RIT3-R: 5'-TGAAGTTATAGCGCAGACC TGA-3'. For each pair, the forward primer was fluorescently labelled with either an FAM or a HEX dye. The position of these novel markers on the physical map of human chromosome 5 is also shown in table 1.

RESULTS

The pedigree of the HBT family is shown in fig 1. The inheritance is autosomal dominant, with no apparent lack of penetrance. Thirteen of 20 family members (four male and nine female) had a definite diagnosis of HBT. Table 2 summarises the clinical features of the affected individuals. The age of onset could not always be determined accurately, but some parents noted the onset of macular telangiectases in their sons during the first months of life. A large variability in size (1 to 6.4 cm) and number (1 to 10) of telangiectases was observed among the affected family members, but

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Pairwise LOD scores between hereditary benign telangiectasia and markers on chromosome 5q14</th>
</tr>
</thead>
<tbody>
<tr>
<td>Markers</td>
<td>cM</td>
</tr>
<tr>
<td>DSS672</td>
<td>86.26</td>
</tr>
<tr>
<td>RST2</td>
<td>no</td>
</tr>
<tr>
<td>RST3</td>
<td>no</td>
</tr>
<tr>
<td>DSS641</td>
<td>92.38</td>
</tr>
<tr>
<td>DSS428</td>
<td>95.40</td>
</tr>
<tr>
<td>DSS517</td>
<td>95.40</td>
</tr>
<tr>
<td>RST3</td>
<td>no</td>
</tr>
<tr>
<td>DSS1725</td>
<td>97.82</td>
</tr>
</tbody>
</table>

cM, position of the microsatellite marker on the genetic map (in centimorgan). This is not available for the three newly generated markers RST2, RST3 and RIT3.

Mb, position of the microsatellite marker on the physical map (in megabases).

Table 1

<table>
<thead>
<tr>
<th>Markers</th>
<th>cM</th>
<th>Mb</th>
</tr>
</thead>
<tbody>
<tr>
<td>DSS672</td>
<td>86.26</td>
<td>79.126</td>
</tr>
<tr>
<td>RST2</td>
<td>no</td>
<td>80.785</td>
</tr>
<tr>
<td>RST3</td>
<td>no</td>
<td>81.387</td>
</tr>
<tr>
<td>DSS641</td>
<td>92.38</td>
<td>82.230</td>
</tr>
<tr>
<td>DSS428</td>
<td>95.40</td>
<td>85.598</td>
</tr>
<tr>
<td>DSS517</td>
<td>95.40</td>
<td>86.270</td>
</tr>
<tr>
<td>RST3</td>
<td>no</td>
<td>87.790</td>
</tr>
<tr>
<td>DSS1725</td>
<td>97.82</td>
<td>89.457</td>
</tr>
</tbody>
</table>

Figure 1 Pedigree of the family and haplotypes of marker loci spanning the linked region on chromosome 5q14. Black symbols denote affected individuals, deceased members are marked with a diagonal bar. A thin horizontal line above symbols indicates members of the family who were examined clinically and had blood samples taken. The arrow indicates the proband. The black bar denotes the haplotype segregating with the disease in the family.
lesions invariably became paler with increasing age. Only one patient (IV:1) had mucous membrane involvement, with a lesion affecting the vermillion border of the upper lip. Histological examination showed normal epidermis and dilatation of the smallest blood vessels of the upper part of dermis (fig 2).

Linkage to ORW1 and ORW2 was excluded, with negative LOD scores across both regions, ruling out the hypothesis that HBT could represent a benign allelic variant of either endoglin or ALK1 genes. Analysis of the 380 markers from the genome-wide search produced negative or non-significant LOD scores for all tested loci, except for nine markers on chromosomes 5, 9, 16, 17, and 18. The regions surrounding these loci were saturated with more microsatellite markers and haplotypes were manually constructed. The segregation of different haplotypes in affected individuals and the negative LOD scores obtained allowed excluding all regions but an 11.5 cM interval on chromosome 5q, where a common haplotype was shared by all affected family members. All genotyped markers within this region generated positive LOD score values with a maximum pairwise LOD score of 5.27 for marker D5S641 (θ = 0; penetrance = 0.95) (table 1).

Calculation of pairwise LOD scores assuming lower penetrance values (0.50 to 0.90) under the assumption “affected individuals only” did not result in a significant change (data not shown). To determine the minimum linked interval, we then genotyped three novel polymorphic markers selected from the human genome working draft. The upper and lower boundaries of the region were determined by recombination events occurred in individuals II:2 and II:4, respectively, refining the locus to a 7 Mb interval flanked by marker D5S641 and D5S1194 (hence the name “port-wine stain”). These are usually unilateral with a fairly sharp midline cut-off. Associated eye and brain abnormalities occur in 8–15% of patients with facial port-wine stains (osseous malformations, glaucoma, Sturge-Weber and Klippel-Trenaunay syndromes). Although a typical port-wine stain was not observed in any of the 13 affected individuals in the present family, atypical lesions—difficult to frame within one or the other disorder—have been reported. Moreover, both conditions share similar histological changes characterised by a dilatation of dermal thin walled blood vessels. These observations suggest that HBT and CM represent variable clinical presentations of the same disorder and linkage of HBT to CMC1 corroborates this hypothesis. In this light, the 7 Mb region identified in the present family could significantly narrow the CMC1 locus, representing an important step towards the identification of the causative gene.

This region could be further narrowed to 6.4 Mb by considering individual IV:4, who is unaffected and carries part of the disease haplotype (fig 1). However, even if penetrance appears to be very high in this family, lack of penetrance in subject IV:4 cannot be excluded with certainty. For this reason, penetrance values used for LOD score calculations have been ranged up to 0.95.

The linked region contains 15 genes, some of which represent suitable candidates for cutaneous vascular anomalies. EDIL-3 (EGF-like repeats and discoidin I-like domains 3) encodes Del1, a protein expressed by endothelial cells during early embryogenesis. Versican is a modular proteoglycan involved in the control of cellular growth and differentiation. Although versican is generally known as a proteoglycan involved in the control of cellular growth and differentiation. Although versican is generally known as a large chondroitin sulphate proteoglycan, the smallest splice variant (V3) consists only of the amino- and carboxy-terminal globular domains. If overexpressed, V3 has been shown to alter arterial smooth muscle cell adhesion, migration, and proliferation in vitro. Another interesting gene is RASA1 (RAS p21 protein activator-1), as mosaic mice composed of wild-type and RASA1 null cells show localised vascular defects at E15. The identification of the gene responsible for HBT and CM will help formulate genotype-phenotype correlations within the wide clinical spectrum of

Table 2: Clinical data of the 13 affected members of the family with hereditary benign telangiectasia

<table>
<thead>
<tr>
<th>Individual</th>
<th>Sex</th>
<th>Age at examination (years)</th>
<th>Number of lesions (size, maximum diameter)</th>
<th>Distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>II:2</td>
<td>M</td>
<td>68</td>
<td>2 (1–2 cm)</td>
<td>Upper back</td>
</tr>
<tr>
<td>II:4</td>
<td>M</td>
<td>71</td>
<td>2 (1–5 cm) and 5 cm</td>
<td>Right shoulder and back</td>
</tr>
<tr>
<td>II:6</td>
<td>F</td>
<td>78</td>
<td>1 (2 cm)</td>
<td>Right periocular region</td>
</tr>
<tr>
<td>II:7</td>
<td>M</td>
<td>80</td>
<td>1 (2 cm)</td>
<td>Forehead</td>
</tr>
<tr>
<td>III:1</td>
<td>F</td>
<td>38</td>
<td>&gt;10 (1–6 cm)</td>
<td>Neck, thorax, back, left arm pit</td>
</tr>
<tr>
<td>III:2</td>
<td>F</td>
<td>35</td>
<td>2 (2–3 cm)</td>
<td>Right foot and right shoulder</td>
</tr>
<tr>
<td>III:3</td>
<td>F</td>
<td>30</td>
<td>2 (1–2 cm)</td>
<td>Right leg, wrist</td>
</tr>
<tr>
<td>III:4</td>
<td>M</td>
<td>41</td>
<td>2 (4–5 cm)</td>
<td>Both legs</td>
</tr>
<tr>
<td>III:5</td>
<td>F</td>
<td>39</td>
<td>4 (1–3 cm)</td>
<td>Left arm and leg</td>
</tr>
<tr>
<td>III:7</td>
<td>F</td>
<td>53</td>
<td>4 (2 cm)</td>
<td>Right ear, right shoulder, neck</td>
</tr>
<tr>
<td>III:10</td>
<td>F</td>
<td>50</td>
<td>6 (1–3 cm)</td>
<td>Right leg, both arms</td>
</tr>
<tr>
<td>III:12</td>
<td>F</td>
<td>40</td>
<td>5 (1–3 cm)</td>
<td>Both legs, left arm</td>
</tr>
<tr>
<td>IV:1</td>
<td>F</td>
<td>6</td>
<td>5 (2–6 cm)</td>
<td>Forehead, neck, upper lip, thorax</td>
</tr>
</tbody>
</table>

M, male; F, female.
these conditions and give insight into the mechanisms leading to vascular malformations.

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REFERENCES


Mutations of the β myosin heavy chain gene in hypertrophic cardiomyopathy: critical functional sites determine prognosis

A Woo, H Rakowski, J C Liew, M-S Zhao, C-C Liew, T G Parker, M Zeller, E D Wigle, M J Sole

Objectives: To assess patients with different types of mutations of the β myosin heavy chain (βMHC) gene causing hypertrophic cardiomyopathy (HCM) and to determine the prognosis of patients according to the affected functional domain of βMHC.

Design and setting: Cohort study of subjects referred to an HCM clinic at an academic hospital.

Patients: 70 probands from the HCM clinic were screened for mutations of the βMHC gene and 148 family members of the genotype positive probands were further assessed. The control group for the genetic studies consisted of 106 healthy subjects.

Main outcome measures: Direct DNA sequencing was used to screen 70 probands for mutations of the βMHC gene. Family members underwent genotypic and detailed clinical, ECG, and echocardiographic assessments. The survival of genotype positive subjects was evaluated according to the type of functional domain affected by the missense mutation and according to phenotypic characteristics.

Results: A mutation of the βMHC gene was detected in 15 of 70 probands (21%). Of 148 family members of the genotype positive probands were further assessed. The control group for the genetic studies consisted of 106 healthy subjects.

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Results: A mutation of the βMHC gene was detected in 15 of 70 probands (21%). Of 148 family members studied in these 15 families, 74 were identified with a βMHC defect. Eleven mutations were detected, including four novel mutations: Ala196Thr, Pro211Leu, Val404Leu, and Arg870Cys. Median survival was 66 years (95% confidence interval (CI) 64 to 77 years) in all affected subjects. There was a significant difference in survival between subjects according to the affected functional domain (p = 0.02). Significant independent predictors of decreased survival were the non-conservative (that is, associated with a change in the amino acid charge) missense mutations that affected the actin binding site (hazard ratio 4.4, 95% CI 1.6 to 11.8; p = 0.003) and those that affected the rod portion of βMHC (hazard ratio 4.8, 95% CI 1.2 to 19.4; p = 0.03). No phenotypic characteristics were associated with decreased survival or cardiovascular morbidity.

Conclusions: The type of βMHC functional domain affected by the missense mutation is predictive of overall prognosis in HCM.

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Mutations of the β myosin heavy chain gene in hypertrophic cardiomyopathy: critical functional sites determine prognosis

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