LETTER TO JMG

Autosomal dominant hereditary benign telangiectasia 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

J Med Genet 2003;**40**:849–853

Telangiectases are characterised by an abnormal permanent dilatation of end vessels—mainly venules but occasionally also capillaries and arterioles—in the subpapillary 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 cutaneous plaque-like, punctate, radiating or arborising telangiectases, which usually appear in early childhood with a random distribution. The young lesions are small and red and tend to increase in size with age, becoming softer and salmon-pink, almost like normal skin.[2 3] Lesions are invariably asymptomatic but can be responsible for mild cosmetic disability. There is no bleeding diathesis or systemic vascular lesions. Thus HBT is considered the benign form 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.[2 3 5–10] However, owing to the small size of these families, no locus has been identified so far.

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.[11–13] 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 in some families.[8 12] A locus for CM has recently been mapped to a 23-cM region on chromosome 5q13–q15 (*CMC1*).[12 13] Here we report clinical and histological features of a large HBT family which showed linkage to *CMC1*.

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 syndrome; OMIM 187300 and 600736).
- 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.

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,[14] 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 genetic map of 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

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

Table 1 Pairwise LOD scores between hereditary benign telangiectasia and markers on chromosome 5q14

			LOD scores at θ =						
Markers	**cM**	**Mb**	**0.0**	**0.01**	**0.05**	**0.1**	**0.2**	**0.3**	**0.4**
D5S672	86.26	79.126	−1.63	1.62	2.22	2.29	1.94	1.29	0.52
RST2	na	80.785	−0.61	2.59	3.00	2.91	2.36	1.58	0.67
RST3	na	81.387	2.74	2.77	2.77	2.64	2.17	1.50	0.69
D5S641	92.38	82.230	5.27	5.18	4.83	4.37	3.37	2.24	0.99
D5S428	95.40	85.598	4.87	4.78	4.45	4.02	3.07	2.01	0.86
D5S617	95.40	86.270	4.54	4.47	4.15	3.74	2.84	1.83	0.75
RIT3	na	87.790	−0.65	2.54	2.94	2.85	2.31	1.56	0.70
D5S1725	97.82	89.457	−1.35	1.89	2.32	2.28	1.85	1.22	0.50

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).
na, not available.

working draft using the tandem repeat finder software.[15] These markers were amplified using the following pairs of primers: RST2-F: 5′-CCCTGCTGCTGTTTATTTTT-3′ and RST2-R: 5′-AGGAGCCATAGCCTCTCTTT-3′; RST3-F: 5′-ACT TCCTAGCAAACTCCCAACC-3′ and RST3-R: 5′-GGCAACT AATATGGCCTTTTCTT-3′; RIT3-F: 5′-CGCAACTGAGGAACC TTTTAAC-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 (1×1 to 6×4 cm) and number (1 to >10) of telangiectases was observed among the affected family members, but

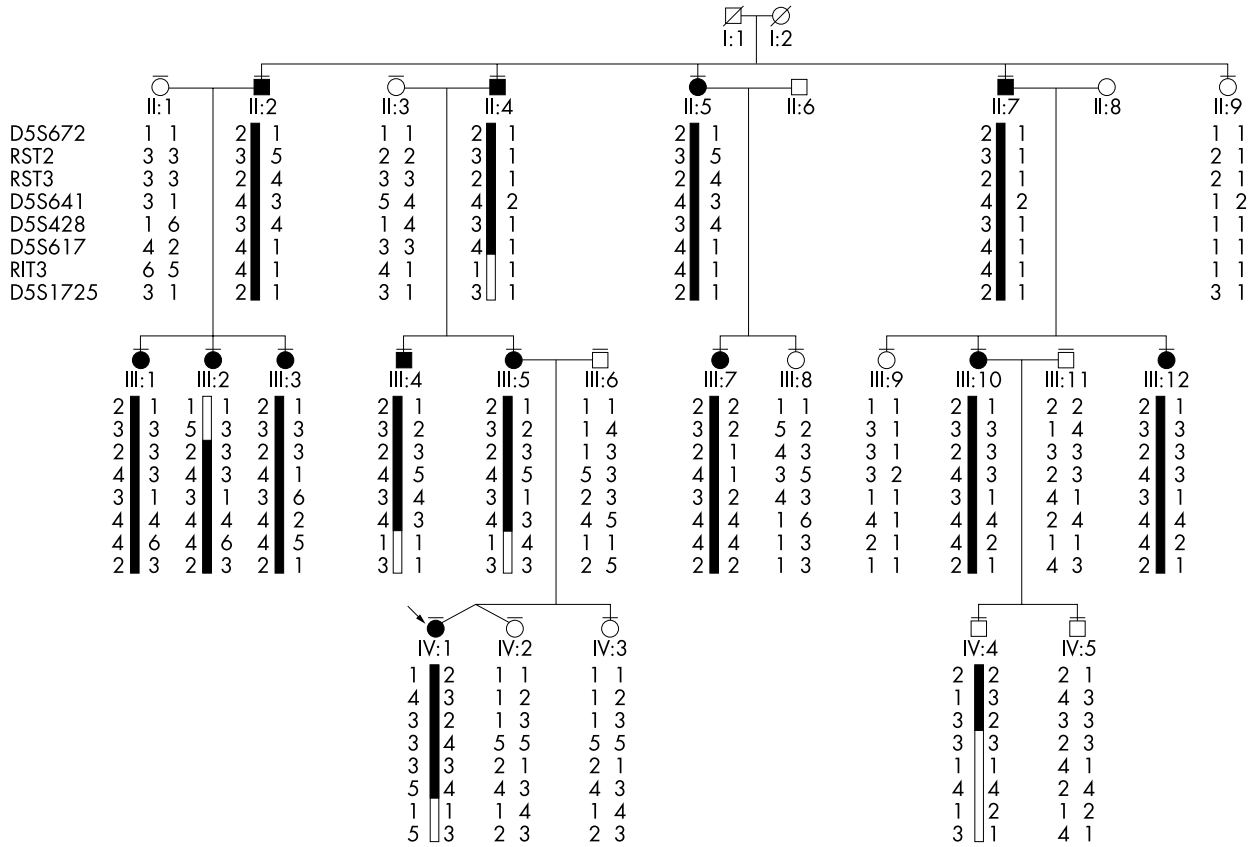

Figure 1 Pedigree of the family and haplotypes of marker loci spanning the linked region on chromosome 5q14. Black symbols denote affected individuals, deceased members are marked with a diagonal bar. A thin horizontal line above symbols indicates members of the family who were examined clinically and had blood samples taken. The arrow indicates the proband. The black bar denotes the haplotype segregating with the disease in the family.

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

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

M, male; F, female.

lesions invariably became paler with increasing age. Only one patient (IV:1) had mucous membrane involvement, with a lesion affecting the vermilion 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 ($\theta = 0$; penetrance = 0.95) (table 1). Calculation of pairwise LOD scores assuming lower penetrance values (0.50 to 0.90) and 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 III:2 and II:4, respectively, refining the locus to a 7 Mb interval flanked by markers RST2 and RIT3. Only one of eight unaffected individuals (IV:4, age 15 years) carried part of the disease haplotype, from D5S672 to RST3 (fig 1).

DISCUSSION

We report the assignment of a locus for HBT to chromosome 5q14 in a large kindred from northern Italy. This is the largest HBT family reported to date; the wide phenotypic variability observed among the 13 affected individuals runs through the spectrum of HBT lesions described so far.[2 3 5–10]

While this work was in progress, the locus for capillary malformation (*CMC1*) was assigned to a 19 Mb region on 5q13–15.[12 13] The linked interval in the present family spans 7 Mb on chromosome 5q14 physical map and coincides to *CMC1*. Among cutaneous vascular anomalies, HBT and CM are usually classified in two distinct groups. In particular, while CM is considered a vascular malformation, HBT and other telangiectases (such as HHT) fall within the group of disorders caused by dilatation of pre-existing vessels.[4]

The typical cutaneous lesion in CM is congenital and appears as a single, cherry-red macular stain, larger in size than HBT lesions. CMs tend to darken slowly but progressively throughout life, becoming raised and thickened plaques (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).[4] 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.[8 12] Moreover, both conditions share similar histopathological changes characterised by a dilatation of dermal thin walled blood vessels.[1] 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.[16] This protein has been shown to regulate the process of primary morphogenesis or vascular remodelling, both in vitro and in vivo.[17] Versican is a modular 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.[18] 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.[19] The identification of the gene responsible for HBT and CM will help formulate genotype–phenotype correlations within the wide clinical spectrum of

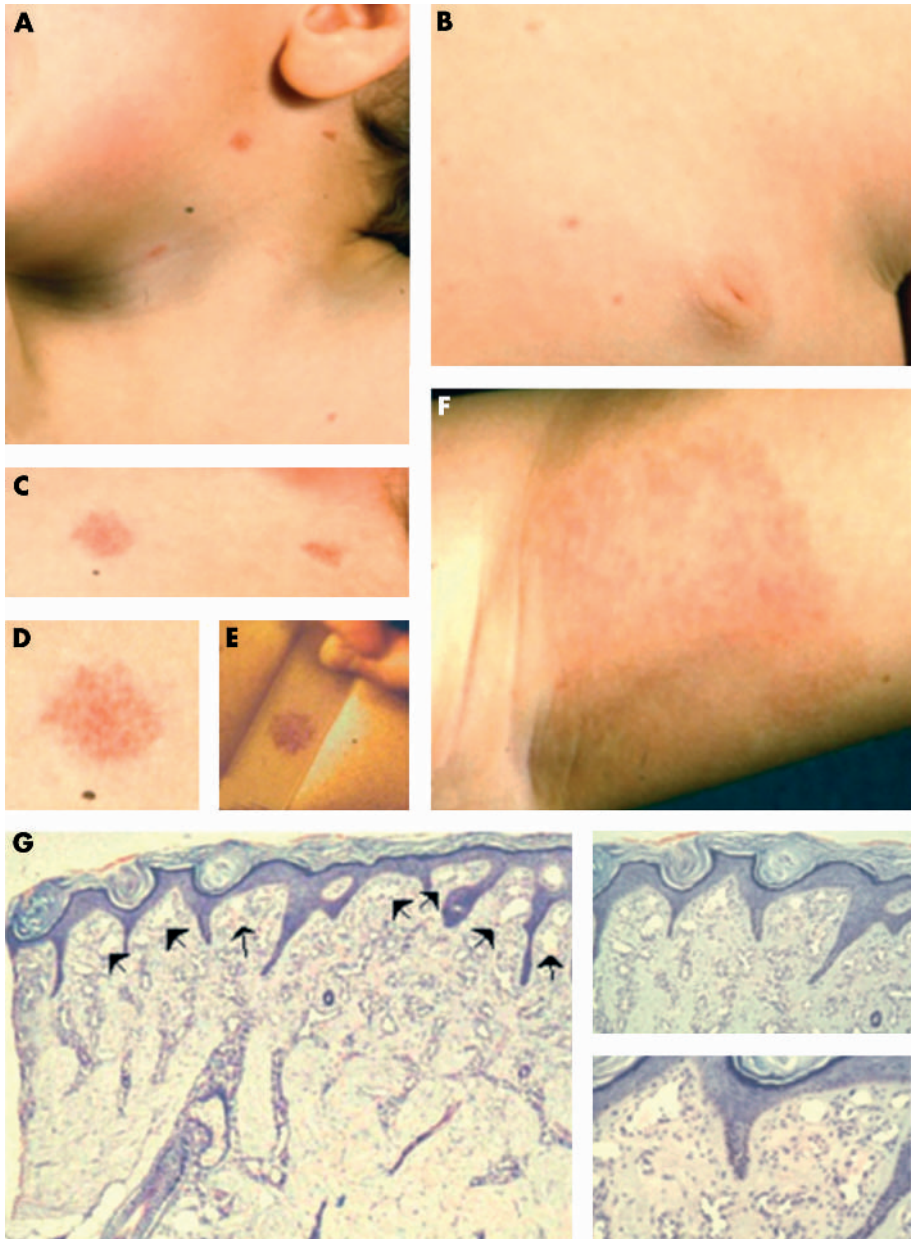

Figure 2 Clinical and histological characteristics of cutaneous lesions in HBT family. Telangiectases distributed on the neck and thorax in subject IV:1 (A–E); large macular telangiectatic lesion on the armpit in subject III:1 (F). Haematoxylin-eosin staining of skin biopsy in patient III:1, showing dilated thin walled vessels (black arrows) lined by a single layer of endothelium within the upper dermis (G).

these conditions and give insight into the mechanisms leading to vascular malformations.

ACKNOWLEDGEMENTS

This work was financed by the Italian Ministry of Health by grant ''Ricerca Corrente 2003''.

Authors' affiliations

F Brancati V Caputo, B Dallapiccola, University of Rome ''La Sapienza'' and IRCCS-CSS, San Giovanni Rotondo and CSS-Mendel, Rome, Italy
E M Valente, IRCCS-CSS, San Giovanni Rotondo and CSS-Mendel, Rome
G Tadini, Malattie Cutanee Ereditarie, Ist di Sc Dermatologiche, Osp Maggiore Policlinico, University of Milan, Italy
C Gelmetti, Dermatologia Pediatrica, Ist di Sc Dermatologiche, Osp Maggiore Policlinico, University of Milan

F Brancati and E M Valente contributed equally to this work.

Correspondence to: Dr Enza Maria Valente, Neurogenetics, CSS Mendel Institute, Viale Regina Margherita 261, I-00168 Rome, Italy; e.valente@css-mendel.it

REFERENCES

1 **Rook A**, Wilkinson D, Ebling J (eds). *Textbook of dermatology*, 5th ed. Oxford: Blackwell Scientific Publications, 1992.
2 **Wells RS**, Dowling GB. Hereditary benign telangiectasia. *Br J Dermatol* 1971;**84**:93–4.
3 **Ryan TJ**, Wells RS. Hereditary benign telangiectasia. *Trans St Johns Hosp Dermatol Soc* 1971;**57**:148–56.
4 **Requena L**, Sangueza OP. Cutaneous vascular anomalies. Part I. Hamartomas, malformations, and dilation of preexisting vessels. *J Am Acad Dermatol* 1997;**37**:523–52.
5 **Gold MH**, Eramo L, Prendiville JS. Hereditary benign telangiectasia. *Pediatr Dermatol* 1989;**6**:194–7.
6 **Zahorcsek Z**, Schneider I. Hereditary benign telangiectasia. *Dermatology* 1994;**189**:286–8.
7 **McNicholl F**, McMullin MF, Nevin NC, *et al*. Hereditary benign telangiectasia: first family in Northern Ireland. *Ulster Med J* 1999;**68**:106–7.
8 **Onishi Y**, Ohara K, Shikada Y, *et al*. Hereditary benign telangiectasia: image analysis of hitherto unknown association with arteriovenous malformation. *Br J Dermatol* 2001;**145**:641–5.
9 **Puppin D**, Rybojad M, Morel P. Hereditary benign telangiectasia: two case reports. *J Dermatol* 1992;**19**:384–6.
10 **Watanabe M**, Tomita Y, Tagami H. Hereditary benign telangiectasia: a congenital type. *Dermatologica* 1990;**181**:152–3.
11 **Mulliken JB**, Young AE (eds). *Vascular birthmarks, hemangiomas and vascular malformations*. Philadelphia: WB Saunders Co, 1988:170–95.

12 **Breugem CC**, Alders M, Salieb-Beugelaar GB, *et al*. A locus for hereditary capillary malformations mapped on chromosome 5q. *Hum Genet* 2002;**110**:343–7.
13 **Eerola I**, Boon LM, Watanabe S, *et al*. Locus for susceptibility for familial capillary malformation (port-wine stain) maps to 5q. *Eur J Hum Genet* 2002;**10**:375–80.
14 **Cottingham RW**, Indury RM, Schaffer AA. Faster sequential genetic linkage computations. *Am J Hum Genet* 1993;**53**:252–63.
15 **Benson G**. Tandem repeats finder: a program to analyze DNA sequences. *Nucleic Acids Res* 1999;**27**:573–80.
16 **Hidai C**, Zupancic T, Penta K, *et al*. Cloning and characterization of developmental endothelial locus 1: an embryonic endothelial cell protein that binds the alphavbeta3 integrin receptor. *Genes Dev* 1998;**12**:21–33.
17 **Rezaee M**, Penta K, Quertermous T. Del1 mediates VSMC adhesion, migration, and proliferation through interaction with integrin alpha(v)beta(3). *Am J Physiol Heart Circ Physiol* 2002;**282**:H1924–32.
18 **Lemire JM**, Merrilees MJ, Braun KR, *et al*. Overexpression of the V3 variant of versican alters arterial smooth muscle cell adhesion, migration, and proliferation in vitro. *J Cell Physiol* 2002;**190**:38–45.
19 **Henkemeyer M**, Rossi DJ, Holmyard DP, *et al*. Vascular system defects and neuronal apoptosis in mice lacking ras GTPase-activating protein. *Nature* 1995;**377**:695–701.

ECHO

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

Please visit the *Journal of Medical Genetics* website [www.jmedgenet.com] for a link to the full text of this article.

Objectives: To assess patients with different types of mutations of the β myosin heavy chain (β MHC) gene causing hypertrophic cardiomyopathy (HCM) and to determine the prognosis of patients according to the affected functional domain of β MHC.
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 β MHC 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 β MHC 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 β MHC gene was detected in 15 of 70 probands (21%). Of 148 family members studied in these 15 families, 74 were identified with a β MHC 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 β MHC (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 β MHC functional domain affected by the missense mutation is predictive of overall prognosis in HCM.

▲ *Heart* 2003;**89**:1179–1185.