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Birt-Hogg-Dubé syndrome: mapping of a novel hereditary neoplasia gene to chromosome 17p12-q11.2

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Birt-Hogg-Dubé syndrome (BHD) is an autosomal dominant neoplasia syndrome characterized mainly by benign skin tumors, and to a lesser extent, renal tumors and spontaneous pneumothorax. To map the *BHD* locus, we performed a genome-wide linkage analysis using polymorphic microsatellite markers on a large Swedish BHD family. Evidence of linkage was identified on chromosome 17p12-q11.2, with a maximum LOD score of 3.58 for marker D17S1852. Further haplotype analysis defined a ~35 cM candidate interval between the two flanking markers, D17S1791 and D17S798. This information will facilitate the identification of the *BHD* gene, leading to the understanding of its underlying molecular etiology. *Oncogene* (2001) **20,** 5239–5242.

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Birt-Hogg-Dubé (BHD [MIM 135150]), characterized by a triad of cutaneous lesions composing multiple fibrofolliculomas, trichodiscomas and acrochordons, was first described by Birt *et al*. (1977). The trichodiscomas and fibrofolliculomas typically appear as multiple small, dome-shaped, yellowish or skin-colored papules, scattered over the face, neck, scalp and upper trunk. They are tumors of the perifollicular mesenchyme and it is not possible to distinguish them clinically. Fibrofolliculomas consist of abnormal hair follicles with epithelial strands extending from the infundibulum of the hair follicle into a hyperplastic mantle of fibrous tissue. The epithelial strands may anastomose or rejoin the infundibulum at several points. Trichodiscomas are hamartomas of the dermal portion of the hair disc and share the histological features of fibrofolliculomas, but contain predominantly the fibrous component. The third skin lesions, the acrochodons, are less specific and reports have shown cases whereby patients with fibrofolliculomas and trichodiscomas did not have acrochodons (Foucar *et al*., 1981; Starink *et al*., 1985; Starink and Brownstein, 1987; Moreno *et al*., 1985). Moreover, it has been argued that the clinically acrochodon-like lesions in BHD patients indeed are histological variations of the trichodiscoma/fibrofolliculoma spectrum and not a separate entity (De la Torre *et al*., 1999). BHD is transmitted in an autosomal dominant fashion and the skin lesions are usually shown in the third decade of life. As BHD is a rare neoplasia syndrome, its population frequency and occurrence in sporadic versus familial cases are yet to be established.

A wide spectrum of BHD-related features has been described and listed by Schulz and Hartschuh (1999). The BHD-related renal tumors, for example, appear to vary in histological features and have been reported as oncocytoma, papillary RCC and clear cell RCC (Roth *et al*., 1993; Toro *et al*., 1999). Lung diseases such as bronchiectasis, bronchospasm (Moreno *et al*., 1985) and spontaneous pneumothorax (Chung *et al*., 1996; Toro *et al*., 1999) have also been associated with BHD patients. In addition, colonic neoplasms (Rongioletti *et al*., 1989; Haimowitz *et al*., 1997), large connective tissue nevus (Weintraub and Pinkus, 1977), multiple lipomas, angiolipomas and parathyroid adenomas (Chung *et al*., 1996) have been described.

To date the locus of the *BHD* gene remains unknown. Here, we performed a genome-wide scan on 17 individuals of a Swedish pedigree (Figure 1) to identify the susceptibility locus of *BHD*. The study was approved by the Karolinska Hospital Ethics Committee in Sweden and the Internal Review Board of the Van Andel Research Institute in USA. To date, 10 family members have been confirmed affected and their clinical features are summarized in Table 1. There are two cases of confirmed renal cell carcinoma (RCC) in the family. One is a unique case of clear cell RCC with focal papillary structures (IV : 4, Figure 2) and the second, bilateral chromophobe RCC (V : 3). The wide spectrum of renal histology found here and among other families (Roth *et al*., 1993; Toro *et al*.,

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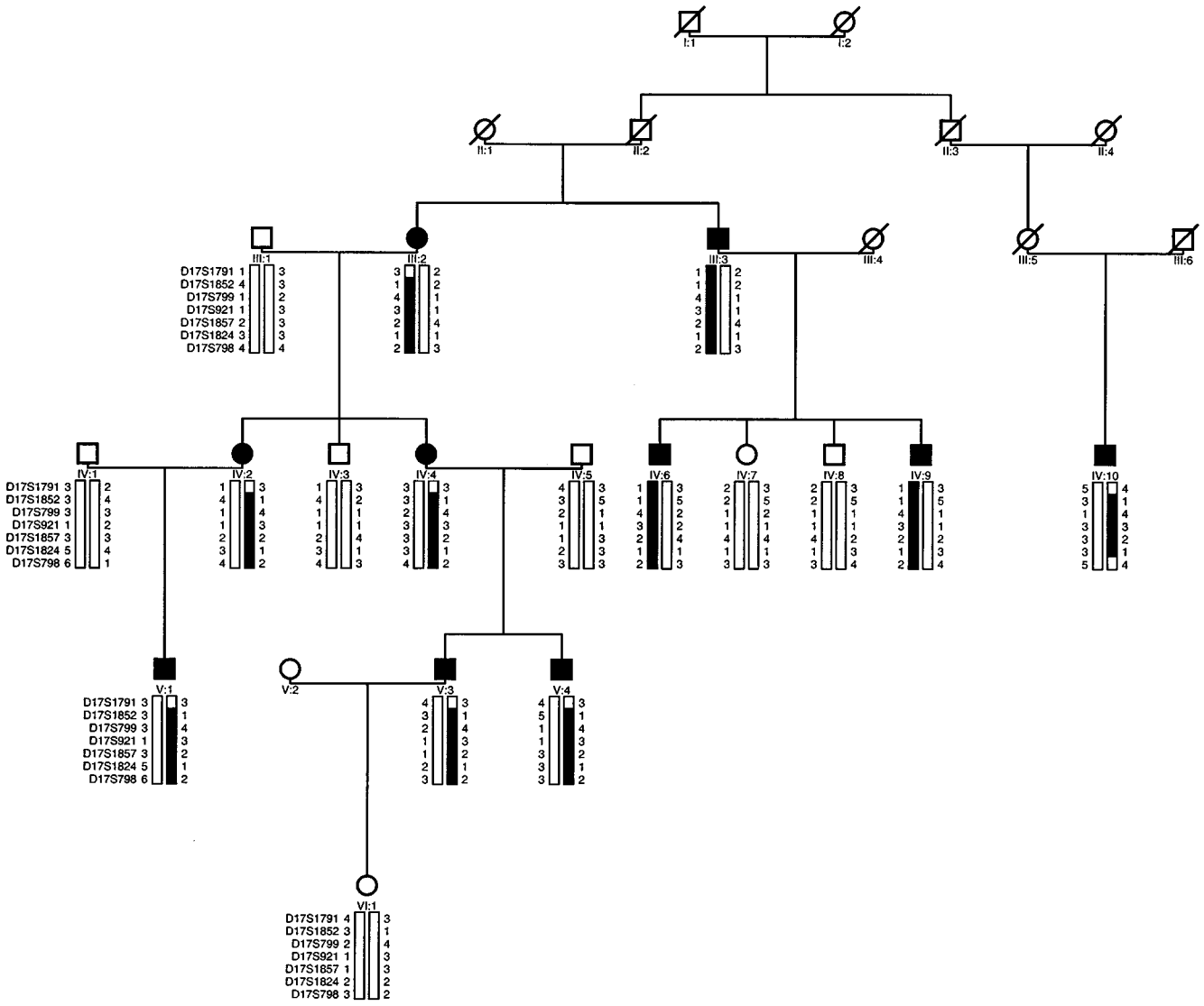

Figure 1 Pedigree of the Swedish family with BHD, showing the candidate interval between proximal marker D17S1791 (individual III : 2, IV : 2, IV : 4, IV : 10, V : 1, V : 3, and V : 4) and distal marker D17S798 (individual IV : 10). Blackened bars represent disease-bearing chromosomes and blackened symbols denote affected individuals

Table 1 Clinical features of 10 individuals with affected status

Individual	*Age*	*Histological verification of FF[a] and/or TD[b]*			*Acrochodons*	*Renal lesions*	*Other findings*
		< 10	*10 – 100*	*> 100*			
III:2	81		x		x	Cysts	Sarcoma dorsi, fibroadenomatosis of breast
III:3	82			x	x	Cysts	None
IV:2	58	x			x	Cysts	Fibroadenomatosis of breast
IV:4	61			x	x	Tumor[1]	Multiple lipomas, eight spontaneous pneumothorax
IV:6	50		x			Cysts	Multiple lipomas
IV:9	52	x				None	Multiple lipomas
IV:10	58	x			x	N/A	None
V:1	30	x				None	None
V:3	35			x	x	Tumor[2]	15 spontaneous pneumothorax
V:4	40	x			x	Atypical cyst	Two spontaneous pneumothorax

FF[a] = Fibrofolliculomas; TD[b] = Trichodiscomas; Tumor[1] = Clear cell RCC with focal papillary structure; Tumor[2] = Bilateral chromophobe RCC; N/A = not available

Table 2 Two-point LOD scores between Birt-Hogg-Dubé syndrome and seven chromosome 17 microsatellite markers

	LOD Score at θ =						
Markers	*.00*	*.01*	*.05*	*.10*	*.20*	*.30*	*.40*
D17S1791	−8.361	−2.707	−0.804	−0.129	0.291	0.296	0.133
D17S1852	3.582	3.517	3.251	2.909	2.194	1.443	0.667
D17S799	3.473	3.408	3.142	2.800	2.086	1.335	0.572
D17S921	2.609	2.553	2.326	2.038	1.455	0.879	0.348
D17S1857	2.679	2.627	2.415	2.143	1.582	1.007	0.445
D17S1824	1.672	1.637	1.497	1.319	0.962	0.617	0.296
D17S798	−3.811	−0.403	0.760	1.077	1.076	0.780	0.360

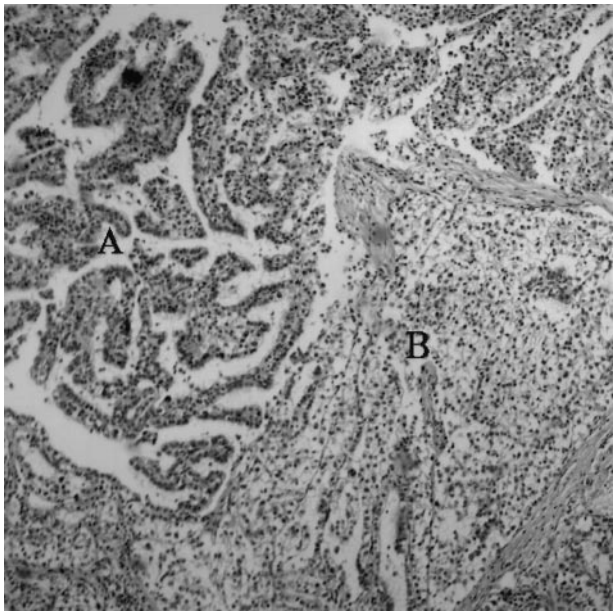

Figure 2 Microphotograph (hematoxylin-eosin staining) showing the mixed histology of the renal cell carcinoma from individual IC : 4; (A) Papillary component. (B) Clear cell component. Original magnification × 100

1999) suggest that the *BHD* gene may play an important role in the differentiation process of renal cells. In addition, two cases of breast fibroadenomatosis (III : 2 and IV : 2) are found in this family. Fibroadenoma of the breast is very common in the general population and therefore its association with BHD needs to be further explored.

Four hundred fluorescence-labeled primer pairs from ABI Prism human linkage mapping set version 2 (PE Biosystem) at an average spacing of 10 cM were used. PCR amplifications were performed according to the manufacturer's recommended protocols and set up in 96-well polypropylene microplates (MJ Research). PCR amplified products were denatured and size-fractioned on a 4.2% polyacrylamide gel on an ABI 377 sequencer (PE Biosystem). Genotypes were determined using Genescan version 3.1 and Genotyper version 2.5 (PE Biosystem). After preliminary analysis suggested its possible linkage to chromosome 17, additional chromosome 17 markers with 5 cM spacing were obtained from the ABI Prism human linkage mapping set-HD5 (PE Biosystem).

Linkage analysis was performed using two-point LOD score by MLink of the Linkage package (Lathrop *et al*., 1985; Terwilliger and Ott, 1994). The disease was modeled as autosomal dominant with complete penetrance. A conservative approach was applied in which age 50 was taken as the cut-off point: healthy individuals above 50 are considered as unaffected whereas those below 50 are considered as unknown. For example, individual VI : 1 (Figure 1) who is 9 years old, is considered as unknown. Genetic distances between microsatellite markers were obtained from the Center for Medical Genetics, Marshfield Medical Research Foundation.

We found a maximum LOD score (Z_{max}) between the disease locus and microsatellite marker (D17S1852 ($Z_{max} = 3.58$ at recombination fraction [θ] 0; Table 2). Additional two-point LOD scores were obtained with six other adjacent markers.

Haplotype analysis (Figure 1) showed a recombination event between markers D17S1791 and D17S1852 in individual III : 2, IV : 2, IV : 4, IV : 10, V : 1, V : 3, and V : 4, as well as between D17S1824 and D17S798 in individual IV : 10. Therefore the disease gene in this BHD family lies within a 35.5 cM region on chromosome 17p12-q11.2.

More than 100 known genes and anonymous ESTs have been assigned to this interval and some of these are interesting candidates for *BHD*. These include integrin β-4 (*ITGB4* [MIM 147557]) and flotillin 2 (*FLOT2* [MIM 131560]) both of which are involved in cell–matrix adhesion. Other candidates include mitogen-activated protein kinases (*MAPK7* [MIM 602521], *MAP2K3* [MIM 602315] and *MAP2K4* [MIM 601335]) which are the Ser/Thr protein kinases that participate in cellular signal transduction pathways. Previously, one of the Ser/Thr kinases, LKB1 or STK11, which is located in 19p has been associated with Peutz-Jeghers syndrome, an autosomal dominant disorder characterized by melanocytic macules on the lips, perioral and buccal regions and by multiple gastrointestinal polyps mostly of the hamartomatous type (Hemminki *et al*., 1998; Jenne *et al*., 1998).

A good way to shorten the list of potential candidates will be to know if the *BHD* gene is a tumor suppressor gene based on loss of heterozygosity (LOH) studies, which will involve the loss of the wild-type alleles in the BHD tumors. Therefore,

we are currently collecting the BHD tumors in this family for investigating their LOH status. In addition, more BHD families will be collected to establish its genetic homogeneity as well as to detect additional recombinations that may further narrow the mapped region.

In conclusion, we have mapped a candidate locus associated with BHD on chromosome 17p12-q11.2. This information can be used to identify individuals at risk in BHD families. It will also facilitate the identification of the *BHD* gene, which will enhance our understanding of the pathogenesis of BHD and its wide spectrum of tumors.

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