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Diaphyseal Medullary Stenosis with Malignant Fibrous Histiocytoma: a Hereditary Bone Dysplasia/Cancer Syndrome Maps to 9p21-22

John A. Martignetti,[1,2] Robert J. Desnick,[1,2] Elias Aliprandis,[1] Karen I. Norton,[2] Philip Hardcastle,[3*] Sydney Nade,[4] and Bruce D. Gelb[1,2]

Departments of [1]Human Genetics and [2]Pediatrics, Mount Sinai School of Medicine, New York; [3]Wembley, Western Australia; and [4]Department of Surgery, The University of Sydney, Sydney, Australia

Summary

Diaphyseal medullary stenosis with malignant fibrous histiocytoma (DMS-MFH) is an autosomal dominant bone dysplasia/cancer syndrome of unknown etiology. This rare hereditary cancer syndrome is characterized by bone infarctions, cortical growth abnormalities, pathological fractures, and eventual painful debilitation. Notably, 35% of individuals with DMS develop MFH, a highly malignant bone sarcoma. A genome scan for the DMS-MFH gene locus in three unrelated families with DMS-MFH linked the syndrome to a region of ~3 cM on chromosome 9p21-22, with a maximal two-point LOD score of 5.49 (marker D9S171 at recombination fraction [θ] .05). Interestingly, this region had previously been shown to be the site of chromosomal abnormalities in several other malignancies and contains a number of genes whose protein products are involved in growth regulation. Identification of this rare familial sarcoma-causing gene would be expected to simultaneously define the cause of the more common nonfamilial, or sporadic, form of MFH—a tumor that constitutes ~6% of all bone cancers and is the most frequently occurring adult soft-tissue sarcoma.

Introduction

Diaphyseal medullary stenosis with malignant fibrous histiocytoma (DMS-MFH) is a rare, autosomal dominant bone dysplasia/cancer syndrome (MIM 112250) of unknown etiology (Arnold 1973; Hardcastle et al. 1986). The bone dysplasia is uniquely characterized by cortical growth abnormalities, including diffuse DMS with overlying endosteal cortical thickening and scalloping, metaphyseal striations, infarctions, and scattered sclerotic areas of the long bones. Clinical features include pathological fractures with subsequent poor healing or nonunion, progressive wasting and bowing of the legs, and painful debilitation. The development of presenile cataracts has also been noted (Arnold 1973; Hardcastle et al. 1986). Most notably, between the second and fifth decades of life, 35% of DMS-MFH gene carriers develop the bone sarcoma, MFH (Arnold 1973; Hardcastle et al. 1986; Norton et al. 1996).

DMS-MFH is the only known familial form of MFH. In its sporadic form, MFH accounts for ~6% of all bone tumors and is the most common adult soft-tissue sarcoma (Mankin et al. 1991; Posner and Brennan 1991). The tumor is believed to be of primitive mesenchymal origin, representing a neoplasm arising from a histiocytic cell or a "facultative fibroblast" (see Unni 1996). Although molecular analyses of these tumors have begun to reveal a number of prognostic markers (Brinck et al. 1995; Reid et al. 1996; Larramendy et al. 1997), no consistent diagnostic molecular markers have been defined (Szymanska et al. 1995; Larramendy et al. 1997; Walter et al. 1997). Because sporadic and inherited cancer forms are often genetically equivalent (see Fearon 1997), identification of the DMS-MFH gene would elucidate the molecular origins of a highly malignant sarcoma.

Since the cause of DMS-MFH is unknown, our studies have focused on identification of the DMS-MFH gene by a positional cloning strategy. Three families with DMS-MFH were recruited, and after a genomewide search, linkage to chromosome region 9p21-22 was established for the syndrome. Localization and characterization of the DMS-MFH gene will permit improved and earlier diagnosis of at-risk patients, will further understanding of normal bone development and metabolism, and will identify a novel tumor-suppressor gene.

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Address for correspondence and reprints: Dr. John A. Martignetti, Department of Human Genetics, Mount Sinai School of Medicine, Box 1498, Fifth Avenue at 100th Street, New York, NY 10029. E-mail: jam@msvax.mssm.edu

* Dr. Hardcastle is in private practice.

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Patients and Methods

Sample Collection

After the subjects gave informed consent, blood samples were obtained from a total of 42 family members and appropriate spouses from the three families with DMS-MFH (Arnold 1973; Hardcastle et al. 1986; Norton et al. 1996). Immortalized lymphoblastoid cell lines were established (Anderson and Gusella 1984), and high-resolution cytogenetic analysis was performed (Verma and Babu 1995) on an index patient from each family. The disease status of each family member included in this study was diagnosed by plain-film x-ray; the findings are shown in figure 1.

Linkage Analysis

Microsatellite markers from the Human Screening Panel, version 4.0 (Research Genetics), were used for the genome scan. Additional markers to further define the critical region were obtained (Research Genetics). PCR amplifications were performed with a radioactively end-labeled primer, as described elsewhere (Gelb et al. 1995). Genotype data were entered into the LABMAN (Adams 1994) custom data management system, which generated the input files for linkage analysis. Two-point linkage analysis was performed with the computer program LINKAGE (version 5.1) and its associated subprograms (Ott and Terwilliger 1994). DMS-MFH was assumed to be a completely penetrant, genetically homogeneous, autosomal dominant disease with an allele prevalence of 0.00001. Sex-averaged genetic recombination maps were used to derive the intermarker distances.

Results

Linkage Analysis

The pedigrees of the three DMS-MFH families recruited for our study were defined (fig. 1). Blood samples were obtained with informed consent from 19, 3, and 20 informative family members (and from relevant spouses) of families 1, 2, and 3, respectively. High-resolution cytogenetic analysis (>550 bands) performed on peripheral leukocytes from an affected member of each family revealed no abnormalities. A genomewide search using PCR-based microsatellite screening markers at a density of 10 cM was performed. Initial linkage to chromosome region 9p21-22 was obtained with marker D9S741, which gave a LOD score of $Z = 2.42$ at recombination fraction (θ) = .10. As shown in table 1, subsequent analysis with 11 additional markers from the region resulted in a maximal LOD score (Z_{max}) for marker D9S171 of 5.49 at $\theta = .05$, exceeding the minimal threshold for linkage of 3.3 (Lander and Kruglyak 1995). Markers D9S1814, IFNA, D9S1846, and D9S1870 had maximal positive LOD scores at $\theta = .00$. The clinical and radiological findings in the three families with DMS-MFH were identical, and positive LOD scores consistent with the hypothesis that DMS-MFH is genetically homogeneous were generated from all three families.

Haplotype Analysis

Haplotype analysis was performed to define the boundaries of the DMS-MFH locus. As shown in figure 2, a common DMS-MFH haplotype was present in four of the affected members of family 1 (V-5, V-7, V-8, and VI-7). The telomeric boundary of the region was defined by a recombinant event between markers D9S1778 and D9S1814 that was observed in affected individual VI-6. Further support for the telomeric border was provided by analysis of unaffected individual IV-6 in family 3, who inherited her familial DMS-MFH haplotype beginning at marker D9S1778 and extending telomeric to it. Accordingly, the DMS-MFH locus was confined to the region centromeric to D9S1778.

Haplotype analysis of affected individuals from family 3 defined the centromeric boundary of the locus. Affected individual III-3 and her two affected descendants, IV-9 and V-19, shared a DMS-MFH haplotype that differed from that of III-1 and her affected children, IV-2 and IV-5, by markers centromeric to D9S171. This indicated an ancestral recombinant event between markers D9S171 and D9S1679/D9S265. The DMS-MFH critical region was further defined as telomeric to D9S171 by a recombinant event between D9S1846/D9S1870 and D9S171 in affected individual IV-7. The two affected individuals in family 2, who shared identical haplotypes, provided no additional information.

Discussion

As part of our overall approach to identify the genetic defect underlying the hereditary skeletal dysplasia/bone cancer syndrome DMS-MFH, a positional cloning strategy was undertaken. A genomewide search of three multigenerational families with DMS-MFH, performed by use of microsatellite markers, localized the DMS-MFH gene to a region of ~3 cM between markers D9S1778 and D9S171 on chromosome region 9p21-22.

The DMS-MFH locus was placed on the chromosome 9 cytogenetic map by reference to the previously positioned interferon gene (*IFNA*). *IFNA,* mapping near the telomeric boundary of the DMS-MFH locus and producing a maximal LOD score of 3.69 at $\theta = .00$ (table 1), had previously been assigned to 9p22 by in situ hybridization (Trent et al. 1982). Molecular studies, performed by means of loss of heterozygosity (LOH) map-

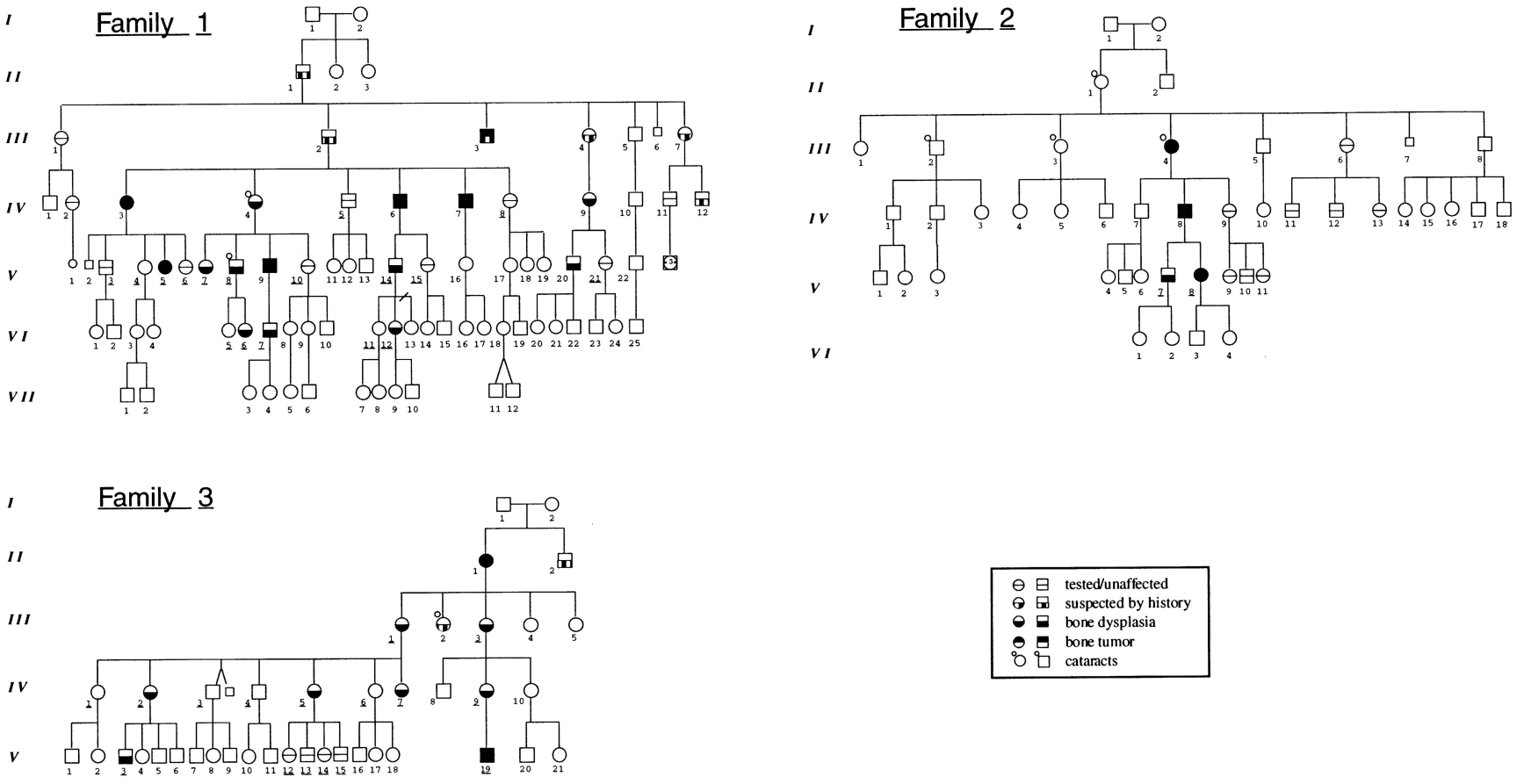

Figure 1 Pedigrees of the three DMS-MFH kindreds. Families 1 and 2 have been described elsewhere as the "American" and "Australian" families, respectively (Arnold 1973; Hardcastle et al. 1986), and findings on their disease status are here updated. Family 3 is the recently described "New York" family (Norton et al. 1996). Family members classified as "suspected by history" were unavailable for radiological diagnosis but had multiple (>2) pathological fractures and/or had children radiologically diagnosed with DMS-MFH. Dysplasia was diagnosed by plain-film x-ray. The identification numbers of individuals from whom blood samples were obtained are underlined.

Table 1

Two-Point Linkage Data between the DMS-MFH Locus and Chromosome 9 Polymorphic Markers

	LOD Score at θ =				
Locus	.00	.01	.05	.10	.20
D9S156	−∞	−1.62	−.34	.09	.31
	−∞	−1.46	−.78	−.52	−.28
	−∞	−.26	.29	.42	.36
	−∞	−3.34	−.83	−.01	.39
D9S157	−∞	.94	1.38	1.35	.95
	.26	.26	.22	.18	.11
	−∞	.02	.57	.67	.57
	−∞	1.22	2.17	2.20	1.63
D9S162	−.56	−.53	−.43	−.34	−.20
	.26	.26	.22	.18	.11
	−∞	.32	.87	.97	.84
	−∞	.05	.66	.81	.75
D9S1778	−∞	.53	.99	.99	.67
	.26	.26	.23	.18	.11
	.62	.73	.92	.95	.79
	−∞	1.52	2.14	2.12	1.57
IFNA	1.07	1.08	1.07	.99	.72
	.26	.25	.22	.19	.11
	2.36	2.32	2.15	1.92	1.44
	3.69	3.65	3.44	3.10	2.27
D9S1814	.48	.46	.39	.30	.13
	.22	.21	.18	.15	.09
	2.60	2.55	2.36	2.11	1.58
	3.30	3.22	2.93	2.56	1.80
D9S1846	−.28	−.26	−.18	−.11	−.04
	.26	.25	.23	.18	.11
	.56	.55	.50	.44	.32
	.54	.54	.55	.51	.39
D9S1870	2.22	2.20	2.10	1.91	1.42
	.26	.25	.21	.18	.11
	2.36	2.32	2.15	1.92	1.44
	4.84	4.77	4.46	4.01	2.97
D9S171	4.82	4.73	4.40	3.96	3.01
	.26	.26	.22	.18	.12
	−∞	.32	.87	.97	.84
	−∞	5.31	5.49	5.11	3.97
D9S1679	2.59	2.52	2.24	1.89	1.14
	.26	.25	.22	.18	.12
	−∞	−1.37	−.12	.27	.44
	−∞	1.40	2.34	2.34	1.70
D9S265	−∞	−.88	−.27	−.06	.05
	.26	.25	.22	.18	.11
	−4.70	−1.13	−.48	−.24	−.07
	−∞	−1.76	−.53	−.12	.09

Note.—Values given are pairwise cumulative LOD scores (*Z*) between the DMS-MFH gene and nine marker loci at different θ values for the three families with DMS-MFH, and their cumulative sums (i.e., in descending order, families 1, 2, 3, and the cumulative sum).

ping of bladder cancers, showed that the locus of the commonly deleted region at chromosomal bands 9p21-22 was between the interferon cluster and D9S171 (Stadler et al. 1994). Therefore, the DMS-MFH locus was assigned to the chromosomal region 9p21-22.

Comparison of the affected haplotypes revealed that the two American kindreds, families 1 and 3, shared an identical haplotype extending through the entire D9S1778-D9S171 region. This finding most likely reflects a shared ancestor, because this haplotype is apparently rare among whites, since it was not observed among 64 unrelated chromosomes in the CEPH database (Murray et al. 1994). The less likely possibility of independent mutations on a common haplotype could not be formally excluded. A detailed historical survey of these two kindreds revealed no known common ancestral surnames; however, the three families shared an English origin. Therefore, although we cannot definitively discriminate between the genetic origins of families 1 and 2, the independent genotype of family 3 and the positive LOD scores generated by this family remain consistent with genetic homogeneity for DMS-MFH.

Haplotype analyses of the members of the families with DMS-MFH had the additional benefit of ordering several polymorphic markers that previously colocalized on the Généthon map. The independent recombinant events between D9S1778 and D9S1814 observed in individuals VI-6 in family 1 and IV-6 in family 3 indicated that D9S1778 was telomeric to D9S1814. Similarly, the ancestral recombination observed in family 1 showed that D9S171 was telomeric to D9S265 and D9S1679, as shown in figure 2. Hence, the order "centromere-[D9S265/D9S1679]-D9S171-D9S1814-D9S1778-telomere" was established for these markers.

On the basis of the Généthon 1996 human genetic map (Dib et al. 1996), the genetic distance between markers D9S1778 and D9S171 is ~7 cM. However, more-precise estimates indicate that the critical region is ~3 cM. Cannon-Albright et al. (1994) estimated the genetic distance between *IFNA* (which colocalized with D9S1778 on the 1996 Généthon human genetic map) and D9S171 to be ~2 cM. Fountain et al. (1992) estimated the distance between *IFNB*, the most telomeric member of the interferon superfamily gene cluster containing *IFNA*, and D9S126, a marker that lies centromeric to D9S171, to be ~4.4 cM. Since D9S126 is 2 cM centromeric to D9S171, the genetic distance between *IFNB* and D9S171 on that map would be ~2.4 cM. Since markers D9S1778 and *IFNB* colocalize on the 1,140-kb CEPH mega-YAC 763h4 (data not shown), the critical region is, at most, 3.5 cM.

The protein products of hereditary cancer genes have been shown to be involved in a number of key aspects of cellular growth and regulation, and, interestingly, chromosome region 9p21-22 contains the tumor-suppressor genes responsible for familial melanoma (Cannon-Albright et al. 1994) and multiple familial trichoepithelioma (Harada et al. 1996). The region itself is one of the most frequently deleted and/or translocated chromosomal regions in human cancer (Mitelman 1994).

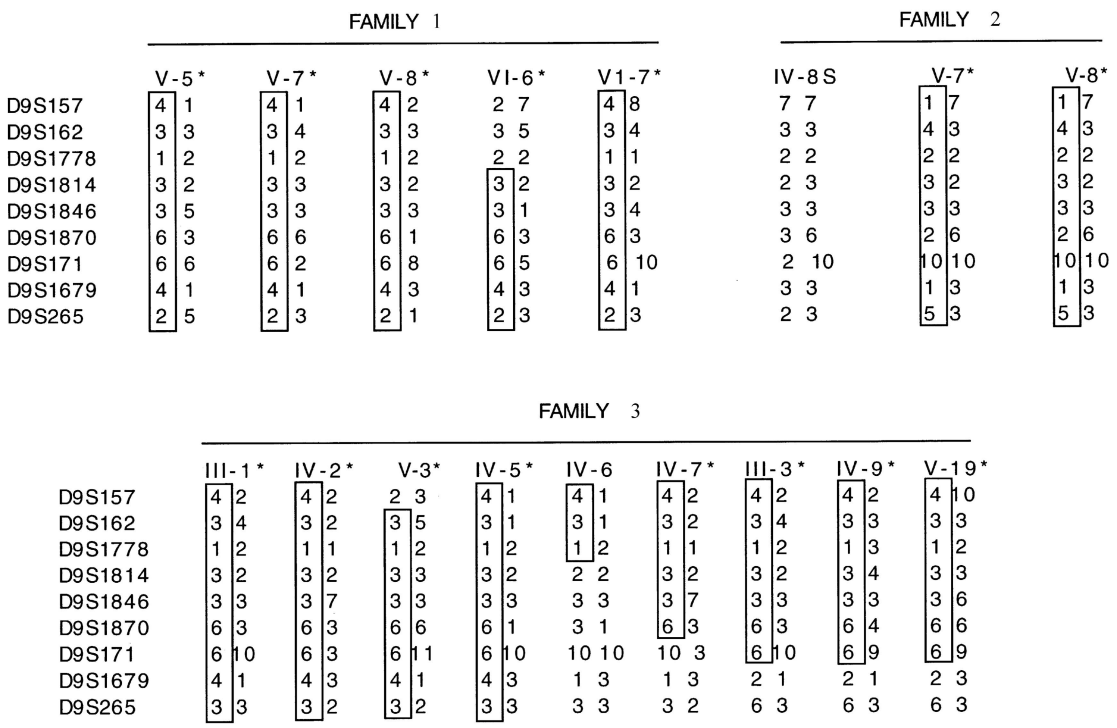

Figure 2 DMS-MFH kindred haplotypes analyzed by nine informative markers (D9S157, D9S162, D9S1778, D9S1814, D9S1846, D9S1870, D9S171, D9S1679, and D9S265) from the linkage region. Family and individual numberings are the same as in figure 1. Affecteds are identified by an asterisk (*). Individual IV-8S in family 2 is the spouse of a deceased affected (IV-8), and her genotypic data were used to determine chromosome origin.

Growth-regulating genes are plausible disease gene candidates for DMS-MFH, whose phenotype includes excessive endosteal bone formation and a predilection for sarcomatous change. Among them, the best-characterized genes are *CDKN2A* and *CDKN2B,* which are cyclin-dependent kinase inhibitors, important negative regulators of cell division. In addition to being deleted on both chromosomes in cell lines derived from lung, breast, brain, bone, skin, bladder, kidney, ovary, and lymphocyte tumors, mutations in *CDKN2A* result in a form of familial melanoma (Gruis et al. 1995). Sequence analysis of the *CDKN2A* and *CDKN2B* genes (as well as their splice variants) from individuals with DMS-MFH failed to reveal any mutations. Similarly, other 9p21-22 candidate genes have been excluded, including *RagA,* a ras-related GTP-binding protein (Bun-ya et al. 1992; Schurmann et al. 1995); *AF-9,* a putative transcription factor in acute leukemia, cloned from a translocated region involving chromosomes 9p22 and 11q23 (Nakamura et al. 1993); and *AK3,* a nucleoside triphosphate-kinase that regulates adenine and guanine nucleotide pools (Xu et al. 1992).

Currently, cloning and analysis of several additional genes in this region, defined by expressed sequence tags, are under way and may facilitate the identification of the DMS-MFH gene. Initial LOH studies on a number of hereditary and sporadic forms of MFH have shown the majority of tumor samples to have deletions within the DMS-MFH locus (J. A. Martignetti, B. D. Gelb, H. Pierce, and R. J. Desnick, unpublished data). These preliminary results not only support a shared genetic etiology between hereditary and sporadic forms of MFH, but also are in accord with the DMS-MFH gene's being a tumor-suppressor gene. Identification of the DMS-MFH gene will therefore allow presymptomatic diagnosis of DMS-MFH in patients and may provide insights into the control of bone growth and the development of bone tumors.

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Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

Généthon, http://www.genethon.fr (for distance between markers D9S1778 and D9S171)

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov.omim (for DMS-MFH [MIM 112250])

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