Am. J. Hum. Genet. 46:661–671, 1990

Unilateral Microfibrillar Abnormalities in a Case of Asymmetric Marfan Syndrome

Maurice Godfrey,*,‡,[1] Susan Olson,† Roberto G. Burgio,‖ Alberto Martini,‖ Maurizia Valli,# Giuseppe Cetta,# Hisae Hori,*,[2] and David W. Hollister*,‡,§,[3]

*Shriners' Hospital for Crippled Children; and Departments of †Medical Genetics, ‡Biochemistry, and §Medicine, Oregon Health Sciences University, Portland; and Departments of ‖Pediatrics and #Biochemistry, University of Pavia, Italy

Summary

The Marfan syndrome is a dominantly inherited connective-tissue disorder characterized by ocular, cardiovascular, and musculoskeletal abnormalities. Although the underlying biochemical and molecular defect(s) of this pleiotropic disease is currently unknown, we have consistently observed apparent diminished content of elastin-associated microfibrillar fibers accumulating in skin, or produced by cultured fibroblasts, from patients with the Marfan syndrome and have documented the cosegregation of these immunofluorescent abnormalities of microfibrillar fibers with the Marfan syndrome phenotype in family studies. Recently, an unusual patient has been described with unilateral phenotypic features of the Marfan syndrome, providing an unique opportunity to compare microfibrillar fibers and other connective-tissue components between the affected and nonaffected sides. In the present report, we demonstrate striking differences in apparent content of microfibrillar fibers, as determined by indirect immunofluorescence of skin and fibroblast cultures, that are revealed when multiple homologous samples derived from different sides of the patient's body are compared. In contrast, no differences in apparent content of type III collagen or in the biosynthesis and apparent structure of types I and III (pro)collagens were found. HLA types and chromosome heteromorphisms were identical in fibroblasts from both sides of the body, eliminating the formal possibility of chimerism and suggesting that a postzygotic mutation accounts for the asymmetric manifestation of the Marfan syndrome in this patient. The observation of striking decreases in microfibrillar fibers on the affected side of the body provides further evidence that abnormalities of this component of the elastic fiber system may be central to the pathogenesis and possibly the etiology of the Marfan syndrome.

Introduction

The Marfan syndrome is a serious heritable disorder of connective tissue associated with multiple manifestations in different organ systems. The ocular findings include subluxation of the lens (ectopia lentis), myopia, and retinal detachment. The cardiovascular features consist principally of progressive dilatation of the aortic root and ascending aorta and prolapse of the mitral valve, leading to aortic regurgitation, aneurysms, dissection, and mitral regurgitation. Musculoskeletal findings include tall stature, long extremities (dolichostenomelia), arachnodactyly, joint laxity, chest deformity (pectus excavatum and/or carinatum, often asymmetric), spine deformities, and congenital contractures. Striae distensae and inguinal hernia are frequent findings in the integument, and pneumothorax and dural ectasia occur in some patients. Median life span is between one-half and two-thirds of normal, and 85% of patients succumb to cardiovascular complications (McKusick 1972; Pyeritz and McKusick 1979; Maumenee 1981; Pyeritz 1983, 1986; Pyeritz et al. 1988).

Received July 31, 1989; final revision received October 30, 1989.

1. Present address and address for correspondence and reprints: Maurice Godfrey, Munroe Center for Human Genetics/Meyer Rehabilitation Institute, Departments of Pediatrics and Pathology, University of Nebraska Medical Center, 444-44th Street, Omaha, NE 68131-3795.

2. Present address: Department of Tissue Physiology, Medical Research Institute, Tokyo Medical and Dental University, Dental University, Tokyo.

3. Present address: Munroe Center for Human Genetics/Meyer Rehabilitation Institute, Department of Pediatrics and Pathology, University of Nebraska Medical Center, Omaha.

© 1990 by The American Society of Human Genetics. All rights reserved.
0002-9297/90/4604-0005$02.00

The cause or causes of Marfan syndrome remain obscure but have not altered the conviction that a dominantly transmitted allele(s) involved in connective-tissue metabolism will ultimately be discovered. Defects in collagens have long been suspected (Laitinen et al. 1968; Priest et al. 1973; Francis et al. 1974; Boucek et al. 1981; Byers et al. 1981; Muller et al. 1987), but recently candidate gene linkage studies have effectively excluded linked regulatory or structural defects of collagen types I–III (Tsipouras et al. 1986; Dalgleish et al. 1987; Ogilvie et al. 1987; Francomano et al. 1988). Similarly, defects of elastin have been proposed because of the fragmentation of elastin seen in Marfan aortas as a feature of cystic medial necrosis and certain biochemical findings (Saruk and Eisenstein 1977; Perejda et al. 1985; Takebayashi et al. 1988). Limited candidate gene linkage studies with elastin gene probes have not, to date, indicated linkage in the families studied (Rosenbloom 1984; Huttunen et al. 1989). Elevated fibroblast glycosaminoglycan biosynthesis has been documented (Lamberg and Dorfman 1973; Appel et al. 1979), but its significance is presently unclear.

We have recently examined the microfibrillar fiber component of the elastic fiber system in skin and fibroblast culture by indirect immunofluorescence (IF) studies using monoclonal antibodies directed against a major structural protein of microfibrils, fibrillin (Hollister et al. 1985; Sakai et al. 1986). In a large single-blind study, 24/27 patients with the Marfan syndrome were correctly identified by apparent deficient accumulation of microfibrillar fibers in skin and/or hyperconfluent fibroblast culture; in contrast, 13/13 normal controls and 19/25 patients with other connective-tissue disorders were correctly identified as "non-Marfan" (Hollister et al., submitted). Further, cosegregation of IF abnormalities with the Marfan syndrome phenotype was uniformly observed in family studies and parent-child and sib-sib pairs (Godfrey et al., 1990). No evidence for antigenic masking was detected in these studies. The widespread distributions of microfibrillar fibers and, particularly, codistribution within those tissues exhibiting abnormalities in the Marfan syndrome suggest that abnormalities in the production, assembly, biomechanical integrity, or degradation of these fibrous structural elements is central to the pathogenesis and etiology of the Marfan syndrome.

Burgio et al. (1988) have recently described a case of asymmetrical Marfan syndrome exhibiting dilated aortic root, unilateral (left) ectopia lentis, and typical skeletal overgrowth; together these features meet the criteria for the Marfan syndrome (Beighton et al. 1988). Because of the apparent unilateral manifestation of the Marfan syndrome in this unusual patient, detailed comparisons of certain connective-tissue components between affected and nonaffected sides of the body were of interest. In the present report, we document striking decreases in the apparent content of elastin-associated microfibrillar fibers in skin and accumulated in fibroblast culture, from the affected side of the body as compared with the nonaffected side.

Material and Methods

Tissue Specimens

Six-millimeter skin punch biopsies were obtained from the patient from non-sun-exposed inner aspects of left and right arms. A previous set of biopsies was obtained from her thighs (Burgio et al. 1988). The biopsies were divided for fibroblast explant (culture media) and IF studies (frozen in −20°C hexanes). Control dermal tissues were obtained from age-matched normal individuals taken from the inner aspects of the arm.

Collagen Analysis

Skin fibroblasts (right and left sides) from patient and controls were maintained in Dulbecco's modified Eagle medium (DMEM) supplemented with antibiotics and 10% fetal bovine serum (GIBCO) in 25-cm^2 flasks. ^{3}H-proline labeled procollagens from medium and cell layer, and collagens obtained by limited pepsin digestion, were analyzed by SDS–5%-PAGE followed by fluorography using a slight modification of a published procedure (Barsh and Byers 1981). Delayed reduction of disulfide bonds was used to distinguish type III collagen chains (Sykes et al. 1976).

Immunofluorescence Studies

Fibroblasts derived from patient and controls were plated at 2.5×10^5 cells/ml/chamber into four-chamber microscope slides (Nunc, Naperville, IL) and incubated for 2 d at 37°C in 1 ml/chamber DMEM supplemented as described above. Monolayers were fixed in −20°C acetone for 10 min, washed for 20 min in PBS, and incubated with supernatant monoclonal antibody to fibrillin (Hollister et al. 1985; Sakai et al. 1986) or negative control (culture media without antibody). Two distinct anti-fibrillin monoclonal antibodies (F2 and 201) reactive with different molecular epitopes were used, both monospecific for elastin-associated microfibrils and related (non-elastin-containing) microfibrils as judged by electron-microscope immunolocali-

zation studies (Hollister et al. 1985; Sakai et al. 1986). Following 1 hr incubation with primary antibody, the monolayers were washed for 20 min in PBS and then incubated for 30 min with a goat anti-mouse immunoglobulin (anti-IgG) conjugated to phycoerythrin (Biomeda, Foster City, CA). Excess secondary antibody was washed away, and the chambers were incubated for 10 min in a dilute solution (0.00025%) of propidium iodide (Biomeda), a nuclear dye, to visualize the fibroblasts. The slides were washed extensively in PBS and mounted in Gel/Mount (Biomeda). Fluorescence was viewed using a fluorescein filter set and barrier filter with an emission wavelength greater than 520 nm. The maximum emission of phycoerythrin is 575 nm (gold-yellow) at an excitation wavelength of 500 nm, whereas propidium iodide appears pink-red under similar excitation. Photographs were taken with high-speed Ektachrome film (Kodak) on a Zeiss Photoscope III. All exposures were for 15 s.

Skin samples frozen in hexane were thawed, oriented and mounted by refreezing in Tissue Tek (Miles Laboratories) and cut in 15-μm cryosections on a Leitz 1720 Kryostat. Sections were air dried on poly-L-lysine-coated glass slides. Tissue fixation, staining, and indirect immunofluorescence analysis was carried out as described above. As a control, monoclonal antibody specific for human type III collagen was used on some sections.

To detect possible antigenic masking, enzymatic digestion of skin sections prior to fixation was performed. Chondroitinase ABC (Seikagaku Kogyo, Tokyo) was suspended in 100 mm Tris-acetate buffer pH 7.6. at 0.0125 units/50 μl/15-μm skin section and incubated for 90 min at 37°C (Poole et al. 1980). Hyaluronidase (Worthington, Freehold, N.J.) was suspended at 8,000 units/ml in 0.1M phosphate buffer pH 5.3; 100 μl was added to each tissue section and incubated for 20 min at room temperature. Elastase (Worthington, Freehold, N.J.) diluted to 0.01% in 0.067 M Tris buffer pH 8.8 was added to skin sections and incubated for 20 min at room temperature (Sakai et al. 1986). Following enzyme digestion, the skin sections were fixed and stained as described above.

Chromosome Analysis

To determine possible karyotypic differences and evaluate chromosome heteromorphisms, fibroblasts derived from right and left sides were harvested and chromosome spreads prepared by a standard protocol. Slides were stained with quinacrine (Caspersson et al. 1971), and representative cells were photographed and serially printed to permit detailed comparison of homologous chromosomes and chromosome heteromorphisms between the two sides (Overton et al. 1976; Olson et al. 1986).

Results

Clinical Findings

The patient is a 6½-year-old, tall, thin Italian girl, the product of an uneventful pregnancy and delivery to unrelated young parents with no stigmata of the Marfan syndrome. Details of her early history and physical findings at age 3½ have been recorded (Burgio et al. 1988) and include marked overgrowth of the left side of the body with limb length inequality, functional scoliosis, high narrow palate, asymmetric left pectus carinatum, left arachnodactyly, pes planus, and bilateral joint laxity. Ocular findings included bilateral myopia and unilateral (left) subluxation of the lens. Echocardiography revealed a floppy mitral valve and aortic root dilatation. Anthropometric measurements at initial evaluation and currently are presented in table 1. At age 6½, the patient presented identical physical findings; figure 1 illustrates the skeletal asymmetry.

Control Immunofluorescence Studies

To permit comparison with IF studies of the patient's fibroblast cultures and skin sections, comparable studies of a clinically normal 5-year-old girl are depicted in figure 2.

Cell Culture Immunofluorescence Studies

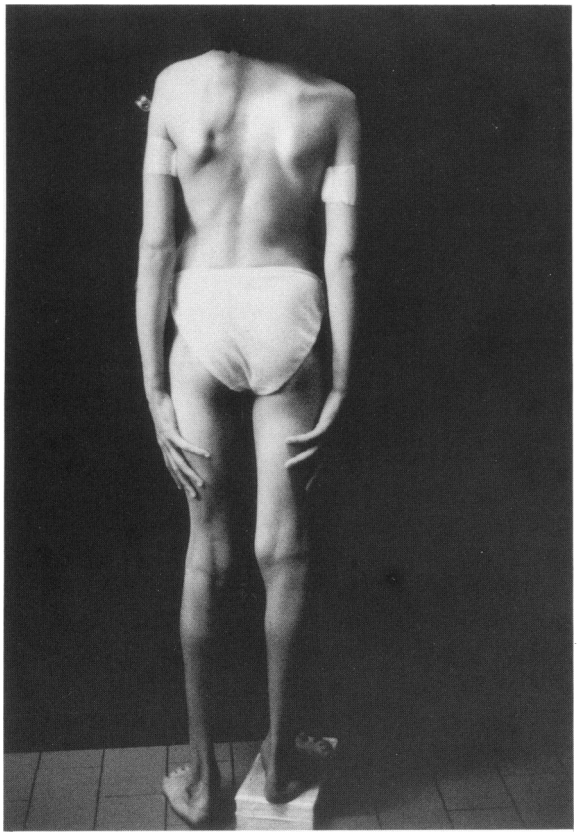
Representative photomicrographs of the fibrous material reactive to monoclonal antibody to fibrillin and accumulating in the 2-d fibroblast culture assay are depicted in figure 3. Fibroblasts derived from the left side of the body are shown in figure 3*A* and those from the right side of the body in figure 3*B*. Cell density, an important determinant of accumulation of immunostainable fibrous materials, is approximately equal as judged by visualization of nuclei with propidium iodide. Fibroblasts derived from the right side of the body (both arm and thigh) uniformly produced a prominent meshwork of immunostainable materials not distinguishable from normal controls (e.g., see fig. 2*A*), whereas those from the left side of the body uniformly exhibited a sharp decrease of immunostainable materials comparable to those previously observed for Marfan syndrome fibroblasts similarly assayed (Godfrey et al. 1990; Hollister et al., submitted). These results were replicated in at least 10 separate experiments (some of which were

Table 1

Patient's Anthropometric Data

Measurement	Age 3 3/12 Years	Age 6½ Years
Height (cm)	103.6 (>97%[a])	124.0 (>97%[a])
Weight (kg)	13.7 (<25%[a])	19.0 (<25%[a])
Upper-to-lower-segment ratio	.95 (<2 SD)	.92 (<2 SD)
Arm-span-to-height ratio	1.03	1.03
Upper limb (cm):[b]		
Right	46.5	53.0
Left	49.0	57.0
Difference	2.5	4.0
Lower limb (cm):[c]		
Right	52.5	65.0
Left	55.0	68.5
Difference	2.5	3.5
Middle-finger-to-hand length (%):		
Right	42.2 (<50%[a])	42.2 (<50%[a])
Left	47.2 (>97%[a])	46.1 (>97%[a])
Aortic root (mm)	26 (11–16[d])	28 (12–18[d])

[a] Percentile for age.
[b] Acromion to tip of middle finger.
[c] Greater trochanter to floor.
[d] Normal limits by body surface area (Henry et al. 1978).



blinded) using fibroblasts derived from both the upper arms and thighs.

Skin Immunofluorescence Studies

Photomicrographs of skin from the left and right sides of the body, stained with monoclonal antibodies to type III collagen and fibrillin, are shown in figures 4 and 5, respectively. Figures 4*A* and 5*A* represent the diffuse and generalized pattern of type III collagen localization in papillary dermis, and no differences in this bright pattern are observed between left and right. Figures 4*B* and 5*B* demonstrate the relative intensity of microfibrillar fiber staining in papillary dermis. Virtually no yellow staining is observed in sections from the patient's left side (fig. 4*B*), whereas the staining in sections from the right side of the body (fig. 5*B*) is clearly present but appears modestly diminished from normal controls (fig. 2*B*). A more striking difference is observed in the reticular dermis. Figure 4*C* shows a virtual absence of staining in skin from the left side of the body, whereas a prominent staining pattern associated with larger fibrous structures is observed on the right (fig. 5*C*).

Figure 1 The patient at age 6½. A 4-cm lift is required to level the pelvis, illustrating the length inequality of the lower limbs. Note the length discrepancy of the upper limbs; skin biopsies of the inner aspects of the arms account for the bandages.

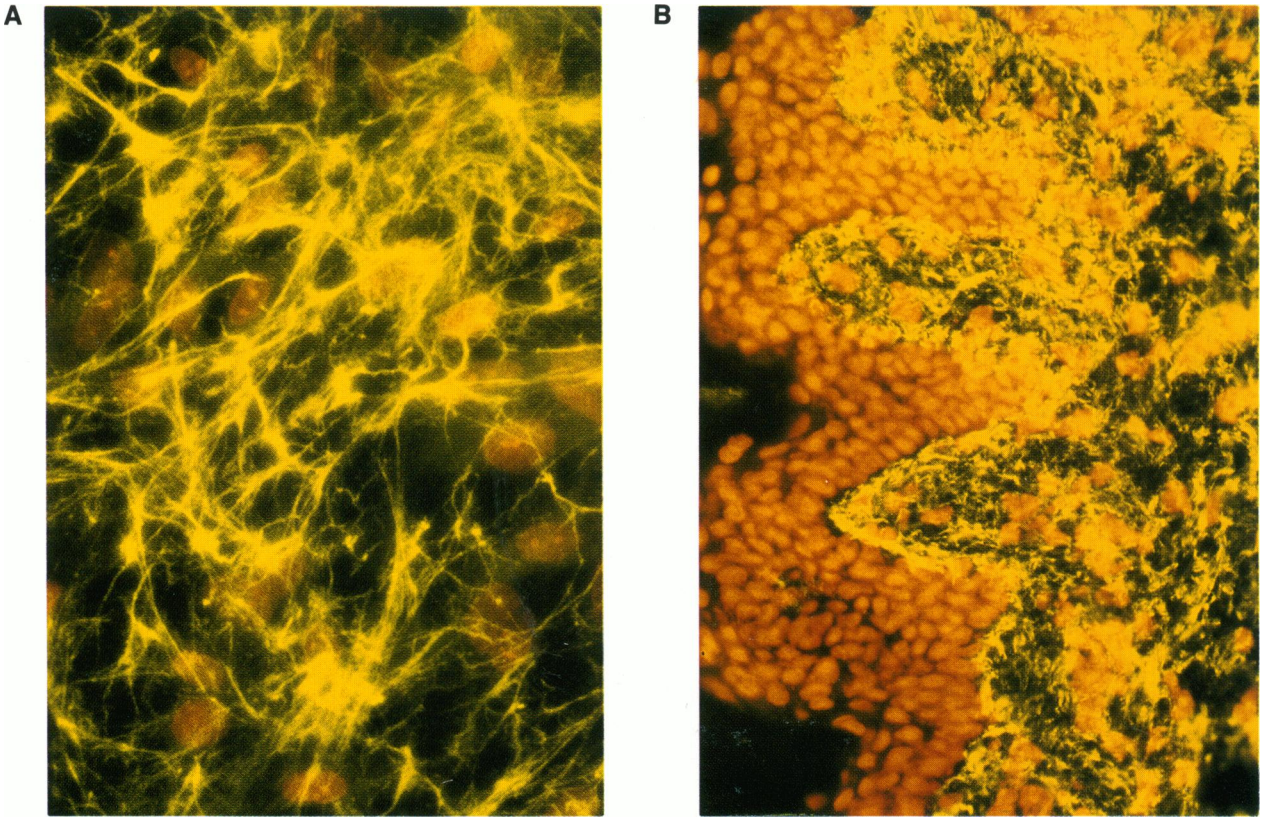


Figure 2 Immunofluorescent staining of an age- and sex-matched normal control individual. Acetone-fixed fibroblast monolayers or 15-μm skin sections were incubated with monoclonal antibodies to fibrillin, then secondary goat anti-mouse IgG conjugated to phycoerythrin (yellow-gold color). Nuclei were visualized with propidium iodide (pink-red color). *A,* Visualization of microfibrillar fiber accumulation in hyperconfluent fibroblast culture. A prominent meshwork of microfibrillar fibers is demonstrated. *B,* Microfibrillar fiber array in the papillary dermis. Bright yellow-gold stained microfibrillar fibers extend from the dermal-epidermal junction into a complex meshwork in the papillary dermis. Original magnification 416×.

Photoquenching experiments in which the phycoerythrin fluorescence is abolished reveal that these larger fibrous structures in reticular dermis contain elastic fibers identified by pale greenish autofluorescence (data not shown).

In order to determine whether masking of antigenic sites was responsible for the decrease in dermal anti-fibrillin staining, skin sections were digested with enzymes (chondroitinase ABC, hyaluronidase, or elastase) prior to staining. In all cases the dramatic differences in anti-fibrillin staining between left and right sides were retained and there was no evidence of antigenic masking.

Collagen Analysis

Electrophoretic analyses of radioactively labeled fibroblast collagens from normal control and the patient's right and left sides are shown in figure 6. No differences in the migration patterns or relative amounts between control and patient's α1 (III) chain of type III collagen or α1 (I) and α2 (I) chains of type I collagen are observed. In addition, no differences in the procollagens from media and cell layer, or in collagens obtained from the cell layer, were observed between control fibroblasts and fibroblasts from either right or left sides of the patient (data not shown).

Chromosomal Analysis

Comparison of homologous chromosomes revealed no differences in banding patterns at the level of 550 bands per haploid karyotype (Harden and Klinger 1985) for fibroblasts derived from differing sides of the body. Similarly, comparison of fluorescent chromosome heteromorphisms on chromosomes 3, 4, 13, 14, 15, 21, and 22 demonstrated identical sets of variants for fibroblasts derived from both sides of the body.

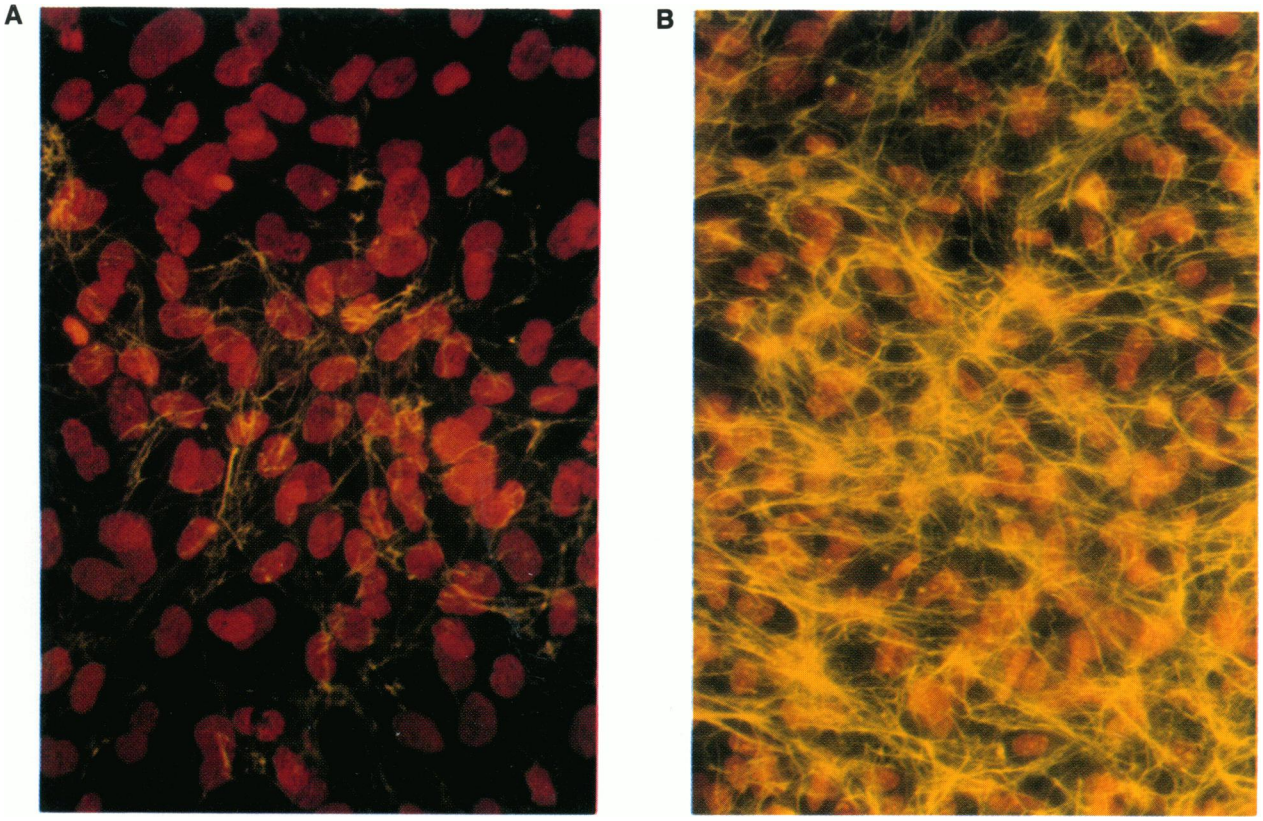


Figure 3 Immunofluorescent visualization of the fibrous materials produced, assembled, and accumulated by hyperconfluent fibroblast cultures. Murine monoclonal antibodies against fibrillin were detected by secondary antibody, and nuclei were visualized as described in the legend to fig. 2. *A*, Fibroblasts from the left side of the body, exhibiting markedly decreased microfibrillar staining. *B*, Fibroblasts from the right side of the body, displaying normal amounts of in vitro microfibrils. Original magnification 312×.

Discussion

Elastin-associated microfibrils form a subgroup among many slender fibrous or filamentous structures visualized in electron micrographs of various tissues. In cross section, microfibrils are typically 10–14 nm in diameter, and in longitudinal section, they appear as long, extended, slightly beaded, fibrous structures, typically organized in a side-by-side fashion into bundles of microfibrils, here referred to as microfibrillar fibers (reviews in Cleary and Gibson 1983; Cleary 1987). Initially identified by association with amorphous elastin profiles (Low 1962), it is now clear that microfibrillar fibers occur in the absence of elastin and form rod-like or branching and arborizing networks in various tissues (Sakai et al. 1986; Cleary 1987). In skin, for example, microfibrillar fibers originate from elastic fibers of the reticular and papillary dermis, form a branching network, and insert into the basement membrane of the dermal-epidermal junction. Such arrangement suggests one functional role for microfibrils, namely, interconnection of elastic fibers with other structural elements whereby elastic recoil may be transmitted (Cotta-Pereira et al. 1976, 1978). Microfibrillar fibers also serve as an apparent scaffolding upon which amorphous elastin is deposited and presumably oriented for cross-linking during the ontogeny of elastic fiber systems (review in Cleary 1987).

The structural components of microfibrils have been experimentally difficult to characterize. To date, three glycoproteins have been purified and localized to microfibrils, including the 350-kD fibrillin (Hollister et al. 1985; Sakai et al. 1986), microfibrillar associated glycoprotein (Gibson et al. 1986), and a 35-kD protein (Serafini-Fracassini et al. 1981); additional proteins are currently under study (Colombatti et al. 1988; Mecham et al. 1988).

Microfibrillar fibers are widely distributed and occur in many tissues. This distribution includes the con-

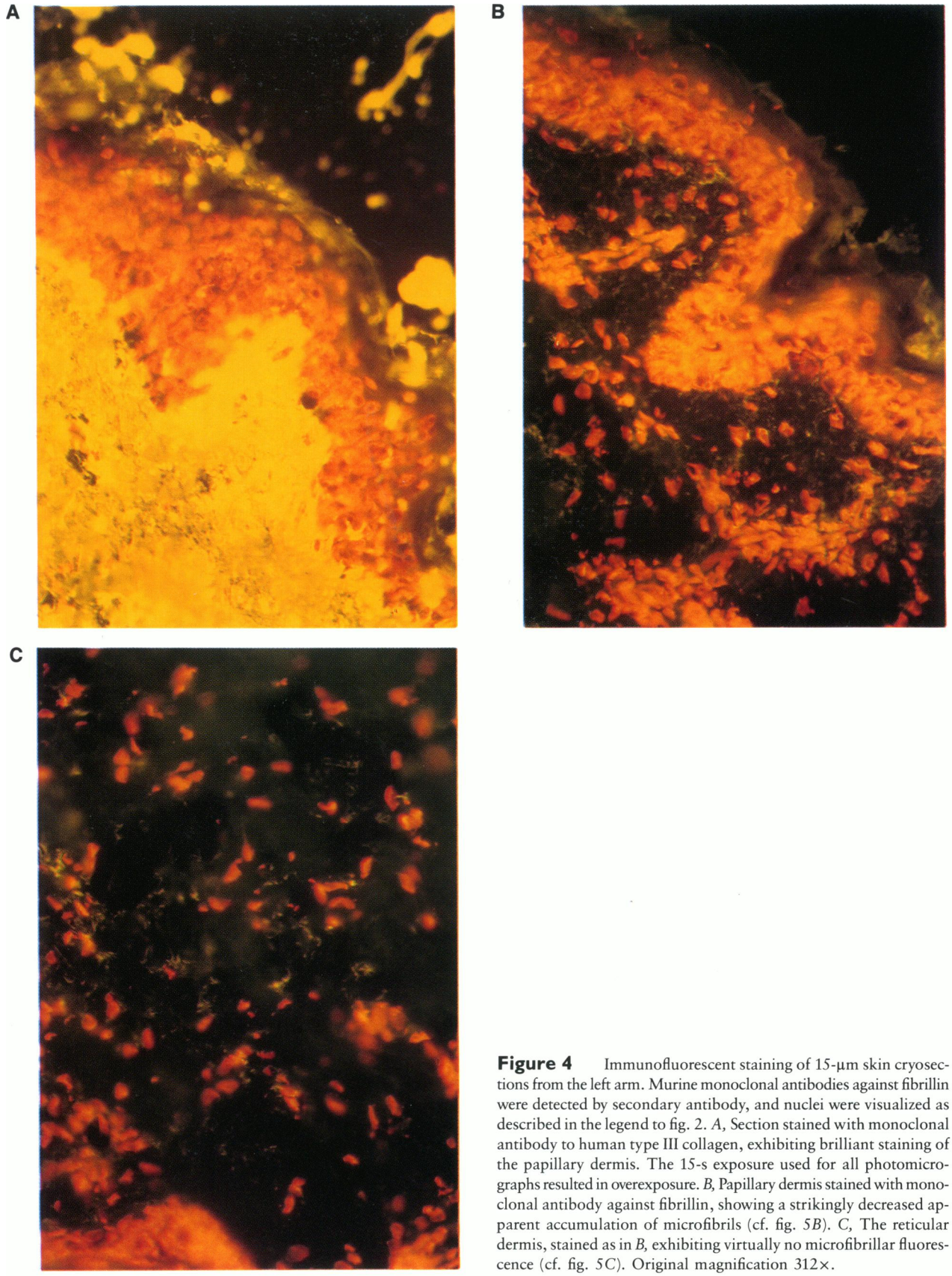


Figure 4 Immunofluorescent staining of 15-μm skin cryosections from the left arm. Murine monoclonal antibodies against fibrillin were detected by secondary antibody, and nuclei were visualized as described in the legend to fig. 2. *A,* Section stained with monoclonal antibody to human type III collagen, exhibiting brilliant staining of the papillary dermis. The 15-s exposure used for all photomicrographs resulted in overexposure. *B,* Papillary dermis stained with monoclonal antibody against fibrillin, showing a strikingly decreased apparent accumulation of microfibrils (cf. fig. *5B*). *C,* The reticular dermis, stained as in *B,* exhibiting virtually no microfibrillar fluorescence (cf. fig. *5C*). Original magnification 312×.

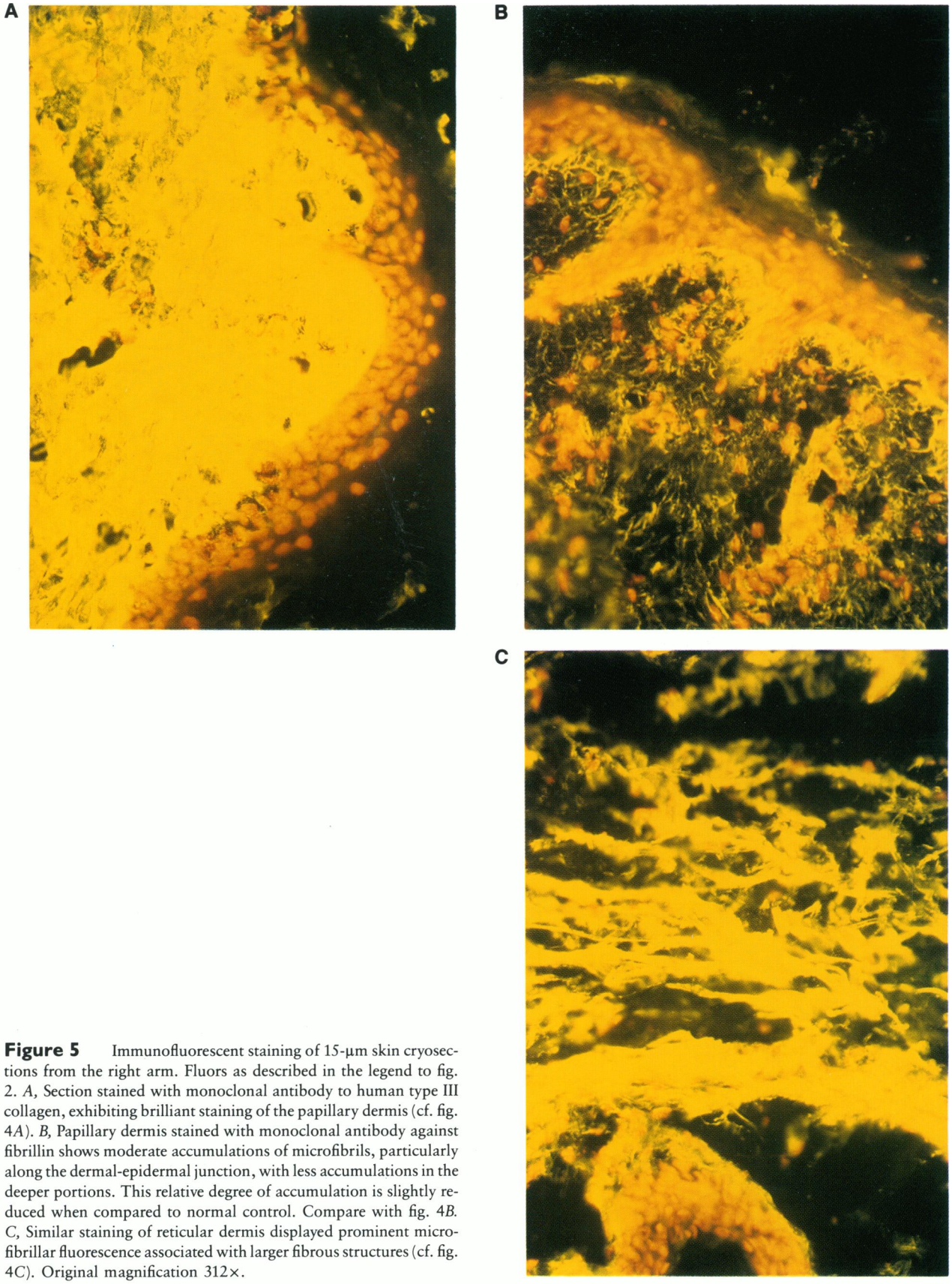


Figure 5 Immunofluorescent staining of 15-μm skin cryosections from the right arm. Fluors as described in the legend to fig. 2. *A,* Section stained with monoclonal antibody to human type III collagen, exhibiting brilliant staining of the papillary dermis (cf. fig. 4*A*). *B,* Papillary dermis stained with monoclonal antibody against fibrillin shows moderate accumulations of microfibrils, particularly along the dermal-epidermal junction, with less accumulations in the deeper portions. This relative degree of accumulation is slightly reduced when compared to normal control. Compare with fig. 4*B*. *C,* Similar staining of reticular dermis displayed prominent microfibrillar fluorescence associated with larger fibrous structures (cf. fig. 4*C*). Original magnification 312×.

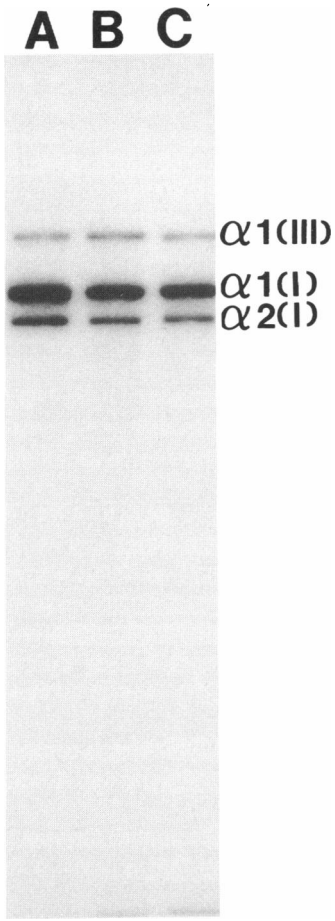


Figure 6 SDS–5% PAGE fluorogram of collagens produced by control fibroblasts (*A*) and fibroblasts derived from the right arm (*B*) and left arm (*C*) of the patient. The identities of the collagen chains are indicated to the right. Both patient samples are equivalent to control.

centric rings of elastin in aorta and major blood vessels, pleura, dura, skin, perichondrium and periosteum; of particular note, the ciliary zonules (suspensory ligaments) of the lens are composed virtually exclusively of microfibrillar fibers without apparent associated elastin (Raviola 1971; Streeten et al. 1983). Biomechanical incompetence of microfibrils (and/or associated elastin) within these tissues offers plausible explanations for the pleiotropic manifestations of the Marfan syndrome, as, for example, ectopia lentis or skeletal overgrowth due to diminished functional tethering by periosteal and perichondrial structures.

The patient studied here appears to be a remarkable experiment of nature, with clinical findings of the Marfan syndrome predominantly manifest on the left side of the body. Asymmetric presentation of dominantly inherited connective tissue disease is rare, but examples have been recorded (cited in McKusick 1972; Fryns and van den Berghe 1986). In the present case, chimerism has been effectively excluded by the finding of identical HLA phenotype (Burgio et al. 1988) and chromosome heteromorphisms in cells derived from both sides of the body, data that strongly support the theory that the patient's phenotype is the result of a postzygotic mutation. The most straightforward interpretation is that the patient is a mosaic of normal and abnormal cells, unequally distributed between body halves. In the absence of detectable chromosomal rearrangement in previous studies (Burgio et al. 1988) and the present study, the mutation appears to be subkaryotypic and may be a structural change in one gene or a small group of genes. Therefore, the abnormal cells are virtually isogenic with the normal cells, and identification of consistent differences between tissues and cells derived from differing body sides may directly reflect the consequences of the mutational event.

As demonstrated here, there is a striking difference in the apparent content of microfibrillar fibers (decreased immunofluorescence) in papillary and reticular dermis from different sides of the body that is not altered by maneuvers designed to detect antigenic masking. Notably, such enzymatic digestions have successfully detected antigenic masking of microfibrils in a patient with cutis laxa (M. Godfrey, unpublished data). Further, the decreased apparent amounts of staining are observed in both undigested and enzymatically treated sections with both anti-fibrillin monoclonal antibodies (known to react with different epitopes of the antigen). These findings make antigenic masking of epitopes unlikely.

In addition to the marked differences in apparent microfibrillar fiber content between sides, we observed a modest deficiency in papillary dermis fluorescence from the right as compared with normal control papillary dermis. These findings, together with the bilateral myopia and ligamentous laxity, suggest that absolute partition of abnormal cells to the left side did not occur, and some degree of admixture of normal and abnormal cells is present on the right side. However, although unlikely, it is also possible that a circulating factor(s) which, in part, inhibits microfibrillar accumulation is present in vivo and accounts for the modestly diminished content on the right side. In contrast to the findings for microfibrillar fibers, the apparent amount and distribution of type III collagen is equivalent in dermis derived from both sides of the body.

In agreement with the skin IF studies, the apparent

accumulation of fibrous material synthesized, assembled, and detectable by monoclonal antibody by hyperconfluent fibroblasts is strikingly different in cells derived from different sides of the body. In all instances, fibroblasts from the right side (derived from either arm or thigh) exhibited substantial accumulations of fibrous material, whereas fibroblasts from the left side (both arm and thigh) were markedly deficient. Unlike the skin IF results, no intermediate accumulations of microfibrils in fibroblast cultures from the right side were observed; these cultures always yielded substantial accumulations comparable to control fibroblast lines. In contrast to the substantial differences in microfibrillar fiber accumulation, the apparent amounts, mobilities, and ratios of the chains of types I and III procollagen and collagen produced by fibroblasts were, within experimental error, equivalent from cells derived from left and right sides of the body.

The IF observations from the patient's left side (in both skin and fibroblast culture) are similar to previous observations in multiple patients with classic manifestations of the Marfan syndrome, and are therefore consistent with the impression of hemi-Marfan syndrome based on clinical phenotypic features alone, as previously reported in earlier studies. Conversely, and more significantly, the striking correlation of deficiency of microfibrillar fibers on the phenotypically affected side of the body provides further evidence that abnormalities in this component of the elastic fiber system may be central to the pathogenesis and, possibly, etiology of the Marfan syndrome. These data imply that molecular defects of the structural glycoprotein(s) of the microfibril are candidates for the defective gene product(s) causing the Marfan syndrome.

Acknowledgments

The authors thank Donna Gaudette for invaluable technical support, Drs. Francesco Ramirez and Reed Pyeritz for valuable discussions, Dr. Lynn Sakai for provision of monoclonal antibodies to fibrillin. We thank Biomeda Corporation, Foster City, CA for a kind gift of phycoerythrin-conjugated antibodies. D.W.H. was supported by a grant from Shriners' Hospitals for Crippled Children and G.C. was supported by a grant from Progetto Finalizzato "Biotecnologie e Biostrumentazione" from C.N.R. Rome.

References

Appel A, Horwitz AL, Dorfman A (1979) Cell-free synthesis of hyaluronic acid in Marfan syndrome. J Biol Chem 254:12199–12203.

Barsh GS, Byers PH (1981) Reduced secretion of structurally abnormal type I procollagen in a form of osteogenesis imperfecta. Proc Natl Acad Sci USA 78:5142–5146

Beighton P, DePaepe A, Danks D, Finidori G, Gedde-Dahl T, Goodman R, Hall JG, et al (1988) International nosology of heritable disorders of connective tissue, Berlin, 1986. Am J Med Genet 29:581–594

Boucek RJ, Noble NL, Gunja-Smith Z, Butler WT (1981) The Marfan syndrome: a deficiency in chemically stable collagen cross-links. N Engl J Med 305:988–991

Burgio RG, Martini A, Cetta G, Zanaboni G, Vitellaro L, Danesino C (1988) Asymmetric Marfan syndrome. Am J Med Genet 30:905–909

Byers PH, Siegel RC, Peterson KE, Rowe DW, Holbrook KA, Smith LT, Chang Y-H, Fu JCC (1981) Marfan syndrome: abnormal α2 chain in type I collagen. Proc Natl Acad Sci USA 78:7745–7749

Caspersson T, Lomakka G, Zech L (1971) The 24 fluorescence patterns of human metaphase chromosomes—distinguishing characters and variability. Hereditas 67:89–102

Cleary EG (1987) The microfibrillar component of the elastic fibers: morphology and biochemistry. In: Uitto J, Perejda AJ (eds) Connective tissue disease: molecular pathology of the extracellular matrix. Marcel Dekker, New York, pp 55–81

Cleary EG, Gibson MA (1983) Elastin-associated microfibrils and microfibrillar proteins. Int Rev Connect Tissue Res 10:97–209

Colombatti A, Bonaldo P, Volpin D, Bressan GM (1988) The elastin-associated glycoprotein gp115. J Biol Chem 263: 17534–17540

Cotta-Pereira G, Rodrigo FG, Bittencourt-Sampaio S (1976) Oxytalan, elaunin, and elastic fibers in the human skin. J Invest Dermatol 66:143–148

Cotta-Pereira G, Rodrigo FG, David-Ferreira JF (1978) Comparative study between the elastic system fibers in human thin and thick skin. Biol Cell 31:297–302

Dalgleish R, Hawkins JR, Keston M (1987) Exclusion of the alpha 2(I) and alpha 1 (III) collagen genes as the mutant loci in a Marfan syndrome family. J Med Genet 24:148–151

Francis MJO, Sanderson MC, Smith R (1974) Skin collagen in idiopathic adolescent scoliosis and Marfan's syndrome. Clin Sci Mol Med 51:467–474

Francomano CA, Streeten EA, Meyers DA, Pyeritz RE (1988) Marfan syndrome: exclusion of genetic linkage to three major collagen genes. Am J Med Genet 29:457–462

Fryns JP, van den Berghe H (1986) An asymmetric type of chondrodysplasia in an adult male. Clin Genet 30:324–326

Gibson MA, Hughes JL, Fanning JD, Cleary EG (1986) The major antigen of elastin-associated microfibrils is a 31-kDa glycoprotein. J Biol Chem 261:11429–11436

Godfrey M, Menashe V, Weleber RG, Koler RD, Bigley RH, Lovrien E, Zonana J, et al (1990) Cosegregation of elastin-associated microfibrillar abnormalities with the Marfan phenotype in families. Am J Hum Genet 46:652–660

Harden DG, Klinger HP (eds) (1985) ISCN: an international

system for human cytogenetic nomenclature. Karger, Basel (also published as Birth Defects, Original Article Series, vol 21, no 1)

Henry WL, Ware J, Gardin JM, Hepner SI, McKay J, Weiner M (1978) Echocardiographic measurements in normal subjects: growth-related changes that occur between infancy and early adulthood. Circulation 57:278–285

Hollister DW, Godfrey M, Sakai LY, Pyeritz RE. Marfan syndrome: immunohistologic abnormalities of the microfibrillar fiber system. N Engl J Med (submitted)

Hollister DW, Sakai LY, Burgeson RE (1985) Identification of novel human basement membrane zone components by monoclonal antibodies. In: Fleischmajer R, Olsen BR, Kuhn K (eds) Biology, chemistry, and pathology of collagen. Annals of the New York Academy of Sciences, New York, pp 449–452

Huttunen K, Kaitila I, Savolainen A, Palotie A, Peltonen L (1989) The linkage analysis with RFLP markers of elastin and type III collagen genes in Finnish Marfan families. Am J Med Genet 32:244

Laitinen O, Uitto J, Iivanainen M, Hannuksela M, Kivirikko KI (1968) Collagen metabolism of the skin in Marfan's syndrome. Clin Chim Acta 21:321–326

Lamberg SI, Dorfman A (1973) Synthesis and degradation of hyaluronic acid in the cultured fibroblasts of Marfan's disease. J Clin Invest 52:2428–2433

Low FN (1962) Microfibrils: fine filamentous components of the tissue space. Anat Rec 142:131–137

McKusick VA (1972) Heritable disorders of connective tissue, 4th ed. Mosby, Saint Louis

Maumenee IH (1981) The eye in the Marfan syndrome. Trans Am Ophthalmol Soc 79:684–733

Mecham RP, Hinek A, Cleary EG, Kucich U, Lee SJ, Rosenbloom J (1988) Development of immunoreagents to ciliary zonules that react with protein components of elastic fiber microfibrils and with elastin-producing cells. Biochem Biophys Res Commun 151:822–826

Muller KP, Nerlich AG, Kunze D, Muller PK (1987) Studies on collagen metabolism in the Marfan syndrome. Eur J Clin Invest 17:218–225

Ogilvie DJ, Wordsworth BP, Priestley LM, Dalgleish R, Schmidtke J, Zoll B, Sykes BC (1987) Segregation of all four major fibrillar collagen genes in the Marfan syndrome. Am J Hum Genet 41:1071–1082

Olson SB, Magenis RE, Lovrien EW (1986) Human chromosome variation: the discriminatory power of Q-band heteromorphism (variant) analysis in distinguishing between individuals with specific application to cases of questionable paternity. Am J Hum Genet 38:235–252

Overton KM, Magenis RE, Brady T, Chamberlin J, Parks M (1976) Cytogenetic darkroom magic: now you see them, now you don't. Am J Hum Genet 28:417–419

Perejda AJ, Abraham PA, Carnes WH, Coulson WF, Uitto J (1985) Marfan's syndrome: structural, biochemical, and mechanical studies of the aortic media. J Lab Clin Med 106:376–383

Poole AR, Pidoux I, Reiner A, Tang L-H, Choi H, Rosenberg L (1980) Localization of proteoglycan monomer and link protein in the matrix of bovine articular cartilage: an immunohistochemical study. J Histochem Cytochem 28:621–635

Priest RE, Moinuddin JF, Priest JH (1973) Collagen of Marfan syndrome is abnormally soluble. Nature 245:264–266

Pyeritz RE (1983) Marfan Syndrome. In: Emery AEH, Rimoin DL (eds) Principles and practice of medical genetics. Churchill Livingstone, Edinburgh, pp 820–835

——— (1986) The Marfan syndrome. Am Fam Physician 34:83–94

Pyeritz RE, Fishman EK, Bernhardt BA, Siegelman SS (1988) Dural ectasia is a common feature of the Marfan syndrome. Am J Hum Genet 43:726–732

Pyeritz RE, McKusick VA (1979) The Marfan syndrome—diagnosis and management. N Engl J Med 300:772–777

Raviola G (1971) The fine structure of the ciliary zonule and ciliary epithelium. Invest Ophthalmol 10:851–869

Rosenbloom J (1984) Biology of disease—elastin: relation of protein and gene structure to disease. Lab Invest 51:605–623

Sakai LY, Keene DR, Engvall E (1986) Fibrillin, a new 350 kD glycoprotein, is a component of extracellular microfibrils. J Cell Biol 103:2499–2509

Saruk M, Eisenstein R (1977) Aortic lesion in Marfan syndrome. Arch Pathol Lab Med 101:74–77

Serafini-Fracassini A, Ventrella G, Field M, Hinnie J, Onyezili NI, Griffiths R (1981) Characterization of a structural glycoprotein from bovine ligamentum nuchae exhibiting dual amine oxidase activity. Biochemistry 20:5424–5429

Streeten BW, Swann DA, Licari PA, Robinson MR, Gibson SA, Marsh NJ, Vergnes J-P, et al (1983) The protein composition of the ocular zonules. Invest Ophthalmol Vis Sci 24:119–123

Sykes B, Puddle B, Francis M, Smith R (1976) The estimation of two collagens from human dermis by interrupted gel electrophoresis. Biochem Biophys Res Commun 73:1472

Takebayashi S, Taguchi T, Kawamura K, Sakata N (1988) Osmiophilic elastolysis of peripheral organ arteries in patients with Marfan's syndrome. Acta Pathol Jpn 38:1433–1443

Tsipouras P, Borresen A-L, Bamforth S, Harper PS, Berg K (1986) Marfan syndrome: exclusion of genetic linkage to the COL1A2 gene. Clin Genet 30:428–432