

Myocardial Storage of Chondroitin Sulfate-Containing Moieties in Costello Syndrome Patients With Severe Hypertrophic Cardiomyopathy

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Costello syndrome is a distinctive multiple congenital anomaly syndrome, characterized by loose soft skin with deep palmar and plantar creases, loose joints, distinctive coarse facial features, skeletal abnormalities, cardiac abnormalities (cardiovascular malformation (CVM), hypertrophic cardiomyopathy, tachycardia), predisposition to malignancy, developmental delays, and mental retardation. Previous studies with cultured fibroblasts from individuals with Costello syndrome demonstrate excessive accumulation of chondroitin sulfate-bearing proteoglycans, associated with both impaired formation of elastic fibers and an unusually high rate of cellular proliferation. Despite multiple clinical reports of cardiac abnormalities, there has been only one previously published report describing post-mortem findings in hearts from Costello syndrome patients. Here we provide a detailed description of the post-mortem findings of the hearts of three children with Costello syndrome. Routine histological examination and results of targeted histochemical and immunohistochemical studies revealed that in addition to cardiomyocyte hypertrophy, these hearts also demonstrated massive pericellular and intracellular accumulation of chondroitin sulfate-bearing proteoglycans and a marked reduction of elastic fibers. Normal stroma was replaced by multifocal collagenous fibrosis. Most peculiar was the finding that the bulk of the

chondroitin sulfate accumulated in these Costello syndrome hearts is a chondroitin-6-sulfate. In contrast, deposition of chondroitin-4 sulfate was below the level detected in normal hearts. We propose that an imbalance in sulfation of chondroitin sulfate molecules and subsequent accumulation of chondroitin-6-sulfate in cardiomyocytes contribute to the development of the hypertrophic cardiomyopathy of Costello syndrome.

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KEY WORDS: chondroitin sulfate; Costello syndrome; elastin; elastin binding protein; hypertrophic cardiomyopathy; MCA/MR syndrome

INTRODUCTION

Costello syndrome is an increasingly recognized multiple congenital anomaly syndrome (MIM 218040) [Hennekam, 2003] with a distinctive phenotype which may resemble a storage disease, overgrowth syndrome, progeroid syndrome, or connective tissue disorder. It has some similarity to Noonan syndrome [Marino et al., 1999] and the cardio-facio-cutaneous (CFC) syndrome [Bisogno et al., 1999; Innes and Chudley, 2000] in terms of the face, skin, and heart [Lin et al., 2002, Table VII]. Cardiac abnormalities have been reported in about two-thirds of individuals with Costello syndrome [Lin et al., 2002], but only one report described post-mortem findings in the heart [Mori et al., 1996]. Cardiovascular malformations (CVMs) occur in about one-third of patients, typically valvular pulmonic stenosis. In addition to structural anomalies, a striking predisposition to cardiac hypertrophy [Tomita et al., 1998] has been observed in about one-third of patients. Though its asymmetric distribution in the left-ventricle in some patients fulfills criteria for asymmetric septal hypertrophy (ASH), it may also appear as concentric biventricular hypertrophy. Less well characterized, but often life threatening, are disturbances of rhythm [Fukao et al., 1996], reported in over half of individuals with Costello syndrome. Atrial tachycardia predominates, in particular chaotic atrial rhythm or ectopic atrial tachycardia. The natural history of these cardiac abnormalities (age of onset, progression, impact on survival), their relationship to each other, and correlation with the overall phenotype has not been completely delineated.

Grant sponsor: Canadian Institute of Health Research; Grant number: PG 13920; Grant sponsor: Stroke Foundation of Ontario; Grant number: NA 4381; Grant sponsor: NIH; Grant numbers: CA90571, CA107300; Grant sponsor: Armand Anzalone Research Fund.

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Received 19 August 2004; Accepted 13 October 2004

DOI 10.1002/ajmg.a.30495

The genetic basis of Costello syndrome is unknown, although a single gene disorder with autosomal dominant transmission is suspected [Lurie, 1994; Bodkin et al., 1999; Ioan and Fryn, 2002]. There have only been a few reports exploring its biochemical basis. Qualitative and quantitative histological analyses of the skin of individuals with Costello syndrome demonstrated impaired elastin deposition [Vila Torres et al., 1994]. Histopathologic studies of autopsy cases revealed that elastic fibers detected in numerous internal organs are thinner than in unaffected patient tissues [Mori et al., 1996]. Despite the fact that individuals with Costello syndrome and Williams syndrome have similar features (thick lips, prematurely aged appearance), molecular genetic analyses have not revealed any deletions of the elastin gene [Tandoi et al., 2001], with normal expression of elastin mRNA [Mori et al., 1996].

Hinek et al. [2000] demonstrated that fibroblasts from individuals with Costello syndrome produce normal levels of the soluble tropoelastin and properly deposit an extracellular microfibrillar scaffold. However, these fibroblasts are unable to assemble mature elastic fibers due to a secondary deficiency in the 67-kDa elastin binding protein (EBP), that normally acts as a molecular chaperone facilitating secretion of hydrophobic and nonglycosylated tropoelastin [Hinek et al., 1988, 1995; Hinek, 1994; Hinek and Rabinovitch, 1994]. The 67-kDa EBP is an enzymatically inactive spliced variant of β -galactosidase (S-GAL) [Hinek et al., 1993; Privitera et al., 1998], which binds to the repeating hydrophobic domains on elastin, but may also bind to moieties containing β -galactosugars through a separate "lectin-like" domain [Mecham et al., 1991]. Binding of β -galactosugar-bearing moieties to the lectin domain of EBP causes conformational changes of the entire 67-kDa protein, such that it loses its affinity for elastin and detaches from the cell surface. It has, therefore, been suggested that abnormal accumulation of chondroitin sulfate bearing moieties in fibroblasts from individuals with Costello syndrome may induce shedding of EBP from the cell surface and prevent normal recycling of this reusable tropoelastin chaperone [Hinek et al., 2002, 2004].

In the present study, we evaluate evidence for these mechanisms occurring *in vivo* based on histochemical findings in the hearts of three children who died with a known diagnosis of Costello syndrome.

MATERIALS AND METHODS

Clinical Reports

Patient 1. A Chinese-Caucasian boy, was described in a review of patients with Costello syndrome and neoplasia [Gripp et al., 2002] and listed briefly as "new patient 10" in the Appendix of Lin et al. [2002]. As a review, polyhydramnios was detected by ultrasonography, and he was delivered by cesarean section. There was elevated birth weight and neonatal hypotonia. Chromosomes were normal (46,XY) by amniocentesis and postnatal blood karyotype. His coarse facial appearance prompted evaluations for storage disease and inborn errors of metabolism. Tests for urine mucopolysaccharides, oligosaccharides and organic acids, and blood amino acids were reported as normal. Skin fibroblast analysis for α -glucosidase, liver and muscle biopsies, including electron microscopy, did not detect classic storage disease. Brain MRI scan was reported as normal.

Feeding problems in early infancy required gastrostomy tube placement. At 5 months, concentric hypertrophic cardiomyopathy was demonstrated by echocardiography, with unspecified tachycardia occurring at 25 months. It was suspected that this was atrial tachycardia, although details are not available. Subaortic myectomy of the ventricular septum was performed at that time. Generalized developmental

delay was present. His facial appearance was consistent with classic Costello syndrome, as well as bilateral cryptorchidism, inguinal hernias, and distinctive ulnar deviation of the hands [Gripp et al., 2002].

The patient developed flu-like symptoms consistent with intermittent bowel obstruction around age 5 and one-half, and biopsy of a pelvic mass showed embryonal rhabdomyosarcoma. The primary tumor filled the pelvis and continued to grow and fill the abdomen. The first cycle of chemotherapy with vincristine, actinomycin, cyclophosphamide, and topotecan reduced the tumor size. Debulking surgery was required because of rapid growth and tumor necrosis. The protocol was continued and was deemed sufficiently successful to withhold radiation. One month after completion of the first protocol, tumor recurrence necessitated a second round of chemotherapy with irinotecan and doxorubicin. The latter drug was administered about 1 week before he died, during which time he had tachycardia (heart rate 180–220 bpm).

This chemotherapy failed to shrink the tumor, although radiographic evidence of necrosis was obtained. Debulking surgery was not performed since the bowel was extensively entrapped by tumor. The patient's condition continued to deteriorate. Comfort measures were instituted, and the patient expired with acid-base imbalances and hyperkalemia. Post-mortem examination was limited to the heart and abdomen.

Patient 2. A Caucasian male, was the product of a 35-week gestation born to a 27-year-old woman who had two previous pregnancies with her 32-year old partner [Allen et al., 2004]. The healthy parents were unrelated. Pregnancy was complicated by ultrasonographically detected polyhydramnios and macrocephaly. Malformations were not suspected. Amniocentesis revealed a 46,XY karyotype. Apgar scores were 3 at 1 min, 4 at 5 min, and 7 at 10 min. Intubation and mechanical ventilation were required for persistent respiratory distress. Birth weight was 3,873 g (>95th centile), length was 50.5 cm (95th centile), and OFC was 36.5 cm (>95th centile).

Physical features at birth included an upturned nose with a prominent nasal tip, deep creases on the soles of the feet, and bilateral cryptorchidism. The liver was enlarged and palpated just above the umbilicus. To evaluate persistent respiratory distress, bronchoscopy was performed which demonstrated a mucocele at the base of the tongue and laryngeal edema, and a lung biopsy revealed pulmonary lymphangiectasia. Tracheostomy was necessary after several failed attempts at extubation. Neurologic exam revealed hypertonicity and frequent opisthotonic posturing. An electroencephalogram was abnormal due to poorly organized sleep states and multifocal sharp transients. Magnetic resonance imaging of the head revealed slightly prominent ventricles, but no significant abnormality was identified.

Echocardiography at 1 day of age showed a patent ductus arteriosus and a persistent foramen ovale. At 1 week of age, he developed ectopic atrial tachycardia and supraventricular tachycardia, as well as ventricular ectopy. By 5 weeks of age, there was significant concentric hypertrophy of the left ventricle with systolic anterior motion of the mitral valve and severe mitral insufficiency. The peak instantaneous gradient from left ventricle to aorta was 150 mmHg. The ensuing severe atrial and ventricular arrhythmias were unresponsive to anti-arrhythmic therapy.

At 2 months of age, the physical exam was remarkable for deep creases in the skin of the hands, feet, and gluteal regions. Coarse facial features were evident prompting a clinical diagnosis of Costello syndrome was suggested. Mucopolysaccharidosis was not suspected nor tested.

Pulmonary and cardiac compromise followed and he died at 2½ months of age. We studied cultured fibroblasts from this patient using methodology similar to what was reported in previous *in vitro* studies [Hinek et al., 2000a]. We demonstrated

increased proliferation, abnormal elastogenesis, and an increased expression of chondroitin sulfate-bearing proteoglycans, features consistent with the previously established Costello syndrome "cultured fibroblast phenotype" (Fig. 3).

Patient 3. This Caucasian male was listed as "new patient 5" in the Appendix of Lin et al. [2002]. He was the product of a 39-week gestation born to an unrelated 33-year-old woman and 35-year-old man. The pregnancy was complicated by second trimester polyhydramnios. Macrosomia and macrocephaly were detected in the third trimester, leading to delivery by cesarean section. Birthweight was 4.53 kg (>95th centile; 50th centile for 5 weeks); length 47 cm (50th centile), and OFC was 40.5 cm (>95th centile; 50th centile for 3 months). Hypoglycemia, hypotonia, bilateral cryptorchidism, and scoliosis were noted postnatally. Respiratory distress led to a bronchoscopy at age 2 months that showed redundant soft tissues in the upper airway and subglottic edema and at 3 months a tracheostomy was performed. Severe failure to thrive led to nasogastric tube placement. Echocardiography showed left ventricular hypertrophy progressing slowly over the next few years. Developmental delay was first documented at age 5 months. Brain MRI showed prominent ventricles. The EEG was normal.

Storage disease had been suspected in the newborn period prompting thin layer chromatography of the urine for oligosaccharides, which was reported as "abnormal." However, subsequent fibroblast analysis for hexoaminidase, GM1 β -galactosidase, fucosidase, α -mannosidase, and sialidase were normal, as was another urine oligosaccharides assay at age 3 years. Also normal were urine organic acids, plasma amino acid analysis and lymphocyte and fibroblast karyotypes.

At 5 years 2 months, the height was 90.1 cm (<5th centile; 50th centile for 2 years 3 months); and OFC was 54.1 cm (98th centile). He had coarse facial findings characteristic of Costello syndrome including thick lips and gingival hypertrophy. The tracheostomy remained patent. Scoliosis and asymmetry of the chest were obvious. The skin had a loose texture, and deep plantar creases were noted. There were no papillomata. The toe nails were deeply embedded in the surrounding soft tissue. Hypermobile fingers and bilateral pronated pes planus were present. Clear speech and vocabulary was limited. The patient died suddenly and unexpectedly at home at age 5 years 4 months, slumped over on the toilet. No arrhythmias had been noted at any time prior to this.

Histochemistry

To extend the histopathological observations initially obtained on the three Costello syndrome patients reported here, additional histochemical and immunohistochemical analyses were performed. To better assess the quality and distribution of extracellular matrix components, the histological sections of cardiac tissues obtained at autopsy of the Costello patients and age-matched normal children (victims of motor vehicle accidents) were stained with Movat's pentachrome. This technique shows elastin as black, glycosaminoglycans as green, collagen as yellow, smooth muscle as red, and nuclei as dark blue [Musto, 1996; Hinek and Wilson, 2000]. Subsequent immunostainings have confirmed that the distribution of black-stained material with Movat's method entirely overlaps with immunodetectable elastin, and the yellow-stained material overlaps with immunodetectable collagen type I.

Immunostaining

Multiple parallel histological sections of cardiac tissues were post-fixed in cold 100% methanol at -20°C for 30 min, and immersed with target antigen retrieval solution (Daco S1700). Sections were routinely incubated with hydrogen peroxide to block endogenous peroxidase and with the protein blocking

solution (Daco X0909), then incubated for 60 min with 10 $\mu\text{g}/\text{ml}$ of each the following primary antibodies: polyclonal antibody to tropoelastin (Elastin Products, Inc., Owensville, MI), polyclonal (anti-S-GAL) which recognizes the EBP (40), monoclonal antibody to chondroitin sulfate (Sigma C-8035), and two monoclonal antibodies specifically recognizing the isoforms of chondroitin 4-sulfate or chondroitin 6-sulfate (both from Seikagaku, Japan). Polyclonal antibodies recognizing human collagen type I (LF 39), human biglycan (LF 112), and human versican (LF 99), were generous gifts from Dr. Larry W. Fisher of The National Institute of Health (Bethesda, MD). Antibody recognizing Ki-67 antigen (Daco) present in proliferating cells was also used. All sections were incubated for 30 min with the appropriate biotinylated goat anti-rabbit (GAR-HRP) and goat anti-mouse (GAM-HRP) secondary antibodies. An additional 20 min incubation with Horseradish peroxidase-conjugated streptavidin was followed by exposure to the DAB substrate chromogen solution from the peroxidase detection kit (Daco LSAB2 System, K0672). Nuclei were counterstained with hematoxylin. The slides were then assessed with a Nikon Eclipse E1000 microscope and photographed with a CCD camera.

RESULTS

Autopsy Examinations and Histological Studies

Patient 1. Post-mortem examination confirmed the clinical diagnosis of bowel encasement and necrosis due to viable and necrotic tumor. Although a chest radiographic had shown an opacity suspicious for lung metastasis, this was not found at autopsy. In addition to bowel obstruction, tumor compressive effects caused right hydronephrosis. The heart weighed 250 g (age- and sex-adjusted mean ~ 110 – 120 g) with concentric hypertrophy but demonstrated the usual anatomic landmarks. A small area of scarring was present in the ventricular septum, below the aortic valve in the left ventricular outflow tract. The routine histological examination of this scarred area indicated that this likely represented fibrosis following the prior myomectomy surgery. Diffuse myocyte hypertrophy and disarray, along with mild fibrosis in the region of the His bundle, was associated with fibrous fragmentation. There was, however, no evidence of recent infarction.

Additional histochemical evaluation of Movat pentachrome-stained heart tissue revealed the presence of multiple foci of severe myocardial fibrosis that consisted of numerous fibroblasts surrounded by yellow-stained bundles of collagen fibers and greenish amorphous deposits of glycosaminoglycans (Fig. 1A). Some of the fibrotic tissue penetrating foci of vacuolated cardiomyocytes was particularly rich in small capillaries and contained numerous fusiform and red stained smooth muscle cells (SMCs) (Fig. 1B). Interestingly, this hyperplastic connective scar tissue contained some Ki-67 positive cells, but did not contain any elastic fibers (Fig. 1C). Scarce and very thin elastic fibers were only visible in the stroma of the mildly affected regions. Moreover, elastic fibers that normally form strong parallel bundles in the pericardium and in the cardiac valves were very short, thin or fragmented in the pericardium (Fig. 1D) and valves (Fig. 1E) of this heart. The remarkable finding in this case was the obvious involvement of intracardial arteries, which regardless of their size, contained advanced hyperplastic intimal thickening consisting of vacuolated smooth muscle cells surrounded by the GAGs-rich extracellular matrix that peculiarly lacked elastin (Fig. 1F,H) and the EBP.

The presence of glycosaminoglycan-containing deposits detected by Movat staining around and inside the individual cardiomyocytes (Fig. 1I) and arterial SMCs was further confirmed by the positive immunostaining with the panel of specific antibodies recognizing chondroitin sulfate-bearing

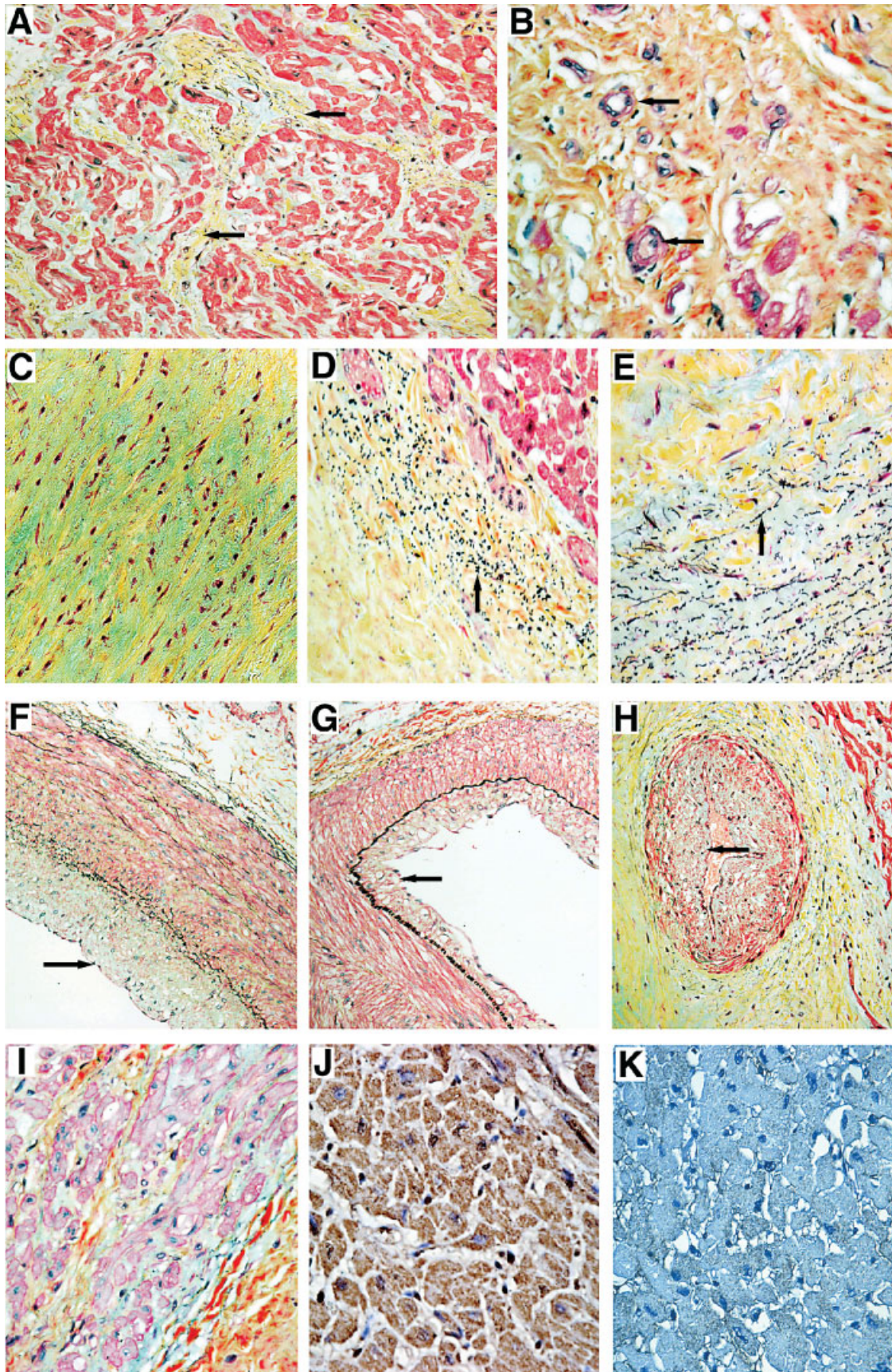


Fig. 1.

GAGs, versican and biglycan, and total chondroitin sulfate. Additional immunostaining with the more specific monoclonal antibodies revealed that GAGs stored by both cardiomyocytes and arterial SMCs contained mostly chondroitin-6-sulfate (Fig. 1J) but not chondroitin-4-sulfate (Fig. 1K)

Patient 2. The post-mortem examination of this large heart did not provide additional information about the distribution of the myocardial hypertrophy. Standard light microscopy noted subendocardial, nodular collections of Purkinje-like cells with sarcoplasmic vacuolization resembling glycogen deposits which did not stain for PAS. Further histochemical examination in addition to vacuolated cardiomyocytes (Fig. 2A) also revealed the presence of multiple focal endocardial fibrotic tissue lacking elastic fibers (Fig. 2B). The thin and fragmented elastic fibers were only observed in pericardium (Fig. 2C).

Immunohistochemistry revealed that the majority of extensively vacuolated cardiomyocytes had a high concentration of material immuno-reactive with antibody to general chondroitin sulfate (Fig. 2D) and chondroitin sulfate-bearing proteoglycans, versican (Fig. 2E) and biglycan (Fig. 2F). Further immunostaining with the more specific monoclonal antibodies showed that accumulated proteoglycans contained chondroitin-6-sulfate (Fig. 2G) but not chondroitin-4-sulfate (Fig. 2H). The higher than normal deposit of chondroitin sulfate proteoglycans were also present in the arterial walls of intramyocardial branches of coronary arteries (Fig. 2I). Despite this abnormality, the coronary arteries did not show intimal hyperplasia (Fig. 2J), but were characterized by lower than normal expression of the EBP (Fig. 2K,L).

Patient 3. Post-mortem examination limited to the chest showed severe hypertrophic cardiomyopathy (weight 210 g). The left ventricle was markedly hypertrophic with focal patchy fibrosis and marked myocyte hypertrophy. The mitral and aortic valve leaflets and cusps were thickened, although the tricuspid and pulmonic valves were normal. The coronary ostia were patent. There were no CVMs.

Histological evaluation showed that patchy abnormal regions were marked with severe collagenous fibrosis (Fig. 4A,C) and pericellular and intracellular storage of glycosaminoglycans immunodetected with antibody to general chondroitin sulfate (Fig. 4D), versican and biglycan (not shown). In fact, vacuolated cardiocytes that stored chondroitin sulfate moieties (Fig. 4D) resembled the dermatan sulfate storing cells in Hurler disease. The entire stroma of this heart contained only sparse thin elastic fibers that were often fragmented. The fragmentation of elastic fibers was particularly visible in the pericardium (Fig. 4B). The intra-myocardial branches of the coronary arteries did not show signs of intimal hyperplasia, but their walls were thicker than normal and contained numerous vacuolated SMCs (Fig. 4E), which also demonstrated higher than normal expression of chondroitin sulfate proteoglycans, accompanied with an obvious deficiency of immunodetected EBP (not shown). Remarkably, higher than normal expression of green-stained proteoglycans was also detected in the pulmonary artery, which elastic lamellae were partially disorganized or fragmented (Fig. 4F). Additional immunostaining with two antibodies selectively recognizing chondroitin sulfate molecules sulfated on position four or six demonstrated that the cardiomyocytes of this heart, similar to our two other

Costello syndrome patients, also had intracellular accumulation of chondroitin 6-sulfate (Fig. 4G) but not 4-sulfate-bearing GAGs (Fig. 4H). It is noteworthy that a normal heart (of a 6-year-old male who died in a motor vehicle accident) detected chondroitin-6-sulfate (Fig. 4I) and chondroitin-4-sulfate (Fig. 4J) only in the stroma.

DISCUSSION

There have been remarkably few pathologic examinations of tissues from individuals with Costello syndrome. An abnormality of elastic fibers was suggested by the detailed skin analyses of Vila Torres et al. [1994]. Mori et al. [1996] reported the only detailed postmortem examination of the heart that included a description of elastic fiber degeneration. This coarse facial appearance, which becomes more apparent over time is reminiscent of certain mucopolysaccharidoses or glycogen storage disease [Cleary et al., 2002; Kaji et al., 2002] and suggests that Costello syndrome might also be a storage disease.

The histological and immunohistochemical analyses of heart tissue obtained from autopsies of three children described in this report demonstrate very similar morphological features. Based on this small series, we propose that such features are typical of individuals with Costello syndrome. However, our experience with this cardiac phenotype is too limited to determine the specificity or sensitivity of this observation. Cardiomyocytes from the hearts of all three children with Costello syndrome were characterized by pericellular and intracellular accumulation of chondroitin-6-sulfate-bearing GAGs and their endocardia showed the presence of the multiple foci of the fibrotic tissue, particularly rich in collagen, but peculiarly lacking elastic fibers. The observation of impaired (thin, short, and fragmented) elastic fibers in the myocardial stroma, pericardium and cardiac valves, coinciding with lower than normal presence of the EBP, delineates another characteristic feature detected in all three hearts analyzed.

In contrast to the heart of patient 1 who died of rhabdomyosarcoma and had been treated with anticancer drugs, the hearts of the two other patients did not reveal obvious hyperplastic intimal thickening in intramyocardial branches of the coronary arteries, or intracardial arteries.

In fact, intracardial arteries of all three analyzed hearts-14 line, regardless of their size, contained higher than normal deposits of chondroitin sulfate proteoglycans and showed lower than normal rate of EBP. Such features, previously detected in intimal cushions of closing ductus arteriosus [Hinek et al., 1991, 1992] and in atherosclerotic arteries [Wight, 1995; Wight et al., 1997; Wight, 2002; Tao et al., 1997; Evanko et al., 1998; Radhakrishnamurthy et al., 1998; Kunecki and Nawrocka, 2001; Chung et al., 2002], might reflect the early stage of arterial disease that could predispose to the later development of occlusive hyperplastic arteriopathy. Since the presence of partially occluded arteries was only observed in the heart of patient 1 who died of rhabdomyosarcoma and had been treated with anticancer drugs, we speculate that this highly advanced hyperplastic arteriopathy could be triggered by circulating growth factors, likely released from the growing tumors or during remodeling of tissues injured by anticancer drugs.

Fig. 1. Representative photomicrographs illustrating histological findings in the heart of patient 1. Movat pentachrome stain of heart tissue revealed the presence of multiple foci of severe myocardial fibrosis (arrows) that consisted of numerous fibroblasts surrounded by yellow-stained bundles of collagen fibers, greenish amorphous deposits of glycosaminoglycans (A). The fibrotic tissue was particularly rich in small capillaries (arrows) and contained numerous red stained smooth muscle cells (B) and did not contain any elastic fibers (C). The elastic fibers were very short, thin or fragmented in the pericardium (D) and valves (E) of this heart. The

intracardial arteries, regardless of their size, contained advanced hyperplastic intimal thickening (arrows) consisting of vacuolated smooth muscle cells surrounded by the GAGs-rich extracellular matrix that peculiarly lacked elastin (F-H). Movat stain detected the presence of greenish glycosaminoglycan-containing deposits around and inside the individual cardiomyocytes (I). Immunostaining (brown deposits) with specific monoclonal antibodies revealed that these stored glycosaminoglycans contain mostly chondroitin-6-sulfate (J) but not chondroitin-4-sulfate (K).

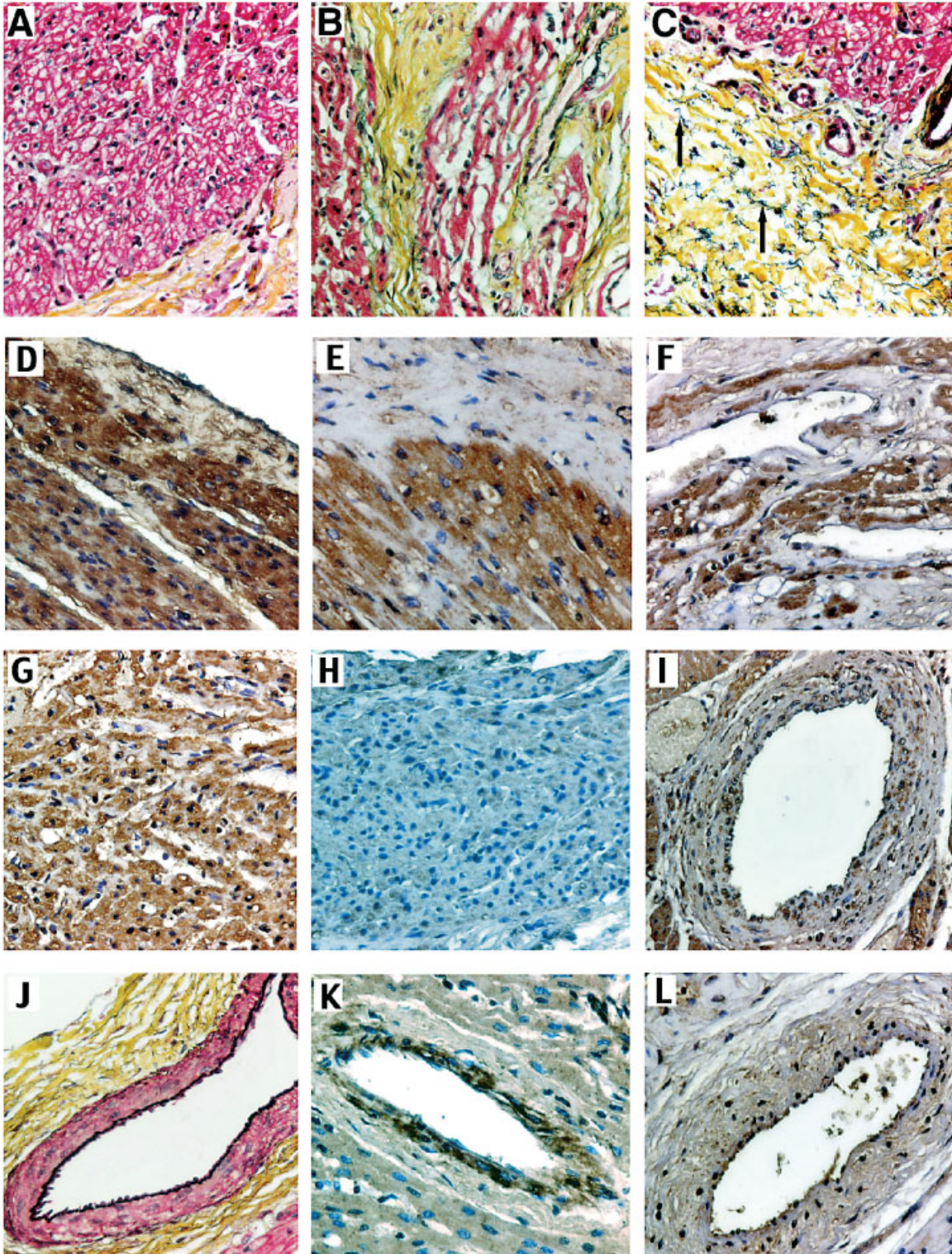


Fig. 2. Representative photomicrographs illustrating histological findings in the heart of patient 2. Movat stained sections of the left ventricle demonstrated myocardium containing highly vacuolated cardiomyocytes (A) and the multiple foci of collagenous fibrotic (yellow) tissue lacking elastic fibers (B). The thin and fragmented elastic fibers (arrows) were only observed in pericardium (C). Immunostaining (brown deposits) of parallel sections revealed that the majority of cardiomyocytes stored moieties that reacted with antibody to general chondroitin sulfate (D) and with antibodies to versican (E) and biglycan (F). Immunostaining with the more specific

monoclonal antibodies demonstrates that material stored by cardiocytes contained chondroitin-6-sulfate (G) but not chondroitin-4-sulfate (H). The higher than normal deposit of chondroitin sulfate proteoglycans were also present in the arterial walls of intra-myocardial branches of coronary arteries (I). Intracardiac arteries of this heart did not show hyperplasia (J). However, in contrast to arteries of the normal heart that demonstrated a high expression of the elastin binding protein (EBP) in their SMC's (K), arterial walls of this heart were mostly characterized by a very low content of this elastin chaperone (L).

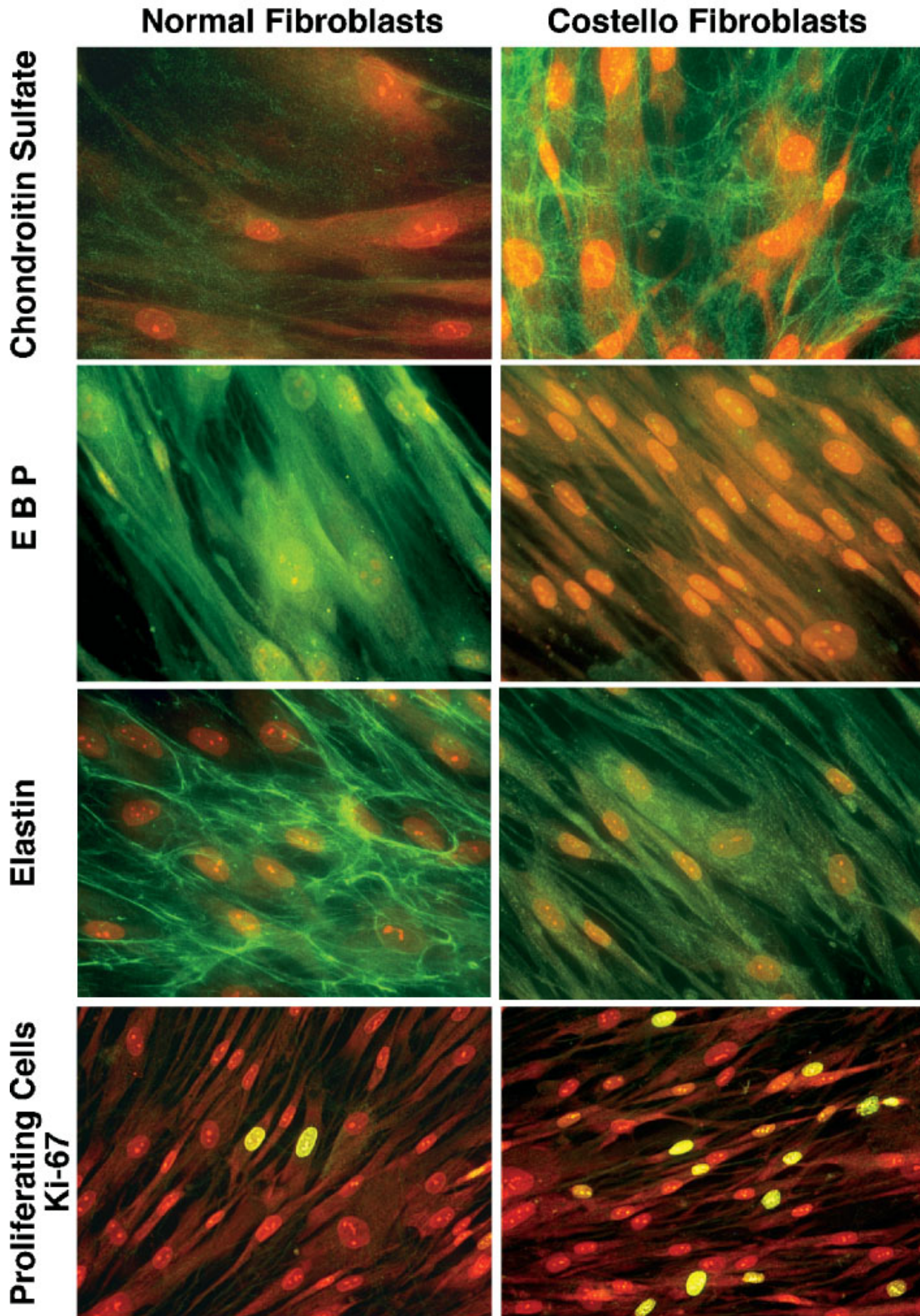


Fig. 3. Representative photomicrographs of 10-day-old cultures of dermal fibroblasts derived from normal individual (**left column**) and from patient 2 (**right column**), immunostained with a panel of specific antibodies. When compared with normal dermal fibroblasts, fibroblasts derived from our Costello syndrome patient demonstrated significantly higher deposition of chondroitin sulfate and lack of EBP. In contrast to normal cells, Costello syndrome fibroblasts did not produce any elastic fibers, but demonstrated higher than normal proliferation, marked with Ki-67 positive cells.

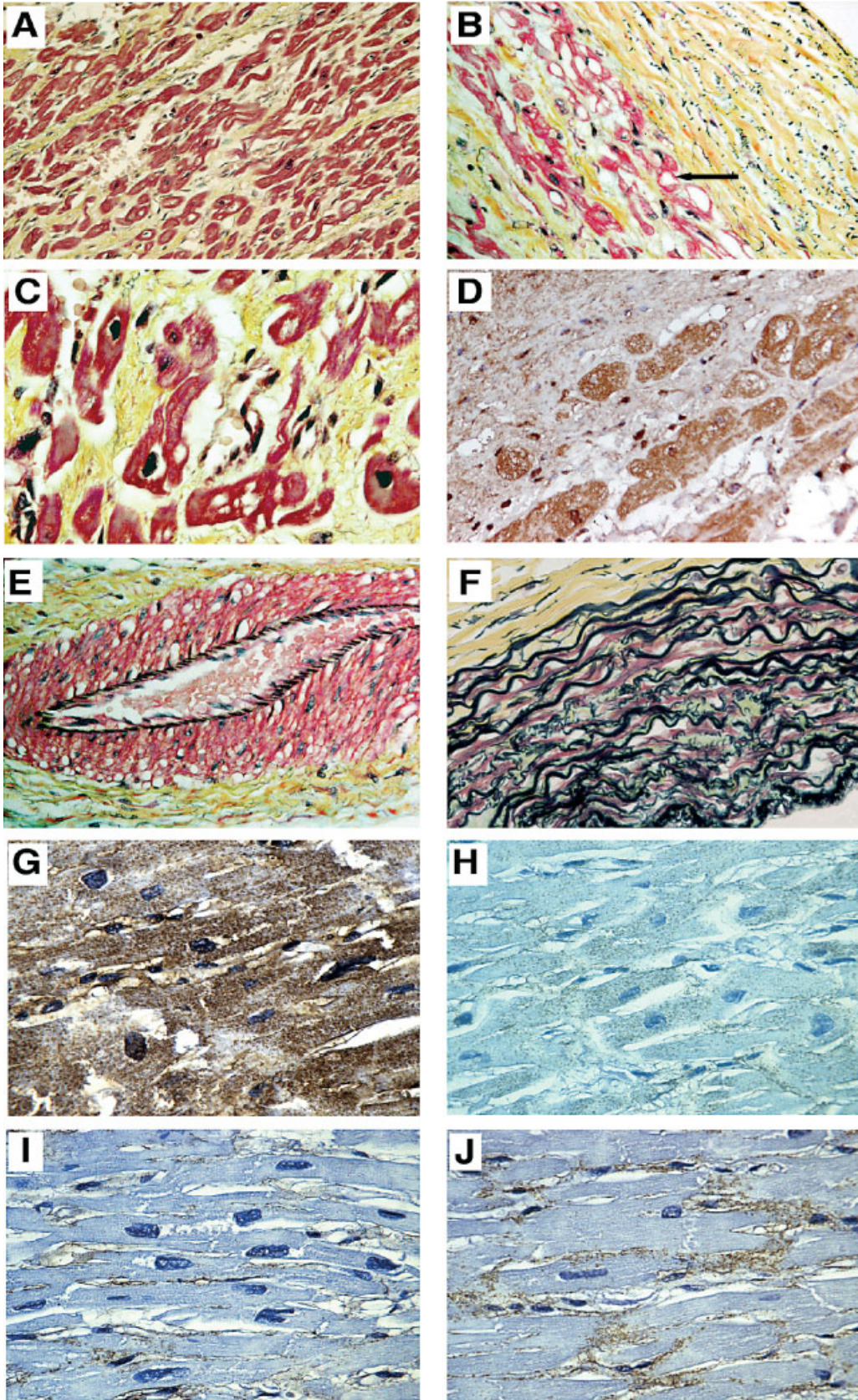


Fig. 4.

Comparison With Hurler Syndrome

It is important to stress that arteriopathy in patient 1, strongly resembled pathomorphological changes characteristic for Hurler disease [Brosius and Roberts, 1981]. Hurler disease (MPS 1) is associated with several cardiac abnormalities including hypertrophic cardiomyopathy, valvular thickening and insufficiency, and striking coronary artery occlusion. Because of a primary deficiency in lysosomal α -L-iduronidase, there is an accumulation of dermatan sulfate and heparan sulfate glycosaminoglycans [Neufeld et al., 1997] coexisting with impaired deposition of elastic fibers [Hinek et al., 2000]. We have also noticed a striking resemblance between the vacuolated cardiomyocytes storing chondroitin-6-sulfate containing moieties in this series of three children with Costello syndrome and vacuolated "Hurler cells," characterized by intracellular and pericellular accumulation of dermatan sulfate (another galactosugar-bearing glycosaminoglycan), and heparan sulfate-containing glycosaminoglycans and glycolipids [Hinek et al., 2000]. While some of the vacuoles detected in the Costello syndrome cardiomyocytes contained green-stained (Movat) or immunodetected GAGs, the others looked empty, since a routine fixation of cardiac tissue may not have properly preserved the glycolipids. While we did not collect material for biochemical assay of α -L-iduronidase from all three patients, we found that activity of this enzyme tested in cultured fibroblasts derived from patient 2 did not differ from normal control. Moreover, we have recently tested fibroblasts from eight living patients diagnosed with Costello syndrome. In all eight cases, activity of α -L-iduronidase (tested in an *in vitro* colorimetric assay) or its intracellular immunolocalization did not differ from normal controls.

Both Hurler disease cardiomyopathy [Haust, 1987; Hinek and Wilson, 2000] and what we describe in our Costello syndrome patients are associated with the impaired elastogenesis. The results of our present studies support our previous hypotheses that accumulation of galactosugar-containing compounds, dermatan sulfate or chondroitin sulfate can both trigger shedding and functional inactivation of the 67-kDa EBP. The consequent impaired elastic fiber deposition may constitute a common pathologic mechanism responsible for similar clinical features in Hurler disease and Costello syndrome [Hinek et al., 2000]. Existence of phenotypic similarities between individuals with Costello syndrome, Hurler disease, and other mucopolysaccharidoses [Belcher, 1972; Haust, 1987; Gross et al., 1988; Hopwood and Morris, 1990; Nelson et al., 1990; Viñalongo et al., 1992; Neufeld and Muenzer, 1997; Dangel, 1998] raises the obvious question whether Costello syndrome might belong to this group of metabolic storage diseases caused by a primary deficiency in enzymes responsible for degradation of chondroitin sulfate moieties. Biochemical and cardiac findings in Costello syndrome and similar conditions are listed in Table I. In addition to those conditions are I-cell disease, sialidosis, and galactosialidosis which have less clinical overlap but are also characterized by elastin-binding protein deficiency. Neuraminidase is deficient in sialidosis and Protective Protein-Cathepsin A is deficient in galactosialidosis [Morrone et al., 2000].

We previously reported that cultured fibroblasts derived from dermal biopsies of patients with Costello syndrome dis-

play accumulation of the chondroitin sulfate-bearing proteoglycans in multiple cytoplasmic vesicles [Hinek et al., 2000]. Our new finding is more precise in that the chondroitin-6-sulfate constitutes the majority of the compound accumulating in cytoplasmic vacuoles of cardiomyocytes from patients with Costello syndrome.

Costello Syndrome as a Possible Enzyme Deficiency

At present, we can only speculate whether this phenomenon might be triggered by an apparent inadequate production of chondroitin 4-sulfate (possible deficiency in one of several known CS4-specific sulfo-transferases), followed by opportunistic over-sulfation and accumulation of chondroitin-6-sulfate, or by the real deficiency of certain lysosomal enzymes specifically responsible for the proper degradation of chondroitin-6-sulfate. Although the primary genetic defect in Costello syndrome is not yet identified, the presented observations substantially narrow and direct the future search for the candidate gene responsible for this disease.

Elastin is often thought to be a component produced in the latest stages of fetal development and in the perinatal period [Parks et al., 1993; Rosenbloom et al., 1993; Christiano and Uitto, 1994; Pasquali-Ronchetti and Baccarani-Contri, 1997; Vrhovski and Weiss, 1998; Debelle and Tamburro, 1999]. However, studies with the developing chick embryo, using *in situ* hybridization revealed, that tropoelastin mRNA is expressed early during development [Holzenberger et al., 1993]. Studies by Hurler et al. [1994] also demonstrated the presence of elastic fibers during early morphogenesis of the limb skeleton *in vivo* and *in vitro*. They suggested that the elastic fiber scaffold plays an important role in coordinating the size and the spatial location of the cartilaginous skeletal elements within the limb buds. They also observed precise patterns of elastic fiber arrangement present in the early developing lung and in the outflow tract and atrioventricular cushion tissue of the heart. These observations pointed to previously unsuspected functions for elastic matrices during embryonic development and support our hypothesis, that impaired elastogenesis in the developing skeleton, skin, and heart may play an important role in the overall pathophysiology of Costello syndrome.

One can speculate that development of the skin, perichondrium, ligaments, connective tissues and cardiac stroma that are deficient in elastic fibers is compromised leading to multiple deformities and lack of resiliency in tissues of patients with Costello syndrome. Lack of elastic fibers in the heart stroma and pericardium may contribute to the development of hypertrophic cardiomyopathy. Although both Costello and Hurler syndrome are frequently associated with hypertrophic cardiomyopathy (Table I), only Hurler syndrome has been associated with endocardial fibroelastosis and congestive cardiomyopathy with ventricular dilation.

We have previously reported that fibroblasts derived from patients with Costello syndrome that lack the ability to assemble normal elastic fibers display heightened rate of proliferation [Hinek et al., 2000]. We also demonstrated that experimental overexpression of a V3 spliced variant of versican, which lacks chondroitin sulfate in cultured fibroblasts

Fig. 4. Representative photomicrographs illustrating histological findings in the heart of patient 3. Movat staining of the left ventricle revealed myocardium marked with severe collagenous (yellow) fibrosis (A) and the presence of vacuolated cardiomyocytes (arrow) (B, C) storing moieties also detected with antibody to chondroitin sulfate (brown deposits) (D). The walls of intracardiac arteries were thicker than normal and contained numerous vacuolated SMCs (E). Accumulation of green-stained GAGs was obvious in pulmonary artery, which elastic lamellae were partially

disorganized (F). Immunostaining (brown deposits) with antibodies selectively recognizing two different forms of chondroitin sulfate molecules showed that cardiomyocytes of this patient accumulated chondroitin-6-sulfate (G) but not chondroitin-4-sulfate-bearing GAGs (H). In contrast, to Costello syndrome heart storing chondroitin-6-sulfate-bearing moieties intracellularly, the normal heart (from a 6-year-old male who died in a motor vehicle accident) shows the equal presence of both chondroitin-6-sulfate (I) and chondroitin-4-sulfate (J) detected only in the supportive stromal tissue.

TABLE I. Impaired Elastogenesis: Comparison of Biochemical and Cardiac Abnormalities in Costello Syndrome and Clinically Similar Disorders

Disease	Enzyme deficiency	Accumulation	Elastin binding protein	Valve abn	HCM	Cardiac abnormalities (% among all)		
						Coronary occlusion	HTN	Atrialtachycardia
Costello	Unknown	Heart: chondroitin sulfate	Functional inactivation, reduced expression, impaired ELN assembly	13%, PVS, Occ. MV	30%, ASH, BVH	No, +	+	30%, SVT, CAR
Hurler (MPS I H)	α -L-iduronidase	Dermatan sulfate (Heparan sulfate)	As above	MV, AoV, TV, PV	ASH, LVH	+	+	No
Morquio B (MPS IV)	Acid Lysosomal β -galactosidase	GMI gangliosides (not in neurons)	As above	MV, AoV	ASH, LVH	+	No	No
Infantile, GMI, gangliosidosis	As above	GMI gangliosides (neurons)	As above	No	+	No	No	No
Sanfilippo (MPS III)	Heparan N-sulfatase	Heparan sulfate	Normal	25%, MV, AoV	+, ASH	No	No	No
Williams syndrome	None	None	Normal (ELN deficiency)	90%, SVAS, +AoVS	No	Yes	Yes	No

The description of the cardiac hypertrophy was not standardized; instead the terms used in the clinical reports were provided.

Abn. abnormality; AoV(S), aortic valve (stenosis); ASH, asymmetric septal hypertrophy; BVH, biventricular hypertrophy; CAR, chaotic atrial rhythm; ELN, elastin; HCM, hypertrophic cardiomyopathy; HTN, hypertension; MPS, mucopolysaccharidosis; MV, mitral valve; PV(S), pulmonary valve (stenosis); SVAS, supra-ventricular aortic stenosis; SVT, supra-ventricular tachycardia; TV, tricuspid valve.

Sources: Belcher [1972], Haust [1987], Gross et al. [1988], Nelson et al. [1990], Hopwood and Morris [1990], Vinallonga et al. [1992], Neufeld and Muenzer [1997], Dangel [1998].

^aAnecdotal reporting of hypertension, currently idiopathic.

from Costello syndrome, caused a competitive loss of chondroitin sulfate from their cell surface and completely reverses impaired elastogenesis, restoring normal proliferation of these cells [Hinek et al., 2004]. These previous observations, together with the current findings in hearts of all three Costello syndrome patients, provide a strong endorsement for a suggested pathophysiological link between accumulation of chondroitin sulfate-bearing moieties, the absence of insoluble elastin and increased cell proliferation.

Increased cellular proliferation detected in the hearts of Costello syndrome patients associated with decreased insoluble elastin in extracellular matrix may result from several overlapping mechanisms. First, constant chondroitin sulfate-induced shedding of the 67-kDa EBP that normally acts as the recyclable tropoelastin chaperone [Hinek et al., 1993; Hinek, 1994, 1996; Hinek and Rabinovitch, 1994] leads to a significant decrease in effective deposition of insoluble elastin in tissues of patients with Costello syndrome. Consequently, a lack of insoluble elastin can eliminate sequestering of the soluble growth factors (e.g., PDGF and bFGF) by this polymer protein, before they can interact with their cell surface receptors [Hinek, 2003]. Secondly, since cell surface residing EBP has been implicated in blocking of interleukin type I receptors [Hinek et al., 1996], its chondroitin sulfate-induced shedding may lead to unmasking of adjacent cell surface receptors that normally transduce mitogenic signals. This latter mechanism does not preclude the possibility that chondroitin sulfate-containing proteoglycans accumulating on the surface of cells from Costello syndrome patients may also act as co-receptors for such major growth factor as bFGF, that has been widely implicated in the growth of numerous human tumors [Herrlich et al., 1998; Sneath and Mangham, 1998; Chiu et al., 1999; Humphrey et al., 1999], including rhabdomyosarcoma which has been reported in Costello syndrome patients [Gripp et al., 2002].

Cardiac Clinical Correlation

The findings discussed in this paper relate to various cardiac manifestations of Costello syndrome, including the cardiac hypertrophy, small vessel disease, and supra-ventricular arrhythmias [Proud et al., 1996]. Abnormal atrial automaticity and triggered arrhythmias have been suggested as underlying mechanisms for a similar arrhythmia in adults [Lin et al., 2002]. It has been also shown that in storage diseases, such as in Pompe disease, conduction may be facilitated either through the normal atrioventricular (AV) node or through abnormal AV conduction pathways similar to these in the familial WPW syndrome with PRKAG2 mutations [Arad et al., 2003]. However, the tachycardia of Costello syndrome is not consistent with AV reentrant mechanisms. Multifocal ventricular reentry is often observed in arrhythmogenic right ventricular dysplasia where fatty and/or fibrotic replacement of ventricular myocytes occurs. Remarkably, abnormalities of ventricular repolarization associated with sudden death have been identified in some patients with Marfan syndrome (a primary fibrillin-1 disorder accompanied with abnormal formation of elastic fibers) [Yetman et al., 2003]. At this moment, we cannot conclude a specific mechanism underlying chaotic atrial tachycardias occurring in patients with Costello syndrome. We can only speculate that storage of chondroitin sulfate in the conductive system cells and other structural changes, similar to those described in the ventricular myocardium may be responsible for the impaired conductivity in the hearts of individuals with Costello syndrome. Future autopsy studies concentrated at the atrial myocardium and AV ring, in addition to ventricular assessment, should shed more light on pathophysiological links between impaired structure and function of Costello patient hearts.

ACKNOWLEDGMENTS

Dr. Hinek is supported by the Canadian Institute of Health Research (grant PG 13920) and by the Stroke Foundation of Ontario (grant NA 4381 and Career Investigator Award, CI 4198). Dr. Teitell is supported by NIH grants CA90571 and CA107300 and CMISE, with a NASA URETI award NCC 2-1364 (to MAT), and is a Leukemia and Lymphoma Society Scholar. Dr. Lin thanks the Armand Anzalone Research Fund. We thank the individuals, families and professionals of the Costello Syndrome Support Group for their continued support, and dedicate this work to the memory of those who are no longer with us. We also are grateful to Kela Liu for his excellent technical assistance.

REFERENCES

- Allen W, Sleater J, McGovern J, Hinek A. 2004. A child with Costello syndrome and fatal cardiomyopathy: Postmortem findings suggest the mechanism of cardiomyopathy might be due to the storage of chondroitin sulfate containing moieties. *Proc Greenwood Genet Ctr* 23:28–30.
- Arad M, Moskowitz IP, Patel VV, Ahmad F, Perez-Atayde AR, Sawyer DB, Walter M, Li GH, Burgon PG, Maguire CT, Stapleton D, Schmitt JP, Guo XX, Pizard A, Kupersmidt S, Roden DM, Berul CI, Seidman CE, Seidman JG. 2003. Transgenic mice overexpressing mutant PRKAG2 define the cause of Wolff–Parkinson–White syndrome in glycogen storage cardiomyopathy. *Circulation* 107:2850–2856.
- Belcher RW. 1972. Ultrastructure of the skin in the genetic mucopolysaccharidoses. *Arch Pathol* 94:511–518.
- Bisogno G, Murgia A, Mammi I, Strafella MS, Carli M. 1999. Rhabdomyosarcoma in a patient with cardio-facio-cutaneous syndrome. *J Pediatr Hematol Oncol* 21:424–427.
- Bodkin NM, Mortimer ES, Demmer LA. 1999. Male to male transmission of Costello syndrome consistent with autosomal dominant inheritance. *Am J Hum Genet* 65(Suppl):A143.
- Brosius FC III, Roberts WC. 1981. Coronary artery disease in the Hurler syndrome. Qualitative and quantitative analysis of the extent of coronary narrowing at necropsy in six children. *Am J Cardiol* 47:649–653.
- Chiu RK, Droll A, Dougherty ST, Carpenito C, Cooper DL, Dougherty GJ. 1999. Alternatively spliced CD44 isoforms containing exon v10 promote cellular adhesion through the recognition of chondroitin sulfate-modified CD44. *Exp Cell Res* 248:314–321.
- Christiano AM, Uitto J. 1994. Molecular pathology of elastic fibers. *J Invest Derm* 103:53–57.
- Chung IM, Gold HK, Schwartz SM, Ikari Y, Reidy MA, Wight TN. 2002. Enhanced extracellular matrix accumulation in restenosis of coronary arteries after stent deployment. *J Am Coll Cardiol* 40:2072–2081.
- Cleary MA, Walter JH, Kerr BA, Wraith JE. 2002. Facial appearance in glycogen storage disease type III. *Clin Dysmorph* 11:117–120.
- Dangel JH. 1998. Cardiovascular changes in children with mucopolysaccharide storage diseases and related disorders—clinical and echocardiographic findings in 64 patients. *Eur J Pediatr* 157:534–538.
- Debelle L, Tamburro AM. 1999. Elastin: Molecular description and function. *Int J Biochem Cell Biol* 31:261–272.
- Evanko SP, Raines EW, Ross R, Gold LI, Wight TN. 1998. Proteoglycan distribution in lesions of atherosclerosis depends on lesion severity, structural characteristics, and the proximity of platelet-derived growth factor and transforming growth factor-beta. *Am J Pathol* 152:533–546.
- Fukao T, Sakai S, Shimozawa N, Kuwahara T, Kano M, Goto E, Nakashima Y, Katagiri Kawade M, Ichihashi H, Masuno M, Orii T, Kondo N. 1996. Life-threatening cardiac involvement throughout life in a case of Costello syndrome. *Clin Genet* 50:244–247.
- Gripp KW, Scott CI Jr, Nicholson L, McDonald-McGinn DM, Ozeran JD, Jones M, Lin AE, Zackai EH. 2002. Four additional Costello syndrome patients with rhabdomyosarcoma: Proposal for a tumor screening protocol. *Am J Med Genet* 108:80–82.
- Gross DM, Williams JC, Caprioli C, Dominguez B, Howell RR. 1988. Echocardiographic abnormalities in the mucopolysaccharide storage diseases. *Am J Cardiol* 61:170–176.
- Haust MD. 1987. Arterial involvement in genetic diseases. *Am J Cardiovasc Pathol* 1:231–285.
- Hennekam RCM. 2003. Costello syndrome: An Overview. *Am J Med Genet Part C (Semin Med Genet)* 117C:42–48.
- Herrlich P, Sleeman J, Wainwright D, Konig H, Sherman L, Hilberg F, Ponta H. 1998. How tumor cells make use of CD44. *Cell Adhes Commun* 6:141–147.
- Hinek A. 1994. Nature and multiple functions of the 67 kDa elastin/laminin binding protein. *Cell Adhes Commun* 2:185–193.
- Hinek A. 1996. Biological functions of the non-integrin elastin/laminin receptor. *Biol Chem* 377:471–480.
- Hinek A. 2003. Impaired elastogenesis and development of the non-atherosclerotic occlusive arterial diseases in children. *Ann Diagnost Pediatr Pathol* 7(1–2):7–14.
- Hinek A, Rabinovitch M. 1994. 67-kDa elastin binding protein is a protective ‘companion’ of extracellular insoluble elastin and intracellular tropoelastin. *J Cell Biol* 126:563–574.
- Hinek A, Wilson SE. 2000. Impaired elastogenesis in Hurler disease. Dermatan sulfate accumulation linked to deficiency in elastin-binding protein and elastic fiber assembly. *Am J Pathol* 156:925–938.
- Hinek A, Wrenn DS, Mecham RP, Barondes SH. 1988. The elastin receptor: A galactoside-binding protein. *Science* 239:1539–1541.
- Hinek A, Mecham RP, Keeley F, Rabinovitch M. 1991. Impaired elastin fiber assembly is related to reduced 67 kDa elastin binding protein in fetal lamb ductus arteriosus and in cultured aortic smooth muscle cells treated with chondroitin sulfate. *J Clin Invest* 88:2083–2094.
- Hinek A, Boyle J, Rabinovitch M. 1992. Vascular smooth muscle cells detachment from elastin and migration through elastic laminae is promoted by chondroitin sulfate-induced shedding of the 67-kDa Cell surface elastin binding protein. *Exp Cell Res* 203:344–353.
- Hinek A, Rabinovitch M, Keeley F, Callahan J. 1993. The 67 kD elastin/laminin-binding protein is related to an alternatively spliced β -Galactosidase. *J Clin Invest* 91:1198–1205.
- Hinek A, Keeley F, Callahan J. 1995. Recycling of the 67-kDa elastin binding protein in arterial monocytes is imperative for secretion of tropoelastin. *Exp Cell Res* 220:312–324.
- Hinek A, Molossi S, Rabinovitch M. 1996. Functional interplay between Interleukin-1 receptor and elastin binding protein regulates fibronectin production in coronary artery smooth muscle cells. *Exp Cell Res* 225:122–131.
- Hinek A, Smith AC, Cutiongco EM, Callahan JW, Gripp KW, Weksberg R. 2000a. Decreased elastin deposition and high proliferation of fibroblasts from Costello syndrome are related to functional deficiency in the 67-kDa elastin binding protein. *Am J Hum Genet* 66:859–872.
- Hinek A, Zhang S, Smith AC, Callahan JW. 2000b. Impaired elastic-fiber assembly by fibroblasts from patients with either Morquio B disease or infantile GM1-gangliosidosis is linked to deficiency in the 67-kDa spliced variant of B-Galactosidase. *Am J Hum Genet* 67(1):23–36.
- Hinek A, Braun KR, Liu K, Wang Y, Wight TN. 2004. Retrovirally mediated overexpression of versican v3 reverses impaired elastogenesis and heightened proliferation exhibited by fibroblasts from Costello syndrome and Hurler disease patients. *Am J Pathol* 164:119–131.
- Holzenberger M, Lievre CA, Robert L. 1993. Tropoelastin gene expression in the developing vascular system of the chicken: An in situ hybridization study. *Anat Embryol (Berlin)* 188:481–492.
- Hopwood JJ, Morris CP. 1990. The mucopolysaccharidoses: Diagnosis, molecular genetics and treatment. *Mol Biol Med* 7:381–404.
- Humphrey G, Hazel DL, MacLennan K, Lewis I. 1999. Expression of CD44 by rhabdomyosarcoma: A new prognostic marker? *Br J Cancer* 80:918–921.
- Hurle JM, Corson G, Daniels K, Reiter RS, Sakai LY, Solursh M. 1994. Elastin exhibits a distinctive temporal and spatial pattern of distribution in the developing chick limb in association with the establishment of the cartilaginous skeleton. *J Cell Sci* 107:2623–2634.
- Innes AM, Chudley AE. 2000. Rhabdomyosarcoma in a patient with cardio-facio-cutaneous syndrome. *J Pediatr Hematol Oncol* 22:546.
- Ioan DM, Fryn JP. 2002. Costello syndrome in two siblings and minor manifestations in their mother. Further evidence for autosomal dominant inheritance? *Genet Couns* 13:353–356.
- Kaji M, Kurokawa K, Hasegawa T, Oguro K, Saito A, Fukada T, Ito M, Sugie H. 2002. A case of Costello syndrome and glycogen storage disease type III. *J Med Genet* 39:e8.
- Kunecki M, Nawrocka A. 2001. Elastin-laminin receptor and abdominal aortic aneurysms. New subject to study? A review. *Pathol Biol (Paris)* 49(4):333–338.

- Lin AE, Grossfeld PD, Hamilton R, Smoot L, Proud V, Weksberg R, Gripp K, Wheeler P, Picker J, Irons M, Zackai E, Scott CI, Nicholson L. 2002. Further delineation of cardiac anomalies in Costello syndrome. *Am J Med Genet* 111:115–129.
- Lurie IW. 1994. Genetics of the Costello syndrome. *Am J Med Genet* 52:358–359.
- Marino B, Digilio MC, Toscano A, Giannotti A, Dallapiccola B. 1999. Congenital heart diseases in children with Noonan syndrome: An expanded cardiac spectrum with high prevalence of atrioventricular canal. *J Pediatr* 135:703–706.
- Mecham RP, Whitehouse L, Hay M, Hinek A, Sheetz MP. 1991. Ligand affinity of the 67 kDa elastin/laminin binding protein is modulated by the protein's lectin domain: Visualization of elastin/laminin-receptor complexes with gold-tagged ligands. *J Cell Biol* 113:187–194.
- Mori M, Yamagata T, Mori Y, Nokubi M, Saito K, Fukushima Y, Momoi MY. 1996. Elastic fiber degeneration in Costello syndrome. *Am J Med Genet* 61:304–309.
- Morrone A, Bardelli T, Donati MA, Giorgi M, DiRocco M, Gatti R, Parini R, Ricci R, Taddeucci G, D'Azzo A, Zammarchi E. 2000. B-galactosidase gene mutations affecting the lysosomal enzyme and the elastin-binding protein in GM1-gangliosidosis patients with cardiac involvement. *Hum Mut* 15:354–366.
- Musto L. 1996. Modified Movat pentachrome stain. *J Histotechnol* 9:173–174.
- Nelson J, Shields MD, Mulholland HC. 1990. Cardiovascular studies in the mucopolysaccharidoses. *J Med Genet* 27:94–100.
- Neufeld E, Muenzer J. 1997. The mucopolysaccharidoses. In: Scriver CR, Beaudet AL, Sly WS, Valle D, editors. *The metabolic basis of inherited diseases*. New York: McGraw-Hill. pp 2465–2494.
- Parks WC, Pierce RA, Lee KA, Mecham RP. 1993. Elastin. *Adv Mol Cell Biol* 6:131–181.
- Pasquali-Ronchetti I, Baccarani-Contri M. 1997. Elastic fiber during development and aging. *Microsc Res Tech* 38:428–435.
- Privitera S, Prody CA, Callahan JW, Hinek A. 1998. The 67-kDa enzymatically inactive alternatively spliced variant of beta-galactosidase is identical to the elastin/laminin-binding protein. *J Biol Chem* 273:6319–6326.
- Proud VK, Keppler-Noreuil K, Lau Y. 1996. Lethal cardiac arrhythmia in Costello syndrome: Two new patients and review of cardiac findings in patients described in the literature. *Proc Genet Greenwood Ctr* 16:210.
- Radhakrishnamurthy B, Tracy RE, Dalferes ER Jr, Berenson GS. 1998. Proteoglycans in human coronary arteriosclerotic lesions. *Exp Mol Pathol* 65:1–8.
- Rosenbloom J, Abrams WR, Mecham RP. 1993. Extracellular matrix 4: The elastic fiber. *FASEB J* 7:1208–1218.
- Sneath RJ, Mangham DC. 1998. The normal structure and function of CD44 and its role in neoplasia. *Mol Pathol* 51:191–200.
- Tandoi C, Botta A, Fini G, Sangiuolo F, Novelli G, Ricci R, Zampino G, Anichini C, Dallapiccola B. 2001. Exclusion of the elastin gene in the pathogenesis of the Costello syndrome. *Am J Med Genet* 98:286–297.
- Tao Z, Smart FW, Figueroa JE, Glancy DL, Vijayagopal P. 1997. Elevated expression of proteoglycans in proliferating vascular smooth muscle cells. *Atherosclerosis* 135:171–179.
- Tomita H, Fuse S, Ikeda K, Matsuda K, Chiba S. 1998. An infant with Costello syndrome complicated with fatal hypertrophic obstructive cardiomyopathy. *Acta Paediat Jap* 40:608–611.
- Viñallonga X, Sanz N, Balaguer A, Miro L, Ortega JJ, Casaldaliga J. 1992. Hypertrophic cardiomyopathy in mucopolysaccharidoses: Regression after bone marrow transplantation. *Pediatr Cardiol* 13:107–109.
- Vila Torres J, Pineda Marfa MP, Gonzalez Ensenat M, Lloreta Trull J. 1994. Pathology of the elastic tissue of the skin in Costello syndrome. An image analysis study using mathematical morphology. *Anal Quant Cytol Histol* 16:421–429.
- Vrhovski B, Weiss AS. 1998. Biochemistry of elastin. *Eur J Biochem* 258:1–18.
- Wight TN. 1995. The extracellular matrix and atherosclerosis. *Curr Opin Lipidol* 6:326–334.
- Wight TN. 2002. Versican: A versatile extracellular matrix proteoglycan in cell biology. *Curr Opin Cell Biol* 14:617–623.
- Wight TN, Lara S, Riessen R, Le Baron R, Isner J. 1997. Selective deposits of versican in the extracellular matrix of restenotic lesions from human peripheral arteries. *Am J Pathol* 151:963–973.
- Yetman AT, Bornemeier RA, McCrindle BW. 2003. Long-term outcome in patients with Marfan syndrome: Is aortic dissection the only cause of sudden death? *J Am Coll Cardiol* 41:329–332.