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Genotyping and Prenatal Assessment of Collagen Lysyl Hydroxylase Deficiency in a Family with Ehlers-Danlos Syndrome Type VI

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SUMMARY

Collagen lysyl and prolyl hydroxylase activities were measured in cultured fibroblasts from a child with clinical features of Ehlers-Danlos syndrome. Lysyl-to-prolyl hydroxylase activity ratios in cells from the proband, mother, father, and control were .24, .86, .52, and 1.00, respectively, providing a biochemical diagnosis of Ehlers-Danlos syndrome type VI and indicating an autosomal recessive mode of inheritance in this family. Prenatal assessment of lysyl hydroxylase deficiency was requested and accomplished for the first time during a subsequent pregnancy in the family. A series of control cultures established lysyl hydroxylase activity to be similar in cultured amniotic fluid cells (AF and F cells) and in cultured dermal fibroblasts. Cultured F and AF cells from the monitored pregnancy had enzyme activity similar to controls, indicating that the fetus should not be affected by lysyl hydroxylase deficiency. This finding was confirmed by demonstration of normal lysyl hydroxylase activity in fibroblasts cultured from the newborn baby. These studies show that cells cultured from second trimester amniotic fluid have collagen lysyl hydroxylase activity similar to that in dermal fibroblasts, making prenatal diagnosis of lysyl hydroxylase deficiency possible.

INTRODUCTION

Ehlers-Danlos syndrome is a heterogenous group of inherited connective disorders. At least 10 distinct types have been defined, based on genetic and phenotypic

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expression [1–3]. Specific biochemical defects in collagen metabolism have been identified for types IV, VI, and VII [4–11]. A recent review of diseases of collagen by Prockop and Kiviriko [12] summarizes other less well-defined molecular defects including abnormal copper metabolism and deficient lysyl oxidase activity reported for type IX.

Type VI Ehlers-Danlos syndrome was the first connective tissue disease to have a molecular defect identified. Reduced lysyl hydroxylase activity in cultured fibroblasts and lack of hydroxylysine in skin was defined as the underlying defect [8, 9]. Several patients with similar clinical and biochemical characteristics were subsequently reported [13–16]. However, data now available indicate that the association of clinical symptoms and biochemical defects in this disease varies from patient to patient. Two additional variant forms of Ehlers-Danlos syndrome type VI have now been described: a form manifesting skeletal, dermal, and ocular abnormalities associated with nearly normal hydroxylysine content in skin, but with only little lysyl hydroxylase activity in cultured fibroblasts; and a predominantly ocular form with unknown biochemical defects in skin or cultured fibroblasts [17–19]. Further insight into the differentiation of this disease among individuals with impaired lysyl hydroxylase activity may be provided by responsivity to vitamin C, a cofactor for this enzyme. Treatment with pharmacological amounts of ascorbate improved the clinical status of one patient and increased his hydroxylysine production in vivo [14–15]. We report here a new family with Ehlers-Danlos syndrome type VI and the first successful prenatal monitoring of collagen lysyl hydroxylase deficiency.

MATERIALS AND METHODS

Cell Culture and Enzyme Preparation

Skin biopsies and amniotic fluid cells were grown in monolayer cultures using Dulbecco and Vogt's basal medium (D&V) supplemented with 15% fetal bovine serum and nonessential amino acids by methods described [20]. Cells from a 75-cm^2 flask harvested 2 days postconfluence were washed twice with phosphate-buffered saline and suspended in 0.75 ml 0.2 M NaCl, 0.1 M glycine, 50 μM DTT, 20 mM Tris-HCl, pH 7.5, and 0.1% Triton X-100. The cell suspension was frozen overnight at −20°C, then thawed and centrifuged at 15,000 *g* for 10 min. The supernatant was used for enzyme assay. Protein content was determined by a modified method of Lowry et al. [21].

Enzyme Assay

Unhydroxylated collagen substrate labeled with L-[4,5-^{3}H]-lysine or L-[3,4^{3}H]-proline was prepared from 14-day-old chick calvaria as described [22]. Radioisotopes were purchased from New England Nuclear (Boston, Mass.). Collagen lysyl and prolyl hydroxylase activities were determined by the tritium-release method of Peterkofsky and DiBlasio [23] as modified by Miller et al. [22].

RESULTS

Clinical Summary

The propositus (II-1, fig. 1) was born January 28, 1978, at full-term without premature rupture of membranes, weighing 8 lbs., 10 oz. At birth, hemifacial asymmetry was present and dislocation of the right wrist and hip were treated.

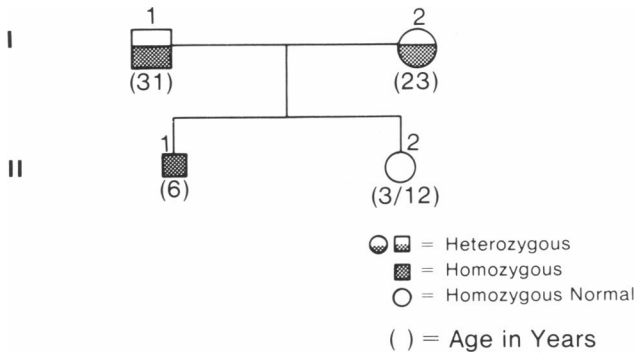

FIG. 1.—Abbreviated pedigree showing the genotype of family T. with Ehlers-Danlos syndrome type VI.

At age 6 months, he underwent bilateral inguinal herniorrhaphy and orchidopexy. At age 9 months, kyphoscoliosis was noted. He had recurrent pneumonia, frequent lacerations, and dislocation of the left shoulder. At age 3 years (1981), he had a Harrington rod placed in his back with surgical revisions in 1981, 1982, and 1983, and required continued support by a Milwaukee brace. At age four years, a diagnosis of Ehlers-Danlos syndrome type VI was suspected on the basis of his clinical findings including amino acid analysis of skin in which no hydroxylysine was found per 1,000 residues (normal is approximately 5/1,000). He was referred to Emory University at age 6 years, where the diagnosis was confirmed by enzyme analysis of cultured skin fibroblasts (table 1). On physical examination, he demonstrated severe kyphoscoliosis with a thoracic curve (55 degrees) apexed to the right causing scapular winging to the right. The rest of his skeletal development was normal except for right hemicranial hypertrophy and joint laxity with pes planus. His eye examination was normal except for mild myopia (OD). His corneal diameters were normal (11.25 mm bilaterally). Skin was soft, velvety smooth, and mildly hyperextensible. There were hypotrophic scars over surgical incisions. No heart abnormalities were present.

Diagnosis of Collagen Lysyl Hydroxylase Deficiency

Collagen lysyl and prolyl hydroxylase activities were examined in cultured dermal fibroblasts from the propositus (II-1), his parents (I-1 and I-2), and controls. Lysyl hydroxylase activity in the proband's cells was 22% of control while fibroblasts from father and mother had 58% and 78% control activity, respectively (table 1). These data established collagen lysyl hydroxylase deficiency in cultured cells from the affected child and indicated an autosomal recessive mode of inheritance in this family. Prolyl hydroxylase activity in lysates from the same cells was similar in all cell lines, demonstrating specificity of lysyl hydroxylase impairment. Previous studies in two other families suggested that genotyping of lysyl hydroxylase deficiency is accomplished best by the ratio of lysyl to prolyl hydroxylase in the same cell lysate [22, 24]. We found this approach useful for genotyping cultured dermal fibroblasts from this family. However, collagen lysyl

TABLE 1

LYSYL AND PROLYL HYDROXYLASE ACTIVITIES IN CULTURED SKIN FIBROBLASTS

CELL SOURCE	CELL TYPE	ENZYME ACTIVITIES (DPM ^{3}H-RELEASE/mg PROTEIN/HR) Lysyl hydroxylase (LH)	Prolyl hydroxylase (PH)	LH:PH (% CONTROL)
Propositus (II-1)	DF	8,455 ± 512 (6)	15,993 ± 1,590 (6)	24
Father (I-1)	DF	22,313 ± 1,103 (6)	19,286 ± 2,279 (6)	52
Mother (I-2)	DF	30,217 ± 3,041 (6)	15,686 ± 703 (6)	86
Control (2069)	DF	38,696 ± 2,165 (6)	17,331 ± 3,003 (6)	100

NOTE: Data are the mean ± standard deviation with the no. enzyme assays in parentheses. All cell lines were assayed at 3–15 passages. DF = cultured dermal fibroblasts. The same substrate was used for all assays. Cultured no. for the control culture is indicated.

and prolyl hydroxylase activities in cultured amniotic fluid cells had not been previously reported. We therefore examined amniotic fluid and dermal fibroblast cultures for comparable collagen hydroxylase activities that could be useful for prenatal monitoring of Ehlers-Danlos syndrome type VI.

Lysyl and Prolyl Hydroxylase Activities in Cultured Amniotic Fluid Cells

In a preliminary study, amniotic fluid and dermal fibroblast cultures exhibited similar collagen lysyl hydroxylase activities (table 2). These data indicated that either amniocytes (AF) or fibroblasts (F) from amniotic fluid cultures may be used for prenatal prediction of this enzyme deficiency. In contrast, prolyl hydroxylase activity in amniotic fluid cells varied considerably between AF and F cells and was usually only a fraction of that observed in dermal fibroblasts (table 2). This finding rendered the lysyl-to-prolyl hydroxylase activity ratio less useful for genotyping Ehlers-Danlos syndrome type IV by use of amniotic fluid cultures. Therefore, for prenatal studies and subsequent comparison with dermal fibroblast cultures after delivery, we relied only on lysyl hydroxylase specific activity to

TABLE 2

COMPARISON OF LYSYL AND PROLYL HYDROXYLASE ACTIVITIES OF CULTURED AMNIOCYTES AND FIBROBLASTS

CELL SOURCE	CELL TYPE	ENZYME ACTIVITIES (DPM ^{3}H-RELEASE/mg PROTEIN/HR) Lysyl hydroxylase (LH)	Prolyl hydroxylase (PH)	LH:PH (% CONTROL)
Propositus (II-1)	DF	2,955 ± 159 (6)	53,996 ± 5,407 (6)	28
Control (3839)	DF	9,800 ± 253 (6)	49,324 ± 1,655 (6)	100
Control (4228)	AF	8,541 ± 439 (6)	10,741 ± 359 (6)	400
Control (4228)	F	11,510 ± 1,638 (6)	18,772 ± 5,641 (6)	309

NOTE: Assay conditions and data handling were as stated in the footnote to table 1. AF and F cells were obtained from the same subject and were at passage nos. 1 and 5, respectively. AF = cultured amniotic fluid cells; F = fibroblasts cultured from amniotic fluid; DF = cultured dermal fibroblasts. Culture nos. for control cultures are indicated. The same substrate was used for all assays and was prepared at a different time than the substrate used to obtain the data in table 1. Different substrate preparations have different specific activities.

determine impairment of this enzyme. Cells cultured from amniotic fluid of the at-risk pregnancy had enzyme activity indistinguishable from that of concurrent control cultures, indicating that the fetus was not affected by collagen lysyl hydroxylase deficiency (fig. 2). Subsequent postpartum studies confirmed this prediction when skin fibroblasts cultured from the propositus (II-1), his newly born sibling (II-2), parents, and controls were examined for collagen lysyl hydroxylase activity (fig. 3). The proband's cells had enzyme activity 13% of control level, and parents had intermediate levels. In contrast, the cells from the newborn sibling had collagen lysyl hydroxylase activity similar to controls. These observations indicated that the child would be unaffected by Ehlers-Danlos syndrome and suggested that she was homozygous for the normal alleles. Her sex at delivery confirmed the prenatal karyotype of 46,XX, and her clinical presentation remains normal at age 12 months.

DISCUSSION

The family described in this study requested prenatal monitoring for the purpose of early management if collagen lysyl hydroxylase deficiency were present. Previous studies in this laboratory indicated that pharmacologic amounts of vitamin C provided an effective means of reducing the clinical manifestation of this disease in another patient with collagen lysyl hydroxylase deficiency [14, 15]. It has been shown that some forms of Ehlers-Danlos syndrome may predispose the affected fetus to congenital dislocation of hips or neurological damage facilitated by excessive joint movement [25]. Although there is heterogeneity in Ehlers-

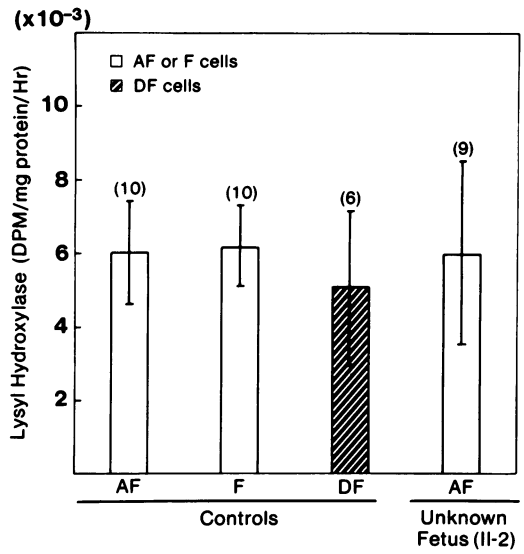

FIG. 2.—Prenatal assessment of lysyl hydroxylase activity using cultured amniotic fluid cells. Enzyme activity was determined by measuring tritiated water produced from unhydroxylated chick collagen. *AF* = cultured amniocytes (lines 3219, 3256, 3317, and 3235); *F* = fibroblasts cultured from amniotic fluid (lines 3362, 3243, and 3312). *DF* = cultured dermal fibroblasts (cell lines 409 and 2069).

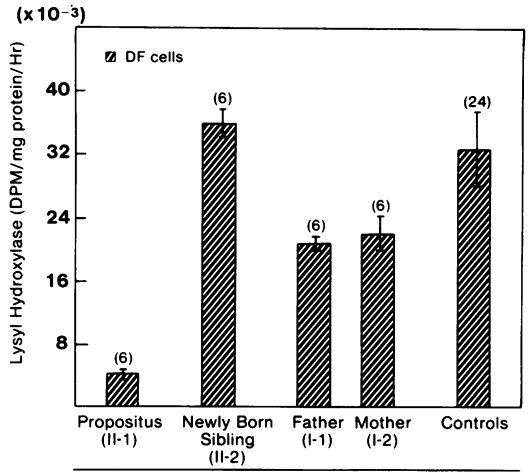

FIG. 3.—Collagen lysyl hydroxylase activity in cultured dermal fibroblasts (*DF*). Prenatal studies summarized in figure 3 were confirmed by measuring enzyme activity in dermal fibroblasts cultured from the newly born sibling, his family members, and controls (cell lines 2809, 1271, and 2069). *Each bar* represents the mean bracketed by 1 SD, with the no. observations in parentheses.

Danlos syndrome type VI, prenatal detection of collagen lysyl hydroxylase deficiency might reduce expression of phenotype and obstetrical risks if ascorbate were administered to the mother early during pregnancy and to the affected newborn.

Prenatal diagnosis of a heritable enzyme deficiency using cultured amniotic fluid cells requires the investigator to know relative levels of enzyme activity in these cells and to be able to distinguish enzyme activity in cells from affected and unaffected fetuses. This study represents the first prenatal evaluation for collagen lysyl hydroxylase deficiency. We compared enzyme activity in AF and F cell types derived from amniotic fluid to that in cultured dermal fibroblasts. In these three cell types, lysyl hydroxylase activity was similar, mitigating any developmental or tissue-specific regulation that might interfere with prenatal diagnosis of this enzyme deficiency. The apparent differential expression of prolyl hydroxylase activity in amniotic fluid cells and dermal fibroblasts is not understood. Other investigators reported a difference in collagen types produced by AF cells and dermal fibroblasts in culture [26, 27]. AF cells from amniotic fluid synthesize a basement membrane-type collagen (type IV), while F cells from amniotic fluid and dermal fibroblasts synthesize type I collagen. While these findings do not explain our data, they suggest existence of other differences that should be considered when amniotic fluid cells are used for prenatal diagnosis of collagen diseases. We found cultured amniotic fluid cells appropriate for prenatal determination of lysyl hydroxylase activity. Enzyme activity predicted prenatally was confirmed by subsequent studies of skin fibroblasts cultured from the newborn.

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THE NEW YORK STATE DEPARTMENT OF HEALTH BIRTH DEFECTS SYMPOSIUM XV on Perinatal Genetics—Diagnosis and Treatment will be held in Albany, New York, at the Albany Hilton Hotel, October 1–2, 1984. Discussions will concern the clinical relevance of recent genetic, biochemical, and epidemiologic findings, and presentations will be given on new technical developments related to carrier detection and to prenatal and postnatal screening in the management of genetic conditions. The symposium will be of particular interest to obstetricians, pediatricians, and geneticists. Abstracts should be sent by August 1, 1984; poster presentations are invited. For more information, contact: Ian H. Porter, M.D., Chief, Laboratory for Human Genetics, Center for Laboratories and Research, New York State Department of Health, Rockefeller Empire State Plaza, Albany, NY 12201.