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CLINICAL AND GENETIC FEATURES OF EHLERS–DANLOS SYNDROME TYPE IV, THE VASCULAR TYPE

MELANIE PEPIN, M.S., ULRIKE SCHWARZE, M.D., ANDREA SUPERTI-FURGA, M.D., AND PETER H. BYERS, M.D.

ABSTRACT

Background Ehlers–Danlos syndrome type IV, the vascular type, results from mutations in the gene for type III procollagen (*COL3A1*). Affected patients are at risk for arterial, bowel, and uterine rupture, but the timing of these events, their frequency, and the course of the disease are not well documented.

Methods We reviewed the clinical and family histories of and medical and surgical complications in 220 index patients with biochemically confirmed Ehlers–Danlos syndrome type IV and 199 of their affected relatives. We identified the underlying *COL3A1* mutation in 135 index patients.

Results Complications were rare in childhood; 25 percent of the index patients had a first complication by the age of 20 years, and more than 80 percent had had at least one complication by the age of 40. The calculated median survival of the entire cohort was 48 years. Most deaths resulted from arterial rupture. Bowel rupture, which often involved the sigmoid colon, accounted for about a quarter of complications but rarely led to death. Complications of pregnancy led to death in 12 of the 81 women who became pregnant. The types of complications were not associated with specific mutations in *COL3A1*.

Conclusions Although most affected patients survive the first and second major complications, Ehlers–Danlos syndrome type IV results in premature death. The diagnosis should be considered in young people who come to medical attention because of uterine rupture during pregnancy or arterial or visceral rupture. (N Engl J Med 2000;342:673-80.)

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THE clinical diagnosis of Ehlers–Danlos syndrome type IV, the vascular type, is made on the basis of four clinical criteria: easy bruising, thin skin with visible veins, characteristic facial features, and rupture of arteries, uterus, or intestines.¹ The diagnosis is confirmed by the demonstration that cultured fibroblasts synthesize abnormal type III procollagen molecules or by the identification of a mutation in the gene for type III pro-

collagen (*COL3A1*). Hypermobility of large joints and hyperextensibility of the skin, characteristic of the more common forms of Ehlers–Danlos syndrome, are unusual in the vascular type.^{2,3} Ehlers–Danlos syndrome type IV, an autosomal dominant disorder, is uncommon (the precise incidence and prevalence are not known), and in part because of its rarity, the diagnosis is often made only after a catastrophic complication or at postmortem examination. As is often the case with rare genetic disorders, physicians' unfamiliarity with the condition may compromise care. Although there are many brief clinical descriptions^{2,3} and case reports focusing on the molecular genetics,^{4,5} the scope of the clinical complications, the results of therapeutic intervention, and information about survival are not readily available. To provide the basis for a better understanding of the course of the disorder and for more informed counseling of patients and their families, we studied the clinical records of 220 index patients, in whom the diagnosis was confirmed by biochemical analysis, and 199 of their affected relatives.

METHODS

Study Subjects

The 220 index patients included all 217 patients whose cultured fibroblasts synthesized abnormal type III procollagen molecules who were evaluated in Seattle between 1976 and 1998 and 3 additional patients who were evaluated biochemically in Zurich, Switzerland, before 1990 (Table 1). We personally examined members of 13 families in Seattle and 3 families in Zurich. From the medical records of each index patient we determined the reasons for the initial referral to a physician and assessed the medical history, family history, physical findings, and when included, autopsy results.

We used three criteria to designate 199 relatives of the index patients as having Ehlers–Danlos syndrome type IV: cultures of dermal fibroblasts synthesized abnormal type III procollagen mol-

From the Departments of Pathology (M.P., U.S., P.H.B.) and Medicine (P.H.B.), University of Washington, Seattle; and the Division of Metabolic and Molecular Diseases, Department of Pediatrics, University of Zurich, Zurich, Switzerland (A.S.-E.). Address reprint requests to Dr. Byers at the Department of Pathology, Box 357470, University of Washington, Seattle, WA 98195-7470, or at pbyers@u.washington.edu.

TABLE 1. CHARACTERISTICS OF 220 INDEX PATIENTS AND 199 RELATIVES WITH EHLERS–DANLOS SYNDROME TYPE IV.*

| CHARACTERISTIC | ALL SUBJECTS (N=419) | MALE SUBJECTS (N=215) | FEMALE SUBJECTS (N=204) |
|---|-------------------------|--------------------------|----------------------------|
| Index patients — no. (%) | 220 | 120 (54.5) | 100 (45.5) |
| Relatives — no. (%) | 199 | 95 (47.7) | 104 (52.3) |
| Mean age at ascertainment — yr† | 28.7±14.8 | 28.0±15.0 | 29.3±14.5 |
| Index patients | 24.9±13.0 | 25.1±13.5 | 24.7±12.4 |
| Relatives | 33.3±15.6‡ | 32.4±17.0‡ | 35.0±15.2‡ |
| Family history of the disease in the index patients — no. (%) | | | |
| Yes | 84 (38.2) | 39 | 45 |
| No | 91 (41.4) | 54 | 37 |
| Unknown | 45 (20.5) | 27 | 18 |
| Age at first complication in index patients — yr | 23.5±11.1 | 23.9±10.9 | 22.8±11.4 |
| No. of patients with data available | 136 | 84 | 52 |
| Type of first complication in index patients | | | |
| Arterial dissection or rupture | | | |
| Age — yr | 24.6±11.0 | 24.8±11.4 | 24.7±10.1 |
| No. of patients with data available | 89 | 60 | 29 |
| Gastrointestinal rupture | | | |
| Age — yr | 20.6±11.0§ | 21.3±9.3 | 19.8±12.9 |
| No. of patients with data available | 41 | 20 | 21 |
| Organ rupture | | | |
| Age — yr | 28.0±7.5 | 28.5±8.2 | 27.0±8.5 |
| No. of patients with data available | 6 | 4 | 2 |

*Plus-minus values are means ±SD.

†The analysis included 207 index patients and 167 relatives.

‡P<0.001 for the comparison with index patients.

§P<0.03 for the comparison with index patients with arterial complications.

ecules in the case of 44 relatives; a familial molecular genetic abnormality was identified in the DNA of 35 relatives; and evidence in the family-history portion of the index patient's records indicated that the relative had had an arterial rupture, dissection, or aneurysm, bowel perforation, or organ rupture in the case of 120 relatives. Additional clinical data were provided for the first two groups of relatives at the time of testing; only data in the medical records of the index patient were available in the case of the remaining relatives.

Relatives were classified as unaffected if they were reported by a physician to be unaffected, if the results of biochemical or molecular genetic studies excluded the diagnosis, or if they had not had a major complication by the age of 50 years. We identified 462 relatives with a 50 percent risk of inheriting the condition on the basis of family-history data, of whom 238 (51.5 percent) were affected. Forty of the 224 apparently unaffected relatives were younger than 16 years of age and had not been tested, so their status could not be confirmed. These data suggest that most affected members of these families were identified.

From available medical records of the index patients and some of their relatives, we determined the number and type of medical or surgical complications, the ages at which they occurred, the cause of and age at death, reported birth defects, and identified complications of pregnancy. The age at testing (i.e., ascertainment) in the index patients was the age at which we confirmed the diagnosis. For their affected relatives, the age at ascertainment was the age at which we identified them with the use of biochemical or molecular genetic studies, their last known age, or their age at death, as recorded in the family history. The age at ascertainment was known for 374 of the 419 subjects (207 index patients and 167 relatives) and ranged from 1 to 78 years. The index patients were identified at a younger age than were their affected relatives, as would be expected when family histories are used to identify

affected members of prior generations (Table 1). Except for the study subjects who lived in our local communities, we did not follow most subjects after the diagnosis of Ehlers–Danlos syndrome type IV.

Seventy percent of the index patients (154 of 220) were referred for evaluation after a major event. Sixty-six index patients who had had no complications had one or more physical findings consistent with the diagnosis (characteristic facial features, thin skin with visible veins, easy bruising, and increased joint mobility of the hands) that led to the evaluation. Thirty-two of these 66 patients also had affected relatives who had had complications.

Biochemical and Molecular Studies

Dermal fibroblasts were obtained from the subjects and cultured, and the synthesis of type III procollagen was studied as described previously.^{6,7} For the molecular studies, RNA and DNA were extracted from cultured fibroblasts, and complementary DNA was synthesized by reverse transcription from RNA.⁸ Overlapping fragments of complementary DNA were amplified by the polymerase chain reaction⁹ and analyzed by electrophoresis on polyacrylamide gels to identify insertions or deletions⁷ or by single-strand conformation polymorphism analysis to detect point mutations in the coding sequence.^{7,10} Abnormal fragments were sequenced by the dideoxy chain-termination method¹¹ with T4 polymerase (Sequenase, U.S. Biochemicals, or Prism model 310 genetic analyzer, Applied Biosystems). All mutations were confirmed by sequence analysis or restriction-enzyme digestion of genomic DNA.

Statistical Analysis

We used a two-sample t-test, assuming that variance was unequal, to compare the mean age at ascertainment and at the time of complications in the index patients and their affected relatives. We used

life-table methods to estimate survival (SPSS statistical software, version 7.5) and included the age at death (including information on two index patients whose deaths were apparently unrelated to any complication of Ehlers-Danlos syndrome type IV) or the last known age of each living subject. We constructed a normal curve from the 1994 age-specific death rates from the Division of Vital Statistics of the Centers for Disease Control and Prevention.¹² We compared Kaplan-Meier survival curves for the index patients and their relatives using a log-rank statistical analysis (SPSS software, version 7.5). We also used Kaplan-Meier analysis to calculate survival free of a first complication for the index patients (SPSS software, version 7.5) by plotting the expression $[1 - (\text{cumulative survival})]$ against age, with survival defined according to the age at the time of the first complication.

We computed standardized incidence ratios to compare the rate of birth defects in our affected subjects with the rate in the general population. The ratios and 95 percent confidence intervals were calculated on the assumption that the values followed a Poisson distribution.¹³ All P values were two-sided.

RESULTS

Survival

A total of 131 subjects died: 26 index patients and 105 relatives. The overrepresentation of relatives probably reflects our method of ascertaining their disease status by using the records of younger index patients and the clinical criteria for diagnosis and inclusion. The median survival for the entire cohort was 48 years. The age at death ranged from 6 to 73 years (Fig. 1A). The median survival of the index patients was longer than that of their affected relatives (Fig. 1A). It is not clear whether this difference reflects the different age distributions in the two groups or recent improvements in medical care.

Causes of Death

Most deaths resulted from arterial dissection or rupture (Table 2). Of 103 deaths caused by arterial rupture, 78 involved thoracic or abdominal vessels and 9 resulted from central nervous system hemorrhage; the artery was unspecified in the case of 16 deaths. About half the remaining deaths resulted from organ rupture; bowel rupture and sepsis accounted for 8 percent of all deaths.

Medical and Surgical Complications

At the time of ascertainment, 287 of the 419 subjects (68 percent) had had a single complication (defined as arterial dissection or rupture, spontaneous bowel perforation, or organ rupture) and 86 (21 percent) had had more than one. Among the index patients the risk of a medical or surgical complication was 25 percent by the age of 20 years and greater than 80 percent by the age of 40 years (Fig. 1B). To determine how often a complication led to death, we assessed the outcome of first and second complications for the index patients. We excluded relatives from the evaluation because their medical histories were less complete or were unavailable. Among 220 index patients, 154 had had at least one complication and 18 (12 percent of those with complications) died af-

ter the first event (Fig. 2). The likelihood of death was greatest after organ rupture (45 percent) and least after bowel rupture (2 percent). The average age at the time of a first complication was 23.5 years, with rupture of the gastrointestinal tract likely to occur at an earlier age than arterial rupture (Table 1). Fifty-two of the 136 index patients who survived a first complication had a second recorded complication, which was fatal in 6 (12 percent). The relative frequencies of arterial complications and of gastrointestinal complications were similar for the first and second complications (Fig. 2). The type of second complication did not reflect the nature of the first complication (Fig. 2).

Arterial Complications and Surgical Outcome

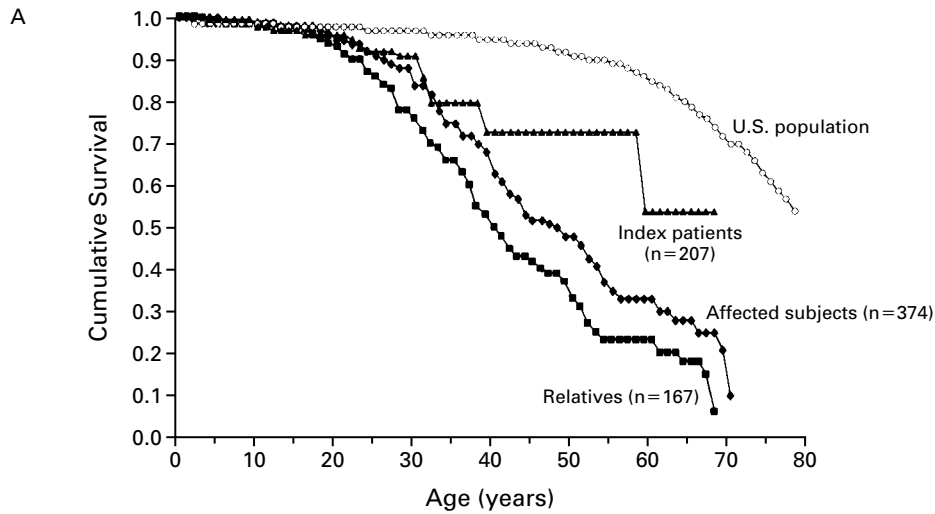
In the entire cohort of 419 subjects, there were 272 identified arterial complications. About half involved the thoracic or abdominal arteries, and the rest were divided equally between those in the head and neck and those in the limbs. Forty-three subjects had 44 arterial complications of the central nervous system between the ages of 17 and 65 years (mean age, 32.8); 17 of these subjects have been described previously.¹⁴ The most common nonlethal central nervous system events were fistulae involving the carotid artery and cavernous sinus (10 subjects), carotid-artery dissection (8), aneurysm (5), and rupture (2).

We had documentation that 98 subjects had undergone an invasive evaluation procedure or surgery: 29 had undergone angiography, 1 of whom died during cerebral studies, and 69 had undergone surgery, 28 of whom died. Among the 28 who died during or after surgery, the underlying diagnosis was not known at the time in most, and often the patient was moribund even before surgery.

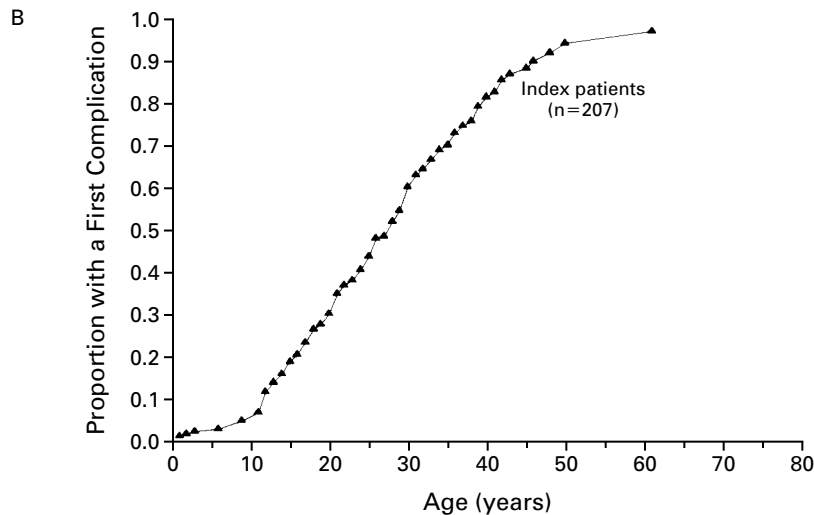
Gastrointestinal Complications and Surgical Outcome

Most of the identified bowel complications (62 of 87) in the index patients and relatives affected the colon, commonly the sigmoid colon (in 29 subjects). Perforation of the small bowel (seven subjects) and gastric perforation (two subjects) were uncommon. Ten deaths were recorded as resulting from rupture of the gastrointestinal tract. Tissue fragility and poor wound healing contributed to surgical complications, death, or both. Dehiscence of the wound, evisceration, hemorrhage of abdominal vessels, fistulas, and adhesions were all described. Recurrent bowel rupture was reported in 15 subjects and occurred between 2 weeks and 26 years after the first event.

Spontaneous bowel perforation was usually treated by partial colectomy. In the case of 42 subjects who underwent partial colectomy, medical records indicated that partial resection was followed by colostomy in 26 subjects and that immediate end-to-end reanastomosis was performed in 8. Closure of the colostomy and reanastomosis of the bowel were successfully



| No. AT RISK | | 0 | 10 | 20 | 30 | 40 | 50 | 60 | 70 |
|----------------|-----|-----|-----|-----|----|----|----|----|----|
| Index patients | 207 | 188 | 130 | 76 | 25 | 10 | 4 | 1 | |
| Relatives | 167 | 158 | 138 | 103 | 56 | 23 | 10 | 5 | |



| No. AT RISK | | 0 | 10 | 20 | 30 | 40 | 50 | 60 | 70 |
|----------------|-----|-----|-----|----|----|----|----|----|----|
| Index patients | 207 | 181 | 118 | 65 | 17 | 3 | 1 | 0 | |

Figure 1. Kaplan–Meier Estimates of Overall Survival among 374 Subjects with Ehlers–Danlos Syndrome Type IV (Panel A) and Age at the Time of a First Complication among 207 Index Patients (Panel B). Panel A also includes a curve derived from a 1994 abridged life-table for persons born in the United States.¹²

performed between one month and one year after initial repair in 22 subjects. Seven of these subjects had a second bowel perforation, whereas at least six survived with no recurrence for an average of 6 years (range, 3 to 16). In the eight subjects who underwent an initial end-to-end reanastomosis, perforations recurred in five within 1 to 22 years. Among the three remaining subjects, one died of peritonitis,

one had an abdominal hemorrhage that led to splenectomy, and there was no information available for the third.

Total colectomy was performed in four subjects after repeated perforations. All were younger than 25 years of age. The duration of follow-up ranged from one to nine years, and there was a single death from arterial rupture during this period.

TABLE 2. CAUSES OF DEATH IN 26 INDEX PATIENTS WITH EHLERS–DANLOS SYNDROME TYPE IV AND 105 AFFECTED RELATIVES.

| CAUSE OF DEATH | TOTAL | MALE | FEMALE |
|--------------------------|-------|-----------------|----------|
| | | SUBJECTS | SUBJECTS |
| | | no. of subjects | |
| All causes | 131 | 77 | 54 |
| Arterial rupture | 103 | 62 | 41 |
| Organ rupture | 13 | 7 | 6 |
| Uterus | 5 | 0 | 5 |
| Heart* | 3 | 2 | 1 |
| Liver or spleen | 5 | 5 | 0 |
| Gastrointestinal rupture | 10 | 7 | 3 |
| Other causes† | 5 | 1 | 4 |

*The cause of death was left ventricular rupture.

†Two relatives died of other causes: an adolescent boy died in a motor vehicle accident at the age of 17 years, and a 70-year-old woman died of an apparent heart attack.

Outcome of Pregnancy

Eighty-one of the women with Ehlers–Danlos syndrome type IV had had a total of 183 pregnancies, with 167 deliveries of live-born infants at term, 3 stillbirths, 10 spontaneous abortions, and 3 voluntary terminations. Twelve women died during the peripartum period or within two weeks after delivery (five of uterine rupture during labor, two of vessel rupture at delivery, and five in the postpartum period after vessel rupture). Five of these pregnancy-related deaths have been reported previously.¹⁵ There were five pregnancy-related deaths among the 81 women who had been pregnant once, three among the 53 who had been pregnant twice, two among the 24 who had been pregnant three times, two among the 13 who had been pregnant four times, and no deaths among the 6 women who had been pregnant five times, the 2 who had been pregnant six times, or the 1 who had been pregnant seven times. The three women who

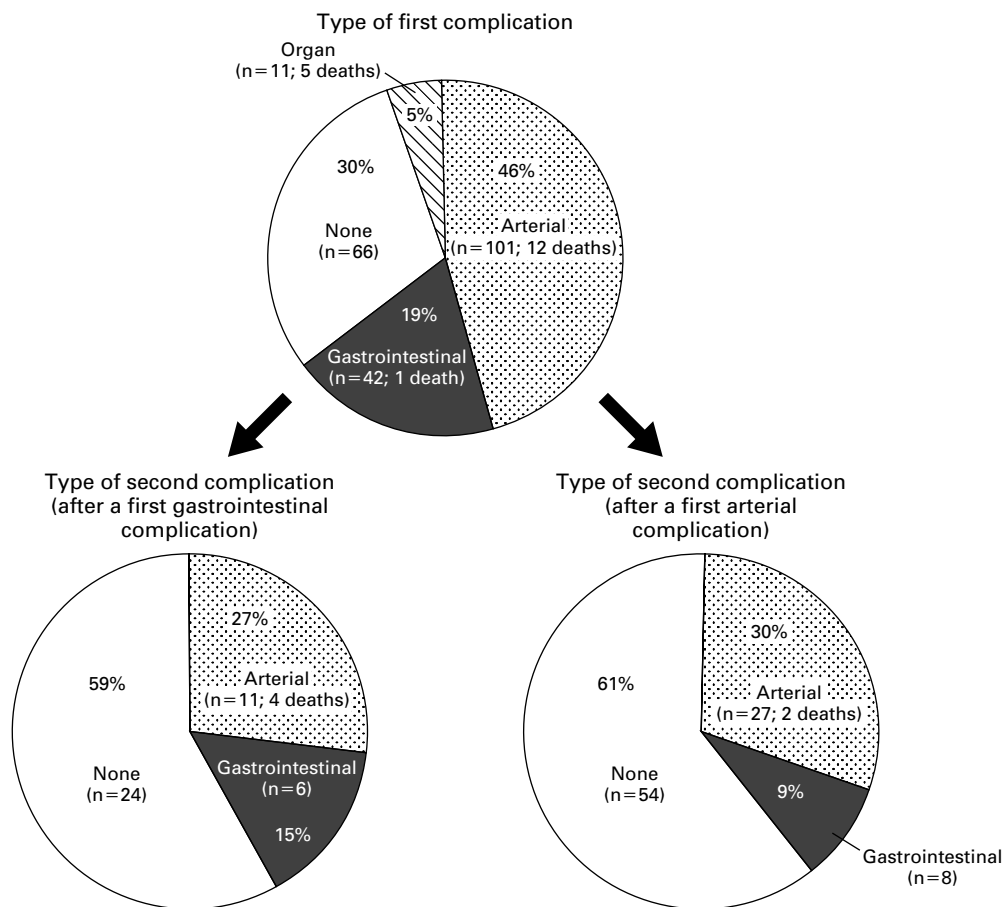


Figure 2. Types of First Complications in Index Patients with Ehlers–Danlos Syndrome Type IV and the Relation between the Type of First Complication and the Type of Second Complication.

underwent voluntary termination of pregnancy were excluded from the analysis.

Congenital Defects

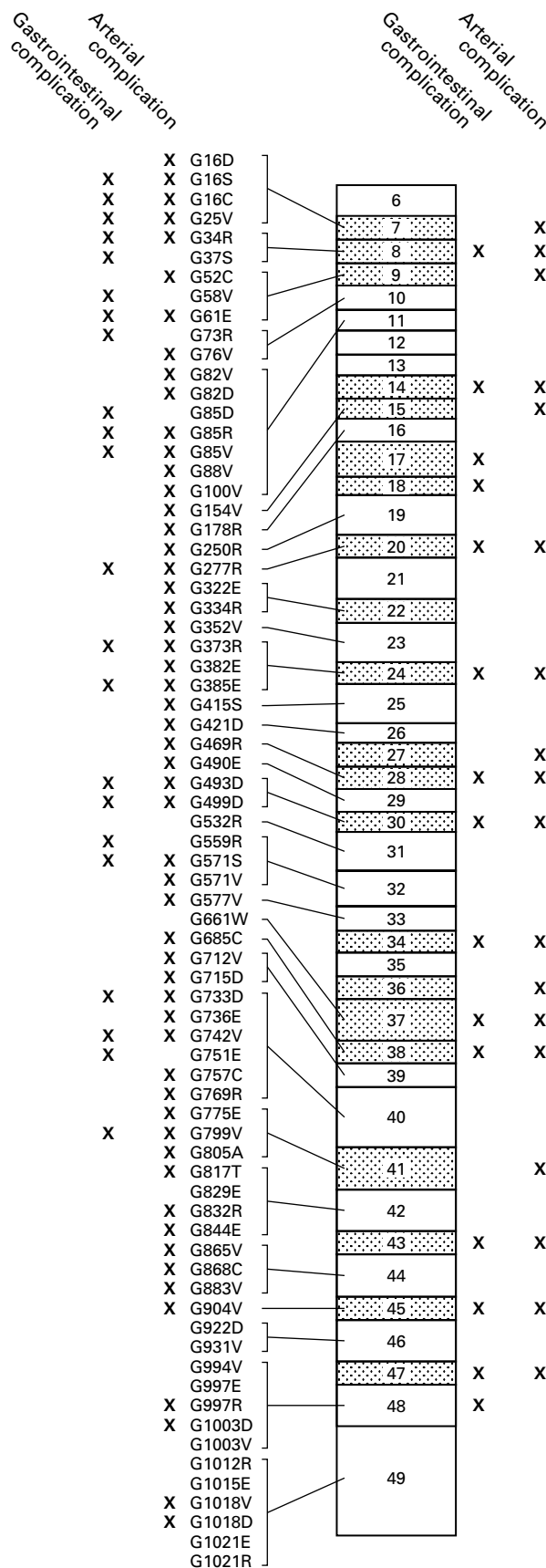
Two congenital defects were reported more frequently than expected among the subjects: club-foot (in 41 subjects, 24 male subjects and 17 female subjects; rate in the general population,¹⁶ 20 per 10,000; $P < 0.001$) and congenital dislocation of the hip (in 8 subjects; rate in the general population, 7.3 per 10,000 [Botto LD: personal communication]; $P < 0.001$). A total of 12.4 percent of index patients (21 of 169) were born prematurely, as compared with 11 percent of the U.S. population.¹⁷

Correlation between Genotype and Phenotype

We identified the causative mutations in the *COL3A1* gene in 135 index patients (Fig. 3). Four mutations led to the deletion of multiple exons (2 of which have been reported previously^{18,19}), and 41 led to the skipping of a single exon.⁷ One mutation (IVS24 + 1G→A) led to the skipping of exon 24 in seven unrelated index patients. Four index patients had splice-site mutations with complex splicing outcomes and multiple messenger RNAs.⁷ In a single index patient a 10-bp deletion in the acceptor site of intron 29 led to the presence of a cryptic site within exon 30 and to the deletion of three amino acids from the triple helix. In the remaining 85 index patients, 73 different point mutations led to the substitution of some other amino acid for glycine throughout the triple-helical domain. A number of mutations — G16S (seven families) and G82D, G373R, G385E, G415S, G499D, and G1021E (two families each) — were identified multiple times in unrelated index patients. We discerned no correlation between the nature or location of the mutation and the type or frequency of major complications (Fig. 3).

Figure 3. Relations between the Nature and Location of Mutations in the Gene for Type III Procollagen (*COL3A1*) and the Types of Complications.

There was no apparent relation between the mutation and the type of complication. The causative mutations in the *COL3A1* gene were identified in 135 index patients. There were 73 different mutations in 85 index patients that resulted in the substitution of some other amino acid for glycine (G) within the triple helix. Mutations in 41 index patients led to the skipping of a single exon (indicated by the stippling). The remaining mutations were more complex (see the Results section). The presence of an X at the site of a mutation indicates that one or more index patients with that mutation had a complication of the indicated type. The glycines are numbered from the first glycine of the major triple helix, which is residue 168 of the prepro α 1(III) chain of type III procollagen. A denotes alanine, C cysteine, D aspartic acid, E glutamic acid, R arginine, S serine, V valine, W tryptophan, and T threonine. The G817T mutation results from a 2-bp substitution in a glycine codon (GGA→ACA). All other substitutions result from single base-pair substitutions in a glycine codon.



DISCUSSION

We identified 220 patients whose cultured dermal fibroblasts synthesized abnormal type III procollagen molecules, a finding that is diagnostic of Ehlers–Danlos syndrome type IV. From the family histories of these index patients we identified 120 affected relatives on clinical grounds, and we identified an additional 79 relatives as affected on the basis of diagnostic biochemical or molecular genetic studies. The value of the data on survival, outcome of complications, and age at the onset of complications depends on the extent to which this large group represents the population of people with this uncommon disorder. The clinical diagnosis of Ehlers–Danlos syndrome type IV rests on the finding of at least two of four diagnostic criteria (thin, translucent skin; arterial, intestinal, or uterine rupture; easy bruising; and a characteristic facial appearance), but laboratory studies are necessary for confirmation.¹

In our study, the diagnosis was confirmed biochemically in all the index patients, so the clinical significance and applicability of the findings in this study depend on whether we were referred a representative group to study. The 220 index patients fell into three groups: 154 were referred for evaluation after a major complication, 32 were evaluated because they had a family history strongly suggestive of Ehlers–Danlos syndrome type IV and they had physical findings characteristic of the condition but no known complications, and 34 were evaluated because they had physical findings of the diagnosis but no affected relatives. The estimated median survival did not differ significantly among the index patients, the affected relatives of index patients who had had a complication, and the affected relatives of index patients who had not had a complication (data not shown), suggesting that the groups were similar. The nature, type, and location of mutations were also similar among the three groups. Thus, although the reasons for evaluation varied, it seems likely that the group as a whole was made up of people at different points in the evolution of the condition and did not represent different subgroups of those with Ehlers–Danlos syndrome type IV. Nonetheless, our results pertaining to the natural history of the disease, the age at the time of a first complication, and the incidence and causes of death may be most relevant to patients identified in a similar manner.

In this population, survival was shortened, largely as a result of vascular rupture. The age at death ranged from 6 to 73 years, with a median life span of 48 years. The median survival was longer for the index patients than for their relatives, but it was not clear whether this difference reflects better medical care or differences in the age distributions of the groups. Major complications were uncommon in childhood, but 25 percent of the index patients had had medical or surgical complications by the age of 20 years, and more

than 80 percent had had such complications by the age of 40 years.

In this group of patients, rupture of any artery into a free space is life-threatening and requires immediate intervention, even though the tissues are friable and repair is often difficult. Rupture into a confined space may be sealed because of tamponade, however, and in such cases, surgical intervention may be deleterious.²⁰ Although a preexisting aneurysm could occasionally be documented, usually either studies had not been done or no aneurysms were documented. Whether there is a role for the repair of unruptured aneurysms in patients with this syndrome is not clear.

Although arterial tears are considered the hallmark of Ehlers–Danlos syndrome type IV, about 25 percent of all complications in this group affected the gastrointestinal tract. Prompt surgical intervention was usually crucial in the treatment of bowel rupture, and colostomy was the preferred treatment. Bowel continuity was restored with little difficulty in most cases. Treatment of bowel perforation with end-to-end reanastomosis after partial colectomy was associated with a higher risk of both immediate failure and later complication than was treatment with colostomy. Because the sigmoid colon is a frequent site of rupture, removal of the distal colon may decrease the risk of recurrence.²¹

Women with Ehlers–Danlos syndrome type IV have an increased risk of complications of pregnancy as well as a 50 percent risk of having an affected child. In one series,²² there were no deaths or clinically significant complications during more than 20 pregnancies in eight women with *COL3A1* mutations, whereas other studies have reported pregnancy-related deaths and complications in such women.^{15,21,23–28} If the 20 women (5 of whom died of pregnancy-related causes) whom we described previously¹⁵ are excluded from the analysis, the mortality rate among women who became pregnant was 11.5 percent (1 death per 23 pregnancies). Women with Ehlers–Danlos syndrome type IV who become pregnant should be considered at high risk and should be followed at specialized centers. Although several pregnant women died of uterine rupture at term, we do not know whether the use of elective cesarean section would decrease mortality.

Fibroblasts from all the index patients synthesized abnormal type III procollagen molecules, and to date we have identified causative mutations in the *COL3A1* gene in more than half. We did not find that different rates of arterial or gastrointestinal complications were associated with different types of mutation or with specific mutations. Mutations that affect the structure of type III procollagen may produce a milder form of disease than that in most of the patients and families we studied,²⁹ and the life span of persons with such mutations can approach normal, as was true in one previously described³⁰ family included in our study. There is no evidence of heterogeneity of ge-

netic loci in Ehlers–Danlos syndrome type IV, and the only specific correlation between genotype and phenotype recognized to date is with minor phenotypic findings.²² The effects of null mutations have yet to be established, although our unpublished evidence suggests that they can cause severe disease.

Although no specific therapies delay the onset of complications in patients with Ehlers–Danlos syndrome type IV, knowledge of the diagnosis may influence the management of surgery, pregnancy and reproductive counseling, and major complications. The diagnosis should be considered and biochemical evaluation performed in young people with unexplained bowel or arterial rupture, especially those with a family history of similar events.

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