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CASE REPORT

Ehlers–Danlos syndrome type IV with a unique point mutation in *COL3A1* and familial phenotype of myocardial infarction without organic coronary stenosis

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Abstract. Nishiyama Y, Nejima J, Watanabe A, Kotani E, Sakai N, Hatamochi A, Shinkai H, Kiuchi K, Tamura K, Shimada T, Takano T, Katayama Y (Nippon Medical School, Tokyo, and Chiba University School of Medicine, Chiba, Japan). Ehlers–Danlos syndrome type IV with a unique point mutation in *COL3 A1* and familial phenotype of myocardial infarction without organic coronary stenosis (Case Report). *J Intern Med* 2001; **249:** 103–108.

We report on a 43-year-old male patient with Ehlers–Danlos syndrome (EDS) type IV with acute myocardial infarction (MI) without organic coronary stenosis. The disease was complicated with pneumothorax, subcutaneous and mediastinal emphysema, and splenic artery rupture. Three of the patient's family members suffered sudden cardiac death or MI. A diagnosis of EDS type IV was confirmed by decreased production of type III collagen by 86%. Mutation analysis revealed a point mutation in the *COL3A1* gene that substituted glycine for aspartate at amino acid position 877. This mutation had not been reported as pathogenic for EDS type IV. These findings suggest close linkage between the mutation and the phenotype with familial MI.

Keywords: acute myocardial infarction, *COL3A1* gene point mutation, Ehlers–Danlos syndrome (EDS) type IV, recurrent pneumothorax, splenic artery dissection, tracheal perforation.

Introduction

Ehlers–Danlos syndrome (EDS) is a heterogeneous group of connective tissue disorders characterized by hyperextensible skin, skin fragility, joint hypermobility and abnormal scarring [1, 2]. The current classification recognizes 11 types [3] and several subtypes of EDS, based on clinical, genetic, biochemical and molecular characteristics [4]. It is important to specify the type of EDS, since the natural history, clinical management and type of inheritance vary considerably depending on the type. Amongst the different forms of EDS, type IV must be considered to be a special, because of the peculiar natural history and poor prognosis. Clinical manifestations specific to EDS type IV include translucent skin, easy bruising, visible subcutaneous veins and the laxity limited to the small joints of the fingers [5]. EDS type IV is inherited in autosomal dominant fashion and the phenotype is a consequence of mutations in the

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COL3A1 gene coding type III collagen, which is a major component of blood vessels, viscera and the uterus [6]. Accordingly, individuals with EDS type IV often experience life-threatening vascular and gastrointestinal complications. Occasionally, death results from iatrogenic trauma caused by diagnostic or therapeutic procedures with subsequent vascular complications [7].

We report a unique mutation in a patient with varieties of clinical manifestations of EDS type IV, and discuss the close relationship between the region of the point mutation and the clinical phenotype.

Case report

A 43-year-old man was transferred to the coronary care unit of Nippon Medical School Hospital on 26 January 1998, with a diagnosis of acute MI. The patient had developed a sudden onset of right-sided chest pain 2 h after thoracotomies for haemopneumothorax, which had developed 2 weeks before.

He had no risk factors of coronary artery disease such as hypertension, hyperlipidaemia or smoking. The past medical history of the patient as well as his family history were also suggestive of EDS type IV. At the age of 3, he developed severe congenital bilateral dislocations of the hip joints and talipes equinovarus. Pneumothorax occurred twice at the age of 43. Many of his family members died suddenly (Fig. 1). His mother had a sudden cardiac death at the age of 50. His elder sister died suddenly of unknown causes in the second trimester of pregnancy at the age of 27. His younger sister, who was 33 years old, developed an acute MI at 24 weeks' gestation. She underwent coronary arteriography, which did not demonstrate any organic stenosis.

His body temperature was 35.7°C, pulse 104 min^{-1}, regular, and blood pressure 82/46 mmHg. His skin displayed thin and visible subcutaneous veins, especially over the anterior chest. The distal interphalangeal joints of both hands were slightly hyperextensive, with acrogenic hands. His facial appearance was characterized by large eyes, 'pinched' nose and thin lips, and he was of short stature (142 cm, 38 kg). On chest radiograph, the cardiothoracic ratio was 54.0% and there was no pulmonary congestion. Arterial blood gas evaluation on mechanical ventilatory assistance showed pH, 7.43: P_aO_2, 184.3 mmHg; P_aCO_2, 37.8 mmHg. An electrocardiogram showed *ST*-

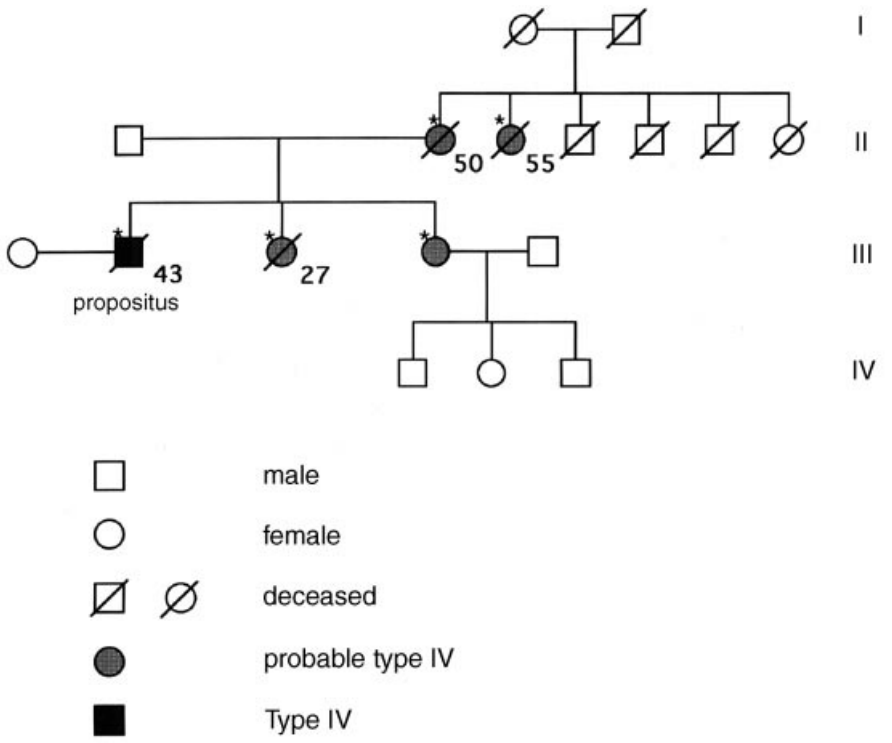

Fig. 1 Pedigree of propositus. This family elicited an autosomal dominant inheritance pattern. The shaded line indicates death at the age indicated. Asterisks indicate individuals with myocardial infarction or sudden death.

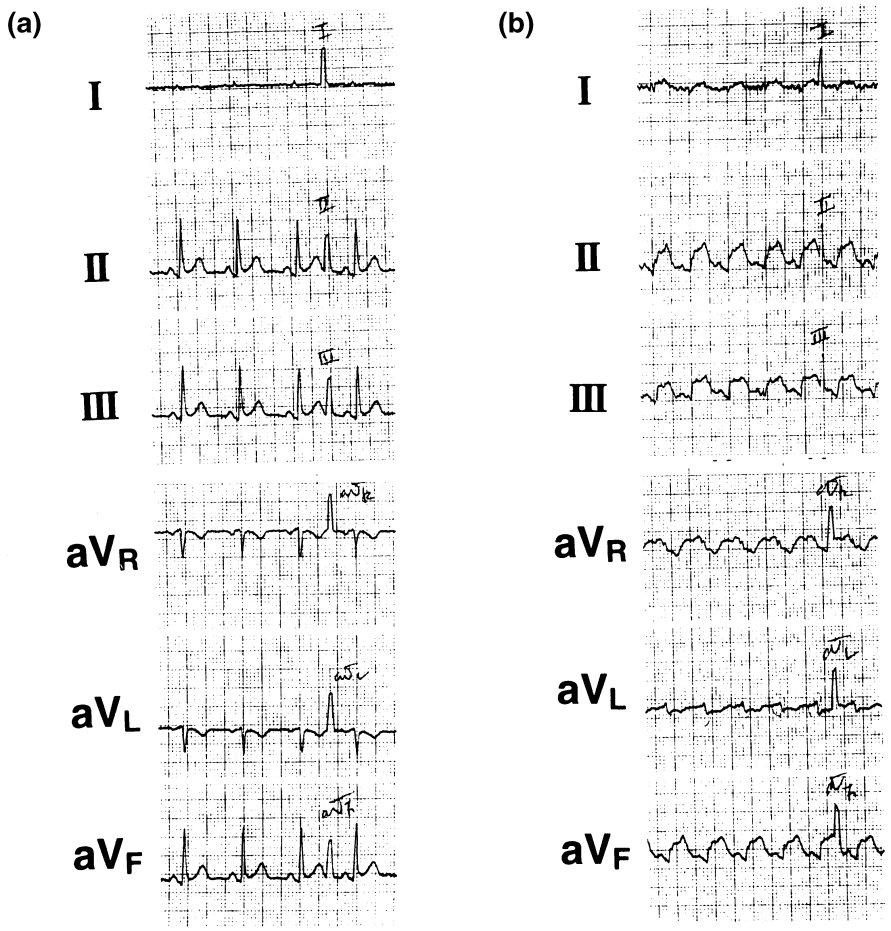

Fig. 2 An electrocardiogram recorded before (a) and after (b) thoracotomy. The electrocardiogram after the thoracotomy showed a pattern representing acute myocardial infarction. Chest leads were not recorded because of bandages covering surgical wounds after thoracotomy.

segment elevation and *R*-wave voltage reduction in leads II, III and aVF (Fig. 2). Total serum creatine kinase (CK) was 1104 U L^{-1} (normal range 35–200) and CK-MB isozyme was 46 U L^{-1}. Plasma troponin-T was 3.5 ng mL^{-1} (reference range < 0.25) and myosin light chain-1 was 5.3 ng mL^{-1} (reference range < 2.5). An echocardiogram demonstrated mild hypokinesis of the inferior wall. According to these findings, we diagnosed him as having an acute MI. Thrombolytic treatment was not indicated because of thoracotomy performed 10 h before. Characteristic physical findings, together with his past and family history, led to the clinical diagnosis of EDS type IV. To confirm EDS type IV, a skin biopsy was performed.

The patient developed progressive subcutaneous and mediastinal emphysema that was attributed to tracheal perforation below the vocal cord. A covered stent was placed on the fistula. The emphysema was clinically improved and the stent was removed. One week later, marked tachycardia and elevation of body temperature with abdominal tenderness developed suddenly. Abdominal ultrasonography demonstrated the findings of a rupture of the abdominal artery in the peritoneal cavity. No antecedent trauma was recognized. His haemoglobin level fell from 10.8 to 6.3 g dL^{-1}, which necessitated blood transfusions. All resuscitative efforts were to no avail, and the patient expired shortly.

Autopsy revealed tissue fragility of liver, spleen and lung. The splenic artery was dissected at the centre of media, and partially ruptured. Myocardial findings revealed focal scattered fibrotic foci surrounding peripheral coronary veins extending into the intercellular space in inferior and lateral myocardial walls. Immunohistochemical study showed a marked decrease in type III collagen in skin, heart, trachea and lungs.

To confirm the reduced production of type III collagen, the collagen synthesis in the patient was

analysed as previously described [8]. Briefly, the patient's skin fibroblasts were obtained from biopsy specimen, and the cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum at 37°C in 5% CO_2 containing 50 μCi mL^{-1} of [2,3^{3}H] proline and 50 μg of L-ascorbic acid 2-phosphate and cultured for 24 h at 37°C. The medium was collected and precipitated by the addition of tricolour acetic acid (final concentration 10%). The precipitate was dissolved in 0.05 mol L^{-1} acetic acid and treated with pepsin (100 μg mL^{-1}) for 3 h at 4°C. Collagen samples were separated by SDS/5% polyacrylamide gel electrophoresis in the presence or absence of 2-mercaptoethanol. The radioactivity of specific bands was determined by scanning autoradiograms with a densitometer. The synthesis of type III collagen was about 14% of that of normal control fibroblasts (Fig. 3).

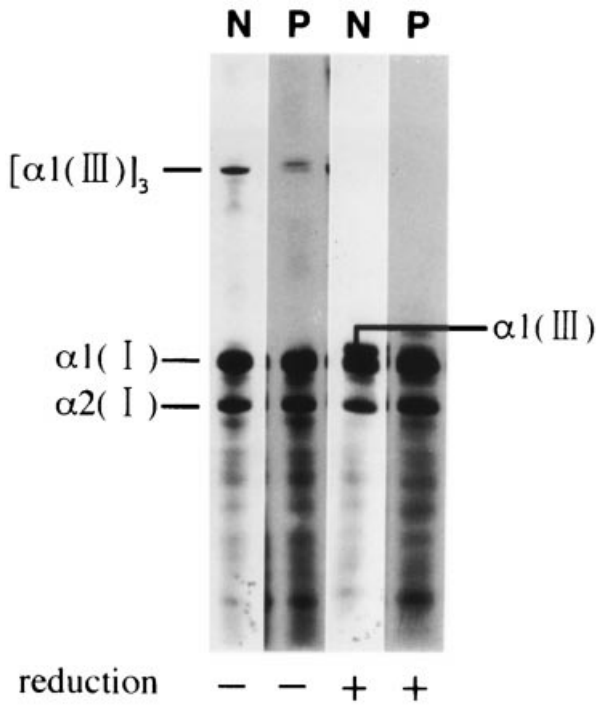

Fig. 3 Production of the type III collagen. Biochemical studies on the patient's fibroblasts (left panel P) provided depressed production of the type III collagen as compared with a normal subject (left panel N) without reduction. These bands of type III collagen (α1(III)) disappeared both in the patient and in the normal control subject, with reduction (right panel). The synthesis of type III collagen was estimated by scanning to be ≈ 14% compared with normal control fibroblasts.

In order to search for mutation in the patient's messenger RNA (mRNA), the reverse transcriptase polymerase chain reaction (RT-PCR) product was prepared from cultured skin fibroblast using Ex-Taq (Takara) and sequenced directly including the 3.3 kb-pair entire sequence encoding the triple-helix domain of type III collagen as previously described [9]. The result of the sequencing revealed a heterozygous missense base mutation in the *COL3A1* gene, which converted the codon of -GGC- for glycine at amino acid position 877 to the codon of -GAC- for aspartate (Fig. 4a). The sequence data confirmed the loss of a HaeII site in the patient by restriction enzyme fragment length polymorphism (RFLP) (Fig. 4b).

Discussion

This is the first report of a unique mutation, in that substitution of aspartate acid for glycine occurred at amino acid position 877 in the *COL3A1* gene, which is involved in the pathogenesis of EDS type IV. This mutation of the position in the triple-helical domain of the type III procollagen has not been previously reported.

The *COL3A1* gene encodes 1467 amino acids, of which 1029 are located within the triple-helical domain consisting of the repetition of Gly-X-Y formation. The glycine is important for formation of the triple helix. Approximately two-thirds of published cases of EDS type IV with genetic analysis had the point mutations of glycine residues [10]. Accordingly, the unique mutation found in the present patient should be considered to be pathogenic. Recent reports indicated that the mutant region of the type III collagen might reflect clinical severity of the phenotype in terms of prognosis [10–13]. There are many reports that the position of the mutation in *COL3A1* may play a role in a common natal complication for each family, such as subarachnoid haemorrhage [14], intraperitoneal bleeding [15] or spontaneous perforation of the iliac artery [8]. In fact, there is a report that a mutation in *COL3A1* caused familial aortic aneurysms [16]. Considering the universal miserable feature of the familial phenotype in the present case, the type of the mutation found in this patient might be responsible for severe phenotype. Further detailed analyses of the mutation in a greater number of

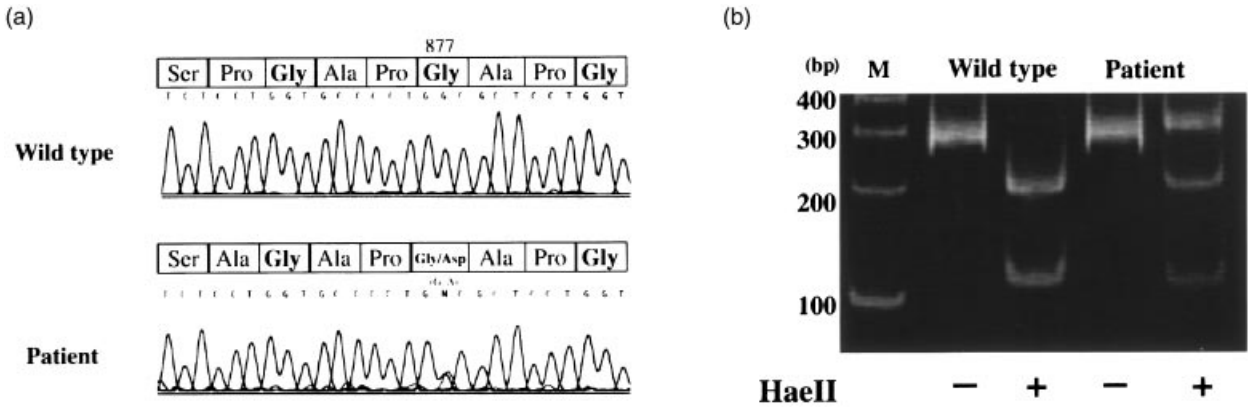

Fig. 4 Detection of mutation in the *COL3A1* gene. (a) Nucleotide sequence of the region of complementary DNA (cDNA) where the mutation was found in the patient (bottom). The top panel shows the sequence of the same region in wild type. The sequence in the patient (bottom), as compared with that in the wild type (top), indicates the single base substitution converting the codon of GGC for glycine at amino acid position 877 of the triple-helical domain to GAC, a codon for aspartate acid. (b) Restriction enzyme fragment length polymorphism (RFLP) analysis. cDNA obtained from fibroblast samples was amplified using primers 5′ and 3′ of the mutation. Amplified DNA was digested with HaeII followed by PAGE (polyacrylamide gel electrophoresis). The mutation abolishes a HaeII restriction site in the amplified cDNA. The shorter two fragments (200 and 103 bp) represent the normal allele, the larger fragment (303 bp) the mutant allele. Because the mutation is present in a heterogeneous state, three bands in digestion are easily detected in the patient (right column), whilst two bands are detected in wild type (centre column).

patients are desirable to enable early prenatal diagnosis and prevent lethal complications.

In previously reported EDS type IV patients, pneumothorax and haemopneumothorax have been the most common clinical features [17, 18]. In previous reports, any deaths have been documented not solely as a result of the pulmonary manifestations of EDS type IV [17], but with vascular complications, which was the case in the present patient. Subcutaneous or mediastinal emphysema was not reported previously in EDS type IV patients. The emphysema would have been caused by the intratracheal tube being in the same position for several days, resulting in a perforation facilitated by the fragility of connective tissue in EDS type IV.

As demonstrated in complications of the present case, acute MI is also an important cause of morbidity and mortality. Acute MI is a rarely reported complication. Eight cases who developed acute MI have been reported [19–25]. Causes of acute MI included coronary dissection ($n = 4$), coronary rupture ($n = 1$), and no coronary disease with sinus of Valsalva ($n = 1$). In two cases, the cause of acute MI was not described. In the present case, there was no evidence of coronary embolism, thrombi, stenosis or dissection leading to MI. Considering his family medical history of sudden death and acute MI, it is conceivable that the point mutation demonstrated in the present patient may be closely linked to a specific phenotype represented by prevalence of acute MI. Plasma levels of total CK were modified by thoracotomy, which could account for the minimal fraction of the CK-MB isozyme.

Finally, we emphasize that young patients or those in early middle age who have recurrent pneumothorax, acute MI or arterial dissection with their characteristic familial history should be suspected of EDS type IV. Currently, there is no specific treatment for EDS type IV. However, early recognition of the disease by clinical manifestations, past medical history and family history is of extreme importance to prevent complications of investigative and surgical procedures [6, 26]. Further information on patients with *COL3A1* mutation in correlation with phenotype would have important implications not only for the management of patients with EDS type IV but also for the advanced care of other family members.

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