Clinical Variability and Molecular Diagnosis in a Four-generation Family with X-linked Emery-Dreifuss Muscular Dystrophy

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Aim. To describe the clinical variability of X-linked Emery-Dreifuss muscular dystrophy (X-EDMD) with cardiac involvement in a four-generation family with a novel mutation in the STA gene.

Methods. Clinical data were provided for 4 affected males and a female carrier. The Western blot analysis of emerin was performed on lymphoblastoid cell lines and followed by sequencing of the emerin gene.

Results. A thymine insertion at nucleotide 417 in exon 2, resulting in a frameshift with a premature stop codon at position 62 and absence of functional protein, was found in one of the three available patients. In ten-year-old proband’s dizygotic twin-nephews the intermittent first-degree A-V block, atrial and ventricular ectopy, atrial runs, and exit sinus block were found, although the echocardiographic findings were normal. One of the twins also had short episodes of atrial fibrillation, idioventricular rhythm, and junctional rhythm.

Conclusion. Cardiac abnormalities in the proband’s ten-year-old dizygotic twins without evident clinical features suggestive of EDMD were remarkable in contrast to the oldest patient in the family, who lived to the age of 63 with a pacemaker, and to the proband who had a very early on set of muscle wasting and weakness, and a pacemaker implantation at the age of 27. This striking intra-familial variability in cardiac involvement as associated with specific null mutation (417 ins T) has practical early diagnostic and possibly preventive implications. It also points at genetic and environmental factors as causes of clinical features in X-EDMD.

Keywords: carrier state; frameshift mutation; heart conduction system; muscular dystrophy, Emery-Dreifuss; mutation, frameshift; mutational analysis, DNA; phenotype

Emery-Dreifuss muscular dystrophy (EDMD; OMIM 310300) (1) was delineated as a separate form of muscular dystrophy nearly 40 years ago (2), on the basis of distinctive clinical features of early contractures, humero-peroneal weakness, and cardiac conduction defects (3,4). The disease was first described as an X-linked muscular dystrophy (X-EDMD; OMIM 310300) (2), although there were reports on autosomal dominant (AD-EDMD; OMIM 181350) (5-8) and autosomal recessive (OMIM; 604929) (9) forms.

Un til recently, only the gene for the X-linked form was known – the STA gene on Xq28 (10), which codes a putative protein, emerin. In 1999, Bonne et al (11) identified the lamin A/C gene (LMNA), responsible for the autosomal dominant form of Emery-Dreifuss muscular dystrophy (AD-EDMD). The lamin A/C gene is located on 1q11-q23 chromosome. Within this locus, another autosomal dominant gene that causes slow progressive limb girdle muscular dystrophy with age-related atrioventricular car diac conduction disturbances and the absence of early contractures (LGMD1B), has been mapped (12). Muchir et al (13) demonstrated that LGMD1B and autosomal dominant Emery-Dreifuss muscular dystrophy are allelic disorders. Moreover, it was found that different mutations in the lamin A/C gene cause both autosomal dominant and autosomal recessive Emery-Dreifuss muscular dystrophy (14).

The onset, course, and severity of X-linked Emery-Dreifuss muscular dystrophy can vary remarkably and not every patient has to develop the full set of clinical features (15-17). The spectrum of the phenotype in patients with null alleles is quite broad and con sists of arrows, intra- and inter-familial phenotypical abnormalities that have been reported (15). Usually, it is a mild but progressive skeletal myopathy with
unique, serious cardiac manifestations, causing sudden death in young affected men (18-22). Female carriers of defective gene may have fully developed heart disease, but without skeletal muscle weakness or contractures (23).

This clinical and genetic heterogeneity of Emery-Dreifuss muscular dystrophy imposes the need for detailed and systematic studies on phenotype-genotype correlation in such patients, especially those from large families, with prospects for better defining each phenotype and the frequency of the major clinical features at different age. These data are particularly useful in reaching differential diagnosis in sporadic cases and should help the approach to the pathophysiological process, which is the prerequisite for accurate management and treatment.

The aim of this study was to describe the clinical variability of this disorder, with emphasis on cardiac involvement in a four-generation family with X-linked Emery-Dreifuss muscular dystrophy (6 affected males, aged 22 months to 62 years, and 3 obligate carriers, aged 39 to 70 years) with novel mutation in the STA gene.

**Patients**

**Patient 1**

Patient 1, a proband (Fig. 1A, III, 3), was first seen at the age of 23 (Fig. 2) because his pregnant sister (Fig. 1A, III, 2) asked for genetic counseling due to several cases of muscular dystrophy in their family. His disease had a violent and very early onset, but presented itself with a classical spectrum of clinical features. The patient started to walk when he was 14 months old. He was generally weak, fell frequently, and was observed to walk with a waddling gait, which led him to his first hospitalization at the age of 5. Examinations performed at that time revealed pronounced wasting and weakness of the proximal lower and upper limb muscles and mild scapular winging. He had a lordotic posture, but contractures were not observed. His intellectual development was normal. When he was 9, he had myopathic face, thoracolumbar scoliosis, elbow contractures, and the tightness of the right Achilles tendon. At age of 23 (Fig. 2), he started to complain of occasional palpitations not related to exertion. ECG revealed a first-degree atrioventricular (AV) block. When he was 27 years old, ECG confirmed the first-degree AV block (PR 0.24 s), in complete right bundle branch block, and left anterior fascicular block. The prophylactic insertion of a diagnostic pacemaker, enabled the follow-up of conduction disturbances without leaving the patient unprotected. As AV and intraventricular conduction defects were more prominent before pacemaker implantation, so did sinus node dysfunction become more important during the follow-up (24). Today, in his late thirties, the proband is still ambulant, although physically very handicapped.

![Figure 1](image-url)

**Figure 1.** Pedigree of the proband’s family. A) Square and circle symbols represent males and females, respectively. Closed squares denote affected patients, and half-closed circles denote carriers of the STA gene. Crossed symbols indicate deceased persons. The arrow indicates the proband; Ins T-, normal sequence; Ins T+, mutated sequence. B) MboII restriction pattern. M, molecular size marker; C, unaflected control; MboII-, no restriction site (longer restriction fragment); MboII+, new restriction site (shorter restriction fragment).
Two of the three proband’s maternal uncles (Fig. 1A, II, 3, and 4) had shown similar clinical features. They had been ambulant until the age of 32 and 34, but soon after both suffered sudden and unexpected death.

Patient 2
When seen at 62 (Fig. 3), patient 2, the third uncle of the proband (Fig. 1A, II, 5), had obvious wasting of the shoulder girdle musculature, with particular involvement of the biceps and triceps muscles, marked elbow, wrist, and Achilles tendon contractures, facial weakness, severe scoliosis, and rigid spine. ECG revealed a complete monofascicular block (41/min) with no discernible atrial activity (probably atrial standstill with escape junctional rhythm) (24). He refused insertion of a pacemaker and suddenly died at 63 years of age.

Patients 3 and 4
Twin A (patient 3; Fig. 1A, IV, 2) and twin B (patient 4; Fig. 1A, IV, 3) were proband’s dizygotic twin-nephews. They were born at term, after an abnormal pregnancy. Their birth weight was 3,200 and 3,250 g, respectively, and they were both 49 cm long. They had normal early milestones. Patient 3 was born with equinovarus of the right foot, but the deformity disappeared after several months of conservative treatment. At 14 months of age, he started to walk. When examined at 22 months of age, he had limited spinal flexion as well as facial weakness with limited smile.

Ten of the three proband’s paternal uncles (Fig. 1A, II, 3, and 4) had shown similar clinical features. They had been ambulant until the age of 32 and 34, but soon after both suffered sudden death.

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Physical examination revealed only heart arrhythmia. ECG showed a wandering (atrial) pacemaker with QT prolongation (QT 480 ms, corrected QTc (QTc) 518 ms) and negative T-waves in the right precordial leads. Echocardiographic findings were completely normal. During submaximal stress test, there was no rise in blood pressure, QT in the val short ended during physical stress from 400 to 320 ms. Beside normal sinus rhythm with pre dominant QT prolongation, 24-hour ambulatory ECG monitoring also showed intermittent PR prolongation, low volt age P waves, wandering atrial pacemaker, junctional rhythms with a frequency of 43/min, sinus bradycardia of 41/min, and supraventricular extrasystolia.

Twin B was able to sit at 6 and walk at 12 months of age. On examination, 22 months of age, he spontaneously and occasionally resumed a position on his toes, as his twin-brother did. By the age of 10 years and 8 months (Fig. 4, shorter person), he was 143 cm tall (50th percentile) and weighed 40 kg (>75th percentile). He had a full range of mobility of all joints, with slight limitation of trunk flexion. There was slight winging of the scapulae, marked weakness of the biceps, but no contracture of the elbows. Wasting of the lower legs with moderate weakness of dorsiflexion of the ankles was also observed. On physical examination, after some knee-bends and limited trunk flexion, the child was tired and mildly dyspneic. His CK was elevated to 4.36 µKat/L, whereas CK-MB was normal. ECG showed sinus rhythm with borderline QT prolongation. Echocardiographic findings were normal. Stress test showed only a borderline QT prolongation, which persisted throughout the testing. Twenty-four-hour ambulatory ECG monitoring revealed a pre dominant sinus rhythm with borderline QT prolongation, short atrial runs with a frequency of about 391 Canki-Klain et al: Emery-Dreifuss Muscular Dystrophy Family Croat Med J 2000;41:389-395

Figure 2. Patient 1 – the proband (III, 3 in Fig. 1A) aged 23 years. Note contractures of the elbows, Achilles tendons, marked wasting of the medial parts of both gastrocnemii and the scapula winging. On examination, he had limited spinal flexion as well as facial weakness with limited smile.

Figure 3. Patient 2 (II, 5 in Fig. 1A) at age 62. Note severe scoliosis, excessive elbow and wrist contractures, with musculature wasting affecting par ticularly the biceps and sparing of the deltoids.
130/min, wandering atrial pacemaker, no junctional rhythm, and episodes of PR-prolongation.

**Obligate Carriers**

Proband’s grand mother (Fig. 1A, I, 1) suddenly died at the age of 39. Proband’s mother (Fig. 1A, II, 2) had never been examined by the age of 72 and was reported to be in good health.

**Patient 5**

Patient 5, a 45-year-old proband’s sister (Fig. 1A, III, 2), had no evidence of any muscle weakness, except difficulty in walking on her heels. She had never been examined by the age of 72 and was reported to be in good health.

**Results**

Direct sequencing of the emerin gene allowed the identification of a novel mutation. A thymin inserted after nucleotide 417 (417 ins T) in exon 2 of the STA gene (Fig. 5) confirmed the di agnosis of EMD in both twin nephews of the proband (Fig. 1A, IV, 2 and 3). This insertion generates a new restriction site (MboII+). The mutant allele was also proved by MboII restriction digestion carried out at 37°C in 30 µL reaction with 15 µL of PCR product, 3 µL buffer MboII, 0.2 U enzyme MboII and 0.3 µL BSA. The products were run in a 6% acrylamide gel for size analysis.

Emerin Analysis

Lymphoblastoid cell lines were established by transfection of peripheral blood lymphocytes with an engineered vector containing the emerin cDNA. DNA methylation-sensitive HhaI restriction near the polymorphic CAG repeat was used to identify the proband (Fig. 1B, IV, 3). The restriction pattern analysis confirmed that his mother was heterozygous (Fig. 1B, III, 2), whereas his father (Fig. 1B, III, 1) and sister (Fig. 1B, IV, 1) carried a normal allele. These findings demonstrated that the twins’ sister was not a carrier of the mutation.

Western blot analysis (Fig. 6) showed a total absence of emerin on lymphoblastoid cell lines in both affected twins (IV, 3 and 2) and non-affected twins (IV, 1). Surprisingly, the emerin protein levels were greatly reduced in their mother (III, 2), to less than 5% of normal. For this reason, we examined the X-inactivation pattern in both affected twins (IV, 3 and 2) and non-affected twins (IV, 1) by using the chemiluminescence method and ECL kits from Amersham International (Buckinghamshire, UK).

**Methods**

DNA Analysis

After the patients gave their informed consent according to guidelines set down by the Zagreb University Hospital, Zagreb, Croatia, genomic DNA was extracted from peripheral blood lymphocyte samples, by use of the standard methods (25). Polymerase chain reaction (PCR) with specific primers (26) was used to amplify exons 1 to 6 of the STA gene. The following primer pair was used for amplification of exon 2: 5’cgcctcagctcgacacccgc3’ (sense) and 30s at 60.5°C, 2 min at 72°C, and 7 min for final extension. The PCR products were run in a 1.5% agarose gel for size analysis.

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In this report, we describe the clinical variability of X-linked Emery-Dreifuss muscular dystrophy in a four-generation family. Pedigree data, together with the classical disease spectrum, allowed the diagnosis of Emery-Dreifuss muscular dystrophy in 1987, when genetic counseling was requested (32). Reinvestigation of this family by molecular methods ten years later permitted 1) the discovery of a novel mutation in the STA gene; 2) confirmation of the diagnosis; 3) early detection of still "presymptomatic" ("paucisymptomatic") proband's dizygotic twin-nephews and proband's sister; 4) carrier exclusion in the proband's adult niece (33); and 5) confirmation of intra-familial phenotype variability, with emphasis on cardiac involvement associated with specific null mutation in STA gene.

The novel mutation (417 ins T; submitted to the EMD Mutation database; http://www.path.cam.ac.uk/emd) detected in the family caused a shift in the reading frame, which resulted in a completely different sequence of amino acids downstream of the mutation. The resulting polypeptide was truncated shortly afterwards by the introduction of a stop codon at position 62 and this resulted in the absence of emerin, as confirmed by the Western blot analysis of lymphoblastoid cell lines of the patient IV-3.

Clinic data available for 4 affected males and a female carrier, together with clinic data in four mutation on two affected males and two female carriers, confirmed a considerable intra-familial phenotype variability as associated with the 417 ins T mutation.

What makes this family unique is the early cardiac involvement in the proband's dizygotic twin nephews (at 10 years of age) still without evident muscle wasting and weakness (but with increased CK, and normal CK-MB), and the survival of the oldest patient until the age of 63 with a pacemaker. This is remarkable, since his two younger brothers (Fig. 1A, II, 3 and 4) who had a similar clinical aspect, died suddenly at the age of 32 and 34 years, respectively.

The proband had a classical disease spectrum with a severe, very early on set resembling limb-girdle muscular dystrophy (17). Atrioventricular (AV) conduction defect was discovered when he was 23 years old, and four years later a permanent pacemaker was implanted.

Of the three obligate carriers, proband's grandmother (Fig. 1A, I, 2) died suddenly at 39 years of age. His mother (II, 2), to day 72 years old, had never been examined because of her good health. Proband's sister (III, 2), examined at the age of 45, collapsed during the exercise test and developed paroxysmal atrial tachycardia and low blood pressure. During the exercise test, paroxysmal ventricular couplets were recorded. In 24-hour ambulatory ECG recording, intermittent QT prolongation and atrial and ventricular ectopy were observed.
Observed clinical similarity between X-linked Emery-Dreifuss muscular dystrophy (2-4) and autosomal dominant Emery-Dreifuss muscular dystrophy (11,13,34), together with remarkable interand intra-familial clinical variability of both Emery-Dreifuss muscular dystrophy forms suggests a common pathophysiological process. Our data are in favor of this hypothesis. Emerin is the protein of the inner nuclear membrane and affected by mutations in classic Emery-Dreifuss muscular dystrophy, an X-linked disease. The autosomal form of the disease is caused by mutations in the lamin A/C gene (11,13,34,35). Recently, Clements et al. (36) demonstrated direct interaction between the recombinant emerin and lamin A molecules, using biochemical interaction analysis and monoclonal antibodies, MANEM5.

In conclusion, the described family is the first report on a remarkable intra-familial variability of cardiac disease in Emery-Dreifuss muscular dystrophy. Further investigation of patients with this disorder is needed to better understand the role of genetic (one or more modifier genes) and environmental factors in causing the observed phenomena.

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