Genotype-Phenotype Correlation of the Childhood-Onset Bethlem Myopathy in the Mediterranean Region of Turkey

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Abstract

Objectives: Collagen-VI-related myopathies are caused by both dominant and recessive mutations in the three collagen-VI-related genes (COL6A1, COL6A2, and COL6A3) and present as two different major clinical entities; Bethlem myopathy and Ullrich congenital muscular dystrophy.

Methods: In this study, we evaluated the clinical, pathologic, and genetic features of 8 patients with Bethlem myopathy from 3 families. Results: We inspected disease course differences with age and mutations. Different variants in COL6A1 and COL6A2 genes were detected. Muscle MRI of the lower limbs showed a specific pattern of muscle involvement with variable severity of fatty infiltration. One family had essential hypertension. Conclusion: Genotype-phenotype correlation studies are critical in determining gene or mutation-targeted therapies, patient follow-up, severity and progression prediction, and genetic counselling.

Keywords: Bethlem myopathy, collagen VI, COL6A2, Ullrich congenital muscular dystrophy

INTRODUCTION

Collagen VI is a ubiquitous extracellular matrix protein that forms a microfibrillar network and is closely associated with the basement membrane in most tissues such as muscle, cartilage, peristeum, ligaments, cornea, skin, tendon, and bone.[1] Collagen-VI-related myopathy is a disease entity caused by mutations, both dominant and recessive, in the three collagen-VI-related genes (COL6A1, COL6A2, and COL6A3).[2] These myopathies include a spectrum of disorders, including Bethlem myopathy (BM), Ullrich congenital muscular dystrophy (UCMD), autosomal recessive myosclerosis myopathy, and autosomal dominant limb-girdle muscular dystrophy (LGMD).[3] Classification of these disorders is based on phenotype; UCMD presents with the congenital onset of progressive muscle weakness, hyperlaxity of the distal joints, contractures of proximal joints, and respiratory insufficiency.[2,4] The BM phenotype is characterized by mild proximal muscle weakness and typical distal contractures of the fingers and ankle joints, with a late-onset and slow progression.[5,6] According to European Neuromuscular Centre’s 229th workshop in 2017, Bethlem myopathy dominant was classified as LGMD D5 collagen 6-related and Bethlem myopathy recessive was classified as LGMD R22 collagen 6-related.[7] Therefore, all of our patients were in LGMD D5 collagen 6-related group. Cardiac and cognitive functions are not affected, ambulation and respiratory functions are preserved in adult life.[8] BM is mostly inherited dominantly, although a few cases of autosomal recessive inheritance have been reported.[9] The main laboratory finding is slightly elevated creatine kinase (CK) level (<1000 IU/L).[6] MRI findings are characteristic for collagen VI-related myopathies, caused by fatty infiltration mostly in anterior thigh muscles.[10]

METHODS

Study population

The study group of 8 patients with collagen VI-related myopathies was retrospectively identified from Neuromuscular Disorders Centre of Antalya Research and Training Hospital in Turkey’s Mediterranean region. Four cases were index cases, and the other four patients were diagnosed by family screening. Demographic data (age, gender, etc.) and clinical features (initial symptom, age on-set, etc.) were reviewed from clinical records. Respiratory function evaluation was made with spirometrical measurements according to international standards.[11] Patients who had mild proximal muscle weakness, preserved ambulation and cognitive, respiratory and cardiac functions with supporting COL6A1-3 mutations were diagnosed with Bethlem Myopathy (BM).

Electroneuromyography (ENMG), echocardiography (ECHO), electrocardiography (ECG), office blood pressure

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measurements were performed for all the patients. Three patients with hypertension had 24-hour Holter monitorization and 24-hour ambulatory blood pressure measurements for further evaluation.

Renal functions tests (RFT) and serum creatine kinase (CK) levels were examined for each patient in six months periods.

Muscle MRI
Magnetic resonance imaging (MRI) with T1 and T2 weighted images were acquired by 1.5 T or 3T MR machines. Axial sections (10-mm slices) were obtained in a T1 weighted spin-echo sequence (repetition time 600-700 msec, echo time 30 msec). Vastus lateralis and rectus femoris muscle involvements were evaluated.

Muscle biopsy
Index cases from each family underwent a muscle biopsy. Histological studies contained the following stainings: Gomori trichrome, periodic acid shift (PAS), oil-red-o (ORO), nicotinamid adenine dinucleotide phosphate (NADH), succinate dehydrogenase (SDH), cytochrome-c oxidase (COX), adenosine triphosphatase (ATPase), myophosphorilase stains were used for histochemical studies and merosin, spectrin, dystrophin 1,2,3, calpain, sarcoglycans (α, b, g and d subunits), merosin/laminin-2, emerin, myotilin and laminin a/c, collagen 6, dystroglycans (α and b subunits).

Genetic analysis
First, DNA was isolated from the peripheral blood of the index cases, and a panel for myopathy-related genes in the Human phenotype ontology (HPO) database was studied using the Next Gene Sequencing (Illumina, NovaSeq, 6000) platform. The obtained variants were evaluated and classified according to the American College of Medical Genetics (ACMG) criteria, and the compatibility of clinical findings and family segregation were performed for variants that were not clearly reported as disease-causing in the literature. For segregation analysis, primers were designed which were specific to the variant that detected in the index case, and the primer design was based on transcripts, which are provided by RefSeq, a database built by the NCBI. NM_001848.3 and NM_001849.4 transcripts were used for COL6A1, and COL6A2, respectively.

The PP4 criterion was added to the classification if the clinical findings were compatible with the literature, and PP1 criterion was added to the classification if it showed segregation in the family. Finally, ACMG criterias were interpreted using the VarSome tool.

Results
Family 1
Patient 1 was a 49-year-old male born of consanguineous parentage presenting with recurrent falls, contractures, and keloids due to his injuries since the age of 10 years. He noticed a predominantly proximal weakness of all four limbs, difficulty in climbing stairs, rising from the floor, and progressed very slowly until 35 years of age. On examination, his proximal muscles were mildly weak (Medical Research Council (MRC) grade 3-4/5 proximally and 4-5/5 distally) with atrophy of palmar muscles. Contractures were noted at metacarpals, elbow, and ankle with hyperextensibility of fingers. There were multiple keloids over the forearm and both hands, along with follicular hyperkeratosis.

Both his son and daughter presented with predominant proximal muscle weakness (MRC grade 4-5/5 proximally) of all four limbs, follicular hyperkeratosis, and distal hyperextensibility of fingers. Patient 2 (his son) was 17 years old with symptom onset at 12 years of age. He had contractures at metacarpals. Patient 3 (his daughter) was 14-years-old with disease onset at 12 years of age [Figure 1]. She had mild proximal weakness of all four limbs and had occasional falls. Despite his brother, she had not had any contractures or keloids yet.

Distinct from usual Bethlem’s myopathy patients, children suffered from headaches around ten years of age and were diagnosed with migraine. Throughout their follow-ups, both developed hypertension at 12 years of age. Father was diagnosed with hypertension in the third decade. Electrocardiography (ECG), echocardiography (ECHO), renal ultrasound, and renal Doppler ultrasound studies were normal for all of them. Laboratory examinations for urine analysis, renin-angiotensin level, and lipid profile were within normal limits. Therefore, causes of secondary hypertension were eliminated.

Mental and motor milestones and respiratory functions were normal in all three patients. Muscle enzymes were normal in the father and mildly elevated in both children.

We detected a heterozygous COL6A2 c.2096G >A variant in all three patients. We classified this variant as pathogenic according to the ACMG criterias (PM1, PM2, PP1 moderate, PP3, PP4_strong) [Table 1].

Family 2
Patient 4 was an 18-year-old female, presented with frequent falls and difficulty in climbing stairs around the age of 7. She...
Table 1: Demographic and clinical data of the patients

| Patient number | Age/ Sex | First symptom age (years) | Ambulation | Contractures | Dermatological findings | CK (IU/L) | Respiratory Evaluation FEV1/FVC, PEF | Cardiovascular Evaluation | Mutation |
|----------------|----------|---------------------------|------------|--------------|-------------------------|-----------|-------------------------------------|--------------------------|----------|
| 1              | 49/M     | 10                        | Preserved  | Present      | Keloids + follicular hyperkeratosis | 1150      | >80%                                | Primary hypertension    | COL6A2 c.2096G>A |
| 2              | 17/M     | 7                         | Preserved  | Present      | Keloids + follicular hyperkeratosis + acne vulgaris | 850       | >80%                                | Primary hypertension    | COL6A2 c.2096G>A |
| 3              | 14/F     | 8                         | Nonpresent | Nonpresent   | Follicular hyperkeratosis + hirsutism + acne vulgaris | 1350      | >80%                                | Primary hypertension    | COL6A2 c.2096G>A |
| 4              | 18/F     | 6                         | Preserved  | Nonpresent   | Keloids + follicular hyperkeratosis + hirsutism + acne vulgaris | 263       | >80%                                | Normal                  | COL6A2 c.736-2A>G |
| 5              | 17/M     | 7                         | Nonpresent | Nonpresent   | Follicular hyperkeratosis + acne vulgaris | 417       | >80%                                | Normal                  | COL6A2 c.736-2A>G |
| 6              | 46/M     | 10                        | Preserved  | Present      | Follicular hyperkeratosis | 850       | >80%                                | Normal                  | COL6A2 c.736-2A>G |
| 7              | 13/F     | 2                         | Nonpresent | Nonpresent   | Keloids + follicular hyperkeratosis + acne vulgaris | 239       | >80%                                | Normal                  | COL6A1 c.1056+1G>A |
| 8              | 55/M     | 15                        | Preserved  | Present      | Keloids + follicular hyperkeratosis | 527       | >80%                                | Normal                  | COL6A1 c.1056+1G>A |

showed proximal muscle weakness (MRC 4+/5), follicular hyperkeratosis on her back and arms, and keloids on physical examination. She was ambulatory and able to resume her daily activities. Her brother, patient 5, also had frequent falls and difficulty in climbing stairs around the age of 6. He had follicular hyperkeratosis on her back and arms and keloids, similar to his sister. However, his proximal muscle weakness (MRC 4/5) was more apparent on physical examination. He had no functional impairment. Patient 6, their father, had muscle weakness; on proximal muscle groups (MRC 3+/5), on long finger flexors, and extensors (MRC 4+/5) [Figure 1]. He had distal contractures on his fingers. He had multiple keloids. Muscle weakness and similar symptoms were noted in family history (grandmother) without clinical diagnoses. COL6A2 c.736-2A>G variant was detected, which was reported as pathogenic in the literature, in all three patients [Table 1].

**Family 3**

Patient 7 was a 13-year-old female with first symptom age of 2 years. She had normal motor mental milestones until the age of 2, then she developed frequent falls, difficulty in climbing stairs, and rising from the floor in time. By the age of 10, significant elbow contractures were noted. On the last visit, physical examination revealed proximal (MRC 3+/5) and distal (MRC 4+/5) muscle weakness, severe contractures, and scoliosis. Patient 8, her father, is a 55-year-old male, presented to our clinic with her daughter and undiagnosed [Figure 1]. In his early childhood, he had frequent falls, difficulty in climbing stairs, and rising from the floor. Muscle weakness and joint contractures worsen progressively, and he needed walking aid at the age of 40. Severe proximal (MRC 2+/5) and distal (MRC 3/5) muscle weakness of the lower limbs, elbow, heel, and finger contractures were noted, and he was in a wheelchair at the last visit. Both patients had keloids caused by frequent falls and follicular hyperkeratosis on the back. Genetic analysis revealed the COL6A1 c.1056+1G>A variant, known to be disease-causing in both patients [Table 1].

**Discussion**

Here we report three unrelated families with clinical features, mutations, and genotype-phenotype correlation for BM and UCMD. Nowadays, it is known that there is genetic heterogeneity in several diseases. In addition, a single gene mutation can lead to different phenotypes. BM and UCMD are one of the examples for this phenomenon. COL6A1, COL6A2, and COL6A3 gene mutations lead to both the BM and UCMD phenotype. Family 1 and 2 had a mutation in the COL6A2 gene, and Family 3 had a mutation in the COL6A1 gene. All of the patients had proximal muscle weakness, follicular hyperkeratosis, and distal hyperextensibility of fingers. However, keloids and contractures were mostly seen in patients who were in more advanced stages of the disease. Respiratory and cardiac evaluations of all patients were unremarkable, which is common in BM.

In family 1, we detected COL6A2 c.2096G>A, which was a novel mutation. The clinical courses of patients 1, 2 and 3 were similar, but unlike her father and brother, Patient 3 had no contractures or keloids. We suggested that because of the patient 3 was in early stage of the disease she had not contractures or keloid formation. All three patients had essential hypertension. To our knowledge, hypertension is not a cardinal feature of Bethlem myopathy, and for the first
time in the literature, we reported a family with Bethlem myopathy and hypertension published previously.\(^{[15]}\) In cohort studies conducted to date, hypertension has not been reported in patients with COL6-related myopathy.\(^{[2,16]}\) van der Kooi and colleagues did not find any cardiac abnormality in a Dutch family with COL6A2 mutation.\(^{[17]}\) Only, Bao and colleagues reported a patient with recurrent hematuria, and after whole-exome sequencing analysis, they speculated that impaired interaction between COL6 and COL4 results in hematuria.\(^{[18]}\) Because collagen is present throughout the body, all organs or systems could be affected by any loss of function.\(^{[19]}\) Therefore, we suggested that the overlapping of clinical findings is not unexpected.

In family 2 we detected the COL6A2 c.736-2A > G mutation that is a known mutation, and these patients’ clinical findings were compatible with the literature.\(^{[8]}\)

Family 1 and 2 had mild features of BM.

In family 3 we detected the COL6A1 c.1056 + 1G > A variant, known to be disease-causing, and both patients had the same disease-onset symptoms, joint contractures, keloid formation, and proximal and distal muscle weakness. Family 3 had the most severe form of BM among our patients. This mutation has been reported previously with both ends of the clinical presentation. The patients present with BM phenotype and c.1056 + 1G > A variant was reported needing walking aid in 5th decade.\(^{[2,8,20]}\)

CK levels were elevated in all patients. CK levels and clinical severity wasn’t correlated in our patients as reported in previous studies. Also, age onset and current age didn’t affect CK levels.\(^{[2]}\)

In the literature, respiratory failure was reported in BM patients with diaphragmatic involvement. In the study of van der Kooi et al., yearly pulmonary investigations including FVC measurement in sitting and supine positions were recommended.\(^{[17]}\) FEV1/FVC measurements were performed in all of our patients, and no pulmonary abnormality was detected.

Muscle MRI findings, fatty infiltration of different muscle groups, could be used in describing different muscular dystrophies. The typical UCMD pattern consisted of diffuse involvement of the thigh muscles with relative sparing of sartorius, gracilis and adductor longus. In BM, there is predominantly anterior thigh involvement. The central part of the rectus femoris and peripheral part of the vastus muscles involvement is the most characteristic finding for BM. “Target” sign is due to severe fatty infiltration in the central region of rectus femoris, surrounding a mid-point of little infiltration.\(^{[3,10]}\) The severity of the muscle MRI involvement in patients with both UCMD and Bethlem myopathy was variable and was always more closely associated with the severity of clinical course than with age. In our patients, MRI findings were seen with advanced age [Figure 2]. We recommend conduct MRI studies in the 3rd decade.

**CONCLUSION**

Genotype-phenotype correlation studies are very important in determining gene or mutation-targeted therapies of diseases, patient follow-up, severity and progression prediction, and genetic counselling. Mutations and clinical findings that have not been previously reported in the literature also can provide information about the functions of proteins, and thus it may be possible to improve the treatment of various diseases.

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**Conflicts of interest**

There are no conflicts of interest.

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