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CASE REPORT

NOVEL MUTATION IN THE *COL11A1* GENE CAUSING MARSHALL-STICKLER SYNDROME IN THREE GENERATIONS OF A BULGARIAN FAMILY

Mladenova M[1,2,*], Todorov T[2], Grozdanova L[3], Mitev V[1], Todorova A[1,2]

[*]**Corresponding Author:** Dr. Mihaela Mladenova, Department of Medical Chemistry and Biochemistry, Medical University Sofia, 15 Acad. Ivan Geshov Str. Sofia, Bulgaria. Tel.: +359-9520-522. Fax: +359-2-9155-049. E-mail: mihaela.mladenova@gmail.com

ABSTRACT

Here we report the first familial case spread through at least three generations, genetically verified cases of Marshall-Stickler syndrome in Bulgaria. The proband, a 2-year-old girl, has craniofacial dysplasia, ocular hypertelorism, small saddle nose with a flat bridge and midface hypoplasia. The pedigree of the proband's family showed that her father has the same clinical manifestations of the disease. In addition, her father presented with a tall, thin stature and mild hearing loss, manifested with aging. The same dysmorphological symptoms were presented by the paternal grandfather. Both patients, the 2-year-old girl and her father, have been diagnosed to carry Marshall-Stickler syndrome. The *COL2A1* gene tested negative in the family. Based on the higher percentage of mutations in the *COL2A1* gene, we analyzed this gene as the first target in the family. The *COL2A1* gene tested negative, and we sequenced the gene further. A novel splice site mutation c.3474+1G>A was found in intron 44. This variant is related to the clinical presentation in the patient and her father. The c.3474+1G>A mutation results in altered splicing affects at the donor splice site of intron 44, which most probably gives a nonfunctional protein. The variant affects the major triple-helical domain that represents a mutation hot-spot for the gene.

Keywords: *COL11A1* gene; Marshall-Stickler syndrome; Midface hypoplasia; Splice-site mutation.

[1] Department of Medical Chemistry and Biochemistry, Medical University Sofia, Sofia, Bulgaria

[2] Genetic Medico-Diagnostic Laboratory "Genica," Sofia, Bulgaria

[3] Department of Medical Genetics, University Hospital "St. George," Plovdiv, Bulgaria

INTRODUCTION

Stickler syndrome, affecting one in 7500 to 9000 newborns, is a hereditary autosomal dominant disorder (MIM 108300). The condition is characterized by typical facial, ocular, articular, and auditory features [1-3]. The most common reported manifestation of the cases with Stickler syndrome are wuth vitreoretinal degeneration, cleft palate, retinal detachment, osteoarthritis, sensorineural-hearing loss, high myopia and midfacial hypoplasia. Marshall's syndrome features are also similar (MIM 154780) [4,5] that provoke continuing debate is a different condition of one syndrome or two single syndromes [5-9]. Some researchers have classified Marshall syndrome as a variant of Stickler syndrome while others consider it to be a separate disorder (MIM 154780) [4,5]. The clinical manifestations of these two conditions are summarized in Table 1.

All the genes that are associated with Marshall-Stickler syndrome provide instructions for making components of collagens. They are complex molecules modeling the structure and affirming the strength of the connective tissue and supporting the body joints and organs [10]. If collagen molecules are irregulated or their amounts are reduced, then collagen impairs the development of connective tissues in many different parts of the body, leading to a wide variety of syndromic features [11]. Mutations in the *COL2A1*, *COL11A1* and *COL11A2* procollagen genes cause Stickler syndrome. Marshall syndrome, caused by a *COL11A1* gene mutation, has clinical overlap with Stickler syndrome.. About 80.0-90.0% of all cases are caused by mutations in the *COL2A1* gene. The remaining ~20.0% of cases result from mutations in the *COL11A1* gene [5].

Here, we report a novel splice-site mutation in the triple-helical domain of the *COL11A1* gene in a Bulgarian patient. This is first genetically verified familial case of Marshall-Stickler syndrome in our country.

Table 1. Clinical manifestations of the Marshall and Stickler syndromes [5] (with modifications).

Findings	Marshall Syndrome	Stickler Syndrome
Head	brachycephaly; thickened calvaria	normocephaly
Midface	flat; retracted	dish-shaped; flat
Nose	small, short saddle nose with flat bridge	long nose with prominent nasal bridge
Ocular hyperterlorism/ other ocular findings	yes/high myopia; glaucome; retinal detachment; no astigmatism	no/high myopia; vitreoretinal degeneration; astigmatism
Hearing loss	frequent; sensorineural	mild
Stature	short and stocky	normal or tall and thin
Skeletal abnormalities	spondyloepiphyseal abnormalities	osteochondrodysplasia; spondyloepiphyseal dysplasia
Joints	hypoextensible	hypoextensible; arthropathy with degenerative arthritis
Inheritance	autosomal dominant	autosomal dominant
COL21A1 mutations	no	yes

Clinical Data. The proband, a 2-year-old girl, has craniofacial dysplasia, ocular hypertelorism, small saddle nose with flat bridge and midface hypoplasia (Figure 1). At this age she does not yet demonstrate hearing loss and she presents with a normal stature.

The proband's father is 38 years old and has ocular hypertelorism and an inner canthal distance of 40 mm (>97th percentile) (Figure 2). His phenotypic features include broad flat nasal bridge, relative mandibular prognathism, midface hypoplasia with primary telecanthus and nasal hypoplasia. He presents a tall thin stature and mild hearing loss. Psycho developmental evaluation demonstrates moderate intellectual disability. Both patients, the 2-year-old girl and her father, have been diagnosed clinically as Marshall-Stickler syndrome. The family photographs, provided by the father, showed the same facial dysmorphism in the paternal grandfather (Figure 3).

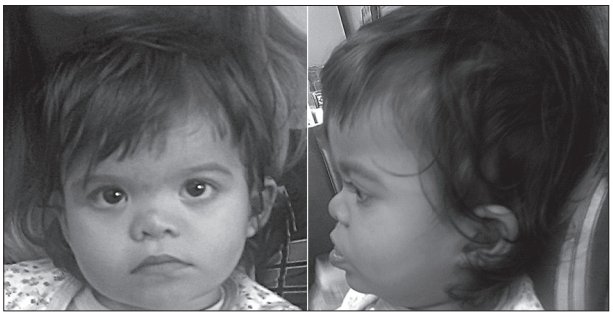

Figure 1. Frontal and profile view of proband.

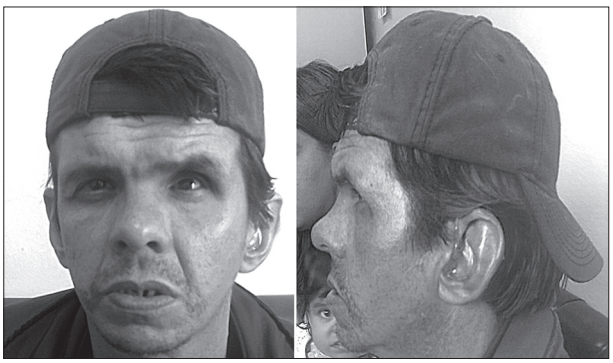

Figure 2. Frontal and profile view of proband's father.

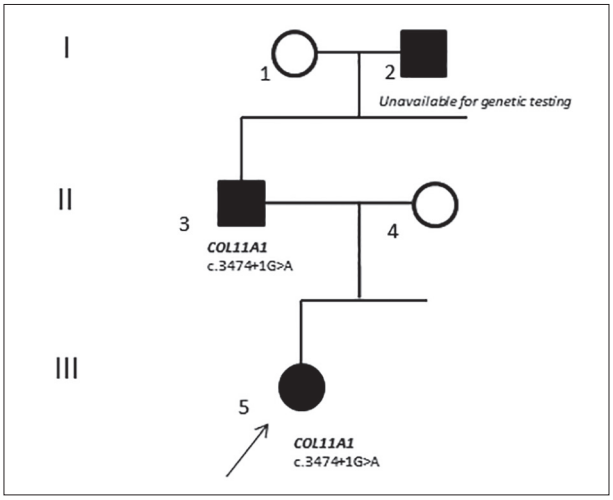

Figure 3. Pedigree of the studied family.

METHODS

Informed consent was obtained from the patient's father prior to genetic testing. The study was approved by the Ethics Committee of Sofia Medical University, Sofia, Bulgaria.

Molecular Genetic Methods. Genomic DNA was extracted from blood leukocytes. Polymerase chain reac-

tion (PCR) and Sanger sequencing were performed in order to screen for germline mutations in the *COL2A1* and *COL11A1* genes. All coding exons and exon-intron boundaries of the primers were designed to specifically amplify. The electrophoretic separation was performed on ABI PRISM® 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). The sequencing reaction was performed by BigDye®Terminator cycle sequencing kit v.3.1 (Applied Biosystems) that includes Thermo Sequenase II DNA polymerase and fluorescently labeled nucleotides. The sequencing profiles were interpreted by the software Sequencing Analysis v5.1.1 (https://assets.thermofisher.com/TFS-Assets/LSG/manuals/cms_041266.pdf). The mRNA reference sequence was based on the information available from Human COL 11A1, RefSeq NM_001854, accession number NM_001854 (https://www.ncbi.nlm.nih.gov/nuccore/1519243093). Human COL2A1, RefSeq NM_001844, accession number NM_001844 (https://www.ncbi.nlm.nih.gov/nuccore/1519243785).

Results and Discussion. Based on the higher percentage of mutations in the *COL2A1* gene, we analyzed this gene as the first target in our family. The *COL2A1* gene tested negative in the family and we further sequenced the *COL11A1* gene. A novel splice-site mutation c.3474+1 G>A was found at intron 44 (Human *COL11A1*, RefSeq NM_001854). The segregation analysis in the family showed that the father is a carrier of the above mentioned variant, c.3474+1G>A, which is related to the clinical presentation of both the proband and her father.

The mutation c.3474+1G>A at intron 44 affects the donor splice-site, and as a result of altered splicing, gives a nonfunctional protein. The variant is localized in the region encoding the major triple-helical domain that represents a hot-spot for mutations on the *COL11A1* gene [12].

In the present genetic variant, the purine nucleotide guanine (G) is substituted by the purine nucleotide adenine (A), an event known as transition. We analyzed the neighboring sequence of 22 bp upstream and 22 bp downstream of the mutation. The surrounding area is abundant with repeated elements (AA, GG and TT) and trinucleotide palindromic sequence AAA/TTT, closely situated to the position of the substitution (Figure 4). The repeated and palindromic sequences might play a role in a G>A substitution and the transition fixation in the genome. The DNA polymerase proof-reading activity at this position might be impaired by a secondary structure formation, making chemically identical substitutions difficult to recognize and remove, thus leading to their fixation in the genome.

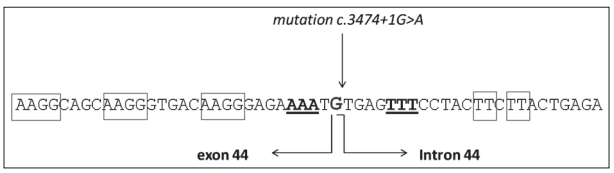

Figure 4. The neighboring sequences 22 bp upstream and 22 bp downstream of the G>A substitution (c.3474+1G>A) at intron 44 of the *COL11A1* gene.

In conclusion, the present report concerns the first familial case spread through at least three generations, genetically verified case of Marshall-Stickler syndrome in Bulgaria, caused by a novel splice-site mutation in the triple-helical domain of the *COL11A1* gene.

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Declaration of Interest. The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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