A novel mutation in collagen transport protein, *MIA3* gene, detected in a patient with clinical symptoms of Ehlers–Danlos hypermobile syndrome

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Abstract

Background. Collagen, the most abundant human protein, is a significant component of the extracellular matrix (ECM) in tissues and organs like skin, bone, ligaments, and tendons. Collagen secretion is a complex, multistage process involving many molecules. A protein playing one of the main functions in this process is TANGO1 encoded by *MIA3* gene. In the hypermobile type of Ehlers–Danlos syndrome (hEDS), one of the most common collagenopathies with no known genetic background, disrupted secretion of many molecules (including collagen) was observed.

Objectives. The aim of this study was the evaluation of the *MIA3* gene role in hEDS patients.

Materials and methods. One hundred patients with clinically diagnosed hEDS and negative next-generation sequencing (NGS) testing for connective tissue disorder (e.g. Ehlers–Danlos syndrome, osteogenesis imperfect (OI), Marfan syndrome, and others) were tested for molecular changes in the *MIA3* gene.

Results. Among the 100 tested patients, 14 single structural changes in the *MIA3* gene were detected. Thirteen were missense benign or likely benign, while 1 variant (c.567dup, p.Leu1880ThrfsTer6) was truncating the TANGO1 protein.

Conclusions. We suppose that the presence of truncating variant (c.5637dup) in the *MIA3* gene and disrupted secretion of connective tissue protein may be one of the pathogenic mechanisms of clinical symptoms present in the tested patient, but these findings require a more comprehensive multidimensional investigation.

Key words: NGS, hypermobility, Ehlers–Danlos syndrome, *MIA3*, TANGO1

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Background

Collagen, the most abundant human protein, is a major component of the extracellular matrix (ECM) in tissues and organs like skin, bone, ligaments, tendons, arteries, veins, as well as the gastrointestinal and respiratory systems. All human proteins, including collagen, require proper protein folding, maturation and secretion processes. Collagen biosynthesis is a complex, multistage process involving many molecules. It takes place in the rough endoplasmic reticulum (rER). In the lumen of the rER, collagen molecule undergoes a series of post-translational modifications, e.g., hydroxylation and glycosylation. Then, the collagen molecule is transported via the Golgi apparatus to the extracellular space.[1]

Efficient transport of procollagen molecules from the rER to the Golgi apparatus involves a special protein transport complex. Procollagen folded in the rER has ~300 nm in length, which is too large to fit conventional coat protein complex II (COPII) vesicles (vesicles have approx. 60–80 nm in diameter).[1] For the secretion of large molecules, another trafficking system is required. Saito et al. showed that transmembrane protein transport and Golgi organization (TANGO1; NP_940953.2), encoded by *MIA3* (NM_198551.4) gene, facilitates the entrance of collagen into COPII vesicles at the rER exit site.[2] TANGO1 is a protein of 1907 amino acids composed of the SH3 domain (N-terminus) that binds collagen molecules, 2 coiled-coil domains (CC1 and CC2), and the proline-rich domain (PRD), which all assist in the formation of collagen-like COPII vesicles (C-terminus) and play a significant role in interactions between the endoplasmic reticulum and the COPII components (Sec23/Sec24). Shortening of this TANGO1 domain may disrupt vesicle formation and result in a reduction of the capability of collagen and other ECM protein transport.[2–4]

The potential function of the TANGO1 molecule was assessed by Wilson et al. in a study on knockout mice. Their analysis revealed that secretion of numerous collagens, including collagen type I, II, III, IV, VII, and IX, from fibroblasts to the ECM was disrupted, most likely because of the defective export of these molecules from the endoplasmatic reticulum.[5] The major role of TANGO1 in collagen molecule transport was also confirmed by Raote et al. and Ishikawa et al.[4,6]

Ehlers–Danlos syndrome (EDS) is a heterogeneous group of heritable connective tissue disorders. The 2017 international classification of EDS recognizes 13 subtypes that are caused by pathogenic variants in 19 different genes, encoding different types of collagen or protein involved in collagen metabolism or functioning. For all types of EDS, the genetic background was determined, except the hypermobile type of EDS (hEDS), which is one of the most common connective tissue disorders.[7] Despite intensive investigation, genes related to hEDS have not been identified yet.

Studying hEDS patients, Chiarelli et al. assessed the presence of some ECM proteins inside and outside fibroblasts. The study showed that ECM proteins, especially collagen type I, III and V, as well as fibrillin, tenascin and fibronectin, were detected only in the cell cytoplasm of hEDS patients, while in the intercellular space they were either not visible or only a few thin, sparse fibrils were present. Contrarily, in the healthy control group, these proteins were visible inside and outside cells.[8] Abnormality in ECM transport protein may be one of the components of the molecular background of clinical symptoms of hEDS. The cause of dysfunction of ECM protein transport from cytoplasm to the intracellular space in hEDS patients is unknown. In the ECM protein transport pathway, many molecules are involved, and one of them may be the TANGO1 protein.

Objectives

The aim of this study was the evaluation of the role of *MIA3* gene role in hEDS patients.

Materials and methods

The study group included 100 hEDS patients of Polish origin, 84 women and 16 men, aged 17–63 years (median: 31 years). Patients were enrolled in the study by experienced clinical geneticists, according to the 2017 international classification of the EDS diagnostic criteria.[7] Joint hypermobility was evaluated on the Beighton scale. Patients were not related. The control group consisted of 100 volunteers from the general Polish population matched by age and sex with the investigated group, healthy (including lack of EDS) at the time of the investigation and without a history of EDS in the family. Enrollment in the control group was based on volunteers' medical history.

All hEDS patients or their parents provided informed consent to participate in the study. Consent to publish clinical/genetics data has been obtained from the patients.

The study was approved by the Ethics Committee of the Collegium Medicum in Bydgoszcz, Nicolaus Copernicus University, Toruń, Poland (approval No. KB485/2013).

The analysis was performed on genomic DNA (gDNA) which was extracted from leukocytes (fibroblasts were not available) with QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) using standard procedures. In all patients, other types of EDS or other connective tissue disorders were excluded by testing them with NGS technology (Illumina, San Diego, USA). The connective tissue congenital defects panel included *COL5A1, COL5A2, COL3A1, COL1A1, COL1A2, HSP47, TNXB, ADAMTS2, PLOD1, FKBP14, ZNF469, PRDM5, B4GALT7, B3GALT6, SLC39A13, CHST14, DSE, COL12A1, C1R, C1S, SEC23A, SEC24D, COL6A1, COL6A2, COL6A3, COL9A1, COL9A2, FBN1,*

FBN2, FLNA, FLNB, ELN, NOTCH1, MYH11, MYLK, TGFB2, TGFB3, and *TGFBR1*. Copy number variations (CNV) analysis was also performed.

Molecular analysis of all exons of the *MIA3* gene was performed with Sanger sequencing according to standard procedure (primer sequences available upon request). The pathogenicity of detected variants was assessed according to the ACMG guideline released by VarSome.[9,10]

Results

All patients were tested using NGS multi-gene panel for connective tissue disorders, EDS, osteogenesis imperfect (OI), Marfan syndrome, and others. In any of them, no pathogenic or likely pathogenic variants were detected. Copy number variations were also not detected. The next step of the investigation was the sequencing of the *MIA3* gene using Sanger sequencing. In 14 patients among 100, 5 variants in *MIA3* were detected. Four of 5 alterations were missense variants (benign or likely benign) and 1 was a frameshift variant assessed as a likely pathogenic (class 4 according to ACMG) (Table 1). All found alterations were heterozygotes. The variants were not detected in the control group.

Frameshift variant c.5637dup, which results in termination of transcription (p.Leu1880ThrfsTer6), was found in a 49-year-old woman. She presented with hypermobility of joints (7/9 Beighton score), recurrent dislocations, arachnodactyly, chronic severe joints and spinal pain since childhood, fatigue, soft, mild, hyperextensible and easy bruising skin, stretch marks, brain aneurysm, astigmatism, blue sclerae, hyperopia, dry eyes, many food products intolerances, and a positive family history. Her 14-year-old daughter suffered from hypermobility (9/9 Beighton score), chronic joint pain, scoliosis, soft and mild hyperextensible skin, and blue sclerae. The first symptoms occurred earlier (at about 10 years of age) and in a more severe form than in her mother. She was also a carrier of the c.5637dup *MIA3* variant.

The proband and her daughter were first clinically diagnosed with rheumatoid arthritis. Later on, the disorder was excluded. Both were of normal height (mother 174 cm, daughter 165 cm), and both also had a normal bone density. Proband's parents were not tested (they did not consent to participate in the study). However, proband indicated that her mother had symptoms similar to her own but in a milder form and with onset in older age (Fig. 1).

Discussion

MIA3 gene encodes a transmembrane protein which plays an important role in the transport of different proteins from fibroblasts and chondrocytes to extracellular space. In an investigation by Wilson et al., mice lacking TANGO1 protein displayed neonatal lethality with a chondrodysplasia, lack of bone mineralization, dwarfism, and defective secretion of numerous collagens (including collagen type I, II, III, IV, VII, and IX).[5] Recently, Lekszas et al. identified a homozygous synonymous variant in *MIA3* (c.3621A>G, p.Arg1207=) that leads to exon 8 skipping, which results in truncating of TANGO1 protein. The affected patient presented with various skeletal abnormalities including short stature, scoliosis, osteopenia, brachydactyly and clinodactyly, dentinogenesis imperfecta, and mild intellectual disability. Another observation was a drastic reduction of collagen I secretion from

Table 1. Variants detected in the *MIA3* gene

No.	Patient assigned lab number	Nucleotide	Amino acid	ExAC frequency
1.	46	c.1099A>T	p.(Thr367Ser)	T = 0.78%
2.	148	c.1099A>T	p.(Thr367Ser)	T = 0.78%
3.	61	c.1099A>T	p.(Thr367Ser)	T = 0.78%
4.	62	c.1099A>T	p.(Thr367Ser)	T = 0.78%
5.	63	c.1099A>T	p.(Thr367Ser)	T = 0.78%
6.	68	c.1099A>T	p.(Thr367Ser)	T = 0.78%
7.	80	c.1099A>T	p.(Thr367Ser)	T = 0.78%
8.	140	c.1099A>T	p.(Thr367Ser)	T = 0.78%
9.	85	c.2566G>A	p.(Asp856Asn)	A = 0.01%
10.	24	c.2637C>A	p.(Asp879Glu)	A = 0.1%
11.	25	c.2637C>A	p.(Asp879Glu)	A = 0.1%
12.	26	c.2637C>A	p.(Asp879Glu)	A = 0.1%
13.	139	c.2687C>T	p.(Aln896Val)	T = 0.09%
14.	90	c.5637dup	p.Leu1880ThrfsTer6	dup = 0.001%

ExAC – Exome Aggregation Consortium.

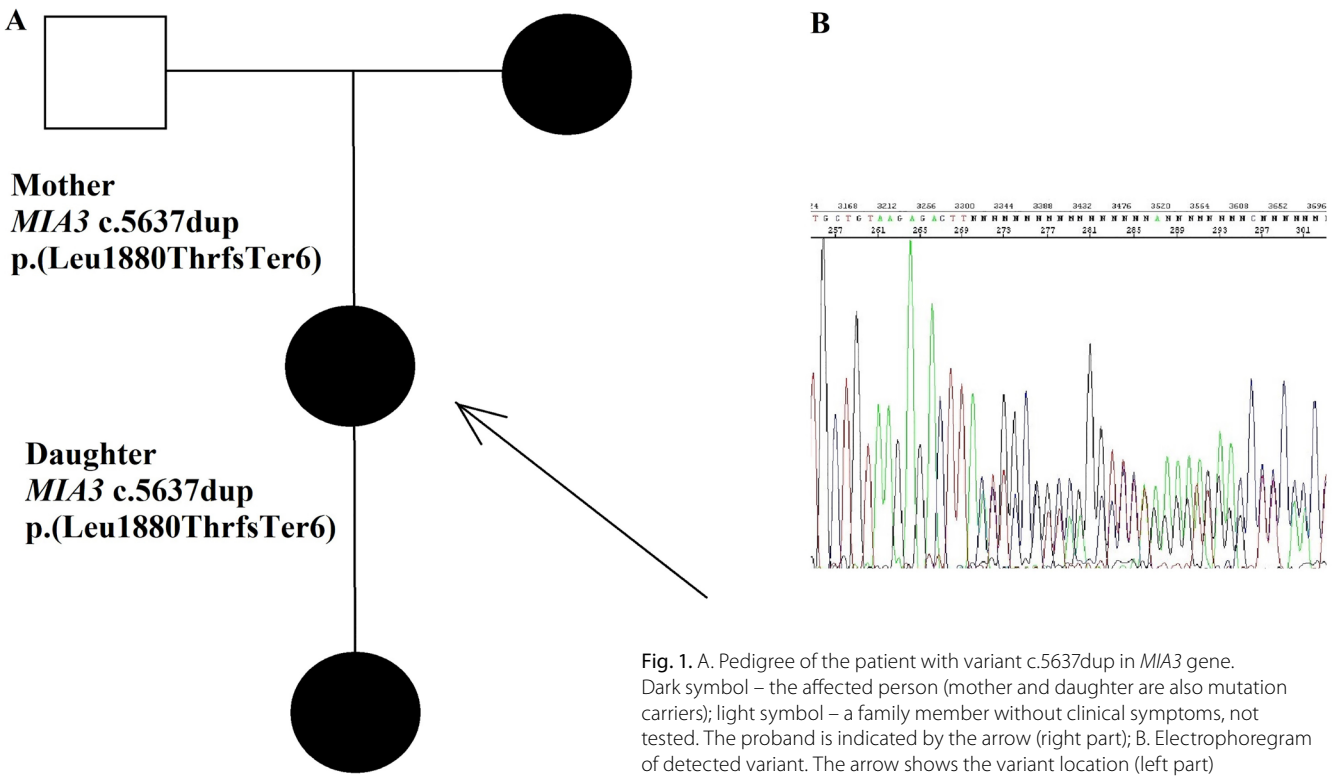

Fig. 1. A. Pedigree of the patient with variant c.5637dup in *MIA3* gene. Dark symbol – the affected person (mother and daughter are also mutation carriers); light symbol – a family member without clinical symptoms, not tested. The proband is indicated by the arrow (right part); B. Electrophoregram of detected variant. The arrow shows the variant location (left part)

cells to the extracellular space. Other connective tissue proteins which are released from fibroblasts were not assessed.[11] In another investigation, by Guillemyn et. al, a novel homozygous frameshift variant (c.2770_2773del) was detected in a consanguineous Indian family, in whom the proband was a fetus presenting with early lethality and almost complete absence of bone formation. The family had a history of 3 induced terminations of pregnancy because of clinical suspicion of lethal OI. Both proband parents were carriers of heterozygous c.2770_2773del variant. After functional analysis, it was revelaed that TANGO1 expression was reduced by about 50%. However, the secretion of collagen I was assessed as normal (secretion of other collagens or connective tissue protein was not evaluated). Parents did not present OI features, and information about their other clinical symptoms was not included.[12]

In a study by Clark and Link, the role of TANGO1 protein was evaluated in zebrafish. The authors reported that homozygous mutants were significantly shorter, had craniofacial defects and did not survive to adulthood. Heterozygous organisms were not tested.[13]

In patients with joint hypermobility syndrome/hEDS investigated by Chiarelli et al., ECM organization was assessed (for the first time in JHS/hEDS patients). In patients' fibroblasts, collagens (I, III and V type) were accumulated in the cell cytoplasm and were not or almost not visible outside the cells. Similarly, fibronectin, tenascin, fibrillin, and elastin necessary for fibrils formation were not released from the cells into the extracellular space, or only thin fibrils were detected outside the cells. The potential mechanism of dysfunctional ECM protein transport was not evaluated in patients tested by Chiarelli et al. and remains unknown.[8]

The patient investigated by us was a carrier of the heterozygous truncating variant in the *MIA3* gene. She presented clinical symptoms of hEDS. Her symptoms were not so severe as it was described in homozygous patients and in the homozygous mice and zebrafish models.[5,11–13]

Based on the results of our and other authors' research, we want to add the next step to the ascertainment proposed by Guillemyn et al. – "TANGO1 pathogenicity path", where the location of gene variants has a significant impact on phenotype severity; variants located in N-terminus cause lethality,[12] while variant located at the beginning of the cytoplasmatic part of protein – a severe but not lethal clinical picture.[11] We suppose that a mild clinical condition of our patients may be the result of the variant location in the cytoplasmatic part of protein as well as the heterozygous status of the *MIA3* gene. We also believe that the potential role of the disrupted pathway of collagen secretion in the mechanism of collagenopathy should be taken into account.

Limitations

We have to consider the fact that other patients analyzed in our study had clinical symptoms of hEDS and were all negative for the NGS-EDS panel, as well as negative for likely pathogenic/pathogenic variants of the *MIA3* gene. These data show that *MIA3* alterations may be only one among many various collagenopathy causes.

Conclusions

The role of *MIA3* or other genes involved in connective tissue protein secretion should be taken into account as potential etiological factors in connective tissue hereditary diseases, including hEDS; however, their role in the process requires much wider investigation.

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