Case report: A Thai patient with mucopolysaccharidosis type IVA (Morquio syndrome type A) - clinical phenotypes and *GALNS* gene mutation analysis

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Background : Mucopolysaccharidosis type IVA (MPS IVA) or Morquio syndrome type A is an autosomal recessive disorder. N-acetylgalactosamine-sulfate sulfatase *(GALNS)* gene is the disease-causing gene of MPS IVA.

Objective : The aim of our study is to study clinical phenotypes, skeletal findings, and molecular analysis in one Thai patient with the severe type of MPS IVA.

Methods : We reviewed the clinical features and family history of the patient. We performed GALNS and B-galactosidase enzyme assay in her blood leukocytes. The *GALNS* gene mutation analysis was performed on gDNA derived from blood leukocytes of the patient and her parents.

Results : A 3 $^{3}/_{12}$-year-old Thai girl has presented with abnormal gait and disproportionate short stature for 18 months. Skeletal survey revealed unique skeletal dysplasia known as dysostosis multiplex. GALNS enzyme assay showed low enzyme activity with normal B-galactosidase level. Direct sequencing analysis of the *GALNS* gene identified the compound heterozygous mutations, c.463G>A (p.G155R) and c.1175C>T (p.A392V). These two missense mutations have been previously reported in patients with MPS IVA.

Conclusion : MPS IVA should be suspected in an individual with disproportionate short stature and genu valgum. Since the *GALNS* gene has a marked molecular heterogeneity, we need to do the molecular study in the larger number of Thai patients with MPS IVA on a national scale to elucidate the clinical phenotypes and mutational spectrum in Thai patients.

Keywords : Mucopolysaccharidosis type IVA, Morquio syndrome type A, *GALNS* gene

Introduction

Mucopolysaccharidosis type IVA (MPS IVA) or Morquio syndrome type A is a lysosomal storage disorder caused by the deficiency of the N-acetylgalactosamine-sulfate sulfatase (GALNS) enzyme.[1] In 1929, Luis Morquio, Uruguayan pediatrician, and James F. Brailsford, British radiologist, independently reported patients with skeletal abnormality that became known as Morquio-Brailsford syndrome.[2-4] The prevalence for MPS IVA ranged from 1:76,000 to 1:640,000 births.

The deficiency of lysosomal GALNS enzyme accumulates keratan sulfate and chondroitin-6-sulfate mainly in bone, cartilage, and cornea leading to unique skeletal dysplasia with mild corneal clouding. The characteristic features consist of short trunk dwarfism, severe pectus carinatum, kyphoscoliosis, knock-knees,

and preservation of intelligence.[5, 6] Skeletal manifestation of MPS IVA includes platyspondyly, anterior beaking of vertebral bodies, odontoid hypoplasia, irregular epiphyses with widened metaphyses of the long bones, shortening and widening of the diaphysis of the humerus, tilting of the radial epiphysis towards the ulna, genu valgum, coxa valgus deformity, and flared iliac wings.[7-9]

The *GALNS* gene located on chromosome 16q24.3 is the only known disease-causing gene of MPS IVA. In 1991, Tomatsu et al firstly cloned and sequenced a full-length cDNA of human placental *GALNS*.[10] This gene contains 14 exons and 13 introns comprised 1,566 bp of cDNA encoding 522 amnion acids.[11] To date, more than 362 pathologic variants in the *GALNS* gene were identified.[12] The aim of our study is to study clinical phenotypes, skeletal findings, and molecular analysis in one Thai female patient with MPS IVA.

Methods

We reviewed the clinical features, family history, and X-ray findings of a female patient seen at Siriraj Hospital, Mahidol University, Bangkok, Thailand. GALNS and B-galactosidase enzyme activities were performed in her blood leukocytes by a fluorimetric enzyme assay technique.[13]

We performed DNA extraction from blood leukocytes of the patient and her parents. We designed PCR primer pairs for exon 1-14 of the *GALNS* gene based on the sequencing chromatograms of reference sequences (NCBI: NG_008667.1). These PCR primers were listed in Table 1. The PCR amplification conditions were shown in Table 2. After purification of the PCR product, exons were sequenced by the fluorescent dideoxy cycle sequencing method with both forward and reverse primers using an ABI Prism 3730XL DNA Analyzer.

Table 1 PCR primers for 14 exons of the *GALNS* gene

Exon	Primer Name	Sense/ Antisense	bp	Products (bp)
1	GALNS1F	GCCCCACTGGTCACGAGGCAGTCCA	25	332
	GALNS1R	CCCACCCCGGCCCTGCCCCGTCCCACCGCCCGCACTCA	38	
2	GALNS2F	ACACGCTCTTGGCACCAT	18	340
	GALNS2R	CCACCCTCCCTGCAGTAGTA	20	
3	GALNS3F	CGTCTGTCACGCGTCTGT	18	294
	GALNS3R	ACCAGCGGTACCCCACCT	18	
4	GALNS4F	CCTGGAAAAATCTTGGGAAGT	21	386
	GALNS4R	GACACCCTCCTCATTTGGAA	20	
5	GALNS5F	CTGGAGGGTGCTCGTCTTAC	20	347
	GALNS5R	ACTTGAGCCCACCAGTGCTA	20	
6-7	GALNS6-7F	AAGCCCATGGCTTTGCTG	18	698
	GALNS6-7R	CCATCTCTGGAGTCAAGCAC	20	
8	GALNS8F	CTGCCTGATCCATTTGTCAC	20	317
	GALNS8R	AGAGGGACCCTTCATGCTCT	20	
9	GALNS9F	CCCTTTGTCCCTATGACCAG	20	327
	GALNS9R	AGGAGAGCGGTGAGGATGAG	20	
10	GALNS10F	GTGGGCGTGTGAGCATGTAT	20	381
	GALNS10R	CCTGTGTCCAGAACCAGGAG	20	
11	GALNS11F	CTTGCGGGCCTTTTTACTTT	20	371
	GALNS11R	GAGTTCCTGCCTGTCTCACC	20	
12	GALNS12F	AGGACACGGGCAGACGAG	18	347
	GALNS12R	CAAGCACGTGTGGGTATGAA	20	
13	GALNS13F	ACATGGTCCCAGTGACTGCT	20	397
	GALNS13R	TGTGCTCTGAGGCACGAG	18	
14a	GALNS14aF	AGCCTGGGTGACAGAGTGAG	20	482
	GALNS14aR	GGTTCACAAAGGCGTGAGAC	20	
14b	GALNS14bF	ATTTCAGGACCCTCCTCCTC	20	653
	GALNS14bR	GAGTCCTGCCCTTCAACAAC	20	

Table 2 PCR amplification conditions

	Temperature	Time	
Pre-denature	94 °C	5 minute	
Denature	94 °C	45 second	
Annealing	T °C	1 minute	35 cycles
Elongation	72 °C	1 min 15 second*	
Final Extension	72 °C	5 minute	
Holding	4 °C	∞	

** Except exon11, 12, and 13, elongation time is 30 second.

Results

A 3 $^{3}/_{12}$-year-old Thai girl has presented with abnormal gait and disproportionate short stature for 18 months. She has been diagnosed with moderate-size patent ductus arteriosus (PDA) since birth. Her development was normal. She was the only child with no history of parental consanguinity. No other family members were clinically affected. On examination, her weight was 8.8 kg. (< 3rd percentile), height was 73.7

cm. (< 3rd percentile). She had short stature, short neck, pectus carinatum, flaring of lower rib cage, ulnar deviation of the wrists, flexion contracture of both elbows and knees, genu valgum (knock knee), kyphoscoliosis, waddling gait, and joint laxity (Figure 1). Neurological examination was normal without any signs of spinal cord compression.

Echocardiography showed only PDA without cardiac valve involvement. Ophthalmologic and hearing examination revealed mild corneal clouding of both eyes and mild conductive hearing loss. Pulmonary function test showed mild restrictive lung disease.

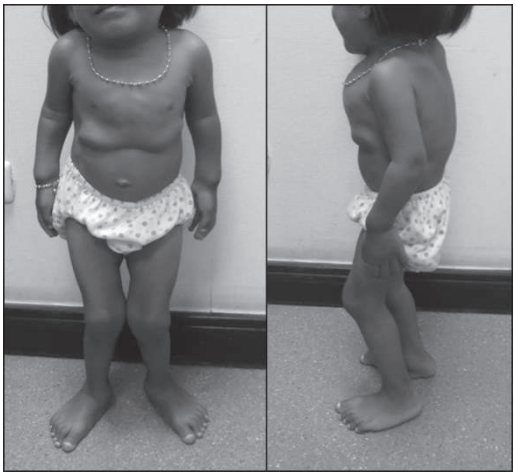

Figure 1. Clinical features of the patient included short stature, pectus carinatum, flaring of lower rib cage, genu valgum, and kyphoscoliosis.

Her skeletal survey revealed characteristic skeletal dysplasia knowns as dysostosis multiplex. The x-ray findings included platyspondyly, anterior beaking of vertebral bodies, incomplete ossification in odontoid process (dens), pectus carinatum, abnormal thoracic cage, kyphoscoliosis, widening of the diaphysis of the humerus, tilting of the radial epiphysis towards the ulna, short metacarpals with pointed proximal ends of 2nd to 5th metacarpals, genu valgum, irregular epiphyses with widened metaphyses of the long bones, genu valgum, and femoral head dysplasia (Figure 2).

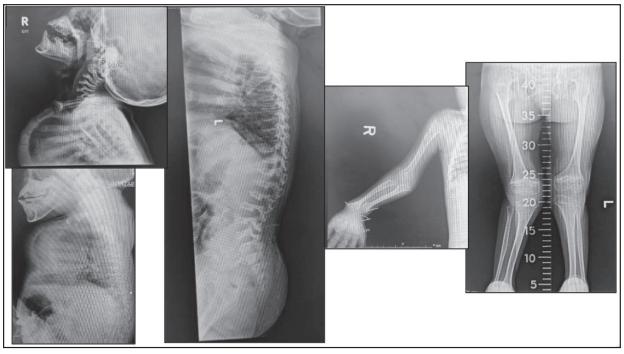

Figure 2. X-ray images of the patient showed platyspondyly, odontoid hypoplasia, pectus carinatum, anterior beaking of vertebral bodies, thoracolumbar kyphosis, tilting of the radial epiphysis towards the ulna, genu valgum, and irregular epiphyses with widened metaphyses of the long bones.

Qualitative urine glycosaminoglycan analysis by thin layer chromatography showed keratan sulfate and chondroitin 6-sulfate. GALNS enzyme activity in her blood leukocytes revealed low enzyme activity with normal B-galactosidase activity. Direct sequence analysis of the *GALNS* gene on gDNA derived from blood leukocytes, identified the compound heterozygous mutations, c.463G>A (p.G155R) and c.1175C>T (p.A392V), in the patient. We also identified c.463G>A in her father and c.1175C>T in her mother (Figure 3).

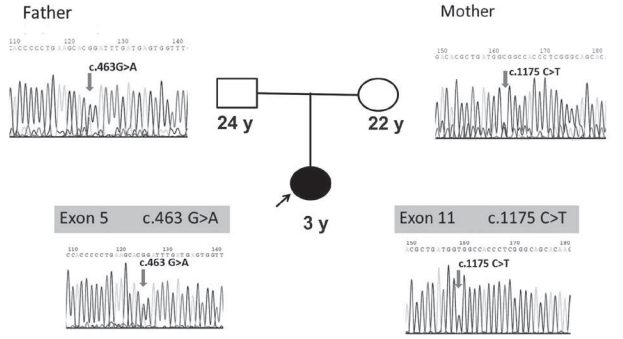

Figure 3. Pedigree and the results of the *GALNS* gene sequencing in the patient and her parents.

Discussion

The diagnosis of MPS IVA is established by combined information from medical history, physical examination, radiographic findings, biochemical tests, and molecular analysis. Patients with MPS IVA typically have normal intellectual ability at the time of diagnosis. MPS IVA is classified in two types, attenuated (slowly progressive) type and severe type. The common symptoms in both types include marked disproportionate short stature, genu valgum, coxa valga, kyphoscoliosis, pectus carinatum, flaring of lower rib cage, waddling gait with frequent falls, and cervical spinal cord compression (cervical myelopathy).[1, 14] Most patients with MPS IVA usually have no distinctive clinical findings at birth. The severe type is usually apparent between ages one and three years, and the attenuated type may have first symptoms in late childhood or adolescence.[15] Extra-skeletal manifestation includes respiratory compromise from restrictive lung disease, obstructive sleep apnea, valvular heart disease, hearing impairment, visual impairment from cloudy cornea, and cervical myelopathy.[16] The management of MPS IVS patients is undertaken by multiple specialists in the care of individuals with complex medical problems, since enzyme replacement therapy and hematopoietic stem cell transplantation do not have enough impact on bone and cartilage lesions.[1, 16]

MPS IVA should be suspected in an individual with disproportionate short stature and genu valgum. We report a Thai patient with the severe type of MPS IVA confirmed by molecular analysis of the *GALNS* gene. The *GALNS* gene has a marked molecular heterogeneity and a different mutational spectrum in patients. The severe type of MPS IVA correlates to some mutations, such as deletions, nonsense mutations, and some missense mutations (p.R90W, p.I113F, p.G155R, p.H166Q, p.G168R, p.G301C, p.A351V, etc.).[17] Some mutations are likely to be associated with the attenuated type, such as p.D60N, p.N204K, p.R259Q, etc.[17] Our patient has compound heterozygous mutations in the *GALNS* gene, c.463G>A (p.G155R) and c.1175C>T (p.A392V). However, these two missense mutations have been previously reported in patients with MPS IVA. The c.463G>A (p.G155R) mutation found in our patient has been reported in MPS IVA patients with severe type.[17, 18] Another mutation found in our patient, c.1175C>T (p.A392V), appears to have variable phenotypes, both in attenuated and severe types of MPS IVA.[17, 18]

This report is the result of the mutation analysis in one MPS IVA patient with severe type. Since the *GALNS* gene has a marked molecular heterogeneity, we need to do the molecular study in the larger number of Thai patients with MPS IVA on a national scale to elucidate the clinical phenotypes and mutational spectrum in Thai patients.

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รายงานผู้ป่วยเด็กโรค mucopolysaccharidosis type IVA (Morquio syndrome type A) ลักษณะทางคลินิกและผลตรวจวิเคราะห์พันธุกรรมของยีน *GALNS*

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บทนำ : โรค Mucopolysaccharidosis type IVA (MPS IVA) หรือโรค Morquio syndrome type A เป็นโรคที่ถ่ายทอดแบบยีนด้อย โดยเกิดจากการกลายพันธุ์ของยีน N-acetylgalactosamine-sulfate sulfatase *(GALNS)*

วัตถุประสงค์ : เพื่อศึกษาลักษณะทางคลินิก ภาพถ่ายเอกซเรย์กระดูก และผลตรวจวิเคราะห์พันธุกรรมของยีน *GALNS* ในผู้ป่วยเด็กไทยที่เป็นโรค MPS IVA

วิธีการศึกษา : ทบทวนข้อมูลและบันทึกทางการแพทย์ทั้งหมดของผู้ป่วย นำเลือดผู้ป่วยมาตรวจวัดระดับเอนไซม์ GALNS และ B-galactosidase ในเม็ดเลือดขาว รวมถึงการตรวจวิเคราะห์พันธุกรรมของยีน *GALNS* ในผู้ป่วยและบิดามารดา

ผลการศึกษา : ผู้ป่วยเด็กหญิงไทยอายุ 3 ปี 3 เดือน มีอาการเดินผิดปกติและตัวเตี้ยไม่สมส่วนมานาน 18 เดือน มีความผิดปกติที่จำเพาะในภาพถ่ายเอกซเรย์กระดูกเรียกว่า dysostosis multiplex ผลตรวจระดับเอนไซม์ในเม็ดเลือดขาวพบเอนไซม์ GALNS มีระดับต่ำ แต่เอนไซม์ B-galactosidase มีระดับปกติ ผลตรวจวิเคราะห์พันธุกรรมของยีน *GALNS* พบการกลายพันธุ์สองอัลลีล คือ c.463G>A (p.G155R) and c.1175C>T (p.A392V) ซึ่งเคยมีผู้รายงานการกลายพันธุ์ทั้งสองอัลลีลมาก่อนหน้านี้

สรุป : แพทย์ควรนึกถึงโรค MPS IVA ในผู้ป่วยที่มาด้วยตัวเตี้ยไม่สมส่วนและเข่าผิดรูปชนกัน รายงานผู้ป่วยนี้มีผู้ป่วยไทยเพียงรายเดียว จึงควรมีการศึกษาลักษณะทางคลินิกและการตรวจวิเคราะห์พันธุกรรมของยีน *GALNS* ในผู้ป่วยไทยที่เป็น MPS IVA ในระดับประเทศให้มีจำนวนมากขึ้น

คำสำคัญ: Mucopolysaccharidosis type IVA, Morquio syndrome type A, ยีน *GALNS*

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