A novel matrix metalloproteinase-2 mutation in two Egyptian siblings with Winchester syndrome

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Background
Winchester syndrome is one of multicentric osteolysis nodulosis and arthropathy spectrum, which is an autosomal recessive skeletal dysplasia. Homozygous or compound heterozygous mutations in matrix metalloproteinase-2 (MMP-2) gene are responsible for the condition. Approximately 21 mutations of MMP-2 gene have been reported to accompany multicentric osteolysis nodulosis and arthropathy.

Patients and methods
In this study, we report two Egyptian siblings diagnosed with Winchester syndrome based on clinical and radiological evaluation and confirmed by finding a novel homozygous mutation at exon 4 of MMP-2 gene by molecular studies. We also reviewed the literature for previously published patients with nonsense mutations of that gene.

Results
The detected MMP-2 mutation is the sixth reported nonsense mutation and the first pathogenic mutation detected in exon 4.

Conclusion
Phenotypic analysis of previously reported patients with different MMP-2 mutations including our present patients showed no genotype/phenotype correlation.

Keywords:
genotype/phenotype correlation, multicentric osteolysis nodulosis and arthropathy, nonsense matrix metalloproteinase-2 mutations, novel gene mutation, Torg syndrome, Winchester syndrome

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Introduction

Torg–Winchester syndrome, also termed multicentric osteolysis nodulosis and arthropathy (MONA), is an autosomal recessive skeletal dysplasia OMIM (259600). The syndrome was originally defined as three separate entities that showed marked clinical and radiological overlap: Torg syndrome, Winchester syndrome (WS), and nodulosis–arthropathy–osteolysis (NAO) syndrome. As these syndromes have common clinical features and pathogenic mechanism, reclassification has combined them as a single entity, Torg–Winchester syndrome, with NAO syndrome as a variant. However, de Vos *et al.* (2019) proposed a novel nosological revision, placing WS, and MONA in a new spectrum named defective collagen-remodeling spectrum. which includes different osteolysis syndromes with overlapping phenotypic features.

The clinical consensus of WS includes multiple peripheral osteolysis, arthropathy, subcutaneous fibro-fatty nodules on palms and soles, and progressive joint contractures, along with other associated features, including short stature, coarse facies, gum hypertrophy, corneal clouding, hyperpigmentation of skin and cardiac defects (Al-Mayouf *et al.*, 2000; Zankl *et al.*, 2007; Tuysuz *et al.*, 2009; Azzollini *et al.*, 2014).

Martignetti *et al.* (2001) were the first to report that mutations in matrix metalloproteinase-2 (MMP-2) gene are the causative gene for NAO syndrome. MMP-2, also known as gelatinase A or type IV collagenase is widely expressed in most tissues and cells. It is involved in diverse functions such as remodeling of the vasculature, angiogenesis, tissue repair, tumor invasion, inflammation, and atherosclerotic plaque rupture. Moreover, it degrades extracellular matrix proteins, in addition to several nonmatrix proteins, which have expanded the biological roles of this important protease (Sariahmetoglu *et al.*, 2007). Membrane type-1 metalloproteinase (MT1-MMP or MMP-14) plays an integral role in the activation of pro-MMP-2 on the cell surface. Later, mutations in

MMP-14 were also reported to cause Torg–Winchester syndrome (Evans *et al.,* 2012). So far, 21 MMP-2 gene mutations have been reported, including 27 families described from India, Saudi Arabia, Egypt, Turkey, Italy, Morocco, South America, Brazil, Algeria, Lebanon, and Korea (Bhavani *et al.,* 2016; Dagher *et al.,* 2018).

In this study, we report an Egyptian family with two affected siblings with WS.

Patients and methods

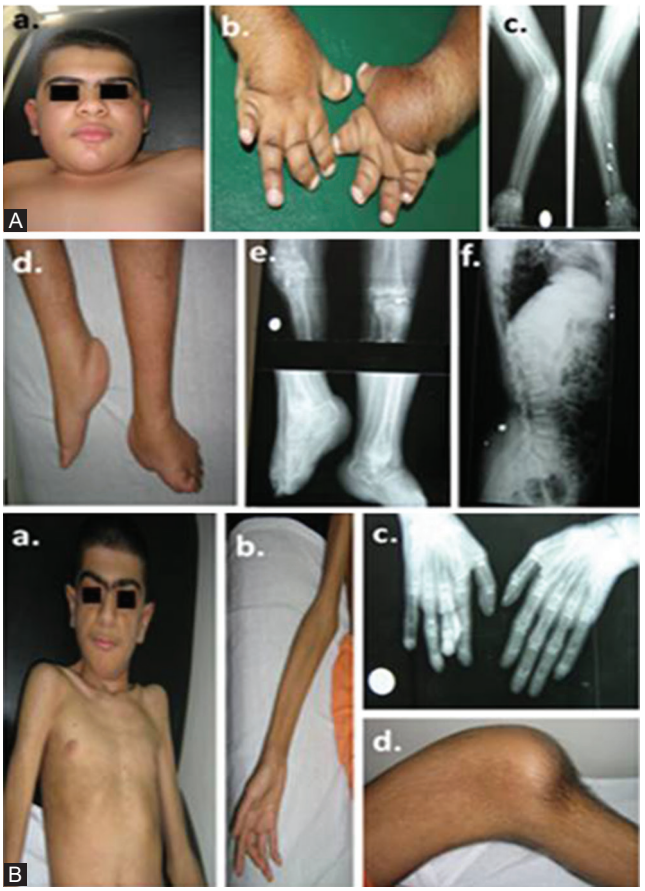
Clinical report

The proband is a 13-year-old male patient who presented to the Limb Malformations and Skeletal Dysplasia Clinic, Center of Excellence for Human Genetics, NRC, because of generalized bone pain and recurrent fractures. He is the third offspring of apparently normal first cousin parents. He has a normal sister and an older similarly affected brother. Pedigree analysis showed absence of other similarly affected family members. Pregnancy and delivery histories were uneventful. His birth weight was average. The condition was noticed by the parents at the age of 4.5 and 5 years of the proband and his affected brother, respectively, when they had the first fracture after major trauma, which recurred 4–5 times per year. Fractures were mostly of lower limbs, of femur and tibia. Frequent surgical repairs were done. Both patients had normal developmental milestones.

On examination of the proband and his affected brother, both were of average intelligence and co-operative but were not able to walk independently. Both had coarse facies, arched and thick eyebrows, synophrys, and trichomegaly, wide palpebral fissures with normal colored sclera, thick lips, large prominent ears, and normal hearing.

Both siblings had bony deformities; the proband had puffy small hands with brachydactyly, radial deviation of first to third fingers and ulnar deviation of fourth and fifth fingers of the right hand, and radial deviation and camptodactyly of the left hand with limited movements of interphalangeal joints. He had bowed femora, small feet, brachydactyly, and lower limb edema (Fig. 1A). The affected brother had bowing of arms and forearms, camptodactyly of third, fourth, and fifth fingers bilaterally and muscle wasting of the thenar and hypothenar eminences. He had generalized lower limb muscle wasting, anterior bowing of femur, and limited extension of knees (Fig. 1B). Both had kyphoscoliosis, hypotonia, generalized hirsutism, and skin pigmentation.

Figure 1



A: The proband (a) showing face with thick eye brows and thick lips. (b) Hands showing fusiform short fingers with dorsal edema. (c) Osteopenic picture of radius, ulna, osteolysis of carpal bones. (d) Feet showing swelling of ankle joints and dorsum of foot, short toes and hirsutism. (e) Osteopenic picture of lower ends of tibia and fibula, osteolysis of tarsal bones (f) Osteopenia of vertebral bodies and irregular upper and lower end plates of vertebrae. B: Affected brother (a) face and trunk with thick eyebrows (b) right arm and hand showing wasted muscles and mild contractures of fingers 3-5 (c) X-ray hands showing osteopenea and carpal osteolysis. (d) Left lower limb showing wasting of leg muscle, knee joint swelling and hirsutism.

Clinical examination did not show any abnormalities in heart or chest. Both siblings showed delayed puberty (P2, A1, prepubertal testicular size) and short stature. Anthropometric measurements at the time of examination of the affected brother and the proband showed weights of 20 and 56.5 kg (–5 and +1 SD), respectively; heights of 132.5 and 132 cm (–6 and – 4.3 SD), respectively; and head circumferences of 51 and 52.3 cm (–2.8 and – 1.3 SD), respectively.

Skeletal survey of the proband showed extensive osteopenic texture of radius, ulna, femur, tibia, and fibula in addition to carpal and tarsal bones with extensive bone erosions of carpal bones, wormian bones in the skull, osteopenia of vertebral bodies, and irregular upper and lower end plates of vertebrae (Fig. 1). The affected brother was subjected to multislice computed tomography (CT) scan of both knees joints at the age

of 21 years, which showed marked diffused cortical bone thinning and medullary osteopenia of the examined lower femora, upper tibia and fibula on both sides associated with diffused bilateral hypoplasia of all muscle groups and soft tissues in thighs and upper legs, and prominent deformity of lower femora and knee joints with minimal synovial collection of right knee joint (Fig. 1B).

Bone densitometry values at the lumbar spine for the affected brother and the proband were 0.391 and 0.280 g/cm^2, respectively, which were 52 and 33% of the mean of the patients' age match, and 3.3 and 4.7 SD below the maximum mean, respectively, denoting definite osteoporosis at the lumbar spine. Bone densitometry values at the femur for both were −3.97 and −2.1 SD, respectively, and at the forearms were −3.57 and −3.82 SD, respectively.

Intelligence quotient (IQ) was done using Wechsler intelligent scales, denoting borderline intelligence (IQ 79) for the older brother and average intelligence for the proband (IQ 90). Serum parathyroid hormone levels were normal for both siblings, whereas calcium and phosphorus levels in 24-h urine were 44 (reference range: 100–300 mg/day) and 356 mg/day (reference range: 400–1300 mg/day), respectively. The proband has shown that serum calcium, phosphorus, and alkaline phosphatase were 9.1, 3.3 mg/dl, and 151 U/l, respectively. Additionally, the growth hormone level of the affected brother using insulin tolerance stimulation test was low (peak below 1.1). Diagnosis of WS was made according to history and clinical manifestations, which was followed by molecular mutational analysis.

Molecular studies

Informed consents were taken from the parents according to the guidelines of the Medical Research Ethics Committee of the NRC. Blood samples of the proband, the affected older brother, and their parents were withdrawn into 0.5 mol/l EDTA tubes. DNA was extracted from peripheral blood leukocytes using PAXgene DNA blood extraction kit (Qiagen, Hilden, Germany). Polymerase chain reaction was carried out with primers covering all the thirteen coding exons and the flanking intronic regions of MMP-2 (Azzollini *et al.*, 2014). Sanger sequencing was performed for purified PCR products using ABI 3500 automated genetic analyzer according to the manufacturer's protocol. Parents of the proband were tested to confirm the biallelic inheritance. In silico analysis was performed to determine the suspected pathogenicity of the novel mutation, using Mutation Taster application, HGMD (The Human Gene Mutation Database), dbSNP (The Single Nucleotide Polymorphism Database), UniProtKB (UniProt Knowledgebase), and RCSB PDB (Protein Data Bank) databases (Schwarz *et al.*, 2014; Burley *et al.*, 2018).

Results

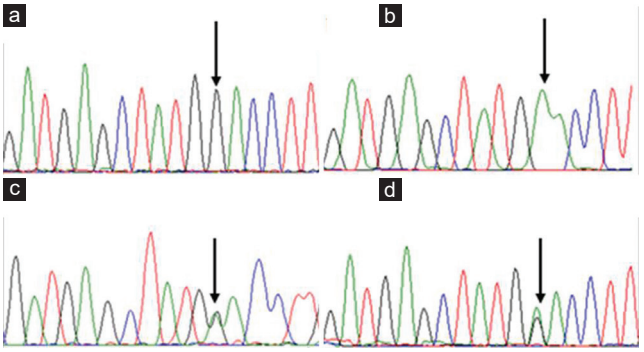
Molecular analysis of MMP-2 gene revealed that both affected siblings had homozygous c.639G>A(p.W213*) mutation (NCBI reference sequence: NC_000016.1). This mutation has resulted in nonsense mutation at exon 4. The parents were heterozygous carriers of the mutation (Fig. 2).

In silico analysis showed that MMP-2 protein (ID: P08253) consists of five domains. The detected mutation (p.W213*) has resulted in premature stop codon at codon 213, which produced a truncated protein that is deprived of fibronectin domains, active site, collagen-binding region, collagenase-like 2 region, and hemopexin-like membrane anchor (Fig. 3). Additionally, mutation taster analysis showed that p.W213* has deleterious and disease-causing effect. Upon reviewing mutation databases (HGMD and dbSNP), the detected mutation was not reported before.

Discussion

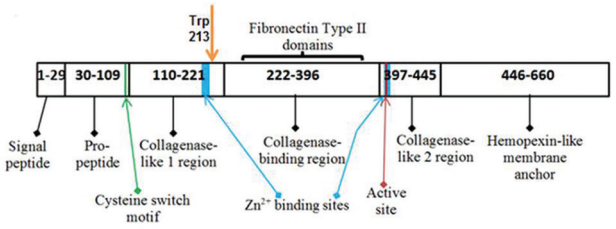
MONA spectrum is a rare genetic chronic skeletal disorder involving Torg syndrome, WS, and NAO syndrome. It is characterized by peripheral osteolysis (especially carpal and tarsal bones), interphalangeal joint erosions, subcutaneous fibrocollagenous nodules, facial dysmorphism, and a wide range of associated manifestations. This disorder is mainly caused by an alteration in the MMP-2 gene. Subsequently,

Figure 2



Sequencing electropherogram shows part of exon 4 of MMP-2 gene. (a) the wild type sequence for a normal individual, (b) the mutant sequence of the proband with homozygous c.639G>A (p.W213*) mutation, (c abd d) the parents sequences with heterozygous c.639G>A mutation. Black arrows direct the site of the mutation.

Figure 3



Domain structure of human MMP-2 protein with the indicated amino acid residues. The upper orange arrow directs the site of the mutant tryptophan at codon 213. The three Fibronectin Type II domains are from residues ? (228-276, 286-334 and 344-392)

homozygous missense mutations in MMP-14 were reported to cause Torg–Winchester syndrome (Evans *et al.*, 2012).

Up to our knowledge, 21 mutations of MMP-2 gene have been reported in association with MONA (Bhavani *et al.*, 2016; Dagher *et al.*, 2018). In this study, we report a novel homozygous nonsense mutation (p.W213*) in two Egyptian siblings. The detected mutation is the sixth reported nonsense mutation and the first pathogenic mutation detected in exon 4. The MMP-2 protein is 72 kDa and composed of 660 amino acids (NCBI Reference Sequence: NP_004521.1). It is gelatinase A or type IV collagenase Zn^{2+} dependent protease, consisting of five domains (collagenase-like 1 region, fibronectin domains, collagen-binding region, collagenase-like 2 region, and hemopexin-like membrane anchor) (Sariahmetoglu *et al.*, 2007) (Fig. 3). The detected mutation (p.W213*) resulted in the production of truncated protein of only 212 amino acids missing four domains of the protein including the active site and Zn^{2+} binding sites, so that loss of enzymatic activity would be expected. Molecular analyses and clinical features have confirmed the diagnosis of WS.

Herein, we have reviewed the reported nonsense mutations, including previously and presently reported Egyptian patients described with WS. The previously reported five nonsense mutations were detected in exons 2, 3 and 5. Including this study, 12 patients were reported with nonsense mutations in MMP-2 gene. All the patients have progressive osteolysis of carpal, tarsal, and phalangeal bones; destructive changes of interphalangeal metacarpo-phalangeal and metatarso-phalangeal joints; generalized osteopenia; coarse facies; and one of the other three symptoms (gum hypertrophy, subcutaneous nodules, or cardiac anomalies). Patients with earlier age at onset usually have subcutaneous nodules, as the onset of this syndrome varies from birth to 22 years. In this study, multiple fractures could be explained by the low bone density and osteoporosis. Although, short stature is reported as one of the manifestations of WS, the affected brother had striking short stature (–6 SD), which could be attributed to the associated deficiency of growth hormone.

Endocrinal abnormalities were noted by Zankl *et al.* (2005), where elevated TSH and diabetes mellitus type I were identified. Additionally, irregular menstruation and polycystic ovaries were reported by Temtamy *et al.* (2012). Low growth hormone in this study may add more evidence raising the question whether endocrine abnormalities are part of the clinical picture of WS or just a coincidental finding. Such observations warrant investigation of endocrinal abnormalities in newly diagnosed cases.

As shown in Table 1, comparison of genotype with phenotype in patients with nonsense mutations, severe mutation causing abolishment of enzyme action, showed that the age of disease onset was early in all previously reported cases ranging from birth to 3 years. However, the currently reported patients were older (4.5 and 5 years). Moreover, most of the patients were manifested with coarse facial features, joint contractures, osteolysis, subcutaneous nodules, and hirsutism. However, gingival hypertrophy, cardiac anomalies, and skin hyperpigmentation were not consistent in all patients. On the contrary, de Vos *et al.* (2019) reviewed 36 patients with WS due to homozygous mutations in MMP-2 gene. All the patients with various MMP-2 mutations, either mild or severe ones, shared most of clinical features of the disease (Table 2). However, hirsutism was remarkably reported in patients with nonsense mutations ranging from mild to severe forms. Gum hypertrophy and short stature were observed in less than 50% of the patients.

Interestingly, MMP-2 enzymatic activity was completely lost irrespective to the site and type of the homozygous nonsense, missense, or frameshift mutations, suggesting lack of genotype/phenotype correlation (Martignetti *et al.*, 2001; Zankl *et al.*, 2005; Zankl *et al.*, 2007; Tuysuz *et al.*, 2009; Jeong *et al.*, 2010; Azzollini *et al.*, 2014). In this study, the older patient (the affected brother) has shown different manifestations and notable growth retardation, whereas the proband had more classic features of WS. This phenotypic difference is probably owing to the associated growth hormone deficiency in the older patient. Other reports showed complexity in the genotype/phenotype correlation even within the same family (Rouzier *et al.*, 2006; Azzollini *et al.*, 2014; Bhavani *et al.*, 2016). This could be explained by the existence of other genetic or environmental modifiers.

Table 1 Clinical and molecular results of previously reported patients with nonsense mutations and Egyptian patients including this study

Origin	Sex	Age of onset	Consanguinity	Mutation	Protein domain	Coarse facies	Joint contractures	Gum hypertrophy	Joint swelling	Reduced bone mineral density	Subcutaneous -nodules	Cardiac anomalies	Short stature	Skin hyperpigmentation	Hirsutism	References
India	Female	3 years	+	p.C102* Exon 2	Cysteine-switch motif	+	+	?	+	+	+	?	?	?	?	Bhavani *et al.* (2016)
India	Male	1.5 years	+	p.Y263* Exon 5		+	+	+	+	+	+	+	?	+	–	Bhavani *et al.* (2016)
India	Male	3 years	+	p. E231* Exon 5	Fibronectin type 2 domain	+	+	–	+	+	–	–	?	–	+	Bhavani *et al.* (2016)
Brazil	Female	?	?							No available clinical data						LOVD MMP-2 database**
Saudi Arabia	Male	8 months	+	p.Y244* Exon 5		+	+	–	+	+	+	–	–	–	Moderate	Al Aqeel *et al.* (2000); Martignetti *et al.* (2001)
	Female	1 year	+			+	+	–	+	+	+	–	+	–	+	
	Four other affected females were reported related to this family			No available clinical data												Martignetti *et al.* (2001)
Egypt	Female	At birth	+	p.W151* Exon 3		+	+	+	+	+	+	+	–	+	+	Temtamy *et al.* (2012)
Egypt	Female	4.5 years	+	p.D180E Exon 3	Collagenase-like 1 region	+	+	+	+	+	+	–	–	–	Mild	Temtamy *et al.* (2012)
	Male	2 years	+			+	+	+	+	+	+	–	–	+	+	
Egypt	Male	5 years	+	p.W213* Exon 4		+	+	–	+	+	+	–	++	–	+	This study
	Male	4.5 years	+			+	+	–	+	+	+	–	+	+	+	

MMP-2, matrix metalloproteinase-2. +, present; ++, severe; –, absent, ?, not mentioned. ***https://grenada.lumc.nl/LOVD2/mendelian_genes/home.php?select_db = MMP2*.

Table 2 Clinical features of patients with Winchester syndrome according to de Vos *et al.* (2019), and the reviewed patients in Table 1

	MMP-2 Winchester syndrome (de Vos *et al.*, 2019) (*n*=36)	Currently reviewed patients (*n*=10)
Coarse facial features	25/33	10/10
Gingival hypertrophy	11/22	4/9
Short stature	18/30	3/7
Joint destruction (contractures)	35/35	10/10
Osteolysis	36/36	10/10
Subcutaneous nodules	28/35	9/10

MMP, matrix metalloproteinase-2.

As other MMPs such as MMP-14 have a key role in activation of pro-MMP-2 and degradation of fibrillar collagen to form the activated MMP-2.

Molecular studies of MMP-2 gene are crucial to avoid misdiagnosis of WS in affected patients till adulthood, in addition to aid in genetic counseling and prenatal molecular diagnosis in subsequent pregnancies of affected families. On the contrary, complexity of the disease (MONA spectrum) with overlapping clinical features and pathogenic mechanism in addition to the lack of genotype/phenotype correlation urge the need of additional molecular studies to determine the specific role of MMP-2 in bone development and the other potential modifiers involved.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Conflicts of interest

There are no conflicts of interest.

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