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ORIGINAL ARTICLE

Kyphoscoliotic type of Ehlers-Danlos Syndrome (EDS VIA) in six Egyptian patients presenting with a homogeneous clinical phenotype

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Abstract The kyphoscoliotic type of the Ehlers-Danlos syndrome (EDS VIA) is a rare recessively inherited connective tissue disorder characterized by bruisable, hyperextensible skin, generalized joint laxity, severe muscular hypotonia at birth and progressive congenital scoliosis or kyphosis. Deficiency of the enzyme lysyl hydroxylase 1 (LH1) due to mutations in *PLOD1* results in underhydroxylation of collagen lysyl residues and, hence, in the abnormal formation of collagen cross-links. Here, we report on the clinical, biochemical, and molecular findings in six Egyptian patients from four unrelated families severely affected with EDS VIA. In addition to the frequently reported p.Glu326_Lys585dup, we identified two novel sequence variants p.Gln208* and p.Tyr675*, which lead either to loss of function of LH1 or to its deficiency. All affected children presented with similar clinical features of the disorder, and in addition, several dysmorphic craniofacial features, not yet described in EDS VIA. These were specific for the affected individuals of each family, but absent in their parents and their unaffected siblings.

Conclusion: Our description of six patients presenting with a homogeneous clinical phenotype and dysmorphic craniofacial features will help pediatricians in the diagnosis of this rare disorder.

Keywords Ehlers-Danlos syndrome · EDS VIA · *PLOD1* · Kyphoscoliosis · Craniofacial dysmorphisms · Microcorneae · Urinary pyridinolines · LP to HP ratio

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Abbreviations

CT	Computer tomography
EDS	Ehlers-Danlos Syndrome
EDS VIA	Kyphoscoliotic type of the Ehlers-Danlos Syndrome
EMG	Electromyography
HP	Hydroxylysyl-pyridinoline
LH1	Lysyl hydroxylase 1
LP	Lysyl-pyridinoline
NCV	Nerve conduction velocity
PLOD1	Gene coding for procollagen-lysine, 2-oxoglutarate 5-dioxygenase 1

Introduction

The Ehlers-Danlos syndrome (EDS) is a genetically and clinically heterogeneous group of connective tissue disorders which have in common joint hypermobility, skin hyperextensibility, and fragility [19]. The latest classification of EDS from 1998, the Villefranche Nosology, recognizes six major subtypes, based on the severity of the clinical symptoms, the pattern of inheritance and the underlying biochemical and molecular defect [2]. Among them, the kyphoscoliotic type of the Ehlers-Danlos syndrome (EDS VIA; OMIM 225400) is a rare autosomal recessive disorder characterized by severe generalized muscular hypotonia at birth, kyphoscoliosis which is progressive and severe, marked joint hypermobility and luxations, and severe skin hyperelasticity. In addition, there is fragility of the skin with abnormal scarring, osteopenia, often a striking Marfanoid habitus, microcorneae, bluish sclerae, and occasionally rupture of arteries [2, 19]. The world-wide prevalence is estimated as low as ~1 in 100,000 live births [13, 15]. Additional variants of EDS with distinct molecular and biochemical abnormalities, such as musculocontractural-EDS (MS-EDS; OMIM #601776) and FKBP14-EDS (OMIM 614547) have been recently identified and have to be considered in the differential diagnosis of EDS VIA [4].

EDS VIA is caused by mutations in procollagen-lysine, 2-oxoglutarate 5-dioxygenase 1 (*PLOD1*; MIM 153454), which encodes lysyl hydroxylase 1 (LH1; E.C. 1.14.11.4) [9]. This enzyme catalyzes the formation of hydroxylysine in collagen chains by the hydroxylation of lysine residues in Gly-X-Lys sequences [10]. The hydroxylysine residues formed are essential for the formation of intermolecular cross-links that provide the collagen fibrils with their tensile strength and mechanical stability, and serve as attachment sites for carbohydrate units [10]. The enzyme deficiency gives rise to an abnormal urinary excretion pattern of lysyl-pyridinolines (LP) and hydroxylysyl-pyridinolines (HP) cross-links [16, 18]. Hence, the diagnosis of EDS VIA relies on the demonstration of a markedly increased ratio of total LP to HP (LP/HP) [5, 16, 18], and may then be confirmed by finding either homozygous or compound heterozygous disease causing variants in *PLOD1*. These are point mutations, insertions, and deletions resulting in premature termination codons that lead to a deficiency of the enzyme LH1, while only six different missense disease associated variants have been described (according to the LOVD Gene Homepage for PLOD1 at https://eds.gene.le.ac.uk/home.php?select_db=PLOD1). The *PLOD1* disease associated variants are mostly private mutations identified in just one or few affected individuals, with the exception of a large duplication between exon 10 and exon 16 of *PLOD1* (p.Glu326_Lys585dup). This duplication, which is the most common of all reported mutations [6, 15], occurs via homologous recombination of a 44-bp identical sequence located in a region of Alu repeats in introns 9 and 16 [7, 8, 14].

Here, we report the clinical, biochemical, and molecular data of six EDS VIA patients from four unrelated Egyptian families who shared a quite homogeneous clinical phenotype in addition to dysmorphic craniofacial features. Finally, we alert the physician to pay attention to facial dysmorphisms in the diagnostic work-up of EDS VIA.

Materials and methods

We studied six individual patients from four unrelated Egyptian families who manifested the characteristic clinical features of EDS VIA. They were referred to one single genetic center from 2010 until 2013 for further diagnostic investigations. Patients were subjected to clinical evaluation, biochemical work-up of EDS VIA, and molecular analysis of *PLOD1*. Clinical examination was performed by the same geneticist.

Total urinary pyridinolines were measured as described [11] and were expressed as the ratio (LP/HP) of lysyl-pyridinoline (LP) to hydroxylysyl-pyridinoline (HP).

Genomic DNA was isolated from peripheral blood leukocytes using standard techniques, and direct PCR and sequencing of *PLOD1* was performed as described [6]. Two 4-day lymphocyte cultures were established from 2-ml heparin-blood sample from P3 in RPMI 1640 (Life Technologies) medium containing phytohaemoagglutinin A (PHA) according to Kretz et al. [13]. Before collection of the cells by centrifugation, one culture was treated for 4 h with 200 mM cycloheximide, in order to inhibit nonsense-mediated RNA decay (NMD). Total RNA extraction and RT-PCR were performed as previously described [6].

Results

The patient cohort consisted of three boys and three girls aged 6 months to 6 years, all born to consanguineous parents, belonging to four unrelated Egyptian families. All families but one included multiple affected members (Fig. 1). All patients fulfilled the major diagnostic criteria for EDS VIA (Table 1) except for absence of scleral fragility and rupture of the ocular globe. They all manifested most of the minor criteria, and presented additionally dysmorphic craniofacial features not yet reported in the literature in association with EDS VIA (Table 1 and Fig. 2).

Family 1 A 5-year-old girl (P1; Fig. 2 A-1–G-1), the first child of healthy consanguineous Egyptian parents (Fig. 1), presented for genetic evaluation of back deformity. Apart from weak fetal movements, pregnancy was uneventful and delivery was at term. At birth, severe muscular hypotonia, back deformity, and adducted thumbs were noted. By the age of 2 years, the severe muscular hypotonia had led to grossly delayed motor development. At that age, she could hardly support her head. A

neuromuscular work-up, including a brain CT, EMG, and NCV, was normal. The patient began walking supported at the age of 3 years and only attained unsupported walking after her fourth birthday. However, despite the markedly retarded motor development, her neurological status and intellectual development were normal. The family sought genetic consultation only after the birth of a younger son who seemed to suffer from the same condition.

The girl showed severe kyphoscoliosis and pectus excavatum (Table 1). She had soft skin, hypermobile joints, long hands with long hyperextensible fingers, interdigital webbing, faint palmar creases, long toes, pes planus, and unusual plantar softness with doughy consistency of the heels. The craniofacial features included faint synophrys, medial flare of eyebrows, remarkable epicanthic folds and down-slanting palpebral fissures, bluish sclerae, microcorneae, malar hypoplasia, depressed nasal bridges and upturned nostrils. The growth parameters were within the normal range.

At examination, her 6-month-old brother (P2; Table 1) presented with severe hypotonia, and was lying in a frog-like position unable to support his head. Mild kyphoscoliosis, pectus excavatum, adducted thumbs, abnormal palmar creases and striking plantar softness were noted. He also had flat supraorbital ridges, narrow almond-shaped palpebral fissures, marked epicanthus inversus, and a small nose with upturned nostrils.

An increased urinary pyridinoline ratio in P1 as well as homozygosity for a *PLOD1* c.622C>T (p.Gln208*) disease causing variant in P1 and P2 confirmed the clinical diagnosis (Table 2).

Family 2 Two sisters, aged 6 (P3) and 4 (P4) years, respectively (patient P4 in Fig. 2 A-2–G-2; Table 1), were referred for evaluation of skin fragility that was observed by the surgeon during stitching of their recurrent skin lesions. They were the second and third child of consanguineous parents (Fig. 1), born at term by normal vaginal delivery. Pregnancy was uneventful, apart from weak fetal movements reported by the mother. Since birth, the mother noticed a peculiar doughy skin consistency, severe hypotonia and poor sucking. In

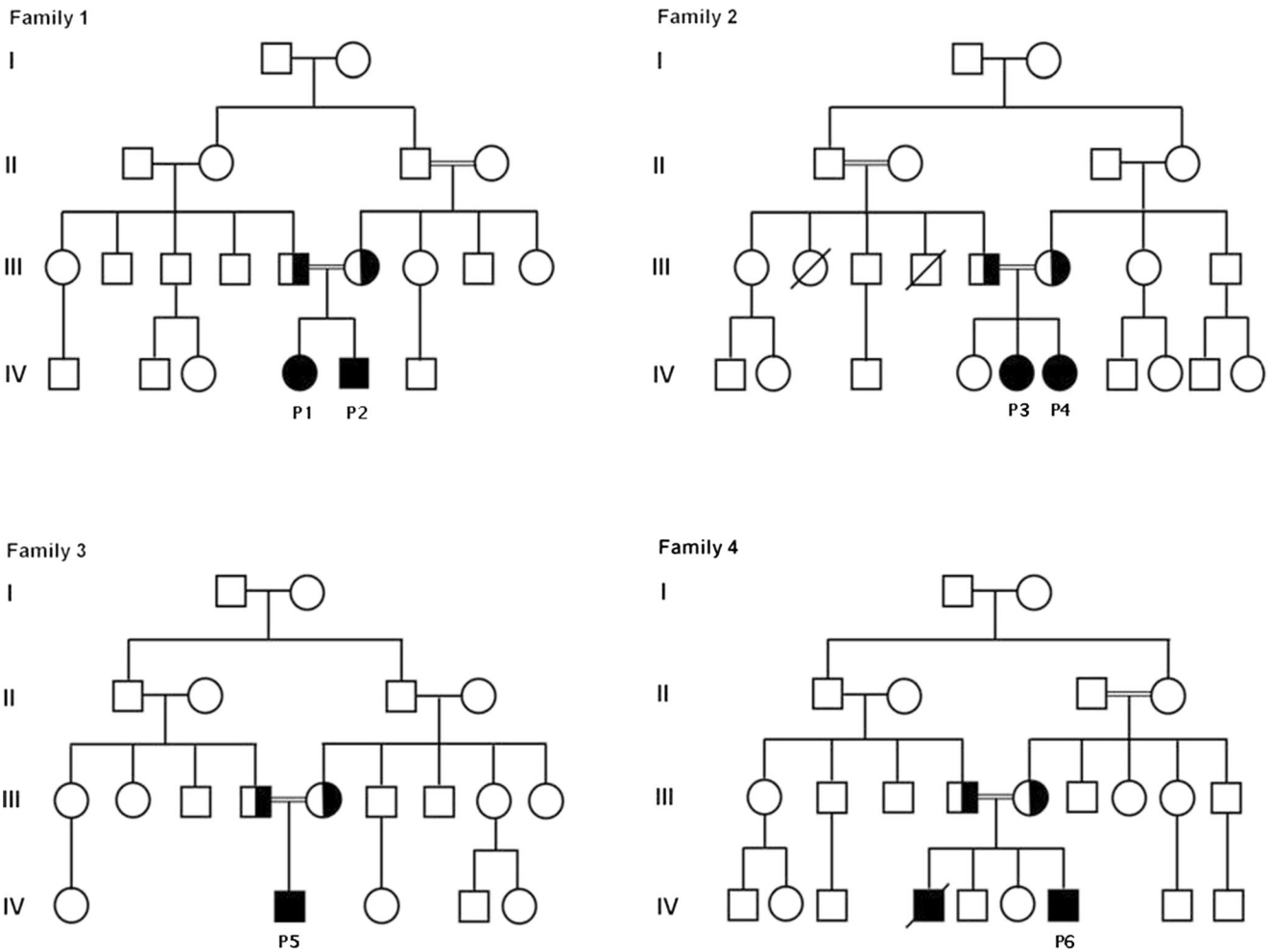

Fig. 1 Pedigrees of the affected individuals showing the degree of inbreeding. The carriers are depicted as *half-solid symbols*, the patients as *solid symbols*

Table 1 Clinical features of six patients affected with EDS VIA, and comparison with the major and minor criteria according to the Villefranche Nosology [1]

Patients	P1	P2	P3	P4	P5	P6
Age at investigation	5 years	6 months	6 years	4 years	4 years	3.5 years
Delivery	NVD	NVD	NVD	NVD	CS	NVD
Weight at birth in g	3250	3500	3200	3350	3000	3500
Length at birth in cm	52	56	54	52	53	55
Villefranche Nosology						
Major criteria						
Severe muscular hypotonia at birth	+	+	+	+	+	+
General joint laxity	+	+	+	+	+	+
Kyphoscoliosis at birth, progressive	+	+	+	+	+	+
Scleral fragility and rupture of the ocular globe	−	−	−	−	−	−
Minor criteria						
Tissue fragility	+	+	+	+	+	+
Easy bruising	+	+	+	+	+	+
Arterial rupture	−	−	−	−	−	−
Marfanoid habitus	+	?	+	+	+	+
Osteopenia[a]	n.e.	n.e.	+	+	+	n.e.
Microcornea[b] (corneal diameter)	+ (10mm)	+ (n.e.)	+ (10.5mm)	+ (10.5mm)	+ (9.5mm)	+ (9mm)
Additional clinical signs						
Craniofacial dysmorphisms	+	+	+	+	+	+
Adducted thumb bilateral	+	+	+	+	+	+

NVD normal vaginal delivery, *CS* cesarean section, + the feature is present, − the feature is absent, *n.e.* not estimated, *?* unclear

[a] Osteopenia assessed on X-rays

[b] In normal individuals the corneal diameter is approximately 11.7 mm (range 11.0–12.5) [17]

addition, back deformity was noted in the first months of life. Both girls also presented bilateral adducted thumbs, which gradually improved and resolved by the age of 2 years. The motor developmental milestones were severely delayed: they began to sit up unsupported by the age of 2 years, and stood alone at age 3 years. Their cognitive development was normal. The girls presented with severe kyphoscoliosis, joint hypermobility, mild skin hyperelasticity with abnormal scarring, pectus excavatum, hypoplastic widely spaced nipples and a protruding abdomen. Bluish sclerae, microcorneae, arched eyebrows with marked medial flare and synophrys were noted. In addition, the girls had long hyperextensible fingers, interdigital webbing, palmar and plantar softness, pes planus, rocker bottom heels, and a striking hallux adductus (sandal gap deformity). Easy fatigability and poor exercise tolerance were reported by the patients.

A significantly increased LP:HP ratio, as well as homozygosity for a novel *PLOD1* nonsense variant p.Tyr675* (c.2025C>G) confirmed the suspected diagnosis (Table 2).

RT-PCR on total RNA extracted from cultured lymphocytes of P3 demonstrated that this premature stop in exon 18 did not undergo NMD (Fig. 3).

Family 3 A 4-year-old boy (P5) was the first child of healthy Egyptian first cousins parents (Fig. 1). He was born at term by cesarean section because of a contracted pelvis. The parents reported kyphoscoliosis from birth and severe hypotonia accompanied by severely delayed motor development: the patient could not sit until age 2 years and began walking after the age of 3. Cognitive development was normal. A neuromuscular disorder was initially considered, however, neuromuscular work-up was normal.

Clinical examination revealed (Fig. 2 A-3–G-3; Table 1) severe kyphoscoliosis, pectus excavatum, hypoplastic widely spaced nipples, protruding abdomen with a small umbilical hernia, hypermobile joints, and soft skin with slightly increased extensibility. Additionally facial asymmetry, synophrys, marked epicanthic folds, microcorneae, and bluish sclerae were noted. Hyperextensible long fingers with digital

Fig. 2 Similar clinical features of Egyptian patients affected with EDS VIA: Multiple atrophic scars on foreheads and microcorneae [*white arrows*; *A-1–4*, front view; *B-1–4*, lateral view], joint hypermobility [*C-1–4*], pectus excavatum and hypoplastic, widely spaced nipples [*D-1–4*], kyphoscoliosis and prominent abdomen [*E-1–4*], abnormal scarring on knees [*F-1–3*] and elbows [*F-4*] and pes planus with rocker bottom heel [*G-1–4*] ▶

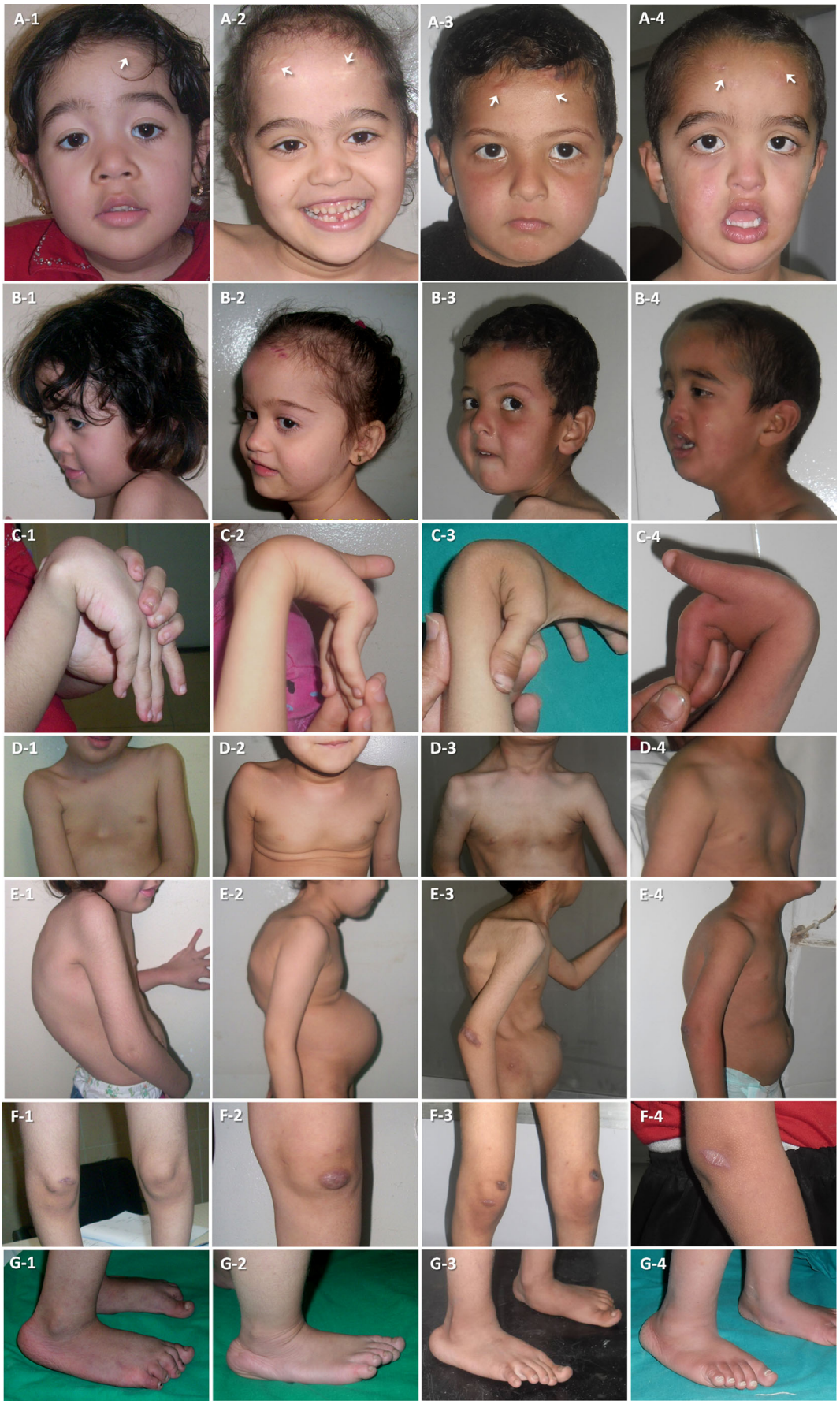
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Table 2 Biochemical and molecular findings in six patients diagnosed with EDS VIA. In all parents, heterozygosity for the corresponding *PLOD1* mutation was proven

Family and patient numbers	LP:HP ratio[a]	Homozygous mutations in *PLOD1*
Family 1 (P1 and P2)	6.00 (P1)[b]	p.Gln208* (c.622C>T)
Family 2 (P3 and P4)	3.88 and 3.89	p.Tyr675* (c.2025C>G)
Family 3 (P5)	6.25	p.Glu326_Lys585dup (c.1067_1546dup)
Family 4 (P6)	7.28	p.Glu326_Lys585dup (c.1067_1546dup)

[a] LP:HP normal range is 0.158–0.218 (n=83; age 1 months to 14 years) [11]

[b] Urine sample of P2 not available

webbing, pes planus, soft soles, and rocker bottom heels were also noted. The patient manifested multiple abnormal atrophic scars all over his body, which, as the mother claimed, had developed after skin injuries induced by minor trauma after the age of 2 years.

After confirming the diagnosis of EDS VIA through the analysis of total urinary pyridinolines, homozygosity for the common large duplication between exon 10 and exon 16 of *PLOD1* was detected (Table 2).

Family 4 A 3.5-year-old boy (P6) born to consanguineous Egyptian parents presented with progressive kyphoscoliosis. He was born at term by vaginal delivery after an uneventful pregnancy. At birth, he was very floppy and back deformity was noticed. Over the years, this deformity became more pronounced and hypotonia persisted leading to motor developmental delay. In fact, he achieved unsupported walking only after the age of 3 years. His parents also complained about skin fragility with easy bruising and poor scar formation. The patient had a similarly affected sibling who suffered from repeated chest infections and died at the age of 4 months for unknown reasons (Fig. 1).

At examination, the patient (Fig. 2 A-4–G-4) showed a prominent high forehead and frontal upsweep of anterior hairline, hypoplastic supraorbital ridges, and marked malar hypoplasia. Striking hypertelorism with epicanthic folds, down-slanting palpebral fissures, faint synophrys and microcorneae were also noted. The nose had a very broad root, a depressed bridge, and a blunt tip. He presented with mild pectus excavatum, hypoplastic widely spaced nipples, pes planus, rocker bottom heels, marked plantar softness, and hallux adductus. Multiple atrophic scars were noticed on the forehead, the lower back, and both elbows and knees. All anthropometric measurements were within average parameters and the cognitive status was normal.

A markedly increased LP:HP ratio and presence of the homozygous *PLOD1* exon 10–exon 16 duplication confirmed the suspected clinical diagnosis (Table 2).

Discussion

Major and minor diagnostic criteria for the kyphoscoliosis type of EDS (EDS VIA) have been defined by the Villefranche Nosology in 1998 [2]. The first include generalized joint laxity, severe muscle hypotonia at birth, scoliosis at birth which is progressive, fragility of the sclerae with occasional rupture of the ocular globe. The minor diagnostic criteria include tissue fragility, easy bruisability of the skin with atrophic scar formation (Fig. 2 A-1–4 and F-1–4), arterial rupture, Marfanoid habitus, microcorneae, and osteopenia.

The Egyptian patient cohort presented here calls attention to the strikingly homogenous clinical phenotype (Table 1).

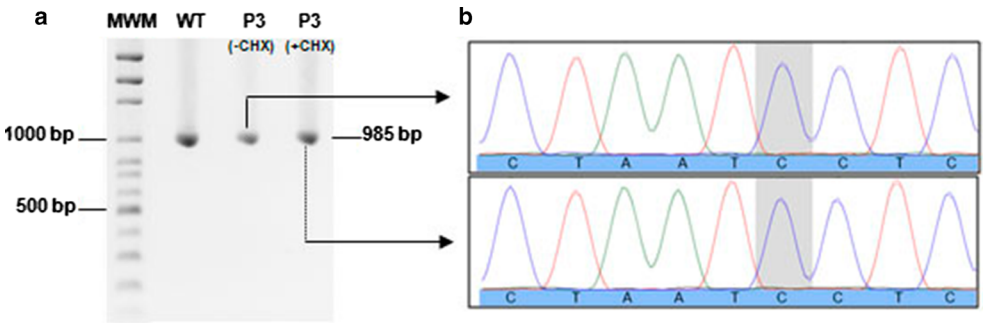

Fig. 3 RT-PCR on total RNA extracted from cultured lymphocytes of a normal control (*lane 2*), P3 without CHX (*lane 3*) (**a**), and P3 with CHX (*lane 4*); and WT sequencing of *PLOD1* between exon 13 and exon 19 in P3 (**b**). Efficient amplification of the exon 13-exon 19 sequence and homozygosity for the c.2025C>G pathologic variant in the RNA sample obtained without CHX treatment, indicates that the mutant *PLOD1* transcript escapes NMD

They all manifested severe neonatal muscular hypotonia, congenital progressive kyphoscoliosis (Fig. 2 E-1–4), pectus excavatum (Fig. 2 D-1–4), severe joint hypermobility (Fig. 2 C-1–4) and pes planus with rocker bottom heel (Fig. 2 G-1–4), microcorneae and dysmorphic craniofacial features (Fig. 2 A-1–4, front view; B-1–4, lateral view). Osteopenia was a less common feature, being identified in about half of the probands. None of the patients had suffered yet any complications related to arterial rupture, or scleral fragility. Normal cognitive development and normal intellectual ability were documented in the patients.

Congenital severe hypotonia accompanied by muscular weakness led, in all probands, to a severe impairment in achieving age-related developmental milestones, and before the present examination was done, it prompted a neuromuscular work-up which gave negative results. Although it is known that neonatal muscular hypotonia in the first years of life leads to a delay in gross motor development in infants affected with EDS VIA, and should prompt the noninvasive investigation of urinary pyridinolines [6, 16, 20], the oldest patients of our cohort were suspected in infancy to suffer from a congenital myopathy. Therefore, they underwent an invasive neuromuscular work-up which gave normal results.

Literature reports on long-term outcome of ambulation in patients with EDS VIA are scarce. Rohrbach et al. [16] reported data on six patients aged 4 to 27 years, showing that all but one patient, who was wheel chair bound, could either walk independently or with assistance. The patients presented here could achieve unsupported walking by an average age of 4 years.

Skin fragility with easy bruising, recurrent cuts on minor trauma, and abnormal scarring (Fig. 2 F-1–4) were invariable complaints of the patients. Velvety skin texture, a feature previously described [16] was absent. However, extreme plantar softness and doughy consistency of the heels, were noted in all patients, and were reported by the parents to be more pronounced in the neonatal period and in early infancy.

Apart from down-slanting palpebral fissures, a feature reported occasionally in the literature [20], there are scarce reports on craniofacial features in EDS VIA patients. All our patients showed dysmorphic craniofacial features (Fig. 2 and Table 1) which were absent in their parents as well as in their unaffected siblings.

Despite being considered a minor diagnostic criterion for EDS VIA, microcorneae [2] was present in all affected children, but not in their unaffected siblings. In the contrary, despite being considered a major diagnostic criterion according to the Villefranche Nosology, scleral fragility with occasional rupture of the ocular globe was not documented in any of our patients. To the best of our knowledge (search in OMIM and PubMed), there are only four reports in the literature of ocular fragility accompanied by either retinal detachment or rupture of the ocular globe: a male patient presenting with rhegmatogenous retinal detachment in his left eye at the age of 47 years, and accidental rupture of the ocular globe with subsequent enucleation of the right eye at the age of 9 years [3]; a female patient who suffered from enucleation of her left eye after a car accident [12]; two siblings who presented with fragility of ocular tissues leading to rupture of the globe or retinal detachment in adult age [1].

Although unusual for EDS VIA, bilateral adducted thumb, which was noted during examination of the two infant patients, was a common finding in all affected children in infancy, and according to their parents it resolved spontaneously by the age of 2 years.

For prenatal purposes, mutational analysis of *PLOD1* was performed (Table 2).

In two patients (P5 and P6) from two unrelated families, homozygosity for the common large duplication of exons 10 to 16 (c.1067_1546dup; p.Glu326_Lys585dup) was detected.

In patients P1 and P2, we identified a novel *PLOD1* disease causing variant in exon 6 (c.622C>T, p.Gln208*) which is expected to lead to complete deficiency of LH1 because of NMD. The second novel nonsense variant p.Tyr675* (c.2025C>G in exon 15), which was found in homozygosity in patients P3 and P4, is localized close to the naturally occurring stop codon in exon 19, and escapes NMD as shown by RT-PCR (Fig. 3). Therefore, p.Tyr675* is expected to lead to a shorter enzyme protein missing the 2-oxoglutarate dioxygenase and the iron-binding domains essential for its catalytic activity. If the shorter enzyme protein preserves partial activity is unclear, although the relatively low LP:HP ratios measured on both siblings (3.88 and 3.89; Table 2) might support this hypothesis; however, the low LP:HP ratios recorded in these severely affected sisters suggest a lack of correlation between the severity of the phenotype and the urinary LP:HP value.

The high degree of inter- and intrafamilial phenotypic variability among individuals affected with EDS VIA and lack of an obvious genotype-phenotype correlation has been well documented [6, 16, 19]. Our report on six patients sharing similar clinical features and nearly absent intrafamilial variability seems to contradict these previous reports. It is tempting to hypothesize that frequent consanguineous marriages leading to a less heterogeneous genetic pool, in addition to the same ethnic background, partially contributes to the documented phenotypic similarity in our cohort of EDS VIA patients.

In conclusion, here, we show that six EDS VIA affected children from four unrelated Egyptian families, who are homozygous for *PLOD1* mutations leading either to loss of function of LH1 or to its deficiency, presented all with similar phenotypical features and craniofacial dysmorphisms not yet reported in the literature. Two new *PLOD1*-disease causing variants, p.Gln208* and p.Tyr675*, were identified. To the best of our knowledge, this is not only the first report of EDS VIA cases from Egypt, but also the first report on so many

cases sharing the same ethnic background. Finally, we propose to consider craniofacial dysmorphisms as possible additional clinical features of EDS VIA that may help the pediatrician in recognizing this rare disorder.

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Ethical standards For all studies written, informed consent of the patients or their parents, in accordance with requirements of the Local Ethics Committee and the Helsinki Declaration of 1975, as revised in 2000, was obtained.

Conflict of interest The authors declare that they have no conflict of interest.

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