Mutation analysis of the arylsulfatase B gene among Egyptian patients with Maroteaux–Lamy disorder

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Received 10 April 2018
Accepted 05 Nov 2018

Middle East Journal of Medical Genetics
2018,7:96–103

Background
Mucopolysaccharidosis type VI (Maroteaux-Lamy syndrome) (OMIM: # 253200), is an autosomal recessive lysosomal storage disorder. It is caused by deficiency of the enzyme arylsulfatase B (*ARSB*), also known as N-acetylgalactosamine-4-sulfatase. *ARSB* is responsible for the degradation of the glycosaminoglycans dermatan sulfate and chondroitin 4-sulfate. Deficiency of the *ARSB* enzyme leads to accumulation of partially degraded dermatan sulfate in the lysosomes especially in connective tissues leading to clinical complications.

Aim
This study aimed at identifying the molecular basis of MPS VI (Mucopolysaccharidosis VI) among 15 Egyptian patients.

Method
Patients were from 15 families, age ranged from 1year and 5 months to 11 years and 9 months(5.19±0.8) with parental consanguinity in 11 families out of 15 (73.3%). All patients were subjected to all necessary clinical, radiological and biochemical assessments. Molecular assessment was carried out by sequencing the 8 coding exons of the *ARSB* gene for the fifteen studied patients.

Results
The disease causing mutations were revealed in 13 patients. Four novel mutations; c.257delA, c.189insA, p.Ser94Leu and p.Leu51Pro were identified as well as four previously reported mutations; p.Leu82Arg, p.Ser96Arg, p.Arg160X and p.Arg160Gln.

Conclusion
The study highlights the heterogeneity of the mutational pattern which was obvious in finding novel mutations in homozygous forms in approximately 40% of the studied patients. Exons 1 and 2 seem to carry most of the mutations in the Egyptian MPS VI patients. Arginine at position 160 seems to be the most abundant mutational hot spot in the Egyptian MPS VI patients.

Keywords:
ARSB gene, dermatan sulphate, MPS VI

Middle East J Med Genet 7:96–103

2090-8571

Introduction

Mucopolysaccharidoses (MPS) are a group of lysosomal storage diseases characterized by deficiency of the enzymes responsible for the step-wise degradation of glycosaminoglycans (GAGs) (Burrow and Leslie, 2008). MPS type VI (Maroteaux–Lamy disorder) (OMIM: # 253200) is an autosomal recessive lysosomal storage disorder first reported in 1963 by Dr Pierre Maroteaux and Dr Maurice Lamy (Maroteaux *et al.*, 1963). It is caused by deficiency of the enzyme arylsulfatase B (*ARSB*), whose deficiency leads to accumulation of partially degraded dermatan sulfate in the lysosomes and detected in urine (Mathew *et al.*, 2015).

Incidence of MPS VI has been determined in few countries and ranges from 0.04: 100 000 live births in Poland to 8: 100 000 live births in Saudi Arabia (Moammar *et al.*, 2010). Although no birth registries were available for Egyptian patients with MPS in general and MPS VI in particular, the high consanguinity rate in our population, which ranges between 29% in urban regions to 39% in rural regions (Temtamy and Aglan, 2012) increases the incidence of rare autosomal recessive disorders in general. In available Egyptian studies, MPS VI represented ~24% of the patients with diagnosed MPS (Shawky *et al.*, 2012; Fateen *et al.*, 2014). This might be attributed to some degree of chance for disorders represented in the founder population.

Patients with MPS VI present clinically with short stature, coarse facial features, corneal clouding, joint stiffness, organomegaly, obstruction, and recurrent infections of the upper airway. Most of the patients have central nervous system (CNS), cardiac, and ear, nose, and throat abnormalities (Giraldo *et al.*, 2016).

Early diagnosis and treatment is of high importance to allow early therapeutic interventions (Lehman *et al.*, 2011). Enzyme replacement therapy is used for patients with MPS VI although it does not cure the disease but only limits its progression (Valayannopoulos and Wijburg, 2011; Quartel *et al.*, 2018).

ARSB gene is located on chromosome 5 and consists of eight exons producing a protein of 533 amino acids. Most of the mutations reported and listed by the Human Gene Mutation Database are missense or nonsense mutations (Lin *et al.*, 2015).

Patients and methods

Patients

The study included 15 unrelated Egyptian patients from 15 families diagnosed with Maroteaux–Lamy disease. The patients were referred from the Clinical and Biochemical Genetics Departments at the National Research Centre. The patients were referred from different areas of Egypt. A written informed consent was signed by the patients' guardians according to the Medical Research Ethical Committee of the National Research Centre.

Methods

Clinical evaluation started with recording family history and pedigree construction. Patients were clinically examined, supported by photography and imaging techniques. Ophthalmological examinations and hearing assessments were done whenever needed.

Biochemical investigations included quantitative determination of total GAGs in the urine samples (De Dong *et al.*, 1989) followed by qualitative two-dimensional electrophoretic separation of the urinary GAGs (Mossman and Patrick, 2005). Finally, biochemical diagnosis is confirmed by quantitative *ARSB* enzyme activity assay (Bhattacharyya *et al.*, 2007).

Molecular assessment included DNA extraction from peripheral blood leukocytes (Miller *et al.*, 1988). This was followed by PCR amplification of the eight exons of *ARSB* gene according to Petry *et al.* (2005). PCR fragments were allowed to run on a 2% agarose gel to confirm successful amplification. The PCR products were purified using QIAquick PCR purification kit (Qiagen, Hilden, Germany). Cycle sequencing PCR was carried out using BigDye Terminator kit (Applied Biosystems, Foster City, California, USA). Removal of dye terminators was done using CENTRI-SEP purification spin columns (Applied Biosystems). Sequencing was performed using a 310 ABI Prism DNA sequencer.

Bioinformatics were performed for the results obtained using different in silico analysis tools. Data files obtained from the sequencer were shown by Finch TV, version 1.4.0 (310 ABI Prism DNA sequencing, ThermoFischer scientific, Waltham, Massachusetts, USA). This was followed by aligning query sequences against those present in BLAST Basic Local alignment search tool (*http://blast.ncbi.nlm.nih.gov*) (Camacho *et al.*, 2009). If a variant was detected, which was previously not reported, bioinformatic predictions of the defects in the protein variant were performed using the following bioinformatic tools: PolyPhen-2 (*http://genetics.bwh.harvard.edu/pph 2/*) (Adzhubei *et al.*, 2010), MutationTaster (*http://www.mutationtaster.org/*) (Schwarz *et al.*, 2014), SNPs and GO (*http://snps-and-go.biocomp.unibo.it/snps-and-go/*) (Calabrese *et al.*, 2009), and Provean (*http://provean.jcvi.org/index.php*) (Choi *et al.*, 2012).

Table 1 Data of the studied patients with mucopolysaccharidoses VI

Patient number	Origin governorate	Sex	Age at presentation	Parental consanguinity	Other affected family members
1	Qalyubia	Male	11 years and 9 months	Same village	–
2	Minya	Female	2 years and 4 months	+	–
3	Dakahlia	Female	4 years and 6 months	+	2 sisters
4	Asyut	Male	11 years	Same village	–
5	Dakahlia	Female	1 year and 5 months	+	2 sisters
6	Giza	Male	5 years and 2 months	+	–
7	Fayoum	Male	6 years	Same village	1 sister
8	Giza	Male	2 years and 4 months	+	–
9	Giza	Male	3 years and 6 months	+	–
10	Qena	Female	3 years	+	–
11	Asyut	Male	4 years and 3 months	+	–
12	Fayoum	Female	3 years	+	–
13	Gharbia	Female	5 years and 10 months	+	–
14	Minya	Female	10 years	–	–
15	Dakahlia	Male	3 years and 11 months	+	1 sister

+ Present.

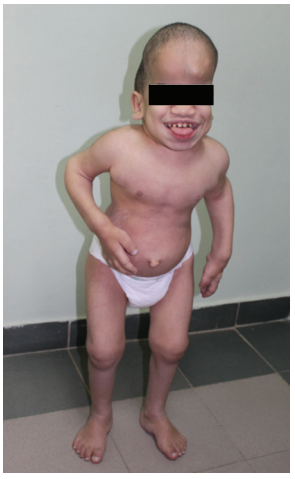
Results

The study included 15 unrelated Egyptian patients suspected of having Maroteaux–Lamy disease, comprising seven (46.6%) females and eight (53.4%) males. Age at presentation ranged from 1 year and 5 months to 11 years and 9 months with positive consanguinity in 11 (73.3%) of 15 families. Genetic data are shown in Table 1.

Clinical examination showed that coarse facial features, short stature (height/length below 2.5 SD), and skeletal and joint deformities with dysostosis multiplex seen by radiological examination were common in all patients. Clinical manifestations are shown in Fig. 1 and Table 2.

Biochemical results illustrated that urinary GAGs analysis was high in all cases apart from patient 3 (Table 3). Two-dimensional electrophoretic separation of the urinary GAGs revealed big dermatan sulfate spot in all patients, which is characteristic of patients with MPS VI. Enzyme activity assay for *ARSB* revealed null activity in most of the studied cases (Table 3).

Figure 1



Frontal view of patient 1 at the age of 11 years and 9 months, showing dolichocephalic head, prominent forehead, coarse facies, short neck, short stature with broad joints, and limited extension of knee joints, pectus carinatum, and umbilical hernia.

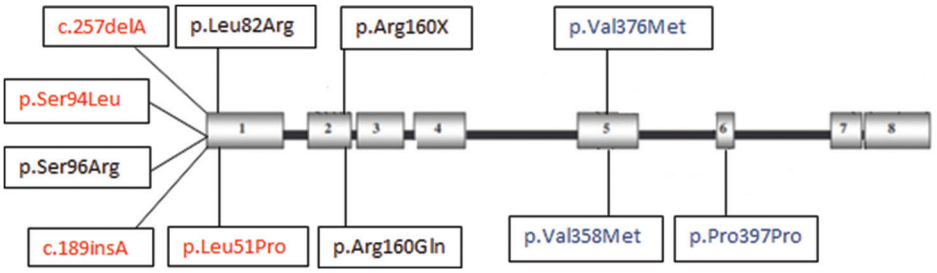
Molecular assessment including sequencing of the eight coding exons of the *ARSB* gene for the 15 studied patients revealed pathogenic mutations in 13 patients, as shown in Table 3. All the mutations found were in exons 1 and 2, as shown in Fig. 2. Four novel mutations were detected, and their pathogenicity was confirmed by in silico functional analysis using different programs (Table 4). The novel mutations detected were c.189insA, c.257delA, p.Ser94Leu (c.281C>T), and p.Leu51Pro (c.152T>C). The frequencies of the individual mutations can be tabulated from Table 3, noting that the p.Arg160X (c.478C>T) was the most common. The DNA reference sequence from the reference sequence database is NM_000046.4. The Genebank protein reference sequence is NP_000037.2. The four previously reported mutations that were detected in the studied patients were p.Leu82Arg (c.245T>G), p.Arg160X (c.478C>T), p.Arg160Gln (c.479G>A), and p.Ser96Arg (c.288C>G). p.Val358Met, p.Val376Met, and p.Pro397Pro are the most common presumed benign variants detected among the studied patients. The novel and reported mutations were submitted to ClinVar database and had ClinVar accessions SCV000609478–SCV000609485.

Discussion

This is the first Egyptian molecular study of patients with MPS VI. The studied patients presented the common reported clinical findings of the disease with varying severity (Mathew *et al.*, 2015; Giraldo *et al.*, 2016). Craniosynostosis was found in one of the studied patients. Although a very rare association with MPS, Al-Sannaa *et al.* (2018) reported a Saudi patient with MPS VI and associated craniosynostosis.

The biochemical analyses were in the form of three complementary tests. Quantitation of urinary GAGs, which showed high amounts for age in all patients, except patient 3 who had most probably provided a diluted urine sample and not an early morning concentrated

Figure 2



Locations of mutations in the *ARSB* gene identified in the Egyptian patients with MPS VI. The exons are represented by boxes. The novel mutations are in red, previously reported pathogenic mutations are in black, and presumed benign variants are in blue. *ARSB*, arylsulfatase B; MPS, mucopolysaccharidoses.

Table 2 Clinical manifestations in the studied patients

Patient number	Short stature	Coarse facies	Skeletal deformities	Organomegaly/hernia	Cardiac problem	Eye problems	ENT problems	CNS problem
1	+	+	Limited joint movements, broad joints, pectus carinatum, and craniosynostosis [Figure 1]	–/umbilical hernia	Mitral valve stenosis and aortic valve stenosis	Corneal clouding	Moderate hearing loss and recurrent otitis media	Hydrocephalic changes
2	+	+	Limited joint movements and kyphosis	Hepatomegaly/ umbilical hernia	ASD secundum	Corneal clouding	Sleep apnea	–
3	+	+	Limited joint movements, pectus excavatum, and kyphoscoliosis	Hepatosplenomegaly/ umbilical and hiatus hernia	Mitral and aortic regurge	Nystagmus and corneal clouding	Sleep apnea and hypertrophied adenoids	Delayed white matter myelination, diffuse brain atrophy, and post-encephalitic parietal areas of encephalomalacia
4	+	+	Limited joint movements, pectus excavatum, and kyphoscoliosis	Splenomegaly/ umbilical and inguinal hernia	Mitral and pulmonary regurge	Corneal clouding	Sleep apnea, and recurrent upper respiratory tract infection	Hydrocephalic changes
5	+	+	Broad joints, kyphoscoliosis, and pectus carinatum	–/umbilical hernia	Mildly thickened mitral leaflets and aortic cusps	–	Sleep apnea	–
6	+	+	Limited joint movements, pectus carinatum, and kyphoscoliosis	Hepatomegaly/ umbilical hernia	–	Corneal clouding	Hypertrophied adenoids, otitis media	–
7	+	+	Limited joint movements, broad joints, and kyphoscoliosis	Hepatosplenomegaly/ umbilical hernia	Hypertrophic cardiomyopathy	Corneal clouding	Sleep apnea and hypertrophied adenoids	Communicating hydrocephalus and delayed white matter myelination
8	+	+	Limited joint movements, broad joints, pectus excavatum, and kyphoscoliosis	Hepatosplenomegaly/ umbilical hernia	Dilated left ventricle and aortic regurgitation	Corneal clouding, nystagmus	Hypertrophied adenoids and recurrent upper respiratory tract infection	Delayed white matter myelination and frontal brain atrophy
9	+	+	Limited joint movements and broad joint kyphosis	Hepatosplenomegaly/ umbilical hernia	–	Corneal clouding	Hypertrophied adenoids and sleep apnea	History of neonatal convulsions
10	+	+	Limited joint movements, broad joints, pectus excavatum, and kyphoscoliosis	–/umbilical hernia	Infiltrative cardiac disease	–	Severe hearing loss	Cerebral atrophic changes with ventriculomegaly
11	+	+	Limited joint movements and pectus carinatum	Hepatosplenomegaly/–	Mild thickening of valvular cusps, and mild mitral and tricuspid regurgitation	Corneal clouding	Bilateral secretory otitis media	Small subarachnoid cyst
12	+	+	Limited joint movements and pectus carinatum	Hepatomegaly/ umbilical hernia	Hypertrophic cardiomyopathy and mitral valve regurgitation	Corneal clouding	Hypertrophied adenoids, sleep apnea, and recurrent otitis media	Mild brain atrophy
13	+	+	Limited joint movements, broad joints, pectus carinatum	Hepatosplenomegaly/ umbilical hernia	Mild dilated left ventricle, myxomatous degeneration of mitral valves	Corneal clouding	Recurrent otitis media	–

Contd...

Table 2 Contd...

Patient number	Short stature	Coarse facies	Skeletal deformities	Organomegaly/hernia	Cardiac problem	Eye problems	ENT problems	CNS problem
14	+	+	Limited joint movements, and pectus excavatum	Hepatomegaly/huge umbilical hernia	Mitral stenosis, aortic stenosis, aortic regurgitation, and pulmonary hypertension	Corneal clouding	Recurrent upper respiratory tract infection, and otitis media	–
15	+	+	Limited extension of joints, broad joints, kyphoscoliosis, and pectus carinatum	–/umbilical hernia	Thickened mitral valve leaflets and mitral regurgitation	–	Recurrent otitis media	Borderline IQ: 73 by WPPSI test

+ Present. CNS, central nervous system; ENT, ear, nose, and throat.

Table 3 Mutations in arylsulfatase B gene and biochemical results in the studied Egyptian patients

Patients	Mutation	GAGs (mg/mmol creatinine)	*ARSB* (nmol/mg protein min)
1	c.257delA[a]	28	0
2	p.Leu82Arg (c.245T>G)	37.6	0
3	p.Arg160X (c.478C>T)	2	0
4	p.Arg160X (c.478C>T)	41.4	0
5	p.Arg160X (c.478C>T)	33.5	0.15
6	p.Ser94Leu (c.281C>T)[a]	19	0
7	c.189insA/p. Leu82Arg[a]	47.7	0
8	p.Arg160Gln (c.479G>A)	229.8	0
9	p.Ser94Leu (c.281C>T)[a]	25	0
10	p.Arg160X (c.478C>T)	32.7	0
11	p.Ser96Arg (c.288C>G)	14.7	5
12	c.189insA[a]	38.4	0.2
13	p.Leu51Pro (c.152T>C)[a]	44	0
14	Not detected	132.5	0
15	Not detected	16	0

ARSB, arylsulfatase B; GAG, glycosaminoglycan.[a]Novel mutation; normal *ARSB* enzyme more than 6.08 nmol/mg protein min.

one. Bidirectional electrophoretic separation of the urinary GAGs showed the big dermatan sulfate spot characteristic for MPS VI (Giraldo *et al.*, 2016) in all of the studied patients. *ARSB* enzyme activity was measured for the patients and was found to be of low activity in all of them. This matches previous biochemical studies on patients with MPS VI, which also showed elevated GAGs and big dermatan spot in all patients with MPS VI (Fateen *et al.*, 2014).

Molecular studies revealed the presence of eight pathogenic mutations in 13 patients as shown in Table 3. Four novel mutations included insertion c.189insA, deletion c.257delA and two missense mutations p.Ser94Leu and p.Leu51Pro. The four novel mutations were detected in homozygous form in five patients, which is optimum for phenotype–genotype correlation. We also reported four previously described mutations (p. Leu82Arg, p.Ser96Arg, p.Arg160Gln, and p.Arg160X). Each of the detected mutations will be discussed in detail starting with the most common mutations found in this group of Egyptian MPS VI patients. No mutations could be detected in two of the patients. As all the mutations found were in exons 1 and 2, this may indicate that these two exons are hotspots among the Egyptian patients with MPS VI. In addition, three previously presumed benign variants (p.Val358Met, p.Val376Met, and p.Pro397Pro) were detected in the analyzed patients. Our findings are in agreement with literature, confirming the great genetic heterogeneity associated with MPS VI (Petry *et al.*, 2005).

Voskoboeva *et al.* (1994) were the first to report the c.478C>T (p.Arg160X) nonsense mutation. It is the most frequent mutation found among the studied group of Egyptian patients with MPS VI (26.6%), where it was found in four unrelated patients (patients 3, 4, 5, and 10) in a homozygous form. This goes well with the postulation that the amino acid Arg160 is a mutational hotspot for the *ARSB* gene (Kantaputra *et al.*, 2014). All of the patients with the c.478C>T (p.Arg160X) nonsense mutation were from consanguineous marriages except for patient 4, whose parents were from the same village. The patients were from different areas in Egypt (Qena, Mansura, and Asyut). The patients had different SNP pattern found on exon 5, which may explain that p.Arg160X is a common mutation and not from a common founder effect. This mutation has a wide clinical presentation.

Patient 3 has a severe disease form and had two affected sisters who passed away at the ages of 1 and 13 years. This may augment the categorization of this genotype as causing severe clinical form of MPS VI. This is in agreement with the literature. p.Arg160X was found in a homozygous form and in compound heterozygosity

Table 4 Predictions of the effect of novel mutations in Egyptian patients with mucopolysaccharidoses VI; all bioinformatics tools showed that the variants are deleterious and cause disease mutations

Mutation	PolyPhen-2	MutationTaster	Provean	SNP&GO
C.257delA	NF	Disease causing probability: 1	NF	NF
p.S94L	Deleterious score: 1	Disease causing probability: 0.99	Deleterious score: 4.7	Disease causing RI: 9
C.189insA	NF	Disease causing probability: 1	NF	NF
p.L51P	Deleterious score: 1	Disease causing probability: 0.99	Deleterious score: 5.7	Disease causing RI: 10

NF, not found because the programs could not perform the prediction of the effect owing to the type of mutation; RI, reliability index.

in patients from other populations as in Portuguese patients as reported by Karageorgos *et al.* (2007), who classified it as being a rapidly progressing mutation. It was found to present with 3.3% allele frequency and 4.8% patient frequency in his study. It was also found in French and German patients. This elects it to be one of the most common mutations in the *ARSB* gene. Compound heterozygosity of both p.Arg160X and p.Arg160Gln was reported in a Belarusian patient who had a severe form of MPS VI, which goes well with our postulation (Jurecka *et al.*, 2012).

The missense mutation c.245T>C (p.Leu82Arg) was found in a homozygous form in patient 2 and in a heterozygous form in patient 7. It was first reported in a homozygous form in a Spanish patient and was classified as causing an intermediately progressive disease (Garrido *et al.*, 2008).

Patients 2 and 7 share some common clinical manifestations; however, patient 7 has a more severe phenotype. Phenotype–genotype correlation might be biased as patient 7 is bearing the mutation in a compound heterozygous form with the frameshift c.189insA mutation, which seems to synergize the pathogenicity of the p.Leu82Arg mutation. This was deduced from the fact that patient 7 had an affected sister, who had the same genotype and passed away. This comparison leads us to conclude that p.Leu82Arg in a homozygous form shows intermediate disease but when combined with c.189insA it might increase the disease severity.

c.288C>G (p.Ser96Arg) missense mutation was found in a homozygous form in patient 11. It was first reported in Portuguese patients. The mutation affects a serine that is highly conserved among sulfatases (Karageorgos *et al.*, 2007). Patient 11 had some residual *ARSB* enzyme activity, which is in agreement with Parini *et al.* (2013), who stated that missense mutations are sometimes compatible with some enzymatic residual activity and then result in a wide range of phenotypes spanning the entire spectrum from severe to attenuated.

c.479G>A (p.Arg160Gln) missense mutation was first reported by Voskoboeva *et al.* (1994). It was found in a homozygous form in patient 8. p.Arg160Gln was previously reported in four unrelated patients, two from Belarus and one each from Russia and Spain, in all of whom, it was in the compound heterozygous state and was also found in an Indian patient in a homozygous form with moderate phenotypic presentation (Mathew *et al.*, 2015).

Patient 8 has the highest GAG level among our studied group of patients (>200 μg/mg creatinine). Most patients with MPS VI who had urinary GAGs more than 100 μg/mg creatinine do not survive beyond the age of 20 years (Swiedler *et al.*, 2005). This drives us to postulate that patient 8 has a severe progressive phenotypic presentation in contrast to what is stated about p.Arg160Gln mutation in literature so far. This may be owing to the presence of another unknown variant in the promotor region.

c.281C>T (p.Ser94Leu) is a novel missense mutation detected in patients 6 and 9. The serine at position 94 is changed to leucine owing to a single nucleotide change. Serine at position 94 is considered one of the evolutionarily conserved amino acid residues along the family of sulfatase enzymes. It lines the active-site pocket of *ARSB* enzyme and is located at the base of a cleft in domain 1 (Bond *et al.*, 1997), which theoretically leads to a rapidly advancing form of the disease and early onset of a severe clinical phenotype as postulated by Karageorgos *et al.* (2007) for any mutations in the active site of *ARSB*. The mutation was found in a homozygous form in both patients, who were from consanguineous marriages in the same region (Giza). The *ARSB* enzyme activity assay revealed abolished enzyme activity for both patients.

c.152T>C (p.Leu51Pro) is a novel missense mutation in patient 13 where the leucine at position 51 located in domain 1 of the protein is changed to proline. It was found in a homozygous form with null enzyme activity. The patient was 5 years and 10 months old and offspring of consanguineous parents. She presented with typical clinical presentations but had normal milestones of development and no associated CNS manifestations, which can lead us to deduce that this mutation does not affect the CNS but affects other body systems.

c.189insA is a novel frameshift mutation detected in patients 7 and 12. It causes an early stop codon

and production of a truncated protein of 126 amino acid instead of 533, owing to insertion of adenine nucleotide at codon 63. This mutation might cause nonsense-mediated mRNA decay, a mechanism known to cause the degradation of mRNA bearing a premature termination codon. Consequently, it is expected to result in early disease onset with rapid progression (Garrido *et al.*, 2008). This mutation was found in compound heterozygosity with p.Leu82Arg, which was previously detected in patient 7 and in homozygous form in patient 12. Both patients were from the same governorate (Fayoum) with an early onset and a severe disease form.

c.257delA is a novel frameshift mutation in patient 1 leading to a truncated protein of 113 amino acid owing to deletion of adenine nucleotide from codon 86. This explains the abolished activity of *ARSB* enzyme in this patient who had the mutation in homozygous form and severe manifestations in addition to hydrocephaly and the rare association of craniosynostosis. The patient was born to nonconsanguineous parents, from the same village, who were found to be carriers for the mutation. The previously reported patient with MPS VI with severe manifestations in addition to craniosynostosis and obstructive hydrocephaly was found to be homozygous for a known pathogenic nonsense c.753C>Gp.Y251X mutation in the *ARSB* gene (Al-Sannaa *et al.*, 2018).

Mutational analysis of the eight exons of *ARSB* gene in patients 14 and 15 showed no mutations in spite of having classical clinical and biochemical findings of Maroteaux–Lamy disease. This could be explained by the presence of mutations in the regulatory region or intronic regions, which were not involved in this study. Such cases will be diagnosed by whole genome sequencing in the near future.

Six (40%) patients of the 15 studied ones showed p.Val358Met, p.Val376Met, and p.Pro397Pro presumed benign variants. p.Val358Met was detected once in homozygous form and once in heterozygous form. p.Val376Met was detected once in heterozygous form and twice in homozygous form. p.Pro397Pro was detected only once in a homozygous form. A functional analysis done by Garrido *et al.* (2008) revealed that p.Val376Met showed equivalent enzymatic activity for both the valine and the methionine alleles, whereas for p.Val358Met, the methionine-bearing enzyme in this position showed only 42% of *ARSB* activity compared with the valine-bearing enzyme. The study proved that p.Val358Met presumed benign variant is a pathogenic one. We reported this variant in patient 7 who was compound heterozygous for c.189insA/p.Leu82Arg, and we suggest that it synergizes the phenotypic severity in this case.

In conclusion, this study showed that MPS VI mutational pattern among Egyptian patients is rich in homozygous forms of different mutations. It also highlights the heterogeneity of this mutational pattern. Finding novel mutations in homozygous forms in ~40% of the studied patients indicates the unique pattern of MPS VI among the studied patients. Larger numbers of patients need to be studied, expecting to show more novel mutations and correlate more with the phenotype presentations. Exons 1 and 2 seem to carry most of the mutations in the Egyptian patients with MPS VI. Arginine at position 160 seems to be the most abundant mutational hotspot in the Egyptian patients with MPS VI, as 33.3% of our patients had mutations in this amino acid.

Acknowledgements

The authors thank the patients and their families for accepting to participate in this study.

Funding: The research was partially funded by the STDF (Science and Technology Development Fund in Egypt) through the project entitled 'Recent Advances in the Diagnosis Management and Research of Genetic diseases' Grant number: 5253 for Center of Excellence of Human Genetics.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

Adzhubei IA, Schmidt S, Peshkin L, Ramensky VE, Gerasimova A, Bork P, *et al.* (2010). A method and server for predicting damaging missense mutations. *Nat Methods* **7**:248–249.

Al-Sannaa NA, Al-Abdulwahed HY, Al-Majed SI, Bouhalaigah IH (2018). The clinical and genetic spectrum of Maroteaux-Lamy syndrome (mucopolysacharidosis VI) in the Eastern Province of Saudi Arabia. *J Community Genet* **9**:65–70.

Bhattacharyya S, Joanne K, Tobacmana B (2007). Steroid sulfatase, arylsulfatases A and B, galactose-6-sulfatase, and iduronate sulfatase in mammary cells and effects of sulfated and non-sulfated estrogens on sulfatase activity. *J Steroid Biochem Mol Biol* **103**:20–34.

Bond CS, Clements PR, Ashby SJ, Collyer CA, Harrop SJ, Hopwood JJ, Guss JM (1997). Structure of a human lysosomalsulfatase. *Structure* **5**:277–289.

Burrow TA, Leslie ND (2008). Review of the use of idursulfase in the treatment of mucopolysaccharidosis II. *Biologics* **2**:311–320.

Calabrese R, Capriotti E, Fariselli P, Martelli PL, Casadio R (2009). Functional annotations improve the predictive score of human disease-related mutations in proteins. *Hum Mutat* **30**:1237–1244.

Camacho C, Coulouris G, Avagyan V, Ma N, Papadopoulos J, Bealer K, Madden T (2009). BLAST plus : architecture and applications. *BMC Bioinformatics* **10**:421.

Choi Y, Sims GE, Murphy S, Miller JR, Chan AP (2012). Predicting the functional effect of amino acid substitutions and indels. *PLoS One* **7**:e46688.

De Dong JG, Wevers RA, Laarakkers C, Poorthuis BJ (1989). Dimethylene-blue based procedure for mucopolysaccharidoses. *Clin Chem* **35**:1472–1477.

Fateen EM, Ibrahim MM, Gouda AS, Youssef ZA (2014). Biochemical diagnosis of mucopolysaccharidoses over 11 years: the Egyptian experience. *Middle East J Med Genet* **3**:16–23.

Garrido E, Cormand B, Hopwood JJ, Chabas A, Grinberg D, Vilageliu L (2008). Maroteaux–Lamy syndrome: functional characterization of pathogenic mutations and polymorphisms in the arylsulfatase B gene. *Mol Genet Metab* **94**:305–312.

Giraldo GA, Ramírez PA, Prieto JC, Robles RG, Acosta JC (2016). Molecular findings of Colombian patients with type VI mucopolysaccharidosis (Maroteaux–Lamy syndrome). *Meta Gene* **7**:83–89.

Jurecka A, Piotrowska E, Cimbalistiene L, Gusina N, Sobczyńska A, Czartoryska B, *et al*. (2012). Molecular analysis of mucopolysaccharidosis type VI in Poland, Belarus, Lithuania and Estonia. *Mol Genet Metab* **105**:237–243.

Kantaputra PN, Kayserili H, Guven Y, Kantaputra W, Balci MC, Tanpaiboon P, *et al*. (2014). Clinical manifestations of 17 patients affected with mucopolysaccharidosis type VI and eight novel *ARSB* mutations. *Am J Med Genet A* **164A**: 1443–1453.

Karageorgos L, Brooks DA, Pollard A (2007). Mutational analysis of 105 mucopolysaccharidosis type VI patients. *Hum Mutat* **28**:897–903.

Lehman TJA, Miller N, Norquist B, Underhill L, Keutzer J (2011). Diagnosis of the mucopolysaccharidoses. *Rheumatology* **50**:41–48.

Lin WD, Ke YY, Chou IC, Wang CH, Tsai FJ (2015). Deletion of exon 4 in the N-acetylgalactosamine-4-sulfatase gene in a Taiwanese patient with mucopolysaccharidosis type VI. *Tohoku J Exp Med* **235**:267–273.

Maroteaux P, Leveque B, Marie J, Lamy M (1963). A new dysostosis with urinary elimination of chondroitin sulfate B. *Presse Med* **71**:1849–1852.

Mathew J, Jagadeesh SM, Bhat M, Kumar SU, Thiyagarajan S, Srinivasan S (2015). Mutations in *ARSB* in MPS VI patients in India. *Mol Genet Metab Rep* **4**:53–61.

Miller SA, Dykes DD, Polesky HF (1988). A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acid Res* **16**:1215–1225.

Moammar H, Cheriyan G, Mathew R, Al-Sannaab N (2010). Incidence and patterns of inborn errors of metabolism in the Eastern Province of Saudi Arabia, 1983-2008. *Ann Saudi Med* **30**:271–277.

Mossman J, Patrick A (2005). Prenatal diagnosis of mucopolysaccharidoses by two-dimensional electrophoresis of amniotic fluid glycosaminoglycans. *Prenat Diagn* **2**:169–176.

Parini R, Bertola F, Russo *P* (2013). Molecular basis, diagnosis and clinical management of mucopolysaccharidoses. *Cardiogenetics* **3**:e2.

Petry MFG, Nonemacher K, Sebben JC, Schwartz IVD, Azevedo ACM, Burin MG, *et al*. (2005). Mucopolysaccharidosis type VI: identification of novel mutations on the arylsulphatase B gene in South American patients. *J Inherit Metab Dis* **28**:1027–1034.

Quartel A, Harmatz PR, Lampe C, Guffon N, Ketteridge D, Lea¯o-Teles E, *et al*. (2018). Long-term galsulfase treatment associated with improved survival of patients with mucopolysaccharidosis VI (Maroteaux-Lamy Syndrome): 15-year follow-up from the survey study. *J Inborn Errors Metab Screen* **6**:1–6.

Schwarz JM, Cooper DN, Schuelke M, Seelow D (2014). MutationTaster2: mutation prediction for the deep-sequencing age. *Nat Methods* **11**:361–362.

Shawky R, Elsayed N, Ibrahim D, Seifeldin N (2012). Profile of genetic disorders prevalent in northeast region of Cairo, Egypt. *Egypt J Med Human Genet* **13**:45–62.

Swiedler SJ, Beck M, Bajbouj M, Giugliani R, Schwartz I, Harmatz P (2005). Threshold effect of urinary glycosaminoglycans and the walk test as indicators of disease progression in a survey of subjects with Mucopolysaccharidosis VI (Maroteaux-Lamy syndrome). *Am J Med Genet* **134A**: 144–150.

Temtamy S, Aglan M (2012). Consanguinity and genetic disorders in Egypt. *Middle East J Med Gen* **1**:12–17.

Valayannopoulos V, Wijburg FA (2011). Therapy for the mucopolysaccharidoses. *Rheumatology* **50**:49–59.

Voskoboeva E, Isbrandt D, von Figura K, Krasnopolskaya X, Peters C (1994). Four novel mutant alleles of the arylsulfatase B gene in two patients with intermediate form of mucopolysaccharidosis VI (Maroteaux-Lamy syndrome). *Human Genet* **93**:259–264