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Clinical, Biochemical, and Molecular Characterization of Metachromatic Leukodystrophy Among Egyptian Pediatric Patients: Expansion of the *ARSA* Mutational Spectrum

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Abstract

Metachromatic leukodystrophy (MLD) is a neurodegenerative disorder characterized by progressive demyelination due to deficiency of the enzyme arylsulfatase A (ARSA) in leukocytes, and consequently leads to impaired degradation and accumulation of cerebroside-3-sulfate (sulfatide). This study aimed to sequence the *ARSA* gene in a total of 43 patients with metachromatic leukodystrophy descendant from 40 Egyptian families. In addition, four carrier parents from two families with children who had died from MLD came to the clinic for genetic analysis. Prenatal diagnosis was performed for four families with molecularly diagnosed MLD sibs. Different mutations were characterized in our cohort, including missense, nonsense, splice, and deletion. Overall, 21 different mutations in the *ARSA* gene were detected, with 12 novel mutations, i.e. p.Arg60Pro, p.Tyr65*, p.Val112Asp, p.Arg116*, p.Gly124Asp, p.Pro193Ser, p.Gln238*, p.Gln456*, p.Thr276Lys, and p.Gly311Arg, in addition to two new acceptor splice-site mutations 685-1G > A and c.954_956 delCTT. The amniotic fluid samples revealed two carrier fetuses with heterozygous monoallelic mutations, and two affected fetuses had the homozygous biallelic mutations. In conclusion, the current study sheds light on the underlying *ARSA* gene defect, with an expansion of the mutation spectrum. To our knowledge, this is the first molecular study of MLD among the Egyptian population.

Keywords Arylsulfatase A · *ARSA* gene · Magnetic resonance imaging (MRI) brain · Metachromatic leukodystrophy (MLD)

Introduction

Metachromatic leukodystrophy (MLD) (MIM #250100) is a severe neurodegenerative disorder inherited as an autosomal recessive trait, with estimated worldwide prevalence of 1.45 per 100,000 births (Giugliani 2012). MLD is caused by deficient activity of the enzyme arylsulfatase A (ARSA) and leads to sulfatide storage within the lysosomes (Cesani et al., 2016). ARSA catalyzes the initial step of the metabolic pathway, sphingolipid 3′-O-sulfogalactosylceramide, known as sulfatide, which accumulates in myelin-producing cells and causes progressive demyelination and neurodegeneration (Ozkan and Ozkara 2016, Dehghan Manshadi et al. 2017 and Issa et al. 2018).

Patients with MLD present with progressive physical and mental deterioration, clumsiness, frequent falls, seizures, and hypotonia. MLD patients are classified into three types, i.e. late infantile, juvenile, and adult forms, based on the age at onset (Biffi et al. 2008). The most severe type is the late infantile onset, which is associated with poor prognosis (death typically occurs within 5–6 years) and manifests as regression of motor skills, gait abnormalities, seizures, ataxia, hypotonia, extensor planters, and optic atrophy (Lynch et al. 2019). The

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juvenile form manifests between 4 and 15 years of age. It is further subdivided into early juvenile and late juvenile subtypes, depending on whether the onset is before or after the age of 6 years, while the adult form manifests after the age of 15 (Kehrer et al. 2014). Magnetic resonance imaging (MRI) of the brain and biochemical assay of ARSA enzymatic activity in leukocytes are used to confirm the diagnosis (Luzi et al. 2013). The first responsible gene defect is *ARSA* (MIM #607574; GenBank accession number, NG_009260), which includes eight exons and maps to chromosome 22q13.33, covering 3.2 kb of genomic DNA (Dehghan Manshadi et al. 2017). The gene is translated into 509 amino acid precursors (GenBank accession number NP_000478). Numerous different mutations have been identified in the *ARSA* gene in the Human Gene Mutation Database, including deletions, splice-site mutations, and mostly missense mutations (Lugowska et al. 2005). The second causative gene defect in MLD patients is the SapB protein derived from the 524 amino acid precursor proteins, encoded by the prosaposin gene (*PSAP*; MIM #176801). The *PSAP* gene is located on chromosome 10q22.1and consists of 15 exons (Cesani et al. 2016).

The ARSA pseudo-deficiency (PD) allele also has common polymorphisms that result in lower than average ARSA activity (Gieselmann et al. 1989). The ARSA-PD allele is characterized by two in-cis A > G transitions. The first c.1049A > G transition alters one of the *N*-glycosylation positions at 350 (p.N350S), resulting in partial mistargeting (approx. 55%) of the enzyme (Harvey et al. 1998). The second *96A > G transition occurs in the first functional polyadenylation signal, leading to a severe deficiency of the major 2.1 kb *ARSA*-mRNA species (Gieselmann et al. 1989), with a consequent reduction in the amount of ARSA protein produced. However, the presence of the modifier PD allele, in either the homozygous or compound heterozygous state, with the disease allele is thought to lower the enzyme activity and possibly increase disease severity (Cesani et al. 2016).

In this study, we investigated 43 patients descendant from 40 families of Egyptian origin by mutation analysis of the complete coding sequences of the *ARSA* gene. To our knowledge, this is the first molecular study of MLD among Egyptian patients.

Patients and Methods

The study included 43 Egyptian MLD patients. Their mean age at presentation was 2.8 years (range 1–7 years). Four parents with a history of deceased MLD children based only on enzyme activity came in for genetic counseling and carrier detection. Additionally, prenatal diagnosis was performed for four mothers with affected patients. The diagnosis was based on clinical features, brain MRI, and *ARSA* enzyme deficiency, followed by molecular testing. All samples were collected after obtaining the guardians' informed consent using a form approved by the ethical committee of the National Research Centre, Egypt.

ARSA Enzymatic Assay

ARSA activity was estimated as μmol/gpt/h protein in leukocytes, using *p*-nitrocatechol sulfate (Wenger et al. 1991).

Molecular Analysis

Genomic DNA was extracted from leukocytes of patients and healthy control subjects, using the standard method, and all eight *ARSA* gene exons and exon–intron boundaries were amplified with primer pairs generated using Primer3 (https://frodo.wi.mit.edu/). Primer sequences and conditions for amplification for each fragment are available upon request. In addition, DNA was extracted from the amniotic fluid (AF) samples of four pregnant mothers with MLD sibs using the QIAamp DNA Blood Mini Kit (QIAGEN Inc., Valencia, CA, USA), according to the manufacturer's protocol. The PCR products were purified using the ExoSAP PCR Cleanup Kit (Fermentas, Germany), sequenced in both directions using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA), and analyzed on the ABI Prism 3500 Genetic Analyzer (Applied Biosystems). Sequencing results were then analyzed on the NCBI website (https://blast.ncbi.nlm.nih.gov/Blast.cgi) and compared with the cDNA sequence of the *ARSA* gene (NM_000487.5). The novel gene variations were confirmed after ruling out polymorphism by the analysis of the exome sequencing data of 50 Egyptian healthy individuals.

In Silico Sequence Variant Validation

To predict the putative effect of the novel missense variants, we used different prediction algorithms including SIFT [Sorting Intolerant From Tolerant] (Kumar et al. 2009), PolyPhen-2 [Polymorphism Phenotyping v2] (Adzhubei et al. 2010) (https://genetics.bwh.harvard.edu/pph2), MutationTaster (https://www.mutationtaster.org/), and I-Mutant2.0 (https://folding.biofold.org/i-mutant/i-mutant2.0.html). In addition, the dbSNP (https://www.ncbi.nlm.nih.gov/snp/), 1000 Genomes (https://browser.1000genomes.org/Homosapiens/Info/Index),

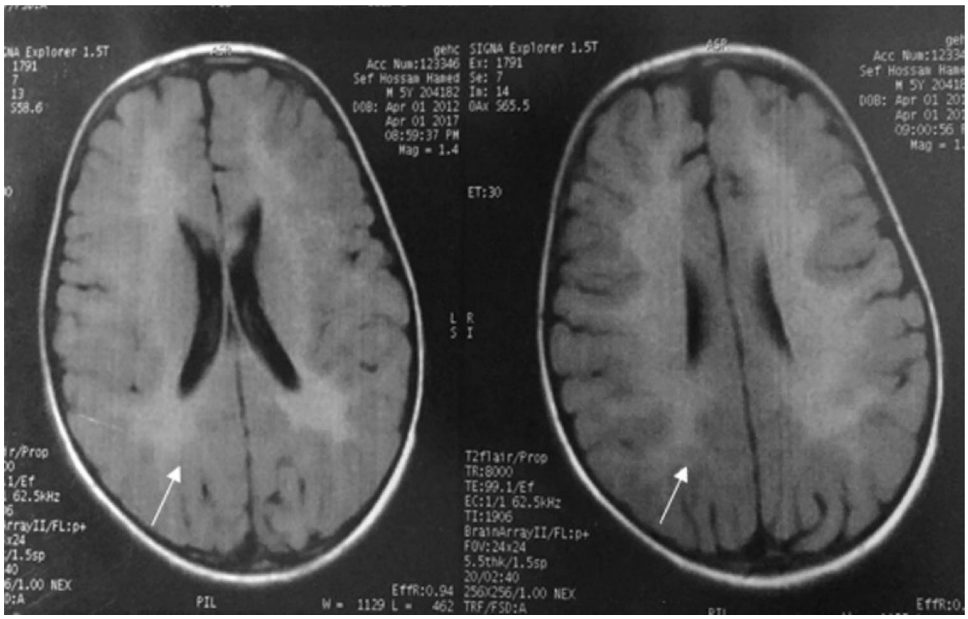


Fig. 1 Brain MRI. T2-weighted MRI of the brain in MLD patient (15) typically reveals white matter hyperintensities (arrow)

Exome Aggregation Consortium (ExAC) (https://gnomad.broadinstitute.org/gene/ENSG00000100299?dataset=gnomad_r2_1), and gnomAD databases were accessed for confirmation of the novel and previously reported variations.

Results

This study included 43 patients from 40 Egyptian families; 27 were female and 16 were male (1.7:1). Thirty-two patients (76.2%) were descendants of consanguineous marriages. Thirty-three patients (76.7%) were classified as late infantile type, and ten (23.3%) were of the juvenile type. All patients presented with regression of motor and mental development. Nine patients (20.9%) showed minor dysmorphic features in the form of depressed nasal bridge, upwards slanting of the eyelids, nystagmus, and low-set ears. Hypertonia was present in 38 patients (90.5%), strabismus in four patients (9.3%), gait disturbance in 10 (23.8%), ataxia in 2 (4.7%), spastic paraplegia in 2 (4.7%), EEG abnormality in 23 (54.8%), microcephaly in 10 (23.3%), cortical atrophy in 7 (16.3%), and white matter demyelination in all patients (Figs. 1

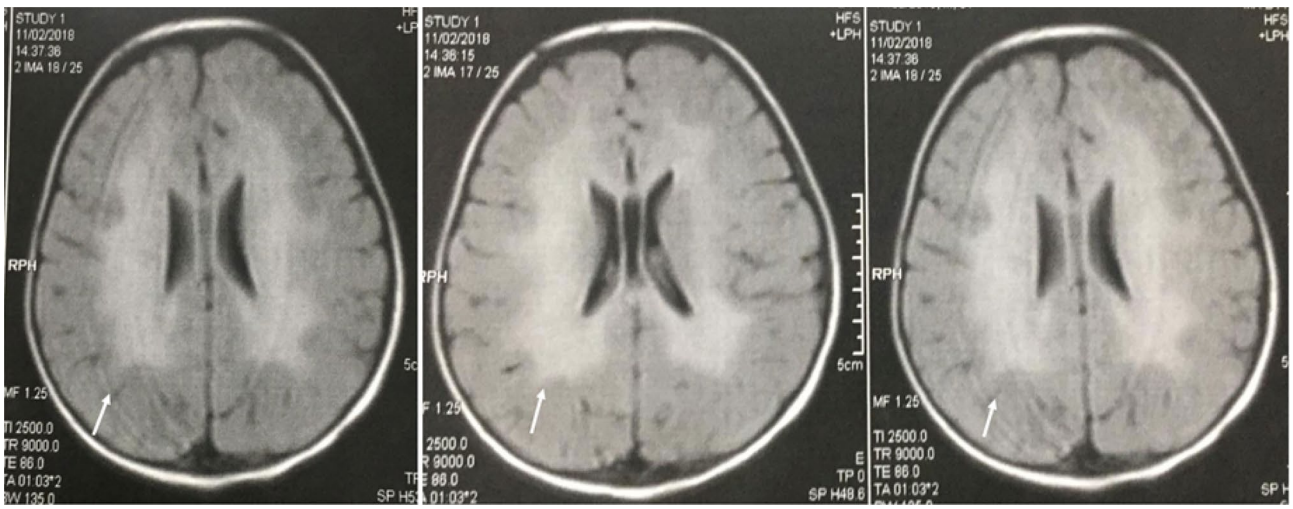


Fig. 2 Brain MRI. T2-weighted MRI of the brain in MLD patient 31 revealed white matter hyperintensities (arrow)

Table 1 Clinical, radiological, biochemical, and molecular characteristics of the MLD patients

Family/patient number	Age in years/Sex	Cons	Positive FH	Dysmorphic features	Neurology	EEG	MRI brain	Arylsulfatase activity	Nucleotide change	Amino acid change	Location	Mutation type	Status	PD allele	References
1/1	3/F	+	-	-	Hypotonia, seizures	-	White matter demyelination	4.5	c.179G > C	p.Arg60Pro	Ex 1	Missense	Homo	Yes	This study
1/2	2/M	+	+	-	Hypertonia	-	White matter demyelination	4.2	c.179G > C	p.Arg60Pro	Ex 1	Missense	Homo	Yes	This study
1/3	1/M	+	+	-	Hypertonia	-	White matter demyelination	4.5	c.179G > C	p.Arg60Pro	Ex 1	Missense	Homo	Yes	This study
2/4	2/F	+	-	-	Hypertonia Hyperreflexia Gait disturbance Microcephaly (−3.8 SD)	Generalized epileptogenic dysfunction	White matter demyelination	0	c.195C > G	p.Tyr65*	Ex 1	Nonsense	Homo	Yes	This study
3/5	1.2/M	+	+	-	Hypotonia, Brisk reflexes	-	White matter demyelination	3.5	c.335 T > A	p.Val112Asp	Ex 2	Missense	Homo	No	This study
3/6	4.2/F	+	+	-	Hypertonia Hyperreflexia Seizures	Generalized epileptogenic dysfunction	White matter demyelination	3.5	c.335 T > A	p.Val112Asp	Ex 2	Missense	Homo	No	This study
4/7	2/F	-	-	-	Hypertonia Hyperreflexia	-	White matter demyelination	4	c.371G > A	p.Gly124Asp	Ex 2	Missense	Homo	No	This study

Table 1 (continued)

Family/patient number	Age in years/Sex	Cons	Positive FH	Dysmorphic features	Neurology	EEG	MRI brain	Arylsulfatase activity	Nucleotide change	Amino acid change	Location	Mutation type	Status	PD allele	References
5/8	2/F	+	-	-	Hypertonia Hyperreflexia	-	White matter demyelination	4	c.371G > A	p.Gly124Asp	Ex 2	Missense	Homo	No	This study
6/9	3/M	-	+	-	Hypertonia Hyperreflexia Gait disturbance	-	White matter demyelination	4.5	c.449C > T c.827C > A	p.Pro150Leu p.Thr276Lys	Ex 2/ Ex 4	Missense Missense	Hetero Hetero	No	Qu et al. ,1999 This study
7/10	1.5/F	-	+	-	Hypotonia Hyperreflexia Seizures	Generalized epileptogenic dysfunction	White matter demyelination	1.5	c.449C > T c.827C > A	p.Pro150Leu p.Thr276Lys	Ex 2 Ex 4	Missense Missense	Hetero Hetero	Yes	Qu et al., 1999 This study
8/11	5/M	+	-	-	Hypertonia Hyperreflexia	Focal epileptogenic dysfunction	White matter demyelination Cortical atrophy	2,2	c.465 + 1G > A	r.?	Intr2	Splice	Homo	Yes	Biffi et al., 2008(6)
9/12	3/F	+	-	-	Hypertonia Hyperreflexia	Focal epileptogenic dysfunction	White matter demyelination Cortical atrophy	2.7	c.465 + 1G > A	r.?	Intr 2	Splice	Homo	Yes	Biffi et al., 2008(6)
10/13	4/F	+	-	-	Hypertonia Hyperreflexia	-	White matter demyelination Cortical atrophy	1.7	c.571G > A	p.Pro193Ser	Ex 3	Missense	Homo	Yes	This study

Table 1 (continued)

Family/patient number	Age in years/Sex	Cons	Positive FH	Dysmorphic features	Neurology	EEG	MRI brain	Arylsulfatase activity	Nucleotide change	Amino acid change	Location	Mutation type	Status	PD allele	References
11/14	3/M	+	-	-	Hypertonia Hyperreflexia	Epileptogenic dysfunction	White matter demyelination	2.5	c.571G > A	p.Pro193Ser	Ex 3	Missense	Homo	Yes	This study
12/15	3/F	+	-	-	Hypertonia Hyperreflexia	-	White matter demyelination	1.5	c.571G > A	p.Pro193Ser	Ex 3	Missense	Homo	Yes	This study
13/16	3/M	+	-	+	Hypertonia Hyperreflexia Strabismus Ataxia Microcephaly (−3.8SD)	Epileptogenic dysfunction	White matter demyelination	1	c583_583delT	p.Trp195fs*5	Ex 3	Deletion	Homo	No	Cesani et al., 2016
14/17	4/F	+	-	+	Hypertonia Gait disturbance Strabismus Ataxia, spastic paraplegia	Generalized epileptogenic dysfunction	White matter demyelination	0	c583_583delT	p.Trp195fs*5	Ex 3	Deletion	Homo	No	Cesani et al., 2016
15/18	3/F	_	+	-	Hypertonia Hyperreflexia Seizures	Generalized epileptogenic dysfunction	White matter demyelination	1.5	c.585G > T c.827C > A	p.Trp195Cys p.Thr276Lys	Ex 3 Ex 4	Missense Missense	Hetero Hetero	Yes	Dehghan et al., 2017 This study
16/19	5/M	-	-	-	Hypertonia Gait disturbance	-	White matter demyelination	1.8	c.585G > T c.960G > T	p.Trp195Cys p.Trp320Cys	Ex 3 Ex 5	Missense Missense	Hetero Hetero	Yes	Dehghan et al., 2017 This study

Table 1 (continued)

Family/patient number	Age in years/Sex	Cons	Positive FH	Dysmorphic features	Neurology	EEG	MRI brain	Arylsulfatase activity	Nucleotide change	Amino acid change	Location	Mutation type	Status	PD allele	References
17/20	3/F	+	-	-	Hypertonia Hyperreflexia	-	White matter demyelination	1	c.685-1G > A	r.?	Intr 3	Splice acceptor	Homo	Yes	This study
18/21	4/F	+	-	+	Hypertonia Hyperreflexia Seizures Microcephaly (−3.2 SD)	Generalized epileptogenic dysfunction	White matter Demyelination	0	c.712C > T	p.Gln238*	Ex 4	Nonsense	Homo	Yes	This study
19/22	3/F	+	-	+	Hypertonia Hyperreflexia Seizures	Generalized epileptogenic dysfunction	White matter demyelination	0	c.712C > T	p.Gln238*	Ex 4	Nonsense	Homo	Yes	This study
20/23	2/F	+	-	+	Hypertonia Gait disturbance Seizures	Generalized epileptogenic dysfunction	White matter demyelination	1	c.712C > T	p.Gln238*	Ex 4	Nonsense	Homo	Yes	This study
21/24	2/F	+	-	+	Hypertonia Hyperreflexia Gait disturbance	Generalized epileptogenic dysfunction	White matter demyelination Cortical atrophy	1	c.712C > T	p.Gln238*	Ex 4	Nonsense	Homo	Yes	This study
22/25	2/M	+	-	+	Hypertonia Hyperreflexia Seizures Microcephaly (−3 SD)	Generalized epileptogenic dysfunction	White matter demyelination	0	c.712C > T	p.Gln238*	Ex 4	Nonsense	Homo	Yes	This study

Table 1 (continued)

Family/patient number	Age in years/Sex	Cons	Positive FH	Dysmorphic features	Neurology	EEG	MRI brain	Arylsulfatase activity	Nucleotide change	Amino acid change	Location	Mutation type	Status	PD allele	References
23/26	4//F	+	+	+	Hypertonia Seizures Gait disturbance	Epileptogenic dysfunction	White matter demyelination	0	c.712C>T	p.Gln238*	Ex 4	Nonsense	Homo	Yes	This study
24/27	7/F	-	+	-	Hypertonia Hyperreflexia Learning problems	-	White matter demyelination	4.5	c.827C>A c.960G>T	p.Thr276Lys p.Trp320Cys	Ex 4 Ex 5	Missense Missense	Hetero Hetero	No	This study Önder et al., 2009
25/28	2/M	+	-	-	Hypertonia Hyperreflexia	-	White matter demyelination Cortical atrophy	5	c.851A>G	p.Asn284Ser	Ex 4	Missense	Homo	No	Grossi et al., 2008
26/29	1/F	+	-	-	Hypertonia Hyperreflexia Seizures	Generalized epileptogenic dysfunction	White matter demyelination	4.7	c.851A>G	p.Asn284Ser	Ex 4	Missense	Homo	No	Grossi et al., 2008
27/30	1/F	+	+	-	Hypotonia Seizures, Microcephaly (−3.9 SD), Strabismus	Generalized epileptogenic dysfunction	White matter demyelination	4.5	c.851A>G	p.Asn284Ser	Ex 4	Missense	Homo	No	Grossi et al., 2008
28/31	3/M	+	+	-	Hypotonia Hyperreflexia Spastic paraparesis Strabismus		White matter demyelination	4.7	c.851A>G	p.Asn284Ser	Ex 4	Missense	Homo	No	Grossi et al., 2008

Table 1 (continued)

Family/patient number	Age in years/Sex	Cons	Positive FH	Dysmorphic features	Neurology	EEG	MRI brain	Arylsulfatase activity	Nucleotide change	Amino acid change	Location	Mutation type	Status	PD allele	References
29/32	2/F	+	+	-	Hypertonia Hyperreflexia Gait disturbance	-	White matter demyelination Cortical atrophy	4.5	c.851A > G	p.Asn284Ser	Ex 4	Missense	Homo	No	Grossi et al., 2008
30/33	3/F	+	+	-	Hypertonia Hyperreflexia	-	White matter demyelination	3.5	c.851A > G	p.Asn284Ser	Ex 4	Missense	Homo	Yes	Grossi et al., 2008
31/34	2/F	+	+	-	Hypertonia Hyperreflexia Seizures	Generalized epileptogenic dysfunction	White matter demyelination	4.8	c.851A > G	p.Asn284Ser	Ex 4	Missense	Homo	No	Grossi et al., 2008
32/35	3/M	+	+	-	Hypertonia Hyperreflexia	-	White matter demyelination	4.7	c.851A > G	p.Asn284Ser	Ex 4	Missense	Homo	No	Grossi et al., 2008
33/36	4/M	+	+	-	Hypertonia Hyperreflexia Gait disturbance	-	White matter demyelination	3.1	c.883 G > A	p.Gly295Ser	Ex 5	Missense	Homo	Yes	Berná et al., 2004
34/37	2/F	+	-	-	Hypertonia Hyperreflexia Gait disturbance	-	White matter demyelination	4.2	c.883 G > A	p.Gly295Ser	Ex 5	Missense	Homo	Yes	Berná et al., 2004

Table 1 (continued)

Family/patient number	Age in years/Sex	Cons	Positive FH	Dysmorphic features	Neurology	EEG	MRI brain	Arylsulfatase activity	Nucleotide change	Amino acid change	Location	Mutation type	Status	PD allele	References
35/38	2/F	+	+	-	Hypertonia Hyperreflexia seizures	Focal epileptogenic dysfunction	White matter demyelination Cortical atrophy	3	c.883 G > A	p.Gly295Ser	Ex 5	Missense	Homo	Yes	Berná et al., 2004
36/39	6/M	+	-	+	Hypertonia Hyperreflexia Microcephaly (−3 SD)	Focal epileptogenic dysfunction	White matter demyelination Cortical atrophy	2	c.931G > C	p.Gly311Arg	Ex 5	Missense	Homo	Yes	This study
37/40	3/M	+	-	+	Hypertonia Hyperreflexia Microcephaly (−3.5 SD)	Generalized epileptogenic dysfunction	White matter demyelination	1.5	c.954_956 del CTT	p.Phe319	Ex 5	Deletion	Homo	No	This study
38/41	1/M	_	-	-	Hypertonia Hyperreflexia Seizures, Microcephaly (−3 SD)	-	White matter demyelination	3.5	c.960G > T c.585G > T	p.Trp320Cys p.Trp195Cys	Ex 5 Ex 3	Missense Missense	Hetero Hetero	Yes	Önder et al., 2009 Dehghan et al., 2017
39/42	2/F	_	-	-	Hypertonia Hyperreflexia Seizures Microcephaly (−3.8 SD)	Focal epileptogenic dysfunction	White matter demyelination	4	c.960G > T c.847G > T	p.Trp320Cys p.Trp195Cys	Ex 5 Ex 3	Missense Missense	Hetero Hetero	Yes	Önder et al., 2009 Dehghan et al., 2017

Table 1 (continued)

Family/patient number	Age in years/Sex	Cons	Positive FH	Dysmorphic features	Neurology	EEG	MRI brain	Arylsulfatase activity	Nucleotide change	Amino acid change	Location	Mutation type	Status	PD allele	References
40/43	3/F	+	-	+	Hypertonia Hyperreflexia Seizures Microcephaly (−3 SD)	Focal epileptogenic dysfunction	White matter demyelination	0	c.1366C > T	p.Gln456*	Ex 8	Nonsense	Homo	Yes	This study

Cons: consanguinity, Positive FH: positive family history, EEG: electroencephalogram, MRI: magnetic resonance imaging, r?: refers to unknown reference sequence, PD: pseudo allele

and 2). The biochemical analysis of the arylsulfatase A enzyme activity in leukocytes (expressed as µmol/gpt/h) was deficient in all 43 patients and ranged between 0 and 5 µmol/gpt/h.

Molecular Results

Sequencing of all coding regions of the *ARSA* gene for the 43 patients and their available parents revealed a total of 21 causative mutations with 12 novel variants (Table 1). The majority of the ARSA-MLD allele types varied between single base substitution, nonsense mutation, small deletion, and splice-site mutation. The six new missense variants were NM_000487.5 p.Arg60Pro, p.Val112Asp, p.Gly124Asp, p.Pro193Ser, p.Thr276Lys, and p.Gly311Arg, four new stop codon mutations (p.Try65*, Arg116*, p.Gln238*, p.Gln456*), one new acceptor-splice acceptor site mutation 685-1 G > A that would be expected to disrupt splicing, and one in-frame deletion c.954_956delCTT. Most of the new characterized missense mutations showed a change in the characteristic chemical nature of the resulting amino acid, including a change from hydrophilic polar to hydrophobic non-polar amino acid of (Arg60Pro), from hydrophobic non-polar to hydrophilic polar of each p.Val112Asp, p.Gly124Asp, p.Pro193Ser, and p.Gly311Arg, and from polar uncharged to polar positive p.Thr276Lys (Figs. 3 and 4). Interestingly, most of the characterized mutations were found in the homozygous state (36/43, 83.7%), with the exception of seven (7/43, 16.3%) patients who had a compound heterozygous causative variant (Table 1). In addition, four parents with children who had died from MLD and were requesting genetic counseling, were found to be carriers (Table 2). Prenatal diagnosis was also performed in four families and revealed that two affected fetuses had the homozygous biallelic mutation and two were carrier fetuses with heterozygous monoallelic mutations (Table 3). All novel mutations were predicted for the functional impact of an amino acid based on the alignment of highly similar orthologous and/or paralogous protein sequences and predicted to be "disease-causing" by in silico analysis (Table 4). Also, the novel potential mutations were defined by exclusion from the Human Gene Mutation Database (https://www.hgmd.cf.ac.uk) and the previously reported mutations on PubMed (https://www.ncbi.nlm.nih.gov/PubMed/).

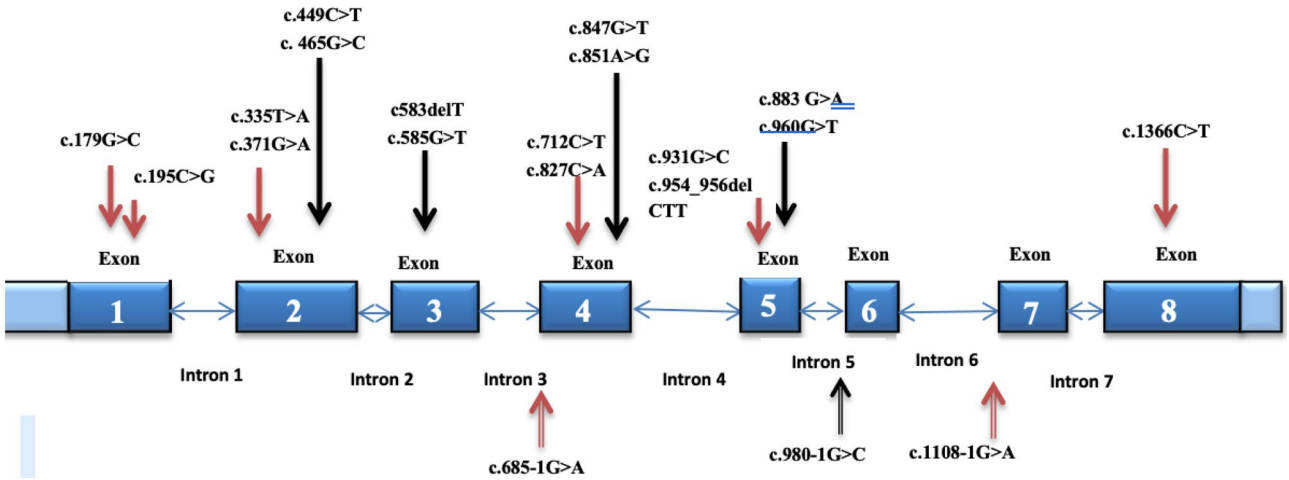


Fig. 3 The distribution of the alleles is reported in relation to the *ARSA* gene. The *ARSA* gene maps to chromosome 22q13 covering 3.2 kb of genomic DNA. The map of the *ARSA* gene depicts the positions of eight exons (blue boxes numbered 1–8) and between numbered introns (pale blue lines). All exonic variants are given above the gene schema, all intronic below. The red arrows point to the novel mutations detected in this study, and the black arrows point to the reported known variants

Discussion

Metachromatic leukodystrophy is a lysosomal storage disease, with more than 264 mutations reported in different populations (https://www.LOVD.nl/ARSA). The diagnosis of MLD depends on the presence of the clinical phenotypic characteristics, the deficiency of the ARSA activity in leukocytes, and the molecular testing of the *ARSA* gene (Liaw et al. 2015). In the current study, we report on the clinical, biochemical, and molecular findings in 43 patients descendant from 40 Egyptian families.

The late infantile variant accounted for 76.7% of the studied group, while 23.3% were of the juvenile type. The late infantile subtype is the most common and presents with severe clinical features, including gait disturbances, frequent falls, and regression of motor and mental functions (Cesani et al. 2016; Wang et al. 2019). Five patients showed mild dysmorphic features in the form of depressed nasal bridge, upward slanting of eyelids, nystagmus, and low-set ears. Nerve conduction studies showed delayed motor and/or sensory conduction velocity, consistent with demyelinating peripheral neuropathy, in all the investigated patients. Cameron et al. (2004) emphasized that slowing of the nerve conduction velocity is the hallmark of MLD.

In this study, the biochemical analysis of the arylsulfatase A enzyme activity in leukocytes was deficient compared to controls. This reduction occurs in certain conditions including classical MLD, the presence of PD allele, compound heterozygosity for the pathogenic variant, and PD alleles of the *ARSA* gene without white matter disease (Wang et al. 2016). The presence of the PD alleles with other pathogenic variants in the *ARSA* gene in the same patient leads to greater deficiency of enzyme activity and possibly increased disease severity (Regis et al. 2004). Based on MRI, all patients showed white matter demyelination, and only four had cortical atrophy consistent with previous reports (Grossi et al. 2008 and Wang et al. 2019).

The sequencing of MLD patients in the present study identified 12 novel causative variants in the *ARSA* gene. MLD is known as a heterogeneous disease, and *ARSA* gene variants are not distributed homogeneously, but tend to cluster around exons 2 and 4. It is worth mentioning that p.Asn284Ser was encountered in eight patients belonging to eight unrelated families, showing the highest density of all identified mutations (8/43, 18.6%), which may be evidence of a founder effect in MLD patients with a common ancestor in an Egyptian population. This mutation, previously reported in an Italian patient, prevents the correct positioning of the sulfate group of the substrate and consequently its hydrolysis, leading to low residual ARSA activity compared to controls (Qu et al. 1999).

Similarly, the novel c.712C > T mutation detected in six patients (6/43, 13.95%) suggests that it might be a founder mutation of the *ARSA* gene in Egyptian families with MLD, and results in severe reduction in enzyme activity due to premature termination at codon Gln238*. In addition, the p.Trp195Cys mutation found in four patients (4/43, 9.3%) was previously reported in a MLD patient from Iran, with 2.8% reduced enzyme activity (Dehghan Manshadi et al. 2017). The new p.Thr276Lys mutation encountered in four patients has not been reported in the current

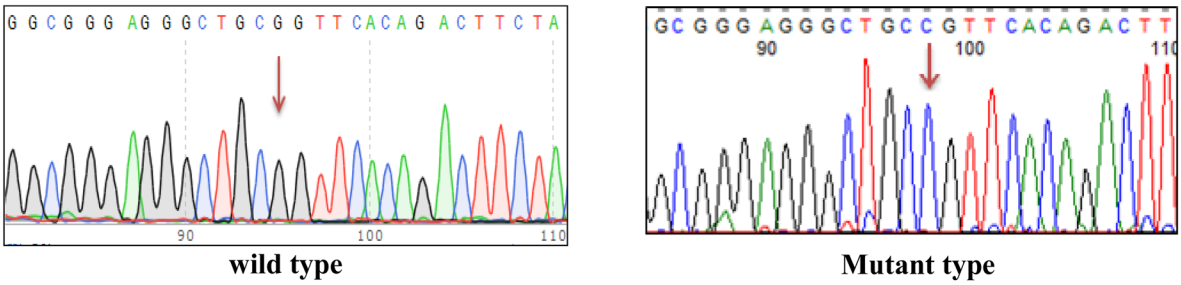


Fig. 4 Portion of the sequencing electrophoregrams displaying the *ARSA* gene variants identified in our patients. The arrow indicates the site of the variant

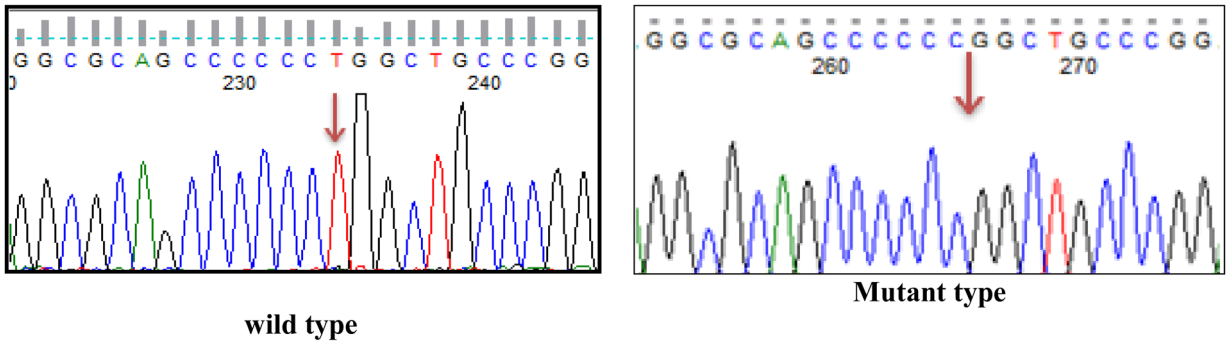
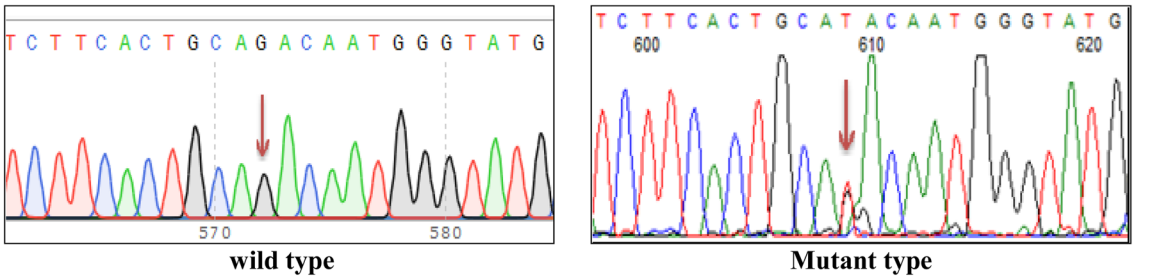
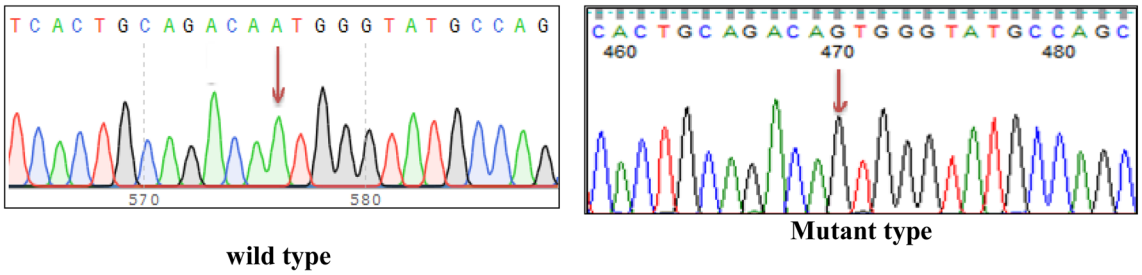


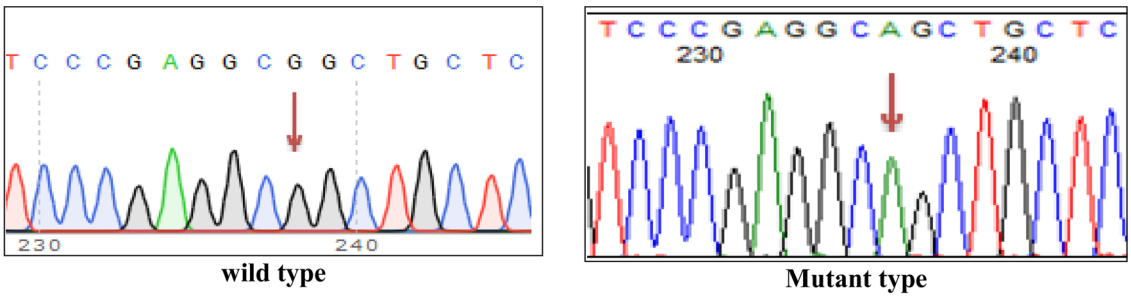
Fig. 4 (continued)



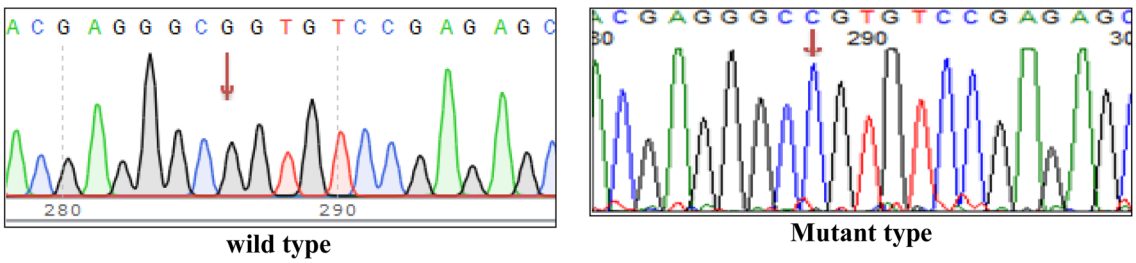
c.847G>T(P.Asp283Tyr)



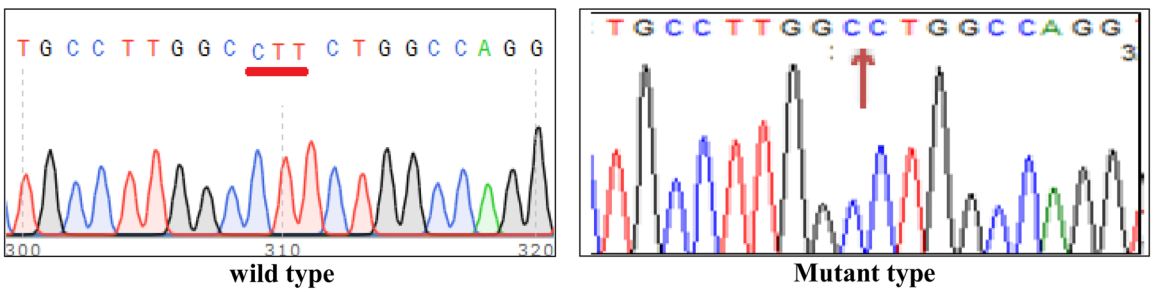
c.851A>G p.Asn284Ser



c.883G>A (p.Gly295Ser)

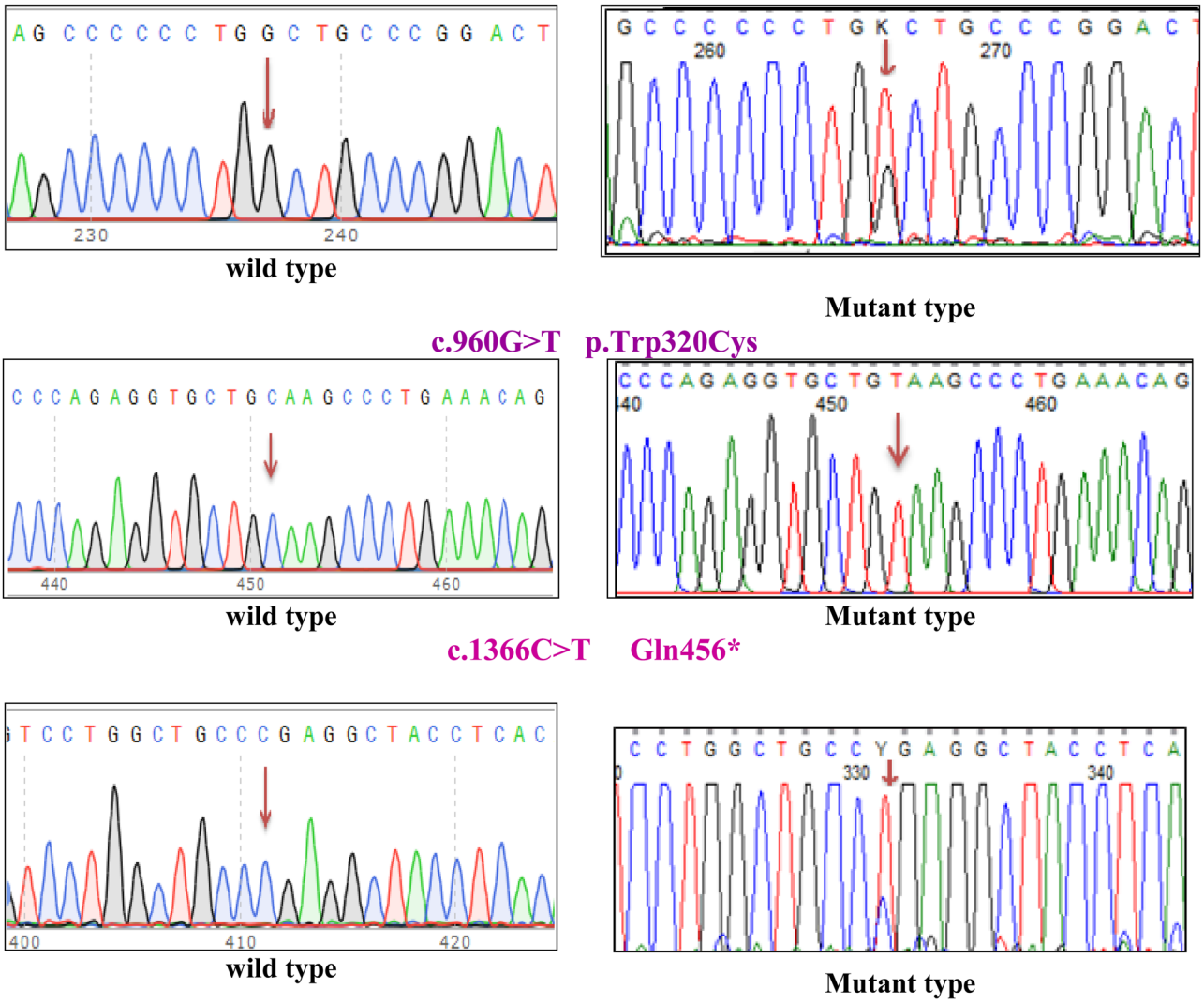


c.931G>C p.Gly311Arg



c.954_956delCTT (p.Phe319)

Fig. 4 (continued)



public databases dbSNP (https://www.ncbi.nlm.nih.gov/SNP/) and gnomAD (https://gnomad.broadinstitute.org/gene/ENSG00000100299?dataset=gnomad_r2_1), nor has it been described in the 1000 Genomes Project data sets (https://www.1000genomes.org), but it was reported in the same codon Thr276, with the substitution of lysine to methionine, in six patients of Lebanese descent (Harvey et al. 1993). The infrequently encountered mutations among our study group such as c.459 + 1G > A, p.Pro426Leu, and p.Iso179Ser were found to be common in the European population, and p.Gly99Asp, p.Gly245Arg, and p.Thr409Ile were found in Japanese populations (Lugowska et al. 2005 and Zhi-Hong et al. 2016). This may be due to differences in ethnic origin.

Table 2 Molecular characteristics for the carrier parents

	Age/Sex	Nucleotide change	Amino acid change	Location	Mutation type	Status	PD Allele	References
1	32/M	c.827C > A	p.Thr276Lys	Ex 4	Missense	Heterozygous	No	This study
	26/F	c.827C > A	p.Thr276Lys	Ex 4	Missense	Heterozygous		This study
2	35/M	c.346C > T	p.Arg116*	Ex 2	Nonsense	Heterozygous	No	This study
	28/F	c.346C > T	p.Arg116*	Ex 2	Nonsense	Heterozygous		This study

Table 3 Molecular analysis of the amniotic fluid samples

	Gestational age	Nucleotide change	Amino acid change in affected proband	Location	Mutation type	Status	PD Allele	References
1	16 weeks	c.712C > T	p.Gln238*	Exon 4	Missense	Heterozygous	No	This study
2	16 weeks	c.346C > T	p.Arg116*	Exon 2	Nonsense	Homozygous	No	This study
3	15 weeks	c.851A > G	p.Asn284Ser	Exon 4	Missense	Homozygous	yes	Grossi et al., 2008
4	16 weeks	c.960G > T	Trp320Cys	Exon 5	Missense	Heterozygous	Yes	Önder et al., 2009

Most of the characterized mutations were present in a homozygous pattern, consistent with the pattern of autosomal recessive inheritance of MLD and the high consanguineous rate among the Egyptian population (Regis et al. 2004). Interestingly, the six unreported missense variants p.Arg60Pro, p.Val112Asp, p.Gly124Asp, p.Thr276Lys, p.Pro193Ser, p.Arg498His, and p.Gly311Arg (Table 1) were suggested to have a pathological nature on protein feature. In silico tools revealed that it might involve an alteration of metal binding. This was supported by the involvement of conserved residues in the ARSA protein and their absence in more than 100 chromosomes of the Egyptian population, confirming the absence of any spurious changes. The enzyme levels of arylsulfatase in these patients were severely affected compared to controls, suggesting that the resulting mutant protein primarily affects its folding and decreases its stability, which is concordant with previous reports (Dorboz et al. 2009; Cesani et al. 2016). On the other hand, the characterized missense variants p.Pro150Leu, p.Trp195Cys, p.Thr276Met, p.Asp283Tyr, p.Asn284Ser, p.Gly295Ser, and p.Trp320Cys in our patients were previously detected in different ethnic groups (Harvey et al. 1993; Tsuda and Hasegawa 1996; Halsall et al. 1999; Qu et al. 1999; Berná et al. 2004; Grossi et al. 2008; Önder et al. 2009; Dehghan Manshadi et al. 2017). Assessment of the phenotypic presentation of this group was marked by variable severity and varying age at onset, with the possible presence of a genotype–phenotype correlation, as a result of enzyme deficiency and altered protein production.

Some of the identified mutations, such as p.Pro150, p.Gly295, p.Asp283, and p.Asn284, exhibited their effect on ARSA activity either through their default binding interactions with other neighboring proteins, or by functionally disrupting the protein folding. They were also found to have an effect on the correction of the folding in the ER or instability of the lysosomes and consequently their hydrolysis. In addition, their presence in cis with the pseudo-allele would ultimately inactivate the enzyme (Halsall et al. 1999; Kay et al. 2000; Berná et al. 2004; Grossi et al. 2008). Furthermore, the unreported nonsense mutations p.Gln238* found in patients 21–26, p.Gln456* in patient 43 (Table 1), and p.Arg116* in two carrier parents screened for carrier detection (Table 2) produce a premature termination codon that could lead to either nonsense-mediated mRNA decay (NMD) or a truncating protein escaping NMD, with dominant negative activity. In the latter process**,** a more severe phenotype is produced as a result of the dominant negative effects of the translated faulty protein (Inoue et al., 2004). Further functional studies are needed to determine whether the detected nonsense mutations may cause NMD.

Table 4 In silico analysis and mutation prediction pathogenicity score for new detected variants

No	Mutation	SIFT*	PolyPhen-2* Probably damaging	MutationTaster Disease-causing	MutPred* Deleterious	Proven as deleterious
1	c.179G > C p.R60P	Tolerated (0.09)	(1.000)	(103)	(0.903)	(−3.382)
2	c.335 T > A p. Val112Asp	Damaging (0. 04)	(0.961)	(152)	(0.873)	(−3.219)
3	c.371G > A p.Gly124Asp	Damaging (0. 0)	(1.000)	(94)	(0.916)	(−5.563)
4	c.571G > A p.Pro193Ser	Damaging (0. 01)	(1.000)	(58)	(0.531)	(−7.701)
5	c.827C > A p.Thr276Lys	Damaging (0.0)	(1.000)	(76)	(0.898)	(−5.862)
6	c.931G > C p.Gly311Arg	Damaging (0.0)	(1.000)	(125)	(0.963)	(−7.904)

SIFT: threshold for intolerance is 0.05; PolyPhen-2, and MutPred score ranges from 0.0 (tolerated) to 1.0 (deleterious); MutationTaster: Scores range from 0.0 to 215; otherwise it is predicted as "Neutral"; and Proven scores ≤ -2.5 are predicted as “Deleterious"

This study also reported a new splice variant, c.685-1G > A, in patient 18, in addition to the reported donor splice c.465 + 1G > A in patients 10 and 11, which are the most frequent mutations detected in Caucasians and Italian patients as described by Biffi et al. (2008). The effect of these two mutations that occurred in splice-site consensus sequences is mis-splicing that does not alter the reading frame but would disrupt the amino acid sequence via either the loss or addition of amino acid residues in c.685-1G > A and c.465 + 1G > A, respectively. Both of these alterations in the primary structure of the protein would likely interfere with folding and lead to protein instability, and mRNA functional analysis is needed to confirm their effect.

Furthermore, our study detected a new in-frame deletion 3bpc.954_956delCTT located in exon 5 in one patient (patient 40), in addition to the previously reported in-frame deletion c.583delT found in patients 16 and 17. This new in-frame deletion was found to be deleterious, with a score of −9.89, using PROVEAN [Protein Variation Effect Analyzer] pathogenicity prediction software (cutoff value of −2.5). Our cohort suggests a genotype/phenotype correlation, as patients with either deletions or nonsense mutations were associated with severe deficiency of ARSA activity and presented with severe clinical manifestations and extremely rapid disease progression. These findings are concordant with previous reports (Biffi et al. 2008; Hettiarachchi and Dissanayake 2020).

In this study, three families underwent prenatal testing of the targeted causative variants and were given genetic counseling. The obvious variability in the clinical phenotype and the correlation with the characterized genotype for predicting the patient's prognosis represents a challenge, especially in those who harbor heteroallelic mutations, one identified allele and one new allele, as in patients 9, 10, 18, 19, and 27. These results suggest that Egyptian MLD patients have a different distribution of ARSA mutations than those found in Caucasian or other Arab populations (Dorboz et al. 2009; Luzi et al. 2013).

Although there is no curative treatment currently available, hematopoietic stem cell transplantation has slowed disease progression in some patients. Future novel therapies, such as enzyme replacement and gene editing, are promising (Böhringer et al. 2017).

Conclusion

Our findings expanded the spectrum of ARSA mutations with 12 novel variants, discovered in Egyptian families affected by MLD. Furthermore, it provided carrier detection, prenatal diagnosis, and proper genetic counseling. To our knowledge, this is the first detailed clinical, biochemical, and molecular testing report of MLD patients in Egypt. The metachromatic leukodystrophy diagnosis should be considered in cases with consanguineous marriages, developmental regression, and slowing of nerve conduction velocity. A genotype/phenotype association was observed with regard to enzyme activity and underlying molecular pathology. Further investigations of the novel mutations through in vitro expression experiments are needed to reveal their impact on protein function.

Declarations

Funding This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Compliance with Ethical Standards

Conflict of Interest All authors declare no conflicts of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration. Informed consent was obtained from all individual participants included in the study.

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