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Spectrum of genetic mutations in methylmalonic aciduria among Iranian patients

Mohadeseh Fathi[1,2,4], Sheyda Khalilian[1,2,4], Mohammad Miryounesi[3]✉ & Soudeh Ghafouri-Fard[2]✉

Methylmalonic aciduria (MMA) is described by high methylmalonic acid concentrations in the blood and urine. This condition can be isolated or in combination with homocystinuria. While variants in the *MMUT*, *MMAA*, *MMAB*, *MMADHC* and *MCEE* genes contribute to the pathogenesis of the isolated form, variants in *MMACHC*, *MMADHC*, *LMBRD1*, and *ABCD4* genes, are responsible for diverse types of combined MMA and homocystinuria. In the current study, we report molecular tests of 15 Iranian patients who had mutations in MMA-related genes. Among the assessed patients, *MMACHC* gene was the most prevalently mutated gene (mutated in 7 patients). Each of *MMAA*, *MMAB*, and *MMUT* genes were mutated in 2 patients, respectively. Finally, we detected variants in each of *ACSF3* and *ABCD4* genes in one case, respectively. Among the identified variants, five variants were not reported before. Cumulatively, the current study provides some data about MMA-related variants among Iranian patients.

Keywords Methylmalonic aciduria, Genetics, Iran

Isolated methylmalonic aciduria (MMA) encompasses a group of metabolic disorders described by elevated methylmalonic acid concentration in the blood and urine[1]. Its frequency is about 1:50 000 people. The underlying cause is a defect in the isomerization (conversion) of methylmalonyl-coenzyme A (CoA) into succinyl-CoA in the course of propionyl-CoA metabolism in the mitochondrial matrix. In the isolated form of disorder, there is no variation in other metabolites, thus there is no associated hyperhomocysteinemia, homocystinuria, or hypomethioninemia. This disorder is caused by complete or incomplete defects in the enzyme methylmalonyl-CoA mutase (encoded by *MMUT* gene)[2], a deficiency in the transport or biosynthesis of its cofactor, 5-deoxy-adenosyl-cobalamin (cblA, cblB, or cblD-MMA, due to variants in *MMAA*, *MMAB*, and *MMADHC* genes, respectively[3]), or deficiency of the enzyme methylmalonyl-CoA epimerase (encoded by *MCEE* gene[4]). *MMUT* gene encodes a key enzyme responsible for the catabolism of the branched-chain amino acids, odd-chain fatty acids and the side chain of the cholesterol, through facilitating transformation of methylmalonyl-CoA to succinyl-CoA as substrate for the Krebs cycle[5]. A number of MMA variants are described as combined MMA with homocystinuria. These variants are caused by defect in the transport or synthesis of MMUT cofactor (adenosylcobalamin) or methionine synthase cofactor (methylcobalamin). Targeted metabolomics shows high level of propionylcarnitine (C3) in dry blood spot in MMA patients .

The long-term prognosis of MMA is determined by the affected gene and the severity of the variant. The majority of MMA patients have variants in the *MMUT* gene[6]. This gene is responsible for coding methylmalonyl-CoA mutase, an enzyme that cooperates with vitamin B12 to break down numerous amino acids, some types of lipids, and cholesterol[7]. Variants that preclude the synthesis of any functional enzyme lead to a form of the disorder nominated as *mut0*[8]. This is the most severe form of MMA with the poorest outcome. Variants that alter the configuration of methylmalonyl-CoA mutase but do not abolish its activity result in a less severe form (*mut-*)[9].

MMA has a significant morbidity and mortality. In fact, the long-term survival of patients is not favorable. While early reports of 1980s demonstrated a mortality rate of 60–88% for mut MMA[10], this rate was shown to be decreased to about 40% in the 2000s[11]. A more recent study showed that the long-term survival of MMA patients differs from normal to life-threatening outcomes based on multiple factors, such as "pre-treatment onset", "diagnosed with or without newborn screening", and the presence of certain *MMACHC* variants[12].

[1]Student Research Committee, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran. [2]Department of Medical Genetics, Shahid Beheshti University of Medical Sciences, Tehran, Iran. [3]Genomic Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran. [4]These authors contributed equally: Mohadeseh Fathi and Sheyda Khalilian. ✉email: miryounesi@gmail.com; s.ghafourifard@sbmu.ac.ir

Proteins encoded by the *MMAA*, *MMAB*, and *MMADHC* genes contribute to the appropriate functions of methylmalonyl-CoA mutase[3]. Finally, methylmalonyl CoA epimerase contributes to the breakdown of amino acids, some types of lipids, and cholesterol. Defects in the function of methylmalonyl CoA epimerase result in a form of MMA with various clinical signs[4].

In addition, there are diverse types of combined MMA and homocystinuria, namely cblC, cblD, cblF, and cblJ, caused by variants in *MMACHC*, *MMADHC*, *LMBRD1*, and *ABCD4* genes, respectively[13–16].

In the current study, we used whole exome sequencing (WES) technique to find the pathogenic variants that lead to MMA among Iranian patients to find mutation spectrum of MMA-related genes in Iranian patients and use the data for genetic counseling of at-risk families.

Case presentation

This study was performed on 15 unrelated Iranian cases of MMA. Cases were obtained through consecutive referrals. The following inclusion and exclusion criteria were considered.

Inclusion criteria

Patients were included in the study if they met the following criteria:

1. Diagnosis: Clinical and biochemical suspicion of MMA based on:

- Elevated methylmalonic acid levels in blood/urine.
- Clinical symptoms (such as metabolic acidosis, hyperammonemia, and developmental delay).

2. Referral: Patients referred to the Comprehensive Genomic Center, Tehran, Iran (2018–2024) for genetic confirmation.
3. Consent: Legal representatives provided written informed consent for genetic testing and study participation.
4. Data Availability: Availability of clinical, biochemical, and follow-up data for analysis.

Exclusion criteria

Patients were excluded if they had one of the following conditions:

1. Incomplete Data: Lack of sufficient clinical or laboratory records for confirmation.
2. Alternative Diagnoses: Confirmed genetic or metabolic disorders other than MMA.
3. Non-Consent: Cases where informed consent was not obtained.

Patient selection process

A structured approach was used to identify and enroll eligible MMA cases:

1. Referral & Initial Screening:

- Patients were referred to the Comprehensive Genomic Center (2018–2024) due to clinical/biochemical suspicion of MMA.
- Preliminary assessment included medical history, metabolic workup (e.g., plasma/urine MMA levels, ammonia, gas analysis), and exclusion of other metabolic disorders.

2. Eligibility Confirmation:

- Cases with elevated methylmalonic acid and compatible symptoms proceeded to genetic testing.
- Patients with incomplete records or unclear diagnoses were excluded.
- Genetic Testing & Enrollment:
  - WES was performed for molecular confirmation.
  - Those with variants in MMA-associated genes and signed consent were included in the final cohort ($n = 15$). All cases were unrelated.

3. Ethical Approval:

- The study was approved by the Ethics Committee of Shahid Beheshti University of Medical Sciences.
- All procedures followed declaration of Helsinki guidelines.

Molecular diagnosis

Genomic DNA was obtained from the peripheral blood of MMA cases using the standard salting-out procedure. The concentration and quality of DNA were evaluated using a NanoDrop 1000 (Thermo Fisher Scientific, USA). Genomic DNA of probands was subjected to WES using an Illumina HiSeq4000 system with paired-end reads of 101 bp and 100X coverage. Exonic and adjoining exon-intron border regions were enriched using SureSelectXT2 V6 kits. After exclusion of low-quality reads, the reads were mapped to the human genome reference (hg37 build) using the Burrows-Wheeler Aligner version 1 (https://bio-bwa.sourceforge.net/). Next, Sequence Alignment/Map (SAM) tools version 1.6 (http://samtools.sourceforge.net) were used for detection and removal of duplicates. Then, recalibration and single nucleotide polymorphism/indel calling were conducted. Variant calling and filtering were done using the Genome Analysis Toolkit version 4 (https://gatk.broadinstitute.org/hc

/en-us). The primary step involved manual filtration process. First, formerly reported variants in ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/) were evaluated for their association with the clinical finding. At that point, we considered the allele state. Finally, the called variants were annotated and ranked using ANNOVAR software version 2019-10-24 (https://annovar.openbioinformatics.org/en/latest/). The identified variants were verified by Sanger sequencing in the probands (Figures S1-S5). Furthermore, segregation analyses were conducted in the families that requested prenatal diagnosis for subsequent pregnancies.

Results

Genetic spectrum of MMA in the Iranian cohort

We identified MMA-associated variants in 15 Iranian MMA cases. Variants were distributed across six genes (*MMACHC*, *MMAA*, *MMAB*, *MMUT*, *ACSF3*, and *ABCD4*). Notably, *MMACHC* was the most prevalent gene in this cohort (7/15 cases, 46.7%), with the recurrent c.394 C > T (p.R132X) variant being detected in 4 cases in homozygote state. A patient (case 10) was a compound heterozygote for two pathogenic variants in the *MMACHC* gene. Five variants were previously unreported (e.g., *MMAA* c.1229T > A, *MMUT* c.309_327del). Finally, variants in *ACSF3* (p.R10Q, VUS) and *ABCD4* (p.L47F, likely benign) were detected but require functional validation. Table 1 summarizes molecular data, including ACMG classifications and population frequencies.

Figure 1 represents the protein domains of this key gene, where the location of each amino acid change is shown.

Genotype-Phenotype correlations

Patients with *MMACHC* variants (cblC Type) had severe neonatal-onset (Cases 5–8). Moreover, homozygosity for p.R132X was associated with metabolic acidosis (3/4 cases), seizures (3/4 cases), developmental delay (4/4 cases), consistent with classic cblC phenotypes. Case 10 (compound heterozygous: p.R206W/p.E92=) had milder phenotype (no acidosis, normal development), suggesting that hypomorphic alleles may attenuate severity.

We had two cases with *MMAA/MMAB* variants (cblA/cblB Types). Case 2 (*MMAA* p.R359X, homozygous) had metabolic acidosis but no neurological deficits, aligning with typical cblA. Case 4 (*MMAB* p.R191W, homozygous) had seizures and respiratory distress at birth, reflecting early-onset cblB. Among patients with *MMUT* variants (Mut Type), case 12 (p.M1?) had severe metabolic acidosis and vomiting, consistent with mut(0) subtype; and case 13 (p.R103Sfs*71) had neonatal hyperammonemia and acidosis, typical of null variants.

Among those with *ACSF3/ABCD4* variants (Atypical MMA), case 14 (*ACSF3* p.R10Q) had recurrent infections but no classic MMA symptoms, suggesting a milder or tissue-specific effect; and case 15 (*ABCD4* p.L47F) had hypotonia and seizures but normal MRI, complicating cblJ classification (variant classified as likely benign).

Finally, 12/15 cases (80%) had consanguineous parents, reinforcing autosomal recessive inheritance. Table 2 details clinical profiles, highlighting correlations with genetic subtypes.

Functional impact of variants (In Silico Predictions)

Table 3 integrates computational analyses. Pathogenic truncating variants (e.g., *MMACHC* p.R132X, *MMAA* p.L410X) were uniformly predicted as deleterious. Among missense variants, conflicting predictions was retrieved for *MMAB* p.Pro53Leu (REVEL: benign; DANN: deleterious), warranting functional studies. The *ABCD4* p.L47F variant was predicted as an uncertain variant by REVEL and SIFT, but deleterious by three other prediction tools. This discordance is possibly due to differences in algorithmic training datasets and the variant's moderate evolutionary conservation. In fact, SIFT relies greatly on sequence homology; and the low score (0.005) may reflect tolerated substitutions at this residue across species. On the other hand, PolyPhen-2, DANN and GenoCanyon tools emphasize structural disruption (e.g., hydrophobic-to-aromatic change at residue 47) and genomic constraint, thus signifying p.L47F as deleterious. Leucine 47 resides in a putative transmembrane domain of ABCD4, a protein critical for vitamin B12 trafficking. While some substitutions may be tolerated, phenylalanine introduces a bulky aromatic side chain, potentially disturbing protein folding or interaction partners. Although ClinVar labels p.L47F as a likely benign variant, its presence in a symptomatic patient suggests either a minor functional role or synergistic effects with other genetic/environmental factors. Functional validation is needed to elucidate its contribution to cblJ-like phenotypes.

Discussion

Molecular diagnosis of MMA has important implications in the treatment of patients, identification of at-risk families, and genetic counseling. It also facilitate reliable classification of MMA patients, a critical step in the treatment, prediction of outcome, and prenatal diagnoses[40]. In the current study, we provided a summarized data of genetic variants in 15 Iranian patients with MMA. The majority of MMA patients in this study presented with clinical signs and symptoms within the first month of life. However, the exact age of onset for most of the cases was not clear.

Among the assessed patients, *MMACHC* gene was the most prevalently mutated gene. Recurrent *MMACHC* p.R132X correlates with severe cblC phenotypes in Iran, mirroring global reports but with higher local prevalence. In line with our study, previous studies demonstrated that the majority of *MMACHC* variants are nonsense and frameshift variants, thus leading to severe clinical phenotypes[41]. We also detected variants in each of *ACSF3* and *ABCD4* genes in one case, respectively. However, the data regarding pathogenicity of the latter two variants was insufficient.

A survey in human gene mutation database and systematic review of databases to explore contribution of different genes to MMA showed global frequencies of causative mutations at the global level are as follows: *MMUT* (more than 64%), *MMACHC* (about 18%), *MMAA* (about 13%), *MMAB* (about 7%), *MMADHC*

Case number	Gene	Transcript	Variant	Associated disease	OMIM	Inheritance	Zygosity	ACMG classification	Clinvar	dbSNP rsID	Reference/ Ethnicity
1	*MMAA*	NM_172250	c.970G > A p.V324I	Methylmalonic aciduria, vitamin B12-responsive	251,100	AR	Het	VUS	VUS	-	-
			c.1229T > A p.L410X				Het	Likely Pathogenic	Pathogenic	-	-
2	*MMAA*	NM_172250.3	c.1075 C > T p.R359X	Methylmalonic aciduria, vitamin B12-responsive, cblA type	251,100	AR	Hom	Pathogenic	Pathogenic	rs999844958	Iranian[17], other populations[18,19]
3	*MMAB*	NM_052845.4	c.158 C > T p.Pro53Leu	Methylmalonic aciduria, vitamin B12-responsive, cblB type	251,110	AR	Hom	VUS	VUS	rs764683053	-
4	*MMAB*	NM_052845.4	c.571 C > T p.R191W	Methylmalonic aciduria, vitamin B12-responsive	251,110	AR	Hom	Pathogenic	Pathogenic	rs376128990	Iranian[17], other populations[20–22]
5	*MMACHC*	NM_015506.3	c.394 C > T p.R132X	Methylmalonic aciduria and homocystinuria, cblC type	277,400	AR	Hom	Pathogenic	Pathogenic	rs121918241	Other populations[23–28]
6	*MMACHC*	NM_015506.3	c.394 C > T p.R132X	Methylmalonic aciduria and Homocystinuria, cblC type	277,400	AR	Hom	Pathogenic	Pathogenic	rs121918241	Other populations[23–28]
7	*MMACHC*	NM_015506.3	c.394 C > T p.R132X	Methylmalonic aciduria and homocystinuria, cblC type	277,400	AR	Hom	Pathogenic	Pathogenic	rs121918241	Other populations[23–28]
8	*MMACHC*	NM_015506.3	c.394 C > T p.R132X	Methylmalonic aciduria and homocystinuria, cblC type	277,400	AR	Hom	Pathogenic	Pathogenic	rs121918241	Other populations[23–28]
9	*MMACHC*	NM_015506.3	c.481 C > T p.R161X	Methylmalonic aciduria and homocystinuria, cblC type	277,400	AR	Hom	Pathogenic	Pathogenic	rs370596113	Other populations[28–31]
10	*MMACHC*	NM_015506.3	c.616 C > T p.R206W	Methylmalonic aciduria and homocystinuria, cblC type	277,400	AR	Het	Pathogenic	Pathogenic	rs538023671	Other populations[29,32–34]
			c.276G > A p.E92=				Het	Pathogenic	Pathogenic	rs556977618	Other populations[35,36]
11	*MMACHC*	NM_015506.3	c.316G > A p.E106K	Methylmalonic aciduria and homocystinuria, cblC type	277,400	AR	Hom	VUS	VUS	rs201617713	Other populations[23]
12	*MMUT*	NM_000255.3	c.2T > C p.M1?	Methylmalonic aciduria, mut type	251,000	AR	Hom	Likely Pathogenic	Pathogenic	rs879253820	Other populations[37–39]
13	*MMUT*	NM_000255.4	c.309_327del p.R103Sfs*71	Methylmalonic aciduria, mut(0) type	251,000	AR	Hom	Likely Pathogenic	NR	-	-
14	*ACSF3*	NM_001243279.3	c.29 G > A p.R10Q	Combined malonic and methylmalonic aciduria	614,265	AR	Hom	VUS	VUS	rs751551226	-
15	*ABCD4*	NM_005050.4	c.141G > C p.L47F	Methylmalonic aciduria and homocystinuria, cblJ type	614,857	AR	Hom	Likely Benign	Conflicting	rs147446660	-

Table 1. Summary of molecular data of MMA patients. rsID: Reference single nucleotide polymorphism; ACMG: American College of Medical Genetics and Genomics.

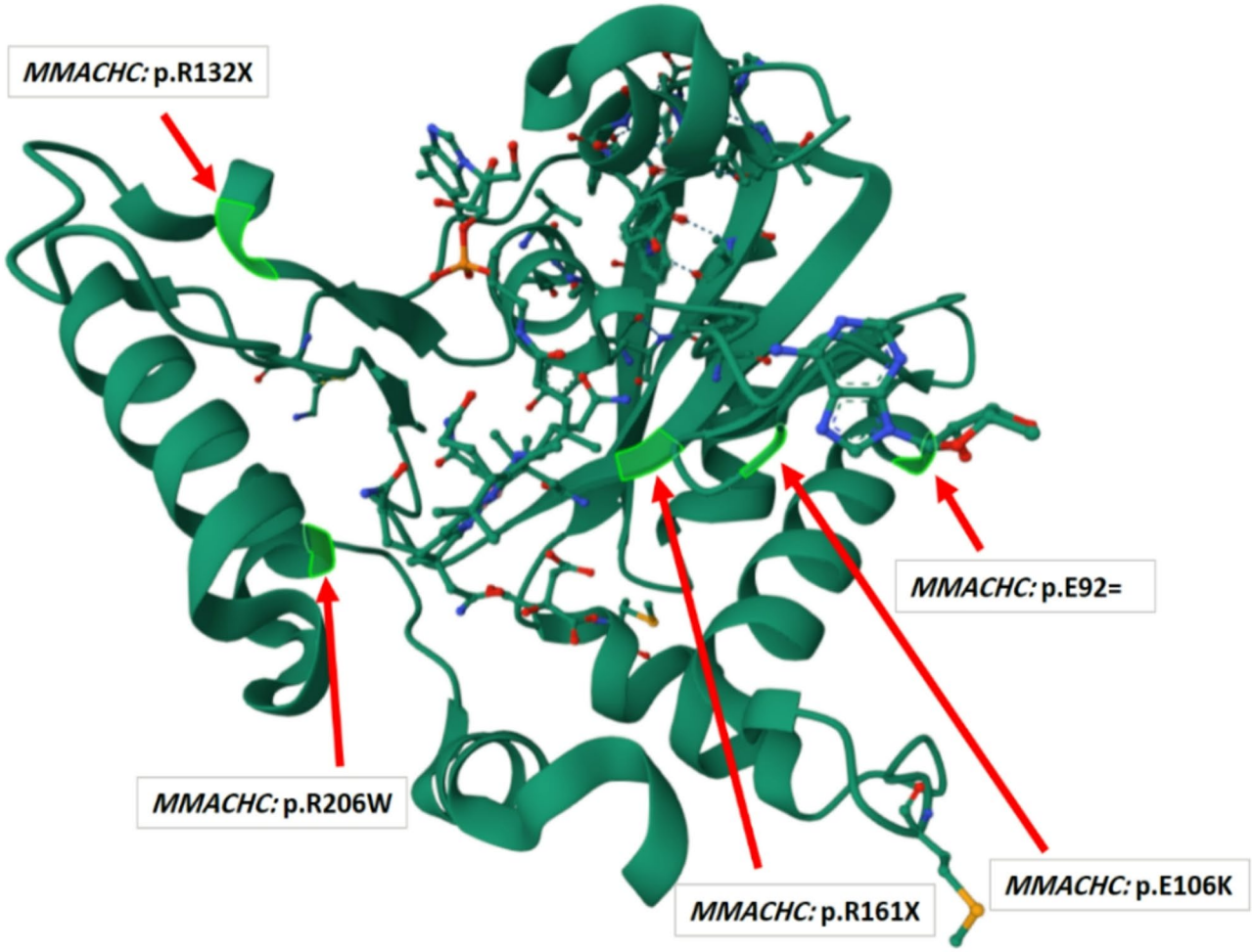

Fig. 1. Protein domains of *MMACHC* gene. The location of each amino acid change in this gene is shown by arrows. (https://www.rcsb.org).

(about 3%), and *MCEE* (less than 1%)[6]. Thus, the distribution of MMA-related variants in the current study was different from what was reported globally. This discrepancy might be due to the nature of our study that included cases from a single referral center. Additional mutational studies in different centers are needed to explore the whole spectrum of MMA-associated variants among Iranian patients. The integrated results of these studies can be used for establishment of a population-specific screening or diagnostic panel for MMA.

Figure 2 shows the frequency of MMA-associated variants among Iranian patients as well as the global frequency of variants.

Moreover, a previous survey in the literature reported 24 MMA mutations in Iranian MMA patients of which 11 mutations (45.8%) were detected only among Iranian patients[6]. In the current study, we found five mutations in our patients which were not reported either among Iranian patients or patients from other populations.

A recent genetics analysis in Iranian MMA cases found six homozygous variants, including five formerly reported variants and one novel variant, in the two MMA-causing genes namely c.577G > C, c.290 + 69G > T, and c.662T > A of *MMAB*, and c.100dupA, c.394 C > T of *MMACHC*[42]. Notably, c.394 C > T variant was also detected in four patients in our study. This variant is among the most frequent variants in cblC leading to defects in a part of the Cbl binding domain. Since it is associated with late-onset presentation, it is expected to maintain residual function. A multi-faceted biophysical approach showed that the secondary structure components in the encoded mutant protein are intact, but the stability is fragile[24].

A missense mutation in *MMUT* gene (c.1055 A > G, p.Q352R) was also reported in an Iranian couple with a history of infant death[43]. Moreover, another study identified two novel mutations in Iranian MMA patients, namely A252Vf*5 and G87R, within the *MMAA* and *MMUT* genes, respectively and concluded that high frequency of mutations within exons 2 and 3 of *MMUT* gene and exon 7 of *MMAB* gene is in line with the overall anticipated frequency of genetic variations among MMA patients[44]. Thus, the spectrum of MMA-related mutations among Iranian patients is quite wide including both previously reported variants and possibly population-specific ones.

Since the majority of presented cases in the current study were born to consanguine parents, one can suggest the inclusion of MMA in newborn screening panels in consanguineous families. Identification of most common

Case Number	Age	Sex	Consanguinity	Indication	Developmental delay	FTT	seizure	Hypotonia	Vomiting	Hepatomegaly	Elevated liver enzymes	Steatorrhea	Metabolic acidosis
1	1 Y	Male	Consanguine	Hepatomegaly, steatorrhea, elevated level of liver enzymes and FTT	+	+	-	+	-	+	+	+	-
2	1 Y	Male	Consanguine	Metabolic acidosis and clinical diagnosis of organic acidemia	-	+	-	-	+	-	+	-	+
3	2.5 Y	Male	Consanguine	Speech delay and developmental delay	+	-	-	-	-	-	+	-	-
4	13 M	Male	Consanguine	Meconium swallow and respiratory distress, seizure, and clinical diagnosis of MMA	+	-	+	-	-	-	N/A	-	NA
5	11 M	Male	Non-Consanguine	Seizure, developmental delay, elevated level of homocysteine	+	-	+	+	-	-	N/A	-	-
6	13 M	Male	Non-Consanguine	Developmental regression, seizure, ataxia and sign of leukodystrophy in brain MRI	+	-	+	-	-	-	N/A	-	-
7	1 Y	Male	Consanguine	Seizure, developmental regression and hyperhomocysteinemia	+	-	+	+	-	+	N/A	-	-
8	4 M	Female	Consanguine	Seizure, reflux, elevated level of ammonia	-	-	+	-	-	-	N/A	-	-
9	10 D	Male	Consanguine	Metabolic acidosis	-	-	-	-	-	-	-	-	-
10	11 M	Male	Non-Consanguine	Clinical diagnosis of organic acidemia									
11	10 M	Male	Consanguine	Seizure, developmental delay, hypotonia, and neck weakness	+	-	+	+	-	-	N/A	-	-
12	9 M	Female	Consanguine	Vomiting and diarrhea at two-month-old and clinical diagnosis of MMA	+	+	-	-	+	-	-	-	+
13	12 D	Female	Consanguine	Metabolic acidosis, hyperammonemia, and clinical diagnosis of organic acidemia	+	-	+	+	-	-	N/A	+	+
14	8Y	Female	Consanguine	Recurrent infection									
15	2.5 Y	Male	Consanguine	Asphyxia during birth, seizures, developmental delay, hypotonia, undescendent testis and normal MRI	+	-	+	+	-	-	-	-	-

Table 2. Summary of clinical data of MMA patients (N/A: not available, FTT: failure to thrive).

Case number	Gene	Variant	Revel*	SIFT&	Polyphen2$	DANN#	GenoCanyon@
1	*MMAA*	c.970G > A p.V324I	Uncertain (0.52)	Uncertain (0.028)	N/A	Deleterious (1)	Deleterious (1)
		c.1229T > A p.L410X	N/A	N/A	N/A	Deleterious (0.99)	Deleterious (1)
2	*MMAA*	c.1075 C > T p.R359X	N/A	N/A	N/A	Deleterious (0.98)	Benign (0)
3	*MMAB*	c.158 C > T p.Pro53Leu	Benign (Moderate) (0.17)	Uncertain (0.038)	Uncertain (0.85)	Deleterious (0.99)	Deleterious (1)
4	*MMAB*	c.571 C > T p.R191W	Deleterious (Moderate) (0.92)	Deleterious (Supporting) (0)	Deleterious (Moderate) (1)	Deleterious (1)	Deleterious (1)
5	*MMACHC*	c.394 C > T p.R132X	N/A	N/A	N/A	Deleterious (1)	Deleterious (1)
6	*MMACHC*	c.394 C > T p.R132X	N/A	N/A	N/A	Deleterious (1)	Deleterious (1)
7	*MMACHC*	c.394 C > T p.R132X	N/A	N/A	N/A	Deleterious (1)	Deleterious (1)
8	*MMACHC*	c.394 C > T p.R132X	N/A	N/A	N/A	Deleterious (1)	Deleterious (1)
9	*MMACHC*	c.481 C > T p.R161X	N/A	N/A	N/A	Deleterious (1)	Deleterious (1)
10	*MMACHC*	c.616 C > T p.R206W	Deleterious (Moderate) (0.89)	Deleterious (Supporting) (0)	N/A	Deleterious (1)	Deleterious (1)
		c.276G > A p.E92=	N/A	N/A	N/A	N/A	N/A
11	*MMACHC*	c.316G > A p.E106K	Deleterious (Supporting) (0.73)	Uncertain (0.01)	Uncertain (0.83)	Deleterious (1)	Deleterious (1)
12	*MMUT*	c.2T > C p.M1?	Deleterious (Supporting) (0.72)	Uncertain (0.001)	N/A	Deleterious (0.99)	Deleterious (1)
13	*MMUT*	c.309_327del p.R103Sfs*71	N/A	N/A	N/A	N/A	N/A
14	*ACSF3*	c.29 G > A p.R10Q	Benign (Moderate) (0.05)	Benign (Supporting) (0.243)	Benign (Supporting) (0.02)	Deleterious (0.99)	Deleterious (1)
15	*ABCD4*	c.141G > C p.L47F	Uncertain (0.64)	Uncertain (0.005)	Deleterious (Supporting) (0.98)	Deleterious (1)	Deleterious (0.99)

Table 3. Functional validation of the variants through in Silico predictions (N/A: not available). * https://sites.google.com/site/revelgenomics/ (version 1). & https://sift.bii.a-star.edu.sg/ (version 3). $ http://genetics.bwh.harvard.edu/pph2/ (version 2). # https://maayanlab.cloud/datasets2tools/landing/tool/DANN (version 1). @ https://zhaocenter.org/GenoCanyon_Index.html (version 1).

mutations in MMA-associated genes among Iranian patients would pave the way for design of population-specific diagnostic/screening panels. The current study facilitates this goal. The importance of this aspect is highlighted by the reported meta-analysis data showing higher disease frequencies for both forms of MMA in the Middle East and North Africa which is possibly explained by high rates of consanguinity in these regions[45]. In addition, the fact that most patients in this study were born to consanguine parents precludes estimation of the frequency of the detected variants.

Cumulatively, the current study provides an overview of MMA-related variants among Iranian patients possibly facilitating the molecular diagnosis of MMA within the Iranian population. But the study has a limitation in terms of detailed clinical and metabolomics data hindering the assessment of genotype-phenotype correlations in the patients. Moreover, the study's conclusions are drawn from a relatively small cohort of 15 patients. While this provides valuable preliminary data, we acknowledge small sample size as the limitation of our study. Finally, while the newly discovered variants are indeed very critical for studying population-specific diagnosis, the possible impact of these novel variants on protein function was not assessed in this study. Thus, we suggest conduction of functional assays on these variants in future studies.

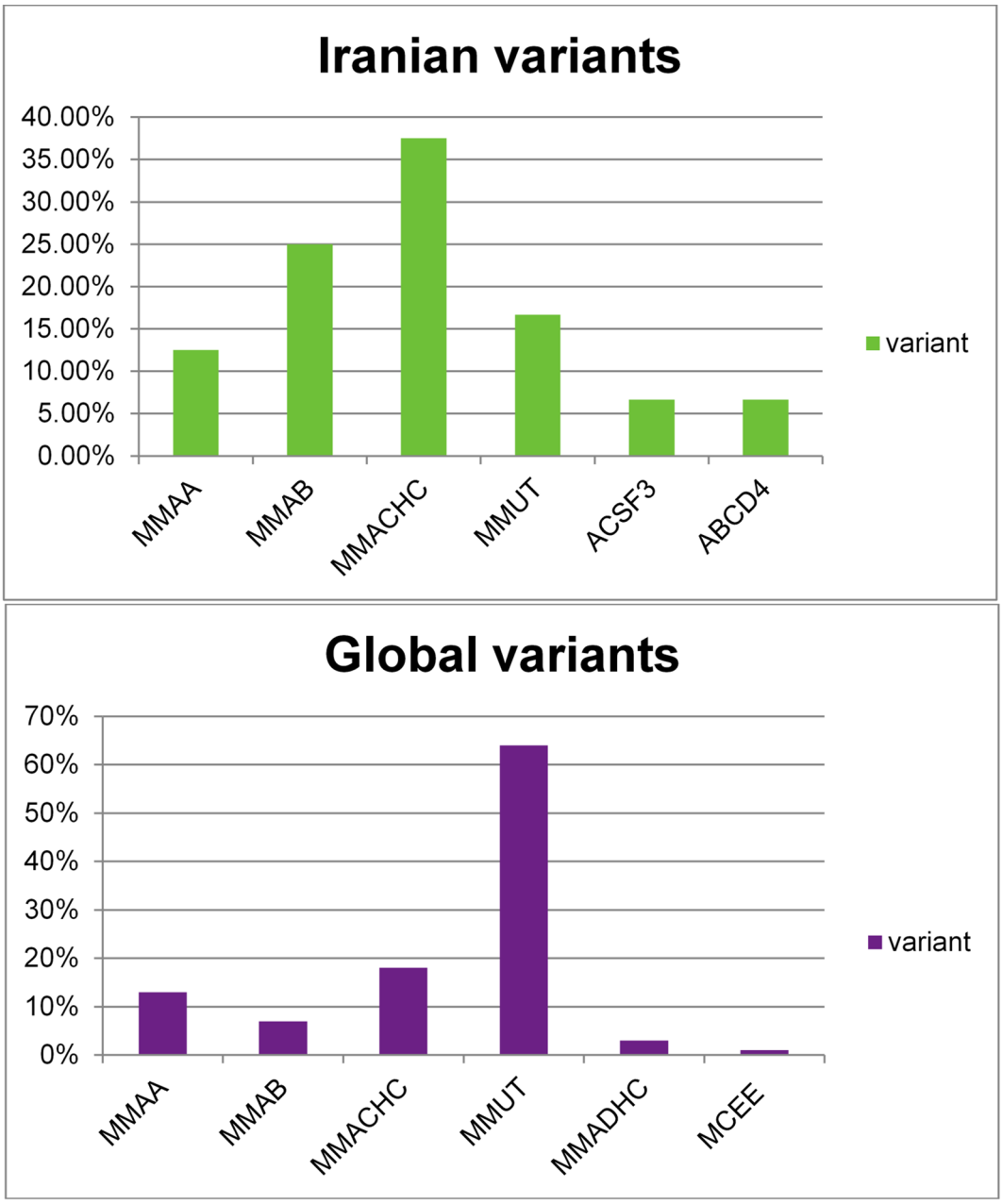

Fig. 2. Frequency of MMA-associated variants among Iranian patients (data of current study in addition to other reports from Iran[42–44]) versus global frequencies[6].

Data availability

The datasets generated and/or analysed during the current study are available in the Clinvar repository (https://www.ncbi.nlm.nih.gov/clinvar/?term=%22MMAA%22%5BGENE%5D&redir=gene).

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References

1. Manoli, I., Sloan, J. L. & Venditti, C. P. Isolated methylmalonic acidemia. In: (eds Adam, M. P., Feldman, J., Mirzaa, G. M., Pagon, R. A., Wallace, S. E. & Amemiya, A.) GeneReviews(®). Seattle WA: © 1993–2024, University of Washington, Seattle. GeneReviews is a registered trademark of the University of Washington, Seattle; (1993).
2. Ledley, F. D., Jansen, R., Nham, S. U., Fenton, W. A. & Rosenberg, L. E. Mutation eliminating mitochondrial leader sequence of methylmalonyl-CoA mutase causes muto methylmalonic acidemia. *Proc. Natl. Acad. Sci. U.S.A.* **87** (8), 3147–3150 (1990).
3. Yang, X. et al. Mutation analysis of the MMAA and MMAB genes in Japanese patients with vitamin B12-responsive methylmalonic acidemia: identification of a prevalent MMAA mutation. *Mol. Genet. Metab.* **82** (4), 329–333 (2004).

4. Bikker, H. et al. A homozygous nonsense mutation in the methylmalonyl-CoA epimerase gene (MCEE) results in mild methylmalonic aciduria. *Hum. Mutat.* **27** (7), 640–643 (2006).
5. Costanzo, M. et al. Label-Free quantitative proteomics in a Methylmalonyl-CoA Mutase-Silenced neuroblastoma cell line. *Int. J. Mol. Sci.* ;**19**(11). (2018).
6. Malekizadeh, G., Jazayeri, O., Alijanpour, M. & Tafrihi, M. Gene frequencies of methylmalonic acidemia disease at the global level and compiling the pathogenic mutations in the Iranian population. *Res. Mol. Med.* **11** (1), 37–48 (2023).
7. Mucha, P., Kus, F., Cysewski, D., Smolenski, R. T. & Tomczyk, M. Vitamin B12 metabolism: a network of multi-protein mediated processes. *Int. J. Mol. Sci.* **25** (15), 8021 (2024).
8. Mansoor, S. et al. Identification of a biallelic MMUT variant (p. Thr230Arg) and its global perspective on clinical management. *Mol. Biol. Rep.* **52** (1), 97 (2025).
9. Waisbren, S. E. Review of neuropsychological outcomes in isolated methylmalonic acidemia: recommendations for assessing impact of treatments. *Metab. Brain Dis.* **37** (5), 1317–1335 (2022).
10. Matsui, S. M., Mahoney, M. J. & Rosenberg, L. E. The natural history of the inherited methylmalonic acidemias. *N. Engl. J. Med.* **308** (15), 857–861 (1983).
11. Harrington, E. A. et al. Neutralizing antibodies against Adeno-Associated viral capsids in patients with mut methylmalonic acidemia. *Hum. Gene Ther.* **27** (5), 345–353 (2016).
12. Hao, L. et al. Long-term follow-up of Chinese patients with methylmalonic acidemia of the CblC and mut subtypes. *Pediatr. Res.* (2024).
13. Lerner-Ellis, J. P. et al. Identification of the gene responsible for methylmalonic aciduria and homocystinuria, CblC type. *Nat. Genet.* **38** (1), 93–100 (2006).
14. Coelho, D. et al. Gene identification for the CblD defect of vitamin B12 metabolism. *N. Engl. J. Med.* **358** (14), 1454–1464 (2008).
15. Rutsch, F., Gailus, S., Suormala, T. & Fowler, B. LMBRD1: the gene for the CblF defect of vitamin B_{12} metabolism. *J. Inherit. Metab. Dis.* **34** (1), 121–126 (2011).
16. Fettelschoss, V. et al. Clinical or ATPase domain mutations in ABCD4 disrupt the interaction between the vitamin B12-trafficking proteins ABCD4 and LMBD1. *J. Biol. Chem.* **292** (28), 11980–11991 (2017).
17. Shafaat, M. et al. Autozygosity mapping of methylmalonic acidemia associated genes by short tandem repeat markers facilitates the identification of five novel mutations in an Iranian patient cohort. *Metab. Brain Dis.* **33**, 1689–1697 (2018).
18. Dempsey-Nunez, L. et al. High resolution melting analysis of the MMAA gene in patients with CblA and in those with undiagnosed methylmalonic aciduria. *Mol. Genet. Metab.* **107** (3), 363–367 (2012).
19. Plessl, T. et al. Protein destabilization and loss of protein-protein interaction are fundamental mechanisms in cblA-type methylmalonic aciduria. *Hum. Mutat.* **38** (8), 988–1001 (2017).
20. Forny, P. et al. Spectrum and characterization of bi-allelic variants in MMAB causing cblB-type methylmalonic aciduria. *Hum. Genet.* **141** (7), 1253–1267 (2022).
21. Agnarsdóttir, D. et al. Early cardiomyopathy without severe metabolic dysregulation in a patient with cblB-type methylmalonic acidemia. *Mol. Genet. Genomic Med.* **10** (7), e1971 (2022).
22. Keyfi, F. et al. Mutation analysis of genes related to methylmalonic acidemia: identification of eight novel mutations. *Mol. Biol. Rep.* **46** (1), 271–285 (2019).
23. Kaur, R., Attri, S. V., Saini, A. G. & Sankhyan, N. A high frequency and geographical distribution of MMACHC R132* mutation in children with cobalamin C defect. *Amino Acids.* **53** (2), 253–264 (2021).
24. Passantino, R. et al. Investigation on a MMACHC mutant from CblC disease: the c.394C > T variant. *Biochim. Biophys. Acta Proteins Proteom.* **1870** (6), 140793 (2022).
25. Wang, C. et al. Mutation spectrum of MMACHC in Chinese pediatric patients with cobalamin C disease: A case series and literature review. *Eur. J. Med. Genet.* **62** (10), 103713 (2019).
26. Wang, C. et al. Rapid screening of MMACHC gene mutations by high-resolution melting curve analysis. *Mol. Genet. Genomic Med.* **8** (6), e1221 (2020).
27. Tong, W. et al. Whole-exome sequencing helps the diagnosis and treatment in children with neurodevelopmental delay accompanied unexplained dyspnea. *Sci. Rep.* **8** (1), 5214 (2018).
28. Hu, S., Mei, S., Liu, N. & Kong, X. Molecular genetic characterization of CblC defects in 126 pedigrees and prenatal genetic diagnosis of pedigrees with combined methylmalonic aciduria and homocystinuria. *BMC Med. Genet.* **19** (1), 154 (2018).
29. Liu, M. Y. et al. Mutation spectrum of MMACHC in Chinese patients with combined methylmalonic aciduria and homocystinuria. *J. Hum. Genet.* **55** (9), 621–626 (2010).
30. Topaloglu, R. et al. Do not miss rare and treatable cause of Early-Onset hemolytic uremic syndrome: cobalamin C deficiency. *Nephron* **142** (3), 258–263 (2019).
31. Nogueira, C. et al. Spectrum of MMACHC mutations in Italian and Portuguese patients with combined methylmalonic aciduria and homocystinuria, CblC type. *Mol. Genet. Metab.* **93** (4), 475–480 (2008).
32. Fofou-Caillierez, M. B. et al. Interaction between methionine synthase isoforms and MMACHC: characterization in CblG-variant, CblG and CblC inherited causes of megaloblastic anaemia. *Hum. Mol. Genet.* **22** (22), 4591–4601 (2013).
33. Zong, Y., Liu, N., Zhao, Z. & Kong, X. Prenatal diagnosis using genetic sequencing and identification of a novel mutation in MMACHC. *BMC Med. Genet.* **16**, 48 (2015).
34. Antony, P., Baby, B., Ali, A., Vijayan, R. & Al Jasmi, F. Interaction of glutathione with MMACHC Arginine-Rich pocket variants associated with cobalamin C disease: insights from molecular modeling. *Biomedicines* ;**11**(12). (2023).
35. Petropoulos, T. E. et al. Renal thrombotic microangiopathy and pulmonary arterial hypertension in a patient with late-onset cobalamin C deficiency. *Clin. Kidney J.* **11** (3), 310–314 (2018).
36. Koenig, J. C. et al. Nephrotic syndrome and thrombotic microangiopathy caused by cobalamin C deficiency. *Pediatr. Nephrol.* **30** (7), 1203–1206 (2015).
37. Chu, J. et al. Next generation sequencing of patients with mut methylmalonic aciduria: validation of somatic cell studies and identification of 16 novel mutations. *Mol. Genet. Metab.* **118** (4), 264–271 (2016).
38. Forny, P. et al. Molecular genetic characterization of 151 Mut-Type methylmalonic aciduria patients and identification of 41 novel mutations in MUT. *Hum. Mutat.* **37** (8), 745–754 (2016).
39. Burda, P. et al. Insights into severe 5,10-methylenetetrahydrofolate reductase deficiency: molecular genetic and enzymatic characterization of 76 patients. *Hum. Mutat.* **36** (6), 611–621 (2015).
40. Keyfi, F., Talebi, S. & Varasteh, A. R. Methylmalonic acidemia diagnosis by laboratory methods. *Rep. Biochem. Mol. Biology.* **5** (1), 1–14 (2016).
41. Su, L. et al. Clinical and genetic analysis of methylmalonic aciduria in 60 patients from Southern China: a single center retrospective study. *Orphanet J. Rare Dis.* **19** (1), 198 (2024).
42. Beyzaei, Z., Moravej, H., Imanieh, M. H., Inaloo, S. & Geramizadeh, B. Clinical spectrum and genetic variation of six patients with methylmalonic aciduria (MMA); a report from Iran. *BMC Pediatr.* **24** (1), 795 (2024).
43. Habibzadeh, P. et al. Pre-implantation genetic diagnosis in an Iranian family with a novel mutation in MUT gene. *BMC Med. Genet.* **21** (1), 22 (2020).
44. Jafari, M. et al. Identification of novel mutations in the MMAA and MUT genes among methylmalonic aciduria families. *Iran. Biomed. J.* **27** (6), 397 (2023).

45. Almási, T. et al. Systematic literature review and meta-analysis on the epidemiology of methylmalonic acidemia (MMA) with a focus on MMA caused by methylmalonyl-CoA mutase (mut) deficiency. *Orphanet J. Rare Dis.* **14** (1), 84 (2019).

Author contributions
M.F. and S.K. evaluated patients' genetic reports. S.GF wrote the manuscript. M.M assessed the patients. S.GF, and MM supervised the study. All the authors read and approved the submitted version.

Declarations

Competing interests
The authors declare no competing interests.

Ethical standards
Informed consent forms were signed by legal representatives of patients. All methods were carried out in accordance with relevant guidelines and regulations. All experimental protocols were approved by ethical committee of Shahid Beheshti University of Medical Sciences.

Consent to participate
Informed consent forms were signed by legal representatives of patients.

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Correspondence and requests for materials should be addressed to M.M. or S.G.-F.

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