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Molecular characterization, clinical phenotype, and neurological outcome of twelve Palestinian children with beta-ketothiolase deficiency: report of two novel variants in the *ACAT1* gene

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Abstract

Background Beta-ketothiolase deficiency (mitochondrial acetoacetyl-CoA thiolase, T2) deficiency (OMIM #203750, *607809) is an autosomal recessive disorder of isoleucine catabolism and ketone body utilization. It is caused by mutations in the *ACAT1* gene and characterized by intermittent ketoacidosis episodes triggered by ketogenic stresses, with no clinical symptoms between the episodes. Neurological complications, particularly extrapyramidal signs may occur as sequelae of the ketoacidosis episodes but may also occur without or before any apparent metabolic crisis. T2 deficiency is characterized by the accumulation of isoleucine metabolites, 2methylacetoacetate, 2-methyl-3-hydroxybutyrate, and tiglylglycine, detected in urine organic acids and blood acylcarnitines with or without hypoglycemia.

Methods This study presents data from twelve patients with T2 deficiency, diagnosed between 7 months and 22 months of age at two tertiary care centers in Palestine. The clinical, biochemical, molecular genetic data, and neurological outcomes are reviewed.

Results We report on twelve patients (6 females and 6 males) from eight families in four different regions of the West Bank and Gaza Strip. All patients were offspring of consanguineous marriages. Ketoacidotic episodes were the predominant manifestations in all patients, and each episode was triggered by either acute gastroenteritis or upper respiratory infections. One patient initially presented with hypotonia and psychomotor delay, later developing a ketoacidotic episode a few months afterward. The characteristic laboratory finding in all patients was the increased urinary excretion of 2-methyl-3-hydroxybutyrate and tiglylglycine. Ten of the twelve patients had favorable outcomes, while two unfortunately passed away at the time of the study. Molecular genetic analysis of the *ACAT1* gene was conducted on nine patients from six families, revealing four different variants, two of which were novel. Additionally, a founder mutation was identified in six patients from three families.

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Conclusions The study underscores the critical role of genetic research in unraveling the complexities of beta-ketothiolase deficiency and related disorders. By identifying haplotype blocks, founder mutations, and novel pathogenic variants, researchers can significantly improve diagnostic precision, enhance genetic counseling, and lay the groundwork for developing targeted therapies. We identified two novel variants and a founder mutation, thereby broadening the genetic spectrum of this rare disease.

Keywords T2, Beta-ketothiolase, Ketoacidosis, 2-methyl-3-hydroxybutyric acid, *ACAT1* gene

Introduction

Beta-ketothiolase deficiency (also known as mitochondrial acetoacetyl-CoA thiolase or T2 deficiency; OMIM #203750, *607809) is an inherited metabolic disorder affecting isoleucine catabolism and ketone body utilization. It is caused by mutations in the *ACAT1* gene and is classified as an organic aciduria or ketolytic defect [1]. Ketone bodies serve as a critical energy source, especially for the brain, during periods of inadequate energy supply. As a result, individuals with T2 deficiency experience episodes of ketoacidosis and exhibit characteristic biochemical abnormalities, including the accumulation of isoleucine metabolites in urine organic acids (such as 2-methylacetoacetate, 2-methyl-3-hydroxybutyrate, and tiglylglycine) and elevated blood acylcarnitines, specifically C5-OH carnitine (2-methyl-3-hydroxybutyryl-carnitine) and C5:1 carnitine (tiglylcarnitine) [1, 2].

Most patients with T2 deficiency typically present within the first two years of life, often experiencing at least one episode of metabolic decompensation. Neonatal onset and onset after the age of six years are uncommon [2, 3]. Ketoacidotic episodes are frequently triggered by factors such as gastroenteritis, infections (particularly upper respiratory infections), fasting, and other stressors that induce physiological ketosis [3, 4]. The frequency of these episodes tends to decline with age and becomes rare after the age of 10 [5]. The most common clinical manifestations include recurrent vomiting, dehydration, dyspnea, hypotonia, and lethargy, which can progress to coma in severe cases [6]. In rare instances, patients may exhibit chronic neurologic symptoms, such as muscular hypotonia, seizures, hyporeflexia, or impaired motor function [2, 7]. Ketoacidosis is the most frequently observed laboratory finding. Other, less common laboratory abnormalities may include mild hyperammonemia, hypoglycemia, and hyperglycemia [1–6]. The majority of patients exhibit normal psychomotor and cognitive development. However, approximately 20% experience complications, which may include psychomotor delay, ataxia, hypotonia, choreoathetosis, dystonia, and nystagmus [2, 6].

The *ACAT1* gene is located in chromosome 11q22.3-23.1 and comprises 12 exons. Both homozygous and heterozygous variants have been identified within this gene. Among the most frequently observed variants are the stop-gain variant *ACAT1* c.622 C > T, p.(Arg208*), the splice site variant c.1006-1G > C (p.Val336fs), and the missense mutation c.949G > A, p.(Asp317Asn) [1, 2, 4].

In this study, we present the clinical phenotypes, molecular genetic findings, and neurological outcomes of 12 patients diagnosed with T2 deficiency. Through comprehensive genetic analysis, we have expanded the mutation profile of the *ACAT1* gene to include *ACAT1* p.Ile323Val, p.Asn414_Gly415delinsSer, and p.Cys142Phe variants and identified two previously unreported variants, thereby broadening the genotypic spectrum associated with this rare disorder.

Materials and methods

Study design and participants

This study presents a retrospective analysis of 12 patients (6 females and 6 males) diagnosed with T2 deficiency between the ages of 1 and 22 months. The diagnosis was confirmed through clinical phenotype evaluation, urine organic acid analysis in all patients, and molecular genetic testing in 9 patients (three patients denied genetic testing). Data were collected from the patients' medical records, supplemented by information provided by parents or the patients themselves during follow-up visits at the metabolic clinic. Clinical examinations were conducted at the time of diagnosis and for ten patients during subsequent follow-up appointments. To our knowledge, this represents the largest cohort of patients with T2 deficiency reported in Palestine.

Informed consent for detailed clinical phenotyping, genetic studies, and publication of the results was obtained from the enrolled individuals and/or legal guardians (IRB approval was given by the Palestinian Health Research Council PHRC/HC/ 518/19) in compliance with the Declaration of Helsinki and local ethical committee guidance.

Molecular analysis

Sample Preparation

Peripheral blood samples (5 mL) were collected from 9 patients diagnosed with autosomal recessive beta-ketothiolase deficiency in EDTA-coated tubes. Genomic DNA was extracted using the Qiagen Flexigene Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The gDNA was then quantified using a Nanodrop spectrophotometer (Thermo Fisher Scientific,

Waltham, MA, USA), and the quality was checked by gel electrophoresis.

Primer design

Twelve pairs of primers were designed using Primer3 software to amplify regions flanking each exon of the *ACAT1* gene. The primer sequences are listed in Table 1, and each primer pair amplified approximately 200–300 bp of DNA.

Sanger sequencing

The 12 exons of the *ACAT1* gene, along with flanking intronic regions, were amplified using custom primers. A touchdown PCR protocol, employing the PCRBIO kit and a 55 °C annealing temperature, was performed using a Veriti 96-Well Fast Thermal Cycler (Applied Biosystems). Briefly, 50 ng of genomic DNA was subjected to 3 cycles of denaturation (95 °C), annealing (63–55 °C), and extension (72 °C), followed by a final extension (72 °C). Amplified fragments were visualized on a 2% agarose gel stained with ethidium bromide, using a BioRad Ultraviolet Imaging System. Following confirmation of successful amplification, PCR products were purified using the EPPiC Fast kit (A&A Biotechnology). Sequencing reactions were prepared using the ABI Prism BigDye Terminator v3.1 kit. Subsequently, sequenced PCR products were purified via EDTA/ethanol precipitation. Dried samples were resuspended in Hi-Di formamide (Applied Biosystems) and subjected to capillary electrophoresis on the ABI 3500 Genetic Analyzer.

The resulting ab1 files were analyzed using DNA Chromatogram software, and sequences were aligned to the human reference genome (UCSC, GRCh37/hg19).

Whole exome sequencing (WES)

Whole-exome sequencing was performed on individual II-1 from Family 2 (Fig. 1) using the Truseq Capture Exome Kit (Illumina), which covers around 45 Mb of exonic content. The captured and amplified library was sequenced on the Illumina Nextseq500 platform according to the manufacturer's protocol. Paired-end sequences were obtained at a read length of 150 bps. Sequence reads were then aligned to the reference human genome (hg19) using a BWA aligner. Alignments then underwent preprocessing steps by PCR duplicate removal, base quality recalibration, and realignment around indels. The variants were called by GATK (Genome Analysis Toolkit and annotated by ANNOVAR (http://annovar.openbioinformatics.org/) Nonsense, frameshift, nonsynonymous, and splice-site variants were prioritized by excluding variants with minor allele frequency of >0.01 in gnomAD (https://gnomad.broadinstitute.org), PopFreqMax datab ases, by our in-house exome database and by excluding variants predicted to be benign by variant effect predictor tools such as PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/) and SIFT (http://sift.jcvi.org/). Copy nu mber variations were called from the exome data using XHMM software (https://atgu.mgh.harvard.edu/xhmm/). Co-segregation analysis was performed by Sanger sequencing on an ABI 3130 Genetic Analyzer (Applied Biosystems).

Table 1 Sequencing primers

Exon	Forward	Reverse
1	ACGTCCTTCACTCGTCAACC	CTGGGTGTTATCGGAGCACT
2	AAGGATGCAGGAAGAAACCA	TTCCTAACTGTTACATAATCCTGCTT
3	TCCTGTAGAGAACTGGGAAGG	GGGAAAGCAGAAATGTAGGC
4	TGCCTACATTTCTGCTTTCC	GGATGGTCTCCATCTCTTGA
5	TTGGCAGAAGAAATGTTGTAGTTT	ATCCTCCTGCCTCGACCTT
6	TGCATGTAGAATACTTGTTTGGTG	GCCAGCTAAGCGTCAGAAAT
7	CACCTCCGGCCTAAGAAATA	TTAACCATAAGCAGTTGAAATGAA
8	TTGGGATGGTTTAAGTGAAGC	CCCTGGGATTTATATATTGAAGGTA
9	ATTTAGCAGCCCAGGCAATA	ATGCCACCATGCTCAGCTA
10	GAAATGTGCATTTAATGGGCTA	CATGGGAAGATTTGAAACCAA
11	TCCTTCCATCTGAAACCAAGA	TGAAAGATGGGTAGGAAGGAAA
12	AGATTCTGCCATTGCACTCC	TGTGTTCTCACAAATGGAAACTT

Haplotype construction

To investigate the genetic background potentially associated with the p.Ile323Val variant in the *ACAT1 gene* (Table 2), four microsatellite repeat markers flanking the gene were selected for haplotype analysis. These markers were amplified using polymerase chain reaction (PCR) with fluorescently labeled primers, and the resulting products were analyzed via capillary electrophoresis (CE). Allele sizing and haplotype reconstruction were performed using GeneScan software. The genotyping was carried out in *ACAT1*-positive families, including affected individuals, both parents, and one heterozygous sibling per family, allowing for segregation analysis and tentative haplotype inference.

The analysis of these microsatellite markers revealed that affected individuals shared a common haplotype surrounding the *ACAT1* locus. In particular, the presence of a conserved region of homozygosity (ROH) across affected individuals suggests that the p.Ile323Val variant may reside on a shared genetic background. While this pattern may reflect a potential founder effect or indicate that the mutation has arisen on a common ancestral chromosome, the interpretation of these findings must remain cautious. The small sample size, coupled with the lack of a broader control population for comparison, limits the ability to make definitive conclusions about the strength or specificity of the association. Moreover, the limited number of informative meioses and the inherent variability of microsatellite markers in different

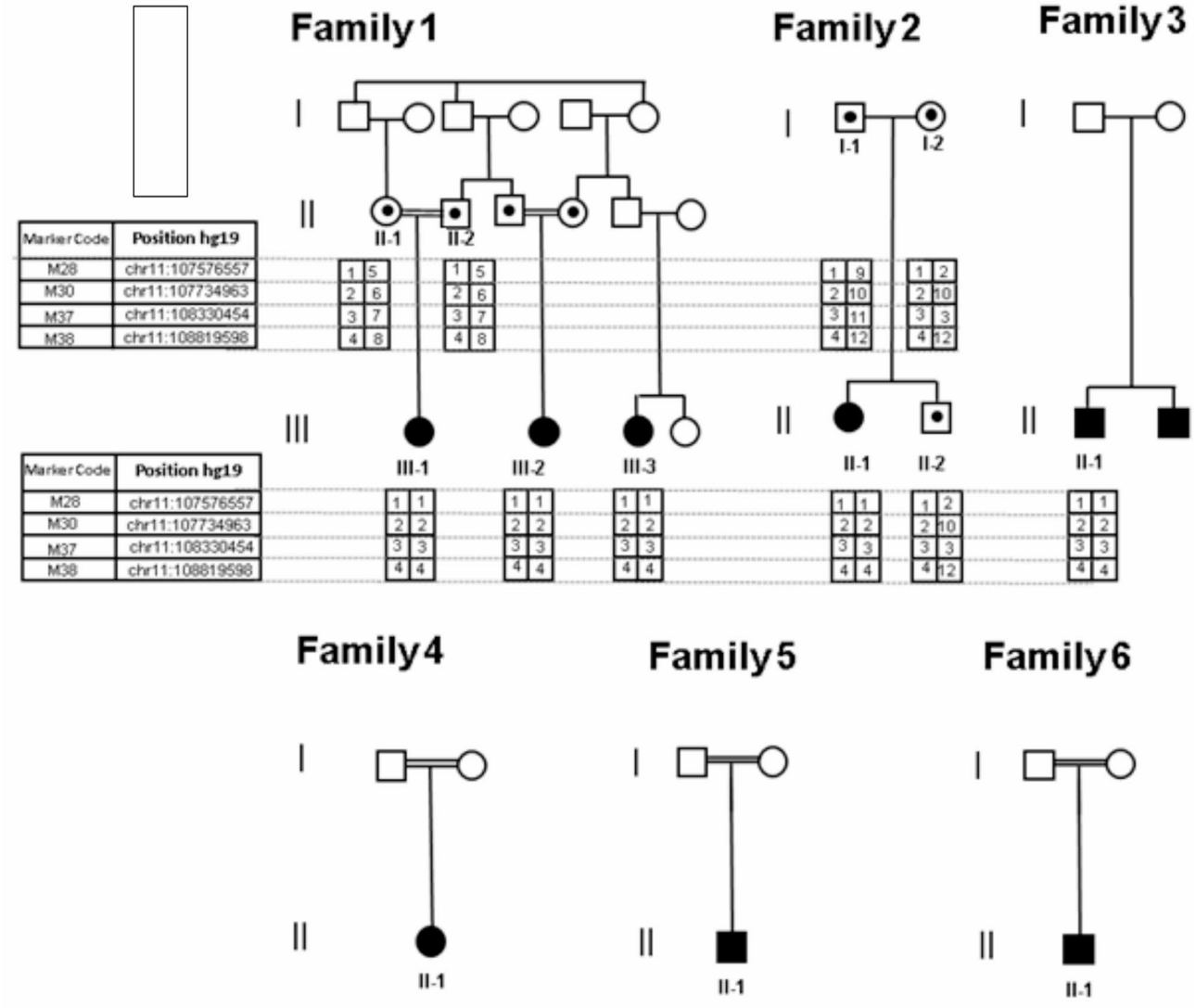

Fig. 1 Family pedigree of the patients enrolled in the study. Four microsatellite repeat (M28, M30, M37, and M38) markers flanking the gene were selected. A significant 1.2 megabase (Mb) haplotype block, encompassing four microsatellite markers tightly linked to the founder mutation p.Ile323Val in the *ACAT1* gene, was identified in six individuals from three families

Table 2 Genotyping of four STR markers flanking the *ACAT1* gene was used to define patient haplotypes

position hg19	Repeat	Marker	Distance from the ACAT1 gene	Amplicon size
chr11:107576557-107576596	20xAC	M28	-415,773	105 bp
chr11:107734963-107735014	26xTC	M30	-257,408	198 bp
chr11:107992294-108018330	***ACAT1*gene**			
chr11:108330454-108330497	22xAC	M37	338,095	139 bp
chr11:108819598-108819642	22xTG	M38	827,280	127 bp

populations further constrain the generalizability of the observed haplotype structure. Without additional population-based data, it remains unclear whether the shared haplotype represents a true founder mutation or is the result of local linkage disequilibrium.

Identifying a potentially conserved haplotype provides a preliminary basis for further investigation. Future studies incorporating high-density SNP genotyping, whole-genome sequencing, or targeted analysis of broader population cohorts would be necessary to confirm the presence of a founder effect, define the extent of the shared haplotype, and assess its prevalence in affected versus unaffected individuals.

Results

Clinical phenotype

We report on twelve patients (6 females and 6 males) from eight families in four different regions of the West Bank and Gaza Strip. All patients were offspring of consanguineous marriages. The detailed clinical manifestations, biochemical findings, and neurological outcomes of the twelve patients enrolled in the study are summarized in Table 3. The age at which symptoms first appeared varied from 4 months to 21 months. Common features included recurrent vomiting, lethargy, and Kussmaul breathing. The characteristic laboratory finding in all patients was the increased urinary excretion of 2-methyl-3-hydroxybutyrate and tiglylglycine. Out of the twelve patients, ten had favorable outcomes. Patient II-1 from Family 4 presented at 7 months of age with symptoms of vomiting, severe metabolic acidosis, and coma, ultimately leading to death at 8 months. Patient II-1 from Family 3 initially exhibited hypotonia and psychomotor delay at 4 months. His first episode of ketoacidosis occurred a few months later, and he passed away at the age of 4 years (Table 3).

Mutation analysis

In this study, we identified four variants in the *ACAT1* gene (Table 4). The first, p.Ile323Val, was found in six patients: Three from Family 1, one from Family 2, and two from Family 3. The second, p.Val359Glnfs*10, is a complex alteration in exon 11, consisting of a 20-nucleotide deletion followed by a 2-nucleotide insertion (c.1074_1094delGGTTGTACTAGCAAACATTAAinsCC). This variant was identified in Family 4. A third mutation, designated p.Asn414_Gly415delinsSer, was observed in Family 5; this variant represents a deletion and insertion of amino acids at positions 414 and 415. Finally, the p.Cys142Phe variant, resulting in a cysteine to phenylalanine substitution at position 142, was identified in Family 6.

Table 4 presents the ACMG classifications for these *ACAT1* variants, highlighting their potential roles in autosomal recessive beta-ketothiolase deficiency. Notably, p.Val359Glnfs*10 and p.Cys142Phe are novel.

In silico prediction tools, including CADD (Combined Annotation-Dependent Depletion), support the potential pathogenicity of both p.Ile323Val and p.Cys142Phe variants with CADD scores of 18.2 and 24.8, respectively. These scores place these variants within the top 0.1% of deleterious variants in the human genome. Both p.Ile323Val and p.Cys142Phe variants were absent from our in-house database of 3000 normal controls and population databases, suggesting they are rare and potentially pathogenic, rather than benign. Furthermore, AlphaMissense, a deep learning model based on the protein structure prediction tool AlphaFold2, classified both p.Ile323Val and p.Cys142Phe as likely pathogenic, with pathogenicity likelihood scores of 0.77 and 0.85, respectively [9]. These findings highlight the potential contribution of these mutations to the pathogenesis of autosomal recessive beta-ketothiolase deficiency and emphasize the importance of genetic testing in affected individuals.

p.Ile323Val is a founder mutation. A significant haplotype block, measuring 1.2 megabases (Mb) and encompassing four microsatellite markers, was identified as being tightly linked to the founder mutation p.Ile323Val in the *ACAT1* gene, in six individuals from three families (Fig. 1). While the relationships between these families may be unknown, this variant resides within a shared haplotype among six affected individuals (diploid haplotypes) and healthy carriers (haploid haplotypes). This finding strongly suggests a genetic association within this region, indicating that it likely originated from a common ancestor and has been conserved across generations due to limited recombination in this genomic segment.

Discussion

Molecular genetic analysis of nine patients from six families diagnosed with beta-ketothiolase (T2) deficiency in our study identified four distinct variants in the *ACAT1* gene. Two of these variants are novel: a frameshift mutation p.Val359Glnfs*10 and a missense variant p.Cys142Phe. Both novel variants are predicted to be pathogenic by in silico tools. Specifically, the Combined Annotation-Dependent Depletion (CADD) scores were 24.8 for p.Cys142Phe and 18.2 for the p.Ile323Val variants, indicating a high likelihood of deleterious impact [8]. Additionally, AlphaMissense, a pathogenicity predictor based on AlphaFold2 structural models, assigned pathogenicity likelihood scores of 0.85 for p.Cys142Phe and 0.77 for p.Ile323Val, supporting their damaging potential [9]. These variants were absent from population frequency databases and an in-house cohort of 3,000 controls, suggesting rarity and probable pathogenicity.

A significant finding was the identification of the p.Ile323Val missense variant as a founder mutation in

Table 3 Clinical manifestations and laboratory findings of the patients enrolled in the study

	Patient Profile			Clinical phenotype					Laboratory findings				Outcome	
Family	**Patient number and initials**	**Sex**	**Consanguinity**	**Age at onset of symptoms**	**Age at diagnosis**	**Clinical manifestations**	**Trigger**	**Number of episodes**	**Blood glucose mg/dL (RR: 60–140)**	**Initial serum Ammonia µmol/L (RR 30–85)**	**Serum amino acid analysis µmol/L**	**Urine organic acid analysis**	**Age at last Follow-up**	**Neurological and cognitive outcome**
Family 1	**III-1**	F	First cousins	6 months	9 months	Recurrent vomiting, severe dehydration, Kussmaul breathing, severe metabolic acidosis	GE, URI	1	68	NA	Normal	Massive excretion of lactic acid, 3-Hydroxybutyric acid, 2-methyl-3-hydroxybutyric, tiglylglycine	21 years	Normal cognition and social interaction. Normal neurological examination
	III-2	F	First cousins	10 months	11 months	Recurrent vomiting, fever, Kussmaul breathing, severe metabolic acidosis, coma	GE, URI	6	60	102	Normal	Massive excretion of 3-Hydroxybutyric, acetoacetic acid, 2-methyl-3-Hydroxybutyric acid, tiglylglycine.	6 0.5 years	Normal cognition and social interaction. Normal neurological examination. At school, first class with good school performance
	III-3	F	First cousins	16 months	18 months	Recurrent vomiting, Kussmaul breathing, lethargy, severe metabolic acidosis	GE	5	58	NA	Elevated plasma valine (786; RR: 74–321), leucine (578; RR: 49–216), isoleucine (288; RR: 22–107), and lysine (438; RR: 48–284).	Moderate excretion of lactic acid. Massive excretion of 2-methyl-3-hydroxybutyric acid. Mild excretion of tiglylglycine	11 years	Normal cognition and social interaction. Normal neurological examination. At school, 5th class with good school performance
Family 2	**II-1**	F	First cousins	18 months	18 months	Convulsions, Kussmaul breathing, severe metabolic acidosis, hyperuricemia	GE	3	72	74	Normal	Massive excretion of 3-hydroxybutyric, 2-methyl-3-hydroxybutyric acids. Mild excretion of tiglylglycine	4 years	Normal cognition and social interaction. Normal neurological examination
Family 3	**II-1†**	M	First cousins	4 months	13 months	Hypotonia and psychomotor delay were followed a few months later by vomiting, Kussmaul breathing, lethargy, severe metabolic acidosis.	URI	1	NA	NA	Normal	Massive excretion of 3-Hydroxybutyric. Moderate excretion of 2-methyl-3-hydroxybutyric acids, tiglylglycine	4 years	Severe head lag, dystonia, psychomotor delay, had a gastrostomy tube. Died at age 4 years
	II-2	M	First cousins	18 months	18 months	Fever, decreased oral intake, dehydration	GE	1	20	80	NA	Massive excretion of 3-Hydroxybutyric. Moderate excretion of 2-methyl-3-hydroxybutyric acids, tiglylglycine	2 years and 9 months	Normal cognition and neurological examination
Family 4	**II-1†**	F	Second cousins	7 months	7 months	Vomiting, severe metabolic acidosis, lethargy, coma	GE	1	NA	54	Normal	Massive excretion of lactic acid, 3-Hydroxybutyric acid, 2-methyl-3-hydroxybutyric, tiglylglycine	7 months	Persistent encephalopathy and coma. Died at age 8 months
Family 5	**II-1**	M	Second cousins	21 months	22 months	Lethargy, Kussmaul breathing	GE	2	24	22	Normal	Massive excretion of 2-methyl-3-hydroxybutyric acid, tiglylglycine. Moderate excretion of 2-methyl-glutaconic acid	3 years	Normal cognition and social interaction. Normal neurological examination. His left lower limb was amputated following a major injury in Gaza
Family 6	**II-1**	M	First cousins	6 months	10 months	Vomiting, lethargy, Kussmaul breathing,	GE, URI	12	NA	NA	Normal	Massive excretion of 2-methyl-3-hydroxybutyric acid, mild excretion of tiglylglycine	6 years	Normal cognition and social interaction. Normal neurological examination

Table 3 (continued)

	Patient Profile			Clinical phenotype				Laboratory findings				Outcome	
Family	Patient number and initials	Sex	Consanguinity	Age at onset of symptoms	Age at diagnosis	Clinical manifestations	Trigger	Number of episodes	Blood glucose mg/dL (RR: 60–140)	Initial serum Ammonia µmol/L (RR 30–85)	Serum amino acid analysis µmol/L	Urine organic acid analysis	Age at last Follow-up	Neurological and cognitive outcome
Family 7	**II-1**	F	First cousins	8 months	13 months	Severe recurrent episodes Kussmaul breathing, recurrent vomiting, lethargy, hypoactivity	URI	4	NA	NA	Normal	Massive excretion of lactic acid, 3-Hydroxybutyric acid, acetoacetic, 2-methyl-3-hydroxybutyric, tiglylglycine	6 years	Normal cognition and social interaction. Normal neurological examination
Family 8	**II-1**	M	First cousins	12 months	12 months	Vomiting, lethargy, coma, Kussmaul breathing, severe metabolic acidosis	URI	1	250	NA	Normal	Massive excretion of lactic acid, 3-Hydroxybutyric acid, 2-methyl-3-hydroxybutyric, tiglylglycine	32 years	Normal cognition and social interaction. Normal neurological examination.
	II-2	M	First cousins	8 months	8 months	Vomiting	GE	1	NA	NA	Normal	Massive excretion of lactic acid, 3-Hydroxybutyric acid, 2-methyl-3-hydroxybutyric, tiglylglycine	24 years	Normal cognition and social interaction. Normal neurological examination.

(†): The patient was deceased at the time of conducting the study

GE: Gastroenteritis

URI: Upper respiratory infection

NA: Not available

F: Female

M: Male

RR: Reference range

six patients from three unrelated families. Identifying a haplotype block associated with *ACAT1*-related disorders provides critical insights into the genetic architecture of beta-ketothiolase deficiency, offering a valuable tool for tracing the inheritance patterns of founder mutations and understanding their evolutionary history. These findings underscore the importance of genetic testing in diagnosing beta-ketothiolase deficiency, particularly given the disorder's clinical severity, which often manifests in early childhood with episodes of ketoacidosis and metabolic decompensation. While most patients exhibit normal psychomotor development, approximately 20% experience long-term complications, highlighting the need for early diagnosis and intervention [2, 6]. Together, these findings advance our understanding of the genetic basis of beta-ketothiolase deficiency, emphasizing the role of population-specific variants and the potential for personalized therapeutic approaches in managing this rare metabolic disorder.

All patients in our study experienced recurrent episodes of ketoacidosis triggered by infections. Patient II-1 from Family 4 experienced a severe ketoacidotic episode at 7 months of age, which progressed to coma and ultimately resulted in death. On the other hand, patient II-1 from Family 3 presented with hypotonia and psychomotor delays starting at 4 months of age, independent of any ketoacidosis episodes, and a few months later, he developed a ketoacidosis episode. Unfortunately, he passed away at age 4 years before the study was conducted (Table 3). Three patients (III-3 from Family 1, II-2 from Family 3, and II-1 from Family 5) had documented hypoglycemia, while patient II-1 from Family 8 had documented hyperglycemia at the initial presentation.

Abdelkareem et al. reviewed 105 *ACAT1* variants associated with T2 deficiency, which have been reported in 149 patients from 134 nuclear families. Most of these variants were missense mutations. The majority of patients experienced episodic ketoacidosis but were generally well between episodes, although there were potential complications such as death or permanent neurological abnormalities [10]. Clinical variability regarding the age of onset and frequency of ketoacidotic episodes has been reported, even among siblings, indicating a lack of genotype-phenotype correlation [11–14]. Neuroimaging abnormalities observed in patients with T2 deficiency include distinctive findings on T2-weighted MRI, such as bilateral hyperintensity in the globus pallida, putamina, and periventricular white matter [15]. A brain MRI was performed on Patient II-1 from Family 3 when the patient was one year old, as part of the diagnostic evaluation for psychomotor delay, and was normal.

The management of ketoacidotic episodes in emergency settings typically involves intravenous glucose infusion, supplemented with appropriate electrolytes, to

Table 4 Genetic variants and in Silico predictions for *ACAT1* mutations in autosomal recessive Beta-Ketothiolase deficiency

Family	Patient	Variant in the ACAT1 gene	Testing Method	Zygosity	ClinVar Classification	ACMG Classification	In Silico Prediction Tools	Supporting Evidence
Family 1	**III-1**	Chr11:108014736 A > G (GRCh37). *ACAT1*: c.967 A > G, p.(Ile323Val).	Sanger Sequencing	Homozygous	Variant of Uncertain Significance (VUS)	Likely Pathogenic	PolyPhen-2: Possibly damaging (score 0.86), SIFT: Tolerated (score 0.63), MutationTaster: Polymorphism CADD Score: 18.2, Alpha Missense Score: 0.77	- PP1: Cosegregation with disease in affected family members. - PM2: Extremely low frequency in gnomAD. - PM5: Amino acid change from known pathogenic variants. - PP2: Missense mutation in a gene where missense mutations are common disease mechanisms.
	III-2		Sanger Sequencing	Homozygous				
	III-3		Sanger Sequencing	Homozygous				
Family 2	**II-1**		Whole Exome Sequencing (WES)	Homozygous				
Family 3	**II-1**		Sanger Sequencing	Homozygous				
	II-2		Sanger Sequencing	Homozygous				
Family 4	**II-1**	Chr11:108016997 delGGTTGTACTAGCAAACATTAA insCC (GRCh37). *ACAT1*: c.1074_1094delinsCC p.(Val359GlnfsTer10)	Sanger Sequencing	Homozygous	Not Reported	Likely Pathogenic	PolyPhen-2: Not applicable (indel), SIFT: Not applicable (indel), MutationTaster: Not applicable (indel), CADD Score: 24.1, Alpha Missense Score: Not Applicable	- PVS1: Null variant with loss-of-function mechanism. - PM2: Very low frequency in gnomAD. - Protein Impact: Premature termination, leading to a truncated protein.
Family 5	**II-1**	chr11:108018074 ATGGA > GT (GRCh37): *ACAT1*: c.1241_1245delATGGAinsGT p.(Asn414_Gly415delinsSer)	Sanger Sequencing	Homozygous	Likely Pathogenic	Likely Pathogenic	PolyPhen-2: Possibly damaging (score 0.97), SIFT: Tolerated (score 0.60), MutationTaster: Disease-causing, CADD Score: 20.4, Alpha Missense Score: 0.79	- Previously reported as likely pathogenic (PMID: 31268215). - PM3: Detected in trans with pathogenic variants. - PM2: Extremely low frequency in gnomAD. - PM4: In-frame deletion/insertions affecting protein length. - PM1: Located in a critical functional domain.
Family 6	**II-1**	Chr11:108005959 G > T (GRCh37). *ACAT1*: c.425G > T, p.(Cys142Phe)	Sanger Sequencing	Homozygous	Variant of Uncertain Significance (VUS)	Variant of Uncertain Significance (VUS)	PolyPhen-2: Probably damaging (score 1.0), SIFT: Damaging (score 0.01), MutationTaster: Disease-causing, CADD Score: 24.8, Alpha Missense Score: 0.85	- PM2: Extremely low frequency in gnomAD. - PM1: Located in a critical functional domain. - PP2: Missense mutation in a gene with low rate of benign variants. - PP3: Computational tools predict deleterious effect.

maintain blood glucose levels within the upper normal range and suppress ketogenesis. In T2 deficiency, severe metabolic acidosis may occur, requiring bicarbonate infusion and, in severe cases, dialysis [1, 16]. In our study, all patients were treated with intravenous glucose infusion, electrolyte correction, and bicarbonate infusion. These interventions were administered under strict monitoring of electrolytes, blood glucose, lactic acid, and ammonia levels. Ten patients successfully recovered from recurrent ketoacidosis episodes. However, Patient II-1 from Family 4 experienced persistent coma and passed away one month after the initial presentation. Subsequent management included mild protein restriction (1.5 g/kg/day), fat restriction, avoidance of fasting, and L-carnitine supplementation.

During follow-up at the metabolic clinic, all 10 surviving patients showed normal cognitive and neurological outcomes. The frequency of ketoacidosis episodes decreased with age. However, Patient II-1 from family 6 experienced 12 episodes by the age of 6, while all other patients had fewer than 6 episodes by the time the study was conducted (Table 3).

This study's limitations include the relatively small sample size and incomplete genetic testing, as three families declined molecular analysis, which may have restricted genotype-phenotype correlation assessment. The founder mutation hypothesis relies on limited haplotype data and lacks comparative analysis with ethnically matched controls. Comprehensive population-wide genotyping and longitudinal studies are crucial to validate the founder effect and to better understand the clinical outcomes over time.

Conclusions

The study highlights the critical role of genetic research in unraveling the complexities of beta-ketothiolase deficiency and related disorders. By identifying haplotype blocks, founder mutations, and novel pathogenic variants, researchers can improve diagnostic accuracy, enhance genetic counseling, and pave the way for targeted therapies. These advancements underscore the importance of integrating genetic testing and bioinformatics tools into clinical practice to better understand and manage rare genetic disorders. The study's limitations include a small sample size and the refusal of families of three patients to undergo genetic testing.

Abbreviations

T2	Mitochondrial acetoacetyl-CoA thiolase
C5-OH	carnitine 2-methyl-3-hydroxybutyryl-carnitine
C51:carnitine	Tiglylcarnitine
gDNA	Genomic DNA
GATK	Genome Analysis Toolkit
CE	Capillary electrophoresis
STR	Satellite repeat
CADD	Combined Annotation-Dependent Depletion
MRI	Magnetic resonance imaging

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12920-025-02175-8.

Supplementary Material 1

Supplementary Material 2

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Author contributions

I.D drafted, critically revised the manuscript, and substantially contributed to the conception and design. H.A, A.Z and R.B contributed to the study design and data acquisition. M.K and H.K substantially contributed to the genetic analysis and data interpretation. All authors reviewed and approved the final manuscript.

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Data availability

All genetic variations analyzed during the current study are submitted to ClinVar with the following accession numbers: SCV005894706, SCV005894708, SCV005894710, and SCV005894711.The datasets generated and/or analysed during the current study are available in the ncbi repository, https://www.ncbi.nlm.nih.gov/sra/PRJNA1238554(Bio sample SAMN47476274).

Declarations

Ethics approval and consent to participate

The study was approved by the Institutional Review Board (IRB) at the Arab American University, Palestine (registration number at FWA IORG0011491) and by the Palestinian Health Research Council (PHRC/HC/ 518/19). Informed consent to participate was obtained from all participants and the parents/legal guardians of minors. The study adhered to the Declaration of Helsinki to this effect.

Consent for publication

Written informed consent was obtained from the patients/or their parents for publication of their clinical and genetic details. We explained that the patients' names would not be attached and no pictures or videos would be obtained. The consent was delivered directly to the study coordinators during the preparation of the manuscript.

Competing interests

The authors declare no competing interests.

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