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Maple syrup urine disease: Characteristics of diagnosis and treatment in 45 patients in Chile

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Abstract

Maple urine syrup disease (MSUD) is an autosomal recessive disorder characterized by deficient activity of the branched-chain alpha ketoacid dehydrogenase (BCKAD) enzymatic complex due to biallelic variants in the alpha (*BCKDHA*) or beta (*BCKDHB*) subunits or the acyltransferase component (*DBT*). Treatment consists in leucine (LEU), isoleucine (ILE), and valine (VAL) (branched-chain amino acids) dietary restriction and strict metabolic control. to determine the characteristics of the Chilean cohort with MSUD currently in follow-up at Instituto de Nutrición y Tecnología de los Alimentos, during the 1990–2017 period Retrospective analytical study in 45 MSUD cases. Measured: biochemical parameters (LEU, ILE, and VAL), anthropometric evaluation, and neurocognitive development. In 18 cases undergoing genetic study were analyzed according to the gene and protein location, number of affected alleles, and type of posttranslational modification affected. Then, 45 patients with MSUD diagnosis were identified during the period: 37 were alive at the time of the study. Average diagnosis age was 71 ± 231 days. Average serum diagnosis LEU concentrations: 1.463 ± 854.1 μmol/L, VAL 550 ± 598 μmol/L and ILE 454 ± 458 μmol/L. *BCKDHB* variants explain 89% cases, while *BCKDHA* and *DBT* variants explain 5.5% of cases each. Variants p.Thr338Ile in *BCKDHA*, p.Pro240Thr and p.Ser342Asn in *BCKDHB* have not been previously reported in literature. Average serum follow-up LEU concentrations were 252.7 ± 16.9 μmol/L in the <5 years group and 299 ± 123.2 μmol/L in ≥5 years. Most cases presented some degree of developmental delay. Early diagnosis and treatment is essential to improve the long-term prognosis. Frequent blood LEU measurements are required to optimize metabolic control and to establish relationships between different aspects analyzed.

KEYWORDS
BCKDHA, BCKDHB, DBT, leucine, MSUD, neurocognitive development

1 | INTRODUCTION

Maple urine syrup disease (MSUD) is an autosomal recessive disorder characterized by deficient activity of the branched-chain alpha ketoacid dehydrogenase (BCKAD) enzymatic complex due to biallelic variants in the alpha (*BCKDHA*) or beta (*BCKDHB*) subunits or the acyltransferase component (*DBT*) (Strauss, Puffenberger, & Carson, 2006). MSUD has a worldwide incidence of 1:185,000 live newborns (Chuang & Shih, 2001). In Latin America, the estimated incidence is 1:60,000 live newborn children (Cornejo et al., 2014; Cornejo, Raimann, Pérez, Desviat, & Arias, 2017).

The *BCKDHA* gene is located in chromosome 19q13.1q13.2, has 27.18 kb and 9 exons, all of them protein-coding (Aken et al., 2016). The encoded protein has 445 amino acids with a single domain that contains the E1 dehydrogenase component, which uses thiamine as a cofactor. It also presents 25 residues with posttranslational modifications, mainly by phosphorylation (Breuza et al., 2016; Mitchell et al., 2015). Here, 45% of MSUD patients are explained by variants in this gene, with 104 known variants (Stenson et al., 2014; Strauss et al., 2006). Then, 35–61% of these are protein-truncating variants while the remaining ones are protein-altering variants, mainly missense (Landrum et al., 2016; Stenson et al., 2014).

BCKDHB gene is located in chromosome 6q13q15, has 239.6 kb and 10 exons, all of them protein-coding (Aken et al., 2016). The encoded protein has 392 amino acids and 2 domains that form the transketolase catalytic site, and 10 residues with posttranslational modifications, mainly by phosphorylation (Breuza et al., 2016; Mitchell et al., 2015). Deficient activity of *BCKDHB* explains 35% of MSUD cases, with 127 known variants (Stenson et al., 2014; Strauss et al., 2006). Approximately 40–63% of them are protein-truncating variants and the remaining ones are protein-altering variants, mainly missense (Landrum et al., 2016; Stenson et al., 2014).

DBT gene is located on 1p31 chromosome, contains 62.92 kb and 11 exons, all of them are protein-coding (Aken et al., 2016). It has 17 residues with posttranslational modifications mainly by phosphorylation (Breuza et al., 2016; Mitchell et al., 2015). There are 84 known variants in this gene explaining around 20% of MSUD cases (Stenson et al., 2014; Strauss et al., 2006) About 50–80% are protein-truncating variants and the remaining ones are protein-altering, mostly missense (Landrum et al., 2016; Stenson et al., 2014).

The BCKAD loss-of-function produces a blockade in the last reaction of the catabolic pathway involving branched-chain amino acids (BCAA) such as leucine (LEU), isoleucine (ILE), and valine (VAL), which results in an increase in their serum plasma concentration, urine keto acids, and alloisoleucine production. The severity of the condition varies depending on BCKAD residual activity level with a wide clinical spectrum among its five recognized clinical variants (Blackburn et al., 2017). Currently, there is no reported evidence of an association between genotype and phenotype in MSUD patients.

Patients with the classical neonatal form present BCKAD residual activity <2%, showing clinical manifestations like irritability, lethargy, and inappetence during the first week of life. This progresses to autonomic dysregulation, respiratory distress, apnea, bradycardia, hypothermia, hypotonicity, MSUD, cerebral edema, and even death if not treated (Abi-Wardé et al., 2017; Cornejo et al., 2017; Cornejo & Raimann, 2005; Knerr, Weinhold, Vockley, & Gibson, 2012; Strauss et al., 2010; Zinnanti et al., 2009).

Treatment consists of dietary restriction of LEU under strict metabolic control to provide adequate protein intake ensuring weight increase, normal range LEU plasma concentrations and avoiding ILE and VAL deficiency (Frazier et al., 2014). In Chile, follow-up of almost all patients with metabolic diseases is carried by the Instituto de Nutrición y Tecnología de los Alimentos (INTA), University of Chile. Therefore, this analysis represents a countrywide cohort, and its objective was to determine the characteristics of the Chilean MSUD cohort currently in follow-up at INTA, University of Chile.

2 | METHODS

This is a retrospective analytical study based on collected data from clinical records of the Chilean MSUD cohort. During the 1990–2017 period, 45 patients with MSUD diagnosis were identified: 37 classical variants and 8 with different clinical presentations. Thirty-seven were alive at the time of the study, which were included for the analysis. The study was approved by the Ethics Committee of INTA University of Chile, and it was conducted in accordance with the principles of the Declaration of Helsinki (approval date September 26, 2020).

2.1 | Biochemical parameters

Confirmation of the diagnosis was made by quantification in plasma of BCAAs through Ion Exchange Chromatography or liquid chromatography tandem mass spectrometry (ref. values: LEU 48-160 μmol/L, ILE 26–91 μmol/L, VAL 86–190 μmol/L). The measurement of the LEU concentration during follow-up was carried out through the bacterial inhibition assay a semiquantitative method in dry blood sample (DBS) (ref value: <152.4 μmol/L).

According to the international management guidelines for MSUD patients in follow-up published in 2014 (Frazier et al., 2014), it was considered good metabolic control when the DBS value was less than 200 μmol/L in children under 5 years of age and in those over 300 μmol/L. Our patients in follow-up send monthly levels throughout their lives, and occasionally they can increase the frequency to weekly in the event of an acute event or occurrence.

The total of samples of LEU concentration considered for the analysis was 2,255, with a mean of samples per patient per year of 10.2 ± 6.6. Metabolic control was defined based the average plasma LEU concentrations and age as follows: adequate LEU 75–200 μmol/L, inadequate LEU 200–300 μmol/L, and poor >300 μmol/L for patients <5 years and adequate LEU 75–300 μmol/L, inadequate LEU 300–400 μmol/L, and poor >400 μmol/L for patients ≥5 years (O'Reilly et al., 2020).

2.2 | Anthropometric evaluation

Weight and height were measured by a digital weight and stadiometer, precision 0.1 kg and 0.1 cm, respectively, with patients in Frankfurt position, and were evaluated to determine the indicators: weight for height

(W/T) and height for age (H/A) for <5 years, body mass index (BMI) for age (BMI/A) and height for age (H/A) for patients 5–19 years, and BMI for >19 years. The z-score was calculated for each indicator to classify nutritional status according to WHO references in 2006 for <5 years (World Health Organization, 2006) and 2007 between 5 and 19 years (World Health Organization, 2007) according to gender and age.

2.3 | Nutritional analysis

A 24-hr dietary recall was made for each patient. It was quantified with the amino acids analyzer software available in our laboratory to estimate the intake of: energy (kcal/day), proteins (g/kg), and BCAA (mg/kg).

2.4 | Neurocognitive development

Neurocognitive development of patients younger than 3 years 6 months was assessed using Bayley Scales of Infant Development II. They were classified according to their mental development index (MDI) as normal 85–115, mild delay 70–84, or major delay <70. Older patients in preschool, scholar, or adult age were evaluated with the corresponding Wechsler Intelligence Scale (WPPSI, WISC-III, WAIS-IV, respectively). Depending on the resulting intelligence quotient (IQ) (average 100, *SD* 15) they were listed as normal 80–109, borderline 70–79, mild delay 55–69, moderate delay 40–54, or severe delay <40.

2.5 | Variant analysis

The sequencing methodology was recently described in a manuscript under review for 18 patients reported in this study. As these analyses were performed through a research project, it was not possible to test all of them due to resource limitations. All genetic variants identified in patients who underwent genetic study were analyzed according to the gene and protein location, number of affected alleles, and type of post-translational modification affected. Protein information of BCKDHA, BCKDHB, and DBT was obtained from UniProtKB database (UniProtKB entries P12694, P21953, and P11182, respectively) (Breuza et al., 2016). Structural conformation (PDBe entry 2bfb) (Velankar et al., 2016) was used to determine the location of known variants and model new variants. The modeling was done with UCSF Chimera v1.12 (Pettersen et al., 2004).

2.6 | Statistical analysis

All statistical analyses were made with SPSS software. Continuous variables with normal distribution were compared to means with the T student test. Continuous variables with a non-normal distribution were analyzed using Mann–Whitney tests. Nominal variables were analyzed with the Chi square test. A *p*-value <.05 was considered significant.

3 | RESULTS

During the 1990–2017 period, 45 patients with MSUD diagnosis were identified: 37 classical variants and 8 with different clinical presentations. Thirty-seven were alive at the time of the study.

Among the eight deceased patients, five had LEU levels over 1,080 μmol/L upon death. Two patients had high levels upon admission to that hospitalization but normal levels at the time of verifying death, so the direct relationship between metabolic decompensation and death was not clear. One patient died at home with a cause identified as cardiorespiratory arrest, without the possibility of having a LEU level close to the date of death. His last reported level was normal (<152 μmol/L). In two patients, we did not have access to levels in relation to the last hospitalization. Twenty-three MSUD are female and fourteen are male, with an average age of 13.2 ± 7.3 years (range 1.2–29.1 years).

Average diagnosis age was 71 ± 231 days (range 1–1,460 days) and median 16 days of life. Deceased patients average diagnosis age was 29 ± 34.6 days, while in the alive patients were 81 ± 257.5 days. Our cohort presented an average plasma LEU concentrations of 1.463 ± 854.1 μmol/L (range 463–3.962 μmol/L). Only 13 presented plasma LEU concentrations <1.000 μmol/L at the time of diagnosis. No significant statistical association was found between age of diagnosis and diagnosis plasma (peak level) LEU concentrations ($p = .294$). Nor did we find a correlation between the age of diagnosis and the intellectual quotient ($R = -.171$).

Table 1 describes the genetic variants distribution in 18 cases with genetic study. *BCKDHB* variants explain 89% cases, while *BCKDHA* and *DBT* variants explain 5.5% of cases each. Variants p.Thr338Ile in *BCKDHA*, p.Pro240Thr and p.Ser342Asn in *BCKDHB* have not been previously reported in literature (Figure 1). Of the 36 affected alleles, 41.7% corresponds to p.Ile214Lys variant and 16.7% to p.Pro200Ter (16.7%). Two variants (p.Ile214Lys and p.Pro240Thr in *BCKDHB*) were located in the internal portion of the protein, three in the surface (p.Gly336Ser, p.Ser342Asn, and

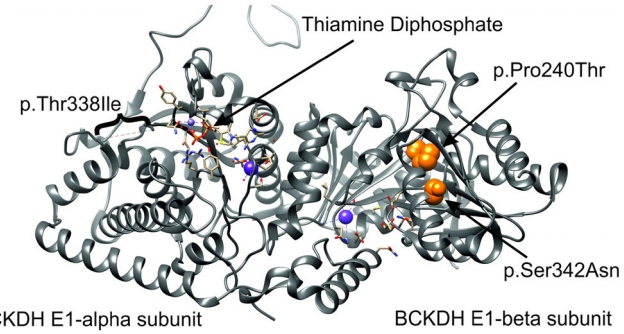

FIGURE 1 Structural representation of new variants detected in Chilean population. Orange spheres represent side-chain atoms of affected residues, big purple spheres represent potassium ions, and small purple sphere represents manganese ion. The parenthesis represents the region in which residue 338 should be located. BCKDH, branched-chain keto acid dehydrogenase

TABLE 1 Description of the variants detected in 18 Chilean MSUD patients according to several characteristics

Gene	Variant	Reported	Number of alleles	Type of variant	Protein location	Protein domain	Posttranslational modification	MAF in control population[a,b]	SIFT score[a]	PolyPhen score[a]	CADD score[a]
BCKDHA	p.Thr338Ile	No	2	Missense	Unknown	Thiamine pyrophosphate BD	Phosphorylation	0	0	1	29.4
BCKDHB	p.Ile214Lys	Yes	15	Missense	Internal	Pyrimidine BD	No	–	–	–	–
	p.Pro200Ter	Yes	6	Frameshift	NA	NA	No	–	–	–	–
	p.Pro240Thr	No	4	Missense	Internal	Pyrimidine BD	No	0	0	0.941	25.7
	p.Gly131Val	Yes	3	Missense	Interface	Pyrimidine BD	No	–	–	–	–
	p.Pro356Leu	Yes	2	Missense	Surface	Transketolase C-terminal	No	–	–	–	–
	p.Gly336Ser	Yes	1	Missense	Surface	Transketolase C-terminal	No	–	–	–	–
	p.Ser342Asn	No	1	Missense	Surface	Transketolase C-terminal	No	0	0.01	0.026	23.3
DBT	p.Gly406Asp	Yes	2	Missense	Unknown	Acyltransferase	No	–	–	–	–

Abbreviations: BD, binding domain; MAF, minor allele frequency; MSUD, maple urine syrup disease; NA, not applicable.
[a]Only described for new variants.
[b]Frequencies reported in Exome Sequencing Project; 1,000 Genomes Project; and gnomAD databases.

p.Pro356Leu in *BCKDHB*) and one in the interphase with subunit alpha (p.Gly131Val in *BCKDHB*). The location of two variants could not be determined as its structure has not been crystalized (p.Thr338Ile in *BCKDHA* and p. Gly406Asp in *DBT*) (Figure 1). Three variants affect the catalytic domains (p.Pro356Leu and p.Gly336Ser in *BCKDHB*, and p.Gly406Asp in *DBT*), and a single variant alters a residue phosphorylated in its native form (p.Thr338Ile in *BCKDHA*).

According to the nutritional status assessment: 3 cases were malnourished, 3 with underweight, 22 were eutrophic, 7 were overweight, and 1 case was obese. It should be mentioned that of the three malnourished subjects, all are over 18 years of age and are metabolically stable. While the three subjects at underweight are under 18 years of age who are in the process of recovering their nutritional status and their metabolic control has not been affected. Eleven cases were classified with a normal-low height, ten cases with low height, and fifteen have normal height and no cases of normal-high of high stature. No significant differences by sex were found in nutritional state and height distribution.

Eleven cases were classified with a normal-low height, ten cases with low height, and fifteen have normal height and no cases of normal-high of high stature. No significant differences by sex were found in nutritional state and height distribution.

Regarding nutritional intake, the cohort was separated by age group and energy intake was compared with recommended dietary allowance, and all comply the requirements of energy (<1 year: 80 kcal/kg, 1–3 years: 84 kcal/kg, 9–13 years: 46 kcal/kg, 14–18 years: 32 kg/kg, and >18 years 30 kcal/kg). The patients received an average: special formula protein 2.0 g/kg/day, representing 97% daily protein intake. All groups presented VAL and ILE average intake according to protocol recommendations (Table 2) (Frazier et al., 2014).

The 37 patients currently in follow-up receive the following supplementation: thiamine (50 mg/day); L-carnitine (50 mg/kg/day); ILE (281 ± 244 mg/day, range 60–1,120 mg/day); and/or VAL (277 ± 221 mg/day, range 30–960 mg/day). Then, 83.3% of patients receive empiric supplementation of thiamine, 78% of L-carnitine, and 55% receive both.

According to control plasma LEU concentrations, the average was 252.7 ± 16.9 μmol/L in 12 cases <5 years (in one case we do not have data) and 209.4 ± 123.2 μmol/L in 24 cases ≥5 years. Twenty-two presented adequate metabolic control, twelve inadequate, and two poor control established by our protocol (Pettersen et al., 2004). Regarding age distribution, in the <5 years group, 1 showed adequate metabolic control, 11 inadequate, and in the group over 5 years; 17 had adequate metabolic control, 5 inadequate, and 2 a poor control. All groups presented mean ILE and VAL plasma concentrations according to international recommendations (200–400 μmol/L) (Frazier et al., 2014).

For neurocognitive evaluation patients were classified according to their IQ or MDI, depending on their age. This evaluation showed that 6 cases had normal IQ, 11 a borderline-mild delay, 1 case had moderate delay, 9 cases with moderate–severe delay, and 10 cases had severe delay. No statistically significant relationship was found between average debut LEU plasma concentrations and

TABLE 2 Comparison of BCAA intake according to age group with international recommendations in the follow-up of 37 Chilean MSUD patients

Age groups (years)	LEU (mg/kg/day) Mean	Recommendation (mg/kg/day) LEU	ILE (mg/kg/day) Mean	Recommendation (mg/kg/day) ILE	VAL (mg/kg/day) Mean	Recommendation (mg/kg/day) VAL
1–3	21.6	40–70	25.6	20–70	30.8	30–70
4–8	32.5	35–65	26.6	20–30	30.6	30–50
9–13	27.3	30–60	33.0	20–30	34.0	25–40
14–18	16.1	15–50	12.8	10–30	14.9	15–30
≥18	12.4	15–50	12.7	10–30	15.0	15–30

Abbreviations: ILE, isoleucine; MSUD, maple urine syrup disease; LEU, leucine; VAL, valine.

neurocognitive outcome ($p = .997$). Neither was there a significant relationship between the last and LEU average follow-up plasma concentrations ($p = .824$). We also analyzed association between diagnostic age and neurocognitive outcome, with no significant findings.

Of the 45 patients, 18 underwent genetic analysis. Comparing this group to patients with no genetic study, we found that the former presented significantly higher debut LEU plasma concentrations (1,674.4 ± 988.8 μmol/L vs. 1,186.8 ± 589.8 μmol/L, p-value < .05). This was not accompanied by differences in the extension of hospital admission, debut presentation, or number of deceased patients (Table 3). When comparing the p.Ile214Lys variant in *BCKDHB* group with other genotypes, no statistically significant differences were found in terms of diagnostic age, anthropometry at birth, diagnostic amino acids plasma levels, LEU plasma follow-up concentrations, clinical presentation, or number of deceased patients (Table 4).

4 | DISCUSSION

Overall our patients presented adequate metabolic control according to established protocols for LEU plasma concentrations and LEU nutritional intake.

Most patients presenting adequate control were ≥5 years, possibly associated with fewer acute medical events. Our group presents a lower mortality rate than the reported in published literature (Ibarra-González, Fernández-Lainez, Belmont-Martínez, & Vela-Amieva, 2007). Of the total of the eight deceased patients, five of them had LEU concentrations >1,080 μmol/L, of which two cases had LEU concentrations in the normal range at the time of death. With these results, it can be concluded that the metabolic decompensation caused by the high concentration of LEU would be the main cause of death in these patients.

No relationship was found between debut and follow-up plasma LEU concentrations with neurocognitive development. As only six patients had a normal neurocognitive outcome, we suggest that random/monthly LEU blood levels in our cohort do not sufficiently represent their degree of metabolic control. Treatment compliance does not seem to directly relate to neurological outcome and multiple variables may intervene in the cognitive delay MSUD patients develop. Study limitations may include that we could not establish a statistical relationship between the exposure variable LEU and clinical outcome. This could be due to the limited number of LEU measurements per patient.

Contrary to expected, no significant statistical association was found between diagnosis age and follow-up plasma LEU concentrations or neurological outcome. This could be attributed to the fact that the average age of diagnosis was 71 ± 231 days, which could have a negative effect on IQ later in life and in turn could explain the lack of correlation between genotype and phenotype in our cohort. Data interpretation in this study is limited by the restricted size of the sample.

In terms of genetic variants detected, the proportion of patients with biallelic variants in *BCKDHB* is much larger than the reported in the Mennonite population (Strauss et al., 2020). Malaysian, Egyptian and Brazilian populations also show a predominance of biallelic variants in *BCKDHB*, being associated with 45–55% MSUD cases (Ali & Ngu, 2018; Khalifa et al., 2020; Margutti et al., 2020). Nevertheless, our population shows an even higher frequency of these variants. Three new variants were detected and the allelic frequency of p.Ile214Lys in *BCKDHB* was 0.47. This variant has a Spanish origin but with a low reported frequency in that population (Rodríguez-Pombo, Navarrete, Merinero, Gómez-Puertas, & Ugarte, 2006). In Brazil, the allelic frequency of the same variant was almost 10-fold lower (0.05) than in our cohort, and the p.Pro200Ter in *BCKDHB* was also less frequent in that population (0.12 in Brazil, 0.17 in Chile) (Margutti et al., 2020). Although it was not possible to identify other Latin American cohorts genetically tested, we can infer that the genetic architecture of Chilean MSUD cohort differs widely from other studied populations. In the future, molecular studies directed to detect frequent variants could be implemented to make a genetic diagnosis to a lower cost. This is a relevant point to consider as our health system does not offer financial support to most of the genetic analyses, and most of them must be carried out abroad, which are expensive for a mean Chilean family (Encina et al., 2019).

A separate analysis of the cohort of patients who underwent genetic study was made. Average diagnostic plasma LEU concentrations were significantly higher compared to patients with no genetic study. As this does not correlate to any clinical finding, the former

TABLE 3 Genetic and clinical features of 18 MSUD patients studied molecularly

Patient #	Genotype	Zygosity	Gene	Age of diagnosis (days)	Clinical onset	Leucine levels (µmol/)[a]	Valine levels (µmol/L)[a]	Isoleucine levels (µmol/L)[a]	IQ range
12	p.Ile214Lys/p.Ile214Lys	Homozygous	*BCKDHB*	22	Classic	2,600	1,180	730	Disability
15	p.Ile214Lys/p.Ile214Lys	Homozygous	*BCKDHB*	9	Classic	440	1,464	759	Disability
18	p.Ile214Lys/p.Pro200Ter	Compound heterozygous	*BCKDHB*	30	Classic	1,716	215	525	Disability
19	p.Ile214Lys/p.Ile214Lys	Homozygous	*BCKDHB*	21	Classic	2,000	369.6	190	Disability
21	p.Ile214Lys/p.Ile214Lys	Homozygous	*BCKDHB*	27	Classic	2,075	726	170	Disability
22	p.Ile214Lys/p.Ile214Lys	Homozygous	*BCKDHB*	90	Intermediate	1,027	117	176	Disability
23	p.Ile214Lys/p.Pro200Ter	Compound heterozygous	*BCKDHB*	17	Classic	1,467	466	419	Normal
29	p.Ile214Lys/p.Gly336Ser	Compound heterozygous	*BCKDHB*	14	Classic	3,560	710	512	Disability
14	p.Ile214Lys/p.Pro200Ter	Compound heterozygous	*BCKDHB*	13	Classic	2,638	585	372	Disability
20	p.Thr338Ile/p.Thr338Ile	Homozygous	*BCKDHA*	10	Classic	741	759	402	Normal
25	p.Pro240Thr/p.Pro240Thr	Homozygous	*BCKDHB*	45	Intermediate	3,962	277	215	Disability
27	p.Pro240Thr/p.Pro240Thr	Homozygous	*BCKDHB*	210	Intermediate	1,653	1,171	586	Disability
34	p.Pro200Ter/p.Ser342Asn	Compound heterozygous	*BCKDHB*	9	Classic	750	512	50.8	Disability
38	p.Pro356Leu/p.Pro356Leu	Homozygous	*BCKDHB*	17	Classic	1,038	133	38	Disability
40	p.Gly131Val/p.Gly131Val	Homozygous	*BCKDHB*	17	Classic	1,090	480	290	Disability
43	p.Ile214Lys/p.Pro200Ter	Compound heterozygous	*BCKDHB*	30	Classic	1,640	174	53	Borderline
45	p.Gly406Asp/p.Gly406Asp	Homozygous	*DBT*	11	Classic	750	726	657	Borderline
36	p.Gly131Val/ p.Pro200Ter	Compound heterozygous	*BCKDHB*	60	Intermediate	993	838	294	Disability

Abbreviations: IQ, intelligence quotient; MSUD, maple urine syrup disease.
[a]Peak level at diagnosis.

TABLE 4 Comparison between clinical characteristics of MSUD patients that had the p.Ile214Lys variant, with those that had another variant

Parameter	p.Ile214Lys ($N = 10$)	Other variants ($N = 8$)	p-Value
Age of diagnosis, days (median ± *SD*)	27.3 ± 23.18	51.7 ± 72.57	$p = .83$[a]
Non classic variant (*N*, %)	1 (10%)	3 (37.5%)	$p = .16$[b]
Debut LEU level μmol/L (median ± *SD*)	1,916.3 ± 882.6	1,419.9 ± 1,166.6	$p = .12$[a]
Debut VAL level μmol/L (median ± *SD*)	600.7 ± 440.3	680.4 ± 290.1	$p = .95$[c]
Debut ILE level μmol/L (median ± *SD*)	390.6 ± 243.5	356.4 ± 210.9	$p = .52$[c]
Duration of first hospitalization, days	17.4 ± 12.0	30.9 ± 30.7	$p = .42$[a]
Follow-up LEU levels μmol/L (median ± *SD*)	253.2 ± 66.3	349.3 ± 170.3	$p = .09$[c]
Deceased (*N*, %)	1 (10%)	3 (37.5%)	$p = .16$[b]

Abbreviation: MSUD, maple urine syrup disease.
[a]Sample analyzed by Mann–Whitney test since it is not normally distributed.
[b]Sample analyzed by chi-square.
[c]Sample analyzed by *t* student given that it is normally distributed.

seems to have little relevance in the clinical outcome of these patients and could be random.

The most frequent allele found in our cohort was p.Ile214Lys in *BCKDHB*. We did not find significant differences among clinical or laboratory parameters of patients carrying this variant compared to different genotypes. However, further genetic confirmation of remaining patients could allow us to perform a genotype–phenotype correlation to confirm this finding.

5 | CONCLUSION

We present a series of 45 patients with MSUD controlled in a single center over several decades. The diversity in socioeconomic and genetic characteristics described in the patients included suggests that these data can be applied to countries that face similar difficulties in the diagnosis and follow-up of patients with MSUD.

The analysis of this Chilean MSUD cohort confirms that MSUD is a condition with heterogeneous clinical presentation. Surprisingly, we did not to find a statistical relationship between blood LEU concentrations and outcome. We suggest that this may be due to the limited availability of blood LEU measurements or at the age of diagnosis that could affect the outcome. We conclude that this pathology should be included as a first priority in Chile's neonatal screening program. And as a second priority, the frequency of LEU measurement should be increased in the follow-up protocol to be able to detect high concentrations early and achieve a better developmental result in MSUD patients.

Although we could not demonstrate a relationship between different exposed variables, we think it is highly significant to share the experience of more than 20 years of follow-up of a controlled cohort in a single center. We hope the data presented here allow for better insight on how to best follow these patients. Further controlled studies with a greater number of patients, more extended follow-up periods, and more frequent plasma LEU measurements are required to establish these and other associations. Collaborative multicentric studies are necessary to provide further information regarding prognosis and outcomes in MSUD.

CONFLICT OF INTEREST

The authors have nothing to declare.

AUTHOR CONTRIBUTIONS

Maria Fernanda Medina: Drafting, design, data analysis, and interpretation. **Gabriela Castro**: Drafting, design, data acquisition, and analysis. **Felipe Falcon**: Data acquisition. **Juan Francisco Cabello**: Critically revised the article. **Víctor Faundes**: Data analysis and interpretation. **Diana Ruffato**: Data acquisition. **María Florencia Salazar**: Data acquisition. **Carolina Arias**: Drafting, data acquisition, and revision. **Felipe Peñaloza**: Data acquisition. **Alicia De la Parra**: Data acquisition, analysis, and interpretation. **Veronica Cornejo**: Critically revised the article. **Juan Francisco Cabello** serves as guarantor for the article, accepts full responsibility for the work and/or the conduct of the study, had access to the data, and controlled the decision to publish.

DATA AVAILABILITY STATEMENT

Data openly available in a public repository that issues datasets with DOIs.

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