Identification of Four Novel Mutations in Chilean Patients with Various Forms of Maple Syrup Urine Disease

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Abstract

Background: Maple syrup urine disease (MSUD) is an autosomal recessive inherited metabolic disorder caused by the deficient activity of the branched-chain α-keto acid dehydrogenase (BCKD) enzymatic complex. BCKD is a mitochondrial complex encoded by four genes: BCKDHA, BCKDHB, DBT, and DLD. MSUD is predominantly caused by mutations in the BCKDHA, BCKDHB, and DBT genes which encode the E1α, E1β, and E2 subunits of the BCKD complex, respectively. The aim of this study was to characterize the genetic basis of MSUD in a cohort of Chilean MSUD patients by identifying point mutations in the BCKDHA, BCKDHB, and DBT genes and to describe their impact on the phenotypic heterogeneity of these patients. This manuscript describes a cross-sectional study of 18 MSUD patients carried out using PCR and DNA sequencing.

Results: Four novel pathogenic mutations were identified: one in BCKDHA (p.Thr338Ile), two in BCKDHB (p.Gly336Ser e p.Pro240Thr), and one in DBT (p.Gly406Asp). Four additional pathogenic mutations found in this study have been described previously.

Conclusion: There were no correlations between the genotype and phenotype of the patients. Thus, if MSUD is diagnosed earlier, with a neonatal screening approach, it might be possible to establish genotype-phenotype relationships more efficiently.

Background

Maple syrup urine disease (MSUD) (OMIM #24860) is an inborn error of the metabolism (IEM) caused by a deficiency in the activity of the branched-chain keto acid dehydrogenase (BCKD) complex which results in the accumulation of branched-chain amino acids (BCAA), leucine (LEU), isoleucine (ILE) and valine (VAL), and their keto acids (BCKA). The BCKD complex is a multienzyme macromolecule with three catalytic components (E1, E2, E3) (1). Deficiency of this complex is responsible for increases in the branched chain amino acids leucine, valine and isoleucine in physiological fluids, as well as their related α-keto acids (2). The accumulation of these amino acids mainly affects the central nervous system (CNS) (3).

There are already a number of described mutations that have been linked in to MSUD most of these

involve disturbance in the catalytic subunits of the branched chain α-keto acid dehydrogenase (BCKD) complex. Based on the analysis of the altered loci, MSUD can be divided into three genetic subtypes: type Ia (MIM # 608348) for mutations in the BCKDHA gene (subunit E1α), type Ib (MIM #248.611) mutations found in the BCKDHB gene (subunit E1β), and type II (MIM # 248610), with mutations in the DBT gene (subunit E2) (5,6). BCKDHA has been mapped to human chromosome 19, at 19q13.2. This gene encompasses approximately 27.2 kb of DNA, with a coding sequence distributed across 9 exons and involving 1791 bp. BCKDHB is situated at 6q14.1, spans approximately 240 kb of genomic DNA, and has 11 exons encoding 1572 bp. DBT is found at 1p31, comprises 63 kb of DNA, also has 11 exons and a coding sequence of 10831 bp (3,7). Between these three genes, researchers have identified more than 140 mutations in the literature. (8).

MSUD is a recessive autosomal disorder with a global incidence rate of approximately one in 185,000 newborns. Although it is a rare defect, in some Mennonite populations settled in Pennsylvania, and a handful of other cities in the United States, the estimated elevated incidence is one in 200 live births (3). A study carried out in Portugal by Quental et al. (2010), which evaluated cases diagnosed by mass spectrometry, and found an incidence of one per 86,800 newborns (9). In Brazil, a study conducted by Margutti (2015) identified 11 new mutations in 25 patients with the disease, with three in the BCKDHA gene (p. Pro39Leu, p. Gly56Arg, and p. Tyr120Ter), six in BCKDHB (p. Arg63Pro, p. Gly131Val, p. Glu146Gln, p.Phe149Cys, p. Cys207Phe, and p. Lys211Asn) and two in DBT (p. Glu148Ter and p. Glu417Val) (10).

The clinical manifestations of patients with MSUD are varied and depend on the levels of residual enzyme activity. The clinical phenotypes associated with MSUD are classified as classic, intermediate, intermittent, responsive to thiamine therapy, and lipoamide dehydrogenase deficiency (E3 subunit), depending on, among other criteria, the age of onset and severity of the disease. In the classical form of MSUD, patients present with less than 3% residual enzyme activity and symptoms appear soon after birth. Patients typically have ketosis and LEU plasma concentrations of more than 2,000 µmol/L. In untreated newborns, maple syrup odor can be detected in the earwax within the first 12-24 h, and in urine 48-72 h after birth, although this characteristic odor is variable and thus not a reliable

diagnostic aide. Elevated plasma concentrations of BCAA, as well as widespread disturbances in the plasma concentrations of amino acids are present at 12-24 h of age; elevation of keto acids and ketonuria and irritability can be observed 24-72 h after delivery; encephalopathy manifesting as lethargy and intermittent breathing difficulties are seen after 4 to 5 days; and coma and central respiratory failure can occur between 7 and 10 days (1).

The intermediate form of MSUD features in infancy and childhood, is characterized by psychomotor developmental delay, failure to thrive, seizures and walking difficulty. Ketosis and plasma concentrations of LEU less than 2,000 µmol/L are typical. Although BCAA elevation is persistent, and there is neurological impairment, severe newborn organ decompensation is not seen like in the the classical form. Enzymatic activity in these cases is between 3 and 30% (2).

The intermittent form appears during infancy or childhood, and the patient usually has normal growth and development, with bouts of ataxia that may be accompanied by ketoacidosis. The increase in BCAA only happens during decompensation events.

The sensitivity to thiamine form has a clinical presentation similar to intermediate and intermittent cases, without acute decompensation. Thiamine is a subunit E1 cofactor, regulating the activity of the enzyme complex. Thus, the administration of thiamine decreases serum levels of BCAA. The doses of thiamine used may vary from 10 to 1000 mg per day (11).

The E3 subunit deficiency form of MSUD is very rare, having been reported in approximately 20 cases from around the world. The prognosis for this form of MSUD seems to be dependent on the residual enzymatic activity which can be between 0% and 25% (11).

There are no studies that describe the genotypic profiles of Chilean MSUD patients.

Objectives

We aimed to identify mutations in BCKDHA, BCKDHB and DBT in a cohort of Chilean patients clinically diagnosed with MSUD, and to analyze the clinical characteristics of these patients, in order to identify possible genotype-phenotype correlations.

Methods

Patients

The nutrition team from the Genetics and Metabolic Diseases Laboratory of the Nutrition and Food Technology Institute, Chile University (INTA), established an agreement with Porto Alegre Clinical Hospital, Brazil, in order to perform a collaborative study designed to identify the novel genetic mutations present in a Chilean cohort of MSUD patients.

A total of 36 patients were recruited to the study from June to August 2012. Their DNA was extracted from 5-10 mL blood samples and used for genetic analysis.

Porto Alegre Clinical Hospital used the Brazilian MSUD Assistance and Research Network to send these samples to Ribeirão Preto Medical School at São Paulo University in order to have the the three main genes involved in MSUD analyzed at a molecular level. To date, DNA from 18 patients has been analyzed.

Molecular Analysis

DNA was extracted from peripheral blood mononuclear cells for the molecular analysis of the three genes involved in MSUD (BCKDHA, BCKDHB, and DBT).

DNA Sequencing

PCR-amplified fragments were sequenced on an ABI 3500xL Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) using a BigDye® Terminator v3.1 cycle sequencing kit (Applied Biosystems).

Mutation Analysis

Sequencing results were visualized using FinchTV® version 1.4.0 software (Geospiza, Seattle, WA, USA) and compared with the relevant reference sequences from the GenBank® database (12). The nomenclature for sequence variant descriptions were derived using the Human Genome Variation Society guidelines (http://www.hgvs.org/mutnomen) (13).

In order to verify the pathogenic potential of the missense mutations, in silico analysis was performed using MutPred® v1.2 (14), Polyphen-2®-Polymorphism Phenotyping v2 software, and SIFT® (15). Sequence variants were also evaluated for their disease-causing potential using the Mutation Taster application (16).

Clinical Data

The nutrition team from INTA, Chile University, provided clinical data regarding the patients in this

study. This data included anthropometry at birth, complications during pregnancy, birth date, age of diagnosis, hospitalization, clinical and laboratory test results for leucine, valine and isoleucine and social information including ancestry and economic class. All these parameters were used to discuss some of the findings.

Statistical Analysis

Fisher's exact test was applied in a sample of patients with the more prevalent mutation, p.Ile214K, in an attempt to assess the degree of correlation between clinical and genetic variation, and to check for the possibility of establishing genotype/phenotype relationships with p-values < 0.05.

Results

Molecular Analysis

Of the 18 patients studied 88% presented with mutations in the BCKDHB gene, one patient had a mutation in the BCKDHA gene, and one patient harbored a mutation in DBT. A total of eight mutations were found in the samples, and four of these (50%) were novel. The novel mutations were c.[1006 G>A] and c.[718 C>A], in BCKDHB, c.[1013 C>T] in BCKDHA, and c.[1217 G>A] in DBT (Table 1). This study was able to identify the highest incidence of mutations in exon 6 of BCDKHB.

Mutation Pathogenicity

Among the mutations already described in the literature, the Ile214Lys mutation, of Spanish origin, had the highest incidence, totaling 61% of the patients, followed by mutation Pro200*, also of Spanish origin, in 33% (Table 2). Mutations p.Gly131Val, p.Pro200Stop, and p.Ile214Lys were found to be heterozygous, while p.Gly131Val, and p.Ile214lys were found to be homozygous.

Novel mutation Pro240Thr was found in 16% of the samples; and was located in exon 6 of BCKDHB. This was the most prevalent of the novel mutations. Following in silico analysis all the novel mutations were classified as pathogenic (Table 3). Mutations p.Thr338Ile, p.Gly406Asp and p.Pro240Thr were detected in heterozygous patients while p.Pro240Thr and p.Gly336Ser were found in homozygous patients.

Clinical Analysis

Anthropometric Assessment

According to clinical data, 72% of the children in this study were born with weight and lengths appropriate for their gestational ages, based on the Intergrowth 21st scale. Patient six was born weighing 4 kg, which is characterized as macrosomia, common in newborns of pregnant women who have gestational diabetes, which was the case here. Patient ten was born with insufficient weight, but her mother had no problems during pregnancy. The mother of child number 14 presented with preeclampsia and this patient had a low birth weight.

Clinical Evaluation

According to the symptom's onset age, 95% of the patients in this cohort were considered to have the classic form of the disease, and 5% the intermediate form. The age at diagnosis ranged from 9 days to 7 months. Leucine levels ranged from 440 to 3962 µmol/L at diagnosis (normal range 35-217 µmol/L). Neuropsychomotor Developmental Delay (NPMDD) occurred in 13 of the 18 children studied, with four presenting with mild NPMDD, four mild NPMDD, three with moderate NPMDD and six with severe NPMDD. Only two children did not have NPMDD, and that information was not available for three participants. Therefore, in children for whom NPMDD data was available, the majority, 40%, had a serious degree of delayed neuropsychomotor development.

The biochemical test values at the time of diagnosis provided by INTA were all relatively high. Leucine levels ranged from 440 to 3962 µmol/L (normal range 35-270 µmol/L), valine ranged from 133 to 1464 µmol/L (normal range 51-325 µmol/L), and isoleucine ranged from 38 to 759 µmol/L (normal range 13-135 µmol /L), as shown in Table 5.

The most prevalent signs and symptoms in this cohort included axial hypotonia, in 83% of the children, followed by NPMDD in 77% of the cases, intellectual deficit in 61%, food intolerance and urine with characteristic odor in 55%. Strabismus, pyramidal syndrome, and encephalopathy were reported in 44% of patients, seizures in 38%, macrocephaly and need for mechanical ventilation in 27%, gastrostomy and ataxia in 22%, apnea, mucous and skin lesions in 16%, attention deficit disorder and hyperactivity in 14%, extrapyramidal syndrome in 11%, and coma in 5%.

Genotype-Phenotype Correlations

INTA staff ranked the phenotypes of their patients, based on age at diagnosis. Intermittent and

sensitive to thiamine phenotypes were not found in this sample. One patient was classified with an intermediate form of MSUD, with diagnosis at 90 days of age, and 17 patients were assigned to the classical form, with diagnosis age ranging from 9 to 30 days (Table 8). The clinical presentation of these patients varied, exhibiting NPMDD and poor prognosis, leading in some cases to death. Among the patients with the classical MSUD phenotype, four died, six had severe NPMDD, two had moderate NPMDD, and two were diagnosed with light NPMDD. In the intermediate patient NPMDD was light. Leucine level values were highly variable. Even in patients with classical presentation these values ranged from 741 µmol/L to 3962 µmol/L, as shown in Figure 1 and Table 6.

In the case of homozygous mutations for c. [1013 C>T] or p.Thr338Ile (novel), c. [1217 G>A] or p.Gly406Asp (novel), c. [392 G>T] or p.Gly131Val, c. [1067 C>T] or p.Pro357Leu, and c. [641 T>A] or p.Ile214Lys 100% of cases presented in as classical MSUD. Homozygous c. [718 C>A] or p.Pro240Thr mutations were associated with intermediate MSUD in 50% of cases. All heterozygous mutations were found in patients with classic phenotypes.

In addition, we attempted to draw a correlation between leucine values and diagnostic age, but this was not possible as a result of the high degree of variability in these values (Figure 2).

Mutation p.Ile214Lys was identified in 11 of the 18 samples, which meant that it was possible to combine clinical information regarding these patients in an attempt to establish genotype/phenotype correlations. The correlation between the p.Ile214Lys mutation and the degree of NPMDD, the mutation and the classic and intermediate phenotypes, the allele type (hetero- and homozygous), and the severity of NPMDD, and the allele type and the classic and intermediate phenotypes were assessed using Fisher`s exact test. None of these values correlated with a probability value of less than the 5% cutoff value. Thus we were not able to draw any conclusions around genotype/phenotype correlations (Table 7).

Discussion

Only 5% of the patients in this study presented with an intermediate form of MSUD, while the majority, 95%, had the classic form of the disease. These classifications were made primarily as a result of the early onset of symptoms, with a high prevalence of homozygous mutations, 61%. It is

interesting that none of the patients had consanguinity involving the parents, although in two cases there were historical reports of illness in the family. This fact might be linked to the greater genetic homogeneity in the Chilean population originating from their indigenous origin. The overwhelming majority of Chileans are the product of varying degrees of admixture between European ethnic groups (predominantly Spaniards) with Amerindian, peoplesindigenous to Chile's territory.

Although the historical mixing of Europeans and Amerindians is evident across all social strata in the Chilean population, there is a strong correlation between the ratio of a Chilean's European and Amerindian genetic components and his or her socioeconomic situation (16).

There is a marked increase in Amerindian ancestry in the lower social classes in Chile, with an increasing European component in the upper classes. Indigenous inheritance, whether cultural or genetic, is most pronounced in rural areas and in aspects of the culture including Chilean cuisine and the Chilean Spanish language (17).

The largest portion of the participants in this study were from Santiago (44%), Chile`s capital. With 73% considered lower class and 27% middle class. Their ancestry was predominantly Spanish (88% of cases). Although none of them had a surname of indigenous origin, it is known that the vast majority of this population is a genetic admix.

An autosomal DNA study from 2014 found that Chile possesses a gene pool with an average admix of 51.85% (± 5.44%) European, 44.34% (± 3.9%) Amerindian, and 3.81% (± 0.45%) African. This study was conducted across all regions of Chile. When this data was stratified by social class and region they were able to show that the average Santiago residents admix was strongly influenced by their social class. With the lower class exhibiting an admix of 51% European and 49% Amerindian, the middle class 70% European and 30% Amerindian and the upper class 91% European and 9% Amerindian (18).

MSUD is initially identified by clinical examinations and is often first suspected following detection of the peculiar maple syrup odor in patient’s urine. BCAA serum analysis is the most convenient method for diagnosis and is primarily done during neonatal screening. If a patient has high levels of leucine, valine, isoleucine, or alloisoleucine (>5 mm/L), MSUD must be part of a clinicians differential

diagnosis (19). In addition, levels of leucine greater than 1,000 µmol/L are considered critical, as they can result in long term organ damage, or even lead to death (20). Leucine levels within our study ranged from 440 to 3962 µmol/L at the time of diagnosis, and most of the participants (72%) presented with leucine values of more than 1,000 µmol/L.

The BCKDHB gene harbored the majority of the mutations, with six of the eight mutations from this cohort located in the E1b subunit. The Spanish mutation Ile214Lys was the known mutation with the highest incidence and was detected in 61% of the patients. The novel mutation with the highest incidence was Pro240Thr which was identified in 16% of the samples. Notably both mutations occur in exon 6 of BCKDHB.

According to Rodríguez-Pombo and collaborators (2006), the p.Ile214Lys mutation is responsible for reducing the stability of the coding protein causing a classic form of the disease. In our findings, we observed symptom variability in patients with this mutation: patients 5, 9, 11, 12, and 18 had a homozygous p.Ile214Lys mutation, while children 9 and 11 presented with serious NPMDD, child five exhibited only mild NPMDD.

Other factors that need to be considered include the level of leucine and the evolution of the disease. It was observed that patient five, with a leucine value of 2600 µmol/L at diagnosis, showed only light NPMDD, while patient 11, with a leucine level of 440 µmol/L had serious NPMDD. Therefore, we found no association between the initial leucine levels and disease severity. Patients 11 and 12 are siblings, have homozygous p.Ile214Lys mutations and their initial values of leucine were very different. Patient 12 is the eldest, born in 1997; and it took 90 days to establish his MSUD diagnosis, while his younger brother, born in 2006, obtained a diagnosis in only 9 days. This rapid diagnosis was probably aided by the history of illness in the family, improvements in diagnostic testing for MSUD, as well as the previous genetic counseling that this family had already received. The earlier diagnosis almost certainly helped reduce the severity of MSUD.

This kind of symptom variability was also observed in the p.Pro240Thr variant. When this appeared as a homozygous mutation it manifested as serious NPMDD in patient 8 but light NPMDD in patient 15. High levels of leucine are seen at diagnosis in most critical patients, and this can result in permanent

organ damage or even death (21). The results of a Brazilian study (20) on the spectrum of MSUD over the last two decades found no significant association between the severity of mental developmental delays and the levels of leucine at the time of diagnosis, which is consistent with our observations. This can be attributed to the fact that long-term metabolic control is a more important factor in cognitive and psychomotor development than initial levels of leucine (21).

When evaluating the novel mutations p.Thr338Ile, p.Gly336Ser, and p.Gly406Asp and the age of diagnosis in the participants, most of these mutations seem to result in classic MSUD, with early onset of symptoms. Only p.Pro240Thr was identified in patients (1) with later disease onset, favoring an intermediate phenotype classification. However, one of the major problems observed here is the time between onset of symptoms and the diagnosis in locations without neonatal screening for MSUD, like in Chile. One of the patients with a homozygous p.Pro240Thr mutation was classified as an intermediate phenotype because of the diagnosis age but this patient remained in hospital for 90 days. His diagnosis was at 210 days, but how much time passed between onset of symptoms and diagnosis is unknown. It is worth noting, as discussed before, that the BCAA values and clinical evolution of this patient was not consistent with that described in the literature for intermediate phenotypes. Thus, a patient’s clinical situation is not necessarily the best way to correlate phenotype and genotype, because neurological deterioration is directly associated with the absence of early diagnosis and, therefore, with the absence of adequate nutritional treatment, which is fundamental to the control of BCAA concentration. In a study by Morton et al. (2002), in which patients had prompt access to a metabolic formula, and where the clinical protocol was followed in the acute early stages of the disease, patient outcomes were better and patients were able to reach more age appropriate developmental milestones (22).

Although some patients were diagnosed in the first month of life, it is known that the time between diagnosis and receipt of metabolic formula can be long and variable.

Therefore, if MSUD was diagnosed more rapidly, by neonatal screening, perhaps it would be possible to establish genotype-phenotype associations more efficiently. MSUD meets most of the criteria from Wilson and Jungner (1968) for neonatal screening (23). In countries where MSUD is included in

neonatal screening, patients are usually diagnosed before the 10th day of life. While in countries where it is not included in public neonatal screening programs, such as Chile, diagnosis is usually delayed.

MSUD treatment includes the application of low protein diets, supplementation with BCAA-free formula and symptomatic treatment during metabolic crises, including ingestion of mannitol to treat brain edema, injection of insulin to lower blood glucose, and use of N-carbamylglutamate to reduce serum ammonia levels (24).

As the liver is responsible for 15% of BCKD production, liver transplantation can restore enzyme activity in patients with MSUD (25). Liver transplantation may benefit patients with the classical form of the disease; however, it does not reverse the disease process (26).

Prenatal diagnosis is important to identify defects before birth, especially with difficult to treat conditions. Accurate genetic analysis of probands has allowed DNA-based prenatal diagnosis of single gene disorders. You et al. (2014) reported a case of a Chinese family with a BCKDHA gene mutation; where the fetus underwent prenatal diagnosis (27). In a study by Li et al. (2015), they were also able to identify a BCKDHB mutation in a prenatal screen (28). In both cases, treatment was started at birth, and diagnosis was possible as a result of the increased awareness of the care givers and family.

Conclusions

We found a total of eight mutations in this patient cohort, four of which were novel: p.Thr338Ile, p.Gly336Ser, p.Gly406Asp, and p.Pro240Thr. Six of the eight mutations are located in exon 6 of BCKDHB, and this gene is the one identified most frequently identified as mutated in this population. Mutation Ile214Lys is present in 61% of the patients and was first described in patients of Spanish origin.

The novel mutations p.Thr338Ile, p.Gly336Ser, and p.Gly406Asp were all identified in patients with early diagnosis and were classified as classical MSUD mutations based on age at diagnosis. Only p.Pro240Thr mutations presented later, allowing patients to be classified as having an intermediate phenotype. The BCAA levels and clinical evolution in those patients were not consistent with those described in the literature for these clinical phenotypes.

We found no association between initial levels of leucine and the severity of MSUD. This can be attributed to the fact that long-term metabolic control can be considered a determining factor that is more important in cognitive and psychomotor development than initial levels of leucine.

If MSUD was diagnosed more promptly, possibly via the application of neonatal screening, perhaps it would be possible to establish genotype-phenotype associations more efficiently.

Declarations

Ethics approval and consent to participate

This research was approved by the Ribeirão Medical School Research Ethics Committee and by the National Commission of Ethics in Research committee (reference number 2.252.930). All collection and analyses were done in accordance with the Helsinki Declaration of 1975. Parents/guardians signed an Informed Consent form giving permission for participation in the study.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analyzed during this study are included in this published article [and its supplementary information files]. For more information, the datasets used and/or analyzed during this study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

DRRC was the PhD studant who performed the biomolecular assays and analyzed the correletions between phenotype and genotype. AVBM standardized the study methodology and helped with the mutation analysis. WASJ helped with the mutations analysis ,DFG helped with the methodology

standardization, GAM helped with the biomolecular assays, AAM did the sequencing reaction, IVDS helped with the mutation analysis , VC, VH and GC were responsible for collecting the blood samples and all the participant’s clinical data. ESB helped with the biomolecular assays and JSCM acted as DRRC’s advisor and helped with the biomolecular analysis and the clinical data interpretation for the genotype/phenotype correlation. All authors read and approved the final manuscript.

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Abbreviations

MSUD: Maple syrup urine disease

BCKD: Branched-chain α-keto acid dehydrogenase

BCKDHA: Branched chain keto acid dehydrogenase E1, alpha polypeptide

BCKDHB: Branched chain keto acid dehydrogenase E1, beta polypeptide

DBT: Dihydrolipoamide branched chain transacylase E2

DLD: Dihydrolipoamide dehydrogenase

IEM : Inborn Error of Metabolism

BCAA: Branched-chain amino acids

LEU: Leucine

ILE: Isoleucine

VAL: Valine

BCKA: Branched-chain keto acids

DNA: Deoxyribonucleic acid

CNS: Central nervous system

INTA: Nutrition and Food Technology Institute, Dr. Fernando Monckenberg Barros, Chile University

PCR: Polymerase Chain Reaction

NPMDD: Neuropsychomotor Developmental Delay

References

1. Strauss, K., Puffenberger, E., Morton, D. (2006). Maple syrup urine disease. Gene Reviews, 30, 1993–2013.
2. Chuang, J. L, Wynn, R. M., Moss, C. C., Song, J. L., Li J., Awad, N., Mandel, H., Chuang, D. T., (2004). Structural and biochemical basis for novel mutations in homozygous Israeli maple syrup urine disease patients: a proposed mechanism for the thiamin-responsive phenotype. Journal of Biological Chemistry. 279, 17792–17800. DOI:10.1074/jbc.M313879200.
3. Chuang, D., Shih, V. (2001). Maple syrup urine disease (Branched-chain ketoaciduria). In: Scriver C, Beaudet A, Sly W, Valle D, editors. The metabolic and molecular bases of inherited disease. (8^{th} ed.). Mcgraw-Hill, 1971–1995.
4. Fisher, C. R., Fisher, C. W., Chuang, D. T., Cox, R. P. (1991). Occurrence of a Tyr393---Asn (Y393N) mutation in the E1 alpha gene of the branched-chain alpha-keto acid dehydrogenase complex in maple syrup urine disease patients from a Mennonite population. Am American Journal of Human Genetics. 49(2):429-34.
5. Rodríguez-Pombo, P., Navarrete, R., Merinero, B., Gómez-Puertas, P., Ugarte, M. (2006). Mutational spectrum of maple syrup urine disease in Spain. Human Mutation. 2006 Jul;27(7):715. DOI:10.1002/humu.9428.
6. McKusick, V. A. (2007). Mendelian Inheritance in Man and its online version, OMIM. American Journal of Human Genetics, 80, 588–604. doi: 10.1086/514346.
7. Stenson, P. D., Mort, M., Ball, E. V., Shaw, K., Phillips, A. D., Cooper, D. N. (2014). The Human Gene Mutation Database: Building a comprehensive mutation repository for clinical and molecular genetics, diagnostic testing and personalized genomic

medicine. Human Genetics 2014 Jan;133(1):1-9. DOI:10.1007/s00439-013-1358-4.

8. Quental, S., Vilarinho, L., Martins, E., Teles, E. L., Rodrigues, E., Diogo, L.(2010) Incidence of maple syrup urine disease in Portugal. Mol Genet Metab, 100, 385–7.doi: 10.1016/j.
9. Margutti A.Genotypic profile of Brazilian patients with maple syrup urine disease. Ribeirão Preto School of Medicine / São Paulo University; 2015.
10. Mitsubuchi, H., Owada, M., Endo, F. (2005). Markers associated with inborn errors of metabolism of branched-chain amino acids and their relevance to upper levels of intake in healthy people: an implication from clinical and molecular investigations on maple syrup urine disease. Journal of Nutrition, 135, 1565–1570.doi: 10.1093/jn/135.6.1565S.
11. Hamosh, A., Scott, A. F., Amberger, J., Valle, D., McKusick, V. A. (2002). Online Mendelian Inheritance in Man (OMIM). Human Mutation, 2000. 15(1):57-61. DOI:10.1002/(SICI)1098-1004(200001)15:1<57::AID-HUMU12>3.0.CO;2-G.
12. Den Dunnen, J. T., Antonarakis, S. E. (2000). Mutation nomenclature extensions and suggestions to describe complex mutations: A discussion. Human Mutation, 2000 15(1):7-12. DOI:10.1002/(SICI)1098-1004(200001)15:1<57::AID-HUMU12>3.0.CO;2-G.
13. Li, B., Krishnan, V. G., Mort, M. E., Xin, F., Kamati, K. K., Cooper, D. N., et al. (2009). Automated inference of molecular mechanisms of disease from amino acid substitutions. Bioinformatics, 25, 2744–2750.
14. Ng, P. C., Henikoff, S. (2001). Predicting deleterious amino acid substitutions. Genome Research.11(5):863-74. DOI:10.1101/gr.176601.
15. Schwarz, J. M., Cooper, D. N., Schuelke, M., Seelow, D. (2014). MutationTaster2: mutation prediction for the deep-sequencing age. Nature Methods Apr;11(4):361-2. doi: 10.1038/nmeth.2890.

16. Census Recociliation. Results of the 2017 Chile Census. 2018;41. Available from: http://www.censo2017.cl/wpcontent/uploads/2017/12/Presentacion_Resultados_Definitivos_Censo2017.pdf.

17. Lizcano Fernández, Francisco. (2005). Composición Étnica de las Tres Áreas Culturales del Continente Americano al Comienzo del Siglo XXI. Convergencia, 12(38), 185-232. Recuperado en 14 de febrero de 2020, de http://www.scielo.org.mx/scielo.php?script=sci_arttext&pid=S1405-14352005000200185&lng=es&tlng=es.

18. Cruz-Coke, R., & Moreno, R. S. (1994). Genetic epidemiology of single gene defects in Chile. Journal of medical genetics, 31(9), 702–706. https://doi.org/10.1136/jmg.31.9.702.

19. Schadewaldt, P., Bodner-Leidecker, A., Hammen, H. W., Wendel, U. (1999). Significance of L-alloisoleucine in plasma for diagnosis of maple syrup urine disease. Clin Chem, 45(10), 1734–1740. doi: http://dx.doi.org/10.1016/j.jped.2014.08.010.

21. Hoffmann, G., Schulze, A. (2009). Organic Acidurias. Pediatric Endocrinology and Inborn Errors of Metabolism.

22. Morton, D.H., Strauss, K.A., Robinson, D. L., Puffenberger, E. G., Kelley, R. I. (2002). Diagnosis and treatment of maple syrup disease: a study of 36 patients. Pediatrics.109 (6) 999-1008; DOI: https://doi.org/10.1542/peds.109.6.999.

23. Wilson, J. M. G., Jungner, G. (1968). Principles and Practice of Screening for Disease [Internet]. world health organization. Available from: http://apps.who.int/iris/bitstream/handle/10665/37650/WHO_PHP_34.pdf?sequence=17&isAllowed=y.

24. Yoshino, M., Aoki, K., Akeda, H., Hashimoto, K., Ikeda, T., Inoue, F., et al. (1999). Management of acute metabolic decompensation in maple syrup urine disease: a

multi-center study. Pediatrics International. Apr;41(2):132-7. DOI:10.1046/j.1442-200x.1999.4121044.x.

25. Díaz VM, Camarena C, De La Vega Á, Martínez-Pardo M, Díaz C, López M, et al. (2014). Liver transplantation for classical maple syrup urine disease: Long-term follow-up. J Pediatrics Gastroenterology Nutrition.Nov;59(5):636-9. doi: 10.1097/MPG.0000000000000469.

26. Feier FHH, Miura IKK, Fonseca EAA, Porta G, Pugliese R, Porta A.(2014). Successful domino liver transplantation in maple syrup urine disease using a related living donor. Brazilian J Med Biol Res, 47(6), 522–526.

27. You, Y., Sun, Y., Li, X., Li, Y., Wei, X., Chen, F., et al. (2014). Integration of targeted sequencing and NIPT into clinical practice in a Chinese family with maple syrup urine disease. Genet Med [Internet]. 16(8), 594–600. Available from: http://www.nature.com/doifinder/10.1038/gim.2013.197%5Cnhttp://www.ncbi.nlm.nih.gov/pubmed/24603436.

28. Li, X., Ding, Y., Liu, Y., Ma, Y., Song, J., Wang, Q., et al. (2015). Eleven novel mutations of the BCKDHA, BCKDHB and DBT genes associated with maple syrup urine disease in the Chinese population: report on eight cases. Eur J Med Genet [Internet], 58(11), 617–623. Available from: http://linkinghub.elsevier.com/retrieve/pii/S1769721215300288

Tables

Table 1 – Pathogenic variants detected in BCKDHA, BCKDHB and DBT genes of Chilean MSUD patients.

Patient	Gene	Nucleotide	Protein Prediction
1	BCKDHA	c.[1013 C>T]† +[1013 C>T] †	p.Thr338Ile + Thr338Ile
2	BCKDHB	c[.595_596delAG] +[641 T>A]	p. Pro200Stop +Ile214Lys
3	BCKDHB	c.[641 T>A] **+ [1006 G>A]**†	p.Ile214Lys **+ Gly336S**
4	BCKDHB	c.[595_596delAG] +[641 T>A]	p.Pro200Stop +Ile214Lys
5	BCKDHB	c.[641 T>A] + [641 T>A]	p.Ile214KLys+Ile214Lys
6	DBT	c.[1217 G>A]†+ c.[1217 G>A]†	p.Gly406Asp+Gly406Asp
7	BCKDHB	c.[595_596delAG]+ [641 T>A]	p. Pro200Stop + Ile214Lys
8	BCKDHB	c.[718 C>A]† + [718 C>A]†	p.Pro240Thr+ Pro240Thr
9	BCKDHB	c.[641 T>A] + [641 T>A]	p.Ile214KLys+Ile214Lys
10	BCKDHB	c.[392 G>T] +[595_596delAG]	p.Gly131Val + Pro200Stop
11	BCKDHB	c.[641 T>A] + [641 T>A]	p.Ile214KLys+Ile214Lys
12	BCKDHB	c.[641 T>A] + [641 T>A]	p.Ile214KLys+Ile214Lys
13	BCKDHB	c.[392 G>T] + [392 G>T]	p.Gly131Val+ Gly131Val
14	BCKDHB	c[.595_596delAG]+ [641 T>A]	p. Pro200Stop +Ile214Lys
15	BCKDHB	c.[718 C>A]† + [718 C>A]†	p.Pro240Thr+ Pro240Thr
16	BCKDHB	c[.595_596delAG]+ [641 T>A]	p. Pro200Stop +Ile214Lys
17	BCKDHB	c.[1067 C>T] + [1067 C>T]	p.Pro356Leu + Pro356Leu
18	BCKDHB	c.[641 T>A] + [641 T>A]	p.Ile214KLys+Ile214Lys

†New Mutations

Table 2 – Mutations already described in the literature found in Chilean MSUD patients.

	Gene	Nucleotide	Protein Prediction	Type	Origin	Phenotype	Reference
Exon 4	BCKDHB	c.[392 G>T]	p.Gly131Val	M	NA	NA	Margutti AV, 2015
Exon 5	BCKDHB	c.[595_596delAG]	p.Pro200Stop	D	Spanish	Classic	Henneke M, 2003
Exon 6	BCKDHB	c.[641 T>A]	p.Ile214Lys	M	Spanish	Classic	Rodriguez-Pombo,2006
Exon 10	BCKDHB	c.[1067 C>T]	p.Pro356Leu	M	Portuguese	Classic	Quental, 2008

NA: Not Available

M: Missense

D: Deletion

Table 3 – Novel mutations found in Chilean MSUD patients.

Region	Gene	Nucleotide	Protein Prediction	Type	Pathogenicity			Cl n
					Sift®	Polyphen-2®	Multipred®	
Exon 8	BCKDHA	c.[1013C>T]	p.Thr338Ile	M	Pathogenic	Pathogenic	Pathogenic	Pa
Exon 6	BCKDHB	c.[718C>A]	p.Pro240Thr	M	Pathogenic	Pathogenic	Pathogenic	Pa
Exon 9	BCKDHB	c.[1006G>A]	p.Gly336Ser	M	Pathogenic	Pathogenic	Pathogenic	Pa
Exon 10	DBT	c.[1217G>A]	p.Gly406Asp	M	Pathogenic	Pathogenic	Pathogenic	Pa

M: Missense.

Table 4 – Characterization of Chilean MSUD patients (n = 18) at INTA.

Patient	Diagnostic Time (days)	Leucine (µmol/L)	NPMDD	First Hospitalization (days)	Presentation a diagnosti
1	10	741	severe	10	Class
2	9	750	severe	10	Class
3	14	3560	moderate	7	Class
4	13	2638	light	32	Class
5	22	2600	light	30	Class
6	11	750	NA	48	Class
7	30	1640	light	5	Class
8	8	3962	severe	2	Class
9	21	2000	severe	30	Class
10	9	993	moderate/severe	20	Class
11	9	440	severe	4	Class
12	16	1027	no	32	Class
13	17	1090	NA	36	Class
14	30	1716	no	10	Class
15	210	1653	light	90	Interme
16	17	1467	moderate	14	Class
17	17	1038	severe	NA	Class
18	27	2075	NA	10	Class

NA: Not Available.

NPMDD: Neuropsychomotor Developmental Delay

Table 5 – Laboratory values at diagnosis of Chilean MSUD patients at INTA.

Pacient	Diagnostic time (days)	Leucine (35-270 µmol/L)	Valine (51-325 µmol/L)	Is (13-1
1	10	741	759	
2	9	750	512	
3	14	3560	710	
4	13	2638	585	
5	22	2600	1180	
6	11	750	726	
7	30	1640	174	
8	8	3962	277	
9	21	2000	369	
10	9	993	838	
11	9	440	1464	
12	16	1027	117	
13	17	1090	480	
14	30	1716	215	
15	210	1653	1171	
16	17	1467	466	
17	17	1038	133	
18	27	2075	726	

Table 6 – Genotype-phenotype correlations of Chilean MSUD patients (n = 18).

Patient	Birth year	First hospitalization (days)	Diagnosis Time (days)	LEU (µmol/L)	Clinical Situation	Phenotype	Genotype
1	2002	10	10	741	Severe	Classic	c.[1013 C>T]§ +[1013 C>T] †
2	2002	10	9	750	Severe	Classic	c[.595_596delAG] + c.[641T>A]
3	1995	7	14	3560	Moderate	Classic	c.[641 T>A] **+ [1006 G>A]§**
4	2006	32	13	2638	Light	Classic	c.[595_596delAG] +c.[641T>A]
5	2012	30	22	2600	Light	Classic	c.[641 T>A] + [641 T>A]
6	2001	48	11	750	Death	Classic	c.[1217 G>A]§+ c.[1217 G>A]¶
7	2004	5	30	1640	Death	Classic	c.[595_596delAG]+c.[641T>A]
8	2004	2	8	3962	Severe	Classic	c.[718 C>A]§ + [718 C>A]§
9	2003	30	21	2000	Severe	Classic	c.[641 T>A] + [641 T>A]
10	1997	20	9	993	Moderate/Severe	Classic	c.[392 G>T] + [595_596delAG]
11	2006	4	9	440	Severe	Classic	c.[641 T>A] + [641 T>A]
12	1997	32	16	1027	NA	Classic	c.[641 T>A] + [641 T>A]
13	1998	36	17	1090	Death	Classic	c.[392 G>T] + [392 G>T]
14	2003	10	30	1716	NA	Classic	c[.595_596delAG]+ [641T>A]
15	1999	90	210	1653	Light	Intermediate	c.[718 C>A]§ + [718 C>A]§
16	1996	14	17	1467	Moderate	Classic	c[.595_596delAG]+ [641 T>A]
17	2000	ND	17	1038	Death	Classic	c.[1067 C>T] + [1067 C>T]
18	2002	10	27	2075	NA	Classic	c.[641 T>A] + [641 T>A]

NA: Not Available.

NPMDD: Neuropsychomotor Developmental Delay.

§: Novel mutation.

Table 7 – Associations between patients with the p.Ile214Lys mutation and specific clinical parameters.

Mutation	NPMDD		Total	P
	Light/Moderate	Severe/Death		
Others	1	6	7	
Ile214Lys	4	4	8	

Mutation	Phenotype		Total	P
	Intermediate	Classic		
Others	1	6	7	
Ile214Lys	0	11	11	

Allele	NPMDD		Total	P
	Light/Moderate	Severe/Death		
Heterozygous	3	2	5	
Homozygous	1	2	3	

Allele	Phenotype		Total	P
	Intermediate	Classic		
Heterozygous	0	6	6	
Homozygous	0	5	5	

p-value<0,05

NPMDD: Neuropsychomotor Developmental Delay

Others: mutations identified in this study other than Ile214Lys.

Figures

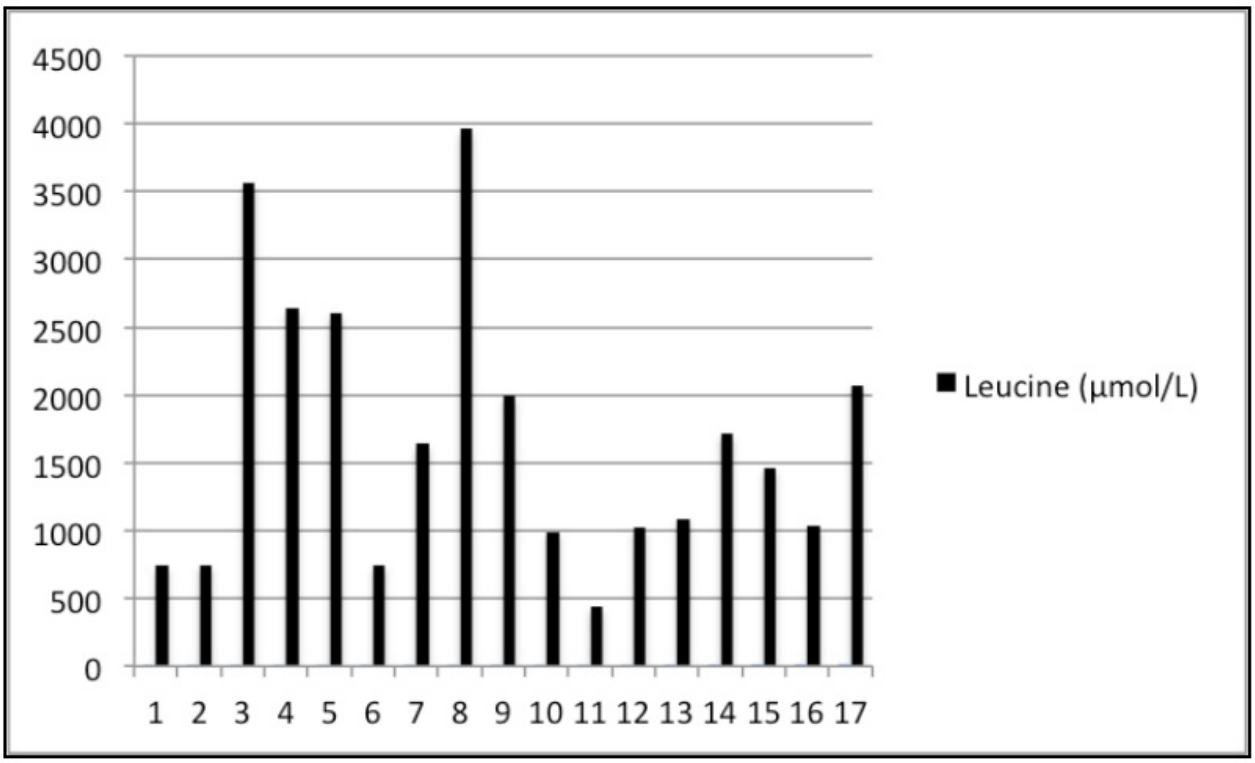

Figure 1

Representation of leucine values in µmol/L at diagnosis in patients classified as classical MSUD, according to age at diagnosis.

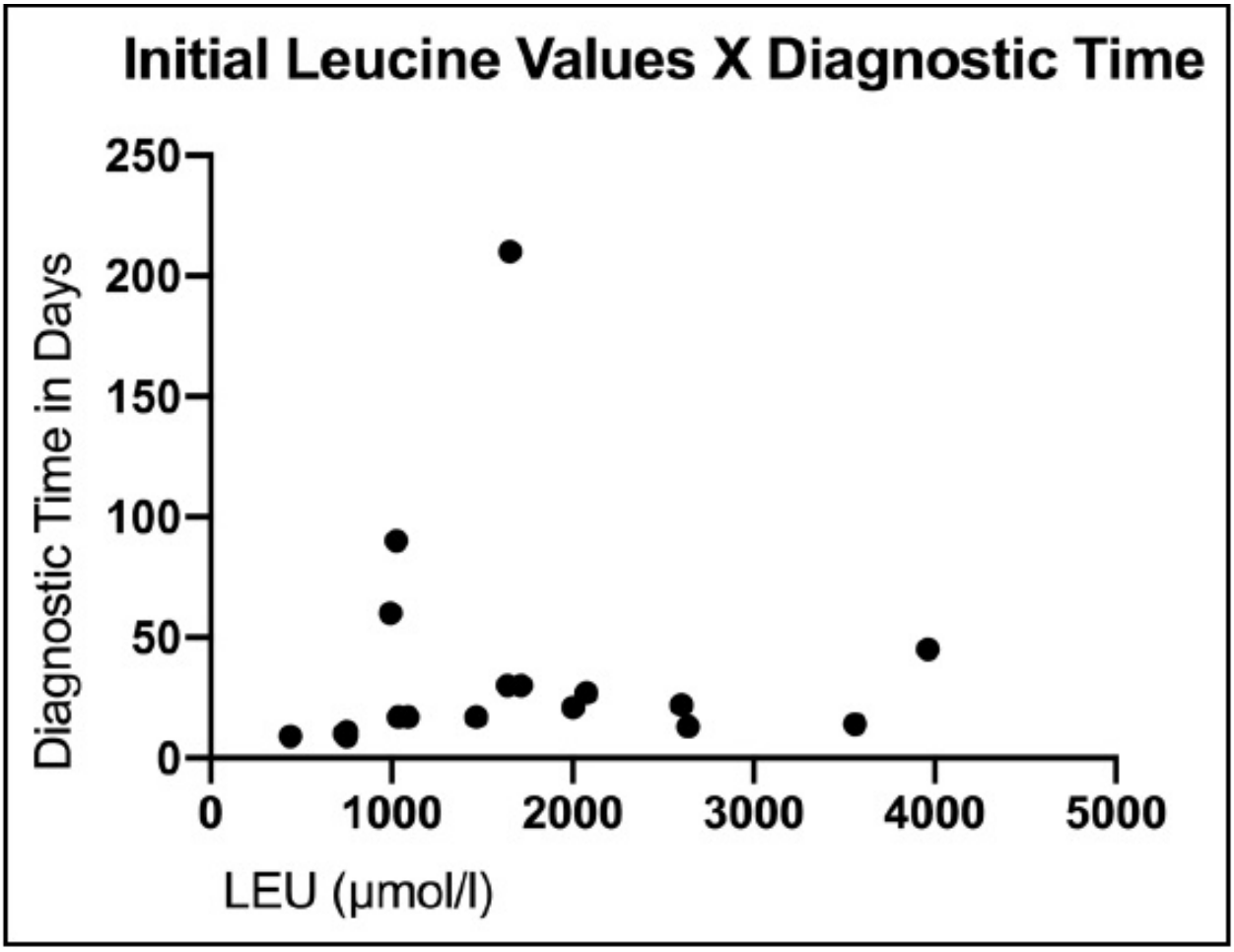

Figure 2

Correlation between leucine values in µmol/L and age at diagnosis in days, with a p value of 0.989.