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ORIGINAL ARTICLE

Analysis of *ASS1* gene in ten unrelated middle eastern families with citrullinemia type 1 identifies rare and novel variants

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Abstract

Background: Citrullinemia type 1 (CTLN1) is a rare autosomal recessive disease caused by argininosuccinate synthetase (ASS) deficiency. Manifestations vary from the acute neonatal or "classic" form to a milder, late-onset, or "unconventional" form. To date, more than 93 variants in the *ASS1* gene located on chromosome 9q43.11 (OMIM #215700) are reportedly responsible for CTLN1. Their incidence and distribution vary according to geographic origins and ethnicity, and a correlation, although not clearly delineated, has been established between the genotype and the phenotype of the disease. Though, in the Middle East, national descriptions of CTLN1 are still lacking.

Methods: A total of ten unrelated Middle Eastern families, five Lebanese, two Syrians, and three Iraqis with citrullinemia index cases, were included in this study. Upon informed consent, DNA was extracted from the whole blood of the index patients as well as their parents and siblings. Genetic analysis was carried out by Sanger sequencing of the *ASS1* gene.

Results: Seven different variants were identified. Two novel variants, c.286C>A (p.(Pro96Thr), RNA not analyzed) in exon 5 and deletion c.685_688+6del(p.(Lys229Glyfs*4), RNA not analyzed) in exon 10, were found in one Lebanese and one Syrian family, respectively, and were correlated with early-onset and severe clinical presentation. Five other known variants: c.535T>C (p.(Trp179Arg), RNA not analyzed) in exon 8, c.787G>A (p.(Val263Met), RNA not analyzed) in exon 12, c.847G>A (p.(Glu283Lys), RNA not analyzed) in exon 13, c.910C>T (p.(Arg304Trp), RNA not analyzed) in exon 13, and c.1168G>A (p.(Gly390Arg), RNA not analyzed) in exon 15, were found in Lebanese, Syrian, and Iraqi families, and were associated with diverse clinical presentations.

Melissa Daou and Mirna Souaid are Equal contribution co 1st authors.

Conclusion: Two novel variants and five known variants were found in a total of ten unrelated Middle Eastern families.

KEYWORDS
argininosuccinate synthetase, *ASS1*, citrullinemia type 1

1 | INTRODUCTION

Citrullinemia is a urea cycle disorder due to *ASS1* gene deficiency, which encodes the argininosuccinate synthetase (ASS) protein. It was first described in 1962 by McMurray et al. and classified into three types: I, II, and III based on biochemical manifestations; it was later reclassified into types I and II based on molecular pathogenesis (Saheki et al., 1987). Citrullinemia type 1 (CTLN1) is caused by urea cycle blockage, which leads to hyperammonia. Its prevalence is estimated to be around 1 in 44,300–200,000 in developed countries (Kasper et al., 2010). This figure may however be higher in communities with greater inbreeding rates, such as the Middle Eastern populations.

CTLN1 may manifest as a severe form during the neonatal period, or later in life, as a mild presentation, but it may as well remain asymptomatic.

The "classic" and most common form is characterized by neonatal onset with severe hypotonia, failure to thrive, and neurological failure, potentially leading to early demise. Less common presentations are hepatic dysfunction (Salek et al., 2010), stroke-like episodes, while rare manifestations are lupus (Pimenta et al., 2018), hypertrophic pyloric stenosis (Rhee et al., 2013), Sandifer syndrome-like (Kılıç et al., 2017), and persistent hiccups (Degirmencioglu et al., 2014). Long-term complications of this classic form include mild to profound mental retardation, seizures, growth deficiency (Maestri et al., 1995), cataracts, and progressive hypertrophic cardiomyopathy (Brunetti-Pierri et al., 2012).

An "unconventional" milder form of the disease with a late onset may manifest in individuals with partial enzyme deficiency.

Asymptomatic homozygous individuals have been as well described; these may be, however, at risk of developing acute metabolic decompensation when exposed to physiological or catabolic stress, such as pregnancy, infection, or trauma (Bachmann, 2003). The clinical course and prognosis of citrullinemia type I are difficult to predict by biochemical means alone. It could be suggested that neonates with high peak ammonia level (>300 μmol), high citrulline level (>1000 μmol), and poor residual enzyme activity (<1%) present with a poor prognosis. Determining the underlying molecular bases is becoming a key to predicting the severity and clinical management of CTLN1. Several studies have already established genotype–phenotype correlations. For instance, c.421-2A>G and c.1194-1G>C variants, which cause aberrant splicing, as well as the missense variants: p.(Gly390Arg), p.(Gly117Ser), p.(Ala118Thr) and p.(Arg127Gln), p.(Arg265Cys), p.(Glu283Lys) and the p.(Gly324Ser) variant are associated with a severe and early onset phenotype.

While, p.(Gly362Val), p.(Trp179Arg), p.(Val263Met), p.(Arg100Cys), p.(Arg157His), and p.(Val141Gly) variants are associated with mild or asymptomatic clinical course (Diez-Fernandez et al., 2017).

2 | MATERIALS AND METHODS

2.1 | Ethical compliance

Ten clinically symptomatic patients presenting with abnormal citrullinemia dosage, determined by mass spectrometry on newborn screening or later through high-performance liquid chromatography, were recruited and referred with their families to our Medical Genetics Unit. All patients' parents signed an informed consent, approved by the ethics committee of Saint Joseph University for participation, sample collection, and data publication.

Genomic DNA was extracted from the whole blood of the patients, parents, and siblings, using Qiagen Blood DNA mini kit (Qiagen®, Hilden, Germany). The primers used for polymerase chain reaction (PCR) and Sanger sequencing are listed in Table 1 and were designed using Primer 3 (https://bioinfo.ut.ee/primer3-0.4.0/) and checked for specificity using BLAST (https://www.ncbi.nlm.nih.gov/tools/primer-blast/). PCR reactions were performed using Multiplex HotStar Taq (Qiagen, Germany) according to the manufacturer's instructions. Bidirectional sequencing of the coding exons flanking intronic regions was performed using the BigDye® Terminator v1.1 Cycle Sequencing Kit (ThermoFisher Scientific, Waltham, MA, USA) under standard conditions and by using the automatic sequencer ABI 3500 Genetic Analyzer sequencing system Analyzer (Applied Biosystems, CA, USA). Electrochromatograms were analyzed using Sequence Analysis Software version 5.4 (ThermoFisher Scientific, Waltham, MA, USA) and compared to reference sequences obtained from the UCSC (https://genome.ucsc.edu/)

TABLE 1 Primers used for PCR and sequencing of *ASS1* gene[a]

Exon	Size	Forward primer (5′ → 3′)	Reverse primer (5′ → 3′)
Exon 3	382pb	ggcactgaaggtgaacatcc	ttgaatgagtgacggtgagc
Exon 4	286pb	ccatggaatggaagctgtct	agacacactggaagggatgg
Exon 5	544pb	tgcagaagaagaagcaccag	ggtggaggtgaggactcaga
Exon 6	526pb	gggtcagatgtgtcctgcac	ctctccgggaagaatgaatg
Exon 7	350pb	ctctcaccctcacaacagca	aagttcaatcccccatcaca
Exon 8	396pb	ggtagccagggtcttgtctg	cccaacaacactcagcagaa
Exon 9	379pb	ccagaatgtttcaggcaggt	ttcaaatgcagcgaggagac
Exon 10	345pb	ggaaatggacagaggagagg	gctgtgcgttaccttgaagc
Exon 11	280pb	catccatttaaggcgtttcg	tcagccacaaccattagctg
Exon 12	246pb	aatgtgtggcctaagctgtg	caaaggggacagggtctcag
Exon 13	391pb	ggcttgtctcctgtcagacg	ctcacatgaggatggcagtg
Exon 14	466pb	tagagggcagggctatgaaa	cactctgtgctctgcatggt
Exon 15	300pb	gagggtttggacccacacag	agtcaaggtcgcatcaaagc
Exon 16	380pb	cgggagagggaatagaaaaa	cccttcccttcgatgacaac

[a]NM_054012.4 (GenBank reference sequence and version number of *ASS1* gene).

database. *ASS1*: NM_000050.4 using ChromasPro version 2.1.9 (Technelysium, Queensland, Australia).

3 | RESULTS

A total of ten Middle Eastern families, five Lebanese, two Syrians, and three families of Iraqi origin with citrullinemia were found to have pathogenic or likely pathogenic variants in the *ASS1* gene (Table 2 and Figure 1).

4 | DISCUSSION

To date, more than 93 variants have been reported in the *ASS1* gene (Engel et al., 2009). However, no data has been reported for Middle Eastern populations.

One of the most widely described variants across different populations and ethnic groups is c.1168G>A, p.Gly390Arg in exon 15, accounting for 17.3% in Germans (Gao et al., 2003), up to 50% in Spanish and Turkish (Engel et al., 2009), and more than 40% in Indians (Bijarnia-Mahay et al., 2018), Argentinians (Laróvere et al., 2009), and people from the Pacific Islands (Glamuzina et al., 2011). This variant located in the protein domain of the arginosuccinate synthetase is classified as pathogenic according to the ACMG and based on functional studies demonstrating loss of argininosuccinate synthase activity (Berning et al., 2008). The variant is reported in dbSNP: rs121908641, Clinvar (RCV001376575.1), and uniport (VAR_000694) (Table 2). Whereas, in Turkish population, the p.(Gly324Ser), p.(Gly362Val), and p.(Gly390Arg) variants in exons 12 and 15 represent approximately 70% of all variants identified (Kose et al., 2017). It has been suggested that a CpG dinucleotide within exon 15 might be responsible for the recurrent occurrence of variants in this region. In our study, only one Iraqi family presented with p.(Gly390Arg) associated with a severe form of the disease. On the other hand, c.847G>A, p.(Glu283Lys) was found in three families of Syrian, Iraqi, and Kurdish origins, all of them presenting with a severe clinical outcome. This variant has previously been reported in isolated cases from China (Wu et al., 2014), Iran (Gao et al., 2003), Germany (Kleijer et al., 2006), and the USA (Miller et al., 2014). p.(Glu283Lys), located in the protein domain of arginosuccinate synthetase within a highly conserved amino acid, is a missense variant classified as pathogenic according to the ACMG, Clinvar (RCV001290024.1), Uniprot (VAR_015902), and also known in dbSNP: rs765338121 (Table 2). It affects the tertiary and quaternary structures, thus altering the intermolecular interactions of this enzyme (Gao et al., 2003). Its high occurrence in our families while it is rarely described in other populations is reflective of the paucity of reports on metabolic diseases in the Middle East.

As for, c.535T>C, p.(Trp179Arg) in exon 8 detected in two of our Lebanese families, it has been widely described in the literature. Indeed, Diez-Fernandez et al. reported this variant in 27 families from different ethnic groups, ranking it among the most common *ASS1* variants worldwide (Raponi et al., 2011). c.535T>C, falls within a highly conserved amino acid at the start of the substrate binding cavity in the protein domain of the arginosuccinate synthetase. It is reported as a missense pathogenic variant by dbSNP: rs121908646, Clinvar (RCV000006707.4), and Uniprot (VAR_015898) (Table 2). The physicochemical difference between tryptophan and arginine is very significant. The variant results in a 13% decrease in enzyme activity compared to the normal protein (Berning et al., 2008), which entails sufficient residual activity to maintain an efficient urea cycle (Häberle et al., 2002). This could explain the mild clinical manifestations described with this variant (Dimmock et al., 2008; Gao et al., 2003), which is in line with both our family's clinical presentations.c.787G>A, p.(Val263Met) in exon 12 has been as well frequently described by Hernandez et al (Diez-Fernandez et al., 2017), notably in European and Turkish populations as well as in the populations of the Pacific Islands (Glamuzina et al., 2011). In our study, c.787G>A was found in two of our Lebanese families. This variant is classified as pathogenic in dbSNP: rs192838388, Clinvar (RCV000078024.13), and Uniprot (VAR_058348) (Table 2), with functional studies confirming a reduction in argininosuccinate synthase activity (Berning et al., 2008). This

TABLE 2 Pathogenic or likely pathogenic variants in *ASS1* gene found in 10 middle eastern families[a]

Family	Origin	Patient: Loation of the variant: cDNA change (NM_000050.4)/ protein change/status	Variant type/novel or described in the litterature	Parents tested	dbSNP ID	Clinvar	Uniprot	Varsome	Ratio pathogenic/ benign predictions by Varsome
1	Lebanon	Exon 5: c.286C>A, p.Pro96Thr, Homozygous	SNV/Novel	Yes/Heterozygous	–	–	–	Likely Pathogenic	19/1
2	Syria	Exon 13:c.847G>A, p.(Glu283Lys), Homozygous	SNV/Described in the litterature	Yes/Heterozygous	rs765338121	Pathogenic	Pathogenic	Pathogenic	20/1
3	Lebanon	Exon 12: c.787G>A, p.(Val263Met), Homozygous	SNV/Described in the litterature	Yes/Heterozygous	rs192838388	Pathogenic	Pathogenic	Pathogenic	17/1
4	Lebanon	Exon 8: c.535T>C, p.(Trp179Arg), Homozygous	SNV/Described in the litterature	–	rs121908646	Pathogenic	Pathogenic	Pathogenic	18/1
5	Lebanon	Exon 8: c.535T>C, p.(Trp179Arg), Heterozygous **&** Exon 12: c.787G>A, p.(Val263Met), Heterozygous	SNV/Described in the litterature	–	rs121908646 and rs192838388	Pathogenic	Pathogenic	Pathogenic	17/1
6 & 7	Kurdistan & Iraq	Exon 13: c.847G>A, p.(Glu283Lys), Homozygous	SNV/Described in the litterature	–	rs765338121	Pathogenic	Pathogenic	Pathogenic	20/1
8	Syria	Exon 10: c.685_688 + 6del, p.(Lys229Glyfs*4), Homozygous	Deletion/Novel	Yes/Heterozygous	–	–	–	Pathogenic	1/0
9	Lebanon	Exon 13: c.910C>T, p.(Arg304Trp), Homozygous	SNV/Described in the litterature	–	rs121908642	Pathogenic	Pathogenic	Pathogenic	18/1
10	Iraq	Exon 15: c.1168G>A, p.(Gly390Arg), Homozygous	SNV/Described in the litterature	–	rs121908641	Pathogenic	Pathogenic	Pathogenic	19/1

[a]NM_054012.4 (GenBank reference sequence and version number of *ASS1* gene).

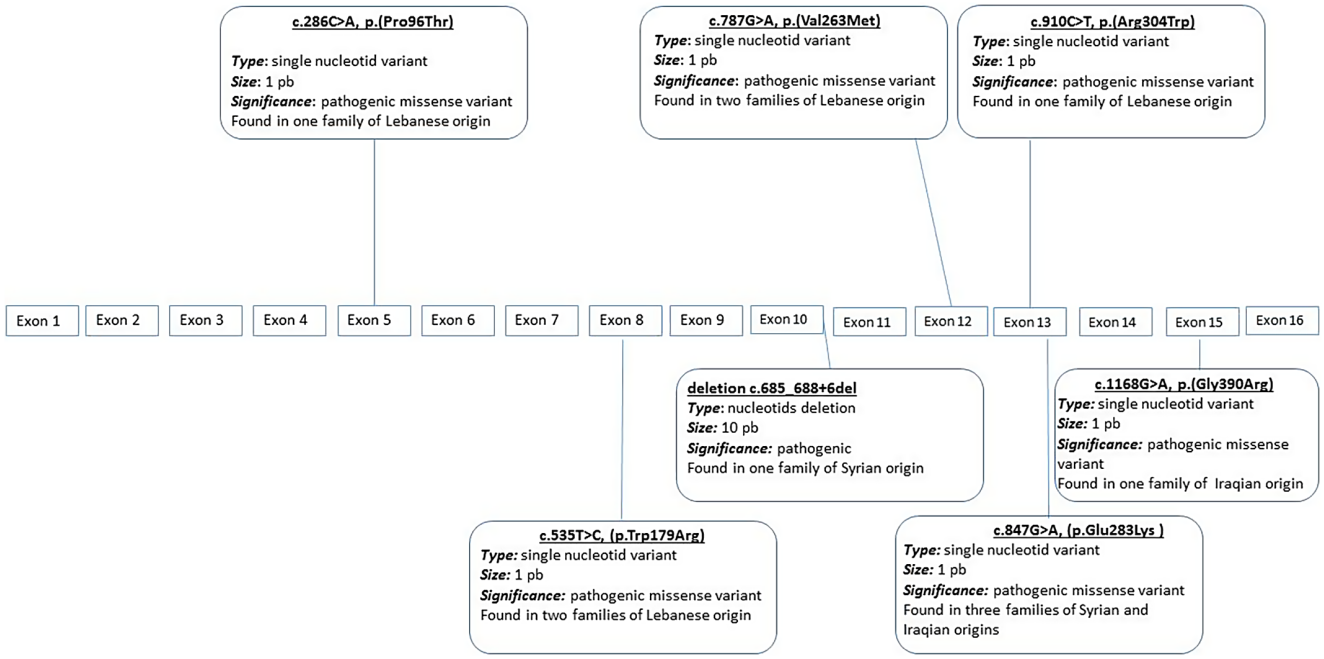

FIGURE 1 Localization of pathogenic or likely pathogenic variants in *ASS1* gene found in 10 middle eastern families†. †NM_054012.4 (GenBank reference sequence and version number of *ASS1* gene)

missense pathogenic variant preserves a quite high residual activity of around 30% (Berning et al., 2008), correlating it with a benign clinical picture, which is corroborated in our study.

The c.910C>T, p.(Arg304Trp) in exon 13 which was previously described in the Japanese (Kobayashi et al., 1994, 1995) and Korean (Lee et al., 2013) populations and in one Turkish patient (Gao et al., 2003), was found in one of our Lebanese families. This missense pathogenic variant, according to ACMG classifications, Clinvar (RCV001376582.1) and Uniprot (VAR_000690), preserves a residual activity of less than 5%, leading to urea cycle dysfunction (Kobayashi et al., 1995), and to a severe clinical form (Shaheen et al., 1994), which is in concordance with our patient's phenotype.

Our study identified as well two novel variants c.286C>A, p.(Pro96Thr) in exon 5 and c.685_688+6del, p.(Lys229Glyfs*4) in exon 10.

c.286C>A, p.(Pro96Thr) was found in one Lebanese family. It is located in the protein domain of the argininosuccinate synthetase and is pathogenic according to the ACMG classification. Alternative variants, p.Pro96His, p.Pro96Leu, and p.Pro96Ser, are classified as pathogenic by Uniprot (VAR_015894). Also, different prediction databases, such as MutationTaster, SIFT, and Polyphen, described this variant as pathogenic. It is not reported in the Genome Aggregation Database (https://gnomad.broadinstitute.org/). Based on the UCSC genome browser, the nucleotide and the amino acid at this location are well preserved in different species. The patient, a female, homozygous for c.286C>A, presented on day seven of life with an alteration of her general condition, including irritability, lethargy, and confusion. She exhibited a total lack of appetite, recurrent vomiting, respiratory distress, and neurological signs of convulsions, coma, alternation of hypotonia and hypertonia, spasticity, and clonus of the ankle. Her biological assessment showed hyperammonia and hypercitrullinemia. An EEG showed abnormal tracing with low amplitude in all regions of the brain, while a brain MRI showed bilateral focal lesions correlated with cytotoxic edema. The patient passed away at 1 month and 5 days of life. Both her parents were confirmed as heterozygous for the variant. The clinical presentation confers a severe form of CTLN associated with this novel c.286C>A, p.(Pro96Thr) variant c.685_688+6del, p.(Lys229Glyfs*4) was found in a Syrian male infant. It affects a donor site on exon 10, which could alter the translation either through exon skipping or through translation of the intronic part according to the Ex-Skip databases (Raponi et al., 2011). The patient presented on day three of life with an alteration of the general condition, lethargy, lack of appetite, and hypertonia. His paraclinical assessments showed hyperammonia, hypercitrullinemia, and abnormal hepatic function. The EEG was extremely abnormal, discontinued, and disorganized across the brain. The patient passed away at 1 year of age. The variant was identified in a heterozygous state in both parents. The clinical presentation for p.(Lys229Glyfs*4) in our patent is correlated with a severe outcome.

5 | CONCLUSION

Neonatal screening is still not routinely carried out in Middle Eastern populations, including Lebanon. Data on citrullinemia from these populations is still lacking, and often a time the disease is underdiagnosed, resulting in inadequate management. This study is the first to describe an array of *ASS1* variants found in patients from the Middle East. Further studies may be required to properly asses the genotypic, demographic, and clinical data of the disease, which would enable adequate, more optimized care and follow-up.

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AUTHOR CONTRIBUTIONS

MD and MS contributed equally to this manuscript, performed molecular genetic testing and analyses; wrote and revised the manuscript. TY and NS performed molecular genetic analyses. IK performed new born screening. HM provided clinical data on patients. AN performed patient data collection. JA and AM provided clinical data on patients. CF corresponding author, study design, wrote and revised the manuscript.

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CONFLICT OF INTEREST

All authors have no conflicts of interest to disclose.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ETHICS STATEMENT

This study was approved by the Ethical Committee of Saint Joseph University (USJ), Beirut, Lebanon. A written informed consent was obtained from both parents of the participant.

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REFERENCES

Bachmann, C. (2003). Outcome and survival of 88 patients with urea cycle disorders: A retrospective evaluation. *European Journal of Pediatrics*, *162*(6), 410–416. https://doi.org/10.1007/s00431-003-1188-9

Berning, C., Bieger, I., Pauli, S., Vermeulen, T., Vogl, T., Rummel, T., Höhne, W., Koch, H. G., Rolinski, B., Gempel, K., & Häberle, J. (2008). Investigation of citrullinemia type I variants by in vitro expression studies. *Human Mutation*, *29*(10), 1222–1227. https://doi.org/10.1002/humu.20784

Bijarnia-Mahay, S., Häberle, J., Jalan, A. B., Puri, R. D., Kohli, S., Kudalkar, K., Rüfenacht, V., Gupta, D., Maurya, D., Verma, J., Shigematsu, Y., Yamaguchi, S., Saxena, R., & Verma, I. C. (2018). Urea cycle disorders in India: Clinical course, biochemical and genetic investigations, and prenatal testing. *Orphanet Journal of Rare Diseases*, *13*(1), 174. https://doi.org/10.1186/s13023-018-0908-1

Brunetti-Pierri, N., Lamance, K. M., Lewis, R. A., & Craigen, W. J. (2012). 30-year follow-up of a patient with classic citrullinemia. *Molecular Genetics and Metabolism*, *106*(2), 248–250. https://doi.org/10.1016/j.ymgme.2012.03.011

Degirmencioglu, H., Oncel, M. Y., Yurttutan, S., Ekmen, S., Suna Oguz, S., Uras, N., & Dilmen, U. (2014). Citrullinemia with an atypical presentation: Persistent hiccups. Case report. *Archivos Argentinos de Pediatria*, *112*(5), e206–e208. https://doi.org/10.5546/aap.2014.eng.e206

Diez-Fernandez, C., Rüfenacht, V., & Häberle, J. (2017). Mutations in the human Argininosuccinate synthetase (ASS1) gene, impact on patients, common changes, and structural considerations. *Human Mutation*, *38*(5), 471–484. https://doi.org/10.1002/humu.23184

Dimmock, D. P., Trapane, P., Feigenbaum, A., Keegan, C. E., Cederbaum, S., Gibson, J., Gambello, M. J., Vaux, K., Ward, P., Rice, G. M., Wolff, J. A., O'Brien, W. E., & Fang, P. (2008). The role of molecular testing and enzyme analysis in the management of hypomorphic citrullinemia. *American Journal of Medical Genetics. Part A*, *146A*(22), 2885–2890. https://doi.org/10.1002/ajmg.a.32527

Engel, K., Höhne, W., & Häberle, J. (2009). Mutations and polymorphisms in the human argininosuccinate synthetase (ASS1) gene. *Human Mutation*, *30*(3), 300–307. https://doi.org/10.1002/humu.20847

Gao, H. Z., Kobayashi, K., Tabata, A., Tsuge, H., Iijima, M., Yasuda, T., Kalkanoglu, H. S., Dursun, A., Tokatli, A., Coskun, T., Trefz, F. K., Skladal, D., Mandel, H., Seidel, J., Kodama, S., Shirane, S., Ichida, T., Makino, S., Yoshino, M., … Saheki, T. (2003). Identification of 16 novel mutations in the argininosuccinate synthetase gene and genotype-phenotype correlation in 38 classical citrullinemia patients. *Human Mutation*, *22*(1), 24–34. https://doi.org/10.1002/humu.10230

Glamuzina, E., Marquis-Nicholson, R., Knoll, D., Love, D. R., & Wilson, C. (2011). Citrullinaemia type I: A common mutation in the Pacific Island population. *Journal of Paediatrics and Child Health*, *47*(5), 262–265. https://doi.org/10.1111/j.1440-1754.2010.01948.x

Häberle, J., Pauli, S., Linnebank, M., Kleijer, W. J., Bakker, H. D., Wanders, R. J., Harms, E., & Koch, H. G. (2002). Structure of the human argininosuccinate synthetase gene and an improved system for molecular diagnostics in patients with classical and mild citrullinemia. *Human Genetics*, *110*(4), 327–333. https://doi.org/10.1007/s00439-002-0686-6

Kasper, D. C., Ratschmann, R., Metz, T. F., Mechtler, T. P., Möslinger, D., Konstantopoulou, V., Item, C. B., Pollak, A., & Herkner, K.

R. (2010). The national Austrian newborn screening program - eight years experience with mass spectrometry. Past, present, and future goals. *Wiener Klinische Wochenschrift*, *122*(21–22), 607–613. https://doi.org/10.1007/s00508-010-1457-3

Kılıç, M., Altınel-Açoğlu, E., Zorlu, P., Yüksel, D., Bülbül, S., & Haeberle, J. (2017). First manifestation of citrullinemia type I as Sandifer syndrome. *The Turkish Journal of Pediatrics*, *59*(6), 696–698. https://doi.org/10.24953/turkjped.2017.06.013

Kleijer, W. J., Garritsen, V. H., van der Sterre, M. L., Berning, C., Häberle, J., & Huijmans, J. G. (2006). Prenatal diagnosis of citrullinemia and argininosuccinic aciduria: Evidence for a transmission ratio distortion in citrullinemia. *Prenatal Diagnosis*, *26*(3), 242–247. https://doi.org/10.1002/pd.1390

Kobayashi, K., Kakinoki, H., Fukushige, T., Shaheen, N., Terazono, H., & Saheki, T. (1995). Nature and frequency of mutations in the argininosuccinate synthetase gene that cause classical citrullinemia. *Human Genetics*, *96*(4), 454–463. https://doi.org/10.1007/BF00191806

Kobayashi, K., Shaheen, N., Terazono, H., & Saheki, T. (1994). Mutations in argininosuccinate synthetase mRNA of Japanese patients, causing classical citrullinemia. *American Journal of Human Genetics*, *55*(6), 1103–1112.

Kose, E., Unal, O., Bulbul, S., Gunduz, M., Häberle, J., & Arslan, N. (2017). Identification of three novel mutations in fourteen patients with citrullinemia type 1. *Clinical Biochemistry*, *50*(12), 686–689. https://doi.org/10.1016/j.clinbiochem.2017.01.011

Laróvere, L. E., Angaroni, C. J., Antonozzi, S. L., Bezard, M. B., Shimohama, M., & de Kremer, R. D. (2009). Citrullinemia type I, classical variant. Identification of ASS-p~G390R (c.1168G>A) mutation in families of a limited geographic area of Argentina: A possible population cluster. *Clinical Biochemistry*, *42*(10–11), 1166–1168. https://doi.org/10.1016/j.clinbiochem.2009.03.024

Lee, B. H., Kim, Y. M., Heo, S. H., Kim, G. H., Choi, I. H., Lee, B. S., Kim, E. A., Kim, K. S., Jhang, W. K., Park, S. J., & Yoo, H. W. (2013). High prevalence of neonatal presentation in Korean patients with citrullinemia type 1, and their shared mutations. *Molecular Genetics and Metabolism*, *108*(1), 18–24. https://doi.org/10.1016/j.ymgme.2012.11.011

Maestri, N. E., Clissold, D. B., & Brusilow, S. W. (1995). Long-term survival of patients with argininosuccinate synthetase deficiency. *The Journal of Pediatrics*, *127*(6), 929–935. https://doi.org/10.1016/s0022-3476(95)70030-7

Miller, M. J., Soler-Alfonso, C. R., Grund, J. E., Fang, P., Sun, Q., Elsea, S. H., & Sutton, V. R. (2014). Improved standards for prenatal diagnosis of citrullinemia. *Molecular Genetics and Metabolism*, *112*(3), 205–209. https://doi.org/10.1016/j.ymgme.2014.05.004

Pimenta, R., Urbano, F., Soares-de-Almeida, L., & Fernandes, S. (2018). Neonatal lupus erythematosus in a newborn with citrullinemia. *Acta Reumatologica Portuguesa*, *43*(3), 235–236.

Raponi, M., Kralovicova, J., Copson, E., Divina, P., Eccles, D., Johnson, P., Baralle, D., & Vorechovsky, I. (2011). Prediction of single-nucleotide substitutions that result in exon skipping: Identification of a splicing silencer in BRCA1 exon 6. *Human Mutation*, *32*(4), 436–444. https://doi.org/10.1002/humu.21458

Rhee, Y., Heaton, T., Keegan, C., & Ahmad, A. (2013). Citrullinemia type I and hypertrophic pyloric stenosis in a 1-month old male infant. *Clinical Practice*, *25*, e2

Saheki, T., Kobayashi, K., Ichiki, H., Matuo, S., Tatsuno, M., Imamura, Y., Inoue, I., Noda, T., & Hagihara, S. (1987). Molecular basis of enzyme abnormalities in urea cycle disorders. With special reference to citrullinemia and argininosuccinic aciduria. *Enzyme*, *38*(1–4), 227–232. https://doi.org/10.1159/000469209

Salek, J., Byrne, J., Box, T., Longo, N., & Sussman, N. (2010). Recurrent liver failure in a 25-year-old female. *Liver Transplantation*, *16*, 1049–53. https://doi.org/10.1002/lt.22118

Shaheen, N., Kobayashi, K., Terazono, H., Fukushige, T., Horiuchi, M., & Saheki, T. (1994). Characterization of human wild-type and mutant argininosuccinate synthetase proteins expressed in bacterial cells. *Enzyme & Protein*, *48*(5–6), 251–264. https://doi.org/10.1159/000474998

Wu, T. F., Liu, Y. P., Li, X. Y., Wang, Q., Song, J. Q., & Yang, Y. L. (2014). Prenatal diagnosis of citrullinemia type 1: A Chinese family with a novel mutation of the ASS1 gene. *Brain & Development*, *36*(3), 264–267. https://doi.org/10.1016/j.braindev.2013.03.005

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