Research Article

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Screening of ass1 gene in two Saudi families from al-madinah al-monawarah with citrullinemia disorder

Abstract

Background: Citrullinemia type 1 is an inherited autosomal recessive disease and a member of Urea Cycle Disorders (UCD),[1] characterized by accumulation of ammonia in the blood as a result of defect in enzyme called "argininosuccinate synthetase" (ASAS), which is responsible for converting citrulline to arginine. Signs and symptoms appear after birth, if untreated CTLN1 may progress to coma or death.

Purpose: To Screen ASS1 gene in two Saudi families with citrullinemia disorder (one affected family and the other is a carrier) from AL- Madinah AL-Monawarah. Define mutations may help in treatment and cure progress for patients.

Methods: Genetic analysis using sequencing technology was carried out to detect mutations in ASS1 gene.

Results: Two Mutations were detected. First family affected and unaffected members (III:3, II:2) have a homozygous missense variant in exon 7 (c.501 C>T, p.166 His>His). While in second family affected member (III:1) has a homozygous splice site mutation in exon 5 (c.364-2 A>G) and unaffected member (II:2) has a heterozygous mutation in exon 5 (c.371 A>T) of ASS1 gene.

Conclusion: Missense and splice site mutations were found in both affected and carrier members, homozygous and heterozygous respectively. The splice site region is important because mutations in these areas may lead to entire exon being spliced out of the mRNA. A heterozygous mutation makes individual a carrier of the disease.

Research Objectives

A. **Main objective:** To Screen ASS1 Gene in Two Saudi Families with Citrullinemia Disorder in AL- Madinah AL-Monawarah

B. **Specific objectives:** To screen ASS1 gene in Saudi families by ABI 3500 genetic analyzer.

To compare ASS1 sequence in affected individuals and his/her unaffected family members.

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Introduction and literature review

Citrullinemia is life-threatening disease, it occurs in 1:57,000 births and usually becomes evident in the first few days of life. It belongs to a small number of conditions called urea cycle disorders (UCD)[1] and it is characterized by accumulation of ammonia in the blood because of defect in enzyme called argininosuccinate synthetase (ASAS), which is either being missing or not working properly. ASAS is responsible for converting citrulline to arginine in urea cycle. Accumulated citrulline and ammonia impair the organ's antioxidant capacity.[2]

In citrullinemia condition, argininosuccinate synthetase enzyme is absent or insufficient due to mutation in Argininosuccinic acid synthetase (ASS1).[3]

Increasing knowledge of mutations that leads to ASAS abnormalities would help in better treatment and cure. This research is trying to define more mutations in ASS1 gene.

Literature review

Citrullinemia definition

Ammonia and other toxic substances can be accumulated in the blood because of an inherited autosomal recessive disorder (Figure 1.1 & Figure 1.2) (Table 1.1) called citrullinemia. Citrullinemia belongs to a family of genetic diseases named urea cycle disorders. Mutations in the ASS1 and solute carrier family 25, member 13 (citrin) SLC25A13 genes cause citrullinemia. The urea cycle is a series of chemical reactions that work at excess nitrogen generated when protein is used by the body in liver cells. The excess nitrogen is used to make a compound called urea, which is excreted in urine.[4,5]

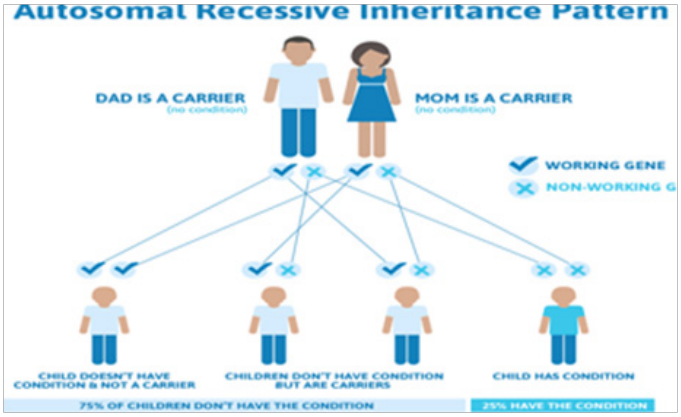

Figure 1.1 Pattern of autosomal recessive inheritance (genetic support foundation, 2015).

Table 1.1 Mutations of the *ASS1* Gene.[10]

1.2.3 Mutations Found in ASS1 Gene: Location	Protein	mRNA	DNA
Nonsense			
Int 0			c.-4C4T
Ex 7	p.G156X		c.459_466del
	p.N158X		c.450_451delCT
Int 11		r.773_774ins47	c.773149C4T
Ex 12	p.G275X		c.823G4T
Ex 12	p.R279X		c.835C4T
Ex 13	p.Q311X		c.931C4T
Ex 14	p.R344X		c.1030C4T
Ex 15	p.Q380X		c.1138C4T
Int 15	p.Q401X		c.1194-21_1213del
Splice site			
Int 4			c.17411G4T
Int 6			c.421-2A4G
Int 7		r.421_495del	c.49511G4T
Int 11			c.77311delG
Int 11			c.77312_77313insT
Int 12			c.83811G4A
Int 13		r.839_970del	c.97015G4A
Int 14/ Ex 15		r.1127_1128ins67	c.11279_1185dup67
Int 15		r.Ex16del7	c.1194-1G4C Turkey
Int 15	p.L399AfsX409	r.1193_1194ins37	
Deletion			
Ex 5	p.R100PfsX139		c.299delG
Int 7/Ex 8			c.496-21_515del
Ex 11/Ex 12		r.689_838del	
Ex 13	p.E298RfsX315		c.892delG
Ex 13	p.A318LfsX375		c.952delG
Missense			
Ex 3	p.G14S		c.40G4A
	p.S18L		c.53C4T
	p.C19R		c.55T4C
Ex 4	p.Q40L		c.119A4T
Ex 5	p.V69A		c.206T4C
	p.S79P		c.235T4C T
	p.R86C		c.256C4T
	p.R86H		c.257G4A
	p.R95S		c.285G4T
	p.P96S		c.286C4T
	p.P96H		c.287C4A
	p.R108L		c.323G4T
	p.G117S		c.349G4A
	p.G117D		c.350G4A
	p.A118T		c.352G4A
	p.T119I		c.356C4T

Table Continued....

1.2.3 Mutations Found in ASS1 Gene: Location	Protein	mRNA	DNA
Ex 6	p.D124N		c.370G4A
	p.R127W		c.379C4T
	p.R127Q		c.380G4A
Ex 7	p.R157C		c.469C4T
	p.R157H		c.470G4A
	p.L160P		c.479T4C
Ex 8	p.W179R		c.535T4C
	p.S180N		c.539G4A
	p.Y190D		c.568T4G
Ex 9	p.E191K		c.571G4A
	p.E191Q		c.571G4C
	p.A192V		c.575C4T
Ex 10	p.A202E		c.605C4A
	p.L206P		c.617T4C
Ex 12	p.V263M		c.787G4A
	p.R265C		c.793C4T
	p.R265H		c.794G4A
	p.V269M		c.805G4A
	p.E270Q		c.808G4C
	p.R272C		c.814C4T
	p.K277T		c.830A4C
	p.R279Q		c.836G4A
	p.G280R		c.838G4C
Ex 13	p.E283K		c.847G4A
	p.T284I		c.851C4T
	p.Y291S		c.872A4C
	p.D296G		c.887A4G
	p.M302V		c.904A4G
	p.R304W		c.910C4T
	p.R307C		c.919C4T
	p.K310Q		c.928A4C
	p.K310R		c.929A4G
	p.G324S		c.970G4A
Ex 14	p.G324V		c.971G4T
	p.S341F		c.1022C4T
	p.V345G		c.1034T4G
	p.G347R		c.1039G4C
	p.Y359D		c.1075T4G
	p.G362V		c.1085G4T
	p.R363W		c.1087C4T
	p.R363G		c.1087C4G
	p.R363L		c.1088G4T
	p.R363Q		c.1088G4A
Ex 15	p.T389I		c.1166C4T
	p.G390R		c.1168G4A

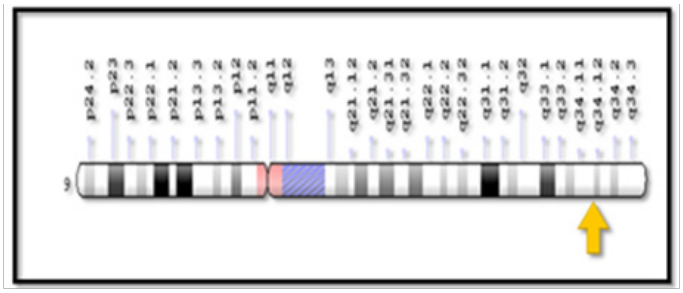

Figure 1.2 Cytogenetic and molecular location of argininosuccinic acid synthetase (ass) gene (genetics home reference, 2006).

Citrullinemia types

1- CTLN2 is a disorder caused by the lack of SLC25A13 gene, which is responsible for making citrin protein. Citrin is a helpful protein because it has four functions, which are build up and breakdown of sugars, proteins synthesis, transportation of urea cycle molecules and it takes part in the production of nucleotides. Any mutations in SLC25A13 gene blocks the functional citrin, it leads to inhibition of urea cycle and production of nucleotides and proteins which causes accumulation of ammonia and other toxic substances in adult-onset.[6]

It is adult-onset (11 to 79 years old) usually from adolescence to adulthood. it's effect on the nervous system suddenly and cause lack of understanding, amnesia, dysfunctional behavior, coma due to increase of ammonia in blood and leads to death because of brain edema.[7]

Diagnosis of CTLN2 depends on two tests, which are biochemical test and mutational study. The result of the biochemical test is hyperammonemia, the amino acids concentration will be increased with the increasing of concentration of citrulline and arginine can be showing in blood profile. While the confirmatory test is done by mutational study of the SLC25A13 gene, allowing genetics counseling.[8]

CTLN2 is a life-threatening disorder because of untreated cerebral edema. The only effective treatment is liver transplantations.[9]

2- Citrullinemia type 1 (CTLN1) is caused by mutations in ASS1 gene, which provides instructions for making an enzyme called argininosuccinate synthase. It is responsible for the third step of Urea Cycle. This step combines two protein building blocks (amino acids), citrulline and aspartate, to form a molecule called argininosuccinic acid. A series of additional chemical reactions uses argininosuccinic acid to form urea.[10]

According to Genetics Home Reference: "Cytogenetic Location of ASS1: 9q34.11, which is the long (q) arm of chromosome 9 at position 34.11" (Figure 1.2).[3]

According to Genetics Home Reference: "Molecular Location of ASS1: base pairs 130,444,707 to 130,501,274 on chromosome 9" (Figure 1.2) (Table 1.1).[3,10,11]

The Prevalence of CTLN1 among the world has been estimated to occur in 1:57,000 births.[12] Newborn screening programs found CTLN1 in different country:[13]

a. Two out of 44,300 individuals in Korea.[14]

b. One out of 200,000 individuals in New England.[15]

c. 1:118,543 In Taiwan.[16]

d. In Austria 1 out of 77,811 individuals.[17]

e. In United States statics of prevalence is one out of 117,000 individuals.[18]

The prevalence of CTLN2 disorder is less common from CTLN1, High frequency of this disorder founded in Japanese population about 1:100,000-230,000. It also recorded in Middle East and East Asia.[19]

Citrullinemia signs and symptoms

CTLN1 symptoms appear soon after birth and caused by increase ammonia level in blood. CTLN1 takes place usually after protein feeding in the first two to three days of life and start with: Absence of appetite refusal to feed, emesis, doziness, lack of energy, irritability, abnormally rapid breathing. In addition, if ammonia level increased more without treatment it may cause low muscle tone (hypotonia). respiratory problems, uncontrolled body temperature, liver enlargement, lack of growth, swelling of the brain due to increasing of fluid, increased intracranial pressure, seizures, ankle clonus, with increased ammonia level patient could go to hyperammonemic coma resulting in Neurological development delay, intellectual disability, learning difficulties, if untreated it could cause death in few weeks after birth.[20]

Citrullinemia diagnosis

Diagnosis of citrullinemia can be confirmed by a detailed patient family history, identification of characteristic findings, and a variety of specialized tests. Excessive amounts of ammonia and citrulline in the blood strongly suggests the diagnosis of CTLN1. Molecular genetic testing for ASS1 gene mutations is available to confirm the diagnosis.[21]

Newborn Screening Results: Elevated citrulline is detected in dried blood spots on newborn screen by tandem mass spectroscopy (MS/MS).[20]

Neonatal presentation: Sign and symptoms classically occur within the first week of life while on a full protein diet:

a. Increasing lethargy

b. Somnolence

c. Refusal to feed

d. Vomiting

e. Tachypnea

f. Stroke

g. Increased intracranial pressure (secondary to hyperammonemia) resulting in increased neuromuscular tone, spasticity, and ankle clonus.[20]

Supportive laboratory findings

1. **Plasma ammonia concentration:** Neonate Initial plasma ammonia concentration in the severe form may be 1000-3000μmol/L (normal: 40-100μmol/L).
2. **Plasma quantitative amino acid analysis:**
   a. Citrulline Usually >1000 μmol/L (normal: <50μmol/L)
   b. Argininosuccinic acid: Absent
   c. Arginine and ornithine Low to normal range

d. Lysine, glutamine, and alanine Increased, these are surrogate markers of hyperammonemia.

3. Urinary organic acids analysis Normal.[20]

Molecular Genetic Testing: Molecular testing is helpful when the phenotype is unclear, when biochemical values are borderline, or to distinguish CTNL1 from CTNL2.[10]

ASS1 is the only gene associated with CTNL1. The majority of causative mutations in the gene are sequence variants, but large deletions have been reported, The Methods to detect mutations of ASS1 gene PCR (Polymerase chain reaction) followed by bidirectional sequencing of the entire coding region and intron/exon boundaries of the ASS1 gene.[10]

Citrullinemia treatment & management

The treatment of hyperammonemia[22] should be applied immediately to prevent and avoid complications which associated with neurologic damage or even death with severe untreated hyperammonemia patients. Intellectual ability is inversely related to the length of time of infant with urea cycle disorder without treatment. Immediate treatment is extremely important in avoiding or minimizing the degree of complications.[23]

Materials and methods

Materials

Ethical consideration: The study proposal was reviewed and ethically approved by the Scientific and the Ethical Committee (Institutional Review Board) at Taibah University and Institutional Review Board committee of health affairs in Madinah. Written informed consents were obtained from all individuals.

Study design: This is a familial study. Following informed consents, individuals with citrullinemia were enrolled in this study.

Study area: The study was conducted in Al-Madinah Al-Monawarah. Patients were recruited from Maternity Hospital. Genetic analysis including DNA extraction, PCR, and sequencing was performed in Center of Genetic and Inherited Diseases (CGID).

Study population: Two families from Al-Madinah Al-Monawarah were included in this study after being fully informed about the aims of the study. The results of the analysis (gene screening and sequencing), have been used for clinical diagnosis, and offered free of charge for all patients participating in the study.

First family was recruited from Al-Madinah Al-Monawarah. It is a three generations autosomal recessive CTLN1 family with 10 members with no history of any other genetic disease. In this family, 8 individuals are unaffected (I:1, I:2, I:3, I:4, II:1, II:2, III:1, III:2) and 2 individuals are affected (III:3, III:4) (Figure 2.1). Both affected individuals are females. Affected individual (III:3) is a 4 years old female diagnosed with CTLN1 at birth. Affected individual (III:4) is a 6 years old female diagnosed with CTLN1 at birth. Blood sample were collected from 1 affected (III:3) and 1 unaffected member (II:2) for deoxyribonucleic acid (DNA) extraction.

The second family was recruited from Al-Madinah Al-Monawarah. It is a three generations autosomal recessive CTLN1 family with 11 members with no family history of any other genetic diseases. In this family, 9 individuals are unaffected (I:1, I:2, I:3, I:4, II:1, II:2, III:3, III:4, III:5) and 2 individuals are affected (III:1, III:2) (Figure 2.2). Both affected individuals are males. The affected individuals (III:1) is a 4 years old male diagnosed with CTLN1 at birth. Affected individual (III:2) is a 7 years old male diagnosed with CTLN1 at birth. Blood samples were collected from 1 affected (III:1) and 1 unaffected member (II:2) for DNA extraction.

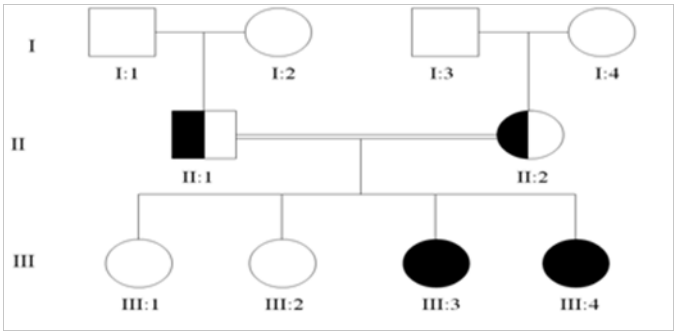

Figure 2.1 Pedigree of the first family from al-madinah al-monawarah. it is three generations autosomal recessive ctln1 family.

Figure 2.2 Pedigree of the second family from Al-Madinah Al-Monawarah. It is three generations autosomal recessive CTLN1 family.

Medical history: The individuals were interviewed by trained team, demographic and clinical data about the patients and their families was collected in a specially designed questionnaire (Appendix 1). Demographic data included age, sex, tribe, weight, height, and address. Clinical data included medical history of citrullinemia, type of treatment, type of diet, ammonia level measurement.

Blood collection: Blood samples (3ml) were collected from available participants in 4 ml EDTA (Ethylenediaminetetraacetic acid) tubes and stored at 4◦C.

Methods

DNA extraction and quantification: The blood collected in EDTA tubes was equilibrated to room temperature and extraction of DNA was carried out using QIAGEN (QIAamp® DNA) Mini Kit according to the supplier's protocols. The DNA was quantified by Nanodrop spectrophotometer (MAESTRO) and diluted to 40ng/µl for PCR (Table 2.1 & table 2.2).

Table 2.1 DNA quality and concentration determined by nanodrop spectrophotometry for the first family

Patients	DNA Purity	DNA concentration
III:3	1.9	22
II:2	2	20
Reference Values	1.7 - 2	20 – 50

Genomic DNA visualization: One percent (1%) of agarose gel was prepared by mixing 50 ml 1X-Tris-borate-EDTA (TBE) buffer and 0.5 g agarose. Solution was heated to boiling, followed by cooling and 3µl of Ethidium Bromide (EtBr) was added. Solution was poured to casting tray.

Table 2.2 DNA quality and concentration determined by nanodrop spectrophotometry for the second family

Primer name	Sequence	Temperature	Product size
ASS1_Ex2F	CAGGAGACAAGGCTGTCC	56.69	318 bps
ASS1_Ex2R	GAGCAGACAGGCTGACAAC	56.96	318 bps
ASS1_Ex3F	ATGGTGTGAACTCAGGGC	56.83	202 bps
ASS1_Ex3R	CAGGAGCATCCACCACTG	58.67	202 bps
ASS1_Ex4F	GGGCTCTGTATGCCAGATG	59.21	320 bps
ASS1_Ex4R	CTCATCTCCTTCCCACACC	57.98	320 bps
ASS1_Ex5F	CTGTCCTTGTCCTCACGTC	56.61	242 bps
ASS1_Ex5R	ATTCTGTGCCTGTCCTGTG	57.1	242 bps
ASS1_Ex6F	CCTCACAACAGCATCCTCTC	58.38	375 bps
ASS1_Ex6R	ACAGAGGCCACGTGTGTAG	57.21	375 bps
ASS1_Ex7F	AGGGTCTTGTCTGAATGGG	57.47	244 bps
ASS1_Ex7R	GTCCTTTGGAATGAGCCC	57.98	244 bps
ASS1_Ex8F	ATGTTTCAGGCAGGTTGG	56.97	231 bps
ASS1_Ex8R	AGCTGCTACCACCAAAGG	56.38	231 bps
ASS1_Ex9F	GTAGGGTGTCCAGGGACTG	57.9	264 bps
ASS1_Ex9R	TAGCTTCCAGGAATGCAGG	58.96	264 bps
ASS1_Ex10F	ATCCATTTAAGGCGTTTCG	57.71	280 bps
ASS1_Ex10R	CTCAGCCACAACCATTAGC	56.83	280 bps
ASS1_Ex11F	GGTGACTCTGAGCCTTGC	56.87	178 bps
ASS1_Ex11R	GATCTCTGGGTTTGCTGG	56.58	178 bps
ASS1_Ex12F	GTCATTTGCTGACAGTTTGG	56.72	265 bps
ASS1_Ex12R	GTACTTTGGGATCCCTTGTG	56.96	265 bps
ASS1_Ex13F	CCCAGGTCTCCCTGTGTC	58.97	308 bps
ASS1_Ex13R	TCAGGCACAGATGTCTTGAG	57.44	308 bps
ASS1_Ex14F	CAGTCCTCCCTTCAAGCAG	58.52	190 bps
ASS1_Ex14R	CAAAGCCTACCCTTTACACC	56.4	190 bps
ASS1_Ex15F	ACCCAGTGTGTGTTGTTATTG	55.89	181 bps
ASS1_Ex15R	TTATCACAACAATTAGCGCC	56.42	181 bps

Three µl of the loading dye was mixed with 3µl of the sample (genomic DNA)[23] and loaded into gel wells. Electrophoresis was performed at 120 voltages for 40 minutes. Gel was visualized by Ultraviolet (UV) in a gel documentation system (Gensys). Ladder size was 10bp (1000-100).

Primers designing: Primers were designed using Primer 3 software from University of California Santa Cruz (UCSC) genome browser (www.genome.ucsc) website. Primer sequences, melting temperature and size of amplified product of all exons of ASS1 gene is available in (Table 2.3). Exons 1 and 16 is non-coding sequences.[25]

Table 2.3 Primer sequences melting temperature and size of amplified product of all exons of ASS1 gene).

Patients	DNA Purity	DNA concentration
III:1	1.8	20
II:2	1.9	21
Reference value	1.7 - 2	20 - 50

Polymerase chain reaction

Exon amplification

ASS1 gene was amplified by PCR. Amplified products were analyzed on 2% agarose and purified by QIAquick Purification kit (QIAGEN). Polymerase chain reaction was carried out in 50µl reaction volume containing 5ul of genomic DNA, 2.5ul of each primer (forward primer and reverse primer), 25ul of master mix (GO) and 15 µl distilled water. The thermal cycler condition used were 35 cycles, 95°C for 1 minute (denaturation), 57-60°C for 1 minute (annealing), 74°C for 1 minute (extension), and final extension of 72°C for 10 minutes. PCR was performed by Veriti 96 well thermal cycler (Applied Biosystems). Annealing temperature for exons 2,3,4,6,8,9,10,11,12,13,14 was 57◦C, 58◦C for exon 15, and 59◦C for exons 5 and 7.[26–30]

First purification of amplified products

Amplified products were purified using USB® ExoSAP-IT®PCR Product Cleanup Kit according to the supplier's protocols.

Second amplification of purified product (second PCR)

One µl of DNA templates (from first purification) were mixed with 1µl 5X sequencing buffer, 6 µl distilled water (dH2O), 1µl of (forward or reverse) primer and 1µl of Big dye terminator. They were mixed together and then centrifuged; they were placed on thermal cycler machine for amplification.

The thermal cycler condition included 35 cycles, 95°C for 3 minutes, 95°C for 1 minute, 57°C for 30 seconds, 60°C for 1 minute and final extension of 60°C for 4 minutes. PCR was performed by Veriti 96 well thermal cycler (Applied Biosystems).

Second purification of amplified products

Amplified products were purified using Big Dye® X-TerminatorTM Purification Kit (Applied Biosystems) according to the supplier's protocols.

Sequencing

After mixing the purified product by Mixmate, the supernatant was transferred to the sequencing plate for sequencing in the capillary electrophoresis and chromatograms were generated on ABI 3500 genetic analyzer (Applied Biosystems).

Results

In the present study, two Saudi families with CTLN1 disease with no history of any other genetic disease were recruited from Al-Madinah Al-Monawarah have been investigated . DNA was extracted from all available individuals. Two blood samples collected from family 1(III:3 affected, II:2 unaffected) and two blood samples collected from family 2(III:1 affected, II:2 unaffected) (Figure 3.1). DNA extraction and Gel Electrophoresis was done on the samples with 1% agarose gel concentration.

DNA for four (III:3, II:2, III:1, II:2) individuals both affected and unaffected were amplified by PCR (Figure 3.2-3.4), purified and sequenced under standard conditions. Sequence analysis using reference sequence from genome browser and Ensemble (which include reference sequence of the exons) identify mutations that are responsible for CTLN1 disease in the families (Figure 3.5-3.8).

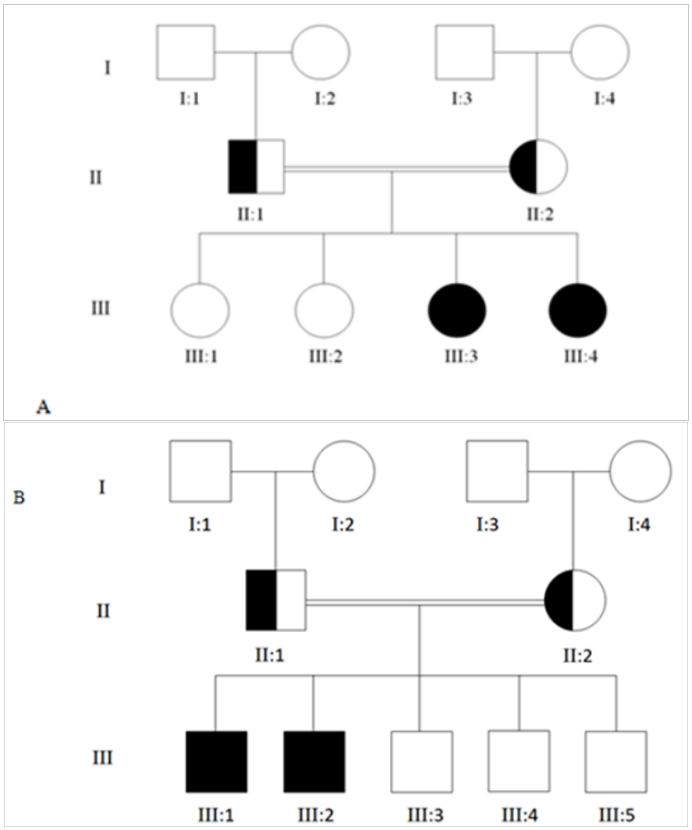

Figure 3.1 Pedigree of Saudi Arabian families segregating CTLN1 in an autosomal recessive fashion. **A** represent the first family, **B** represent the second family, circle represents female individuals, while square represents male individuals. Filled symbols represent affected members.

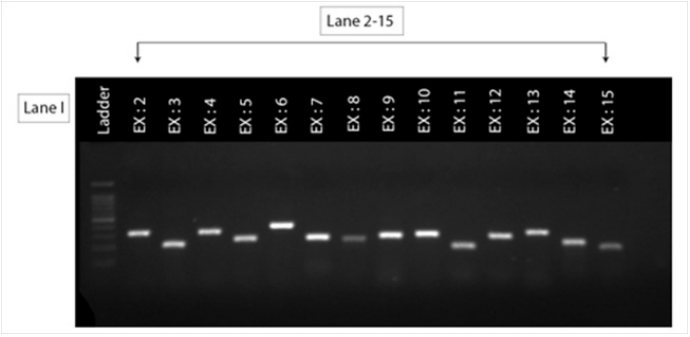

Figure 3.2 1% Agarose gel electrophoresis pattern of PCR amplified coding exons of the *ASS1* gene for the first family, patient (III:3). Lane I (ladder size= 10bp) , lane 2-15 (exons 2-15), the size of exon 7 (300bp).

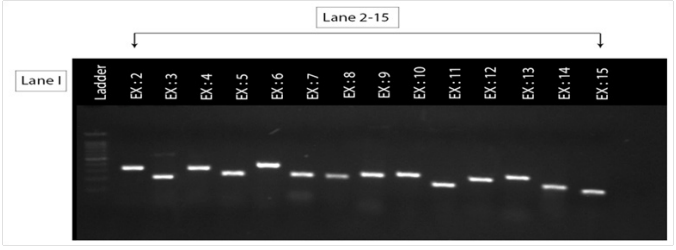

Figure 3.3 1% Agarose gel electrophoresis pattern of PCR amplified coding exons of the *ASS1* gene for the second family patient (III:1). Lane I (ladder size= 10bp), lane 2-15 (exons 2-15), the size of exon 5(350bp).

Citation: Algarni BA, Almugari BF, Sorour RS, et al. Screening of ass1 gene in two Saudi families from al-madinah al-monawarah with citrullinemia disorder. *Int J Mol Biol Open Access.* 2018;3(5):222–233. DOI: 10.15406/ijmboa.2018.03.00081

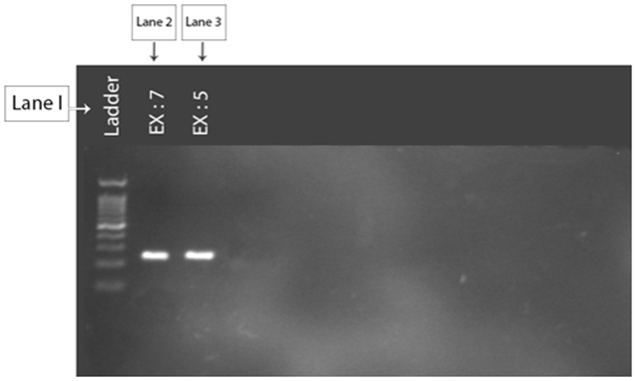

Figure 3.4 1% Agarose gel electrophoresis pattern of PCR amplified coding exons of the *ASS1* gene for unaffected individuals, Lane 1 (ladder size=10bp) lane 2 (exon 7) for the first family (II:2) lane 3 (exons 5) for the second family (II:2), the size of exon 5 and 7 (250bp).

Family one: Affected member 3 of third offspring of first family (III:3) (pedigree 3.1)

Exon (7-forward)

C>T

Discussion

Citrullinemia type 1 is an inherited autosomal recessive disease. It is a member of urea cycle disorders. It is characterized by accumulation of ammonia in the blood because of defect in enzyme ASS, which is either being missing or not working properly.[2] The Prevalence of CTLN1 among the world has been estimated to occur in 1:57,000 births[11] ASS1 gene is the responsible gene of producing argininosuccinate synthase 1 enzyme. Thus, the mutations in this gene lead to CTLN1.

In the present study, which is the first study on ASS1 gene carried out in Al-Madinah Al-Monawarah, two families were investigated with three generations autosomal recessive CTLN1 and severe phenotypic expression of the disorder. The 14 exons of the ASS1 gene -which is the gene that is responsible for CTLN1- was screened in two families. Missense and splice site mutations were found in affected individuals of family 1 and 2 respectively. In the affected individual (III:3) of first family, the homozygous variant was in exon 7 (c.501 C>T, p.166 His>His). The mother (II:2) of this affected individual was also found to be homozygous for same variant (c.501 C>T, p.166 His>His). While the affected individual (III:1) of second family has a splice site homozygous mutation affecting exon 5 (c.364-2 A>G). The mother has a heterozygous mutation in exon 5 (c.371 A>T).

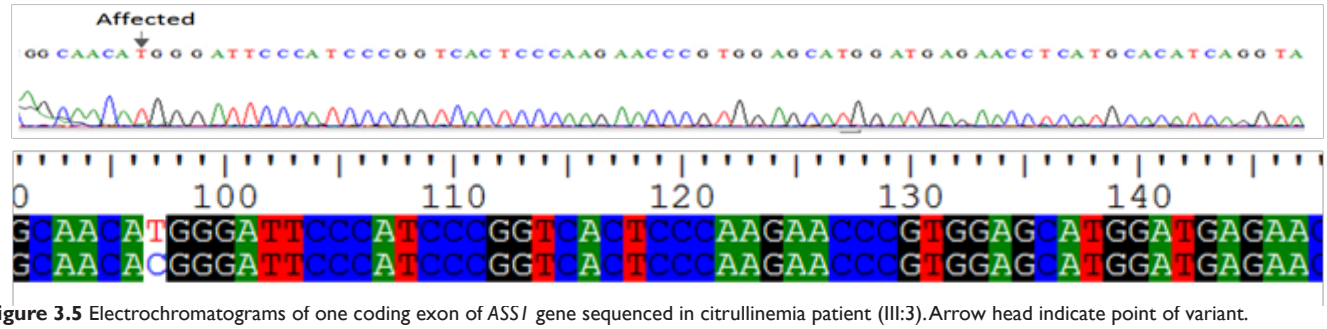

Figure 3.5 Electrochromatograms of one coding exon of *ASS1* gene sequenced in citrullinemia patient (III:3). Arrow head indicate point of variant.

Carrier member 2 of second offspring of first family (II:2) (pedigree 3.1)

Exon (7-forward)

C>T

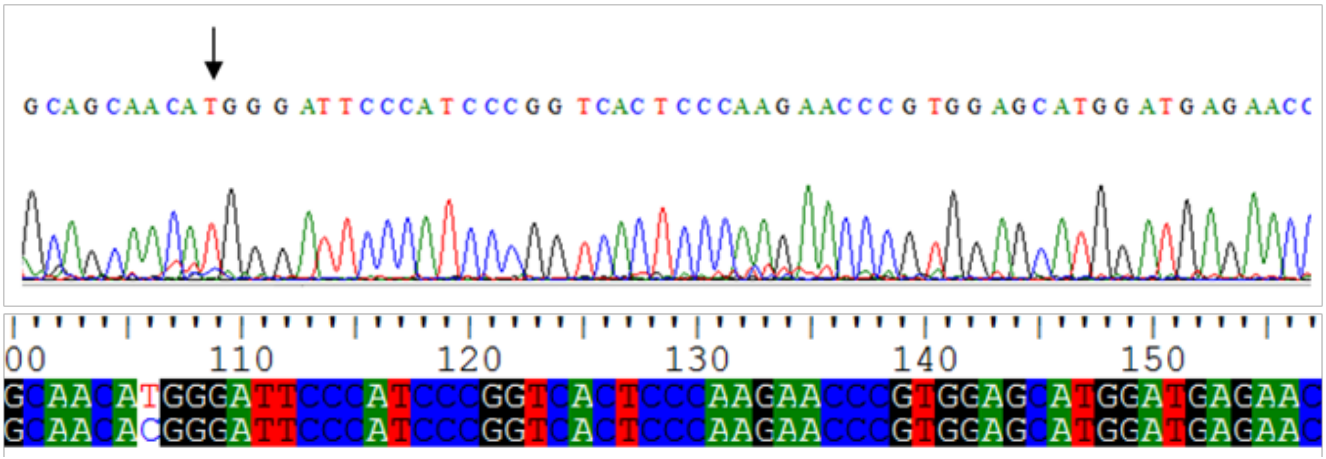

Figure 3.6 Electrochromatograms of one coding exon of *ASS1* gene sequenced in citrullinemia patient (II:2). Arrow head indicate point of variant.

Family two: Affected member 1 of third offspring of second family (III:1) (pedigree 3.1)

Exon (5-forward)

A>G

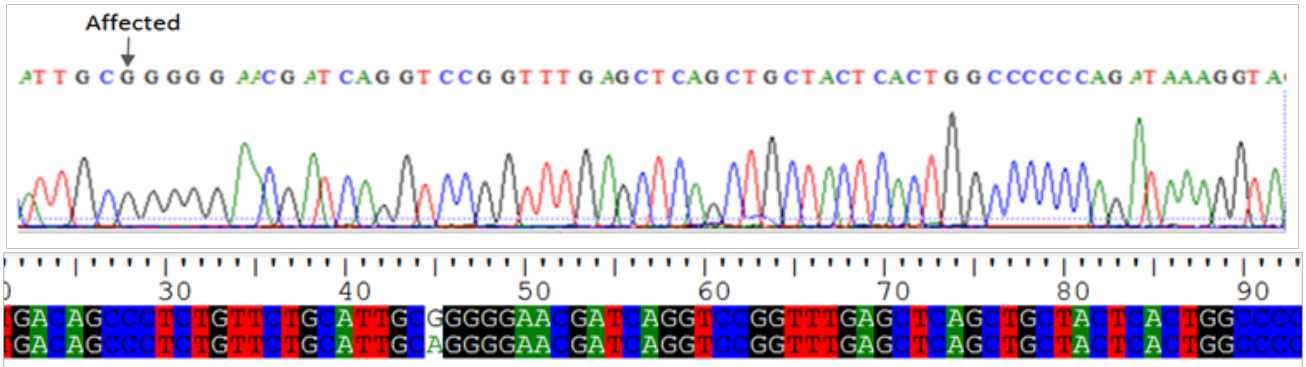

Figure 3.7 Electrochromatograms of one coding exon of *ASS1* gene sequenced in citrullinemia patient with homozygous mutation (III:1). Arrow head indicate point of mutation.

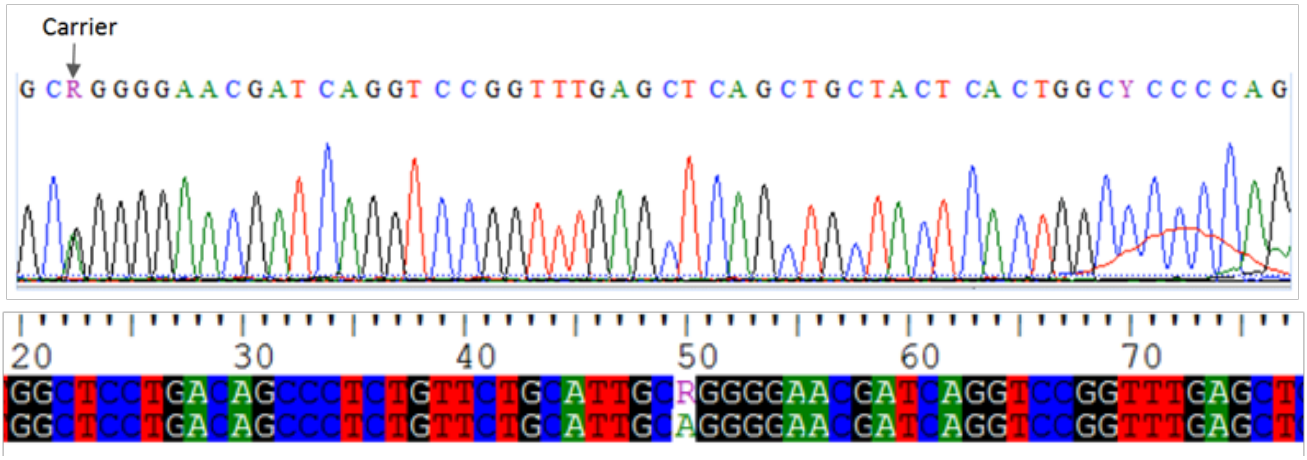

Figure 3.8 Electrochromatograms of one coding exon of *ASS1* gene sequenced in heterozygous individual (carrier) (II:2). Arrow head indicate point of mutation.

Carrier member 2 of second offspring of second family (II:2) (pedigree 3.1)

Exon (5-forward)

A>T

ASS1 gene transcribe into a mRNA of 56,568 bp. It translates into a protein product of 412 amino acids (UCSC, 2017).[25] Previous study conducted in China found missense mutation located in exon 13 (c.970 G>A, p.223 Gly>Ser).[31] Another study conducted on Indian people found missense mutation in exon 3 (c.835 C>T, p.279 Arg>stop codon).[32] ASS1 gene harbor different mutations and the affected individuals show variable phenotype based on lifestyle, race and population. Previous review of mutations in the ASS1 gene listed 87 mutations, including 27 novel mutations in patients with citrullinemia. Mutations are distributed throughout the gene, and it is usually difficult to predict the phenotype based on genotype. However, the p.G390R mutation in exon 15 was found to be the single most common mutation in patients with the classic phenotype [33] Another study found that since most patients with citrullinemia express stable mRNA in fibroblasts, the disorder is ideally suited for gene amplification with PCR and sequence analysis of mutant cDNA. They sequenced cDNA from 11 independent chromosomes and identified 9 different mutations: 3 showed absence of exon 5, 6 or 7, and 6 showed point mutations. Five of the 6 involved G>T transitions in CpG dinucleotides, and 3 of these resulted in loss of MspI sites.[34]

In the course of studying the molecular nature of mutations in Japanese patients with classic citrullinemia, researchers found that 10 of 23 affected alleles had the same mutation, i.e. deletion of exon 7.[35] This differed from the situation in the United States, where far greater heterogeneity of mutations had been found. Another study reported that 20 mutations had been identified in ASS1 mRNA in classic citrullinemia, including 14 single base changes causing missense mutations, 4 mutations associated with an absence of exons 5, 6, 7, or 13 in mRNA, 1 mutation with a deletion of the first 7 bases in exon 16 (caused by abnormal splicing), and 1 mutation with an insertion of 37 bases between the exon 15 and 16 regions of mRNA.[36–38] Moreover, a splice site mutation (IVS6-2A>G) has also been reported in 23 families (20 from Japan and 3 from Korea).

Mutations of The ASS1 Gene: (Table 3.1)

Table 3.1 Mutations of the ASS1 Gene

Location	Protein	mRNA	DNA	Ethnicity
Nonsense				
Int 0			c.-4C4T	Germany

Table Continued....

Location	Protein	mRNA	DNA	Ethnicity
Ex 7	p.G156X		c.459_466del	Germany
	p.N158X		c.450_451delCT	Switzerland
Int 11		r.773_774ins47	c.773149C4T	Germany, Turkey
Ex 12	p.G275X		c.823G4T	Netherlands
Ex 12	p.R279X		c.835C4T	USA
Ex 13	p.Q311X		c.931C4T	Italy
Ex 14	p.R344X		c.1030C4T	Germany
Ex 15	p.Q380X		c.1138C4T	Greece
Int 15	p.Q401X		c.1194-21_1213del	Mexico
Splice site				
Int 4			c.17411G4T	Netherlands
Int 6			c.421-2A4G	USA
Int 7		r.421_495del	c.49511G4T	Scotland
Int 11			c.77311delG	Japan
Int 11			c.77312_77313insT	Spain
Int 12			c.83811G4A	France
Int 13		r.839_970del	c.97015G4A	Japan
Int 14/ Ex 15		r.1127_1128ins67	c.1127-9_1185dup67	Japan
Int 15		r.Ex16del7	c.1194-1G4C Turkey	Turkey
Int 15	p.L399AfsX409	r.1193_1194ins37		Japan
Deletion				
Ex 5	p.R100PfsX139		c.299delG	Japan
Int 7/Ex 8			c.496-21_515del	Italy
Ex 11/Ex 12		r.689_838del		Poland
Ex 13	p.E298RfsX315		c.892delG	Spain
Ex 13	p.A318LfsX375		c.952delG	Germany
Missense				
Ex 3	p.G14S		c.40G4A	USA
	p.S18L		c.53C4T	USA
	p.C19R		c.55T4C	Japan
Ex 4	p.Q40L		c.119A4T	Germany
Ex 5	p.V69A		c.206T4C	Spain, Germany
	p.S79P		c.235T4C T	Thailand
	p.R86C		c.256C4T	USA, Italy
	p.R86H		c.257G4A	Japan
	p.R95S		c.285G4T	Turkey
	p.P96S		c.286C4T	Turkey
	p.P96H		c.287C4A	Italy
	p.R108L		c.323G4T	Spain, Germany
	p.G117S		c.349G4A	Austria, Thailand
	p.G117D		c.350G4A	Spain
	p.A118T		c.352G4A	Japan, Morocco
	p.T119I		c.356C4T	Spain

Table Continued....

Location	Protein	mRNA	DNA	Ethnicity
Ex 6	p.D124N		c.370G4A	Netherlands
	p.R127W		c.379C4T	UK/Arabic
	p.R127Q		c.380G4A	USA, Germany
Ex 7	p.R157C		c.469C4T	Japan
	p.R157H		c.470G4A	USA
	p.L160P		c.479T4C	Germany
Ex 8	p.W179R		c.535T4C	Turkey, Italy, Libya.
	p.S180N		c.539G4A	USA
	p.Y190D		c.568T4G	Germany
Ex 9	p.E191K		c.571G4A	Israe
	p.E191Q		c.571G4C	N. Ireland
	p.A192V		c.575C4T	Japan
Ex 10	p.A202E		c.605C4A	Germany
	p.L206P		c.617T4C	Germany
Ex 12	p.V263M		c.787G4A	Turkey
	p.R265C		c.793C4T	India
	p.R265H		c.794G4A	Japan, Germany
	p.V269M		c.805G4A	Germany
	p.E270Q		c.808G4C	Spain, Germany
	p.R272C		c.814C4T	Japan
	p.K277T		c.830A4C	France
	p.R279Q		c.836G4A	Japan
	p.G280R		c.838G4C	Japan
Ex 13	p.E283K		c.847G4A	Belgium
	p.T284I		c.851C4T	Germany
	p.Y291S		c.872A4C	USA, Germany
	p.D296G		c.887A4G	Italy
	p.M302V		c.904A4G	Italy
	p.R304W		c.910C4T	Japan, Turkey
	p.R307C		c.919C4T	Germany
	p.K310Q		c.928A4C	Canada
	p.K310R		c.929A4G	USA
	p.G324S		c.970G4A	USA, Korea, Turkey
Ex 14	p.G324V		c.971G4T	Mexico
	p.S341F		c.1022C4T	Turkey
	p.V345G		c.1034T4G	Germany
	p.G347R		c.1039G4C	Spain
	p.Y359D		c.1075T4G	Germany
	p.G362V		c.1085G4T	Netherlands, Turkey, Germany
	p.R363W		c.1087C4T	USA, Germany
	p.R363G		c.1087C4G	Greece
	p.R363L		c.1088G4T	Japan
	p.R363Q		c.1088G4A	USA
Ex 15	p.T389I		c.1166C4T	Canada
	p.G390R		c.1168G4A	USA, Spain, Turkey, Bolivia, Isreal, Canada, Austria

Citation: Algarni BA, Almugari BF, Sorour RS, et al. Screening of ass1 gene in two Saudi families from al-madinah al-monawarah with citrullinemia disorder. *Int J Mol Biol Open Access.* 2018;3(5):222–233. DOI: 10.15406/ijmboa.2018.03.00081

Conclusion

From the present study, the following could be concluded: Mutations in ASS1 gene were found using the sequences analysis. In the first family affected member III:3 and unaffected member II:2 have a variant in exon 7 (c.501 C>T, p.166 His>His).

In the second family affected member III:1 has a splice site mutation in Exon5 (c.364-2 A>G). The splice site region is important because mutations in these areas may lead to entire exon being spliced out of the mRNA. The unaffected member II:2 of the second family has a heterozygous mutation in exon 5 (c.371 A>T). A heterozygous mutation makes individual a carrier of the disease.

Ethical approval: The research have two ethical approvals. The first from Taibah University. And the second from Ministry of Health

Recommendations

a. The main cause of the inherited citrullinemia is the consanguineous marriage, therefore it should be avoided as much as possible or doing a genetic test before marriage.

b. Further studies including more affected families are required.

c. Also, another studies could beheld that involve other genes associated with citrullinemia.

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Conflict of interests

Author declares there are no conflict of interests.

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