First Report of Congenital Short Bowel Syndrome in an Iranian Patient Caused by a Mutation in the *CLMP* Gene

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Abstract

Keywords

- congenital short bowel syndrome
- CXADR-like membrane protein
- whole exome sequencing

Congenital short bowel syndrome (CSBS) is a rare congenital neonatal disorder. CSBS results from intestinal impairment during embryogenesis. Mutated *CXADR-like membrane protein (CLMP)* and *Filamin A* genes are involved in the cause of CSBS. In this study, due to our misdiagnosis, we had to perform whole exome sequencing on the patient, and also we implemented cosegregation analysis on his parents with consanguineous marriage and also parents' mothers. We identified a homozygous loss of function mutation in the *CLMP* gene in exon 5 (c.664C > T, p.R222X). Also, both parents and grandmothers of the proband were heterozygous for this mutation. Loss of function mutation in *CLMP* causes CSBS, leading to impaired intestinal development.

Introduction

Short bowel syndrome (SBS) is characterized by a sum of functional alternations that are the consequence of a critical loss in the small bowel length. In neonates, SBS is generally known as an acquired disorder and leads to surgical resection of the small intestine due to intestinal atresia or enterocolitis. In a small minority of cases, the SBS can be congenital. The etiopathogenesis of congenital SBS (CSBS) is poorly identified.¹ CSBS is a heritable, complex, clinical disorder of the newborn with a high mortality rate. In addition, this feature is characterized by malabsorption and diarrhea as a result of the shortened small intestine with a mean length of 50 cm in comparison with the normal length (190–280 cm).^{2,3} In several cases, the colon is affected too.^{4–6} Malabsorption

may lead to nutritional deficiencies. Also, the short bowel results in poor growth, fatty stools, malnutrition, abdominal pain, and dehydration.⁷ The developmental defect can cause malrotation of the intestine, which is always present in these patients. They usually have normal mental ability.^{8,9} Patients with CSBS are born with functional or anatomical loss of adequate small bowel length that is a consequence of a developmental problem of the small intestine.¹⁰ Moreover, receiving parenteral nutrition (PN) as a life-saving therapy is required for patients with SBS, whether it is congenital or acquired and this strategy should be continued for the first 2 years of life.^{8,11} PN might lead to fatal and acute complications including liver disease and sepsis.¹² SBS in children could be improved by surgical interventions through intestinal lengthening or transplantation to increase intestinal area and minimize the complications of PN therapy. Intestinal adaptation and pharmaceutical therapies are other therapeutic

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methods to ameliorate the performance of remnant bowel.¹³ As time passes, length and absorption capacity of the small intestine improve causing better absorption of nutrients. In spite of significant efforts in dealing with the disease, great numbers of patients die of starvation in the early stage of life. Regarding genetics studies on the basis of disease have shown that CSBS is frequently observed in consanguineous families. In previous studies, *CLMP* and Filamin A (*FLNA*) were reported to be involved in the pathogenesis of the recessive and X-linked form of CSBS, respectively.^{14–16} Here, we present the first case of CSBS in a family without the history of SBS in Iran.

Case Presentations

The patient (IV-5) was a Turkish boy born at 38 weeks of gestation from consanguineous parents (III-8 and III-9)

(►Fig. 1). The patient was the first child of a first cousin marriage without a familial history of the specific disease and there was no family history of CSBS (►Fig. 2). The body weight was 3 kg, and body length was 50 cm at birth. He was referred to our center 50 days after birth with acute symptoms including vomit and diarrhea. Clinical examinations and checkup revealed intestinal atresia, dehydration, paralysis, metabolic acidosis, and protein intolerance. The patient has no dysfunction in the ureteral system. At laparotomy, the small intestine of the patient was measured to be 70 cm in length. After 32 days of hospitalization, bilious vomiting, intestinal malrotation, and abdominal distention were observed and the baby was treated with serum therapy. During 5 months, the patient had only 300 g weight gain regarding minimum weight (3.09 kg). At first, the patient was suspected to be suffering from Hirschsprung and

Fig. 1 Family pedigree after genetic counseling.

Fig. 2 Affected child at 7 months of age.

Noonan disease, and surgical resection of the short intestine was performed. After the operation, the patient had sepsis symptoms that represent the possibility of necrotizing enterocolitis. About 2 months after hospitalization, different signs such as hepatosplenomegaly, parathyroid malfunction, thrombocytopenia, hypocalcemia, and reduction in Na^+ and K^+ levels were detected. Abdominal radiography indicated the absence of gaseous dilation in intestine lobes and pneumoperitoneum. In addition, increased opacity in the upper part of the abdomen showed the probability of fluid existence in the abdomen. Thorax radiography indicated slight opacity in the right parahilar, paracardiac area, and atrophic thymus. No brain lesion was detected in brain computed tomography scan. The patient condition got worse and failed to be treated. Therefore, clinician's pediatricians suspected CSBS and the patient has been referred to our medical genetic division for genetic counseling.

Materials and Methods

Whole Exome Sequencing

Genomic DNA was extracted from 200 μL peripheral blood lymphocytes from the proband (IV-5) and his parents (III-8 and III-9) as well as the patient's grandmothers (II-4 and II-6) using Geneall kit. Proband genomic DNA was subjected to whole exome sequencing (WES). The exome library of the proband was prepared using the Agilent SureSelect V6-Post kit (Agilent Technologies, Inc., Santa Clara, California, United States), according to the manufacturer's protocol. Subsequently, the sample was sequenced via Illumina HiSeq4000 instrument. Sequence reads were then aligned to the reference human genome (hg19 from UCSC) using the Burrows-Wheeler Alignment Tool (BWA-0.7.12).

In Silico Analysis of Whole Exome Data

Polymerase chain reaction (PCR) duplicates were excluded using Picard (picard-tools-1.130). Variant calling was done by using the Genome Analysis Toolkit (GATKv3.4.0), and local realignment and quality recalibration were performed. Finally, annotation of variants was performed using wANNOVAR (<http://wannovar.wglab.org/>). Minor allele frequencies greater than 1% were considered as common and unlikely to be pathogenic variants and these variants were removed by using 1000 Genomes Project, Exome Aggregation Consortium, Genome Aggregation Database, and Exome Sequencing Project. Variant prioritization followed the use of multiple prediction algorithms such as SIFT, MutationTaster, PolyPhen-2, CADD, GERP ++, and ClinVar.

Sanger Sequencing Validation and Segregation Analysis

Validation and segregation testing of the *CLMP* (c.664C > T, p.R222X) variant in exon 5 of the gene was performed using PCR on the proband (IV-5) and his parents and grandmothers (II-4 and II-6) using standard cycling conditions with a forward (5'-TTGTGCAGACTACAACCACCC-3') and reverse (5'-GGTGATAGCCATATCCTTCTGG-3') primer. Primers were designed using Primer3 and PCR was done using Taq DNA

Polymerase Master Mix RED 2x (AMPLIQON Company, DK-5230 Odense M, Denmark). Then, amplicons proceeded Sanger sequencing and the data were aligned against human reference genome UCSC build GRCh37 (hg19) and analyzed using CodonCode Aligner software (v 6.0.2).

Results

We performed WES to find the genetic cause of the disease in the patient (IV-5). A mutation in *CLMP* was detected in the affected infant. Precisely, we identified a substitution of thymine to cytosine in the first nucleotide of codon 222 (c.664C > T) in exon 5 of the gene, which predicts the transition of arginine to a premature stop codon (p.R222X). According to MutationTaster, this frame-shift mutation causes a stop codon which is known as pathogenic and deleterious mutation. This variant has been confirmed by Sanger sequencing in the affected boy of the family. We also implemented cosegregation analysis to see whether this variant was inherited or not. The Sanger sequencing results showed that both parents (III-8 and III-9) are heterozygous for this variant. In addition, regarding consanguineous marriage, heterozygous patterns were observed in both grandmothers (II-4 and II-6) (► Fig. 3).

Discussion

In the current study, a homozygous mutation in *CLMP* gene, p.R222X, was identified in a 2-month-old infant from a Turkish family in Iran, who is suffering from specific symptoms of CSBS and this mutation has also been reported before in two CSBS patients from a Canadian family.¹⁵ The mutant allele was present in all unaffected family members in a Mendelian recessive manner. Furthermore, an in-silico analysis of the structural and functional impact of R222X in the *CLMP* gene revealed that it causes an in-frame nonsense mutation which created premature stop codon in mRNA copies. Arginine residue at position 222 in CLMP protein locates in the immunoglobulin (Ig)-like C2-type 2 domain is similar to the antibody constant domain. Changing of the arginine to a stop codon at this position disrupts the Ig-like domain. These domains might be involved in a variety of cellular processes, including cell-cell recognition, muscle structure, cell-surface receptors, and the immune system.¹⁷ It is suggested that this single specific ancestral mutation can disturb gene function and can be responsible for clinical symptoms revealed in the affected patient with congenital SBS.

According to reports of both, congenital and acquired SBS, this complex clinical condition has overlapping features with other disorders. In our case, at first, the patient was misdiagnosed with Noonan syndrome and Hirschsprung disease. Based on our knowledge, no study has been performed about the relation between Noonan syndrome and short intestine.¹⁸ On the other hand, Hirschsprung disease and SBS seem to have common characteristics. Lien et al, in 2012, described a case with Hirschsprung disease with SBS and malnutrition.¹⁹ SBS is a consequence of necrotizing

Fig. 3 Tracing ancestry mutation by Sanger sequencing in affected child (A), father (B), mother (C), paternal grandmother (D), and maternal grandmother (E), respectively.

enterocolitis and Hirschsprung disease.²⁰ Enterocolitis is also observed in patients with Hirschsprung disease.²¹ Necrotizing enterocolitis is caused by intestinal tissue damage. Typical symptoms include abdominal distention,

feeding intolerance, and bloody stool.²² Vomiting, abdominal distention, and enterocolitis are common features of these two phenomena.^{23,24} However, there is no study about direct relation of CSBS and Hirschsprung disease. They just share

some common characteristics which misled the physicians (► **Table 1**).

CSBS is a malabsorption syndrome caused by shortened small bowel length with approximately high morbidity and mortality.²⁴ The severity depends highly on the length and function of the remaining portion of the small intestine. It is also important to know, which part of the small bowel has been shortened in these patients, since different parts have different function and histology, as a result, the residual length determines the outcome of disease.^{25,26} Neonates with short bowel usually present electrolyte deficiency, nutrient deficiency, weight loss, and osmotic diarrhea. The main cause of a short bowel in newborns is not fully understood.¹

Hamilton et al described CSBS for the first time in 1969.⁷ The genes *CLMP* and *FLNA* were recently identified as causes of CSBS. *CLMP* encodes CXADR-like membrane protein that acts as an adhesion molecule and colocalizes with tight junction-associated proteins. *CLMP*, a 373 amino acids protein, is a member of cortical thymocyte marker in *Xenopus* (CTX) family and shares a similar overall structure as the other family members with an extracellular region, a transmembrane part, and a cytoplasmic tail. Several other homologues members of this protein have been identified in human and mouse orthologues to the CTX (CTH/CTM), the junctional adhesion molecule-1, 2, and 3, the A33 antigen, and the coxsackie and adenovirus receptor.²⁷ *CLMP* mRNA is expressed in several cells, generally in epithelial cells in different tissues.²⁸ *CLMP* expression has

also been confirmed in intestinal epithelial cells and hence, loss of function of this gene may cause proliferation defects in the small intestine.¹⁵

The role of *FLNA* in intestinal development is still unclear. CSBS patients with loss of function mutations in *FLNA* presented multiple congenital abnormalities in addition to a shortened small bowel, while patients with a mutation in *CLMP* have phenotypes mostly limited to the intestinal failure.²⁶ These genes were observed in the recessive and X-linked form of CSBS, respectively. In some cases, the small intestine in patients with *CLMP* mutations is longer and diagnosis is performed earlier in life in comparison to patients with *FLNA* mutations.^{26,29,30} Actin-binding domain of *FLNA* protein is located in the terminal region. A study showed that a deletion in its region could cause abnormal actin arrangement in the lymphoblastic cell line of a CSBS case.³¹ *CLMP* interacts with actin filaments with the assistance of interacting proteins such as ZO-1 which is involved in cell proliferation.^{32,33} Therefore, both gene products are involved in the same protein network. This interaction is crucial for intestinal development (► **Fig. 4A**).

The *CLMP* and *FLNA* knockout animal models can shed light on the underlying pathophysiology of CSBS. While knock-down of zebrafish ortholog (*CLMPa*) causes a considerable reduction in intestinal length and approved the importance of *CLMP* in intestinal embryogenesis, recalculation of length of intestine versus body length shows that there is no difference between knock-down zebrafish and controls.¹⁵ In addition, mutant mice are frequently used to

Table 1 Prevalence and featured symptoms of short bowel syndrome, Hirschsprung disease, and Noonan syndrome

Syndromes	Prevalence	Key features	References
Short bowel syndrome	1 in million	Shortened small intestine Diarrhea and steatorrhea Fluid depletion Necrotizing enterocolitis Abdominal pain Weight loss and malnutrition Malabsorption Fatigue Intestinal atresias	13
Hirschsprung disease	1:5000	Delayed of passage of meconium Abdominal distension Fecal retention Constipation Vomiting Neonatal enterocolitis.	37,38
Noonan syndrome	1:1000–1:2500	Craniofacial dysmorphism Pulmonary valvular stenosis Restrictive lung function Chest deformities Failure to thrive Intestinal malrotation Postnatal growth retardation Lymphatic dysplasias Developmental and cognitive delay Congenital hypotonia	39,40

Fig. 4 (A) Schematic picture of CXADR-like membrane protein (CLMP) showing a signal sequence and two Ig loops (the V and C₂ domains), an extracellular region of 233 amino acids (aa), a transmembrane portion (22 aa), and a cytoplasmic tail (118 aa). The site effect of CLMP mutations is also shown. Position of previously reported mutations and p.R222X mutation identified in this study is indicated in the figure. Co-localization of CLMP with the tight junction marker ZO-1 and actin is also shown. (B) Schematic representation of CLMP variants that have been detected to date. Previously reported variants and p.R222x mutation are shown in the figure.

examine the function of a protein. CLMP-deficient mice display a delay in body growth and inability to absorb nutrients and consequently show dehydration and starvation. However, the intestinal length is not reduced in mutant mice in comparison to wild-type mice as shown for the human *CLMP* gene.³⁴

Recently, Gonnaud et al described a CSBS patient with intestinal malrotation and short intestine with 35 cm length and also chronic intestinal pseudo-obstruction syndrome. They identified two previously unreported mutations in the *CLMP* gene. The patient had a compound heterozygous genotype for (c.28 + 1G > C) in intron 1 and (c.502C > T, p.R168X) in exon 4 inherited paternally and maternally, respectively.³⁵ In another study, Alves et al have identified novel *CLMP* variants in three female

patients. Two of them were siblings of a consanguineous family and displayed a homozygous nonsense variant (c.508C > T) in exon 4 that was inherited from their parents. The third one was found to be heterozygous for two variants c.410G > A and c.29-2A4G located in exons 4 and 2 (→Fig. 4B), respectively.³⁶

Conclusion

In sum, the patient presented here has different clinical manifestations related to CSBS and we report this for the first time in Iran. The genetic test, using WES, identified a homozygous nonsense mutation in the *CLMP* gene in this patient. Our further analysis exhibited the inherited pattern of the mutation in the family.

Conflict of Interest

None declared.

Ethical Approval

All procedures performed in the study involving human participants were in accordance with the ethical standards of the institutional and with the 1964 Helsinki declaration and its later amendments.

Informed Consent

Informed consent was obtained from parents of participant included in the study.

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