CLMP Is Required for Intestinal Development, and Loss-of-Function Mutations Cause Congenital Short-Bowel Syndrome

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BACKGROUND & AIMS: Short-bowel syndrome usually results from surgical resection of the small intestine for diseases such as intestinal atresias, volvulus, and necrotizing enterocolitis. Patients with congenital short-bowel syndrome (CSBS) are born with a substantial shortening of the small intestine, to a mean length of 50 cm, compared with a normal length at birth of 190–280 cm. They also are born with intestinal malrotation. Because CSBS occurs in many consanguineous families, it is considered to be an autosomal-recessive disorder. We aimed to identify and characterize the genetic factor causing CSBS. **METHODS:** We performed homozygosity mapping using 610,000 K single-nucleotide polymorphism arrays to analyze the genomes of 5 patients with CSBS. After identifying a gene causing the disease, we determined its expression pattern in human embryos. We also overexpressed forms of the gene product that were and were not associated with CSBS in Chinese Hamster Ovary and T84 cells and generated a zebrafish model of the disease. **RESULTS:** We identified loss-of-function mutations in *Coxsackie- and adenovirus receptor-like membrane protein* (*CLMP*) in CSBS patients. CLMP is a tight-junction–associated protein that is expressed in the intestine of human embryos throughout development. Mutations in CLMP prevented its normal localization to the cell membrane. Knock-down experiments in zebrafish resulted in general developmental defects, including shortening of the intestine and the absence of goblet cells. Because goblet cells are characteristic for the midintestine in zebrafish, which resembles the small intestine in human beings, the zebrafish model mimics CSBS. **CONCLUSIONS: Loss-of-function mutations in *CLMP* cause CSBS in human beings, likely by interfering with tight-junction formation, which disrupts intestinal development. Furthermore, we developed a zebrafish model of CSBS.**

Keywords: ASAM; Animal Model; Genetic Analysis; Embryology.

Patients with congenital short-bowel syndrome (CSBS) are born with a shortened small intestine. The mean length of the small intestine in CSBS patients is approximately 50 cm, compared with a normal length at birth of 190–280 cm.[1–3] Patients with CSBS may develop severe malnutrition as a result of the hugely reduced absorptive surface of the small intestine. This is similar to acquired short-bowel syndrome (SBS), which results from surgical resection of the small intestine for diseases such as intestinal atresias, volvulus, and necrotizing enterocolitis. CSBS usually is diagnosed by barium-contrast radiographs and confirmed by exploratory laparotomy. Infants with SBS, whether congenital or acquired, need parenteral nutrition to survive, although parenteral nutrition itself causes life-threatening complications such as sepsis and liver failure, and a high rate of mortality early in life. However, some long-term survivors of CSBS have been reported.[4–7] Because consanguinity frequently is seen in families in whom CSBS occurs, an autosomal-recessive pattern of inheritance is suspected. Until now, nothing was known about the genetic cause of this disease.

Here, we report the identification and characterization of the *Coxsackie- and adenovirus receptor-like membrane protein* (*CLMP*) as a gene underlying CSBS.

Abbreviations used in this paper: CHO, Chinese hamster ovary; CLMP, Coxackie and adenovirus receptor-like membrane protein; CSBS, congenital short-bowel syndrome; hpf, hours post fertilization; PCR, polymerase chain reaction; RT, reverse transcription; SBMO, splice-blocking morpholino; SBS, short-bowel syndrome; SNP, single-nucleotide polymorphism; TBMO, translation-blocking morpholino; WT, wild type; ZO-1, zonula occludens 1.

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Table 1. Clinical and Molecular Data From All Congenital Short-Bowel Syndrome Patients

Family	Patient	Ethnicity	Consanguinity	Sex	Length of small bowel at birth, *cm*	Additional features	Mutations
1	1-1 (ref 5)	German-American	Unknown	Female	30		c.230delA (p.E77Gfsx24), exon 3 Heterozygous frameshift c.821G>A, exon 6 Heterozygous splice site mutation
2	2-1	Italian	+	Male	Unknown		c.371T>A (pV124D), exon 3 Homozygous missense mutation
3	3-1 (ref 7)	Turkish	+	Male	47	Intestinal neuronal dysplasia	Homozygous deletion (with presumed inversion) in intron 1
	3-2	Turkish	+	Female	Unknown	Intestinal neuronal dysplasia	Homozygous deletion (with presumed inversion) in intron 1
4	4-1 (ref 4)	Dutch	Unknown	Female	54		Homozygous deletion of 12483 bp (including exon 2)
5	5-1 (ref 6)	Canadian	Unknown	Male	50		c.666C>T (p.R222X), exon 5 Homozygous nonsense mutation
	5-2	Canadian	Unknown	Female	Unknown		c.666C>T (p.R222X), exon 5 Homozygous nonsense mutation

Patients and Methods

Research Subjects

The CSBS patients included in this study, aged 0–26 years, were either described previously in the literature or were known to physicians in the field.[4–7] Patients were born with a shortened small intestine with a length of 30 to 54 cm (Table 1). Patients, of whom some were seen by an experienced clinical geneticist, did not show any other clinical features besides CSBS. All parents were reported as normal. Patients 2-1, 3-1, and 3-2 were from consanguineous families. All patients were Caucasians, except for patients 3-1 and 3-2, who were of Turkish ancestry. The study protocol was approved by the institutional and national ethics review committees at the University Medical Centre Groningen (NL31708.042.10), and written informed consent was obtained.

Homozygosity Mapping

Genomic DNA of all participants was extracted from peripheral lymphocytes by standard methods. A genome-wide scan was performed on 5 patients of families 1–4 using the Illumina 610,000 single-nucleotide polymorphism (SNP) array (Illumina, San Diego, CA) according to the manufacturer's instructions. Homozygosity mapping was performed by an automatic search for a minimum of 400 markers in a row (~2–3 MB) that were homozygous in at least 3 of the 4 families, and identical for patients 3-1 and 3-2 (because they were from the same consanguineous family).

Mutation Screening

Analysis of the 7 exons of *CLMP* (NM_024769.2) and the flanking intronic regions was performed in all patients and their parents as well as in 77 Caucasian control individuals (154 control chromosomes). For primer sequences see Supplementary Table 1. Sequencing was performed (forward and reverse) with dye-labeled primers (Big Dye Terminator v3.1 Sequencing Kit; Applied Biosystems, Foster City, CA) on an ABI 3730 automated sequencer (Applied Biosystems).

In Silico Analysis of the Missense Mutation

After analysis of the CLMP protein sequence (NP_079045.1) with the blastp algorithm (available: http://blast.ncbi.nlm.nih.gov/Blast.cgi), homologous sequences were obtained. The program M-Coffee was used to align them (available: http://www.tcoffee.org).[8]

The effect of the missense mutation was evaluated by the Russell method at European Molecular Biology Laboratory (available: http://www.russell.embl-heidelberg.de/aas/),[9] the polymorphism phenotyping algorithm (http://genetics.bwh.harvard.edu/pph/), and the Sort Intolerant From Tolerant algorithm (http://sift.jcvi.org/).

Functional Analysis of the Splice Site Mutation

To determine the effect of the splice site mutation found in patient 1-1, we performed an exon trapping assay. We first generated polymerase chain reaction (PCR) 2.1-TOPO plasmids (Invitrogen, Carlsbad, CA), containing the sequences of the exon of interest (wild type or mutant) and the flanking intronic sequences. The sequence of interest was PCR-amplified using either a control or the patient's genomic DNA as the DNA template. We used the primers GGCG-Ecor1, 5′-AAACCTG-CAAATACTCATTC-3′, and GACG-BamH1, 5′-AAGTGTTTGT-TGAGGATAAG-3′. The amplification was performed using Pushion High-fidelity DNA polymerase (Finnzymes, Helsinki, Finland). The PCR products were inserted into the PCR 2.1 Topo constructs and thereafter digested with BamH1 and EcoR. The inserts from control and mutant subsequently were cloned into the exon trapping vector pSPL3 (Invitrogen). The inserts were checked by direct sequencing.