Genetic screening of Congenital Short Bowel Syndrome patients confirms *CLMP* as the major gene involved in the recessive form of this disorder

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Abstract

Congenital Short Bowel Syndrome (CSBS) is an intestinal pediatric disorder, where patients are born with a dramatic shortened small intestine. Pathogenic variants in *CLMP* were recently identified to cause an autosomal recessive form of the disease. However, due to the rare nature of CSBS, only a small number of patients have been reported to date with variants in this gene. In this report, we describe novel inherited variants in *CLMP* in three CSBS patients derived from two unrelated families, confirming *CLMP* as the major gene involved in the development of the recessive form of CSBS.

Keywords

Congenital Short Bowel Syndrome; malrotation; CLMP; variant

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Introduction

During embryogenesis, the small intestine experiences major growth expanding several times the total length of the whole body. This is a complex process that starts around the fifth week of human development, when the midgut forms a simple linear tube that runs down the midline of the embryo, and is completed by the twentieth week of development1. Several pathways have been reported to play a role in intestinal elongation2, but to date the molecular mechanism(s) responsible for this process remains unclear.

In Congenital Short Bowel Syndrome (CSBS) intestinal elongation is impaired, and patients are born with a substantially shortened small intestine (approximately 50 cm in length instead of 250 cm in a normal counterpart)3. It is a rare disorder for which no cure is available. Total parenteral nutrition is required for long-term survival, but despite considerable efforts to improve treatment, most patients die of starvation or sepsis within the first few days of life4. CSBS was first described by Hamilton *et al.* in 1969, and it has always been considered to have a genetic cause3. However, it was only recently that two genes were identified as the cause of CSBS: *CLMP* and *FLNA*5,6. *CLMP* encodes for the Coxsackie-and adenovirus receptor-like membrane protein, an adhesion molecule that co-localizes with tight junction proteins, but whose function is still unknown7. *FLNA* encodes for Filamin-A, an actin binding protein known to regulate cell shape and to control cell signalling and migration8. Pathogenic variants present in *CLMP* and *FLNA* have been linked to the recessive and X-linked forms of CSBS, respectively.

Here, we report the genetic screening of three CSBS patients derived from two unrelated families, and describe the identification of three novel variants in *CLMP*.

Material and Methods

Patient information

In this study, three female patients diagnosed with CSBS were investigated (Table 1). Two of these patients were siblings of consanguineous descent (P1 and P2). The third patient (P3) has been previously described, when a *de novo* translocation was found between chromosome 2 and 11 (46,XX,t(2,11)(q32.2,p12))9. However, no gene was identified as the causative factor at that time. A complete description of the patients can be found in supplementary data.

Written informed consent was given by the two families reported, and ethical approval was obtained from the Erasmus Medical Center ethical committee (Medisch Ethische Toetsings commissie - METc 2009/364, ABR nr: NL31708.042.10).

Genetic analysis

Genomic DNA was isolated from peripheral blood lymphocytes using standard methods. Exons 1-7 of *CLMP* (ENST00000448775) were amplified using 30 ng of genomic DNA, as described before5. PCR products were purified (ExoSap it – GE Healthcare, Eindhoven, The Netherlands), and Sanger sequencing was performed with dye labelled primers (forward and reverse; Big Dye Terminator v3.1 Sequencing Kit, Applied Biosystems, Bleiswijk, The

Netherlands) on an ABI 3130XL genetic analyzer. Sanger reads were analysed using SeqScape software and compared to the reference *CLMP* genomic sequence (ENSG00000166250). Genetic data was submitted to ClinVAr (http://www.ncbi.nlm.nih.gov/clinvar/) and the following accession numbers were obtained: SCV000264793 [p.(R170*)] and SCV000264792 [p.(C137Y) and (c.29-2A>G)].

Expression vectors

Generation of the expression vectors used is described in supplementary data.

Immunofluorescence

Chinese Hamster cells (CHO-K1) were cultured as described before5. Five hundred thousand cells were seeded in a 6-well plate, and transiently transfected with pcDNA-HA-CLMP WT or pcDNA-HA-CLMP C137Y expressing vectors, using GeneJuice (Millipore, Amsterdam, The Netherlands) as transfection reagent. Twenty four hours after transfection, immunofluorescence was performed as previously described5. Images were taken in a Leica (AOBS) microscope, and analysed with the Leica LAS AF Lite software.

Results and Discussion

As the three patients included in this study are females, an X-linked pattern of inheritance involving *FLNA* was considered unlikely, and only *CLMP* was screened. In all patients we identified previously unreported variants in *CLMP*. None of these variants is listed in any of the available human genome variant databases. Patients P1 and P2 possess a homozygous nonsense variant in exon 4 [c.508C>T; p.(R170*)], leading to the appearance of an early stop codon (Fig.1A). This nonsense variant was inherited from the parents, who were found to be heterozygous. Patient P3 has two unphased heterozygous variants in *CLMP*. Although we suspect that both variants were inherited from the parents, we were unable to investigate segregation in the family due to unavailability of parental DNA. In this patient a missense variant located in exon 4 was identified leading to an amino acid substitution [c.410G>A; p.(C137Y)], together with a possible splice site variant located two base pairs upstream of exon two (c.29-2A>G) (Fig.1A). Prediction programs used to infer pathogenicity (PolyPhen-2, MutationTaster, SIFT, Human Splicing Finder and Alamut Visual) showed that both variants are likely to disturb protein function. The C137Y variant is located in a highly conserved region present in all mammals (Fig.1B), and *in vitro* assays showed that it affects the cellular localization of CLMP to the tight junctions (Fig.1C). The c.29-2A>G variant is predicted to disrupt normal splicing of exon 2 of *CLMP* (Fig.1D). Considering that both variants likely affect CLMP function, we believe that the *de novo* translocation previously identified in this patient9 is an independent event unrelated to CSBS.

Currently, only seven patients have been described carrying pathogenic variants in *CLMP*5. All the variants reported were loss of function, leading to the absence of CLMP, or to the expression of a mutant protein with a disrupted function (Fig.1E). In this report, we expand the mutational spectrum of *CLMP* variants involved in CSBS, consolidating the involvement of this gene in the development of the recessive form of this disease. We also show that it might be worthwhile to screen previously described patients for whom an X-linked pattern

of inheritance has been disregarded, for the presence of variants in *CLMP*, as this might shed some light on the genetic basis of the disease in these cases. To date, the role of *CLMP* in intestinal elongation is still largely unknown, making it difficult to determine the molecular mechanisms underlying the development of CSBS. We believe that the results here described reinforce the need to further study the role of *CLMP* in intestinal elongation, as this might contribute for the development of new therapeutic strategies for CSBS patients.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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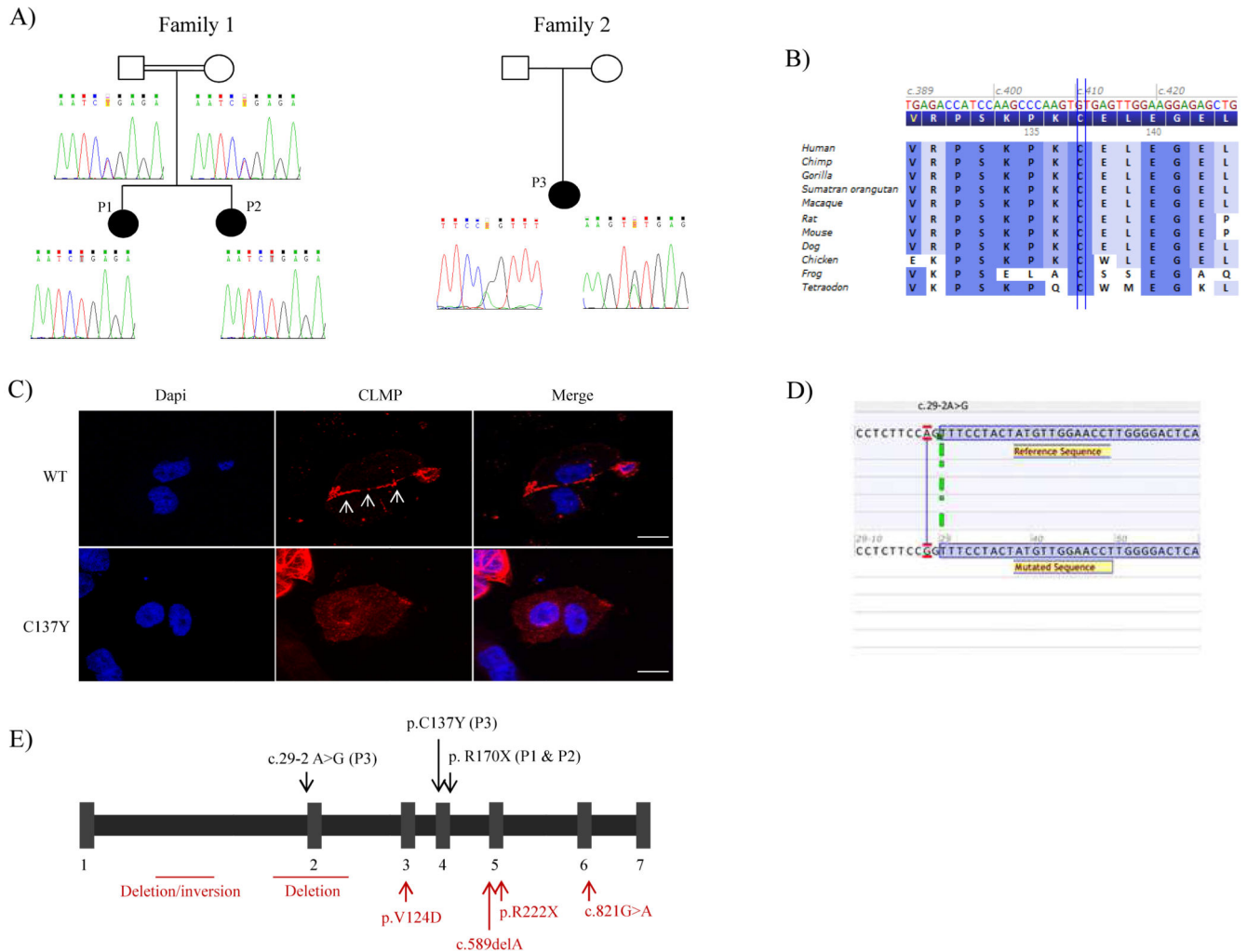
Fig.1.

Genetic analysis of three CSBS patients. **A)** Pedigree of the two families included in this study with Sanger sequencing results showing the presence of variants in *CLMP*. **B)** Conservation alignments showed that the missense variant p.(C137Y) leads to a change of a cysteine residue highly conserved among vertebrates. **C)** CHO-K1 cells transfected with constructs expressing wild-type (WT) CLMP or the p.(C137Y) variant, show different cellular distribution of CLMP. While the WT protein normally localizes to the tight junctions (see arrows), the mutated protein has a punctuated distribution through the cytoplasm. Scale bars: 30μm. **D)** Splicing predictions suggested that the intronic variant identified in patient 3 likely affects normal splicing, as it is predicted to disrupt an acceptor-splicing site (c.29-2A>G). **E)** Schematic overview of all the variants identified to date in *CLMP*. In black are the variants reported in this report, while in red are the variants previously described. Figures 1B and 1D were generated using Alamut prediction software.

Table 1

Clinical and molecular features of the CSBS patients included in this study.

	P1	P2	P3
Gender	female	female	female
Age of diagnostic	3 months	38 days	1 week
Ethnicity	Iranian	Iranian	Caucasian
Consanguinity	+	+	No
Malrotation	+	+	+
Length of the small intestine (cm)	76	26	50
Additional features	Intestinal dysmotility UPJO[*]	Intestinal dysmotility UPJO[*]	Very mild mental retardation
CLMP variants	c.508C>T; p.(R170[*])	c.508C>T; (p.R170[*])	c.410G>A; p.(C137Y) c.29-2A>G
Zygosity	Homozygous	Homozygous	Heterozygous (two unphased variants)

[*] UPJO: Ureteropelvic junction obstruction