Biallelic variants of the first Kunitz domain of SPINT2 cause none syndromic form of congenital diarrhea and tufting enteropathy

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Short Report

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Abstract

Biallelic \textit{SPINT2} pathogenic variants cause a syndromic form of congenital diarrhea and enteropathy (OMIM 270420). To date, all 34 patients reported presented with additional extra-intestinal syndromic features. We report on a 5-year-old girl who presented early in life with congenital diarrhea and was found to have a novel homozygous variant in \textit{SPINT2}. She presented with congenital diarrhea, pathological studies confirmed tufting enteropathy, and at the age of 5 years, she did not develop any extra-intestinal syndromic features. The variant (NM\_021102.4: c.203A > G (p. [Tyr68Cys])) detected in homozygosity status is the first missense mutation reported in the 1st Kunitz domain (KD1) of \textit{SPINT2} in humans. Previously in-vitro functional studies of this variant confirmed the deleterious effect leading to complete loss of inhibitory activity of the intestinal serine proteases. Furthermore, this is the first description of \textit{SPINT2}-related diarrhea in a patient who lived without the need for long-term total parenteral nutrition. This study expands the clinical and molecular characteristics of SPINT-related conditions.

INTRODUCTION

Infantile diarrhea and malnutrition are typical occurrences that can be attributed to several acquired or maternally derived factors, including congenital infections and dietary protein intolerance. Congenital diarrheas and enteropathies (CDEs), a rare cause of severe, life-threatening diarrhea, are monogenic intestinal epithelial disorders. They usually present within weeks of life and are associated with feeding intolerance and malabsorption [1]. Infants with congenital diarrheas usually require parenteral nutrition to maintain proper growth, electrolyte, and nutrient requirements [1]. Although many of these disorders are linked to altered immune system function, disruption of intestinal epithelial structure and function is a characteristic that unites all these diseases [2]. The etiology of CDEs includes the following five categories: intestinal epithelial electrolytes transport defect, dysfunction of epithelial enzymes and metabolisms, disorders of epithelial trafficking and polarity, defects of enteroendocrine cells, and anomalies in the regulation of the intestinal immune response [1, 3].

The \textit{SPINT2} gene, also known as \textit{HAI2} (hepatocyte growth factor activator inhibitor-2), encodes a protein that contains two Kunitz domains [Kunitz Domain 1 (KD1) and Kunitz Domain 2 (KD2)] and is expressed in most epithelial cells. It acts as an inhibitor of several proteases, including matriptase-1, a serine protease. Biallelic mutations of \textit{SPINT2} as a cause of a syndromic form of congenital sodium diarrhea (SCSD) (OMIM: 270420) was first described in 10 families [4]. Extra-intestinal syndromic features included choanal or anal atresia, hypertelorism, corneal erosions, double kidney, cleft palate, digital anomalies and anal atresia. Recently Holt-Danborg and colleagues reviewed all 34 patients reported to date. It is obvious that all had syndromic form, and they all needed total parental nutrition (TPN), but one patient managed to be weaned off at the age of 12 years. Of the 13 different \textit{SPINT2} variants reported, four are missense variants and are localized to the second Kunitz domain (KD2) of \textit{SPINT2}. No patient with biallelic stop mutations was identified, suggesting that at least one \textit{SPINT2} allele encoding a protein with residual function is necessary for survival [5].
We report on a 5-year-old girl with novel biallelic variants of SPINT2 (NM_021102.4: c.203A > G (p. [Tyr68Cys]) which was previously confirmed to cause a loss-of-function effect [6]. This is the first missense mutation in 1st Kunitz domain (KD1) of SPINT2 to be identified. Our patient presented with non-syndromic SPINT2 related congenital diarrhea and enteropathy. She required interpreted short term TPN initially but never been in long term or home TPN, yet she survived.

CASE PRESENTATION

A term female neonate born to apparently healthy parents related as first cousins. Her birth weight was 3.6 kg (50th centile), length was 54 cm (75th centile) and head circumference was 37 cm (75th centile). There was no antenatal history of polyhydramnios. The patient has two healthy older siblings and there was no family history of chronic diarrhea or malabsorption syndromes. At the age of 3 months, she started to have frequent episodes of non-bloody, non-mucoid diarrhea up to seven time per day. It was associated with vomiting initially but no fever. She was managed at the local hospital as acute gastroenteritis with intravenous fluids. However, as the symptoms persist, she started to lose weight and her weight centile dropped to -2 SD. At the age of 8 months, she was referred to a tertiary hospital for further investigations. At the time, examination revealed a small for age child with no dysmorphic features. She had parched buccal mucosa, depressed anterior fontanel and sunken eyes. Her weight was 5.8 kg (-3 SD) and height 66 cm (normal for her age). Abdominal examination revealed distended but soft abdomen with no organomegaly. Eye examination was normal.

Blood investigation showed normal full blood counts, electrolytes showed low sodium; 128 mmol/L (135–145), normal potassium; 4 mmol/L (3.5–5), and chloride 100 mmol/L (98–107). Her urea and creatinine were within normal range. Her albumin level was low at 26 g/L (35–50), elevated alkaline phosphatase at 571 U/L (90–270), and normal bilirubin and transaminase levels. She was investigated for all possible causes of chronic diarrhea in infancy, and her work revealed a normal sweat test, normal celiac serology, normal serum immunoglobulin levels, and normal lymphocytes subset test. Her stool tests were negative for any pathogens. The trial of elemental formula for possible cow’s milk protein allergy was unsuccessful. She underwent a gastroscopy and colonoscopy. Histological examination of the duodenal biopsy reveals villous blunting with associated disorganization of the surface epithelium without an increase in the intraepithelial lymphocytes. The surface reveals pseudo-stratification and multifocal tufted enterocytes (Fig. 1A). Immunohistochemistry with Ber-Ep4 shows positive staining (Fig. 1B). The histological features are consistent with diagnosing Tufting enteropathy with positive Ber-Ep4. Hence, the patient was put on parenteral nutrition, which resulted in weight gain. However, her parents declined the home TPN. So, she was sent home on oral feed (extensive hydrolyzed formula), multivitamins supplements, and oral bicarbonate. Whole exome sequencing revealed a homozygous mutation in the SPINT2 gene (NM_021102.4): c.203A > G (p.[Tyr68Cys]). Both parents were heterozygous for the same mutation. This variant is absent in the public genomic database and in-house exome database. In-silico pathogenicity score predicated damaging effect. Previously, Faller and colleagues functionally confirmed the deleterious effect of the Y68C mutation in the 1st Kunitz domain (KD1) of SPINT2 and showed to cause a complete loss of the inhibitory activity of the intestinal serine proteases [6].
Currently, the patient is 5-year-old, and she still has semi-formed stools up to four times per day with intermittent diarrhea that requires hospitalization. She continued to have bloated abdomen. Her weight centile remains at the 10th centile, but her height is below the 3rd centile with normal developmental milestones. She underwent a complete medical checkup, and no other syndromic features have been identified so far.

**DISCUSSION**

*SPINT2* mutations can lead to CSD-predominant features or Congenital Tufting Enteropathy (CTE) features. The genotype–phenotype correlation in *SPINT2*-associated disease is not well understood, and further studies are necessary to determine the molecular etiology of CTE, CSD, or mixed CTE–CSD phenotype [2]. CTE is characterized by chronic watery diarrhea that occurs within weeks to months after birth. It can be due to mutations in the epithelial cell adhesion molecule (*EPCAM*) or *SPINT2* genes [7]. It has a heterogeneous clinical manifestation [1, 3]. Generally, it is characterized by watery diarrhea (> 50 ml/kg/day), eventually leading to malabsorption of macro and micronutrients. Eventually, it will lead to failure to thrive and impaired growth [3, 7]. Some patients might develop vomiting and abdominal distention [7], as what has happened to our patient. In syndromic CTE, the patients have extra-intestinal symptoms, including ophthalmologic signs (photophobia, cataracts, corneal erosions, and superficial punctuated keratitis), choanal atresia or anal atresia, dermatological anomalies, bone malformations, cholestatic liver disease, chronic arthritis and skeletal dysplasia [3]. Our patient has no extra-intestinal manifestations.

CTE is generally diagnosed by esophagogastroduodenoscopy, noting duodenal histological abnormalities [7]. The most pathognomonic feature is the presence of disorganized surface epithelial cells with crowding and formations of epithelial tufts without an increase in intraepithelial lymphocytes. The latter feature excludes other enteropathy, which sometime can show focal surface epithelial changes like celiac disease, infection, and cow milk allergy. Immunohistochemistry plays an essential role in the diagnosis as loss of Anti-EPCAM immunohistochemistry (MOC-31, Ber-Ep4) along with the morphological features show a sensitivity and a specificity of 100% [8]. However, these stains are usually normal in patients with *SPINT2* mutations [9]. The diagnosis is generally confirmed by identifying mutations in *EPCAM* or *SPINT2* genes [7]. Genetic testing can occur in parallel or early in the diagnostic algorithm, especially if there are apparent factors in the clinical assessment pointing towards monogenic diarrheal disease, such as consanguinity and positive family history [1].

Management of *SPINT2* deficiency is dependent on the predominant phenotype. Main management is via parenteral nutrition and, in severe cases, intestinal transplant [2, 10]. In reviewing the literature, total autonomy was never achieved in CTE or CSD before the age of 11 [5, 10]. Current guidelines recommend avoiding early intestinal transplantation and preserving as much enteral nutrition as possible. Although previous publications in CTE suggest that possibility of parenteral nutrition weaning occurs only after several years [10], our patient was able to survive without TPN support which points to the heterogeneity of the disease.
In conclusion, our study expands the clinical and molecular phenotype of SPINT2-related conditions and emphasizes the presentation of a none syndromic form and achieving autonomy without the need long term or home TPN.

**Declarations**

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Conflicts of interests: None

**Authors’ contribution:**

YAR and AAM designed the study and wrote the manuscript and supervised the project

MAM analysis of the pathology studies

OAS, AAJ, DRA, clinical care, reviewed the manuscript and critically reviewed the data

All authors reviewed the manuscript and approve it

**References**


**Figures**

Figure 1

A: high power view showing focal pseudo-stratification (Red arrow) and tufted epithelium (black arrow).

Figure 1B: high power view showing positive Ber-Ep4 immunohistochemistry.