Non-syndromic Hirschsprung's disease as a result of a RET gene variant

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ABSTRACT

Introduction. Hirschsprung's disease (HD) is characterized by the absence of ganglion cells in the submucosal and myenteric plexuses of the colon as a result of disorders in the migration and differentiation of enteric neural crest cells during embryogenesis. It is a cross-factor condition, with more than 11 genes identified in its pathogenesis, including the RET proto-onco gene.

Case report. We present the case of two siblings with total colon HD where a potentially pathogenic variant of the RET gene was found. Their father also had this condition.

Discussion. Prenatal diagnosis through genetic testing allows for informed decisions and care planning for the newborn, thus reducing delayed diagnosis and treatment, and minimizing long-term complications. Mutations such as the RET gene variant highlight the importance of the genetic approach in understanding and managing HD.

KEY WORDS: Hirschsprung's disease; Genetics; Proto-onco gene proteins c-ret.

ENFERMEDAD DE HIRSCHSPRUNG NO SINDRÓMICA POR VARIANTE EN EL GEN *RET*

RESUMEN

Introducción. La enfermedad de Hirschsprung (EH) se caracteriza por la ausencia de células ganglionares en los plexos submucoso y mientérico del intestino grueso, resultante de deficiencias en la migración y diferenciación de las células de la cresta neural entérica durante la embriogénesis. Es una condición multifactorial, con más de 11 genes identificados en su patogénesis, incluyendo el protooncogén *RET*.

Caso clínico. Se presenta el caso de dos hermanos con EH de colon total, cuyo padre también padeció la enfermedad, y en quien se encontró una variante potencialmente patogénica en el gen *RET*.

Comentarios. El diagnóstico prenatal mediante pruebas genéticas permite decisiones informadas y la planificación de cuidados para el neonato afectado, reduciendo demoras en el diagnóstico y tratamiento, y minimizando las complicaciones a largo plazo. La identificación de mutaciones como la variante en el gen *RET* destaca la importancia del enfoque genético en la comprensión y manejo de la EH.

PALABRAS CLAVE: Enfermedad de Hirschsprung; Genética; Proto oncogén *RET*.

INTRODUCTION

Hirschsprung's disease (HD) is a complex development disorder characterized by the absence of intrinsic parasympathetic ganglion cells in the submucosal and myenteric plexuses of the colon, with distal-to-proximal involvement.

A series of genes, such as the RET proto-onco gene, have been identified as important factors in the pathogenesis of this condition.

We present the case of two siblings diagnosed with total colon HD where a variant of the RET gene was found. Their father also had this condition.

CASE REPORT

The first case is a 34-month-old full-term patient who was not able to evacuate meconium and had abdominal distension and bile vomit at 72 hours of life. His father had been diagnosed with total colon HD during childhood, which led to Lester Martin surgery when he was 2 years old.

In this context, a barium enema was carried out. It revealed a reduced caliber colon along its whole length, without a clear transition area, associated with absence of haustra and dilatation of small bowel loops (Fig. 1). Rectal biopsy demonstrated the absence of ganglion cells. At 12 days of life, intestinal mapping confirmed total colonic

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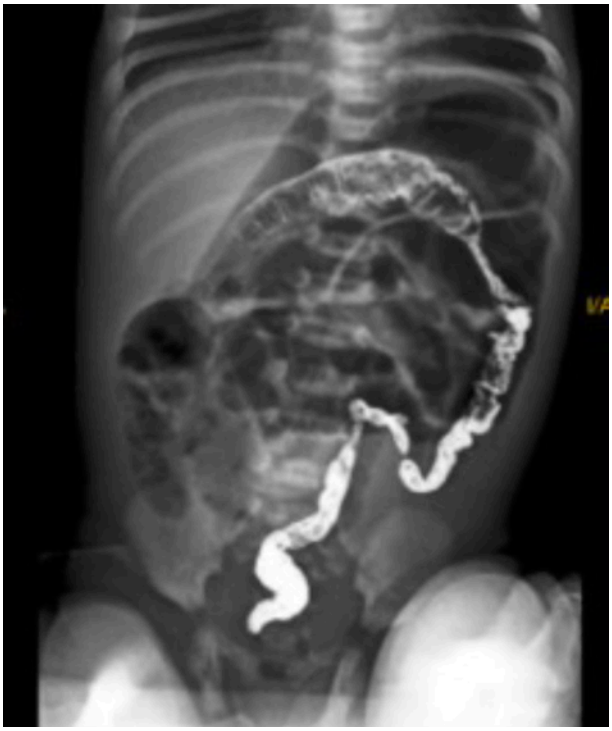

Figure 1. Reduced caliber colon, without a clear transition area, without haustra, and with dilated small bowel loops.

aganglionosis with ileal compromise and presence of ganglion cells at the small bowel, so decision was made to perform ileostomy 40 cm away from the ileocecal valve. Subsequently, he had two episodes of intestinal subocclusion, which led to new intestinal biopsies that showed ganglion cells at the jejunal level. Consequently, a jejunostomy was conducted 100 cm proximally towards the ileostomy. Progression was favorable, so laparoscopic total colectomy was performed 8 months later, with abdominoperineal descent and ileoanal anastomosis of the proximal ileal segment, as well as presence of ganglion cells (Georgeson surgery).

He was assessed by the Genetics department, which conducted genealogy and physical exploration. Given the family history and hypothesizing non-syndromic HD, a panel of genes associated with such condition was requested. The study revealed an autosomal dominant inheritance heterozygous variant of the RET gene classified as probably pathogenic (c.1523-1G>C). In light of this finding, his parents were offered genetic family counselling. Given that it was an autosomal dominant inheritance genic entity, the risk of recurrence at each future gestation was established at 50%, and various reproductive options were provided.

Subsequently, the couple had a new unplanned gestation. Considering the risk of recurrence, they were given the option to search for the family variant prenatally, which turned out to be present in that gestation. In light of this, the newborn was admitted at the Neonatal Intensive Care Unit to monitor potential clinical manifestations of HD. At 24 hours of life, she had abdominal distension and gastric vomit, and she had not been able to evacuate meconium since birth. Therefore, a barium enema was carried out at the operating room on her second day of life (Fig. 2). It revealed a colon without caliber changes, a total loss of haustra, and a dilated ileum without a clearly visible transition area. In light of these findings, rectal biopsy and laparotomy with intestinal mapping were performed. The primary preliminary intraoperative conclusions were the absence of ganglion cells at the sigmoid colon and the cecum, and the presence of ganglion cells at the ileum 10 cm away from the ileocecal valve, which led to ileostomy at that level. Postoperative progression was favorable, with adequate tolerance to enteral feeding and intestinal transit through ileostomy. Therefore, the patient was discharged 14 days later without complications.

At 4 months of age, she underwent surgery for ileostomy closure, as well as laparoscopic total colectomy with transrectal ileoanal descent, while preserving the seromuscular sleeve. Progression was favorable, without postoperative complications, so she was discharged 6 days later. The patient is now 12 months old, tolerates enteral feeding, has a good weight progression, and daily frequency of depositions is progressively decreasing.

DISCUSSION

HD is a complex condition characterized by the absence of intrinsic parasympathetic ganglion cells in the submucosal and myenteric plexuses of the posterior colon[1,2]. It is believed to be caused by the fact enteric neural crest cells are unable to migrate, proliferate, or differentiate at the intestinal wall during embryogenesis, thus leading to aganglionosis in the lower gastrointestinal tract[3].

Incidence is approximately 1 in 5,000 live newborns, and it is more common in boys (4:1.35)[4].

Clinically speaking, it is classified as short segment HD (S-HD) when the aganglionic segment involves the rectum and sigmoid colon (80% of the cases), and as long segment HD (L-HD) when involvement extends proximally to the sigmoid colon (15% of the cases). L-HD can be categorized as total colonic aganglionosis (TCA), or as total colonic aganglionosis with small bowel aganglionosis (TCSA)[5].

The genetic factors predisposing to HD are heterogeneous and have complex interactions that determine penetrance and severity, such as aganglionic segment length and obstructive symptom severity[6]. Variants have been identified in more than 11 different genes involved in HD pathogenesis, such as RET, EDNRB, EDN3, and TTF-1.

The RET proto-onco gene, located at the long arm of chromosome 10, codifies one of the receptors of the tyrosine kinase protein –a cell surface molecule that translates

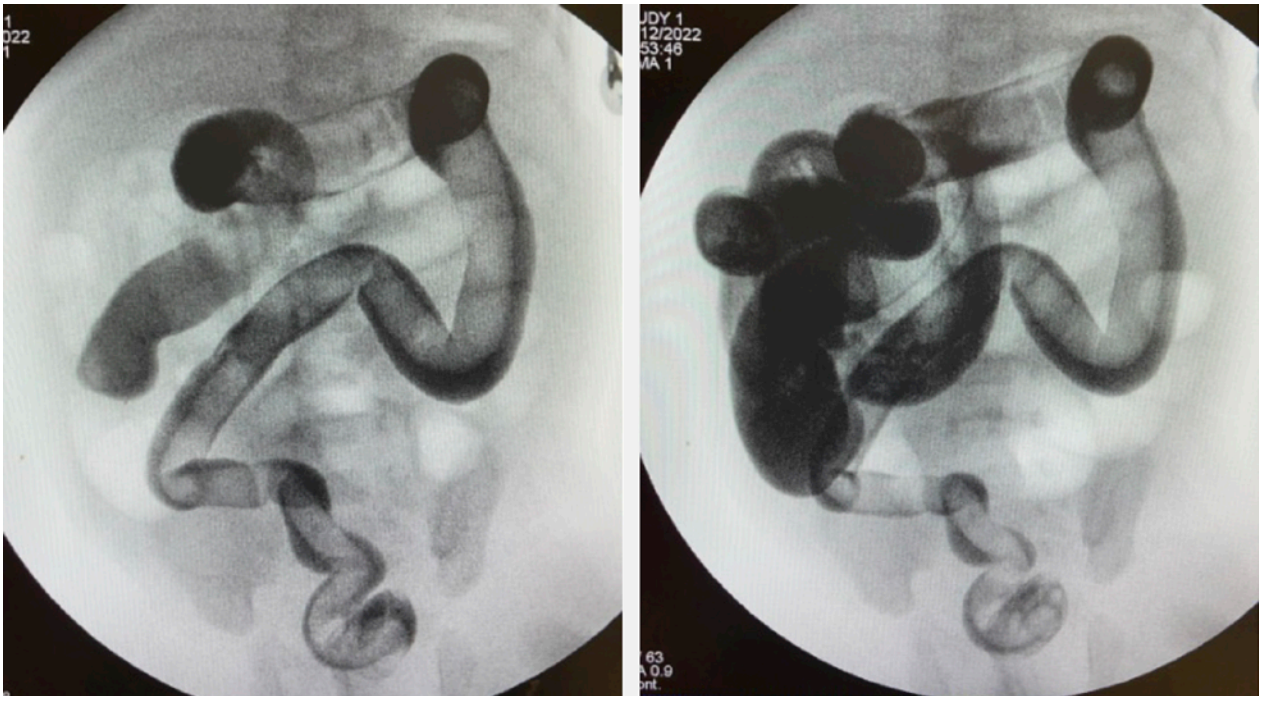

Figure 2. Colon without evidence of caliber changes, with loss of haustra, adequate passage to the small bowel, and visualization of the dilated ileum without a clearly visible transition area.

cell growth and differentiation signs– and plays a critical role in the normal proliferation of the enteric nervous system. RET mutations represent up to 35% of sporadic HD cases and 50% of familial cases[7].

In our case, the gene panel requested to the father showed that the c.1523-1G>C replacement was in heterozygosis in intron 7, of a total of 19, of the NM_020975.6 transcript of the RET gene. Given the position of this variant in the splicing accepting site, it represents an alteration of messenger RNA processing, and as such, a modification/absence of the protein structure. This variant could lead to loss of the exon, shortening/inclusion of the intronic sequence, or total absence of gene product due to lack of transcription or degradation of the altered transcript. However, up until now, there are no scientific publications specifically assessing the impact of this variant on the structure and functionality of the protein. In addition, null RET gene variants are considered pathogenic. Specifically, mutations in the critical splicing sequences have been reported in HD patients. Therefore, this c.1523-1G>C change in the RET gene could be classified as probably pathogenic.

Other genetic variants associated with HD have been identified in the EDNRB, GDNF, NRTN, and SOX10 genes, which play a role in the development of the enteric nervous system[8,9].

Regarding TCA, as it was the case in our patients, incidence has been reported to be higher in family series, which suggests a likely genetic link[10]. In addition, a high degree of heritability and genetic penetrance, as well as progression in the length of the segment involved among generations, have been observed[11]. Recessive and polygenic patterns have also been described, particularly in L-HD cases. Generally speaking, L-HD and TCA seemingly have an autosomal dominant inheritance pattern, with incomplete penetrance (mainly RET), whereas S-HD appears to be transmitted in an autosomal recessive fashion[12]. Segregation studies have shown that the risk of recurrence in siblings ranges from 1.5% to 33% according to sex, aganglionic segment length, and sex of the sibling involved[13].

Early HD diagnosis by means of prenatal genetic testing allows for informed decisions to be made, and for a full care plan to be designed. The molecular characterization of this pathology provides a solid basis for an individualized and precise therapeutic approach, which contributes to prevent delayed diagnosis and treatment, and to reduce the impact of long-term medical complications.

As for the second patient, the fact prenatal diagnosis –which identified the RET gene variant– was available allowed for a highly specialized management since birth. This early approach led to adequate diagnosis and optimal treatment of HD, which helped improve clinical results and patient quality of life.

The availability of prenatal genetic testing in familial HD cases has demonstrated to be an invaluable tool for early detection, thus allowing for the implementation of

adequate and customized medical treatments. This not only reduces short- and long-term medical complications, but also improves patient quality of life and provides families with the support required throughout the whole process.

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