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Spectrum of Congenital Myenteric Hypoganglionosis and Aganglionosis in Patients with Waardenburg Syndrome Type 4

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Abstract

Introduction: Waardenburg Syndrome (WS) is a rare, genetically and phenotypically heterogeneous disorder caused by abnormal migration or differentiation of neural crest cells during embryonic development. WS is classified into four types: WS Type 1 (WS1), WS2, WS3, and WS4. WS4 is characterized by the association of intestinal aganglionosis, pigmentation abnormalities and sensorineural hearing loss. Variability in the severity of the phenotypes has been reported among patients and different animal models. WS4 is typically characterized by aganglionosis, however hypoganglionosis can clinically mimic aganglionosis in Hirschsprung disease (HSCR), most cases of which are limited to the distal colon and treated by surgical resection, with a pullthrough operation. The most severe form, total colonic aganglionosis, is treated with total colectomy. Congenital myenteric hypoganglionosis (CMH) typically involves both small and large bowel and cannot be cured surgically. Thus accurate histopathologic diagnosis is key to distinguishing hypoganglionosis from aganglionosis seen in HSCR in patients with WS4. **Aim**: We describe clinical features and intestinal histology in three patients with WS4. **Conclusion:** Waardenburg syndrome type 4 is a rare genetic disease that presents with a more severe phenotype than types 1-3. Diagnosis is often suspected after a water-soluble contrast enema followed by a rectal suction biopsy. It is ultimately confirmed by a thorough physical examination that is often aided by family history and genetic testing. It remains critical to distinguish these two conditions (aganglionosis and hypoganglionosis) in patients with WS4 in order to avoid unnecessary resections and it seems that enteral autonomy maybe possible to occur over time in those with hypoganglionosis.

Keywords: Waardenburg; Hypoganglionosis; Aganglionosis; Hirschsprung

Introduction

Waardenburg Syndrome (WS) is a rare, heterogeneous genetic disorder caused by abnormal migration or differentiation of neural crest cells during embryonic development [1,2]. Based on its clinical features, WS is classified into four types: WS Type 1 (WS1), WS2, WS3, and WS4. To date, six genes have been shown to be associated with WS including *PAX3, SOX10, MITF, SLUG, EDN3* and *EDNRB* [1-3]. WS4 is characterized by the association of intestinal aganglionosis, pigmentation abnormalities and sensorineural hearing loss [4]. The requirement for SOX10 and endothelin-3/EDNRB signaling pathway during enteric nervous system (ENS) and melanocyte development, as well as their alterations in Waardenburg–Hirschsprung disease

(hypopigmentation, deafness, and absence of enteric ganglia) are well established [5]. WS Type 4 is composed of three etiological subtypes: WS4-A, WS4-B and WS4-C, caused by mutations in the *SOX10, EDNRB* and *EDN3* genes respectively [6,7]. Variability in the severity of the phenotypes has been reported among patients and different animal models [6].

WS4 is typically characterized by the aganglionosis, however hypoganglionosis can clinically mimic Hirschsprung disease (HSCR) [8]. Certain patients with WS4 present with hypoganglionosis or chronic intestinal pseudo-obstruction (CIPO) instead of HSCR [9]. The phenotypic variability is likely multifactorial, for example SOX10 expression is regulated by numerous enhancers leading to the hypothesis that variations affecting certain identified regulatory sequences could be the cause of WS or isolated HSCR [9]. Congenital myenteric hypoganglionosis (CMH) is an uncommon birth defect associated with severe and typically unremitting intestinal pseudo-obstruction. In contrast with HSCR, myenteric and submucosal ganglion cells are present along the entire length of the gastrointestinal tract, but the number of myenteric ganglion cells is markedly diminished due to a combination of a true paucity of neurons and poorly differentiated neurocytologic features [10]. CMH is a rare condition, and even more rarely has been seen with WS4, which typically presents shortly after birth with clinical findings similar to Hirschsprung disease. Unlike the aganglionosis in Hirschsprung disease, which usually is limited to the distal large intestine and is amenable to resection and pull through surgery, CMH typically involves small and large bowel and cannot be cured surgically [10]. Thus accurate histopathologic diagnosis is key to distinguishing hypoganglionosis from CMH or HSCR and true aganglionosis seen in HSCR in patients with WS4.

Results

Patient A

Patient A is a Caucasian male infant born via C-section at 40 weeks gestation due to prolonged labor. He required a 3-week NICU stay due to lack of spontaneous bowel movements. Symptoms improved for 2-3 weeks with the help of rectal dilations.

Prompted by ocular spasmus nutans and visible cranial nerve abnormalities, a brain and spinal MRI revealed, at 2 months of age, malformations of the right inner ear structures, a conjoined cranial nerve VII and VIII, and a tethered cord. ENT and audiology later confirmed hearing loss. Genome sequencing confirmed the diagnosis of Waardenburg Syndrome type 4 due to a pathologic SOX10 mutation (c.829delG,p.D277TfsX9).

Shortly after discharge to home at 3 weeks of age, signs of intestinal obstruction recurred, which responded to daily rectal irrigations. This helped him tolerate enteral feeds for short periods of time, but he often needed periods of gut rest and increased frequency of irrigations, with eventual poor oral intake. He required re-admission to the hospital at 2.5 months of age for failure to thrive, as he had fallen from the 39th percentile for weight at birth to the 0.18th%. Recurrent periods of abdominal distention and vomiting were concerning for chronic intestinal pseudo-obstruction (CIPO), necessitating care by a colorectal surgery team. He underwent sigmoid resection with Hartmann pouch and end-colostomy creation before he was 3 months old. Surgical pathology from the resected specimen showed hypoganglionosis and ganglion cell immaturity, with no hypertrophic nerves seen and absent calretinin staining. However, symptoms persisted, and he failed to pass spontaneous stools from the colostomy for an additional 2 weeks. He underwent repeat colostomy creation and during 4 weeks of inpatient stay, he was started on TPN. On a combination of TPN and enteral feeds, he reached the 10th% on the growth curve within 2 months and has been consistently tracking around the 50th% since he was 8 months of age. He was also started on a variety of medications (including erythromycin and senna) to help with feeding tolerance and passage of stools. He eventually achieved enteral autonomy by 10 months of age.

Multiple imaging studies and endoscopic evaluations revealed normal colon anatomy on a water-soluble contrast enema at 4 months of age and abnormal manometric testing (including antro-duodenal (AD) and colonic manometry) at 9 months of age AD manometry demonstrated post prandial antral hypomotility but high-pressure antral contractions (over 350mmHg) after erythromycin was administered. Small bowel manometry showed normal MMC after octreotide administration. Colonic manometry did not show any high amplitude propagating contractions (HAPC) and simultaneous contractions throughout the colon length were noted after stimulation with 5mg bisacodyl. Pathology from colonic biopsies taken at 4 months showed diffuse hypoganglionosis and ganglion cell immaturity (Figure 1). Biopsies obtained at 9 months of age during endoscopic placement of a manometry catheter showed cluster areas of normal ganglion in the rectum (Figure 1). Additionally, throughout the colon and distal ileum there were segments of fully ganglionated bowel, hypo ganglionated bowel, and immature ganglion cells present) as well as absence of hypertrophic nerves, normal calretinin staining pattern, and normal choline transporter staining pattern.

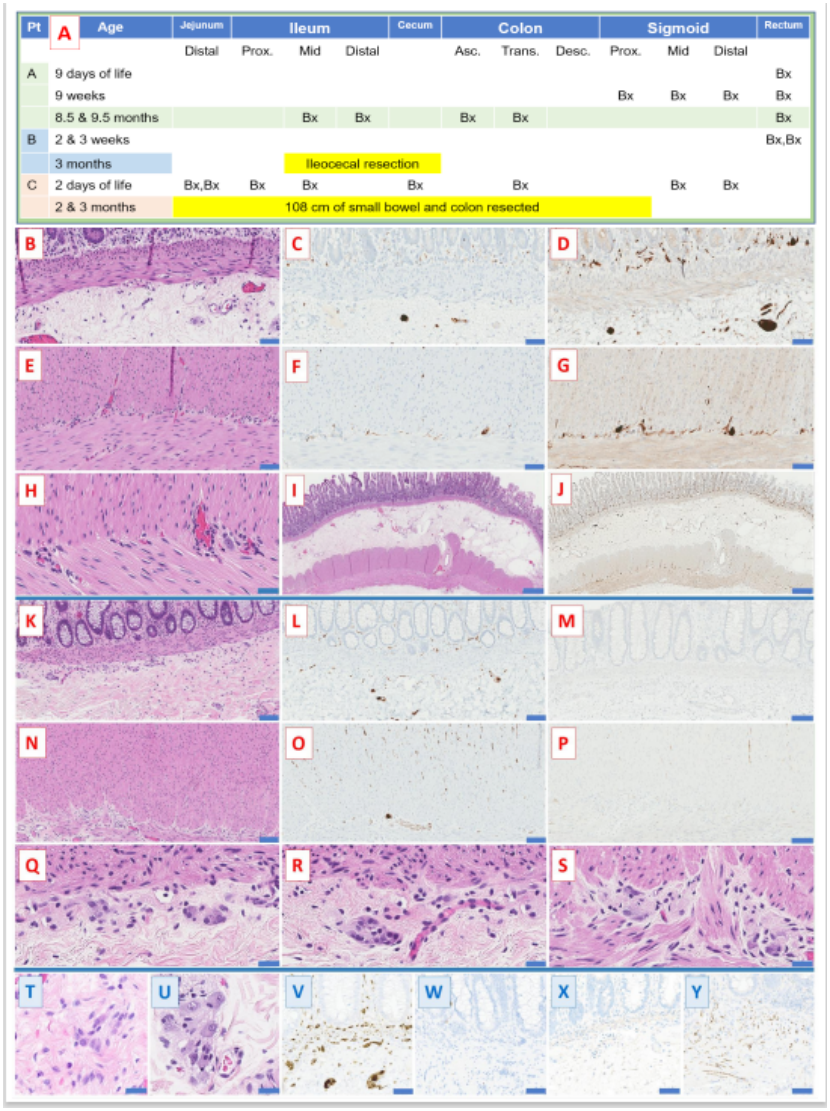

Figure 1 : Timeline (A) of histologic evaluation on biopsies (Bx) and resections, for patients' (Pt) age and location. (Prox.: proximal; Asc.: Ascending; Trans.: Transverse; Desc.: Descending.); Histology of congenital myenteric hypoganglionosis (CMH) in the small intestine (B-J) and large intestine (K-S), with close-up images of the submucosal plexi (B-D; K-L; Q-R) and myenteric plexi (E-H; N-P and S), demonstrating proportional reduction of ganglion cells and innervation patterns (nerve twigs and neuropil), in uneven distribution, with controls (T-Y); Only rare individually scattered ganglion cells are seen in both plexi of this ileal biopsy from patient A, at 9 weeks of age, with only scant traces of delicate nerve twigs on immunostain for calretinin. The myenteric plexus is markedly underdeveloped, essentially inconspicuous on H&E, with closely juxtaposed inner and outer layers of the muscular wall and marked global reduction of innervation on immunostains (PGP9.5 and Gap43); While the enteric nerves system remains globally underdeveloped, irregular distribution of ganglia, is evident throughout the sampled biopsies in the same patient, which may pose diagnostic pitfalls. In colonic biopsies, innervation patterns remain abnormal (on immunostains for calretinin and choline transporter,) though ganglion cells are more readily identified, occasionally in small clusters and with variably "immature" to mature cytomorphology; Controls: Ganglion cells with immature (T) and mature (U) cytomorphology (in a neonate and a 2-year-old, respectively). Innervation patterns in ganglionated neonatal rectum (V,X) and in neonate with Hirschsprung's disease (W,Y) ; Stains: H&E (B,E,H,I,K,N,Q,R,R-U,) and immunohistochemical stains for Calretinin (C,F,L,V-W,) PGP9.5 (D,G,) Gap43 (J), and Choline Transporter (M,P, X-Y); Scale bars: B-G: 50 micrometer (20x-equivalent); H: 25 micrometer (40x-equivalent); I-J: 415 micrometer (2.5x-equivalent); K-P: 100 micrometer (10x-equivalent); Q-Y: 25 micrometer (40x-equivalent).

Patient B

Patient B is an African female born at 40 weeks gestation and admitted to the NICU at 19 hours of life for emesis and abdominal distension with enteral feeds. Rectal suction biopsy performed on day 11 showed no ganglion cells and abnormal calretinin staining. She underwent an appendectomy and leveling colostomy at the ascending colon. Resulting pathology showed aganglionosis from the rectum to the transverse colon with a transition zone near the hepatic flexure.

Her physical exam demonstrated striking blue eyes as well as swirling hypopigmentation with confluent patches of hypopigmentation on her chest, legs, and face. She also demonstrated generalized hypotonia and delayed motor development. Persistent hypoxia while sleeping was found to be related to obstructive (rather than central) sleep apnea – the patient required supplemental O2 overnight to prevent desaturations. Additionally, she failed her newborn hearing screen and required conduction hearing devices. Genetic evaluation revealed a SOX10 mutation (c.685g>T,p.Glu229) consistent with the diagnosis of Waardenburg type 4C. At three months of age the patient continued to have extremely poor gut motility. This manifested in the form of bilious emesis and chronic intestinal pseudo-obstruction (CIPO) regardless of whether feeds were attempted in the stomach, duodenum, or jejunum. She was taken back to the OR for lysis of adhesions, repeat biopsies (small bowel, proximal colon, and colostomy), ileo-colonic resection, and ileostomy creation. Her pathology showed no ganglion at the level of the colostomy and diffuse hypoganglionosis. There were no hypertrophic nerves; calretinin staining was abnormal from the proximal colon to the transition in the ileum twenty-five cm proximal to the cecum (Table 1).

Table 1		Patient A	Patient B	Patient C
Demographics	Mutation (*variant*)	Sox 10 c.829delG,p.D277TfsX9	Sox 10 *c.685g>T,p.Glu229*	EDN3 *c.266del & c.329A>C*
	Gender	Male	Female	Female
	Current Age	3 years 9 months	Deceased, 15 months	5 years 7 months
	Ethnicity	Caucasian	African	Hispanic
Pigmentation	Hair	No	white forelock	white forelock
	Skin	No	swirling hypopigmentation	N/A
	Iris	Blue Color	Blue color	Heterochromia
Hearing Loss	Sensorineural	Yes	Yes	Yes
Other Symptoms	Vomiting	Yes	Yes	Yes
	Gagging/Choking	Yes	Yes	Yes
	Aspiration	None	Yes	None
	Constipation	Yes	Yes	Yes
	Renal involvement	N/A	L sided pelvocaliectasis	N/A
	CNS	horizontal nystagmus, hypotonia, tethered cord, conjoined cranial nerve VII and VIII	horizontal nystagmus, hypotonia, sleep apnea	No
Diagnostic Evaluation	Contrast Study (UGI and WSCE)	UGI: normal WSCE: normal	SBFT: Contrast transit was slow through the dilated distal small bowel loops; WSCE normal	UGI: Malrotation WSCE: No colon
	ADM	Normal	N/A	N/A
	Colonic Manometry	Abnormal (No HAPCs)	N/A	N/A
GI Management	Age of ostomy creation	Distal colostomy at 2 months; ileostomy at 9 months; both present	Ileostomy at DOL 16,	ileostomy at DOL 3, converted to end jejunostomy at 5 months, s/p jejunostomy takedown
	Length of Small bowel remaining bowel	Full length	100cm	53cm
	Progression of Feeding Regimen	Weaned off TPN, Currently on G-tube + oral feeds	Weaned off TPN, continuous GJ-tube feeds	Weaned off TPN, Currently on G-tube + oral feeds
	other stoma (mucus fistula)	Mucus fistula present	N/A	N/A

ADM= Antroduodenal Manometry; HAPC=High Amplitude Propagated Contractions; N/A: Not Applicable

Table 1: Clinical History.

Patient C

Patient is a Latina female born at 40 weeks gestation. She was transferred to the NICU in the early hours of life due to feeding intolerance that presented as abdominal distension, delayed passage of stool, and bilious emesis. She underwent surgical exploration on her third day of life. Histology revealed no ganglion cell in the distal sigmoid colon to the mid ileum as well as hypoganglionosis from the proximal ileum to distal jejunum. This overall lack of ganglion cells met criteria for long segment Hirschsprung's disease. She underwent a colectomy with Duhamel pouch (residual sigmoid colon and rectum left intact), gastrostomy placement, and diverting ileostomy.

Physical exam in the NICU revealed heterochromia, white forelock, and white eyelashes (like her father). Patient failed her newborn hearing screen and had cochlear implantation. Genetic testing diagnosed Waardenburg type 4 syndrome with compound heterozygosity (a pathogenic c.266del causing frameshift mutation with premature protein termination and a c.329A>C) in EDN3 gene.

At 5 months of age, she experienced recurrent enterocolitis presenting as frequent emesis and decreased ostomy output. She was taken back to the OR for a takedown of the Duhamel pull-through and creation of an end-jejunostomy resulting in 53 cm of small bowel remaining. During the early months she was fed in a variety of ways which all resulted in pseudo-obstruction. This presented as abdominal distension, frequent explosive and occasional bloody jejunostomy stool output, poor weight gain (meeting failure to thrive criteria), failure to advance enteral volume, and TPN-dependence. She was started on subcutaneous teduglutide at 22 months of age. Within 6 months she was able to wean off TPN (by 28 months of age). She achieved enteral autonomy by 32 months of age.

Summary

We report three patients with WS4, two with a SOX10 mutation and one with EDN3 type B mutation who had similar abnormalities on histology. They all did not have the typical features of HSCR, but in some cases showed features consistent with congenital hypoganglionosis affecting the entire colon and various parts of the small bowel in all three. Patient A still has his ileostomy and colostomy present but is growing and thriving on all enteral feeds. Unfortunately, Patient B had multiple recurrences of pseudo-obstruction and sepsis which may have contributed to her sudden cardiopulmonary arrest. While cardiac manifestations like septal defects are not typically seen in WS4, neural crest cells contribute to heart precursors and cardiac defects can be seen with PAX3 gene mutations in WS1 and WS3. Patient C has done well since her jejunostomy takedown, and she has been able to maintain her enteral autonomy for over 2 years.

The wide spectrum of histologic abnormalities noted from complete aganglionosis to hypoganglionosis and lack of nerve hypertrophy in our patients could reflect defects at all levels of ENS development: multiplication, migration, and maturation. It is unclear if the motor function of the colon would have recovered over time since these patients had early surgical colonic diversion or resection. Furthermore, in patient C, it is unclear whether teduglutide facilitated enteral autonomy from adequate intestinal adaptation and ENS development or whether it would have occurred spontaneously over time.

Conclusion

Waardenburg syndrome type 4 is a rare genetic disease that presents with a more severe phenotype than types 1-3. Patients initially present with gastrointestinal manifestations shortly after birth including abdominal distension, vomiting, poor weight gain, and delayed passage of stool. Diagnosis is often suspected after a water-soluble contrast enema followed by a rectal suction biopsy and ultimately confirmed, by a thorough physical examination that is often aided by family history and genetic testing. Even though Hirschsprung Disease (aganglionosis with hypertrophic nerves) is reported in WS4, all three of our cases had hypoganglionosis with no hypertrophic nerves in the small bowel and/or colon, with aganglionosis at least in the rectum of one patient. In HSCR a highly damaging ultra-rare loss of-function variant in a key ENS gene (e.g., SOX10) is sufficient to cause the rarer form of the disease in a person with less sensitized genetic background. On the other hand, for a rare variant with moderate effect size, a highly sensitized genetic background is needed for the phenotypic expression of HSCR [11]. This may be true in patients with WS, the genetic background may predispose carriers of the same genetic variant to have variable genetic expressivity and phenotypic variability. Furthermore, the continued postnatal development of the immature ENS can be influenced by inputs from extrinsic nerves, glia cell-derived neurotrophic factors, and interactions with the microbiota. It remains critical to distinguish these two conditions (aganglionosis and hypoganglionosis) in patients with WS4 in order to avoid unnecessary resections and it seems that enteral autonomy may be possible to occur over time in those with hypoganglionosis [8,12–14].

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