Supplemental Data

Patients

Family F1

The proband, a male, is the third child of four siblings, born at term after an uneventful pregnancy to healthy French non-consanguineous parents. He was diagnosed with short-segment HSCR on the seventh day of life due to intestinal obstruction. The diagnosis was made by histological examination of rectal biopsies showing aganglionosis. Postoperative histology confirmed aganglionosis in the distal segment of 18.5cm. Parenteral nutrition was started from the seventh month due to digestive intolerance, associated with recurrent pancreatitis and consequent hepatic fibrosis. Intestinal dysmotility persisted with chronic obstructive symptoms (abdominal pain, nausea and vomiting) without mechanical obstruction of the gut lumen. He has had two to three pseudo-obstructive episodes per year since 4 years of age, requiring a gastrostomy for decompression. A diagnosis of CIPO was made. At 13 years old, colectomy was performed along with histopathological analysis revealing various neuromuscular alterations shown in Figure 2. To date, the patient is totally dependent on parenteral nutrition and GI transplantation was considered. The patient also developed a progressive neuropathy from 8 years of age. Electromyography studies displayed a progressive axonal motor and sensory neuropathy with decreased nerve amplitude and nerve conduction velocities in the normal range. Other manifestations included dysautonomia with tachycardia, hyperesthesia and hyperhidrosis, anosmia with absence of the olfactory bulbs confirmed by brain MRI, bilateral conductive hearing loss and hypoplasia of the external auditory canal on the left, retrognathia, myopia with retinal detachment, hypopigmentation and osteoporosis. Despite his severe illness, the patient had normal cognitive development when last seen at 15 years of age.

Family F2

Family F2 is of Egyptian origin. The proband (F2:II-2) was diagnosed with HSCR and underwent ileostomy at two weeks of age. As shown on Figure 2, histological analysis of post-operative specimens revealed a short distal aganglionic segment followed by a long segment of hypoganglionic bowel. Gastro-jejunal tube feeding was started at approximately six weeks of age due to recurrent microaspirations, feeding intolerance, intestinal bacterial overgrowth and chronic diarrhea. His sister (F2:II-3) also had HSCR and underwent colectomy at the age of 3 years. Histological findings were similar with a short aganglionic segment of 12mm and a long hypoganglionic segment extending to the small intestine with rare and abnormal ganglion cells (Figure 2). At two years of age, after a prolonged period of emesis and failure to thrive, abdominal CT scan was performed and showed enterocolitis but no obstruction. Both siblings also presented developmental delay, 2/3 toe syndactyly and dysmorphic facial features, including microtia, unilateral or bilateral ptosis and retro/micrognathia.

Family 3

Family F3 is consanguineous, of Pakistani origin. For F3:II-1, termination of pregnancy was performed at 16 WG. Three years later, intrauterine fetal death was detected for F3:II-3 at 15 WG, again requiring termination of pregnancy. Both fetuses presented with multisystemic malformations, including intrauterine growth retardation, fetal akinesia, arthrogryposis multiplex congenita, unilateral anotia and congenital heart defects (transposition of the great vessels and hypoplastic left ventricle in F3:II-1 and tetralogy of Fallot in F3:II-3). In F3:II-1, a medial cleft lip and palate was also observed, which is a more complex finding than the posterior cleft palate typically observed in akinesia. Histology of deltoid muscles of both fetuses showed numerous myotubes with a centered nucleus, without muscular degeneration

or interstitial fibrosis (Figure 4). Retrospective analysis of the intestinal tract of F3:II-1 showed total colonic aganglionosis extending up to the stomach (Figure 3). The smooth muscle layers appeared normal.

Family 4

In Family F4, from France, termination of pregnancy of F4:II-1 was performed at 18 WG. The fetus presented with intrauterine growth restriction, arthrogryposis multiplex congenita with axillary, inguinal, elbow and knee pterygia, clubfeet, large clitoris, hypoplasia of the olfactory bulbs, unilateral external auditory canal agenesis and craniofacial anomalies (retrognathia, posterior cleft palate, midface hypoplasia). At necropsy, the motoneurons in the ventral horn and sensory neurons in the dorsal root ganglia appeared normal while analysis of skeletal muscle (psoas) showed sparse-nuclei myotubes with presence of type I and type II myotubes. Ultrastructural analysis by electron microscopy revealed a poverty of type II myotubes, suggesting skeletal myopathy (Figure 4). Retrospective analysis of the intestinal tract showed total colonic aganglionosis extending to the small intestine (Figure 3). The smooth muscle layers appeared normal.

Family 5

The two affected children were born to consanguineous parents of Turkish ancestry. The proband (F5:II-1) had severe constipation, but histopathological examination was not conducted. The gastrointestinal concerns eased after 5 years of age. She additionally presented peripheral axonal neuropathy, hypotonia, mild intellectual disability, unilateral ptosis, sensorineural hearing loss, and clubfeet. Her brother (F5:II-2) displayed similar clinical features with short-HSCR confirmed by histological examination. Indeed, analysis of colon biopsies showed a lack of ganglion cells at the anal resection site combined with a

hyperplasia of nerve fibers in the submucosa. Above the aganglionic segment, a reduced quantity of ganglion cells and hyperplasia of nerve fibers were observed. The proximal colon biopsies showed normal composition of submucosal and myenteric plexuses. He has persisting GI problems with very variable defecation pattern despite daily colonic lavations. Micropenis was also noted.

Supplemental tables

Supplemental table 1. Clinical description of patients with *ERBB3* and *ERBB2* mutations.

See excel sheet

Supplemental Table 2. List of primers used in the study.

ERBB3 resequencing	**Forward primer** (5'-3')	**Reverse primer** (5'-3')
Exon 5-6	AGGTGCCTGTGTACTGACATC	CCCTACCTCCTCCTCAAAGG
Exon 18-19	CCTGATCTCATGAGCACAAA	CTGAGAAGCACAGGACCCTGC
Exon 20	GCAGGGTCCTGTGCTTCTCAG	CACTTTTTCCTGGCTGGCCC
Exon 22	GAATTTGGGACTTGGAAATCC	GGTAAATTTGAAACTGGG
Exon 23	GCATCTGGATGCCCTCTCTAC	GCCAAGATTGATTGCACCAT
Exon 27	GGCAGTGAACAACCCAATAT	GACAGAGAGGCATATGATCA
RT-qPCR	**Forward primer** (5'-3')	**Reverse primer** (5'-3')
ERBB3 mRNA	GCAACATTGATGGATTTGTG	CTCCCGTACTGTCCGGAAGAC
GUSB mRNA	GCGGTCGTGATGTGGTCTGT	GTGAGCGATCACCATCTTCAAGT
pcDNA3.1-HAERBB3 plasmid resequencing	**Forward primer** (5'-3')	**Reverse primer** (5'-3')
ERBB3-CDS1	TAATACGACTCACTATAGGG	CCCTCCAGTCAATTGTGTCC
ERBB3-CDS2	GTCATGTTGAACTATAACACC	TACGCCCAGCACTAATTTCC
ERBB3-CDS3	GCAACATTGATGGATTTGTG	GCCAAGCACTTTAAGCTTCC
ERBB3-CDS4	GGGCTATGAGGCGATACTTG	GTGTTCCAACTGGTAGGCTG
ERBB3-CDS5	GATTGATGAGAACATTCGCCC	TAGAAGGCACAGTCGAGG
Backbone_1	GTTCATAGCCCATATATGGAG	GACGCTCTGCAGGTGCTGGGC

Backbone_2	CGCTGTTGAGATCCAGTTCG	GCCAATAGGGACTTTCCATTG
Backbone_3	CCCACGCTCACCGGCTCCAG	GGGAATAAGGGCGACACGGA
Backbone_4	CCACTGGCAGCAGCCACTGG	GCTAGAGTAAGTAGTTCGCC
Backbone_5	GAATCGGCCAACGCGCGGGG	GGTATCTGCGCTCTGCTGAAG
Backbone_6	CCTCGTGCTTTACGGTATCG	GCGGCGAGCGGTATCAGCTC
Backbone_7	GTTCCGGCTGTCAGCGCAGG	GACCGACCAAGCGACGCCCA
Backbone_8	CGCCCTTTGACGTTGGAGTC	GGCTATCGTGGCTGGCCACG
Backbone_9	CGAGTCTAGAGGGCCCGCGG	TTGGGGATTTCGGCCTATTGG

Mutagenesis primer	**Forward primer** (5'-3')	**Reverse primer** (5'-3')
ERBB3-Thr787Pro	CATCTCTGCAGCTTGTC**C**CTCA ATATTTGCCTCTG	CAGAGGCAAATATTGAG**G**GACA AGCTGCAGAGATG
ERBB3-Thr873Ser	CTATACAGTGAGGCCAAGA**G**T CCAATTAAGTGGATGGCC	GGCCATCCACTTAATTGGA**C**TCT TGGCCTCACTGTATAG
ERBB3-Val899Met	GATGTCTGGAGCTATGGT**A**TGA CAGTTTGGGAGTTG	CAACTCCCAAACTGTCA**T**ACCAT AGCTCCAGACATC
ERBB3-Gln932Arg	GGGAGCGGTTGGCAC**G**GCCCC AGATCTGCAC	GTGCAGATCTGGGGC**C**GTGCCA ACCGCTCCC

Replacement of HA tag by FLAG tag	**Forward primer** (5'-3')	**Reverse primer** (5'-3')
Generation of a linearized vector	ACCCAAGTGTGCACCGGCAC	CTCGGAGCCCCGGGCCAGGC
Generate FLAG + 15bp extensions homologous to vector ends	GCCCGGGGCTCCGAGGACTAC AAAGACGATGACGACAAGACC CAAGTGTGCACC	GGTGCACACTTGGGTCTTGTCGT CATCGTCTTTGTAGTCCTCGGAG CCCCGGGC

The FLAG sequence was cloned in to the vector using In-Fusion HD Cloning Kit following the manufacturer's instructions (Takarabio).

pcDNA3.1-FLAG-ERBB2 plasmid resequencing	**Forward primer** (5'-3')	**Reverse primer** (5'-3')
ERBB2-CDS1	CGACTCACTATAGGGAGACCC	CCAGGGCTGGGCAGTGCAGC
ERBB2-CDS2	CGCGCACTGTCTGTGCCGGTG	GCAGTGCCCTCGGGCGCACAGC
ERBB2-CDS3	GCAGTGGACTGGCCCTCATCC	CACACCAGCCATCACGTATGC
ERBB2-CDS4	GAAGGTGCTTGGATCTGGCGC	GATACTCCTCAGCATCCACC
ERBB2-CDS5	CAGCGCTTTGTGGTCATCCAG	GGGTTAGGGATAGGCTTACC

Mutagenesis primer	**Forward primer** (5'-3')	**Reverse primer** (5'-3')
ERBB2-Ala710Val	GCGATGCCCAACCAGG**T**GCAG ATGCGGATCCTG	CAGGATCCGCATCTGC**A**CCTGGT TGGGCATCGC

Mouse genotyping	**Forward primer** (5'-3')	**Reverse primer** (5'-3')
Cre	GCCTGCATTACCGGTCGATGC	CAGGGTGTTATAAGCAATCCCC
ErbB3	TCTCCTTGTTGATCATGAAGAA CTTG	TTGCAGTGAGTCACACAGACAC CT