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CELLULAR AND MOLECULAR GASTROENTEROLOGY AND HEPATOLOGY

EDITORIAL

Mice Are Not Small Furry People! A New Hirschsprung Disease Model Lets Us Pretend This Is Not True

In 1888, Harald Hirschsprung[1] described 2 children with intractable constipation, massive abdominal distension, malnutrition, and new-onset explosive diarrhea. These unfortunate children probably died from sepsis as colon bacteria escaped epithelial and immune cell barriers in leaky aganglionic distal colon. *Aganglionic* means "lacking enteric nervous system (ENS) ganglia," the clusters of intestinal neurons and glia that control bowel motility, and epithelial and immune cell function in response to local stimuli. Fortunately, for most people, the 600 million enteric neurons, more than 20 neuron types, and specialized enteric glia work so well that we can eat what we want and pursue our passions without consciously controlling approximately 30 feet of amazing bowel. When ENS is missing or defective, misery ensues, a connection first shown in 1949 by Swenson et al[2] in children with Hirschsprung disease (HSCR). Although distal HSCR bowel looks normal, it lacks enteric ganglia and lacks propagating contractions. Swenson et al[2] correctly surmised that aganglionic bowel caused functional obstruction and invented the Swenson pull-through surgery to remove aganglionic bowel and reattach normal bowel near the anal verge. Children with HSCR often are dramatically better after pull-through surgery, but some have persistent constipation, stool leakage, or "Hirschsprung-associated enterocolitis" (abdominal distension, explosive diarrhea, lethargy, and sepsis risk). We still need new treatments and prevention strategies.

One major mystery is why outcomes vary so much after pull-through surgery. In part, the answer may lie in differences in bowel physiology between affected children. Even before treatment, some neonates are critically ill with a distended abdomen, bilious vomiting, and fever (±bowel perforation), and need urgent surgery. Other children with HSCR appear well for years with minimal therapy. In fact, a 53-year-old man in Japan was diagnosed recently with HSCR.[3] He had chronic constipation (weekly bowel movements) on a magnesium-based laxative. After stopping his medicine he went a month without passing stool and was diagnosed with HSCR. This remarkable range of symptoms suggests genetic or nongenetic disease modifiers exist that could be targeted to improve outcomes.

HSCR occurs when neural crest–derived ENS precursors fail to fully colonize bowel during the first trimester of pregnancy.[4] ENS precursors depend on the tyrosine kinase receptor rearranged during transection (RET) for survival, proliferation, and efficient migration (first shown in 1994).[5–7] RET transmembrane tyrosine kinase activity usually is low in people with HSCR. RET is activated in the ENS by GDNF and NRTN via GFRA1 and GFRA2. RET transcription depends of PHOX2B, SOX10, RARB, GATA2, and PAX3. These genes are linked to HSCR. SOX10 competes with *SRY* (the male sex–determining gene) for RET regulatory elements, perhaps explaining the 4:1 male/female ratios in HSCR. RARB is activated by retinoic acid, a vitamin A derivative made by RALDH2. Vitamin A deficiency causes HSCR-like disease in mice (and might increase human HSCR risk). *Raldh2-/-* mice have total intestinal aganglionosis (similar to Ret-/- mice and RET-/- human beings). *SOX10* mutations cause HSCR with deafness, patchy skin depigmentation, and peripheral neuropathy (Waardenburg–Shah syndrome, WS4C). *PHOX2B* mutations cause HSCR with congenital central hypoventilation syndrome (Haddad syndrome). RET, GDNF, GFRA1, and retinoid signaling partially explain why 20% of children with HSCR have congenital anomalies of the kidneys and urinary tract. Thus, RET is central to HSCR pathogenesis.

In human HSCR, aganglionosis is limited to the distal colon 80% of the time, suggesting that machinery needed to make ENS is present but not working efficiently. Typically, in human beings, combinations of mild risk alleles conspire to prevent full-bowel colonization by ENS precursors. More than 30 genetic loci (including trisomy 21) impact HSCR occurrence.[8,9] Maternal medicines, nutrition, and illness all seem likely to impact HSCR incidence. To prevent HSCR and find new cures, we need great model systems.

In this issue of *Cellular and Molecular Gastroenterology and Hepatology*, Sunardi et al[10] describe a model based on a human RET mutation (*S811F*) in which the mouse (*RetS812F*) closely mimics human disease. The *RETS811F* human has HSCR, unilateral kidney agenesis, and oligomeganephronia (reduced nephron numbers). $Ret^{S812F/+}$ mice have distal colon aganglionosis (50%) or hypoganglionosis (50%), small kidneys, and 10% unilateral renal agenesis. Combining Ret (*S812F*) with Ret9 (hypomorphic) or *Ednrb+/-* (HSCR risk allele) increased aganglionosis to 100%. RETS811F probably prevents adenosine triphosphate binding to the kinase domain, generating dominant-negative RET that homodimerizes without phosphorylating wild-type RET. $Ret^{S812F/+}$ had reduced proliferation, reduced migration, and increased apoptosis of ENS precursors, with abnormal enteric neuron subtype ratios. It is easy to say, "I knew that would happen," but it took nearly 30 years to generate Ret-variant mice closely mimicking human HSCR. Thankfully, $Ret^{S812F/+}$ live many weeks, facilitating promising enterocolitis, stem cell, and regenerative medicine (GDNF, 5-HT4–receptor agonist) studies. Although we need to remember that mice are not small furry people, we now can pretend as we seek new cures.

EDI 5.6.0 DTD ■ JCMGH1164 proof ■ 13 March 2023 ■ 5:22 pm ■ ce DVC

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Conflicts of interest
The author discloses no conflicts.

2352-345X
https://doi.org/10.1016/j.jcmgh.2023.02.012