Hirschsprung’s disease, Down syndrome, and missing heritability: too much collagen slows migration

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Hirschsprung’s disease: congenital absence of distal bowel enteric nervous system

Hirschsprung’s disease (HSCR) is a deadly birth defect in which the enteric nervous system (ENS) is missing from the end of the bowel (1, 2). Because the ENS controls most aspects of bowel function (3), even a short region of bowel without neurons and glia (i.e., aganglionosis) can be fatal. Over the past few decades many genetic and nongenetic causes of distal bowel aganglionosis have been identified, including mutations in RET, EDNRB, SOX10, PHOX2B, and ZFHX1B (4, 5), but identifiable genetic problems do not explain HSCR occurrence in many affected children (6). Down syndrome also predisposes to HSCR.

The first definitive description of what is now called HSCR is attributed to Frederick Ruysch in 1691 (7), but the ancient Ayurvedic Sushruta Samhita describes “Baddha Gudodaram,” a disorder remarkably like HSCR, as early as 2000 BCE (8). In 1886, Harald Hirschsprung reported two infants with the disorder that now bears his name (9, 10). The link between distal bowel aganglionosis and the massive proximal bowel distension that occurs with HSCR was not understood, however, until the pioneering studies of Swenson and Bill, Swenson et al., Bodian et al., Zuelzer and Wilson, and Whitehouse and Kernohan in 1948 and 1949, which showed that the absence of enteric neurons causes tonic contraction and functional obstruction (11–15). HSCR in a child with Down syndrome was first reported in 1956 (16), and increased occurrence of HSCR in individuals with Down syndrome was recognized in 1963 (17), suggesting that one or more genes on chromosome 21 contribute to HSCR etiology. Up to 10% of children with HSCR have Down syndrome, and 1% to 2% of children with Down syndrome have HSCR. Thus, Down syndrome increases HSCR risk about 50- to 100-fold above the general population risk (~1:5,000) and is a common underlying partially penetrant cause of HSCR (4).

Over the past few decades there have been dramatic advances in our understanding of HSCR anatomy, embryology, physiology, and genetics (4, 5, 18). Early studies by Yntema and Hammond showed that the ENS forms from enteric neural crest-derived cells (ENCDCs) that originate primarily in the vagal region of the neural tube (19). These ENCDCs proliferate vigorously as they colonize fetal bowel in a rostral to caudal progression, pursuing one of the longest migratory routes of any cell population during fetal development (Figure 1A). ENCDCs then differentiate into diverse neuron and glia subtypes that form a network in the bowel wall with about as many neurons as the spinal cord and every transmitter in the central nervous system (3, 18). Despite these advances in our understanding of ENS development, the causal link between trisomy 21 and increased HSCR incidence has remained elusive. Analysis of partial trisomy 21 phenotypes suggested that an extra copy of at least one gene in the interval from 33.5 to 46.25 Mb increased HSCR risk (20). This interval contains 122 known genes, including DSCAM, BACE2, COL18A1, and COL6A1. A recent SNP association study suggested that excess DSCAM may be important for HSCR pathogenesis in Down syndrome, but excess DSCAM has not been confirmed experimentally to cause HSCR-like disease in model systems (21).

Unbiased approach to identify genes critical for ENS development

In this issue, Soret and colleagues took an unbiased, forward genetics approach and used insertional mutagenesis to identify potential regulators of neural crest–derived cell (NCC) migration (22). To simplify the screening process, Soret et al. randomly inserted a tyrosinase (Tyr) minigene, which rescues pigment production in NCC-derived melanocytes, into albino FVB/N mice and then evaluated animals with nonuniform pigment patterns. Because melanocytes and the ENS are both neural crest derivatives, some mice with pigmentation defects were also expected to have ENS defects, mimicking the human “neurocristopathy” called Waardenburg-Shah syndrome (Waardenburg syndrome type...
Hol Tg / Tg mice have delayed colonization of fetal bowel by ENCDCs due to reduced cell migration, but there was no effect on the direction of ENCDC migration, rates of neuronal differentiation, proliferation, or cell death. In contrast, Hol Tg / Tg mice had a smaller percentage of enteric glia-fated ENCDCs (SOX10+, HuC/D–, S100β+) than WT mice at E15.5 and a larger percentage of undifferentiated ENCDCs (SOX10+, HuC/D–, S100β–), suggesting that the mutation slows glial differentiation, an effect unlikely to influence bowel colonization, which is complete by this time.

4 [WS4]) that is characterized by a combination of skin or eye pigmentation defects, sensorineural hearing loss, and HSCR. Known causes of WS4 include inactivating mutations in EDNRB, EDN3, and SOX10, but these gene defects account for only a small percentage of HSCR cases (5% EDNRB, 4% SOX10, <1% EDN3), and most people with HSCR have normal pigmentation. Surprisingly, the insertional mutagenesis strategy used by Soret et al. led to increased expression of genes adjacent to the transgene insertion site and three new models of HSCR (Holstein [Hol])

ref. 22), TashT (23), and Spot (22). The genes identified by this approach would have been difficult to predict based on known ENS biology and would never have been discovered by traditional “knockout” approaches, because the affected genes are overexpressed in these models of HSCR and WS4, and the insertions involve non-coding regions of the genome.

Holstein homozygote (Hol Tg / Tg) animals exhibit distal colon aganglionosis that is typical of 80% of human children with HSCR, but these animals also have quite extensive albinism, unlike typical human HSCR (22). Hol Tg / Tg mice have delayed colonization of fetal bowel by ENCDCs due to reduced cell migration, but there was no effect on the direction of ENCDC migration, rates of neuronal differentiation, proliferation, or cell death. In contrast, Hol Tg / Tg mice had a smaller percentage of enteric glia-fated ENCDCs (SOX10+, HuC/D–, S100β+) than WT mice at E15.5 and a larger percentage of undifferentiated ENCDCs (SOX10+, HuC/D–, S100β–), suggesting that the mutation slows glial differentiation, an effect unlikely to influence bowel colonization, which is complete by this time.
The transgene insertion site in HolTg/Tg mice is between collagen-6α4 (Col6a4) and glyc erate kinase (Glyctk) in a region that is syntenic to human chromosome 3q22. RNA sequencing (RNA-Seq) analysis on isolated E12.5 ENCDCs revealed that Col6a4 mRNA is markedly increased (about 250-fold) but that the mRNA levels of other nearby genes, including Glyctk, were not affected (22). The increase in Col6a4 mRNA was not detectable by RNA-Seq when the whole bowel was used instead of ENCDCs. This observation suggests that the transgene causes a cell-autonomous increase in Col6a4 mRNA within migrating ENCDCs without affecting Col6a4 mRNA levels in other bowel cells. Consistent with these data, hetero-topic grafting using WT and HolTg/Tg tissues confirmed that HolTg/Tg ENCDCs do not migrate efficiently into WT colons, moving at about half the speed of WT ENCDCs. Immunohistochemical staining confirmed increased collagen VI deposition around HolTg/Tg ENCDCs as they colonize fetal bowel but no increase in collagen VI in regions of the bowel that had not been colonized by ENCDCs. Intriguingly, collagen VI protein levels in HolTg/Tg bowels are only about 3-fold higher than those in WT bowels, an observation ascribed to the need to incorporate COL6A4 protein into trimeric collagen monomers that also contain COL6A1 and COL6A2, which are encoded by genes that are not overexpressed in HolTg/Tg mice. Consistent with the hypothesis that excess collagen VI accounts for the HSCR-like phenotype, cultured WT ENCDCs migrated more slowly on collagen VI, and added collagen VI can reduced migration on fibronectin-coated dishes (Figure 1B). One explanation for this observation is that collagen VI and fibronectin may compete for β1 integrin binding since β1 integrin is required for ENCDC migration (24). Alternatively, altered matrix stiffness or porosity might slow ENCDC migration and reduce glial differentiation.

Increased collagen VI links HSCR and Down syndrome

Soret et al. next hypothesized that their observations in mice may be relevant for human HSCR and could explain the increased HSCR occurrence in children with Down syndrome (22). The translation of observations made in Holstein mice to human biology required a substantial intuitive leap of faith. The human COL6A4 gene on chromosome 3 generates nonprocessed pseudogenes. In contrast, two of the genes encoding human collagen VI are on chromosome 21 (COL6A1 and COL6A2), and COL6A1 is within a region defined as important for HSCR risk in human partial trisomy analyses (20). These collagen genes are expressed in ENCDCs and overexpressed in the skin of human fetuses with trisomy 21, in which they probably exclusively interact with COL6A3 to form classical α1-α2-α3 trimeric collagen monomers. Soret and colleagues analyzed human colon muscle with myenteric ganglia from a cohort of patients with HSCR and healthy controls to test their hypothesis that excess collagen VI might increase HSCR risk (22). Remarkably, collagen VI was more abundant in tissue surrounding myenteric ganglia of children with HSCR compared with that in control specimens (2-fold higher) and present at even higher levels surrounding myenteric ganglia of children with Down syndrome and HSCR (3-fold higher). Interestingly, there was no correlation between the age of the child and the level of collagen VI detected by immunohistochemistry.

Conclusions and future directions

Collectively, the studies by Soret and colleagues fit well with the hypothesis that some of the missing heritability in children with HSCR results from noncoding variants that alter gene expression (6). HSCR risk, however, remains challenging to define because so many genetic and nongenetic factors influence signaling pathways that control ENCDC survival, proliferation, differentiation, and migration (5), leading to complex gene-gene interactions that affect ENS development (18, 26). The observation that elevated collagen VI levels reduce the speed of ENCDC migration fits well with long-standing observations that extracellular matrix molecules and matrix metalloproteinases influence ENS development (5). The hypothesis that increased collagen VI production by migrating ENCDCs in children with Down syndrome underlies increased HSCR risk is provocative and interesting. Studies to more formally test this hypothesis are challenging in humans, however, because HSCR is rarely diagnosed before birth. Moreover, ENCDCs migrate through the bowel during the first trimester of pregnancy, and only 1% to 2% of children with Down syndrome have HSCR. Nonetheless, this remarkable work provides new insight into ENS development, highlighting the need to understand the interaction of ENCDCs with the extracellular matrix, especially as we consider new cell-based therapies to treat enteric neuropathy.

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