Hirschsprung disease — laying down a suitable path

Heather M. Young and Sonja J. McKeown

For many patients with Hirschsprung disease, the underlying pathogenic mechanisms are unknown. A new study using a novel line of transgenic mice and tissue from patients with Hirschsprung disease suggests that overproduction of collagen VI could contribute to pathogenesis and the increased incidence of Hirschsprung disease in patients with Down syndrome.

Hirschsprung disease is a congenital disease in which there is functional bowel obstruction due to an absence of enteric neurons from the distal bowel. This condition is fairly easy to diagnose, but despite extensive study the genetics and pathogenic mechanisms remain poorly understood in many patients. Mutations in >15 genes have been associated with Hirschsprung disease, but mutations in known genes account for the disease in only a minority of individuals. Furthermore, although some candidate genes on chromosome 21 have been suggested, it is still unclear why Hirschsprung disease is over 40 times more common in patients with Down syndrome than in the general population.

Enteric neurons originate from neural crest cells in the hindbrain that migrate into, and then along, the developing gut. As the gut is growing in length as the cells migrate, enteric neural crest cells (ENCCs) probably migrate further than any other embryonic cell population. That ENCCs do not always complete their migration along the entire bowel when their development is perturbed, resulting in Hirschsprung disease, is perhaps not surprising. The control of ENCC migration is complex, and requires expression of several transcription factors by ENCCs themselves, as well as molecules produced by the gut mesenchyme that bind to receptors on the cell surface of ENCCs to activate particular intracellular signalling pathways. A variety of extracellular matrix (ECM) molecules are present in the embryonic gut including fibronectin, laminins, tenascin and collagens, and evidence exists that interactions between ENCCs and several components of the ECM, some of which are produced by ENCCs, are also important for ENCC migration. For example, loss of β1-integrin, which is a transmembrane adhesive protein that links the ECM to the cytoskeleton of the cell, severely retards ENCC migration in the colon of mice. A new study from the laboratory of Nicolas Pilon at the University of Montreal has now identified a key role for collagen VI in ENCC migration and hence potentially in Hirschsprung disease. Importantly, two of the genes encoding the polypeptide chains that contribute to mature microfibrils of collagen VI are located on chromosome 21q22, which is the chromosomal region known to contain genes responsible for the pathogenesis of Down syndrome.

The researchers performed an insertional mutagenesis screen in mice for loci affecting neural crest cells and have now characterized two strains with a Hirschsprung disease phenotype as enteric neurons are missing from the distal colon. In the first line reported, termed the TashT mouse, the gene involved was not conclusively identified, but there was an associated deregulation of several genes already known to be associated with Hirschsprung disease, including genes encoding members of the Gdnf-Ret signalling pathway; of note, RET is the most common gene mutated in patients with Hirschsprung disease.

In the latest study, Soret et al. characterized a second mouse line with a Hirschsprung disease phenotype, which they have called the Holstein mouse as it has a similar pigmentation pattern to the Holstein breed of dairy cows. The researchers provide in vivo and in vitro evidence in mice, and from patients with Hirschsprung disease, for a role of collagen VI produced by ENCCs in the pathogenesis of Hirschsprung disease. The researchers showed that homozygous Holstein mice exhibit intestinal obstruction, lack enteric neurons in the distal bowel and do not live beyond weaning age. A variety of approaches were then used to demonstrate that the transgenic insertion in these mice was in non-coding regions upstream of the Col6a4 gene, which encodes a component of collagen VI. Moreover, collagen VI protein levels were around three times higher in ENCCs from homozygous Holstein mice than ENCCs from wild-type mice, and immunofluorescence studies revealed increased abundance of collagen VI protein specifically associated with ENCCs in the mutants. Thus, the transgene insertion in Holstein mice resulted in overexpression of collagen VI associated with ENCCs.

The control of ENCC migration is complex...

As with other mouse models of Hirschsprung disease, the migration of ENCCs along the embryonic gut was already delayed in Holstein mice at embryonic day 11.5, which is when ENCCs first enter the colon. To examine whether the ENCC migration defect was cell autonomous, Soret et al. cultured ENCCs from Holstein mice with segments of hindgut from wild-type mice. The mutant ENCCs showed retarded migration even when migrating within wild-type hindgut segments, showing that cell autonomous defects in ENCC migratory behaviour are the cause of the absence of neurons in the distal bowel.
Colon of Holstein mice. Cell death, reduced proliferation and premature neuronal differentiation of ENCCs can indirectly retard their migration\(^1\), but none of these processes was shown to be defective in Holstein mice. Experiments performed in vitro showed that increasing concentrations of collagen VI lead to a dose-dependent reduction in the distance migrated by ENCCs, consistent with the increased collagen VI protein and retarded ENCC migration observed in Holstein mice in vivo (FIG. 1). Moreover, high levels of collagen VI retarded the ability of ENCCs to migrate on a fibronectin substrate in vitro, which demonstrates the complexity of the interactions between migrating ENCCs and ECM molecules. The new data are consistent with a previous study in chickens\(^6\), suggesting that ENCCs secrete ECM molecules that influence their migration through the microenvironment.

In the final, very fascinating, section of the study, Soret et al.\(^2\) used immunofluorescence microscopy to examine collagen VI distribution in samples of colonic muscle from three groups of infants: those with Hirschsprung disease only, those with combined Hirschprung disease and Down syndrome, and controls (infants with gastrointestinal malformations that were not enteric neuropathies). The intensity of collagen VI immunostaining associated with myenteric ganglia in patients with Hirschsprung disease only, and combined Hirschprung disease and Down syndrome, were two times and three times higher, respectively, than in samples from controls. Importantly, COL6A1 and COL6A2 loci are on human chromosome 21q, although it is intriguing that patients with Hirschsprung disease without Down syndrome also had increased collagen VI associated with myenteric ganglia.

The new study findings are highly important for several reasons. First, it has identified a new mechanism by which migration of ENCCs can be perturbed — overproduction of ECM molecules by ENCCs. This novel mechanism was identified by a random insertional mutagenesis approach in mice, and would not have been identified by conventional gene knockout strategies. Second, there is already strong evidence that non-coding variants can influence the risk of Hirschsprung disease; for example, non-coding RET variants influence Hirschsprung disease susceptibility by reducing RET transcription\(^12\). The new data from the Holstein mice confirm that non-coding variants can have a crucial role in determining whether ENCCs colonize the entire bowel. Although it is an attractive idea, the challenge now remains to establish conclusively whether overproduction of collagen VI is the mechanism by which patients with Down syndrome are more susceptible to Hirschsprung disease. Investigation of the regulation of collagen VI genes in patients with Hirschsprung disease (with or without Down syndrome) will be a new and exciting avenue of research.

Heather M. Young and Sonya J. McKeown are at the Department of Anatomy & Neuroscience, University of Melbourne, Victoria 3010, Australia. Correspondence to H.M.Y. b.young@unimelb.edu.au


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Competing interests statement

The authors declare no competing interests.

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**Figure 1** | **Over-production of collagen VI by ENCCs retards their migration.** ENCCs migrate along the length of the gastrointestinal tract and give rise to enteric neurons. Failure of ENCCs to completely colonize the gastrointestinal tract results in Hirschsprung disease. ECM molecules, including fibronectin, laminins, tenascin and collagens regulate ENCC migration by binding to integrins (grey), which are linked to the intracellular cytoskeleton (not shown). Compared with wild-type ENCCs, ENCCs in Holstein mice produce more collagen VI (red) and migrate slower. The mechanism by which high levels of collagen VI retards ENCC migration is not known, but as collagens and fibronectin bind to different integrins it is unlikely to be competition for the same integrins. ECM, extracellular matrix; ENCC, enteric neural crest cell.