Received: 15 March 2023 | Accepted: 16 March 2023

DOI: 10.1002/ctd2.185

CLINICAL AND TRANSLATIONAL DISCOVERY Open Access WILEY

COMMENTARY

More than missing neurons: Intestinal fibrosis in Hirschsprung disease

Maria M Alves | **Erwin Brosens**

Department of Clinical Genetics, Erasmus University Medical Centre - Sophia Children's Hospital, Rotterdam, The Netherlands

Correspondence
Erwin Brosens, Department of Clinical Genetics, Erasmus University Medical Centre - Sophia Children's Hospital, Rotterdam, The Netherlands.
Email: e.brosens@erasmusmc.nl

Proper functioning of the gastrointestinal tract requires the coordinated action of the enteric nervous system (ENS), smooth muscle, and interstitial cells of Cajal. Disturbance of any of these components can lead to intestinal motility disorders, characterized by the absence or significant reduction of intestinal peristalsis.[1] Management of intestinal disorders can be complex and involves multiple disciplines. Symptoms can vary, but usually include abdominal pain and distension, constipation, vomiting, and nausea, with some persisting throughout life even after the original clinical defect has been treated or corrected.[2]

Hirschsprung disease (HSCR) is the most common congenital intestinal disorder, characterized by the absence of an ENS in a variable length of the distal colon. It is caused by failure of the enteric neural crest cells (ENCCs) to either migrate, proliferate, differentiate or survive during embryonic development. As a consequence, three distinct regions can be identified in these patients. A ganglionic region composed by a normal ENS, a transition region characterized by ganglion hypoplasia, and an aganglionic region with no ENS.[3] HSCR is a complex genetic disorder caused by a combination of genetic alterations, including rare coding variants, predisposing common haplotypes, and copy number variation.[4] To date, surgical resection of the aganglionic segment is the main treatment option for HSCR, but it does not prevent further complications such as enterocolitis, fecal incontinence, or chronic constipation.[3] New therapies are thus, needed to improve care of these patients, but a better understanding of the molecular mechanism underlying disease pathogenesis is required, especially concerning the involvement of non-neural components of the intestine.

The recent study of Shiwei He and colleagues shed light on this subject by identifying cellular changes that occur in the aganglionic segment of HSCR patients.[5] By using single cell RNA sequencing (scRNA-seq), they provide for the first time, the transcriptomic profile of different cell populations in both aganglionic and ganglionic segments. Single cell suspensions were prepared from these regions and analysed using the 10X Chromium system. scRNA-seq data from about 60.000 cells isolated from aganglionic segments and about 30.000 cells from seemingly unaffected regions of seven HSCR patients, were compared with scRNA-seq data from seven healthy donors available in the Gut Cell Atlas database.[6] Although no differences were found in cellular composition between aganglionic and ganglionic segments, biological processes related to extracellular matrix (ECM) organization, negative regulation of nervous system development, and negative regulation of cell migration was up-regulated. Interestingly, abnormal expression of ECM proteins had already been reported for HSCR patients, suggesting that altered composition of ECM correlates with abnormal innervation.[7] A larger proportion of fibroblasts in aganglionic segments were also identified by scRNA-seq. Since fibroblasts are one of the major producers of ECM,[8] known to contribute to fibrosis in several tissues, the authors speculate that the aganglionic segments are fibrotic. To further elucidate the fibrotic alterations and mechanisms that contribute to ECM production in these patients, a

single-cell ECM expression score that included collagens, glycoproteins, and proteoglycans was established, and a clear shift towards higher ECM-expressing cells (such as fibroblasts, myofibroblasts, smooth muscle, pericytes, and enteric glial cells) was identified in aganglionic segments. ECM-related genes like collagens, fibronectins, and laminins were also found to be increased in aganglionic segments, and immunohistochemical analysis of unaffected and affected segments, as well as from the transition zone, indicated that this abnormal expression extends into the transition zone.

Authors further explored the stromal component, analysing a total of approximately 23.000 cells. An increased proportion of proliferating fibroblasts and myofibroblasts were identified in aganglionic segments, suggesting fibrotic changes in the absence of an ENS. Pseudotime analysis revealed an earlier differentiation of myofibroblasts in the aganglionic segments, which was supported by the earlier expression of the myofibroblast markers *ACTA2* and *ACTG2*, as well as the myofibroblast-regulator *TGFB1*. Also, the fibrosis-related growth factor *POST* as well as *ANXA1* and *HSP70* was over-expressed at an earlier stage in ganglionic and aganglionic segments of HSCR patients when compared to healthy controls, indicating a tendency for fibrosis especially in the aganglionic region. Interestingly overexpression of these genes was also detected in the transition zone.

Next, authors zoomed into enteric glial cells (EGCs), known to be a primary source of ECM. In total they were able to capture 1265 EGCs, clustered into four different subsets. Similar to what was described for the stromal component, up-regulated genes identified in these subsets were associated with abnormal ECM expression and ganglion cell apoptosis, in aganglionic segments. The authors also identified the transcription factor NR2F1 in EGC subsets and suggested its involvement in regulating ECM expression and tissue fibrosis in aganglionic segments. Interestingly, the authors found increased *ZEB2* expression in EGCs and stromal subsets in aganglionic segments. *ZEB2* is the causal gene for Mowatt-Wilson syndrome, which includes HSCR among its associated symptoms.[9] It encodes a crucial transcription factor involved in the development, migration, and differentiation of ENCCs, but it is also known to regulate fibrotic pathways such as transforming growth factor beta (TGF-β).[9]

This study highlights new molecular insights in the behaviour of specific cell populations present in the aganglionic, ganglionic and unaffected healthy intestines, showing that various ECM and fibrosis-related genes were overexpressed in the aganglionic segments. It also showed that activation of fibroblasts and myofibroblasts were increased, elucidating the mechanisms behind intestinal fibrosis in HSCR. One of the limitations of this study is that enteric neurons were not directly analysed and HSCR-related genes such as *RET*, *ZEB2*, *SOX10*, *PHOX2B*, *PAX3* and *EDNRB* were only evaluated in other cellular subsets. To gain a better understanding of the changes that occur in the intestine of HSCR patients, additional research is required. This research should concentrate on the isolation of both neuronal and non-neuronal intestinal cells simultaneously, by improving dissociation protocols and optimizing fluorescence-activated cell sorting.[10] Nevertheless, the findings of this study provide a better understanding on the relationship between fibrotic changes, ENS and Hirschsprung-associated enterocolitis and may lead to the development of new treatment options for severe co-morbidities commonly seen in HSCR patients.

ORCID

Erwin Brosens https://orcid.org/0000-0001-8235-4010

REFERENCES

1. Brosens E, Burns AJ, Brooks AS, et al. Genetics of enteric neuropathies. *Dev Biol*. 2016;417(2):198-208.
2. Kyrklund K, Sloots CEJ, de Blaauw I, et al. ERNICA guidelines for the management of rectosigmoid Hirschsprung's disease. *Orphanet J Rare Dis*. 2020;15(1):164.
3. Heuckeroth RO. Hirschsprung disease - integrating basic science and clinical medicine to improve outcomes. *Nat Rev Gastroenterol Hepatol*. 2018;15(3):152-167.
4. Tilghman JM, Ling AY, Turner TN, et al. Molecular genetic anatomy and risk profile of Hirschsprung's disease. *N Engl J Med*. 2019;380(15):1421-1432.
5. He S, Wang J, Huang Y, et al. Intestinal fibrosis in aganglionic segment of Hirschsprung's disease revealed by single-cell RNA sequencing. *Clin Transl Med*. 2023;13(2):e1193.
6. Elmentaite R, Kumasaka N, Roberts K, et al. Cells of the human intestinal tract mapped across space and time. *Nature*. 2021;597(7875):250-255.
7. Ji Y, Tam PK, Tang CS. Roles of enteric neural stem cell niche and enteric nervous system development in Hirschsprung disease. *Int J Mol Sci*. 2021;22(18):9659.
8. Deng C-C, Hu Y-F, Zhu D-H, et al. Single-cell RNA-seq reveals fibroblast heterogeneity and increased mesenchymal fibroblasts in human fibrotic skin diseases. *Nat Commun*. 2021;12(1):3709.
9. Teraishi M, Takaishi M, Nakajima K, et al. Critical involvement of ZEB2 in collagen fibrillogenesis: the molecular similarity between Mowat-Wilson syndrome and Ehlers-Danlos syndrome. *Sci Rep*. 2017;7(1):46565.
10. Windster JD, Sacchetti A, Schaaf GJ, et al. A combinatorial panel for flow cytometry-based isolation of enteric nervous system cells from human intestine. *EMBO Rep*. e55789. https://doi.org/10.15252/embr.202255789

How to cite this article: Alves MM, Brosens E. More than missing neurons: Intestinal fibrosis in Hirschsprung disease. *Clin. Transl. Disc.* 2023;3:e185. https://doi.org/10.1002/ctd2.185