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The enteric nervous system: From embryology to therapy

This special issue of *Developmental Biology* on the "Development of the enteric nervous system" comprises ten articles representing the major topics discussed at the international conference "Development of the enteric nervous system: cells, signals, genes and therapy" held in Rotterdam, the Netherlands in April 2015 (see Supplementary Fig. 1 for participants). This was the fourth conference of its kind, the others being in New York in 2006, London 2009 and Hong Kong 2012.

To encompass the variety of disciplines that study of enteric nervous system (ENS) development entails, the conference in Rotterdam included sessions ranging from basic developmental biology of the ENS, diseases associated with ENS malformation, including Hirschsprung disease (HSCR) and non-HSCR enteric neuropathies, genetic variations associated with neurointestinal diseases, "developmental physiology" of the ENS, the role of the microenvironment, with a particular focus on medications and the microbiome, and the development of novel therapies for ENS developmental disorders. This latter topic prompted the addition of the word "therapy" in the title of the 2015 conference to reflect the significant growth of research in the field (over 50 papers during the period 2012–2015) that is currently being directed toward the development of an ENS stem cell therapy for enteric neuropathies such as HSCR (Burns and Thapar, 2014). The inclusion of sessions on "Cell therapy for enteric neuropathies" attracted a wider audience than the previous conferences, including clinicians and basic scientists interested in translational research. Although this area has been growing steadily since the early 2000s, significant advances have been made in the last few years at the pre-clinical level. For example, Hotta et al., reported transplantation of ENS progenitors into the gut of postnatal mice *in vivo* and demonstrated the generation of functional enteric neurons (Hotta et al., 2013). Other work presented at the meeting and recently published in *Nature*, reported use of ENS lineages, derived from human pluripotent stem cells, as a source of stem cells for rescue of disease-related mortality following transplantation into the *Ednrb*$^{-/-}$ mouse model of HSCR (Fattahi et al., 2016). The meeting included a forum discussion where researchers with an interest in ENS stem cell therapy agreed to write a White Paper that provides expert opinion on the best approaches currently available to identify, isolate, purify, expand and optimize ENS stem cells/progenitors, transplant them into gut, and ultimately restore gut function. This White Paper, written by 30 authors, also appears in this current Special Issue (Burns et al., 2016).

Another area of intense interest is the genetics of diseases associated with the absence or malfunction of the ENS. For HSCR, there is now strong evidence that variations and mutations in the *RET* gene are present in almost all HSCR patients (Alves et al., 2013). However, it is also clear that these variations usually only contribute to a small part of the overall genetic risk, with the "missing heritability" increasingly being accounted for by new genes and loci, as well as epigenetic modifications (Torroglosa et al., 2016). Recent studies found that several HSCR genes also affect the central nervous system (CNS), reinforcing the concept of the ENS as the "second brain" (Avetisyan et al., 2015; Gershon, 1999). Indeed, in a paper published in *Cell*, misexpression of the autism spectrum disorder-associated gene, CHD8, in zebrafish resulted in brain defects as well as a reduction in enteric neuron number, revealing unexpected comorbidities between CNS and ENS development (Bernier et al., 2014).

While HSCR has been the major disease examined by ENS development researchers, the etiologies of non-HSCR neuropathies, such as chronic intestinal pseudo-obstruction (CIPO), are now receiving increasing attention (reviewed by Goldstein et al. (2016)). This group of diseases is considered very heterogeneous and difficult to diagnose. Perhaps not surprisingly, genetic studies have identified many different disease-causing genes, although it could be hypothesized that related mechanisms underlie the pathophysiology of these diseases. Data to support this idea are reviewed in the article by Brosens et al. (2016).

Finding genetic changes in patients is one thing, but proving that they are disease-causing is another. Animal models including chick, mouse and zebrafish are essential for unravelling how newly identified genes are involved in ENS development (see articles by Bondurand and Southard-Smith (2016), and Heanue et al. (2016)). Animal models are not only extremely powerful as reverse genetic tools where phenotypes are examined after DNA sequences are altered, but they can also be used for forward genetic screens as recently shown by Pilon and colleagues and presented at the meeting. These authors, using an insertional mutation screen for loci affecting neural crest cell development in mice, identified several new genes that were shown to be crucial for ENS development and play an important role in hindgut aganglionosis (Bergeron et al., 2015; Soret et al., 2015).

As well as inherited mutations, environmental factors can also influence ENS development. This is supported by interesting studies from the Heuckeroth group showing that non-genetic factors, including vitamin A deficiency and certain medications, contribute to ENS defects. These authors demonstrated that the immunosuppressive medicine mycophenolate, an inhibitor of *de novo* guanine nucleotide biosynthesis, causes major ENS malformations and HSCR-like pathology in a mammalian model (Lake et al., 2013). Moreover, very recent studies show that ibuprofen causes a HSCR-like absence of enteric neurons in the distal bowel (Schill et al., 2016). Based on these findings, Heuckeroth and colleagues propose that HSCR might be preventable in some genetically susceptible children. This topic is reviewed by Heuckeroth and Schafer (2016).

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A possible glimpse into the future for the ENS development field was given by the keynote speaker, John Cryan (see article by Hyland and Cryan (2016)), who provided an overview of the influence of the gut microbiota on the brain and ENS. Resident microorganisms can influence the development and function of the ENS via interactions involving microbe-derived components, metabolites or other signals that affect enteric neuronal activity and gut function (Carabotti et al., 2015). This is not a one-way street, as the ENS also can also affect the microbiota, and a bi-directional pathway of communication between the microbiota and the brain via the microbiome-gut-brain axis is proposed to have wide-ranging implications for health and disease (Borre et al., 2014; Mayer et al., 2015).

Although much progress has been made in our understanding of the development of the neural crest-derived precursors that form the intrinsic ENS (their migration, proliferation, differentiation and survival within the gut), as well as the development of the extrinsic gut innervation (see Uesaka et al. (2016)), we know much less about how enteric neurons become electrically active, communicate with each other, form circuits, and innervate target cells to regulate gastrointestinal functions including motility. Nevertheless, some of these aspects of ENS development are beginning to be unraveled by Pieter Vanden Berghe and colleagues (see article by Hao et al. (2016)), and by Vassilis Pachis and colleagues (Kabouridis and Pachnis, 2015). Hopefully there is now a critical mass of researchers with expertise in developmental biology, physiology, genetics, and the microbiome to address these and other key issues in the coming years. It will be exciting to hear about the next series of advances, including translational approaches and stem cell therapies for enteric neuropathies, when the ENS development field reconvenes its international conference in April 2018 in Boston, USA, hosted by Allan Goldstein. We hope to see you there.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.ydbio.2016.08.013.

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Alan J. Burns *
Stem Cells and Regenerative Medicine, Birth Defects Research Centre, UCL Great Ormond Street Institute of Child Health, London, UK
Department of Clinical Genetics, Erasmus Medical Center, Rotterdam, The Netherlands
E-mail address: alan.burns@ucl.ac.uk

Robert M.W. Hofstra
Stem Cells and Regenerative Medicine, Birth Defects Research Centre, UCL Great Ormond Street Institute of Child Health, London, UK
Department of Clinical Genetics, Erasmus Medical Center, Rotterdam, The Netherlands

* Corresponding author.