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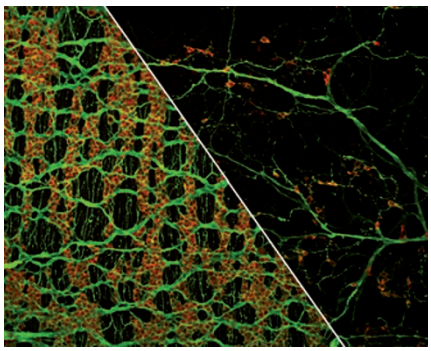
PAEDIATRIC GASTROENTEROLOGY

Role for nongenetic factors in the development of Hirschsprung disease?

Robert Heuckeroth and colleagues have found that the immunosuppressant mycophenolic acid (MPA) causes a Hirschsprung-like pathology in mouse models of the disease, providing hope that some cases of Hirschsprung disease (HSCR) might be preventable.

1 in 5,000 infants are born with HSCR, which is characterized by the absence of the enteric nervous system (ENS) at the end of the bowel (aganglionic bowel). "Although surgical resection of the aganglionic bowel dramatically reduces the risk of death and reduces morbidity, death rates from HSCR in the modern era are about 5%," explains Heuckeroth.

Heuckeroth's team investigated the role of nongenetic factors because none of the many genetic defects known to predispose to HSCR are completely penetrant. In addition, his group previously found that GSK3β antagonists and vitamin A deficiency slow ENS precursor migration through the bowel. As ENS precursors have a long migratory route and most children with HSCR have only a short region of aganglionic bowel, modest increases in ENS precursor colonization efficiency could prevent this life threatening disease. "GSK3 is the target of lithium and is inhibited by several other medicines," says Heuckeroth. "I realized at this point that some cases of HSCR might be induced by nongenetic factors."



A normal ENS (left) versus aganglionic bowel (right) in a mouse model of Hirschsprung disease. During gestation the mouse with the aganglionic bowel received mycophenolic acid. Image courtesy of R. O. Heuckeroth.

Initially, a small-scale experiment was performed to show that drug screening in zebrafish worked. Drugs were added to fish eggs fertilized *in vitro* shortly after bowel formation, but before entrance of neural-crest-derived ENS precursors into the bowel; the fish were kept in the drugs until the time neurons are normally detected at the end of the bowel in wild-type fish. A much larger screen with 1,508 drugs followed and 9 systemically administered compounds consistently inhibited ENS development in zebrafish.

Jonathan Lake in Heuckeroth's lab then focused his efforts on MPA because it is frequently used, is known to work primarily by inhibiting *de novo* GMP synthesis, and its median toxic dose is within therapeutic levels. MPA was shown to cause extensive aganglionosis in mice with genetic mutations predisposing to Hirschsprung-like disease, even when the mutation or drug individually did not cause disease.

Further experiments demonstrated that MPA did not affect the ability of the ENS precursors to 'move' but did affect their ability to divide. "Since cell proliferation is essential for ENS precursors to migrate through the bowel, we now believe that anything that reduces ENS precursor proliferation will increase HSCR risk," explains Heuckeroth. "This work provides powerful evidence that gene–environment interactions may be very important for determining if HSCR occurs in a genetically susceptible child."

As well as using model systems to investigate which other nongenetic factors might cause defects in ENS development, the team plans to extend their current observations to humans. As such, they are partnering with Allen Mitchell and colleagues at Slone Epidemiology Center in Boston to undertake a national study of HSCR epidemiology.

"We are now quite convinced that some cases of HSCR can be prevented. We hope when human studies are completed to be able to provide practical advice to at-risk families about changes they can make to reduce HSCR risk in their future children," Heuckeroth concludes.

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Original article Lake, J. I. *et al.* Hirschsprung-like disease is exacerbated by reduced do novo GMP synthesis. *J. Clin. Invest.* doi:10.1172/JCI69781