Hirschsprung’s disease, first described in 1888 by Dr Harald Hirschsprung, is a congenital abnormality characterised by the blockage of lower gastrointestinal tract with an incidence of 1/5000 live births. It is also called congenital megacolon or intestinal aganglionosis. The diseased is typically presented as an inability to pass meconium and vomiting in neonates or in less severe cases, as chronic constipation and abdominal bloating in an older child. The common morphological change is the absence of nerve cells (the enteric nervous system, ENS) from the wall of affected gut.

It has become clear that apart from blocked gut, some patients are accompanied by various neurological disorders. These include Downs’s syndrome (trisomy 21), congenital central hypoventilation, nerve related deafness, seizures and mental retardation (Moore 2011). This is not surprising since Hirschsprung’s disease is the results of chromosome anomalies and the mutations of a dozen known genes and probably more uncharacterised genes. Strictly speaking, Hirschsprung’s disease is not a single well defined disease but a part of different syndromes caused by chromosomal anomalies and gene mutations. The common feature of these genes is the regulation of the development of neural crest cells that give rise to the ENS and other cells. Furthermore, the perturbation of these gene products results in not only the mal-development of ENS but also the central nervous system.

A large number of chromosomal anomaly (e.g. trisomy 21) and gene mutations have been described in patients with Hirschsprung’s disease. The mutated genes include those for endothelin receptor gene B (EDNRB), endothelin 3, glial cell line derived neurotrophic factor, receptor tyrosine kinase, and several others. It is important to note that these genes account only for about 50% of known cases of Hirschsprung’s disease. More genes or combination of genes that have not been described so far must be responsible for the remaining cases of Hirschsprung’s disease. Each of these gene products often has widespread distribution in the body and hence their mutation will affect multiple systems. For instance, EDNRB is expressed in human cerebral cortex, cerebellum, intestine, adrenal gland, lung and kidney. It is not surprising that EDNRB mutation may have adverse effects on various organs where it normally expresses such as the brain.

Brain anomalies have been reported in about 7% patients, including microcephaly, poor brain growth and development, and absent corpus callosum (Moor 2011). However, the histological changes in the brain have never been studied, obviously due to the inaccessibility of the brain tissues from Hirschsprung’s patients. To address this problem, animal models have been introduced. Spotting lethal (sl) rat is an established rat model of Hirschsprung’s disease with similar gene mutation (EDNRB) and presentation as in human cases. The homozygous sl rat carries a mutation of EDNRB and hence lacks a functional EDNRB in the body. Previous studies of others and ours revealed a significant cell loss in brain regions including the cerebellum, hippocampus and cerebral cortex of neonatal sl rats (Riechers 2004, Vidovic et al. 1998, Song et al. 2013). The cell loss is due to an increase in cell death and a decrease in cell proliferation. At this stage, our two labs (Zan-Min Song at ANU and David Croaker at the Canberra Hospital) are investigating the morphological and functional changes.
in the central nervous system of adult sl rats and explore the possibility of treating brain cell deficit with stem cell therapy.

The structural abnormalities and functional consequences in the brains of adult sl rats have not been studied due to the premature death of sl rats. To overcome this problem, we have developed a surgical procedure (colostomy) to make an opening to the gut through the abdominal wall of 5-7 day-old sl rats. The operated animals gained weight similar to their littermates and lived to adulthood. This operation makes our proposal of studying the adult brains of sl rats possible for the first time. We will compare between diseased and normal rats, including the volumes and histological changes in each brain region, as well as functional changes in key brain regions that structural anomaly has been identified. Our current focus is on two regions: the cerebellum that controls the motility and balance of the body and the hippocampus that regulates learning and memory.

To expand this project further, we aim to correct the nerve cell deficit by transplanting healthy stem cells that can develop into nerve cells and glial cells in diseased cerebellum. We have demonstrated active cell proliferation in the postnatal cerebellum of normal rat (Vidovic et al, 2008). We also established a method of isolating stem cells from the cerebellum of normal neonatal rat and genetically label the cells with a green protein. The labelled cells will grow into a ball of cells called neurosphere, which can be transplanted into the cerebellum of sl rat. Cells in neurospheres are likely to develop into functional nerve cells and glial cells in brains of recipient sl rats. Following transplantation of neurospheres into defective cerebellum, we will assess the restoration in cerebellar functions. We will also examine the extent to which the introduced stem/progenitor cells proliferate, migrate and differentiate into neurons and glial cells in the cerebellum of recipient sl rat.

We expect that our research will demonstrate, for the first time, the structural changes in various brain regions in adult rats with Hirschsprung’s disease. We will further demonstrate any functional deficit related to the structural abnormality. Most importantly, our results will reveal structural integration of transplanted neural stem/progenitor cells in recipient rats and possible functional recovery. The results will provide important information on how the stem cell technique be developed for treatment of brain anomalies in human Hirschsprung’s disease.

References:
Song Z-M et al. (2013) 3rd International Neural Regeneration Symposium, Shenyang, China.