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4.1 Introduction

Humans are not the only mammals to suffer from aganglionosis. Aganglionosis has also been described in mice, rats, horses, cats and dogs. Rodent animal models have contributed significantly to our understanding of Hirschsprung’s disease (HSCR). Over the last decade, the understanding of the genetics and cell biology of the development of the enteric nervous system (ENS) has made great progress. Rodent animal models have shown many points of correlation with humans in regard to ENS development, both normal and abnormal. Nevertheless, the link between the genotype and the phenotype is often indirect, and so many questions have yet to be answered. This chapter deals with the characteristics of aganglionosis in rodents with emphasis on how knowledge of the animal models has contributed to our understanding of the genetics and pathogenesis of HSCR and allied disorders.

4.2 History

4.2.1 Rodents

The first description of aganglionosis in mice was by Derrick and St George-Grambauer in 1957 [1]. They found approximately 3.2 per 1000 of their colony developed aganglionosis. The average length of aganglionosis was 15–20 mm with colonic distension extending into the first few millimeters of aganglionic colon. There was no association with a white or patched color coat. The next description was by Bielschowsky and Schofield in 1962 [2]. In this colony 10% of the offspring were affected and there was an association with a white colored coat. These mice also had a high incidence of mammary cancer and pituitary adenomas. Outbreeding experiments suggested an autosomal recessive trait with modification of the trait by other genetic factors.

In 1966, Lane described two strains of mice which developed aganglionosis as an autosomal recessive condition [3]. The lethal spotting (ls) mice have approximately 2 mm of aganglionosis with a patched coat, and later studies linked the defect to chromosome 2. Piebald lethal (s’i) mice had approximately 10 mm of aganglionosis and linkage studies suggested that the defect was on chromosome 14. In 1979, Ikadai et al. [4] described aganglionosis in spotting lethal (sl) rats. The animals had two lengths of aganglionosis: total colonic aganglionosis (TCA) and mid colon. These animals again showed autosomal recessive inheritance and had a white colored coat. A fourth rodent model is the Dominant megacolon (Dom) mouse
in which the aganglionic colon has a long hypoganglionic transition zone (Table 4.1) [5].

### 4.2.2 Other Mammals

There have been isolated reports of aganglionosis in a range of animals including cats [6, 7], horses [8–11] and pigs [12, 13].

### 4.3 Histologic Anatomy

Are rodents good histologic models of HSCR? The first histologic studies were performed by Lane [3]. These were restricted to showing there was aganglionosis in the terminal bowel, and in documenting the length of aganglionosis.

Bolande and Towler [14] and Bolande [15] investigated the lethal spotting mouse using histochemical and ultrastructural studies. Histology showed hypoganglionosis in the distal bowel but there was no dense ingrowth of nerve fibers. Boley [16] suggested that these findings of no hypertrophied nerve trunks indicated that these mice were not a good model of human disease. In the distal narrowed segment there was a reduction in adrenergic and cholinergic fibers. In the dilated part of the bowel there was an increase in adrenergic fibers. The ultrastructural studies showed that just above the transition zone, there were secondary degenerative changes in the ganglion cells, which increased with age, resulting in what appeared to be secondary cell death and abiotrophy. Webster [17, 18] performed detailed studies in lethal spotting and piebald lethal mice using cholinesterase stains and fluorescence to delineate the adrenergic nerves. In postnatal mice of both strains he demonstrated normal innervation in the proximal bowel followed by a transition zone and then an aganglionic zone with increased nerve trunks and a decrease in the innervation of the circular muscle fibers. In the most distal aganglionic colon, just above the anal sphincters, there appeared to be a variable, but denser, innervation to the circular muscle involving cholinergic nerves. Bu’Lock et al. [19] found a selective depletion of substance P in the transitional zone in piebald lethal mice. However, the change was from 10% in normal mice to 5% in mutant mice, and the study failed to confirm a previous report from the same laboratory of a decrease in substance P in the mutant ileum, indicating that the variability between animals and sensitivity of the techniques can make conclusions difficult.

In the spotting lethal (sl) rat model Ikadai et al. [4, 20] and Horie et al. [21] studied the length of aganglionosis and found that there were two subgroups, one in which there was TCA and a second, less numerous group, in which ganglion cells extended to the proximal half of the colon. The visible cone was often distal to the commencement of aganglionosis. In a histologic study of sl rats using whole-mounts and AchE, tyrosine hydroxylase and substance P, Nagahama et al. [22] showed aganglionosis of the colon along with increased nerve trunks. These changes are similar to those seen in humans with TCA. However, even in the proximal ganglionated duodenum there were changes in the two dimensional structure of the enteric plexus, with the lattice pattern being irregular. This raises the question as to whether the proximal gut is entirely normal, and if there is histologic abnormality in some of the subtle architecture, does this mean diminished function? The bowel also has many functions, so it may be possible for example that water absorption is affected while propulsive activity is normal.

An ultrastructural study in the sl rat model confirmed that almost no nerve terminals were present in the circular muscle layer of any regions of the constricted intestine, but some terminals were observed in the longitudinal muscle layer of that segment. The authors concluded that the denervated circular muscle layer is related to the production of a constricted segment, irrespective of the presence or absence of nerve terminals in the longitudinal muscle layer [23].

<table>
<thead>
<tr>
<th>Name</th>
<th>Length of aganglionosis</th>
<th>Pigment</th>
<th>Inheritance</th>
<th>Locus</th>
<th>Genetic defect</th>
<th>Percent in human HSCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lethal spotting mice (ls)</td>
<td>2mm</td>
<td>Patched</td>
<td>Recessive</td>
<td>Chr.2</td>
<td>Point mutation of EDN3</td>
<td>5</td>
</tr>
<tr>
<td>Piebald lethal mice (sl)</td>
<td>10mm</td>
<td>White</td>
<td>Recessive</td>
<td>Chr.14</td>
<td>Absent EDNRB</td>
<td>&lt;10</td>
</tr>
<tr>
<td>Spotting lethal rat (sl)</td>
<td>TCA</td>
<td>White</td>
<td>Recessive</td>
<td>Chr.6</td>
<td>301 bp deletion in EDNRB</td>
<td>&lt;10</td>
</tr>
<tr>
<td>Dominant megacolon (Dom)</td>
<td>Variable</td>
<td>White</td>
<td>Dominant</td>
<td>Chr.15</td>
<td>Point mutation of SOX10</td>
<td>&lt;1</td>
</tr>
</tbody>
</table>

**Table 4.1** Naturally occurring rodent animal models of HSCR