Brief papers

Hirschsprung's disease associated with a deletion of chromosome 10 (q11.2q21.2): a further link with the neurocristopathies?

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Abstract

We report a patient with total colonic aganglionosis in association with a deletion of part of the long arm of chromosome 10: (del(10)(q11.2q21.2)). This deletion includes the ret proto-oncogene, which has recently been implicated in multiple endocrine neoplasia type 2A (MEN 2A). The possible links between Hirschsprung's disease and the neurocristopathies and the aetiological role of abnormalities of neural crest development in these conditions are discussed.

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Hirschsprung's disease (HD) occurs in approximately 1 in 5000 infants with an overall male predominance (5:1). However, the sex ratio decreases and the recurrence risk to sibs increases as the aganglionosis becomes more extensive. In the 4% of patients with total colonic aganglionosis (TCA) the sex incidence is equal. Badner *et al*¹ have suggested, on the basis of a large pedigree analysis, that a dominant gene or several different dominant genes with incomplete penetrance play a role in the aetiology of HD extending beyond the sigmoid colon. However, the mode of inheritance for shorter segment HD is less clear. Several conditions have been noted in association with HD, mostly in single case reports. Among these are reports of HD in patients with neurocristopathies, including MEN type 2A.²⁻⁴ We report a case with TCA and a deletion in the proximal long arm of chromosome 10, which includes the gene for MEN type 2A.⁵ We are aware of one previously reported similar case.⁶

Case report

A female infant was born at term weighing 2700 g (below the 3rd centile), the only child of healthy, unrelated parents, with no significant family history. At the age of 24 hours she developed bile stained vomiting and had not passed meconium. Laparotomy showed a volvulus, apparently associated with meconium ileus, and 20 cm of terminal ileum was

resected. The infant was thought to have cystic fibrosis, although initial sweat testing was equivocal, and she was negative for the common gene mutations. Postoperatively she thrived, although with intermittent vomiting.

At the age of 3 months the child presented with small bowel obstruction. Laparotomy showed a dilated small bowel with an apparently normal colon. Rectal biopsy was thought to contain ganglion cells, although the specimen was poorly preserved. An ileostomy was formed, and the child thrived. Reversal of ileostomy was performed at the age of 8 months, but small bowel obstruction occurred in the immediate postoperative period, necessitating a further ileostomy. Colonic biopsy showed complete absence of ganglion cells at all sites sampled, confirming a diagnosis of total colonic Hirschsprung's disease. Sweat test at this stage was normal.

Resected ileum was cultured for DNA investigations because of persisting uncertainty over the possible diagnosis of cystic fibrosis. Cytogenetic analysis, performed routinely on cells cultured for molecular studies, showed an interstitial deletion from the proximal long arm of chromosome 10 (46,XX,del(10)(q11)). This was subsequently confirmed on a blood specimen as del(10)(q11.2q21.2) (figure). Both parents had normal karyotypes.

The limits of the deletion were further refined by DNA hybridisation analyses using DNA markers mapped to chromosome 10q. High molecular weight DNA isolated from cultured fibroblasts was digested with appropriate restriction enzymes, fractionated by gel electrophoresis, transferred to nylon membranes, and hybridised to probes for the chromosome 10 region, as previously described.^{7,8} The probe loci examined and their chromosomal positions are indicated in the table. Densitometric comparisons of allele intensities for loci on chromosome 10q and on chromosome 17 (NF1) were performed for the patient and for three normal control samples. Two copies of each locus were present in each control DNA sample. In DNA from the patient, a single copy of loci distal to D10S141 and proximal to D10S5 were detected. Two copies of all other chromosome 10 loci and of control loci on chromosome 17 were present.

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Partial karyotype of the patient showing *del* (10)(q11.2q21.2).

These data are summarised in the table. Our results suggest that the proximal deletion limit in the patient lies in 10q11.2 between the D10S141 locus and the *ret* proto-oncogene. The distal breakpoint lies in 10q21 between D10S5 and D10S22. The molecular size of this deletion is difficult to estimate, but it spans a genetic distance of more than 25 cM (sex averaged).¹⁰ Recent genetic linkage analyses have suggested that a gene for familial Hirschsprung's disease may lie within this region.^{11,12}

The patient underwent a Martin-Duhamel pull through at the age of 18 months, with closure of her ileostomy eight weeks later. Her subsequent course was complicated by mal-absorption requiring a prolonged period of parenteral nutrition. However, she is now fully enterally fed with weight just below the 3rd centile.

Apart from her bowel problems, the patient is healthy with no dysmorphic features. However, her occipitofrontal circumference increased from the 50th centile at birth to above the 90th centile at 1 year, and cranial CT

scan showed mildly enlarged lateral and fourth ventricles. The occipitofrontal circumference has increased steadily along the 97th centile, and further intervention has not been required. Developmental assessment at the age of 30 months indicated a cognitive level of 21 months with a greater motor delay. At least part of this is felt to be because of her prolonged hospitalisation.

Discussion

Hirschsprung's disease results from congenital absence of intramural ganglia affecting the rectum with variable proximal extension. Despite advances in the treatment of the condition, its pathogenesis remains uncertain. Current evidence favours an abnormality in the microenvironment of the embryonic bowel resulting in abnormal migration or differentiation of primitive neural crest cells.¹³ In one mouse model of HD, the lethal spotted mouse, the distal 2 mm of bowel fails to innervate normally¹⁴ and this section of the bowel is not colonised by neural crest cells from a variety of sources.¹⁵

Congenital megacolon occurs in the mouse, rat, and horse, and is considered to be a model for HD in man. In each case, a single gene mutation is responsible. Interestingly, in the piebald-lethal mouse model the locus is on chromosome 14, whose proximal long arm is homologous to human chromosome 10q11q21.¹⁶ In humans, familial cases of HD are well recognised, and a recent analysis of 487 patients and their families concluded that the mode of inheritance is compatible with one or more defective single genes with variable penetrance, at least for long segment disease.¹ HD has been reported in association with various conditions, including a deletion of chromosome 13.^{17,18} It has also been described in association with neuroblastoma, neurofibromatosis type 1, pheochromocytoma, and congenital central hypoventilation,^{2,3} as well as with multiple endocrine neoplasia type 2A (medullary carcinoma of the thyroid, pheochromocytoma, and parathyroid hyperplasia).⁴ All of these conditions may be classified as neurocristopathies, or conditions originating from abnormal development of neural crest cells.

To our knowledge, our patient is the second case of total colonic HD reported with a deletion in the long arm of chromosome 10. The other patient, an Italian girl with a 46,XX,del(10)(q11.21q21.2) karyotype, is developmentally normal at the age of 2½ years, with no other obvious abnormalities apart from total colonic aganglionosis.⁶ These cases are of particular interest as the chromosome 10 deletion includes the *ret* proto-oncogene. Mutations of *ret* are the underlying cause of MEN 2A and familial medullary thyroid carcinoma. Verdy *et al*⁴ found HD in eight of his series of 92 patients with MEN 2A, but we are not aware of any cytogenetic analyses in these cases. Interstitial deletions of 10q are uncommon. Three patients have been reported with deletions in the same region as our patient. The

Deletion of chromosome 10 markers in our patient with Hirschsprung's disease

Locus*	Location*	Hirschsprung's patient
ZNF33B	10q11.2	+
D10S97	10q11.2	+
D10S141	10q11.2	+
RET	10q11.2	—
D10S94	10q11.2	—
D10S102	10q11.2	—
D10S30	10q11.2	—
RBP3	10q11.2	—
D10S15	10q11.2	—
D10S5	10q21.1	—
D10S22	10q21.1	+
CHAT	10q11.2-q22.1	+
D10S170	10q21	+
NF1	17q11.2	+

+ = locus not deleted, — = locus present in only one copy.

*Information regarding these loci is described by Williamson *et al*.⁹

children described by Lobo *et al*¹⁹ (del(10)(q11.1q22.1)) and Ray *et al*,²⁰ (del(10)(q11q21)) both had dysmorphic features including a broad forehead and hypertelorism, together with hypotonia and psychomotor delay. The patient described by Holden and MacDonald²¹ (del(10)(q11.2q21)) had developmental delay, hypotonia, and seizures. However, bowel abnormalities were not reported in any of the cases. It is difficult to be certain from the cytogenetic studies presented how these deletions correspond to that in our patient, and it would be interesting to define them further by DNA analysis.

Attention has focused recently in mice, and other animals, on the role of homeobox and Pax genes in regulating development and cellular differentiation. Wolgemuth *et al*²² have shown overexpression of the homeobox containing gene Hox-1.4 in the embryonic gut of mice, associated with congenital megacolon. More recently, Tassabehji *et al*²³ have shown that some families with Waardenburg's syndrome (a condition probably resulting from abnormal neural crest development) have mutations in the human homologue of the mouse Pax-3 gene. Mutation in the murine gene affects structures derived from the neural crest. The deletion in our patient spans the ret proto-oncogene, mutation of which has been implicated in the neurocristopathies MEN 2A and familial medullary thyroid carcinoma. Recently, genetic linkage analyses in families with HD have shown that a gene for an inherited form of the disease lies in 10q11.2, close to the ret locus.^{11 12} Taken together these data suggest that the ret proto-oncogene is a strong candidate gene for HD. Therefore, further analysis of chromosome 10 in patients with HD, particularly those with total colonic involvement or associated neurocristopathies, is warranted to investigate this possibility.

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