Long segment and short segment familial Hirschsprung's disease: variable clinical expression at the RET locus

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Abstract

Hirschsprung's disease (aganglionic megacolon, HSCR) is a frequent condition of unknown origin (1/5000 live births) resulting in intestinal obstruction in neonates and severe constipation in infants and adults. In the majority of cases (80%), the aganglionic tract involves the rectum and the sigmoid colon only (short segment HSCR), while in 20% of cases it extends toward the proximal end of the colon (long segment HSCR). In a previous study, we mapped a gene for long segment familial HSCR to the proximal long arm of chromosome 10 (10q11.2). Further linkage analyses in familial HSCR have suggested tight linkage of the disease gene to the RET proto-oncogene mapped to chromosome 10q11.2. Recently, nonsense and missense mutations of RET have been identified in HSCR patients. However, the question of whether mutations of the RET gene account for both long segment and short segment familial HSCR remained unanswered.

We have performed genetic linkage analyses in 11 long segment HSCR families and eight short segment HSCR families using microsatellite DNA markers of chromosome 10q. In both anatomical forms, tight pairwise linkage with no recombinant events was observed between the RET proto-oncogene locus and the disease locus ($Z_{\max}=2.16$ and $Z_{\max}=5.38$ for short segment and long segment HSCR respectively at $\theta=0\%$). Multipoint linkage analyses performed in the two groups showed that the maximum likelihood estimate was at the RET locus. Moreover, we show that point mutations of the RET proto-oncogene occur either in long segment or in short segment HSCR families and we provide evidence for incomplete penetrance of the disease causing mutation. These data suggest that the two anatomical forms of familial HSCR, which have been separated on the basis of clinical and genetic criteria, may be regarded as the variable clinical expression of mutations at the RET locus.

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Hirschsprung's disease (aganglionic megacolon, HSCR) is a congenital disorder of unknown origin characterised by the absence of parasympathetic intrinsic ganglion cells in the submucosal and myenteric plexuses of the hindgut.¹ This common condition (1/5000 live births) results in intestinal obstruction in neonates² and in severe constipation in infants and adults.³ HSCR is regarded as the consequence of the premature arrest of the craniocaudal migration of neural crest cells towards the anal end of the rectum, between the fifth and twelfth week of gestation.⁴ The earlier the cessation of migration, the larger the aganglionic segment. In the majority of cases (80%), the aganglionic tract involves the rectum and the sigmoid colon only (short segment HSCR) while in 20% of cases it extends towards the proximal end of the colon (long segment HSCR).

We and others have previously mapped a gene for long segment familial HSCR to the proximal long arm of chromosome 10 (10q11.2).^{5,6} Further linkage analyses in familial HSCR have suggested tight linkage with the RET proto-oncogene locus.^{7,8} Shortly thereafter, we identified nonsense and missense mutations in the extracellular domain of the RET proto-oncogene in four HSCR families linked to chromosome 10q11.2 and in two other families unsuitable for linkage.⁹ Point mutations affecting the tyrosine kinase domain of the RET proto-oncogene have been reported consistently.¹⁰ However, the question of whether short segment familial HSCR maps to the same region remained unanswered.

Here, we show that long segment and short segment familial HSCR are tightly linked to the RET locus and that mutations of the RET proto-oncogene occur in both clinical forms, suggesting that short segment and long segment familial HSCR may be considered as the variable clinical expression of mutations at the RET locus.

Patients

A total of 45 patients and 77 relatives belonging to 19 non-consanguineous multiplex families were included in the study (figs 1 and 2). Partial linkage data on families 1-5, 7, and 12-17 have been presented in a previous study.⁵ Histopathological criteria for inclusion in the study were: (1) increased acetylcholinesterase histochemical staining in nerve fibres on suction biopsies of

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Long segment HSCR

Figure 1 Pedigrees of the long segment HSCR families. Persons are presented with their genotypes at the RET locus (microsatellite sTCL-2).

the rectal submucosa¹¹ and (2) absence of neuronal ganglia on operative histochemical or histological evaluation of the aganglionic tract. Families were divided into two groups according to the length of the aganglionic tract: (1) long segment HSCR (families 1–11, fig 1) when aganglionosis extended beyond the sigmoid colon (total colonic aganglionosis and aganglionosis involving the small intestine were included in this group),¹² and (2) short segment HSCR (families 12–19, fig 2) in rectal and rectosigmoid HSCR. When intrafamilial heterogeneity was noted, families were classified according to the most severely affected person. Subjects with severe constipation starting in childhood or radiological megacolon with disparity of calibre or both were considered to be affected, even when no histopathological data were available (nine persons). Finally, atypical forms associated with intestinal neuronal dysplasia, hearing loss, pigmentary disorders, dysmorphic features, malformations, or chromosomal anomalies were excluded from the study.

Methods

HSCR families were genotyped using one pericentromeric microsatellite DNA marker

(probe AFM183xg1 at locus D10S208) and three microsatellite DNA markers assigned to the proximal long arm of chromosome 10 (probes sTCL-2, AFM115xf2, and AFM175xh10 at the RET, D10S196, and D10S207 loci respectively) according to standard procedures.^{13–15} DNA of CEPH subject 1331-01 was included in each experiment as a control of allele size. Allele frequencies were based on the genotypes of a subset of a CEPH panel.¹³ Familial HSCR was assumed to be an autosomal dominant trait with an estimated gene frequency of 1.2×10^{-5} and a penetrance of 0.66 and 0.51 in males and females respectively.¹⁶ We used the Linkage package (version 5.1) to calculate lod scores (MLINK program).¹⁷ Two point lod scores were calculated for linkage between HSCR and each microsatellite DNA marker.

Multipoint lod score analysis (LINKMAP program)¹⁸ was used to estimate the position of the HSCR gene with respect to the intervals on the following genetic map (recombination fraction between adjacent loci in parentheses): 10p-D10S208-(0.06)-RET-(0.04)-D10S196-(0.09)-D10S207-10q.

Finally, the programs HOMOG (version 3.33), HOMOG2 (version 2.91), and M.TEST were used to perform heterogeneity tests between short segment and long segment HSCR, both for pairwise and multipoint linkage analyses.

Screening for mutations of the RET proto-oncogene using the single strand conformation polymorphism (SSCP) technique and fluorometric sequence analyses were performed as described.⁹

Results

LINKAGE ANALYSES

Using four microsatellite DNA markers mapping to the pericentromeric region and the proximal long arm of chromosome 10, at loci D10S208, RET, D10S196, and D10S207, we performed genetic linkage analyses in 11 long segment and eight short segment HSCR families (figs 1 and 2). The table shows pairwise lod scores between microsatellite DNA markers of chromosome 10 and the disease loci, when computed according to the autosomal dominant model. In both groups, maximum pairwise lod scores were obtained at the RET locus (probe sTCL-2) with no recombination event between the disease gene and the polymorphic marker ($Z_{\max} = 2.16$ at $\theta = 0\%$ and $Z_{\max} = 5.38$ at $\theta = 0\%$ for short segment and long segment HSCR respectively, figs 1 and 2, table). Positive lod scores were also obtained at loci D10S208,

Pairwise lod scores between short segment and long segment familial HSCR and polymorphic loci of chromosome 10

Locus	Short segment HSCR				Long segment HSCR			
	Recombination fraction (θ)				Recombination fraction (θ)			
	0.00	0.10	0.20	$Z_{\max}$ (θ)	0.00	0.10	0.20	$Z_{\max}$ (θ)
D10S208	-2.84	0.63	0.49	0.63 (0.10)	2.34	1.84	1.30	2.34 (0.00)
RET	2.16	1.58	1.02	2.16 (0.00)	5.38	4.16	2.89	5.38 (0.00)
D10S196	-2.26	0.91	0.72	0.91 (0.09)	4.95	3.93	2.83	4.95 (0.00)
D10S207	-11.85	-0.38	0.04	0.07 (0.30)	-7.22	0.66	0.75	0.78 (0.17)

Short segment HSCR

Figure 2 Pedigrees of the short segment HSCR families. Symbols are as described in fig 1. Persons are presented with their genotypes at the RET locus (microsatellite sTCL-2).

Figure 3 Multipoint linkage analysis in short segment and long segment HSCR using four polymorphic loci of chromosome 10. Genetic distances are given in centimorgans (cM) with respect to the RET locus. Dotted line: short segment HSCR families. Thin line: long segment HSCR families. Bold line: total.

D10S196, and D10S207 (table). Multipoint linkage analyses were performed in the two groups to estimate the best position of the HSCR gene. Using this procedure, the maximum likelihood estimate for the position of both short segment and long segment familial HSCR genes was at the RET locus (maximum multipoint lod scores in log base 10 of 2.29 and of 5.98 for short segment and long segment HSCR respectively, fig 3). Heterogeneity tests provided evidence for homogeneity of the two conditions (not shown).

GENOTYPE-PHENOTYPE CORRELATIONS IN HSCR FAMILIES

We have previously reported on point mutations of the RET proto-oncogene in four HSCR families linked to chromosome 10q11.2 and two additional families.⁹ Fig 4 shows that these mutations resulted in either amino acid substitutions (mutations R330Q, S32L, and P64L; families 2, 5, and 15 respectively) or protein termination (mutation R180X, family 3).

Interestingly, the R180X nonsense mutation in family 3 was observed in two patients with long segment HSCR and in their unaffected mother (figs 1 and 4A). Similarly, the P64L missense mutation was observed in a proband with short segment HSCR and in two persons with severe constipation (family 15, figs 2 and 4B). In family 2, the R330Q missense mutation was found in one patient with short segment HSCR, one patient with long segment HSCR, and in three unaffected subjects (figs 1 and 4C). Finally, the S32L missense mutation in family 5 was found in a patient with long segment HSCR, in two patients with short segment HSCR, in a subject with severe constipation (not shown) and in an unaffected subject (family 5, figs 1 and 4D).

Discussion

Hirschprung's disease is a frequent and severe cause of intestinal obstruction which has long been regarded as a sex modified multifactorial condition (sex ratio = 3.9 boys/1 girl).²⁰ Indeed, several lines of evidence suggest the involvement of genetic factors in this disease¹⁶ including (1) the increased risk to sibs (4–19%),^{16,21} (2) the dominant pattern of inheritance in several pedigrees of long segment HSCR,²² (3) the frequent association with malformation syndromes (for example, Waardenburg syndrome)²³ or chromosomal anomalies of chromosomes 21, 13q, and 10q,^{24–26} and (4) the existence of mendelian models for colonic aganglionosis in rodents.²⁷ Extensive segregation analyses have suggested that familial HSCR could be accounted for by incompletely penetrant dominant genes in long segment HSCR while short segment HSCR could be best accounted for by a multifactorial model of inheritance.¹⁶

We have mapped a gene for long segment familial HSCR to the proximal long arm of chromosome 10, in the genetic interval defined by loci D10S208 and D10S196.^{5,6} Shortly

Figure 4 Fluorometric sequence analysis of RET exons in HSCR patients. Direct genomic DNA sequences of RET exons are presented: (A) family 3, exon 3; (B) family 15, exon 2; (C) family 2, exon 5; (D) family 5, exon 2. The base substitution is indicated (*). All probands proved to be heterozygous for their respective mutation as shown by a double peak on fluorometric tracks (arrows). The positions of codons are numbered as described previously.¹⁹

thereafter, we and others provided evidence for close proximity of the disease gene to the RET locus.^{7,8} Recently, we have identified mutations of the RET proto-oncogene (a protein-tyrosine kinase gene overexpressed in tumours derived from neural crest cells^{28,29}) in four HSCR families linked to chromosome 10q11.2 and two other HSCR families unsuitable for linkage.⁹

However, the question of whether this gene also causes short segment familial HSCR remained unanswered. The present study provides genetic evidence for linkage of short segment familial HSCR to the RET locus. Moreover, we show that mutations of the RET proto-oncogene may cause either short segment or long segment HSCR and that the two clinical forms may occur within the same family. The recent report of a RET mutation in short and long segment HSCR patients within the same family supports our observations.¹⁰ It appears therefore that short segment and long segment familial HSCR, which have been differentiated on the bases of both anatomical features and segregation analyses, may be regarded as the variable clinical expression of mutations at the RET locus. Thus, the incomplete penetrance and the variable intrafamilial expression of HSCR could be ascribed either to modifier genes or to environmental factors.

However, missense cysteine mutations in the extracellular juxta-transmembrane domain and in the tyrosine kinase domain of the RET proto-oncogene have been reported in patients with multiple endocrine neoplasia type 2A and type 2B respectively (MEN2A, MEN2B).³⁰⁻³² Taken together, these data suggest that germline mutations of RET can result in either MEN2

or familial HSCR, depending both on the location and the nature of the mutations.

In conclusion, the present study supports the view that familial Hirschsprung's disease is accounted for by one single gene locus, regardless of the clinical form of the disease. These results are consistent with the inheritance of an incompletely penetrant dominant gene whose clinical expression varies among and within families. These data should prompt one to look carefully for minor signs of aganglionosis in subjects related to children with HSCR, regardless of the length of the aganglionic segment in the proband. Whether all familial forms of HSCR are accounted for by this disease gene is still questionable and additional linkage studies in HSCR families from other ethnic origins or presenting various associated malformations are required before any conclusion can be drawn as to the genetic homogeneity of this condition (E Dow *et al*, in preparation). Finally, since familial HSCR only accounts for a minority of cases (10%), the question of whether this gene plays a role in sporadic HSCR is open to debate, especially as chromosomal abnormalities involving various other chromosomes have been reported in sporadic forms of the disease.

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