Novel mutations of the endothelin-B receptor gene in isolated patients with Hirschsprung’s disease

Takeshi Kusafuka, Yiping Wang and Prem Puri*

Children’s Research Centre, Our Lady’s Hospital for Sick Children, Crumlin, Dublin 12, Ireland

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Hirschsprung’s disease (HSCR) is characterized by the absence of autonomic ganglion cells in the terminal bowel and is a relatively common cause of intestinal obstruction in the newborn. The incidence of HSCR is estimated to be 1 in 5000 live births. Recently, the endothelin-B receptor (EDNRB) gene has been shown as a susceptibility gene for HSCR by the production of aganglionic colon in mice with a null mutation of this gene and by demonstrating a missense mutation in a large inbred kindred with a high incidence of HSCR (Mennonite pedigree). However, no further mutations have been demonstrated in other clinical cases. We analysed alterations of the EDNRB gene in 41 isolated patients of HSCR. Two novel mutations were detected: a G to A transition at nucleotide 824 and an insertion of T at nucleotide 878. Both mutations resulted in stop codons, predicted to produce a truncated and non-functional endothelin-B receptor. These observations indicate that dysfunction or loss of function of endothelin-B receptor may be involved in the aetiology of some isolated patients with HSCR.

INTRODUCTION

Hirschsprung’s disease (HSCR) is characterized by the absence of autonomic ganglion cells in the terminal bowel and is a relatively common cause of intestinal obstruction in the newborn. The incidence of HSCR is estimated to be 1 in 5000 live births (1). Recently, mutations of the RET proto-oncogene have been demonstrated in both familial and isolated cases of HSCR (2,3). However, the existence of some HSCR families with no linkage to RET suggests that additional susceptibility genes for HSCR may exist. More recently, it has been demonstrated that targeted disruption of the endothelin-B receptor gene (EDNRB) produced aganglionic colon in mice (4). Additionally, in a large inbred kindred with a high incidence of HSCR (Mennonite pedigree), a missense mutation was demonstrated in exon 4 of the EDNRB gene (5) which was mapped on chromosome 13 (6). There have also been some reports describing some HSCR patients associated with de novo interstitial deletions of chromosome 13 (7,8). This evidence suggests that EDNRB may be another HSCR gene. However, the role of the endothelin-B receptor in the aetiology of sporadically occurring HSCR is not well understood. The EDNRB gene spans 24 kilobases and encompasses seven exons (6) that encode a 442 amino acid protein belonging to the G protein-coupled heptahelical superfamily (9). We analysed seven exons of the EDNRB gene in 41 isolated patients of HSCR by the single-strand conformational polymorphism (SSCP) method. Novel mutations were detected in exon 4 in two patients and both resulted in stop codons, which are predicted to produce a non-functional endothelin-B receptor.

RESULTS

On analyses of exons 1, 2, 3, 5, 6 and 7, we observed no aberrant band patterns except those of mutational controls (data not shown). In exon 4, however, two HSCR patient samples demonstrated aberrant SSCP band patterns which corresponded to novel mutations (Fig. 1). The other 39 samples showed one of three distinct band patterns. These three patterns represented combinations of normal and/or polymorphic strands (Fig. 1). The two novel mutations were a G to A transition at nucleotide 824 and an insertion of T at nucleotide 878. Both mutations resulted in stop codons, predicted to produce a truncated and non-functional endothelin-B receptor. These observations indicate that dysfunction or loss of function of endothelin-B receptor may be involved in the aetiology of some isolated patients with HSCR.

DISCUSSION

We performed molecular analysis of the EDNRB gene in 41 isolated patients with HSCR and detected two novel mutations in two patients. These are the first observations so far suggesting a possible involvement of the EDNRB gene in the development of

*To whom correspondence should be addressed
aganglionosis in isolated cases. The two novel mutations produced stop codons causing early termination of translation and are predicted to produce truncated endothelin-B receptors. Both mutations cause the partial or complete loss of the fifth, sixth and seventh transmembrane domains as well as the C-terminal intracellular domain. Although we have not performed functional studies, it is very likely that the truncated receptors predicted from the mutations are non-functional.

Both patients with these novel mutations were heterozygous for the mutation. Considering the previous observation which demonstrated that 21% of the individuals heterozygous for a missense mutation in a Mennonite pedigree developed HSCR (5), it is likely that the impairment of even one copy of the EDNRB gene through these novel mutations had a critical effect on expression of HSCR in our cases. In EDNRB knockout mice, aganglionosis occurred exclusively in homozygous animals and not in heterozygous mice (4). Therefore, a discrepancy exists between human and mouse regarding the allelic status responsible for developing HSCR. However, a similar species-related difference has been observed in the case of mutations of the RET gene. The reported human HSCR cases associated with RET mutations were heterozygous for the mutation (2,3), while in the mouse, only animals homozygous for disrupted RET gene showed aganglionosis (10).

Regarding the extent of aganglionosis, the majority of reported patients with RET gene mutations have had long-segment HSCR (2,11), although recent reports have described some patients with short-segment HSCR (11,12). Additionally, targeted disruption of the RET gene in mice was reported to generate total aganglionosis throughout the digestive tract (10). In contrast, Hosoda et al. (4) described that, in a majority of EDNRB knockout mice, the aganglionic segment was confined to the rectosigmoid colon. In our patients, the aganglionosis of the patient with a G to A transition at nucleotide 824 was confined to the rectosigmoid colon, while that of the other patient was confined to the descending colon. Neither patient had any associated disease. Considering the fact that 75% of HSCR patients show aganglionosis confined to rectosigmoid (1), it is important to clarify the aetiology of short-segment HSCR. Dysfunction of endothelin-B receptor may be closely associated with short-segment HSCR rather than with more severe forms of the disease.

In conclusion, the detection of two novel mutations of the EDNRB gene in our patients strongly suggests that dysfunction or loss of function of the endothelin-B receptor has a role in the aetiology of some isolated cases with HSCR.

MATERIALS AND METHODS

Clinical description

The aganglionosis in the 41 isolated patients of HSCR was confined to the rectosigmoid in 28 patients, the descending to transverse colon in 10 patients and the total colon in three patients. Two patients had minor craniofacial dysmorphism.

Sample acquisition and processing

The resected intestinal specimens obtained at pull-through operation were embedded in OCT compound and stored at –70°C. DNA was extracted from 10 µm sections of the fresh frozen specimens by digestion with proteinase K (20 µg) in 100 µl of digestion buffer (50 mM Tris, pH 8.5, 1 mM EDTA, 0.5% Tween 20) at 55°C for 1.5 h.

DNA was prepared from blood samples of 70 normal individuals.

PCR–SSCP and sequencing analysis

Initially, we constructed mutational control DNA for each EDNRB exon. PCR was performed using either genomic DNA or mutational control DNA as template, together with primers corresponding to each exon. Two to five µl of each PCR product
was electrophoresed on a 15% nondenaturing polyacrylamide gel (Clean Gel, Pharmacia Biotech) in a Multiphor II system (Pharmacia Biotech). Two different discontinuous buffer systems were used to analyse each exon. In both buffer systems, with different ionic concentrations, acetate was used as leading ion and glycine was used as trailing ion. The gel was stained by silver staining (13). When abnormal SSCP patterns were observed, dideoxy sequencing was performed on genomic DNA by using agarose gel-purified PCR products. Both strands were sequenced and stained by silver staining. The stained sequence results were transferred to APC film (Promega) to gain permanent copies.

Detailed methodology regarding mutational controls, primers, PCR conditions and discontinuous buffer systems is available from TK.

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ABBREVIATIONS

HSCR, Hirschsprung’s disease; EDNRR, endothelin-B receptor; SSCP, single-strand conformational polymorphism.

REFERENCES