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

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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.

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 in one and an insertion of T at nucleotide 878 in the other sample (Fig. 2). Both samples with these mutations were of heterozygous status for the mutation; one combined with a normal strand and the other one combined with a polymorphic strand.

In the screening of 70 normal individuals, we observed no aberrant SSCP band patterns in exon 4, except those representing the polymorphism.

Regarding clinical findings, 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.

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...
demonstrated that 21% of the individuals heterozygous for a mutation. Considering the previous observation which ruled out the possibility of null alleles, it is very likely that the truncated receptors predicted from the intracellular domain are predicted to produce truncated endothelin-B receptors. 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 short-segment 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 protease 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; EDNRB, endothelin-B receptor; SSCP, single-strand conformational polymorphism.

REFERENCES