Point Nucleotidic Changes in Both the RET Proto-Oncogene and the Endothelin-B Receptor Gene in a Hirschsprung Disease Patient Associated with Down Syndrome

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Sakai, T., Wakizaka, A., Nirasawa, Y. and Ito, Y. Point Nucleotidic Changes in Both the RET Proto-Oncogene and the Endothelin-B Receptor Gene in a Hirschsprung Disease Patient Associated with Down Syndrome. Tohoku J. Exp. Med., 1999, 187 (1), 43-47 —— A short-segment Hirschsprung disease (HSCR) patient associated with 21 trisomy showing point nucleotidic changes in both the receptor tyrosine kinase (RET) proto-oncogene and the endothelin-B receptor (EDNRB) gene is reported. A T to A heterozygous transition at the splicing donor site of the intron 10 in the RET proto-oncogene, and a G to A heterozygous substitution in non-coding region in the exon 1 of the EDNRB gene were observed. The familial analysis with these genes revealed that the origin of the former mutation was de novo and the latter one was maternal. No patient has been reported with two points mutations in different pathogenetically susceptible loci for HSCR. There is genetic evidence that the RET and EDNRB genes may interact in their susceptibility leading to HSCR. ——— Down syndrome; Hirschsprung disease; receptor tyrosine kinase RET proto-oncogene; endothelin-B receptor gene; mutation © 1999 Tohoku University Medical Press

Hirschsprung disease (HSCR) is a congenital malformation regarded as a multigenic neurocrisopathology involving neural crest-derived cells (Bolande 1974), affects one out of 5000 live births and is the common cause of intestinal obstruction in newborns (Puri 1993). The association of HSCR and 21 trisomy have been shown a 10% to 15% incidence (Quinn et al. 1994). Four genes have been identified as pathologically susceptible loci for HSCR; the receptor tyrosine kinase (RET) proto-oncogene (Romeo et al. 1994), the endothelin-B receptor

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(EDNRB) (Puffenburger et al. 1994), the endothelin-3 (EDN3) (Baynash et al. 1994), and the glial cell line-derived neurotrophic factor (GDNF) (Angrist et al. 1996). Here, we report a short-segment HSCR patient associated with 21 trisomy showing point nucleotidic changes in both the RET proto-oncogene and the EDNRB gene.

**Materials and Methods**

A 1 year-old male patient with short-segment sporadic HSCR who was associated with 21 trisomy, his healthy parents not consanguineous and two healthy sisters were examined for their genomic DNA extracted from peripheral leukocytes. We obtained informed consent from the family to this study.

Mutation analyses were performed by a direct sequencing with 20 exons of the RET proto-oncogene, 7 exons of the EDNRB, and proteolytic cleavage sites and mature EDN3, and 2 exons of the GDNF. Twenty-four normal individuals were also examined for their genomic DNA with these genes by the same direct sequencing techniques. Primers used to amplify these genes contain the exons and the flanking intronic sequences. Polymerase chain reaction (PCR) mixture containing 10 μl of PCR buffer, 8 μl of 2.5 mM each dNTPs, 66.5 μl of autoclaved ultrapure water, 0.5 μl of Taq DNA polymerase (2.5 unit) (Takara Shuzo Co., Ltd., Kyoto), 5 μl of each sense and antisense primers (20 pmol), and 5 μl of genomic DNA (200 ng) was primed by a template melting step at 95°C for 5 minutes, followed by 40 cycles of the serial temperature changes consisting of 95°C for 1 minute, 56°C to 72°C for 1 minute, 72°C for 1 minute in a DNA Thermal Cycler (mode 480, Perkin Elmer, Norwalk, CT, USA). PCR products were electrophoresed on an A.L.F. red DNA sequencer (Amersham-Pharmacia Biotec, Uppsala, Sweden).

**Results**

The analytical results of this HSCR patient in Fig. 1A shows a point nucleotidic change in the RET proto-oncogene at nucleotide number 2 of the intron 10, a T to A heterozygous transition at the splicing donor site. Another heterozygous substitution of G to A was found in non-coding region as shown in Fig. 1B in the exon 1 of the EDNRB gene (26 bases upstream of the coding region). Fig. 2 shows the results of sequence analysis of the same regions shown in Fig. 1 in this family. The origin of these mutations in this case were de novo and maternal, in the RET proto-oncogene and in the EDNRB gene, respectively. No nucleotidic change was observed in the EDN3 or GDNF genes in this patient. We found no mutation among 24 normal individuals in the same regions by the same sequencing procedure.

**Discussion**

The RET proto-oncogene, which mapped to chromosome 10q 11.1 (Ishizaka et
Fig. 1. Sequential alteration in the RET proto-oncogene (A) and the EDNRB (B) in a male sporadic Hirschsprung patient identified by the direct sequencing technique. Arrows indicate the mutational points. *Indicates transcriptional initiation point.

al. 1989), is a member of the RET superfamily and its protein product (RET) consists of an extracellular ligand binding domain with cysteine-rich region, a single transmembrane domain, and an intracellular kinase domain (Takahashi et al. 1989). Endothelins mediate their effects through two receptors, termed endothelin-A and endothelin-B receptors, which have sequence and structural similarity to the family of G-protein coupled heptahelical receptors (Arai et al. 1993). The EDNRB gene is mapped to chromosome 13q22 and its protein is expressed in brain, kidney, lung, heart, endothelial cells and colon (Arai et al. 1993).

Currently approximately 50 RET mutations have been reported in HSCR patients and it is estimated that RET mutations account for 50% of familial and 15% to 20% of sporadic cases of HSCR (Chakravarti 1996). Twelve EDNRB gene alterations, including large scale deletions, have been identified in HSCR and mutation rates of this gene are less than 5% (Chakravarti 1996). A substitution in non-coding region in exon 1 of the EDNRB gene observed in this case, supposedly interfering with the initiation of the normal transcription, has been previously reported by Amiel et al. (1996). However, a transition at the splice donor site of intron 10 in the RET proto-oncogene, presumed to produce an abnormal spliced transcript, has not been previously identified. In the EDNRB gene mutations heterozygous one would predispose to isolated HSCR with incomplete penetrance, while homozygous one would result in more complex neurocristopathies associating HSCR (Amiel et al. 1996). One HSCR patient who had two point mutations
in the RET proto-oncogene has been reported (Shimotake et al. 1997), but no patient with mutations in both the RET and EDNRB genes has been appeared. Receptor tyrosine kinase and G-protein coupled pathways have been thought to be independent, however, recent studies suggest that these two pathways interact via an adaptor protein Shc (Lev et al. 1995; van Bissen et al. 1995). If these nucleotidic changes observed in this study turn out to be true disease causative mutations, then it is of interest to note that there is genetic evidence that the EDNRB and RET genes may interact in their susceptibility leading to HSCR.

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References


