Analysis of the *RET* Gene in Subjects with Sporadic Hirschsprung's Disease

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Background: Hirschsprung's disease (HSCR), or aganglionic megacolon, is a hereditable disease of the enteric nervous system. It is an embryonic developmental disorder characterized by the absence of ganglion cells in the lower enteric plexus. Gut motility is compromised in HSCR, with consequent risk of intestinal obstruction.

Methods: We sequenced the *RET* gene and characterized the clinical manifestations in 15 unrelated Chinese patients (9 males, 6 females; age range, 2–21 years) with sporadic HSCR. Genomic DNA extraction, PCR and DNA sequence analysis were performed according to standard procedures.

Results: We identified heterozygous *RET* gene mutations in 2 patients. The mutations included a missense mutation in exon 2 (CGC → CAC) resulting in a substitution of arginine by histidine at codon 67 (patient 1), and a missense mutation in exon 3 (TAC → AAC) resulting in a substitution of tyrosine by asparagine at codon 146 (patient 2). The pathological findings disclosed short-segment HSCR in patient 1 and long-segment HSCR in patient 2, respectively.

Conclusion: We identified *RET* gene mutations in 2 of 15 patients with HSCR in Taiwan. The Y146N mutation we identified was novel. [*J Chin Med Assoc* 2008;71(8):406–410]

Key Words: Hirschsprung's disease, novel mutation, *RET* gene, Taiwan

Introduction

Hirschsprung's disease (HSCR), also called aganglionic megacolon, is a hereditable disease of the enteric nervous system.[1,2] It is an embryonic developmental disorder characterized by the absence of ganglion cells which are derived from the neural crest in the lower digestive tract. Due to the absence of enteric plexus, gut motility is seriously compromised in HSCR patients, resulting in intestinal obstruction. There is a racial and ethnic variation in the prevalence of HCRS, and it is more often found among Asians as compared with Caucasians (2.8 *vs.* 2 per 10,000 live births). About 20% of HSCR cases are familial ones, with considerable genetic diversity in HSCR arising either because of locus or allelic heterogeneity. Studies of the genetic bases of HSCR have identified several disease-causing genes, including *RET* (receptor tyrosine kinase, 10q11.2),[3–7] GDNF (glial cell derived neurotrophic factor, 5p12-3.1),[8,9] NTN (neurturin, 10q13.3),[10] EDN3 (endothelin 3, 20q13.2),[11] and EDNRB (G protein-coupled–endothelin receptor B, 13q22).[12] In the case of *RET* mutation in several families, the HSCR phenotype was found to cosegregate with MEN2A, particularly when the *RET* gene mutation is in codon 618 or 620.[3] Thus, the clinical manifestations of *RET* mutations are highly dependent on the location of the mutation.

Heterozygous germline mutations in the *RET* gene that act as dominant inhibitors account for around 50% of familial HSCR and 7–35% of sporadic HSCR.[5,7,13,14] These mutations include missense, nonsense, deletion, insertion and frameshift mutations and occur throughout the *RET* gene.[5,7,13–15] Most missense mutations result in *RET* inactivation, therefore implying that

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Table 1. Oligonucleotide primers used for PCR amplification, PCR product sizes and annealing temperatures

Exon	Forward primer	Reverse primer	Product size (bp)	Annealing temperature (°C)
1	GGGCGGCCAGACTGAGCGC	AACTTCGCCCTGGCCCTGCGG	207	67
2	AGCCTTATTCTCACCATCCC	ATAAGGGCGGCTTGAGGAAG	373	56
3	GGTTTACACCAGCCCTGGAG	TGTGTCAAGGGCTCGCAGAG	456	56
4	CTTCCCGAGGAAAGCGGCTG	CACGGACACTAAACCGACCG	393	56
5	CATCCTAAGGTCTCTGGTTTT	GAGCGAGCACCTCATTTCCT	331	56
6	CAGAGCAGCTTGGTGGTCA	AGTCTACTCTGTGCTGGTTGG	349	56
7	GAATCTCTACCCTCAGGCCATT	ACCCTCCCTCCCTGGAG	358	56
8	GCACTAGCTGGACGCTGG	GAGACCATCCCAGGCTGGC	280	67
9	TAGAGGGGCAGGATCTGC	GCAACTCTGGCTGAAGTGC	282	67
10	AGAGAATGGTCAGTAGGGACACT	GGACCTCAGATGTGCTGTT	531	56
11	ATGAGGCAGAGCATACGCAG	AACGGCACCTCATCACAGTC	535	56
12	CTTTTCCCCCCTCTTCTCC	GCATTGGGGGCTCTTCAGGGT	293	56
13	GCAGGCCTCTCTGTCTGAACTT	GGAGAACAGGGCTGTATGGA	296	56
14	TGTGTCCACCCCCTTACTCATTGG	CGTGGTGGGTCAGGGTGTGG	399	67
15	CCCCCGGCCCAGGTCTCAC	GCTCCACTAATCTTCGGTATCTTT	358	56
16	CCTCCTTCCTAGAGAGTTAG	CCCCACTACATGTATAAGGG	191	56
17	GGCTCTGTGAGGGCCAGGT	CCCTTCCCAAGTGAGGCT	232	56
18	GGCTGTCCTTCTGAGACC	ACTGCCCTGGGGTGAGGCT	233	56
19	TAGTTGTGGCACATGGCTTG	CAGAGCAGACTTTGGTTTTG	313	56
20	TGCACTTGAAGTTTTGGTTCTT	CTCAGAGCTCTTACCCGGTGT	439	56
21	CCGGGCCCACCACATCATC	AGCCCAAATTAGAGCCAGGTTACG	835	56

HSCR is due, in part, to the loss of RET function or a reduced amount of RET protein. Previous studies found the prevalence of *RET* gene mutations among patients with sporadic HSCR to be 14.3% in Japanese[16] and 19% in southern Chinese.[15] In contrast, the prevalence of *RET* gene mutations among patients with sporadic HSCR has been reported to be as low as 3.6% in Taiwan,[17] far below the global average. To address this discrepancy, we investigated the *RET* gene in 15 Chinese with HSCR in Taiwan.

Methods

Patients

Fifteen unrelated Han Chinese (9 males, 6 females; age range, 2–21 years) with sporadic HSCR were studied. Some of them have been described in a previously published report.[18] The clinical diagnosis of HSCR was based on previously described criteria.[2] An additional 50 unrelated normal Han Chinese subjects were recruited as controls to determine whether any sequence changes might be a common polymorphism. Demographic data and a detailed family history were obtained by an interview with each patient and their family members. This study was approved by the institutional review board of the hospital, and informed consent was obtained from each individual.

Analysis of the RET gene

Genomic DNA was isolated from EDTA-preserved whole blood using the GFX Genomic Blood DNA purification kit (Amersham Biosciences, Piscataway, NJ, USA). The 21 coding exons of the *RET* gene were amplified by polymerase chain reaction using primers and conditions as described previously[16,19,20] or modified empirically (Table 1). For all reactions, a 25-μL reaction mixture contained 200 ng of genomic DNA, 2.0 mM $MgCl_2$, 0.2 mM of each dNTP, 0.15 μM of each primer, 1× reaction buffer and 1 unit of FastStart Taq DNA polymerase (Roche, Indianapolis, IN, USA). PCR products were purified by spin column using the GFX PCR DNA and Gel Band Purification Kit (Amersham Biosciences) and sequenced by automated DNA sequencing analysis with fluorescence-labeled dideoxyterminators (BigDye Terminator V3.1 Cycle Sequencing Kits, Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions (ABI 377-36 Autosequencer; Applied Biosystems).

Mutation confirmation

We used restriction analysis to confirm the presence of mutations in the *RET* gene. Restriction endonucleases were selected on the basis of whether a mutation created or destroyed a restriction endonuclease site. Digested PCR products were analyzed by electrophoresis on 12.5% polyacrylamide gels with fragments visualized