Congenital Central Hypoventilation Syndrome: A Novel Mutation of the RET Gene in an Isolated Case

Masayo Kanai, Chikahiko Numakura, Ayako Sasaki, Emi Shirahata, Kazuhiro Akaba, Motoya Hashimoto, Hisaya Hasegawa, Senji Shirasawa and Kiyoshi Hayasaka

Department of Pediatrics, Yamagata University School of Medicine, Yamagata 990–9585,
1Department of Neonatal Intensive Care Unit, Saiseikai Yamagata Saisei Hospital, Yamagata 990–8345,
2Department of Neonatology, Matsudo City Hospital, Chiba 271–0092, and
3Department of Genetics, Medical Institute of Bioregulation, Kyushu University, Fukuoka 812–8582

Kanai, M., Numakura, C., Sasaki, A., Shirahata, E., Akaba, K., Hashimoto, M., Hasegawa, H., Shirasawa, S. and Hayasaka, K. Congenital Central Hypoventilation Syndrome: A Novel Mutation of the RET Gene in an Isolated Case. Tohoku J. Exp. Med., 2002, 196 (4), 241–246 —— Recently, a few genetic abnormalities were identified in congenital central hypoventilation syndrome (CCHS or Ondine’s curse). CCHS is often associated with other neurocristopathies, especially with Hirschsprung’s disease (HSCR). Mutations of the genes involved in the receptor tyrosine kinase RET (REarranged during Transfection) (RET)-glial cell line-derived neurotrophic factor (GDNF) and/or endothelin 3 (EDN3)-endothelin receptor-B (EDNRB) signaling pathway have been found in some of HSCR patients. In this study, we analyzed candidates for HSCR, namely the RET, GDNF, EDN3 and EDNRB genes in three isolated CCHS patients to confirm the hypothesis that some CCHS patients have a common genetic abnormality with patients having HSCR or other neurocristopathies. We found a novel R114H mutation of the RET gene in one patient. The R114H mutation is unlikely to be a polymorphism and appears to be associated with CCHS. In addition, we also examined the HOX11L2 (RNX) gene, for which knock-out mice showed CCHS-like syndrome in these isolated CCHS patients and did not detected any mutation. Further cases should be analyzed for more candidates to clarify the pathophysiology of CCHS. —— congenital central hypoventilation syndrome (CCHS or Ondine’s curse); RET-GDNF signaling pathway; EDN3-EDNRB signaling pathway; HOX11L2 gene (RNX)

© 2002 Tohoku University Medical Press

Received January 28, 2002; revision accepted for publication April 13, 2002.
Address for reprints: Kiyoshi Hayasaka, M.D., Department of Pediatrics, Yamagata University School of Medicine, 2-2-2, Iida-nishi, Yamagata 990–9585, Japan.
e-mail: hayasaka@med.id.yamagata-u.ac.jp
Congenital central hypoventilation syndrome (CCHS or Ondine's curse) is rare and is defined as an idiopathic failure of the automatic control of breathing, especially during sleep. The pathophysiological mechanisms of CCHS remain unknown, but the functional lack or loss of a central chemoreceptor differentiated from neural crest cells may be concerned with the symptoms of CCHS. Approximately 16% of CCHS patients were associated with Hirschsprung's disease (HSCR), and small numbers of CCHS patients were associated with other neurocristopathies such as neuroblastoma, ganglioneuroma or ganglioneuroblastoma (Swaminathan et al. 1989; Wese-Mayer et al. 1992, 1993; Verlos et al. 1993; El-Halaby and Coran 1994; Stovroff et al. 1995; Gozal 1998; Gozal and Harper 1999). In addition, there was one report that a mother who had neuroblastoma during infancy had borne a daughter who had CCHS without any other neurocristopathies (Devriendt et al. 2000). So, it is reasonable to consider that at least a subset of CCHS is one of the neurocristopathies and some patients may have a common genetic abnormality with HSCR and/or other neurocristopathies. As for the etiology of HSCR, several gene abnormalities have been identified in patients (MIM [Online Mendelian Inheritance in Man 2001] 142623). Most were mutations of the receptor tyrosine kinase RET (REarranged during Transfection) proto-oncogene (RET gene), and others were mutations of the glial cell line-derived neurotrophic factor (GDNF) gene, endothelin 3 (EDN3) gene, endothelin receptor-B (EDNRB) gene, and so on. RET gene mutations were also reported in other neurocristopathies (MIM 164761). Recently, it was reported that three cases of CCHS with HSCR (CCHS-HSCR) had RET gene mutations (Amiel et al. 1995, 1996; Sakai et al. 1998) and two CCHS patients not accompanied by HSCR had a mutation of the EDN3 or GDNF gene (Bolk et al. 1996a; Amiel et al. 1998).

In this study, we examined candidates for HSCR; the RET, GDNF, EDN3 and EDNRB genes, in three isolated CCHS patients (CCHS patients without HSCR and/or other neurocristopathies). We also analyzed the HOX11L2 (RNX) gene in these isolated CCHS patients, because Shirasawa et al. (2000) reported that RNX gene knock-out mice show CCHS-like congenital central hypoventilation and the RNX gene would play an essential role in the development of the central respiratory control system.

**MATERIALS AND METHODS**

**Case reports**

Three Japanese patients with isolated CCHS were analysed. Patients 1 to 3 were a 8-year-old girl, an 12-year-old boy and a 1-year-old girl, respectively. All three patients required home ventilation therapy only during sleep and presented normal psychomotor development. All subjects were born at term having neither congenital cardiopulmonary anomalies nor external malformation. They showed hypoventilation and/or apnea soon after their birth, especially during sleep, and all of them required endotracheal intubation and mechanical ventilation within a few hours after birth. Their respiratory function tests performed during sleep showed extremely low or no response to hypercapnia; no increase in minute ventilation, even when blood carbon dioxide levels increased, although results of the respiratory function test during the awake state were normal. They had no signs of neuromuscular or metabolic disorders causing hypoventilation as detected by cranial CT, MRI or other laboratory investigation. Their diseases were diagnosed as CCHS based on these findings within 6 months after birth. Their screening tests for neuroblastoma were all negative and symptoms suggesting that patients having HSCR or tumors of neural crest origin were not detected. Patient 1 had very mild constipation (treatment was not needed), strabismus and incomplete right bundle branch block. Patients 2 and 3
have had no signs of other neurocristopathies or autonomic nervous system dysfunction (i.e., severe constipation, arrhythmia, ptosis, dysfunction or abnormality of pupils) until now. None had any history of CCHS, HSCR, tumors of neural crest origin or sudden infant death syndrome for their three generations. In their siblings, parents and grandparents, there were no patients who had any of the aforementioned neurocristopathies or symptoms of autonomic nervous system dysfunction.

Genetic analysis

Patients. With informed consent from the patients’ families, we extracted genomic DNA from the white blood cells using a Wizard® Genomic DNA Purification Kit (Promega Corporation, Madison, WI, USA). Mutation analysis was performed by direct sequencing.

All coding regions of the RET, GDNF, EDN3, EDNRB and RNX genes were analysed in the patients. Polymerase chain reaction (PCR) primers were designed based on the published sequences or previous reports (Ceccherini et al. 1994; Puffenberger et al. 1994; Hofstra et al. 1996; Grimm et al. 1998; Sakai et al. 2000). Sequences were determined by the DyeDeoxy Terminator Cycle method on an ABI PRISM® Genetic Analyzer 310 (PE Applied Biosystems, Foster City, CA, USA).

Healthy controls and patients’ families. DNAs of healthy controls were collected from Japanese medical students and coworkers who agreed to the substance of this study. The parents of Patients 1 and 3 agreed with our analysis of their genomic DNAs regarding the mutated part of the RET gene detected in their daughters. Screening of the R114H mutation was performed by PCR and Sac II restriction analysis. The PCR primers were 5’-ACAGACCTGACTTCTCTCTG-3’ and 5’-CAGAGCAAGACCAGCAGTAG-3’. SacII digested the 369 base pairs (bp) PCR product from the normal allele into 341 bp and 28 bp fragments. The R114H mutation abolished a SacII restriction site. Digested fragments were resolved by electrophoresis using a 10% polyacrylamide gel. Screening of D489N was also performed by PCR and Eam 1105I restriction analysis. The PCR primers were 5’-TCTGGTGCCAAGCAGCAC-3’ and 5’-TGGAATGCGCTAGAGGACTGC-3’. One base mismatch was introduced to the reverse primer to abolish an Eam1105I restriction site. Eam1105I digested the 158 bp PCR product from the normal allele into 138 bp and 20 bp fragments. The D489N mutation abolished an Eam 1105I restriction site. Digested fragments were resolved by electrophoresis using a 10% polyacrylamide gel.

RESULTS

Patient 1 had a G to A heterozygous transition at the second nucleotide of codon 114 (341G > A) in exon 3 of the RET gene. This transition substitutes Arg to His (R114H). The R114H mutation has not previously been reported in any other studies including those of HSCR. The mutation in the proband was inherited from her healthy father (Fig. 1) and was absent in 50 Japanese healthy controls. In Patient 3, we found a G to A heterozygous transition at the first nucleotide of codon 489 (1465G > A) in exon 7 of the RET gene, changing Asp into Asn (D489N). The D489N was inherited from her healthy mother, but this D489N was also detected in 3 of 100 chromosomes from 50 Japanese healthy controls, suggesting that it is a polymorphism. There were no other mutations in the coding regions of the RET, GDNF, EDN3, EDNRB and RNX genes in the three patients.

DISCUSSION

Many mutations of the RET gene were reported in patients with neurocristopathies such as multiple endocrine neoplasia types 2A and 2B, medullary thyroid carcinoma, and especially HSCR. Recently, three cases with CCHS-HSCR carrying RET gene mutations
were reported (Amiel et al. 1995, 1998; Sakai et al. 1998). All were heterozygous missense mutations and two cases of them were inherited from the healthy parents similar to the present case. In CCHS patients without HSCR, genetic abnormalities were previously reported in the only two cases; EDN3 mutation in an isolated CCHS patient and GDNF mutation in a patient with CCHS and growth hormone deficiency (Bolk et al. 1996a; Amiel et al. 1998). The mutation of the latter case was inherited from the healthy mother. The present case (Patient 1) is the first report of a RET gene mutation in an isolated CCHS patient. The R114H mutation of the RET gene in Patient 1 was inherited from her healthy father, however, it was not detected in 50 healthy controls, suggesting that the mutation is probably not a polymorphism. It is likely that the penetrance of the RET gene mutation is low in CCHS as well as in HSCR. The genes involved in the RET-GDNF signaling pathway and as those in the EDN3-EDNRB signaling pathway had been considered good candidates for CCHS. However, only a few cases had mutations in these genes (Amiel et al. 1996, 1998; Bolk et al. 1996a, b; Sakai et al. 1998, 2001; Urushihara et al. 1999). So, it is probable that other genetic and/or epigenetic factors would be involved in the pathogenesis of CCHS. On the other hand, the finding that RET gene knock-out mice showed a low response to hypercapnia (Burton et al. 1997), suggested that RET plays a very important role in the development of respiratory CO_{2} sensitivity and prompted us to consider that the R114H mutation of the RET gene is associated with the CCHS in Patient 1. The D489N mutation of the RET gene in Patient 3 had not been reported before as a polymorphism (Ceccherini et al. 1994; Bolk et al. 1996b; Fitze et al. 1999; Sancandi et al. 2000), but this mutation is probably a polymorphism specific for Japanese.

RXN gene knock-out mice showed
hypoventilation similar to CCHS (Shirasawa et al. 2000). We examined the RNX gene in isolated CCHS patients, but we could not find any mutations in the RNX gene. Amiel et al. (2000) also analyzed the coding region of the RNX gene in 26 CCHS patients and 4 patients with CCHS-SCCR, and did not find any mutation. So it appears that the RNX gene is not associated with CCHS in humans.

In the present study, we found a novel mutation of the RET gene in one of the isolated CCHS patients. Recently, Marazita et al. (2001) and Weese-Mayer et al. (1993, 2001) reported on the relation between CCHS and symptoms of autonomic nervous system dysfunction and on incidence of autonomic nervous system dysfunction in CCHS families. Their findings suggested that genetic factors would certainly be concerned with the pathogenesis of CCHS, and that CCHS might be a phenotype of systemic autonomic nervous system dysfunction. Accumulation of genetic abnormalities in CCHS patients will help clarify the common pathogenesis of CCHS. Therefore, further cases and more candidates concerned with the development of autonomic nervous system should be analyzed.

References


