CASE REPORT

Congenital Central Hypoventilation Syndrome with *PHOX2B* Gene Mutation in a Taiwanese Infant

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Congenital central hypoventilation syndrome (CCHS) is a rare disease that is characterized by failure in the autonomic control of breathing. Recent reports have identified mutation of the paired mesoderm homeobox protein 2b (*PHOX2B*) gene as playing a major role in CCHS. Increasing polyalanine repeat number is associated with a more severe clinical phenotype. We report a newborn male infant with the clinical manifestations of apnea and cyanosis requiring immediate endotracheal intubation at the age of 1 day. Recurrent hypoventilation with hypercapnia and hypoxemia occurred during sleep after weaning from the ventilator. No primary cardiopulmonary disease was identified. These clinical manifestations are compatible with CCHS. *PHOX2B* gene mutation analysis performed at the age of 4 months revealed expanded alleles containing polyalanine 26 repeats, further supporting the diagnosis of CCHS. Continuous ventilator support was necessary and tracheostomy was ultimately performed at the age of 5 months due to ventilator dependence. He was discharged with home ventilator support at the age of 6 months. [*J Formos Med Assoc* 2007;106(1):69–73]

Key Words: congenital central hypoventilation syndrome, neonate, paired mesoderm homeobox protein 2b

Congenital central hypoventilation syndrome (CCHS, Ondine's curse, Mendelian Inheritance in Man (MIM) 209880) is a rare disease with an estimated prevalence of 1 in 200,000 live births.[1] CCHS, first reported by Mellins et al in 1970, is characterized by dysfunctions in the autonomic nervous system with failure of ventilation regulation.[2] The clinical manifestations of CCHS present with variable degrees of severity, which may range from complete apnea during sleep, severe hypoventilation during wakefulness, to mild hypoventilation during quiet sleep only.[3] Previous reports showed that CCHS may be associated with Hirschsprung's disease or tumor of neural crest origin.[1,3–5] A common pathogenesis involving neural crest-derived cell lineages has been suggested.[5] Specifically, the paired mesoderm homeobox protein 2b (*PHOX2B*) gene is important in the development of the autonomic nervous system, including all derivatives from the autonomic neural crest.[6] Recent studies have identified the *PHOX2B* gene as the major gene involved in CCHS.[6–9] Here, we report a neonate with typical manifestations of CCHS with *PHOX2B* gene mutation detected by polymerase chain reaction (PCR).

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Case Report

A male infant was born to a 34-year-old, G4P1SA3 mother at the gestational age of 39 weeks at a local hospital. Body weight, body length and head circumference at birth were appropriate for gestational age. After delivery, endotracheal tube intubation was required because of frequent apnea and cyanosis. Extubation was performed on the next day after his condition stabilized. However, recurrent apnea with cyanosis while sleeping was noted later on the same day and intubation was performed again. Abdominal distension was also noted. Due to persistent apnea and ventilator dependence, he was transferred to our hospital for further evaluation and management.

Physical examination on admission revealed a generally normal newborn. However, frequent hypercapnia with $PaCO_2 > 60$ mmHg was noted even under a low rate ventilator setting. Bronchoscopic examination showed normal airway structure except for subglottic erosion at the anterior cricoid level with a mild degree of granuloma in the bilateral vocal cord process. A series of studies including cardiac echography, brain magnetic resonance imaging, chest X-ray, and metabolic survey with tandem mass spectrometry and urine gas chromatography revealed normal findings. CCHS was suspected. Polysomnography was arranged but the family refused this examination due to frequent apnea and cyanosis when ventilator support was discontinued. Because of abdominal distension, lower gastrointestinal series was performed to exclude the possibility of Hirschsprung's disease associated with CCHS. Intestinal suction biopsy was performed and the presence of ganglion cells with normal thioacetylcholinesterase stain on pathology ruled out the diagnosis of Hirschsprung's disease. Prokinetic agents and continuous feeding were used to improve gastrointestinal symptoms. The diagnosis of neuroblastoma was also excluded due to normal 24-hour urinary vanillylmandelic acid.

During the following months of hospitalization, the family was initially reluctant to allow tracheostomy for their baby. Assisted ventilation via nasal prong or endotracheal tube was required due to frequent apnea and hypoventilation. These episodes were frequently complicated with hypercapnia or hypoxemia. Tracheostomy was ultimately performed at the age of 5 months after discussion with the family. The infant was discharged 1 month later with home ventilator support. He demonstrated normal growth and development. *PHOX2B* gene mutation analysis was performed to confirm the diagnosis of CCHS.

DNA preparation, PCR amplification and sequencing

A DNA sample was extracted from peripheral blood by using the Puregene DNA Isolation Kit (Gentra Systems Inc., Minneapolis, MN, USA) according to the manufacturer's instructions. To detect the *PHOX2B* gene, the intronic primer pair 5′-CTCGGGCAAAAAGTCTGA-3′ (forward) and 5′-GTCTTTGGAGCGAAGATAGG-3′ (reverse) spanning exon 3 of the *PHOX2B* gene was used. This primer pair combination generated a 389-bp fragment. PCR techniques for the DNA fragments were performed in a total volume of 25 μL containing 50 ng of genomic DNA, 0.12 μM of each primer, 100 μM dNTPs, 8% DMSO, 0.5 units of AmpliTaq Gold enzyme (PE Applied Biosystems, Foster City, CA, USA), and 2.5 μL of GeneAmp 10× buffer II (10 mM tris-HCl, pH = 8.3, 50 mM KCl), in 2 mM $MgCl_2$ as provided by the manufacturer.

Amplification was performed in a multiblock system (MBS) thermocycler (ThermoHybaid, Ashford, UK). PCR amplification was performed with an initial denaturation step at 95°C for 10 minutes, followed by 35 cycles consisting of denaturation at 94°C for 30 seconds, annealing at 56°C for 45 seconds, and extension at 72°C for 45 seconds, and then a final extension step at 72°C for 10 minutes.

For sequence analysis, the PCR products were purified by solid-phase extraction, and bidirectionally sequenced using the Taq DyeDeoxy terminator cycle sequencing kit (Applied Biosystems) according to the manufacturer's instructions. Sequencing reactions were separated on an Applied Biosystems 3100 sequencer. The pedigree and

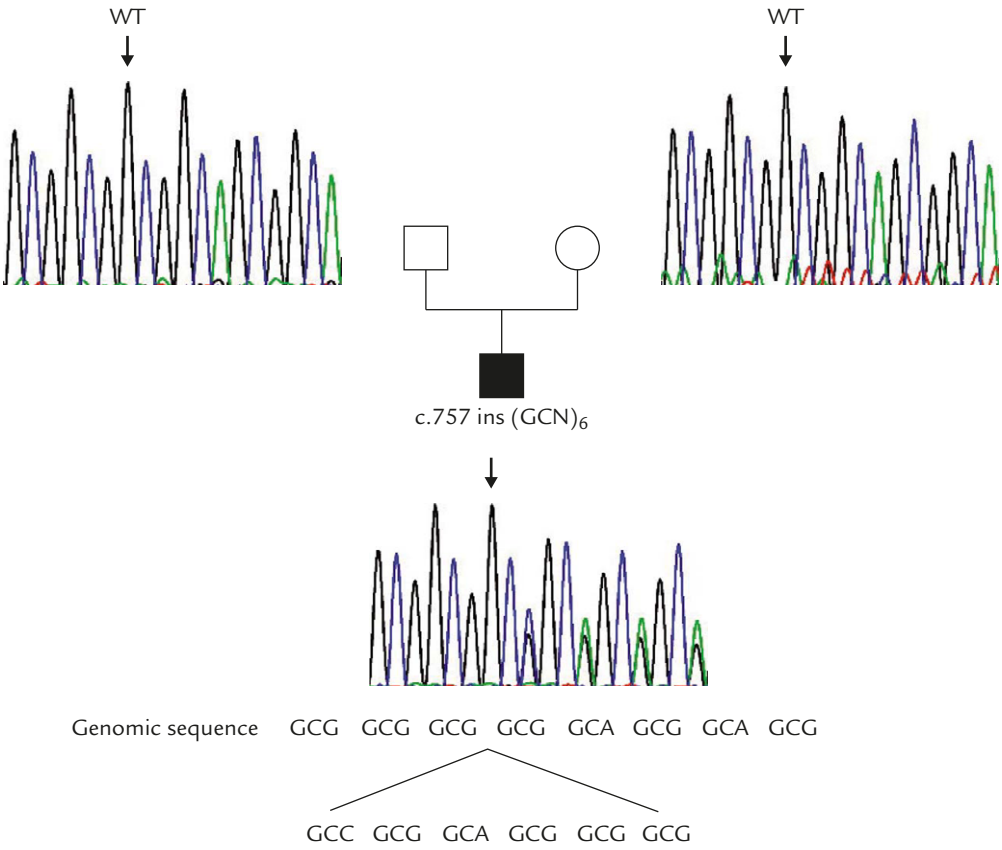

Figure. DNA sequencing of the *PHOX2B* gene shows expanded alleles containing polyalanine 26 repeats in the patient.

results of the sequence analysis are shown in the Figure. *PHOX2B* gene study yielded expanded alleles containing polyalanine 26 repeats in this patient.

Discussion

CCHS is a rare disease that is characterized by the absence of adequate autonomic respiratory control. The International Classification of Sleep Disorders proposed diagnostic criteria for CCHS,[10] which include the following: (1) the patient exhibits shallow breathing or cyanosis and apnea, the signs are worse during sleep than in wakefulness and their onset is during the perinatal period; (2) hypoventilation is worse during sleep than in wakefulness; (3) ventilatory response to hypoxia and hypercapnia is absent or diminished; (4) polysomnographic monitoring during sleep demonstrates hypercapnia and hypoxia, predominantly without apnea; (5) no primary lung disease or ventilatory muscle dysfunction can explain the hypoventilation; and (6) the symptoms are not due to any other sleep disorder, such as infant sleep apnea. The minimal set of criteria to be met for the diagnosis are (1), (2), (5) and (6).[10]

The clinical outcome of CCHS is variable. An overall mortality rate of 38% was reported in a French series of 70 patients with CCHS.[1] Infants with CCHS may present with hypoventilation of variable severity ranging from complete apnea during sleep, severe hypoventilation during wakefulness, to mild hypoventilation during sleep. These infants also tend to show a lack of ventilatory sensitivity to hypercapnia and hypoxia that may result in progressive pulmonary hypertension, cor pulmonale and central nervous system hypoxic damage.[11] Sudden infant death syndrome may be related to this rare disease. Monitoring of respiratory physiology using methods such as polysomnography has been recommended to

identify if there is spontaneous breathing during rapid eye movement (REM) sleep, non-REM sleep and wakefulness.[3,10] In our patient, polysomnography was difficult to perform because of the manifestations of severe apnea and cyanosis when ventilator support was discontinued.

CCHS has been reported in association with several other disorders such as Hirschsprung's disease (15–20%),[1,3,4] neuroblastoma (3%) and growth hormone deficiency (< 1%).[1] This reflects a common molecular pathogenesis involving defects of one or more genes that control the development of neural crest-derived cell lineages.[5] In our patient, abdominal distension was noted in early infancy. Hirschsprung's disease was suspected initially but was then excluded by the pathologic evidence of ganglion cells in the rectum. The patient also had normal 24-hour urinary vanillylmandelic acid that excluded the possibility of neuroblastoma.

Genetic roles in the pathogenesis of CCHS have been widely investigated. Genes involved in the development of the autonomic nervous system, such as the rearranged during transfection (*RET*) proto-oncogene, glial-derived neurotrophic factor gene (*GDRF*), the endothelin 3 gene, the brain derived neutrotrophic factor (*BDNF*) and T-cell leukemia, homeobox 3 (*Tlx-3*) genes, have previously been examined. However, only a few mutations were found to be related.[12–16] Amiel et al first demonstrated that a significant number of individuals with CCHS showed mutations in the *PHOX2B* gene.[6] In addition, the mutation detection rate was over 90% among patients who had CCHS.[7,9] The *PHOX2B* gene in humans is located in chromosome 4p12, encoding a 314 amino acid paired box homeodomain transcription factor, with two short and stable polyalanine repeats of nine and 20 residues, which is expressed in the developing hindbrain and the peripheral nervous system as well as in all noradrenergic centers and visceral motor and branchiomotor neurons of the cranial nerves.[9] The inheritance pattern is autosomal dominant.[7] *De novo* mutation was noted in most cases. Polyalanine expansions are the most common mutations in patients who have CCHS.[6,7,9] Frameshift mutation, nonsense mutation or missense mutation have also been reported in CCHS.[6] In addition, genotype–phenotype correlation has been described in patients who have CCHS. Increasing polyalanine repeat number is associated with a more severe clinical phenotype.[7,9] A somatic mosaicism has been detected in 4.5% of parents whose children have CCHS.[17] This information is important for genetic counseling. Because the percentage of germline mosaicism is unknown, the risk of recurrence cannot be predicted accurately.[18] Our patient had an expanded allele containing polyalanine 26 repeats in the *PHOX2B* gene. Both of his parents had a normal *PHOX2B* gene polyalanine pattern. The assay for *PHOX2B* polyalanine repeat mutation represents a highly sensitive and specific technique for confirming the diagnosis of CCHS.[7,17] Our review of the literature found no previous reports of CCHS confirmed by *PHOX2B* gene mutation analysis from Taiwan.

In summary, diagnosis of CCHS in the past has usually relied on the patient's clinical manifestations. Analysis of *PHOX2B* gene mutation is a precise and rapid method for the diagnosis of CCHS. Further application of gene study in the diagnosis of patients who may have CCHS and in genetic counseling of the parents is highly recommended in Taiwan.

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