# Molecular genetics of congenital central hypoventilation syndrome and Haddad syndrome

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Congenital central hypoventilation syndrome (CCHS) is a disorder of the autonomic nervous system characterized by a decreased response to hypercarbia. CCHS is frequently associated with congenital megacolon; the combination is called Haddad syndrome. CCHS is associated with dysfunction in respiratory features of the autonomic nervous system and with other disorders, including facial deformities, cardiovascular symptoms, and tumors. Patients with CCHS frequently have a mutation in the homeobox protein 2b (*PHOX2B*) gene. Most mutations involve heterozygous expansion of alanine repeats (GCN). Interestingly, a higher polyalanine repeat number is associated with a more severe clinical phenotype. To clarify the role of *PHOX2B* in disease pathogenesis, we introduce and review the clinical and molecular features of CCHS and Haddad syndrome.

**Key words:** Congenital central hypoventilation syndrome, Haddad syndrome, *PHOX2B*.

## Introduction

Congenital central hypoventilation syndrome (CCHS), first reported in 1970 by Mellins et al. [1], is characterized by sleepassociated respiratory insufficiency and markedly impaired ventilatory responses to hypercarbia and hypoxemia. The syndrome is also known as Ondine's curse (online mendelian inheritance in man [OMIM] 209880). Similar cases have been reported by many investigators [2-5]. CCHS has been associated with several disorders, including neuroblastoma, ganglioneuroma, and, most frequently, Hirschsprung disease (HSCR) because of broader structural and functional impairments of the autonomic nervous system [6-8]. HSCR (congenital megacolon; OMIM 142623) occurs in 16% of CCHS patients. The combination is referred to as Haddad syndrome [6]. Haddad syndrome is extremely rare, with under 100 cases reported in the literature [9].

In 2003, molecular genetic approaches showed that CCHS results from mutation of the homeobox protein 2b (*PHOX2B*) gene [10]. Many patients with CCHS have a heterozygous mutation consisting of 5 to 9 alanine expansions within a 20-residue polyalanine tract. CCHS genotypes, comprising various mutations in the *PHOX2B* gene, are associated with different degrees and mechanisms of cellular dysfunction, which have implications for the severity of CCHS [8,11-13]. The genotypephenotype association has been investigated, and the *PHOX2B* genotype is now a useful genetic marker for the assessment of CCHS [14]. Children with CCHS now survive into adulthood, and the clinical value of the *PHOX2B* mutation in CCHS continues to be evaluated by following these patients.

In Korea, Ahn et al. [15] reported the first case of CCHS in 1993. Several additional cases have been reported to date [11,16-19]. With early diagnosis and immediate intervention, the prognosis of CCHS patients has improved in the last decade. However, further research is needed to help Korean patients and their families. To this end, we review the clinical and genetic characteristics of CCHS and Haddad syndrome. We also discuss the clinical and genetic importance of research with a larger number of cases and long-term follow-up in Korea.

## Clinical Features

The first study by Mellins et al. [1] and subsequent reports focused primarily on CCHS as a disorder of ventilatory control in which the automatic control of breathing is absent or impaired.The International Classification of Sleep Disorders proposed diagnostic criteria for CCHS, which include the following: (1) the patient exhibits shallow breathing or cyanosis and apnea in the perinatal period; (2) hypoventilation is worse during sleep than in wakefulness; (3) the 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 showing hypoventilation; and (6) no sleep disorder, such as infant sleep apnea. The minimal set of criteria to be met for diagnosis is (1), (2), (5), and (6).

CCHS has been reported in association with several other disorders. Haddad et al. [6] described a combination of CCHS and HSCR, named Haddad syndrome. Patients with Haddad syndrome had reduced esophageal motility and control of heart rate. The incidence of HSCR in CCHS cases varies between 16% and 50%, and the incidence of CCHS is 1.5% among HSCR cases [10,20-22]. A CCHS patient with distinctive facial features (antimongoloid slanting eyes, triangular mouth, small nose, and low-set, posteriorly rotated ears) was reported by Minutillo et al. [23]. A study of numerous cases with long-term comprehensive follow-up showed that CCHS patients manifested a spectrum of clinical symptoms that reflect dysfunction of the autonomic nervous system, such as severe constipation, difficulty feeding, decreased perception of discomfort, pupillary abnormalities, decreased perception of anxiety, profuse sweating, and decreased basal body temperature [24]. A high percentage of CCHS patients had cardiovascular symptoms (decreased heart rate variability, vasovagal syncope, cardiac dysrhythmias) and

ophthalmologic abnormalities (sluggish or unreactive pupils, abnormal tearing, strabismus, anisocoria, miosis) [24]. Tumors of autonomic neural crest derivatives, such as neuroblastoma, ganglioneuroblastoma, and ganglioneuroma, were also found in approximately 5-10% of CCHS cases, which represents a 500-fold increased risk for such tumors in patients with CCHS, when compared with a rate of 1 in 10,000 among the general population [8,25]. These tumors typically present before 2 years of age in multiple locations including adrenal glands, chest, spinal cord, or mediastinum.

Cases of late-onset CCHS with pulmonary hypertension or clinically significant, persistent alveolar hypoventilation following an acute respiratory illness have been described [26-30]. However, the patients had subclinical or unrecognized diseases when they were children. Therefore, molecular genetic tests should be performed promptly when there is suspicion of late-onset CCHS. The number of reported cases may increase as recognition of the disease grows. A worldwide epidemiological survey estimates there are 500 living patients with CCHS [31].

## Molecular Genetics

The human *PHOX2B* gene (OMIM 603851) maps to chromosome 4p12 and encodes a highly conserved, 314-aminoacid homeobox transcription factor with polyalanine repeats of 9 and 20 residues. *PHOX2B* regulates the development of the autonomic nervous system and the determination of autonomic neural crest derivatives. Given these roles of *PHOX2B*, Amiel et al. [10] investigated *PHOX2B* as a candidate gene in CCHS. *De novo* mutations of the *PHOX2B* gene were found in 62% (18/29) of CCHS patients. The most common *PHOX2B* mutation in CCHS is a heterozygous expansion of alanine repeats (GCN). In individuals with CCHS, 1 of the 2 alleles contains too many repeats (between 25 and 33), whereas the other allele has 20 repeats (the normal number) (Fig. 1).

Weese-Mayer et al. [32] found alanine expansions in 65 of 67 CCHS patients, and further study identified *PHOX2B* mutations, not involving alanine expansion, in the 2 remaining CCHS cases. Thus, all patients had a *PHOX2B* mutation, indicating that *PHOX2B* is the disease-defining gene for CCHS. Interestingly, the results suggested an association between repeat length and the severity of the CCHS phenotype. Matera et al. [33] investigated the *PHOX2B* mutation in 27 CCHS patients, including 3 with associated HSCR and 3 with late-onset CCHS. They also showed that phenotype severity increased with increasing polyalanine
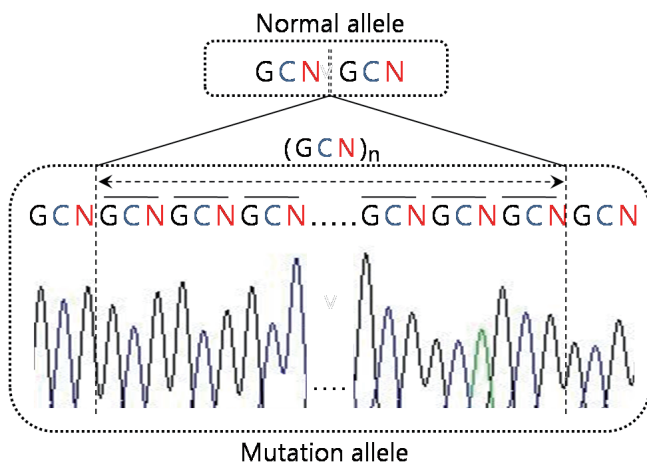
**Fig. 1.** Heterozygous mutation of the *PHOX2B* gene in the congenital central hypoventilation syndrome. Mutated alleleshave a greater number of alanine repeats (GCN) than the normal allele.



**Fig. 2.** *PHOX2B* mutation and the congenital central hypoventilation syndrome (CCHS) severity. On the x-axis, plus signs (+) indicate the number of supernumerary alanine expansions. Higher expansion number and missense mutations (MS) or frameshift mutations (FS) mutation induce more severe symptoms in Hirschsprung disease (HSCR) patients, including tumors (neuroblastomas). However, the association between these severe symptoms and other genes in the circle is still unknown.

expansion size. Increased repeat length was associated with severe respiratory symptoms, a long R-R interval in Holter monitoring, and facial phenotypes [34,35]. Results by Trochet et al. [8] strongly supported this genotypephenotype interaction. Short alanine expansions (+5 to +7) were found in patients with isolated CCHS, but were rare in patients with Haddad syndrome (CCHS+HSCR). Haddad syndrome patients with tumors tended to have longer alanine expansions (>+8). Interestingly, CCHS patients with malignant tumors of the sympathetic nervous system carried either a missense mutation or a heterozygous frameshift mutation in the *PHOX2B* gene. Review of these results demonstrated that HSCR and neural crest tumors were more frequently associated with missense or frameshift mutations of *PHOX2B* than with the polyalanine expansion. This led to the conclusion that missense or frameshift mutations produce more severe dysfunction in *PHOX2B* [8,32-35]. However, the relationship was not strict, and further study of additional cases is needed to confirm the hypothesis.

In HSCR, mutations in *RET* and in the endothelin signaling pathway, including the endothelin B receptor gene (*EDNRB*) and the endothelin 3 gene (*EDN3*), have been reported [36-39]. Because CCHS and HSCR share a common molecular pathology, mutations in *RET, EDNRB,* and *EDN3* were investigated in CCHS and Haddad syndrome. Bolk et al. [40] found mutation of *EDN3* in CCHS and Haddad syndrome. However, further study identified no *EDNRB* or *EDN3* mutations [41]. Subsequently, mutations in the rearranged during transfection (RET)–glial cellderived neurotrophic factor (GDNF) pathway were reported in 14% (1/7) of CCHS and 14% (1/7) of Haddad syndrome patients by Amiel et al. [41]. Although these mutations were
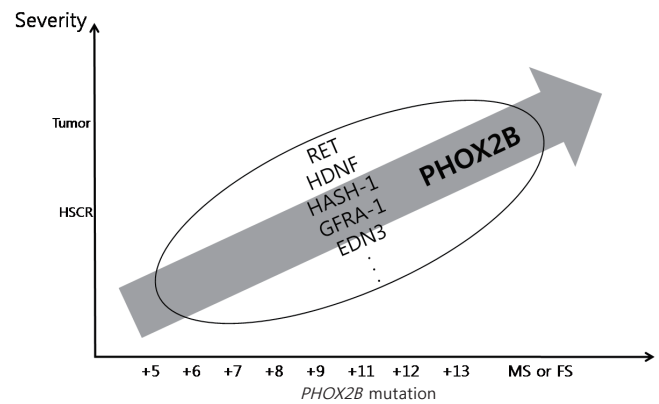
present in a minority of patients, their occurrence suggests that interactive polygenic inheritance (at least 3 genes belonging to distinct signaling pathways) is involved in CCHS.

Mutation of brain-derived neurotrophic factor (*BDNF*) was studied in 14 CCHS patients and 5 Haddad syndrome patients. *BDNF* mutation was identified in a CCHS patient whose father did not have CCHS but presented with postural hypotension and vasovagal syncope [32]. As newborns, *Mash-1+/-*heterozygous mice exhibit impaired ventilator responses to hypercarbia. The human ortholog of *Mash-1* (*HASH-1*) has been investigated as a candidate gene for CCHS [42]. Mutation in *HASH-1* was found in 2 patients with CCHS (10.5%, 2/19) and 1 patient with Haddad syndrome (9.1%, 1/11) [43].

Sasaki et al. [22] studied *RET, GDNF, GFRA1, PHOX2A, PHOX2B, HASH-1, EDN1, EDN3, EDNRB,* and *BDNF* in 7 patients with CCHS and 3 patients with Haddad syndrome. They found no mutations in the EDN3-EDNRB signaling pathway or in the *BDNF* gene. However, mutations in *RET, GFRA1, PHOX2A,* and *HASH-1* were found, supporting the possibility of their involvement in the pathogenesis of CCHS. To clarify the pathogenesis of CCHS and Haddad syndrome, further analysis of additional cases and candidate genes is required.

## Conclusion

In a relatively short period of time, molecular genetic studies have defined the clinical characteristics of CCHS for diagnosis

and treatment. As a result, the prognosis and quality of life of CCHS patients have improved. Patients now survive into adulthood, and their offspring represent a new generation of CCHS patients. Analysis of *PHOX2B* mutations may predict disease phenotype and prognosis. However, its association with other mutations is still unknown (Fig. 2). In Korea, there are few reports describing CCHS and Haddad syndrome [11,17-19]. Further data on patients with CCHS and Haddad syndrome in Korea should be gathered.

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