

Significant phenotype variability of congenital central hypoventilation syndrome in a family with polyalanine expansion mutation of the *PHOX2B* gene

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Background. Congenital central hypoventilation syndrome (CCHS) is a rare genetic disorder resulting from mutations in the *PHOX2B* gene located on chromosome 4p12.3, characterized by hypoventilation secondary to missing responses to both hypercapnia and hypoxia.

Case report. Proband. A girl, hospitalised 5 times for respiratory failure from 6 weeks old, presented at 4 years of age severe cyanosis related to pneumonia. Tracheostomy was done, and she was discharged home using a portable positive pressure ventilator during sleep. Proband's father: The father was retrospectively found out to suffer from severe headache and excessive daytime sleepiness. Molecular genetic evaluation of *PHOX2B* gene was performed and casual polyalanine repeat expansion mutation c.741_755dup15 in exon 3 was found both in proband and her father in heterozygous form. The proband's grandmother died of respiratory failure after administration of benzodiazepine at the age of fifty years. Considering the grandmother's history, she is highly suspected of having had CCHS as well.

Conclusion. Repeated respiratory failure of girl was explained by *PHOX2B* mutation and Ondina curse. Proband's father has incompletely penetrated *PHOX2B* heterozygous mutation as well and proband's grandmother died probably from the consequences of drug interaction with *PHOX2B* mutated background as well. Both daughter and father currently require overnight mechanical ventilatory support. Although most *PHOX2B* mutations occur *de novo*, our case is a rare three generation family affected by autosomal dominant inheritance with incomplete penetrance manifested as the late-form of CCHS and proven *PHOX2B* mutation in two generations.

Key words: congenital central hypoventilation syndrome, *PHOX2B* gene, late-onset form, respiratory failure

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INTRODUCTION

CCHS is a rare genetic disorder with an estimated incidence of 1 in 50 000 – 200 000 live births (ref.^{1,2}). CCHS typically presents in the newborn period as hypoventilation due to missing responses both to hypercapnia (central chemoreceptors) and hypoxia (peripheral chemoreceptors) most pronounced during sleep (ref.^{3,4}). In rare late-onset forms of CCHS (LO-CCHS), the diagnosis is usually established after the newborn period in connection with respiratory tract infection, or administration of depressant drugs such as opioids, benzodiazepines, general anesthetics, and anticonvulsants. All patients with CCHS need life-long respiratory support either during sleep or for the whole day in severe cases.

The *PHOX2B* gene located on chromosome 4 (specifi-

cally 4p12) was proposed to be the disease-defining gene for CCHS in 2003 (ref.⁵). *PHOX2B* is a homeodomain transcription factor, characterized by two polyalanine repeats of 9 and 20 residues in the C-terminal region; the most frequent mutations associated with CCHS are heterozygous in 2 frame duplications leading to alanine expansion within the normal 20-alanine stretch from +5 to +13 alanines; less frequent mutations are frameshift mutations leading to aberrant C-terminal region, nonsense mutations and missense mutations within the homeodomain (ref.⁶). This gene encodes a transcription factor involved in neural crest differentiation including central respiratory control system development. This is in line with the fact that CCHS is often associated with other autonomic nervous system dysfunction, in particular Hirschsprung's disease, tumours of neural crest origin, cardiac asystoles etc.

CASE REPORT

A rare familial case of CCHS caused by polyalanine expansion mutation of *PHOX2B* gene associated with significant phenotype variability is described. Written parental consent was given for publication of this report.

The proband was a four-year-old girl. She was born as one of two dizygotic twins by Caesarean section at 37 weeks of gestation after pregnancy conceived with *in-vitro* fertilization to seemingly healthy parents without consanguinity. Her examination showed normal looking female-newborn with no apparent abnormalities. Within the first six weeks of her life, she was sleepier than the other twin, and feeding difficulties were observed as well.

She experienced two severe cyanotic attacks related to viral pneumonia at the age of 6 weeks and 4 months. In spite of hypoxemia (pulse oxygen saturation was < 80%) and severe hypercapnia ($p_a\text{CO}_2 = 125$ mm Hg) no respiratory distress or tachypnoea were expressed. Both infections led to mechanical ventilation. Each time, she was successfully extubated and discharged home looking generally well.

At the age of one year, she was diagnosed with severe pulmonary hypertension resulting in acute right ventricle failure. After administration of benzodiazepine, she developed respiratory failure. Only then, CCHS became the highly probable cause. Additional blood tests including liver, renal, thyroid function, lactate, ammonia, pyruvate and carnitine measurements were performed. She underwent magnetic resonance imaging of the brain and spinal cord together with electroencephalography and electromyography. Primary pulmonary, cardiac, neuromuscular and metabolic disease responsible for hypoventilation was excluded. Overnight videopolysomnography revealed non-apnoeic oxygen desaturation (55% of total sleep time showing oxygen saturation below 90%). This time the parents refused tracheostomy and mechanical ventilatory support.

After further upper respiratory tract infection at the age of two years, she remained dependent on a ventilator while asleep. Non-invasive ventilatory support by full-face mask with bi-level positive airway pressure during sleep was started.

At the age of three years and nine months, she experienced four syncopes caused by sinus nodal arrest with pauses of up to 20 s. Since permanent epicardial pacemaker implantation was performed, no syncopes occurred during a further follow-up.

At the age of four years, she failed extubation and conversion to overnight non-invasive ventilatory support after pneumonia. Tracheostomy was done, and she was discharged home using a portable positive pressure ventilator during sleep.

At that time, molecular genetic evaluation of *PHOX2B* gene revealed heterozygosity for a polyalanine repeat expansion mutation involving the second polyalanine repeat sequence in exon 3 of the *PHOX2B* gene (casual mutation c.741_755dup15 in exon 3 of *PHOX2B*). Expansions were

in-frame, and resulting in expansion of the normal 20-repeat polyalanine tract to 25 alanine repeats to produce genotypes of 20/25.

As of the time of this report, the patient was eight years old, attending a primary school and doing well at school.

After identifying the *PHOX2B* gene mutation in the girl, molecular genetic evaluation was performed on all family members, i.e. her parents, sister (dizygotic twin), father's sister and her two offspring. The same mutation was confirmed in her father only. It was discovered retrospectively that he had presented with a history of a severe headache and excessive daytime sleepiness for his whole life. Videopolysomnography confirmed a moderate obstructive sleep apnoea desaturation (75% of total sleep time showing oxygen saturation below 90%) requiring nocturnal continuous positive airway pressure.

Afterwards, it was also discovered that father's mother died of respiratory failure after administration of benzodiazepine (diazepam) at the age of 50 years. She is therefore highly probable of having CCHS as well.

DISCUSSION

While more than 90% of CCHS cases are associated with heterozygous in frame polyalanine repeat expansion mutation (PARM) in the *PHOX2B* gene, it is only the minority of such patients who carry nonpolyalanine repeat mutation (NPARM), including frameshift, missense and nonsense mutations. Whereas the majority of NPARMs occur *de novo*, approximately 25% of PARMs cases are inherited from apparently asymptomatic parents carrying either full *PHOX2B* gene mutation or mosaicism for this gene. The mode of inheritance is autosomal dominant with incomplete penetrance and variable expressivity (ref.^{5,7,8}). According to the literature, NPARMs usually produce very severe phenotype with the need for continuous ventilatory support, often associated with both Hirschsprung's disease, and increased tumour risk (ref.⁹).

In PARMs patients, a relationship between the length of polyalanine expanded stretches and ventilatory support dependence has been described (ref.^{1,8-12}). The highest probability of the need for 24-hour per day ventilatory support is expected in individuals with genotypes from 20/27 to 20/33, whereas patients with LO-CCHS usually have the 20/24 or 20/25 genotype. These patients are typically managed with mechanical ventilation during sleep only (ref.^{9,11,13}).

In our three-generation family, 20/25 PARM without mosaicism was identified in two out of three affected family members. Sadly, the genetic testing could not be carried out in the proband's grandmother as she had died before the link between *PHOX2B* gene mutation and CCHS was demonstrated (ref.⁵). The individual phenotype severity differed, ranged from the seemingly asymptomatic father, then the father's mother, who died of complication of benzodiazepine administration at the age of 50 years,

Table 1. Previously reported at least three-generation familial cases of LO-CCHS with proven *PHOX2B* mutation.

No. of generations	Family member	Age of diagnosis	Clinical manifestation	PHOX2B gene mutation	Ref
4	*Male	16 yrs.	Apnoeic spells, Hirschprung disease	PHOX2B gene non-polyalanine repeat mutation	16
	4 males in four generations	5, 13, 42, 80 yrs	Very mild respiratory and gastrointestinal symptoms		
	4 females in three generations	28, 44, 46, 48 yrs.	Very mild respiratory and gastrointestinal symptoms		
	Cousin's son	Newborn period	Hirschprung disease, abnormal sleep study		
3	*Female	3 yrs.	Cor pulmonale and central hypoventilation following upper tract infection	PHOX2B gene expansion mutation 20/24	14
	Proband's mother	?	Snoring, otherwise asymptomatic		
	Proband's maternal grandmother	42 yrs.	Hypoventilation after general anaesthesia		
3	*Male	Newborn period	Hirschprung disease. Respiratory failure at 4 wks. of age	PHOX2B gene non-polyalanine repeat mutation	17
	Proband's mother	Adulthood	Central hypoventilation during sleep		
	Proband's grandfather	Adulthood	Daytime somnolence, short central apnoeas		
3	*Male	Newborn period	Respiratory symptoms	PHOX2B gene expansion mutation 20/25	15
	Proband's mother	35 yrs.	Polysomnography		
	Proband's grandmother	68 yrs.	Polysomnography		
	Proband's mother uncle	31 yrs.	Cardiac failure		
	Proband's half-brother	14 yrs.	Cerebral palsy		
	Proband's mother	?	Snoring, otherwise asymptomatic		
	Proband's maternal grandmother	42 yrs.	Hypoventilation after general anaesthesia		
3	*Female	Newborn period	Respiratory failure	PHOX2B gene expansion mutation 20/25	18
	Proband's father	Newborn period	Respiratory failure		
	Proband's paternal grandmother	?	Mild respiratory symptoms		

*Proband

to the proband presenting with respiratory failure after pneumonia in her early childhood. Our findings reflect the typical variable penetrance of *PHOX2B* gene mutation with the 20/24 and 20/25 genotype (ref.¹⁴).

Just five familial cases with proved *PHOX2B* gene mutation expressed in more than two generations have been described so far (ref.¹⁴⁻¹⁸). Therefore, we reviewed them through PubMed search in (Table 1).

As expected, PARMs were detected in three out of five families, one of them with 20/24, two with 20/25 genotype. The hypoventilation was the major symptom in these families. Surprisingly, further two familial cases were associated with the NPARMs. NPARMs were detected even in only mild symptomatic family members. It is in full contradiction to the generally accepted hypothesis that NPARM carriers depend on continuous ventilatory support (ref.⁹). Instead, Hirschsprung's disease was present in NPARMs families only, confirming thus the finding that association of CCHS and Hirschsprung's disease is a strong predictor of NPARM phenotype (ref.⁹).

CONCLUSION

We found a three-generation family with CCHS, with *PHOX2B* mutation being proved in two family members. Proband with the most severe disease expression pointed to an examination of her father and revealed probable cause of death of her grandmother. With regard to the extreme clinical variability in both PARM and NPARM patients, it is possible that the prevalence of CCHS in the general population is much higher than previously estimated. Molecular genetic testing should be done in seemingly asymptomatic relatives of CCHS individuals as they are at an increased risk of complications related to respiratory infections, and depressant drugs.

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