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## Later-onset congenital central hypoventilation syndrome due to a heterozygous 24-polyalanine repeat expansion mutation in the *PHOX2B* gene

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### Abstract

Aim: to describe a family with later onset congenital central hypoventilation syndrome (LO-CCHS) and heterozygosity for a 24-polyalanine repeat expansion mutation in the *PHOX2B* gene, rendered phenotypically apparent with exposure to anesthetics.

Case summary: An otherwise healthy 2.75-year-old boy presented with alveolar hypoventilation after adenoidectomy and tonsillectomy for obstructive sleep apnea, requiring invasive ventilatory support during sleep. He had a heterozygous 24-polyalanine repeat expansion in the *PHOX2B* gene (20/24 genotype), a genotype that has not been previously described in association with CCHS or LO-CCHS symptoms. Clinical findings in members of the family with the same 20/24 genotype ranged from asymptomatic to prolonged sedation after benzodiazepines.

**Conclusion:** CCHS should be suspected in individuals presenting with unexplained hypoventilation and/or seizures after anesthetics or sedatives. This is the first report of LO-CCHS in a kindred with the *PHOX2B* 20/24 genotype. The incomplete penetrance observed in this family suggests a gene–environment interaction.

### INTRODUCTION

Congenital central hypoventilation syndrome (CCHS) is an uncommon autosomal dominant disorder, characterized by alveolar hypoventilation and decreased sensitivity to hypercapnia and hypoxemia, particularly during sleep, coupled with diffuse autonomic dysregulation (1). Paired like homeobox gene 2b (*PHOX2B*), located on chromosome region 4p12, is the disease-defining gene for CCHS (1). More than 90% of individuals affected with CCHS are heterozygous for an expansion mutation in a polyalanine-coding tract within

exon 3 of *PHOX2B* resulting in 25–33 alanine repeats in the protein, with the normal length being 20 alanines (genotype range 20/25–20/33). The remaining 10% of individuals with CCHS are heterozygous for missense, nonsense and frameshift mutations in *PHOX2B* (1). Typically, individuals with CCHS present in the newborn period with apparent alveolar hypoventilation and cyanosis on falling asleep. However, a growing number of cases have been diagnosed in later infancy, childhood and adulthood with the *PHOX2B* 20/25 genotype or most recently with nonpolyalanine ex-

pansion mutations in *PHOX2B* (1–7). We report a case of later-onset CCHS (LO-CCHS) in a child with obstructive sleep apnea who presented with severe hypoventilation and seizures after exposure to anesthetics and has a polyalanine expansion mutation coding for 24 alanine repeats. Several variably affected family members were identified with the same mutation in *PHOX2B*. To our knowledge, this is the first report of a family with LO-CCHS and the 20/24 *PHOX2B* genotype.

## CASE REPORT

A 2.75-year-old Chilean boy, born to healthy nonconsanguineous parents after a normal pregnancy and delivery, with normal postnatal growth and development, presented with adenoidal hypertrophy and obstructive sleep apnea (OSA). Preoperative hematocrit was 39% and hemoglobin saturation (SpO<sub>2</sub>) was 98–100% in room air, awake. One hour after adenoidectomy with appropriate weight-determined dosages of fentanyl and sevoflurane anesthesia, the boy had a tonic clonic seizure, with recovery SpO<sub>2</sub> of 78% and arterial blood gas (ABG) pCO<sub>2</sub> of 127 mmHg with pH of 6.99. After mask ventilation the seizure terminated and he awakened to continue spontaneous breathing (pCO<sub>2</sub> 34 mmHg, 5 h later). EEG and brain MRI were normal. During the next 2 days, the child had 2–3 brief daily desaturation episodes to 85%, awake. Asleep, he experienced frequent desaturations with SpO<sub>2</sub> < 70%, audible snoring, but no respiratory distress. Echocardiogram (to assess for cor pulmonale indicative of long-standing hypoxemia) was normal. Overnight polysomnography 5 days after adenoidectomy revealed severe OSA (overall apnea/hypopnea index: 25 episodes/h; REM apnea/hypopnea index: 56 episodes/h; longest OSA 36 sec with SpO<sub>2</sub> nadir 50%) and alveolar hypoventilation (SpO<sub>2</sub> nadir 85% without apnea). Mean SpO<sub>2</sub> was 92% in non-REM sleep and 83% in REM sleep. SpO<sub>2</sub> values were <90% during 43% and 92% of non-REM and REM sleep time, respectively. Bronchoscopy revealed a mildly hypoplastic pharynx and moderate tonsillar hypertrophy. Consequently, tonsillectomy was performed with appropriate weight-determined dosages of remifentanyl and sevoflurane as anesthetics. The boy had another generalized seizure 3 h after discontinuation of anesthesia, with an ABG pCO<sub>2</sub> of 216 mmHg and pH of 6.9. After immediate intubation and mechanical ventilation, the boy awakened with a normal ABG, but several attempts to extubate after weaning from sedatives (propofol and chloral hydrate) failed in the following days because of hypoventilation: pCO<sub>2</sub> peak of 120 mmHg and SpO<sub>2</sub> nadir of 70%, asleep. He failed non-invasive mask ventilation (pCO<sub>2</sub> peak 90 mmHg), so an uncuffed, Shiley 5 mm internal diameter tracheostomy tube was placed for nocturnal ventilatory support with a rate of 18 bpm. Two weeks after the initial adenoidectomy, the child could maintain normal ventilation breathing spontaneously in room air awake (pCO<sub>2</sub> 40 mmHg, SpO<sub>2</sub> ≥ 95%) but he required nocturnal ventilatory support via the tracheostomy at the time of home discharge.

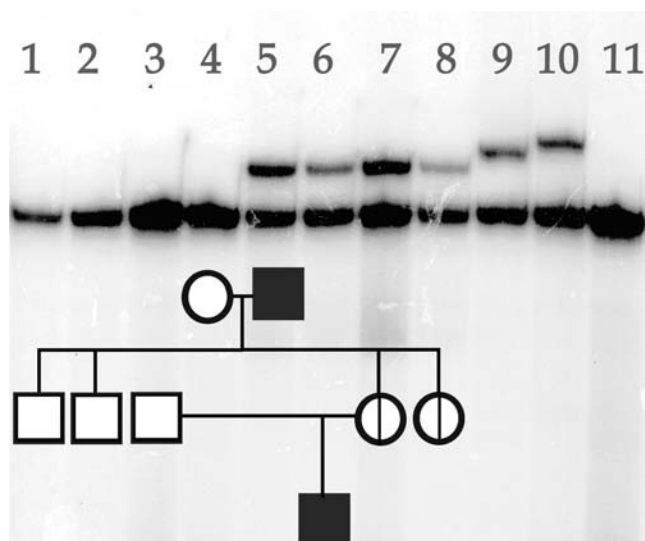
The child returned to normal activity levels (walking, playing, running), breathing spontaneously with a Passy–Muir valve during wakefulness (SpO<sub>2</sub> > 95% in room air, spontaneous respiratory rate 18–20 bpm). Polysomnography during spontaneous breathing repeated 4 months after hospital discharge did not reveal any apnea, E<sub>T</sub>CO<sub>2</sub> peak was 50 mmHg asleep, SpO<sub>2</sub> was >95% during more than 98% of the study time (nadir was 89%). Nine months after discharge, the boy was decannulated without sedation and successfully placed on nocturnal noninvasive mechanical ventilation. A 48-h Holter recording showed no asystoles. He had no history for constipation or profound sweating. Though not formally evaluated, the child's intellect is considered to be normal.

Molecular analysis of the *PHOX2B* gene, performed as described previously (11), showed a heterozygous polyalanine repeat expansion mutation coding for 24 alanine repeats, confirmed by sequencing (Fig. 1): genotype 20/24. No other mutations were found in the gene. His healthy mother and maternal aunt, neither of whom ever received sedatives nor anesthetics, had the same 20/24 genotype. The maternal grandfather, with a history of snoring asleep and several episodes of prolonged somnolence after benzodiazepines or antihistamines, also had the 20/24 genotype (Fig. 1). The study was approved by the IRB at Rush University Medical Center and written informed consent was obtained from studied family members.

## DISCUSSION

To our knowledge, the child and family reported here represent the first description of a kindred with later-onset CCHS and the 20/24 *PHOX2B* genotype. Individuals with CCHS are very sensitive to central nervous system (CNS) depressants (8,9), so it is likely that anesthetic exposure tipped the precarious balance of our patient with OSA and subtle hypoventilation, thus triggering acute respiratory decompensation and seizures. This report emphasizes the need for increased clinical awareness of the later presentation CCHS in childhood and adulthood, maintaining a high index of suspicion in clinical care. This can expedite the diagnosis and avert potentially life-threatening decompensation as well as risk for neurocognitive compromise.

Molecular analysis of *PHOX2B* is a sensitive method to confirm the diagnosis of CCHS. It is a clinically available test offered as an inexpensive *PHOX2B* screening test first, and in the small subset of negative cases, the *PHOX2B* sequencing test as a sequel to the screening test (www.genetests.org). The later-onset and apparent incomplete penetrance in the adult carriers described in our kindred with the 20/24 genotype is similar to what has been described in adults with the 20/25 *PHOX2B* mutation (2–7). The 24 alanine repeat mutation has recently been reported in homozygous form (24/24) in a case of CCHS (10), with the heterozygous parents being asymptomatic. Though Trochet et al. proposed that the 24 alanine repeat behaved as a recessive allele, and our presented kindred supports the previously reported autosomal dominant inheritance pattern of CCHS mutations (11,12), these observations are not necessarily mutually exclusive. The seemingly disparate



**Figure 1** Polyacrylamide gel electrophoresis showing results of *PHOX2B* analysis for the proband and his family. Lane 1 Proband's Maternal Uncle 1 (*PHOX2B* genotype 20/20); Lane 2 Proband's Maternal Uncle 2 (*PHOX2B* genotype 20/20); Lane 3 Proband's Father (*PHOX2B* genotype 20/20); Lane 4 Proband's Maternal Grandmother (*PHOX2B* genotype 20/20); Lane 5 Proband's Maternal Grandfather (*PHOX2B* genotype 20/24); Lane 6 Proband with later-onset presentation of CCHS (*PHOX2B* genotype 20/24); Lane 7 Proband's Mother (*PHOX2B* genotype 20/24); Lane 8 Proband's Maternal Aunt (*PHOX2B* genotype 20/24); Lane 9 Unrelated Subject with neonatal presentation of CCHS (*PHOX2B* genotype 20/25); Lane 10 Unrelated Subject with neonatal presentation of CCHS (*PHOX2B* genotype 20/26); Lane 11 Unrelated Control Subject (*PHOX2B* genotype 20/20). Solid black symbol indicates altered breathing with exposure to sedating medications; vertical lines show asymptomatic mutation carriers, not exposed to sedating medications.

findings might be explained by the need for environmental factors such as anesthetics or sedatives or alternatively the homozygous condition with the 24-polyalanine repeat mutation to cross the threshold necessary for phenotypic manifestation.

Though *in vitro* effects of the different *PHOX2B* mutations have demonstrated that increasing polyalanine repeat expansion size correlates with decreasing transactivation potential of the dopamine beta-hydroxylase (*DBH*) and *PHOX2A* promoters (known targets of *PHOX2B*), decreasing DNA binding capabilities and increasing extranuclear localization of the *PHOX2B* protein, and increasing intracellular aggregation of the mutant *PHOX2B* proteins (12,13), these and other functional assays have produced variable results with respect to amount of disruption of transcriptional activity caused by *PHOX2B* mutations containing 24 or 25 alanines. Toyota et al. (14) demonstrated a significant reduction of transcriptional activation of *DBH* by a 25 repeat expansion relative to wild type *PHOX2B*. Bachetti et al. (13) showed decreased transcriptional activation of both *DBH* and *PHOX2A* as well as increased extranuclear localization with the 25 repeat *PHOX2B* mutant relative to wild type, although much less dramatic than with longer repeats or nonpolyalanine repeat mutations. However, Trochet et al. (12) showed no disturbance of transcription by *PHOX2B* mutations containing 24 or 25 alanines

compared with the significant decrease in the other CCHS-causing mutations studied. These studies taken together suggest that there is cellular dysfunction with the *PHOX2B* 24- and 25-polyalanine repeat mutations, but less severe and more easily compensated than other CCHS-associated mutations described, correlating with the milder clinical disease with the 25 and our newly reported clinically relevant 24 alanine repeat expansions. In fact, lack of previous identification of the heterozygous 24-repeat mutation in typical CCHS patients may suggest that effects of this allele are sufficiently mild that, unlike the 25 repeat allele, it does not ever present in the newborn period in heterozygous form, but only later due to dependence on environmental or additional genetic factors to manifest the mildest CCHS phenotype. As such, this mutation may be heavily under-ascertained because it may predominantly produce ventilatory insufficiency and prolonged recovery from anesthesia or respiratory illness well beyond the newborn period, confounding recognition of its relationship to CCHS.

In summary, we present clinical and molecular information on a family with later-onset CCHS and the 20/24 *PHOX2B* genotype, illustrating incomplete penetrance and variable expressivity. Members of this family show the smallest heterozygous *PHOX2B* polyalanine expansion mutation associated with symptoms described to date and exemplify the interaction of this predisposing genotype with environmental agents such as sedatives and anesthetics and pre-existing obstructive sleep apnea. Further molecular analyses of *PHOX2B* in cohorts of individuals presenting with prolonged recovery from anesthesia or unusually extreme reactions to sedatives or respiratory illness are needed to determine the contribution of the 24-repeat polyalanine expansion mutation to these clinical presentations. Once identified, we propose that individuals with the 20/24 genotype should be counseled to avoid central nervous system depressants, have serial comprehensive physiologic assessments awake and asleep, and consider the diagnosis and implications in family planning.

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## Hypocalcemia impacts heart failure control in DiGeorge 2 syndrome

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Chromosome 10p, DiGeorge syndrome, Heart failure, Hypocalcemia

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### Abstract

Chromosome 10p terminal deletion accounts for a rare subset among patients presenting with DiGeorge syndrome, and is designated as DiGeorge 2 syndrome. We report a neonate with DiGeorge-like phenotype having a deletion of distal 10p (p13-pter) and a duplication of terminal 3q (q29-qter) derived from paternal balanced translocation between 3q29 and 10p13. She had facial dysmorphism, atrial and ventricular septal defect, impaired T-cell function, hypoparathyroidism, sensorineural hearing loss, renal abnormalities and developmental delay. Her phenotype corresponded well with the typical characteristics of partial monosomy 10p and the small duplication of terminal 3q did not involve the critical region of 3q duplication syndrome. Clinically, hypoparathyroidism-related hypocalcemia lasted for three weeks and resulted in repeated episodes of heart failure. It was not until the calcium level was normalized that her heart failure improved markedly.

**Conclusion:** Cytogenetic analysis can help to recognize patients early on who have terminal 10p deletion when microdeletion of 22q11.2 is not the cause of DiGeorge syndrome. Hypoparathyroidism-related hypocalcemia impacts heart failure control in partial monosomy 10p and should be managed aggressively on critical care.

## INTRODUCTION

DiGeorge syndrome (DGS, MIM 188400) is a developmental defect of the third and fourth pharyngeal pouches characterized by hypoplasia of thymus with T-cell deficiency, hypoparathyroid hypocalcemia, conotruncal heart defects, facial dysmorphism, palate anomalies and developmental delay. The clinical features are diverse, with many patients exhibiting only a subset of these traits. Over 90% of patients with DGS are the result of microdeletion of 22q11.2

(DGS1) (1). Another rare chromosome abnormality that presents DiGeorge-like phenotype is terminal deletion of chromosome 10p (DGS2, MIM 601362) (1,2). The prevalence of DGS2 is estimated to occur in 1 out of every 200 000 live births, which is 50 times less frequent than DGS1 (3,4). Partial duplication of 3q has been described in more than 40 patients, and localized the critical region to 3q26.3 (5). We report a 9-day-old girl who demonstrated DGS with partial monosomy 10p and terminal 3q duplication in the absence