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Homozygous *DBX1* Nonsense Variant in a Case of Atypical Congenital Central Hypoventilation

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Abstract

Objectives

Congenital central hypoventilation syndrome (CCHS) is a rare breathing disorder, predominantly caused by deleterious alterations in the *PHOX2B* gene. This report describes a rare case with *PHOX2B*-negative CCHS.

Methods

We conducted a 10-year follow-up, including a clinical evaluation, polysomnography, brain MRI, analyses of blood and CSF, electrodiagnostic testing, and comprehensive genetic analyses including trio-whole exome sequencing (trio-WES).

Results

In a female patient necessitating artificial ventilation immediately postnatally, trio-WES revealed a homozygous deleterious variant in the candidate gene *DBX1* (p.Ala114His*fs*Ter133), likely resulting in a complete loss of DBX1. Additional symptoms included central hypotonia, global developmental delay, seizures, and marked autoaggressive behavior.

Discussion

Dbx1 (developing brain homeobox 1) has an established critical role for mammalian inspiration, dramatically illustrated by the rapid postnatal demise of Dbx1 null mice because of asphyxia. Here, we describe the first human patient with atypical CCHS harboring a deleterious variant in the *DBX1* gene. Surprisingly, over time, our patient gradually achieved the capability of ventilator-independent respiration, although with an irregular rhythm and only during the wake state. These findings suggest that DBX1-deficient individuals are able to install alternative neuronal circuits that maintain inspiratory drive during the wake state.

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Supplementary Material

Introduction

Breathing is an unconscious rhythmic behavior that is generated by a complex neural network within the ventral respiratory column of the brainstem. In mammals, breathing is composed of 3 phases: inspiration, postinspiration, and active expiration.[1] Inspiration is mainly governed by the preBötzinger complex (preBötC), a heterogeneous neural network within the ventrolateral medulla.[2] Specifically, the rhythm-generating glutamatergic preBötC interneurons are derived from progenitors expressing the homeobox gene *DBX1* (developing brain homeobox 1), encoding for a homeodomain transcription factor. DBX1 is involved in early embryonic neuron differentiation throughout the brain with a critical role in establishing the distinction of the neuronal fate of V0 and V1 interneurons deriving from adjacent progenitor domains of the ventral pallidum.[3] Dbx1 knockout mice lack V0 interneurons and the preBötC, resulting in inability to breathe and lethality within the first minutes of life.[3,4] Notably, the current knowledge on the role of DBX1 in preBötC-governed inspiration is indeed restricted to findings in rodents.

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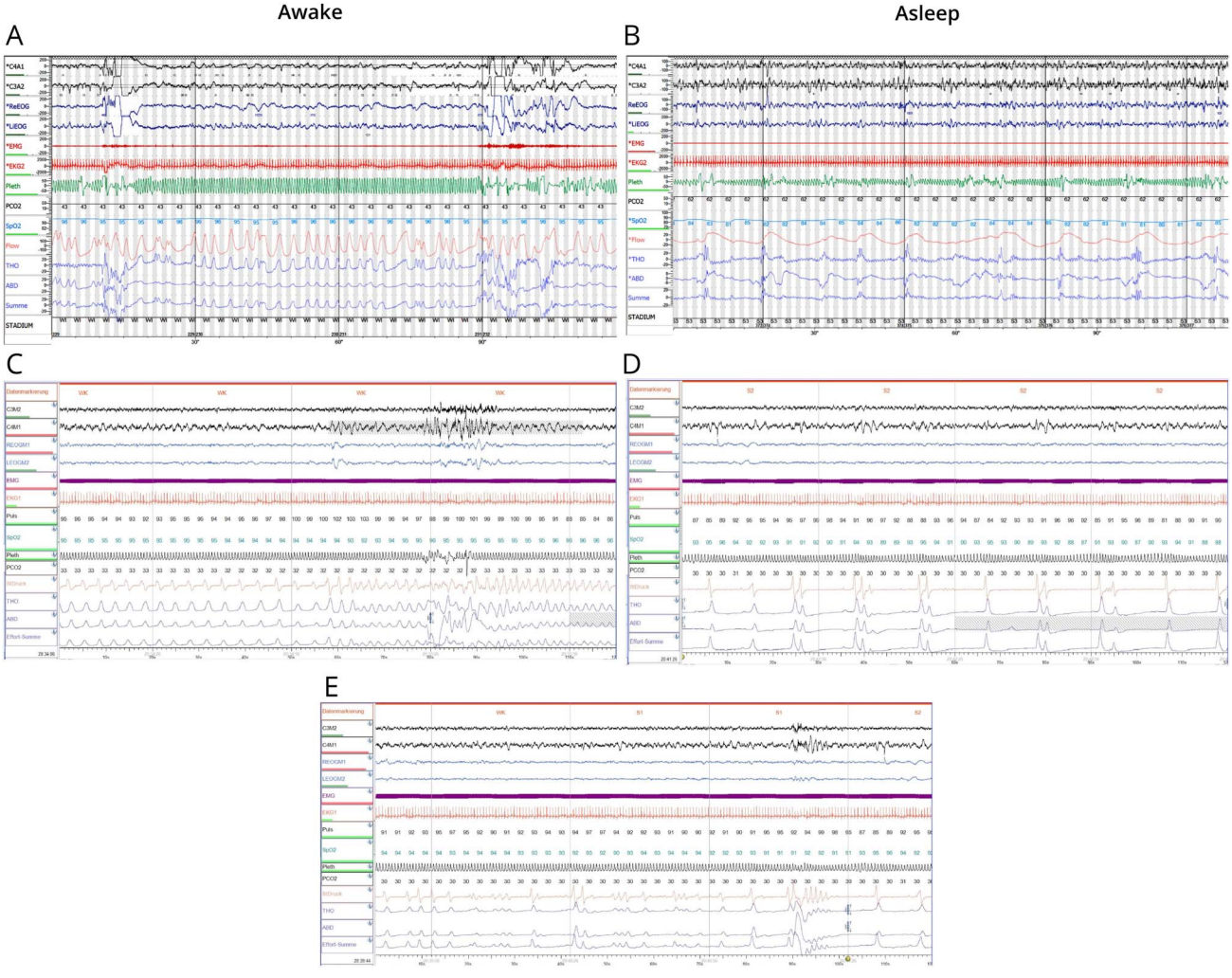
Methods and Results

Biological samples and images were obtained following written informed consent and consent to disclose from the parents of the affected individual, in accordance with protocols approved by the Ethics Committee of the Hamburg Chamber of Physicians: PV 3802. We present a 10-year-old girl whom we have followed-up since birth. She is the only joint child of healthy, first cousins of White descent. The pregnancy was complicated by polyhydramnios and completed after 41 weeks by cesarian section because of fetal distress. Birth measurements were within normal limits (weight 69th percentile, height 45th percentile, and head circumference 68th percentile). APGAR was 3/6/6, cord blood pH was 7.30. Because of severe, global respiratory insufficiency, she required invasive mechanical ventilation immediately after birth. Attempts to extubate the infant failed; thus, a congenital central hypoventilation syndrome (CCHS) was suspected. In addition, profound central hypotonia was apparent. Sucking and swallowing reflexes were absent. Tracheostomy and gastrostomy were implemented by the age of 4 weeks. Generalized tonic seizures were repeatedly observed throughout the first months of life. The seizures persisted for less than a minute and responded to diazepame and/or high-flow oxygen. A prophylactic anticonvulsive treatment with levetiracetam was discontinued by the age of 2 years. Initial, interictal EEG examinations did not identify distinct epileptiform discharges neither did subsequent EEG examinations at 4 and 6 years.

Figure 1 Polysomnography Findings in the Patient During the Wake State and While Asleep



Events were scored following the guidelines of the American Academy for Sleep Medicine. At the age of 33 months, central dysregulation of breathing with prolonged central apneas and mild hypercapnia were observed during the wake state and long-lasting central apneas were present during sleep (*not shown*). At the age of 3.5 years, expanded phases of normal variable breathing interrupted by short apneas and episodes of monotonic breathing with a respiratory rate of 26/min were present while awake (A), whereas long-lasting central apneas occurred during sleep (B). At the age of 10 years, because of agitation, only a brief period could be recorded during the wake state showing a variable breathing pattern without apneas (breathing rate 16–40/min) (C). Clinically, no relevant apneas were observed during this period. Signs of a severe central breathing disorder with onset of numerous central apneas and subsequent oxygen desaturation shortly after falling asleep (D). Some central apneas were long-lasting (up to 29 seconds) (*not shown*). Note the increasing frequency of hypopnea and beginning central apnea during the transitional period between wake state and definite sleep at the age of 10 years (E). C4A1 and C3A2 represent the EEG; REEOG and LIEOG–electrooculogram on the right and left site, respectively. ABD = abdominal breathing movements; Flow = airflow; PCO_2 = transcutaneous CO_2 partial pressure of CO_2; Pleth = plethysmogram; SpO_2 = oxygen saturation; THO = thoracic breathing movements.

At the age of 1 year, the girl gradually achieved the ability to respire spontaneously during the wake state, starting with a few hours, while inspiratory movements remain infrequent and irregular during sleep at the present age of 10 years (Figure 1). The patient continues to be exclusively tube-fed. The central hypotonia persists, but antigravity movements are possible, tendon reflexes are bilaterally present, no spasticity. Motor development was delayed but displayed continuous progress; unaided gait was achieved by the age of 4.5 years but remains broad-based and insecure. Involuntary movements have not been observed neither have abnormal eye movements reminiscent of nystagmus. The visual system appears unimpaired and visually evoked potentials have not been tested to date. Expressive language is limited to 1 word, and the receptive language comprises approximately 50 words. Brainstem auditory evoked potentials at the age of 2 years revealed bilateral latencies for wave III and IV in the upper limit range, thereby implying a mild disturbance within the auditory pathway. Since infancy, she displayed pronounced self-injurious behavior with the head area as the primary target region (hair pulling, skin picking, head banging); her arms are hence constantly preventively fixed to her trunk. The girl displays dysmorphic features (Figure 2).

A cerebral MRI (1.5 T) at the age of 10 months was suspicious of delayed myelination whereas the myelination status was determined as age appropriate at 29 months. Repeat MRIs at 6 and 10 years were normal. Repeated analyses of blood and CSF along with electrodiagnostic testing yielded unremarkable results, as did karyotyping, array-CGH as well as a complete *PHOX2B* genetic analysis (Sanger sequencing of *PHOX2B*, analysis of the polyalanine-repeat region in exon 3 and *PHOX2B* deletion/duplication analysis).

To elucidate a potential genetic cause, trio-whole exome sequencing (trio-WES) with DNA samples of both parents and the index patient was performed, as described previously.[5] Bioinformatic filtering (minor allele frequency MAF <0.01) detected no variants in a known disease gene that could explain the symptoms. In line with parental consanguinity, we identified 25 regions of homozygosity altogether spanning 168 Megabases (eTable 1), including 4 rare homozygous variants, of which only 1 is supposed to have a deleterious effect (eTable 2). The 2 base pair deletion (c.340_341delGC) in *DBX1* (NM_001029865.4) is predicted to change the reading frame, thereby inducing a premature stop codon (p.Ala114His*fs*Ter133). This variant likely causes a complete loss of DBX1, or, if resulting in a stable protein, is predicted to lead to the loss of the important DNA-binding homeodomain (eFigure 1). No homozygous loss-of-function *DBX1* variant was identified in a large sequencing data set, of more than 750.000 individuals (gnomAD V4.1.0[6]) strongly suggesting that humans are intolerant to biallelic *DBX1* loss-of-function variants, and further indicating the causality of the here identified frameshift variant.

Data Availability

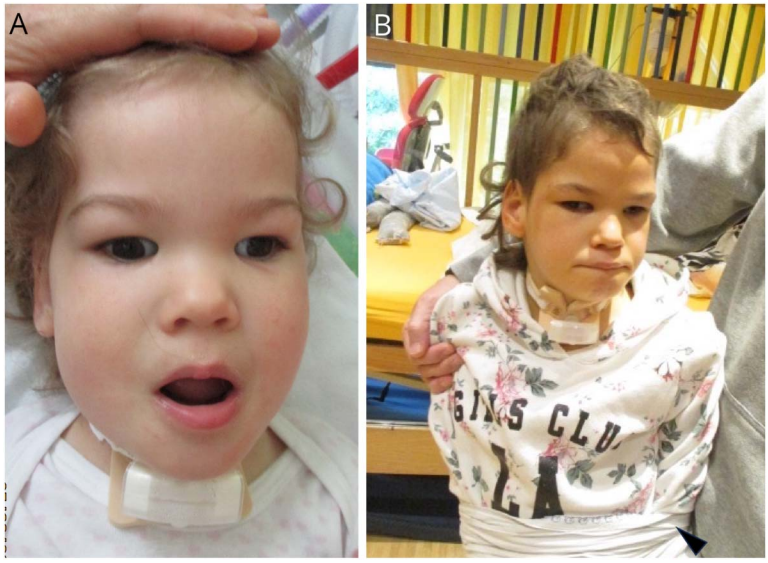
The data that support the findings of this study are available from the corresponding author on reasonable request.

Discussion

Using trio-WES, we identified a homozygous deleterious variant in *DBX1* in a girl with CCHS, central hypotonia, global developmental delay, and autoaggressive behavior.

Dbx1-derived interneurons in the preBötC constitute 1 of the 3 excitatory rhythmogenic microcircuits regulating breathing:

Figure 2 Photographs of the Index Patient



Frontal photographs at the age of 29 months (A) and at the age of 9 years (B). Note the prominent forehead, periorbital fullness, deep-set eyes, upslanting palpebral fissures, telecanthus, broad nasal bridge, and nasal tip and smooth philtrum. Please note the coarsening of facial features over time. The preventive "belt construction" fixing the upper extremities to the trunk (arrow) is necessary because of pronounced autoaggressive behavior.

Table 1 Comparison of Key Features in Different Forms of Congenital Central Hypoventilation Syndrome

Type	DBX1-related congenital central hypoventilation	CCHS (OMIM #209880)	CCHS2 (OMIM #619482)	CCHS3 (OMIM #618483)
Causative gene	*DBX1*	*PHOX2B*	*MYO1H*	*LBX1*
Mode of inheritance	AR	AD	AR	AR
Reported patients	1 (this study)	90%–92% PARM[a] 8%–10% NPARM[b]	3 from 1 consanuineous family	2 from 1 consanuineous family
Neonatal respiratory features	Central hypoventilation, AV during wake and sleep	Hypoventilation during wake and sleep	No	Central hypoventilation, AV during wake and sleep
Infantile respiratory features	AV during sleep	Hypoventilation during wake and sleep	Tracheostomy starting from 6-14 mo	?
Childhood respiratory features	AV during sleep	Hypoventilation during wake and sleep	AV during sleep and/or wake	?
Neurocognitive impairment	Profound	Variable	+ (2/3)	?
Behavioral problems	Marked autoaggression	Mild to severe if inadequate ventilatory support	?	?
Seizures	+	—	+ (2/3)	—
Autonomic nervous system dysregulation: Pupillary response to light	—	Attenuated to absent response to light	?	?
Neural crest tumor	—	PARM = no NPARM = common (neuroblastoma 50%, ganglio-neuroblastoma/-neuroma <5%)	—	—
Esophageal dysmotility/dysphagia	+	+ (1st y of life)	+	?
Constipation	+	+ (severe in >50%)	?	+ (1st y of life)
Hirschsprung disease	?	PARM 20%–30% NPARM 80%–90%	?	?
Cardiac symptoms	—	NPARM = no PARM = cardiac arrhythmia in 20%–80%	Cardiac arrest (1/3)	

Abbreviations: AD = autosomal dominant; AR = autosomal recessive; AV = artificial ventilation; CCHS = congenital central hypoventilation syndrome; NPARM = nonpolyalanine repeat expansion mutation (within *PHOX2B*); OMIM = online mendelian inheritance in man; PARM = polyalanine repeat region (within *PHOX2B*); ? unknown. Modified from reference 15.
[a] PHOX2B has 2 polyalanine repeat regions (PARM) in exon 3. Expansion of the second PARM causes CCHS.
[b] Nonpolyalanine repeat expansion mutation (i.e., missense, nonsense, frameshift, stop codon, splice site).

the rhythm generation core for mammalian inspiration.[7,8] Biallelic loss of murine Dbx1 causes inability to breathe and immediate postnatal lethality.[3,4] A selective destruction of Dbx1 expressed preBötC neurons in slice models of breathing also impairs respiratory rhythm and inspiratory related motor output.[9] A transient suppression of Dbx1-expressed interneurons within the preBöt complex furthermore interrupts the inspiratory rhythm in sedated adult mice and reduced and prolonged the inspiratory frequency and duration in awake mice.[10]

A comparable pattern of breathing was observed in the presented patient harboring a homozygous deleterious *DBX1* variant. Directly on birth, she displayed no respiratory movements, therefore necessitating artificial ventilation. Intriguingly, over months, she gradually achieved the capability of sufficient inspiration, although with an irregular rhythm and only during the wake state. While asleep, she continues to display marked apnea, therefore requiring artificial respiration (Figure 1). This observation suggests an unexpected plasticity of DBX1-regulated neural networks within the preBötC in humans which is reminiscent to what has increasingly been observed in DBX1-independent regions of the preBötC.[11]

Apart of its predominant role in preBötC, Dbx1 participates in the specification of subtypes across hypothalamic nuclei which are involved in multiple types of innate behavior.[12] In the inferior colliculus of the tectal plate, Dbx1 governs the survival of the critical integration center of the central auditory pathway.[13] Thus, the identified *DBX1* variant could also be the cause of our patient's hearing impairment and marked self-injury. Because Dbx1 was shown to control the fate of spinal interneurons involved in locomotor activity,[14] the muscular hypotonia with

impaired sucking and swallowing and the motor developmental delay, could also be due to the DBX1 dysfunction.

Notably, we did not observe symptoms indicating a classic autonomic dysregulation or neurocristopathy, which are commonly observed in individuals with other forms of CCHS (Table 1). This, along with the presence of muscular hypotonia, profound global developmental delay, and behavioral abnormalities, strongly distinguishes our patient from other known CCHS's. We therefore suggest that pathogenic variants in *DBX1* should be considered in individuals with CCHS with negative *PHOX2B*-screening and without classic features of abnormal autonomic regulation or neurocristopathies.

Taken together, despite presenting here a single patient, the combination of (1) the systematic genetic analysis by trio-exome sequencing, (2) the deleterious effect of a homozygous *DBX1* frameshift mutation, and especially, (3) the strong phenotypic similarities to Dbx1 rodent models,[3,4] fulfill previously proposed criteria for identification of novel disease genes in single patients.[16] Clearly, identification of additional patients is needed to further delineate the particular *DBX1*-associated phenotype. Moreover, our findings establish a fruitful ground for further research aiming to dissect the plasticity of neural circuits governing the inspiratory rhythm in humans.

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Author Contributions

A.T. van der Ven: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; analysis or interpretation of data. M. Hempel: major role in the acquisition of data; study concept or design; analysis or interpretation of data claas kruse: major role in the acquisition of data. M. Blohm: major role in the acquisition of data; analysis or interpretation of data. B. Grolle: major role in the acquisition of data; analysis or interpretation of data. C. Kubisch: drafting/revision of the manuscript for content, including medical writing for content; study concept or design. D. Lessel: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; study concept or design; analysis or interpretation of data.

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