Congenital Central Hypoventilation Syndrome

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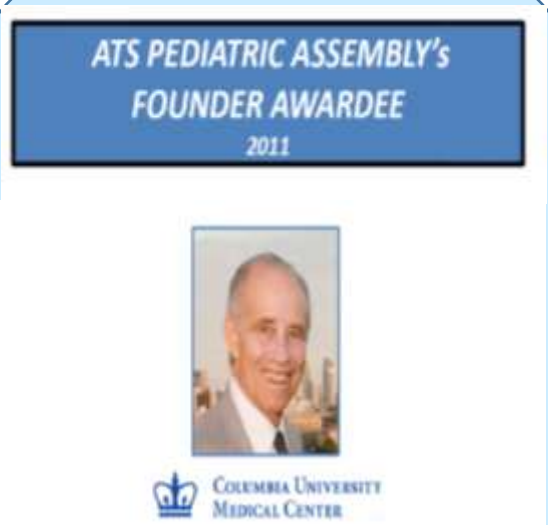
Congenital Central Hypoventilation Syndrome (CCHS)

- 1:200,000 births
- AD

- lifelong primary dysfunction of ANS
- failure of autonomic control of breathing ➡ alveolar hypoventilation mainly during sleep.

Historical milestones



Mellins RB. 1970 - Failure of automatic control of ventilation (Ondine's curse).

Association with Hirschprung dis (20%)

Neural crest tumors (2-5%) [neuroblastoma, ganglioneuroma, ganglioneuroblastoma].

Haddad G et al. 1978

Gene discovered *PHOX2B*

Amiel J et al. 2003

Weese-Mayer DE et al. 2003

CCHS - mutations in the *PHOX2B* gene.

PHOX2B gene regulates a protein synthesis that acts early in development:

1. help promote the **formation** of neurons.
2. regulate maturation and **differentiation** of neurons.
3. **function** of neurons.

The protein is active in the neural crest cells that migrate to form parts of the ANS to many tissues.

Mutations interfere with **neuron formation** and **differentiation**, especially in the ANS resulting in problems regulating breathing and other autonomic body functions.

Pathphysiology - Effect of sleep state

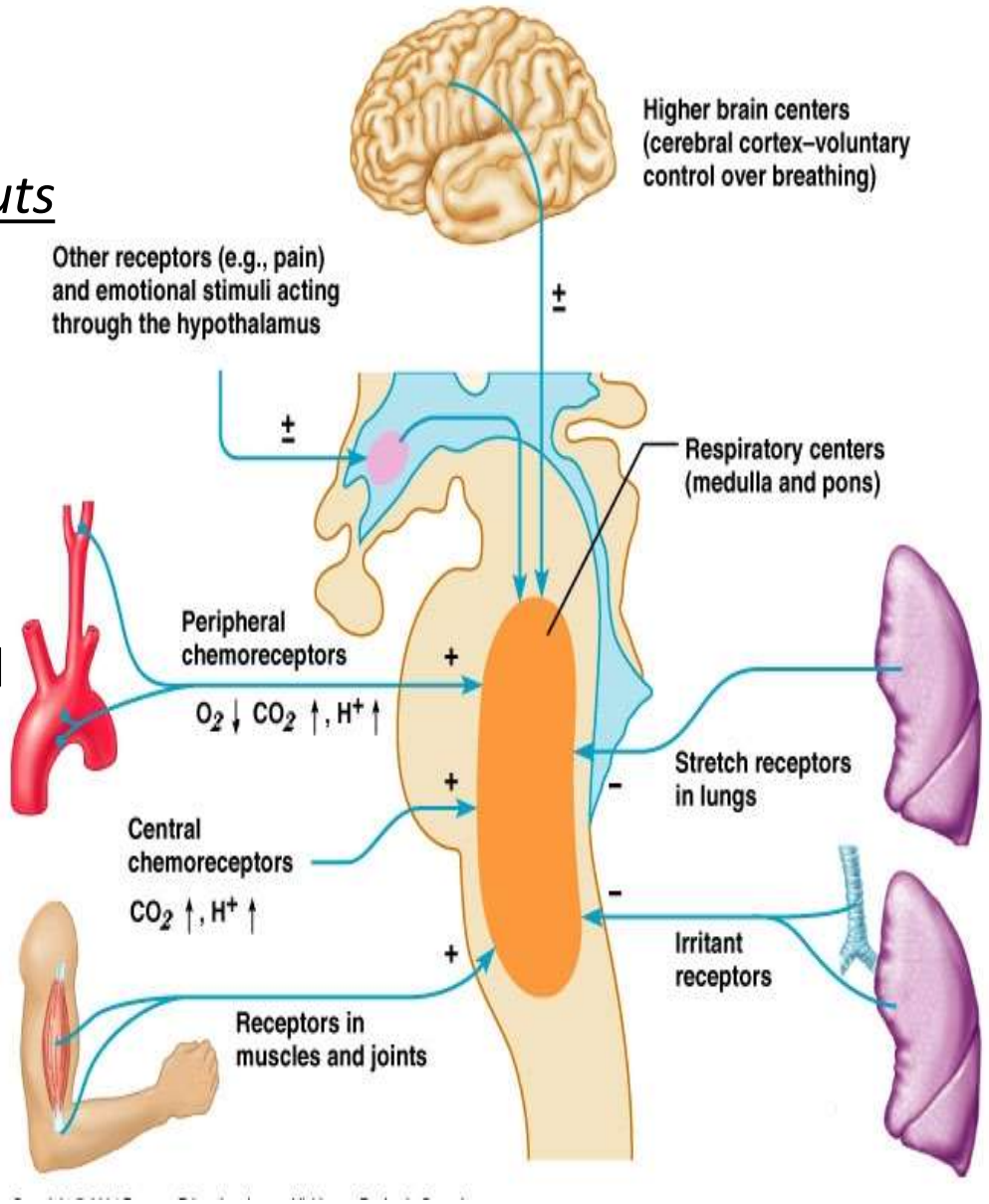
During wakefulness

Additional *non-chemoreceptive inputs*

During sleep

chemoreception regulates ABG

Hypoventilation is more pronounced during **quiet or NREM** (breathing is almost entirely under chemical control than in **REM** sleep (tonic excitatory inputs to the respiratory system)



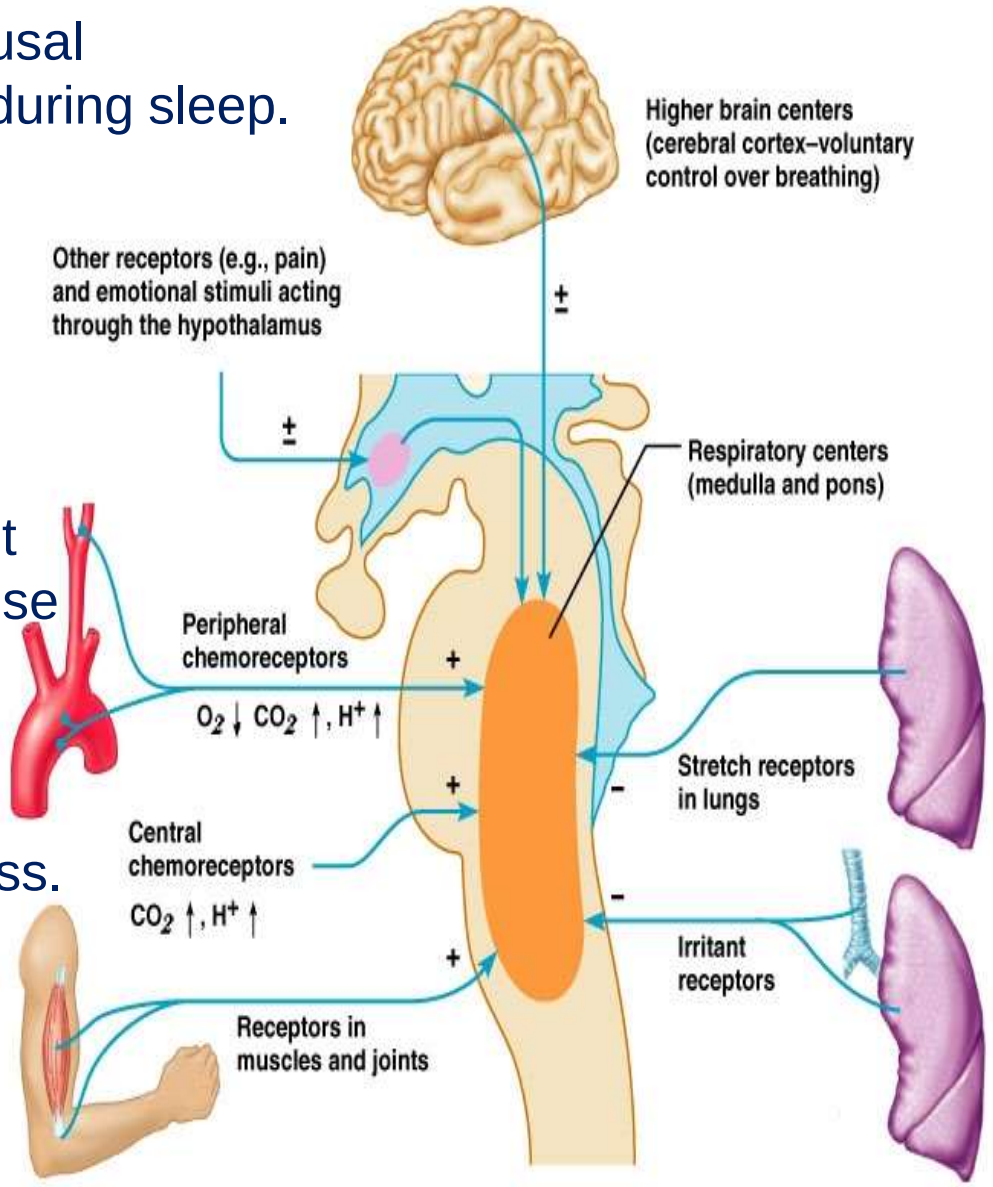
CCHS

Ventilatory responsiveness and arousal response to ⬆CO_2 is blunted mainly during sleep.

Altered or absent perception of shortness of breath when awake.

Respiratory challenges (infection) during sleep, ➡ unable to augment ventilation to meet demands or arouse (no resp. distress).

More severely affected patients hypoventilate also during wakefulness.



<u>Pathophysiology</u>:

Defective <u>integration</u> of chemoreceptors input to central ventilatory controllers (chemoreceptors are normal)

ventral medulla respiratory column

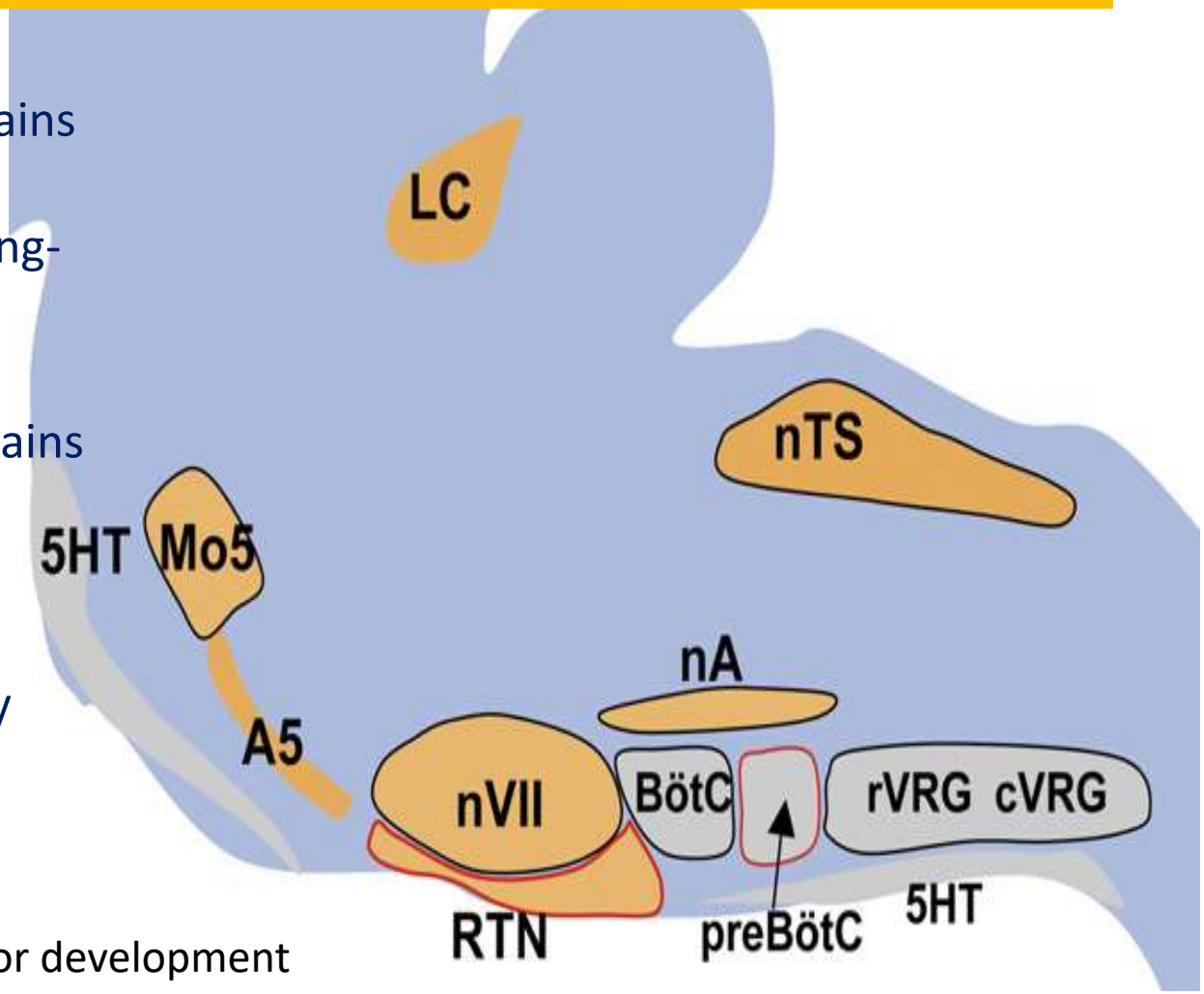
PHOX2B **is controls neuron differentiation early during pregnancy and is required in the development of :**

Also in:

nodose ganglion - contains neurons responsible for pulmonary stretch or Herring-Breuer reflex

carotid body which contains O_2 and CO_2 sensors

petrosal ganglion - innervates the carotid body

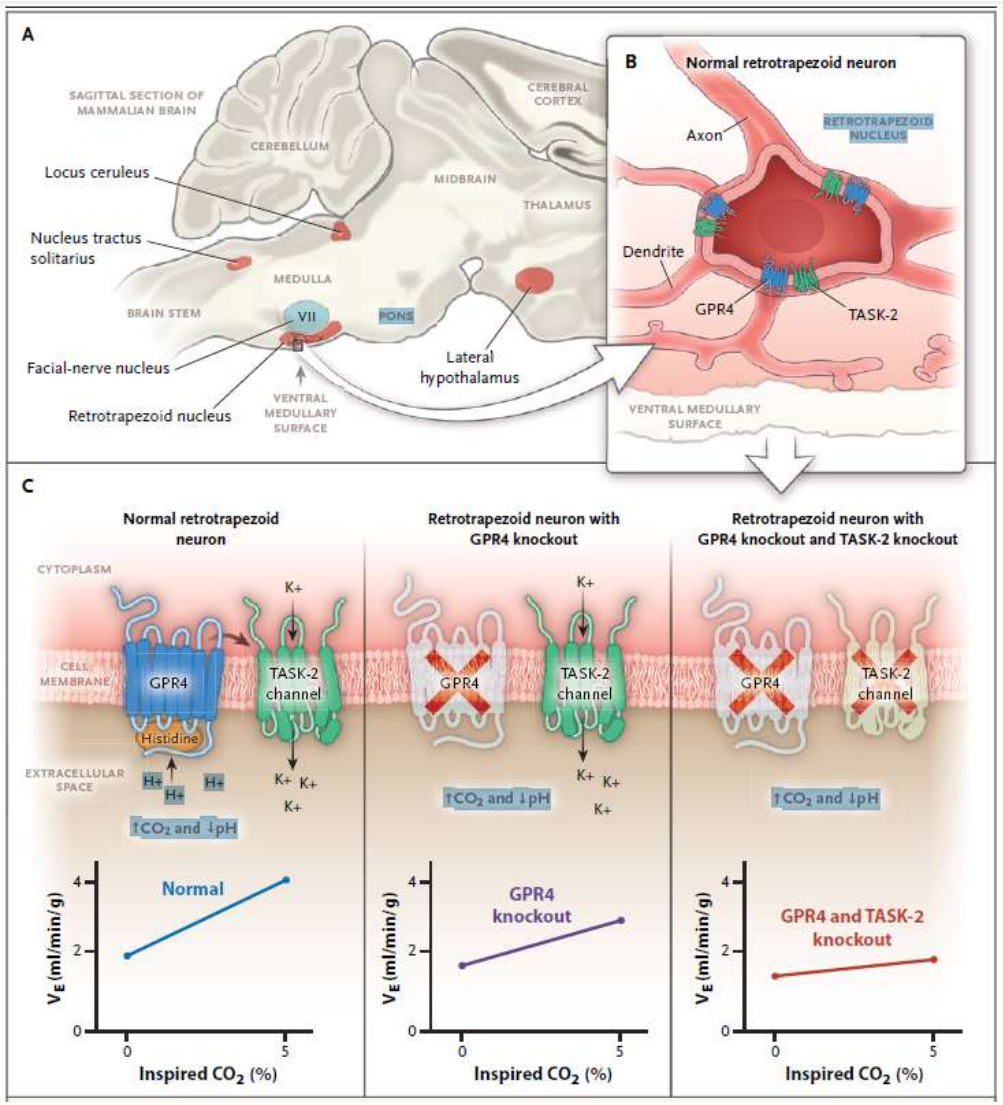


N Depend on *PHOX2B* for development

GPR4 occurs in neurons expressing *PHOX2B* plays an essential role in detecting blood CO_2 and pH levels

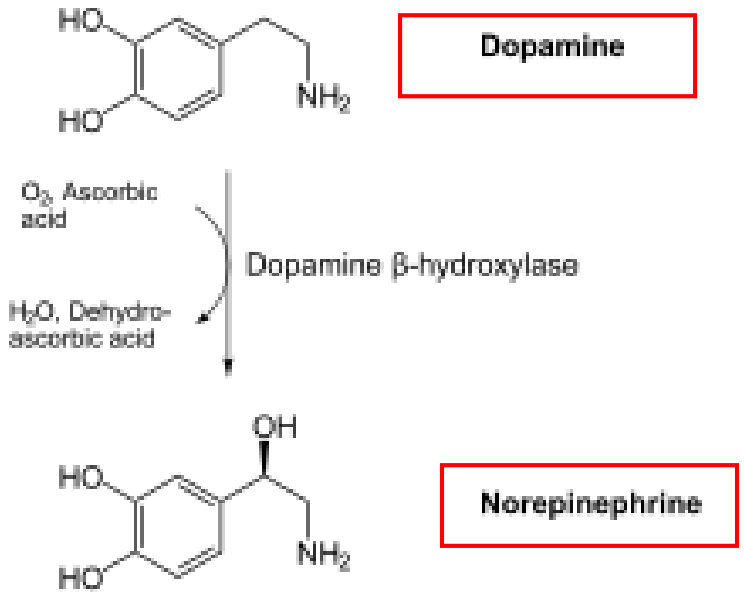
G-protein coupled receptor 4 is a protein that in humans is encoded by the *GPR4* gene

G protein coupled receptors and TASK-2 channels are activated when extracellular **pH** falls into the range of 6.4-6.8 and when **CO_2** rises.



Function reversed by reintroduction of GPR4 (via a lentivirus vector). A path to potential therapies?

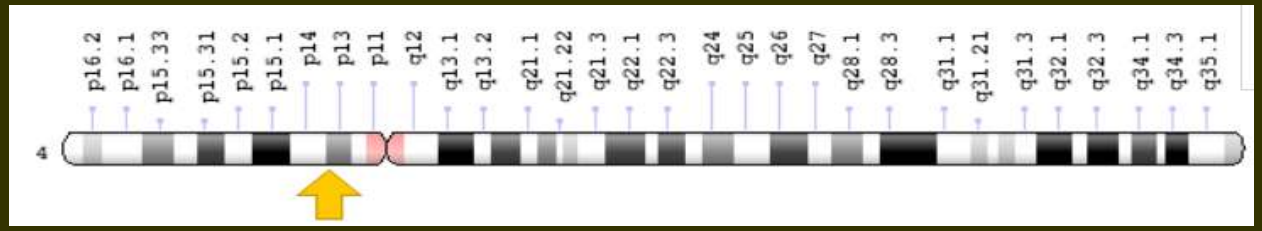
PHOX2B protein activates Dopamine beta-Hydroxylase

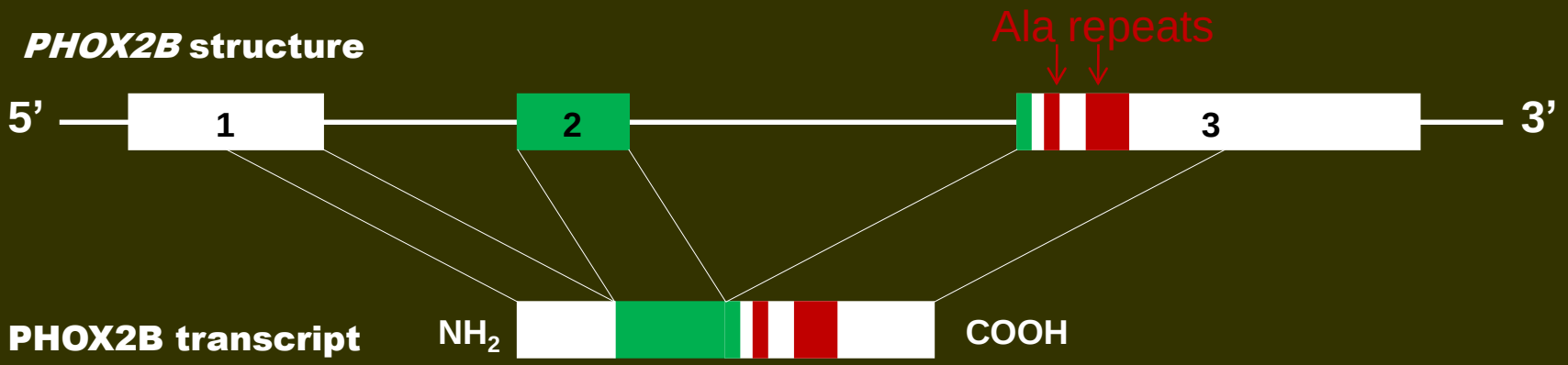


Norepinephrine is a modulator of RTN chemoreceptors and therefore important in the control of respiration and chemoreception.

The *PHOX2B* Gene

4p13

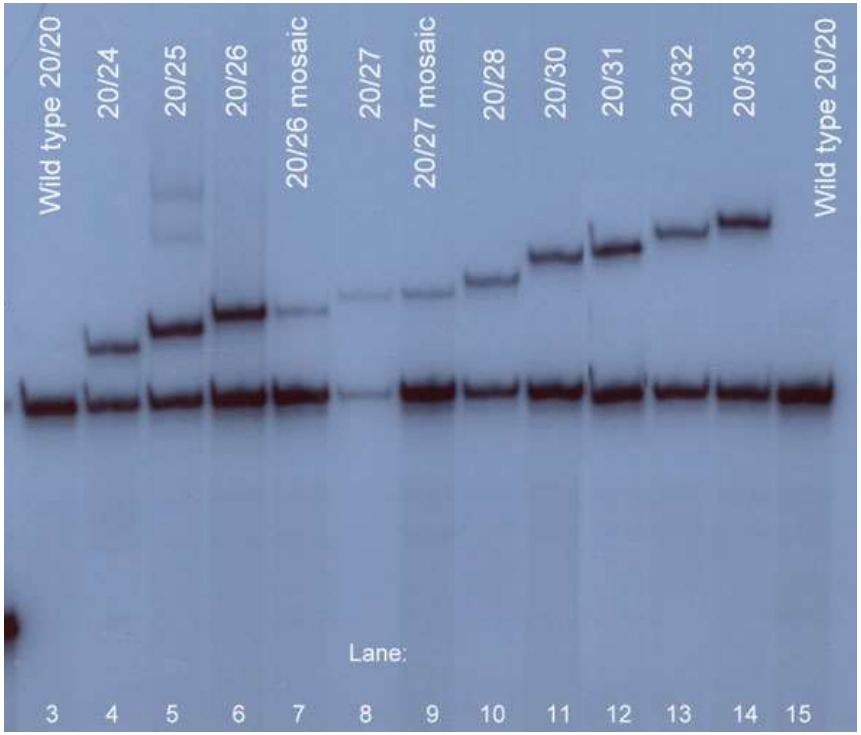


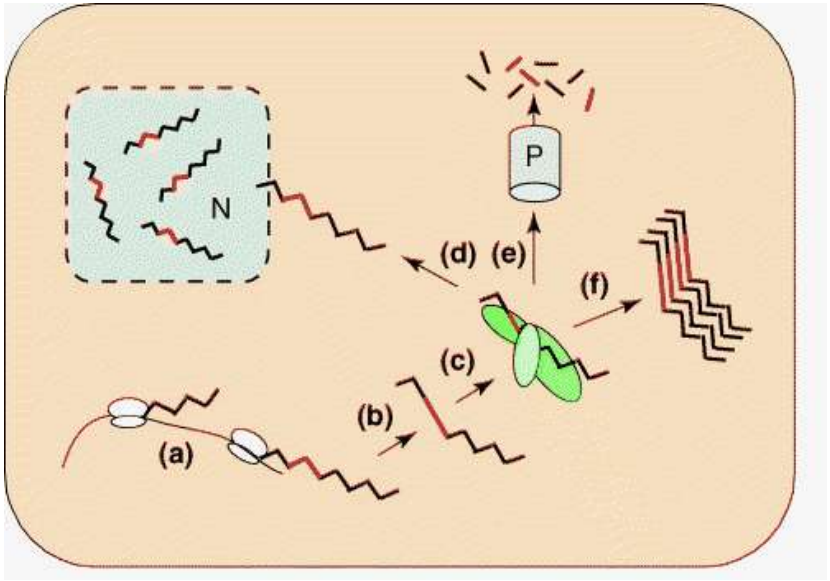


Adapted : Amiel et al. (2003) Nature Genetics 33(4), 459-461

- Paired-Like Homeobox
- 4p13, 3 exons, 314 amino acids
- 2 polyalanine repeat tracts (9 and 20 Ala)

PHOX2B Alanine Expansions

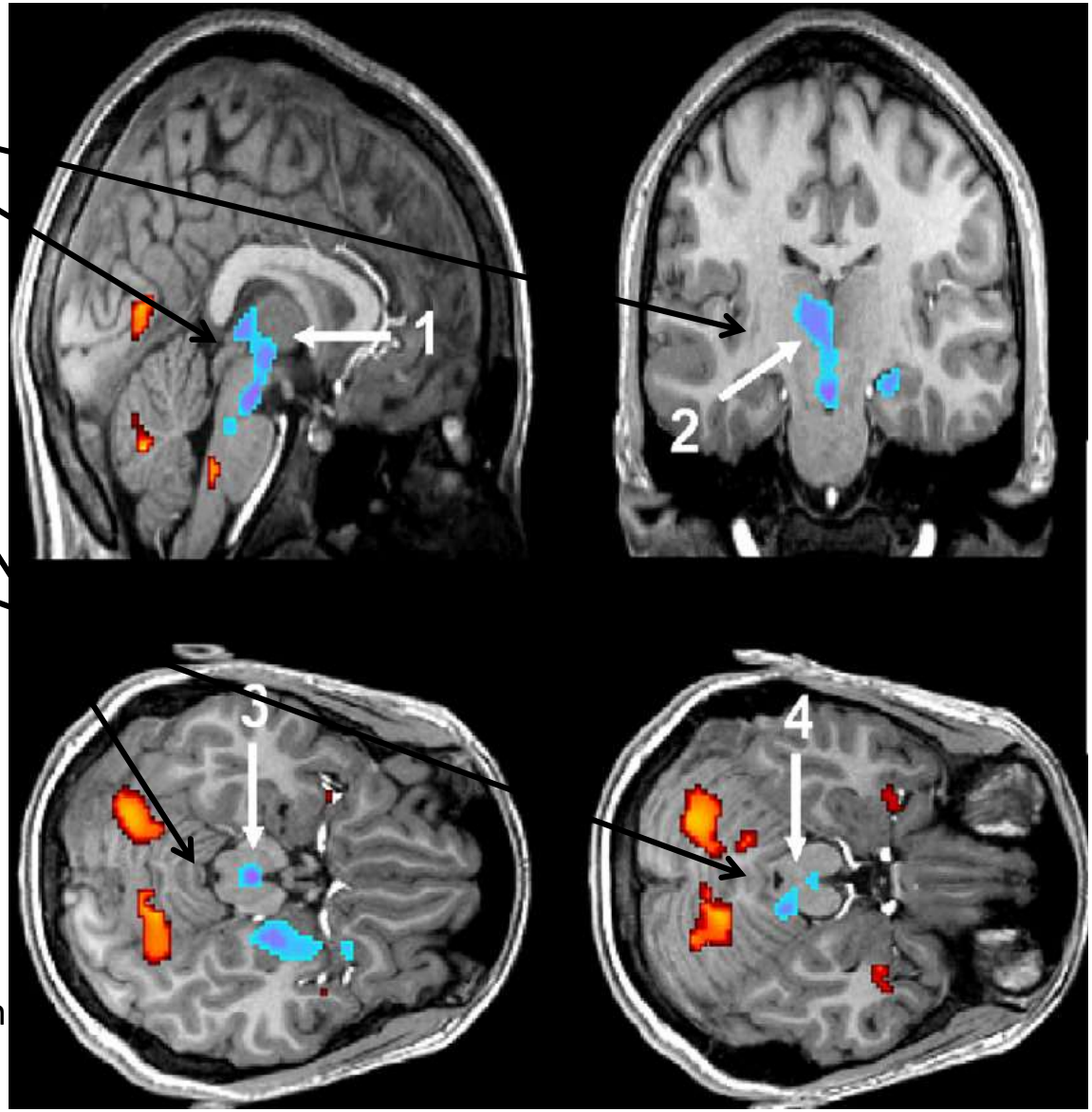




protein misfolding (oligomers instead of dimers), aggregation, reduced mobility within cell

Polyglutamine (polyQ) disorders:
Huntington dis, Kennedy dis

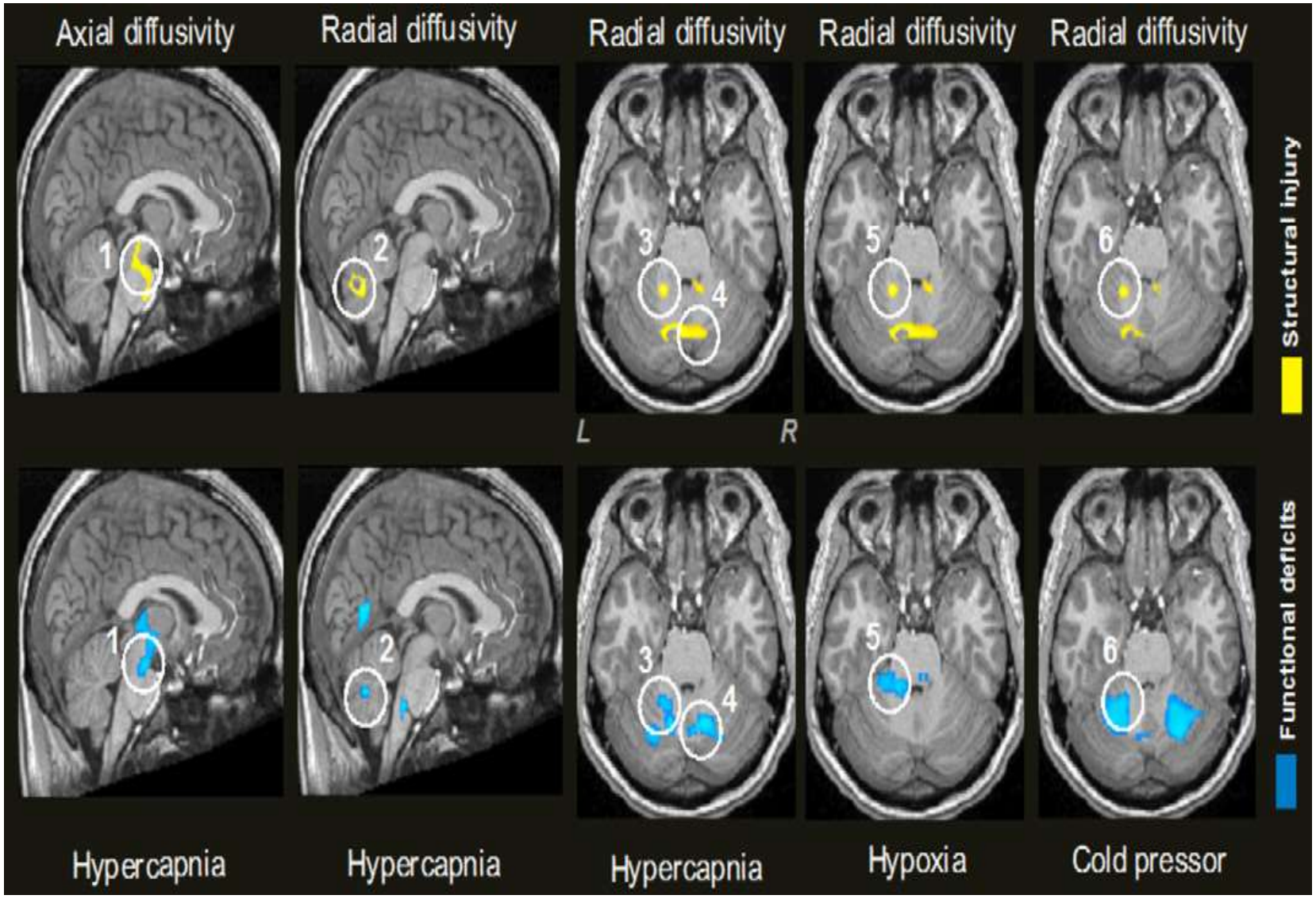
PARM = 90%
NPARM = 10%



These structures serve functions:

- Response to hypercapnia
- sensation (and thus, drive) to breathe from shortness of breath
- switching from inspiration to expiration

fMRI **responses to 5% CO2** in CCHS subjects, compared to age- and gender-matched control adolescents. Signals increase in CCHS subjects, in the dorsal medulla, cerebellum, and amygdala. In the parabrachial pons/locus coeruleus, midbrain and hippocampus, signals decline. **The warm colors represent an increase in signal responses in CCHS cases compared to controls, the cool colors represent a greater decline in CCHS over values in controls.** **Harper et al. (2005)**



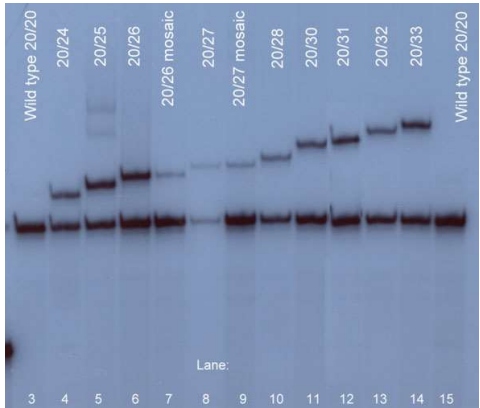
Structural injury and functional deficits appear in cerebellum, lateral medulla, and a region of tissue extending from the posterior thalamus through the midbrain [Harper et al. (2005), Kumar et al. (2008), and Macey et al. (2005)].

Clinical presentation

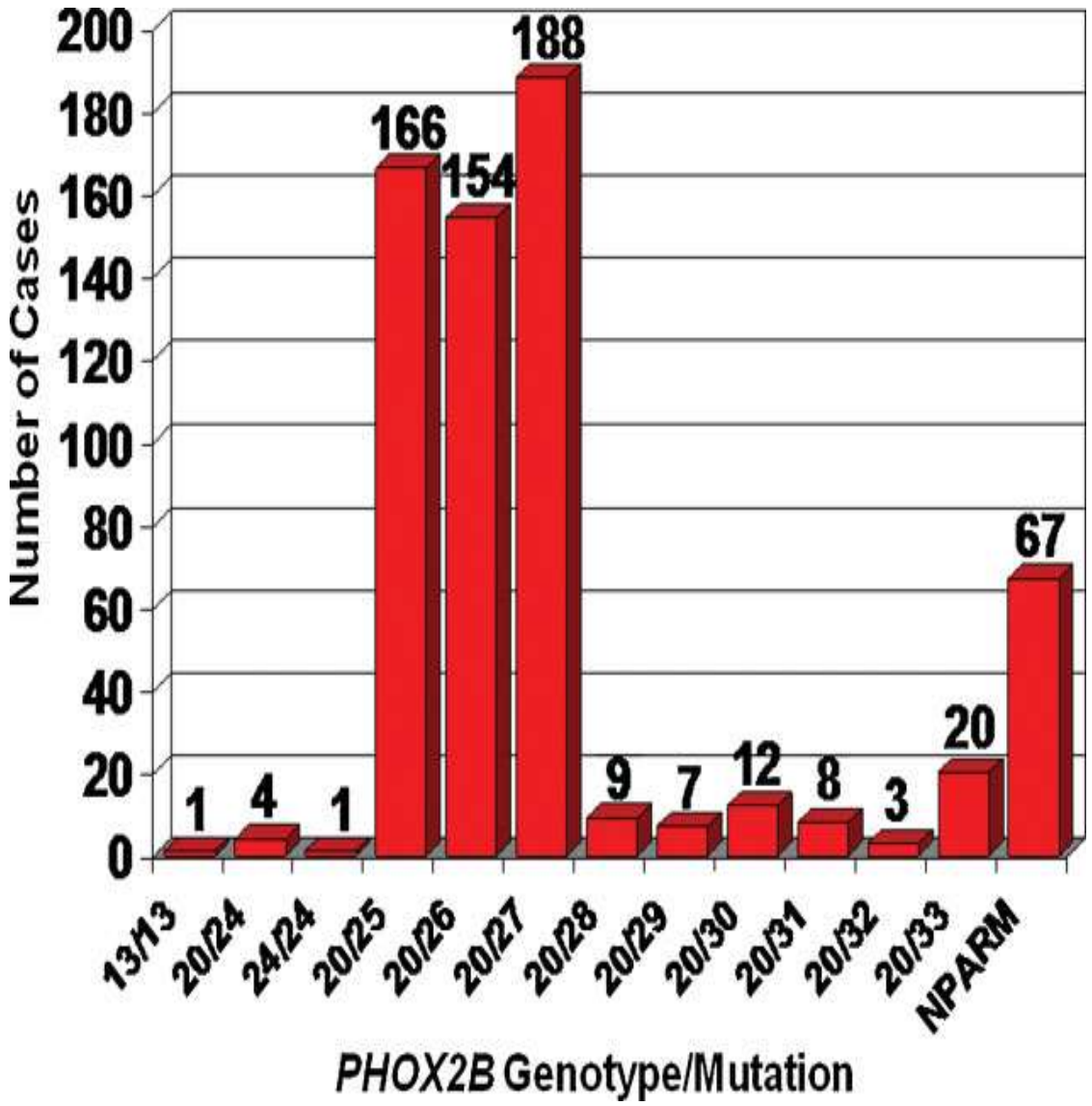
- Most present immediately after birth as cyanosis or cyanotic spells, shallow breathing, bradypnea.
- Some present in the first few months of life with (A)LTE.
- Absence of hyperventilation in response to hypercapneic challenge.

Genotype (AD) – phenotype relationships

- Higher repeats - greater severity of the respiratory phenotype.
- 20/25 genotype usually require only nocturnal ventilation, risk for ventilatory decompensation during anesthesia, resp. infections.
- 20/28 to 20/33 usually require ventilatory support also during wakefulness.
- Later onset cases documented with 20/24 or 20/25 genotypes.
- Hirschsprung's > 20/27 (15-20%) and NPARM (>85%).
- Neuroblastoma occurs in NPARMs mutations (animal studies: *PHOX2B* has strong anti-proliferative effects on sympathetic neuroblasts – acts as tumor suppressor).
- Ganglioneuroma, ganglioneuroblastoma develop in a small subset of those with the longest PARMs.



Feature	**Polyalanine expansion** (+4 to +13 Ala)	**Frameshift / missense mutation** (NPARM)
Location in PHOX2B	20 alanine tract in exon 3	Exon 3 or end of exon 2 (most)
Proportion of all pathogenic variants	~90%	~10%
Present with Hirschprung's Disease	<20%	>87%
Develop neural crest tumour	~1% (all ≥+11 Ala)	~50% over 1 year old
Parent carries mutation	Up to 14%	Almost all *de novo*
Predicted effect on protein	Misfolding, cytoplasmic aggregate formation, nuclear exclusion	Nuclear sequestration



Over 1,000 CCHS cases ave been reported

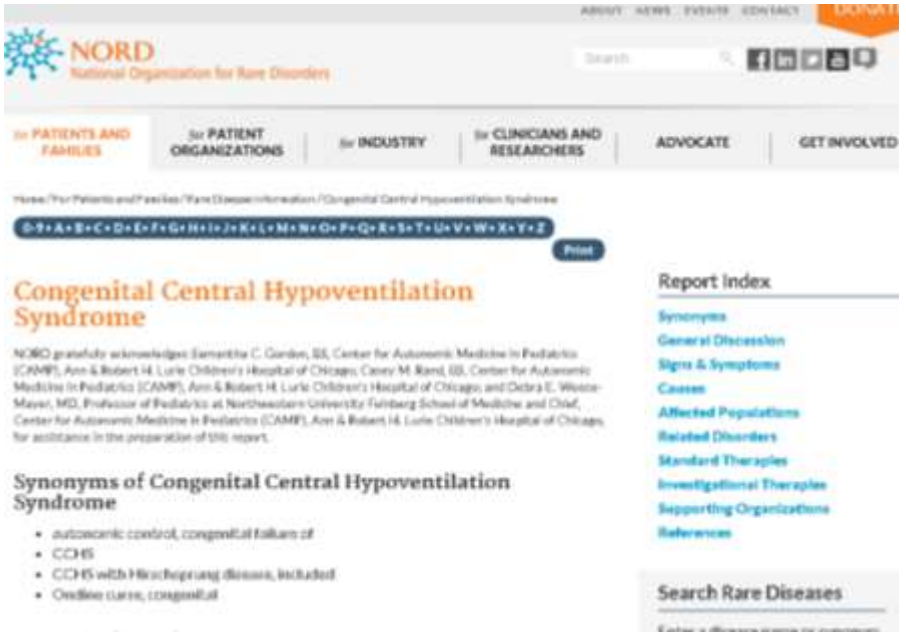
In most cases the mutation arise **de novo (AD)**.
15 to 20% of healthy parents show **somatic mosaicism**. (chance for inheritance depends on degree of mosaicism in germ cells).

As a germ line mosaicism cannot be ruled out, parents with no somatic mosaicism detected are counseled at 1% recurrence risk in siblings.

Alanine **contractions** (−5, −7 and −13 alanines) - no phenotypic consequences reported to date.

Associated autonomic dysfunctions

- temperature instability (low basal body temp)
- excessive sweating
- decreased perception of discomfort and anxiety
- ≥ 26/20 – HRV↓, BHS, syncope, cardiac arrhythmia, bradycardia / asystoles (27/20 – 83% risk for asystole, 0% in 25/20, 19% in 26/20]
- blood pressure does not fall during sleep
- ophthalmologic (sluggish pupils, altered lacrimation, anisocoria)
- characteristic facial features (increase with the length of PARM)
- esophageal dysmotility
- glucose metabolism



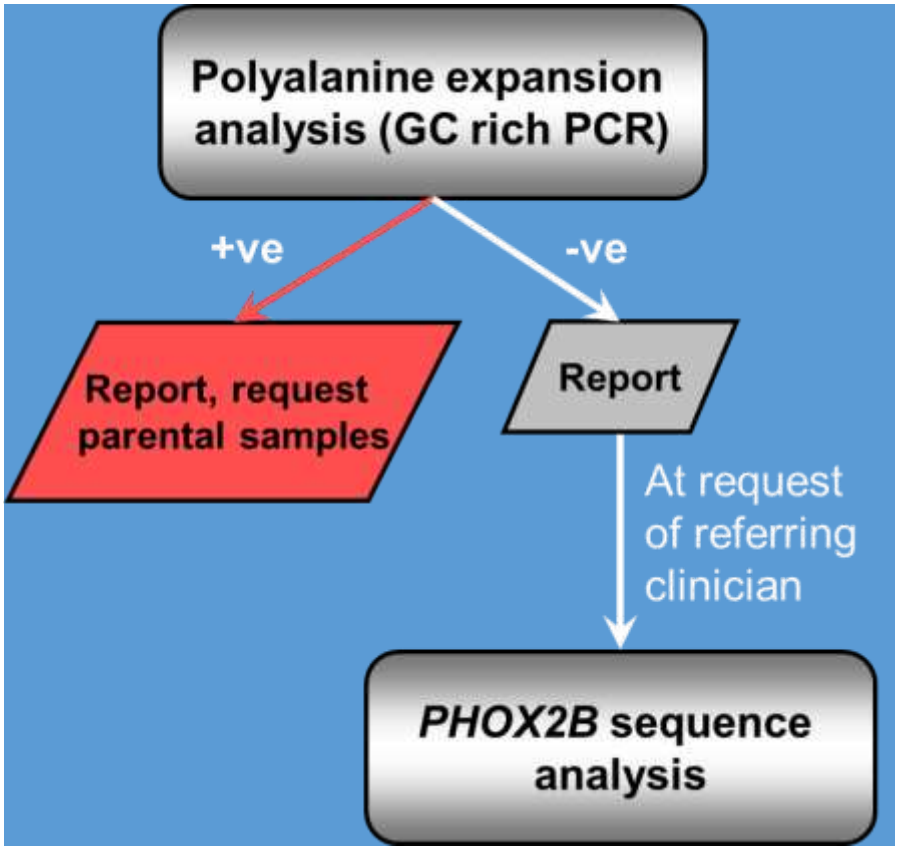
יד לנשימה

החלום שלי זה לנשום - CCHS ישראל

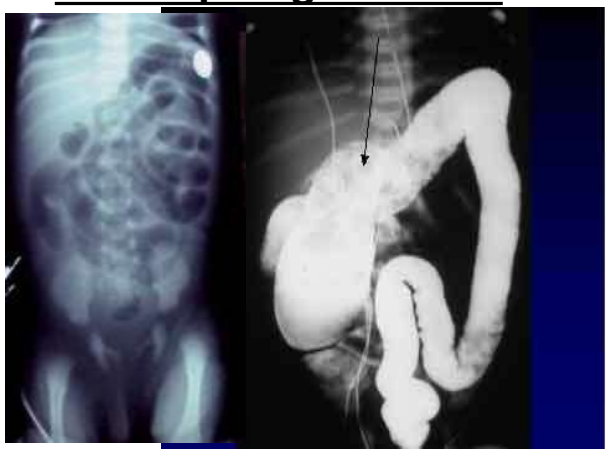
Initial investigations

PHOX2B testing confirmation is now **required** for a diagnosis of CCHS (ATS statement on CCHS 2010).

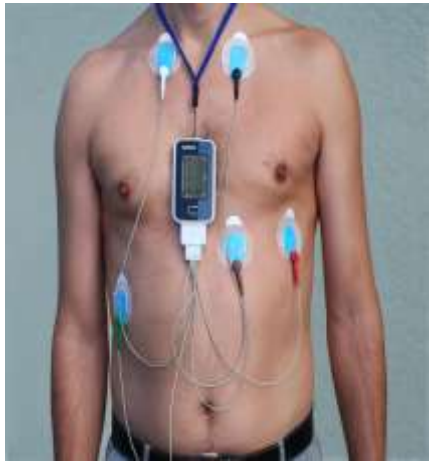
PHOX2B Testing Strategy

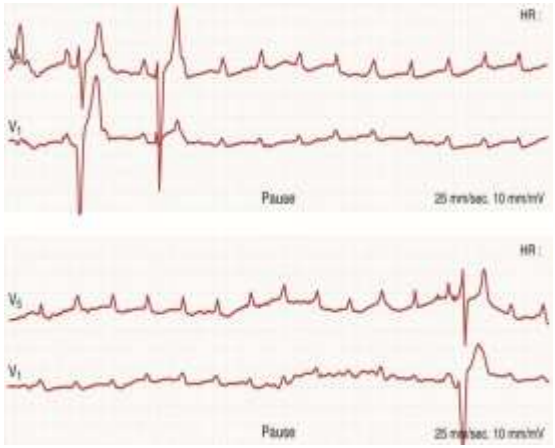


Hirschsprung's disease



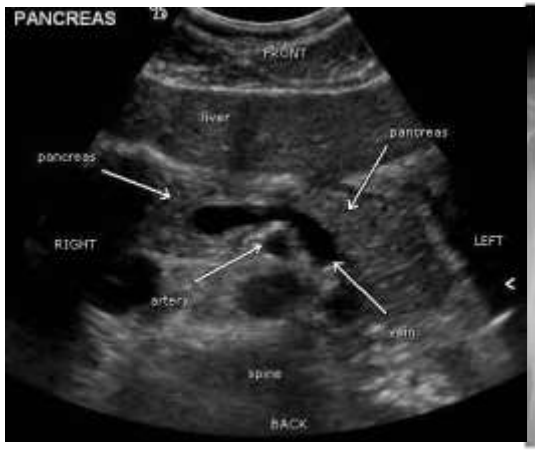
Look for constipation/abd. distension

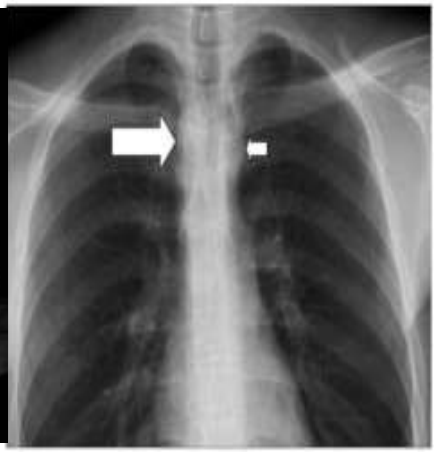




- asystole
- criteria for pacing (compared to general population have not been established)

tumors





particularly in patients with the corresponding mutations.

Other investigation – family genetic screening

Prenatal diagnosis

אבחון גנטי טרום השרשה

Preimplantation Genetic Diagnosis (PGD)

Management

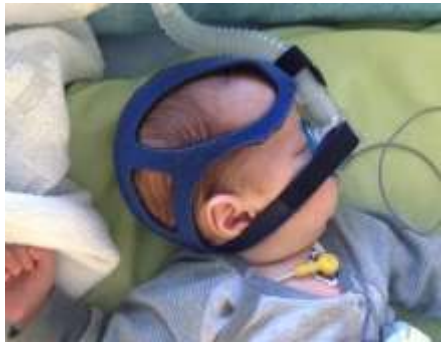
The goal of treatment for CCHS is to ensure adequate oxygenation and ventilation during both wakefulness and sleep.

Oxygen administration alone will improve spO_2 but will not prevent hypoventilation and the ensuing complications including PAH.

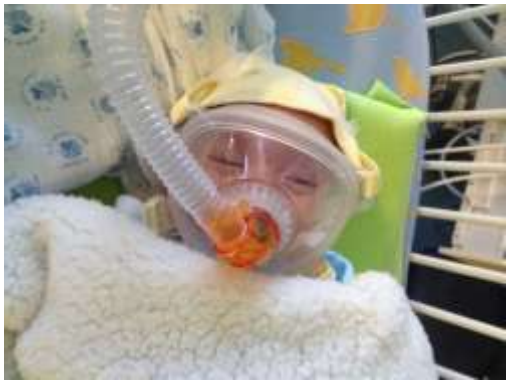
Keeping $paCO_2$ at 30-35 mmHg and $sPO_2 \geq 95\%$ allows for better spontaneous ventilation during the day.

Home CO_2 and O_2 monitoring – essential. May need to augment support during illnesses.

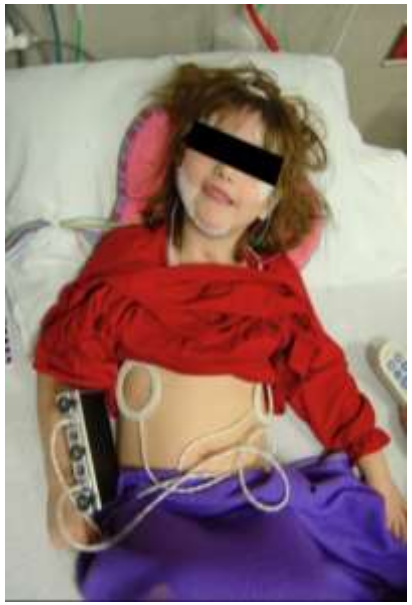
PPV via tracheostomy



NIPPV



Diaphragmatic pacing



Medications

Desogestrel: facts and hope

Response to CO2 was restored in two female patients with CCHS (+5 ala, +6 ala) taking desogestrel as a contraceptive pill.

Respir Physiol Neurobiol 2010

Desogestrel - a potent progesterone receptor agonist induces changes in respiratory control by activating autonomic CO_2 chemosensitive regions that are **unaffected** by *PHOX2B* mutations, such as chemoreceptors in the hypothalamus and the peripheral nervous system.

Progress in studies identifying candidate drug treatments that modulate the expression of the *PHOX2B* gene.
Several in vitro studies have investigated drugs that promote the clearance of mutant proteins.

children with CCHS already demonstrate reduced neurocognitive performance.
Do deviations in neurocognitive performance are intrinsic to the CCHS genotype or due to diffuse central nervous system insult (e.g, hypoxia).

8 of the 19 cases (42%) with 25 PARM were complicated by mental retardation

Shimokaze et al. Journal of Human Genetics 6.2015

Are LO-CCHS children at greater risk for ND deficiencies due to unrecognized sleep hypoxemia?

Table 4. Mental and Motor Development Scores for CCHS-related *PHOX2B* Genotype Groups

	Mental Score*	Motor Score**
Genotype (frequency)		
Polyalanine Repeat Expansion Mutations (PARMs)(25)		
20/25 (7)	103.29 ± 13.76	93.33 ± 2.33[a]
20/26 (9)	76.89 ± 22.17	70.22 ± 19.24
20/27 (8)	66.00 ± 13.58	65.13 ± 15.08
20/33 (1)	83.00 ± N/A	54.00 ± N/A
Non-PARMs (NPARMs) (5)	84.00 ± 29.40	62.60 ± 18.99
Whole Gene Deletion (1)	138.00 ± N/A	120.00 ± N/A
Values provided as mean ± SD		
*P < 0.001; **P < 0.006 (for comparison of 20/25, 20/26, and 20/27 PARM genotypes) [a]N = 6 for Motor Score in 20/25 group		

Shimokaze et al. Journal of Human Genetics 6.2015

Table 2 Clinical features of the cases with 25 polyalanine repeat expansion mutations

Case	sex	GA (wk)	Birth weight (g)	Apgar score 1 min/5 min	Age at presentation of CH	Age at diagnosis of CCHS	Ventilatory management (periods)	DQ or IQ (assessed method) age	Other clinical features
1	M	40	3046	9/na	1 mo	5 mo	CPAP (1 mo–10 mo) Tracheostomy and IMV (10 mo–4 yr) BiPAP (4 yr–)	DQ 99 (*K*-test) 5.6 yr	
2	M	39	2900	8/na	10 mo	15 yr	LTOT and IMV (10 mo–11 mo) Tracheostomy and IMV (11 mo–)	DQ 71 (Enjoji) 10 mo	
3	M	38	2902	9/10	<1 mo	1 mo	Intubation and IMV (<1 mo–)	na	
4	M	33	2282	8/9	<1 mo	<1 mo	BiPAP (<1 mo–)	IQ 85 (WISC-III) 8.1 yr	Familial case
5	F	38	3000	9/na	<1 mo	1.6 yr	Intubation & IMV (<1 mo–2 mo) Tracheostomy & IMV (2 mo–7 yr) BiPAP (7 yr–)	DQ 88 (WPPSI) 5.7 yr	
6	F	41	2786	9/10	<1 mo	1 mo	Intubation and IMV (day 7–1 mo) Tracheostomy and IMV (1 mo–)	DQ 117 (Enjoji) 3 yr	
7	M	37	na	na/na	1.2 yr	1.2 yr	HOT (1.2 yr–5 yr) BiPAP (5 yr–)	DQ 48 (*K*-test) 5 yr	Cor pulmonale reported case (ref. 25)
8	F	39	2758	9/10	<1 mo	2 mo	HOT (<1 mo–3 yr) BiPAP (3 yr–)	DQ 51 (*K*-test) 3.6 yr	
9	M	39	2802	na/na	1 mo	4 yr	BiPAP (1 mo–)	na	Pulmonary hypertension, cor pulmonale
10	M	39	2450	9/9	<1 mo	3 mo	CPAP (<1 mo–3 mo) Tracheostomy and IMV (3 mo–)	normally developed	Ventricular septal defect
11	M	40	3436	Asphyxia	<1 mo	<1 mo	HOT (<1 mo–3 yr) BiPAP (3 yr–)	IQ 60 (WISC-III) 8 yr	Pulmonary hypertension, constipation older brother of case 12
12	M	37	3312	5/6	<1 mo	3 mo	HOT (<1 mo–)	MR	Hypoxic-ischemic encephalopathy younger brother of case 11
13	M	36	2600	Asphyxia	<1 mo	10 yr	HOT (<1 mo–1 yr) BiPAP (10 yr–)	IQ 60 (WISC-III) 6.9 yr	Autism
14	M	na	na	na/na	<1 mo	11 yr	CPAP (<1 mo–1 mo) CPAP (11 yr–)	IQ 85 (WISC-III) 15.6 yr	
15	M	na	na	na/na	1 mo	na	BiPAP (6 mo–)	MR	Pervasive developmental disorder, familial case
16	M	37	2740	8/9	<1 mo	<1 mo	NPPV (<1 mo) HOT (4 yr–12 yr) BiPAP (12 yr–)	DQ 67 (*K*-test) 6 yr	Acute encephalopathy (12 yr 5 mo)
17	F	40	3050	9/10	3 yr	3 yr	BiPAP (3 yr–)	IQ <45 (WISC-III)	

Long-term prognosis

Mortality = 10-38%, causes – cor pulmonale, aspirations, pneumonia
common age – small infants

QOL – most = good.
Home settings, treatment, resources and support – **crucial**

Periodic assessment – some children may be weaned from daytime ventilation – needs sprint training.

Most maintain regular schools. many of these patients now live full adult lives with careers and families.

The first generation of children with CCHS is now surviving to adulthood.

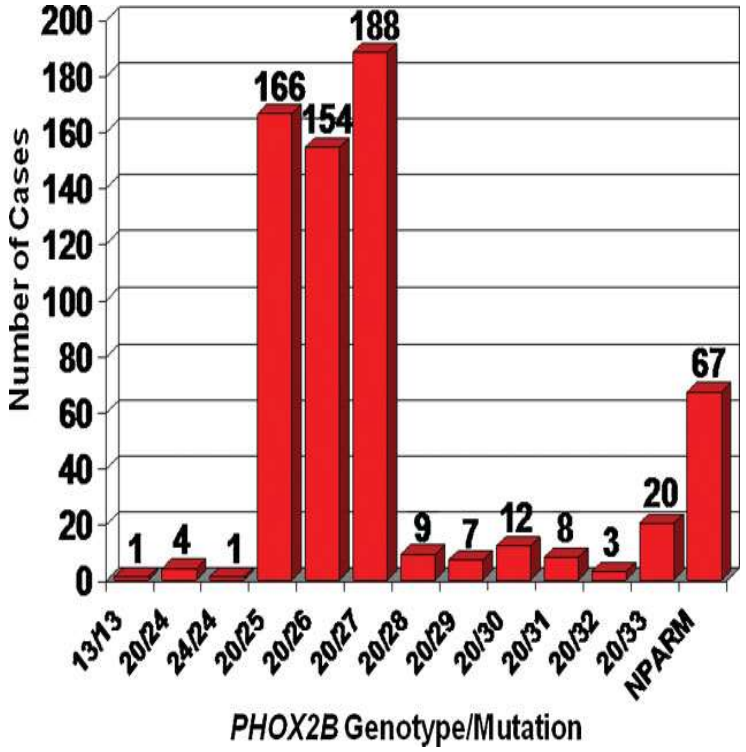
CCHS in Israel

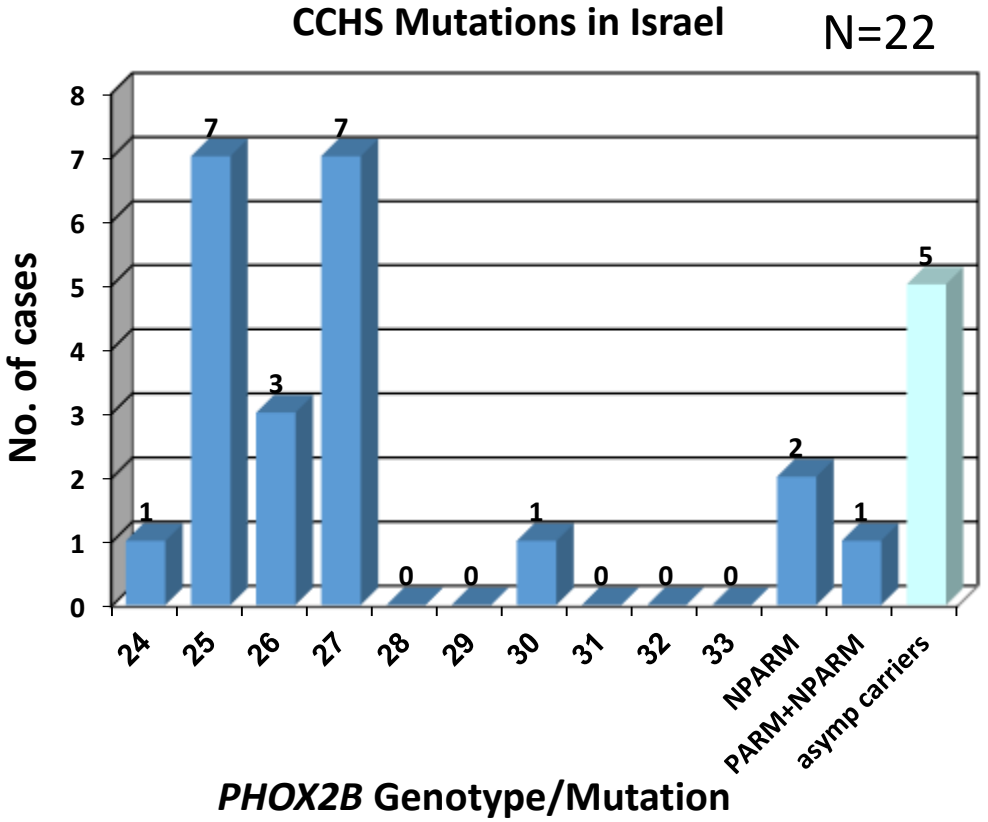
בית החולים אדמונד וליli ספרא לילדים
המרכז הרפואי ע"ש ח. שיבא, תל השומר
בית החולים של המדינה

CCHS Center

1:200,000

1:80,000 – 1:100,000





CCHS in Israel

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בית החולים של המדינה

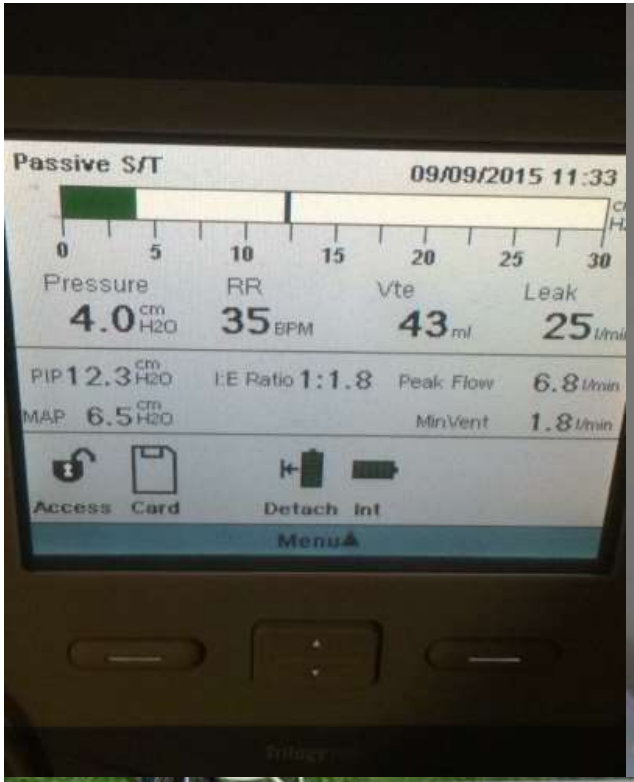
CCHS Center

n	22
Age (y) mean, median, range	5.5 (0.4 – 30)
Nocturnal ventilation	20
Daytime ventilation	2
Tracheostomy	16
Non-invasive ventilation	4
Hirschprung	*5
Cardiac pacing	2
Diaphragmatic pacing	1
Neuroblastoma	1
Developmental delay	3
ASD	3
Seizures	4 (2 in ASD)
Other neurologic problems	6
Eye problems	8

CCHS – follow-up protocol

CCHS Center
בית החולים אדמונד ולילי ספרא לילדים
המרכז הרפואי ע"ש ח. שיבא, תל השומר
בית החולים של המדינה

בדיקה נדרשת	שיטת בדיקה	תדירות
רמת דו תחמוצת הפחמן בערות	קפנוגרפיה – end-tidal CO2	אחת לחצי שנה עד גיל שנתיים אח"כ אחת לשנה
רמת סטורציה בערות	Pulse oximeter	כנ"ל
72 שעות הולטר ECG	הולטר	אחת לשנה
אקו לב		אחת לשנה
מעבדת שינה פוליסומנוגרפית	כולל טיטרציה של הנשמה (מכשיר ביתי) לערכי CO_2 = 33-36	אחת לשנה
מילוי שאלון מערכת אוטונומית של שיקגו	Autonomic Nervous System Dysregulation questionnaire	גיל שנה, שנתיים, 4, 6
תגר נשימתי	תגובה לדו תחמוצת הפחמן	אם אין צבירת CO_2
צילום חזה	מכון דימות	אחת לחצי שנה עד גיל 3 ואחר כך אחת לשנה עד גיל 7
אולטרסאונד בטן	מכון דימות	אחת לחצי שנה עד גיל 3 ואחר כך אחת לשנה עד גיל 7


Passive S/T
09/09/2015 11:33
0 5 10 15 20 25 30
Pressure
4.0 cmH2O
RR
35 BPM
Vte
43 ml
Leak
25 l/min
PIP 12.3 cmH2O
I:E Ratio 1:1.8
Peak Flow
6.8 l/min
MAP 6.5 cmH2O
MinVent
1.8 l/min
Access
Card
Detach
Int
Menu


36.9
100
89


MGB-3800
AVR
BRONCO
CONSTRUCTION
EQUIPMENT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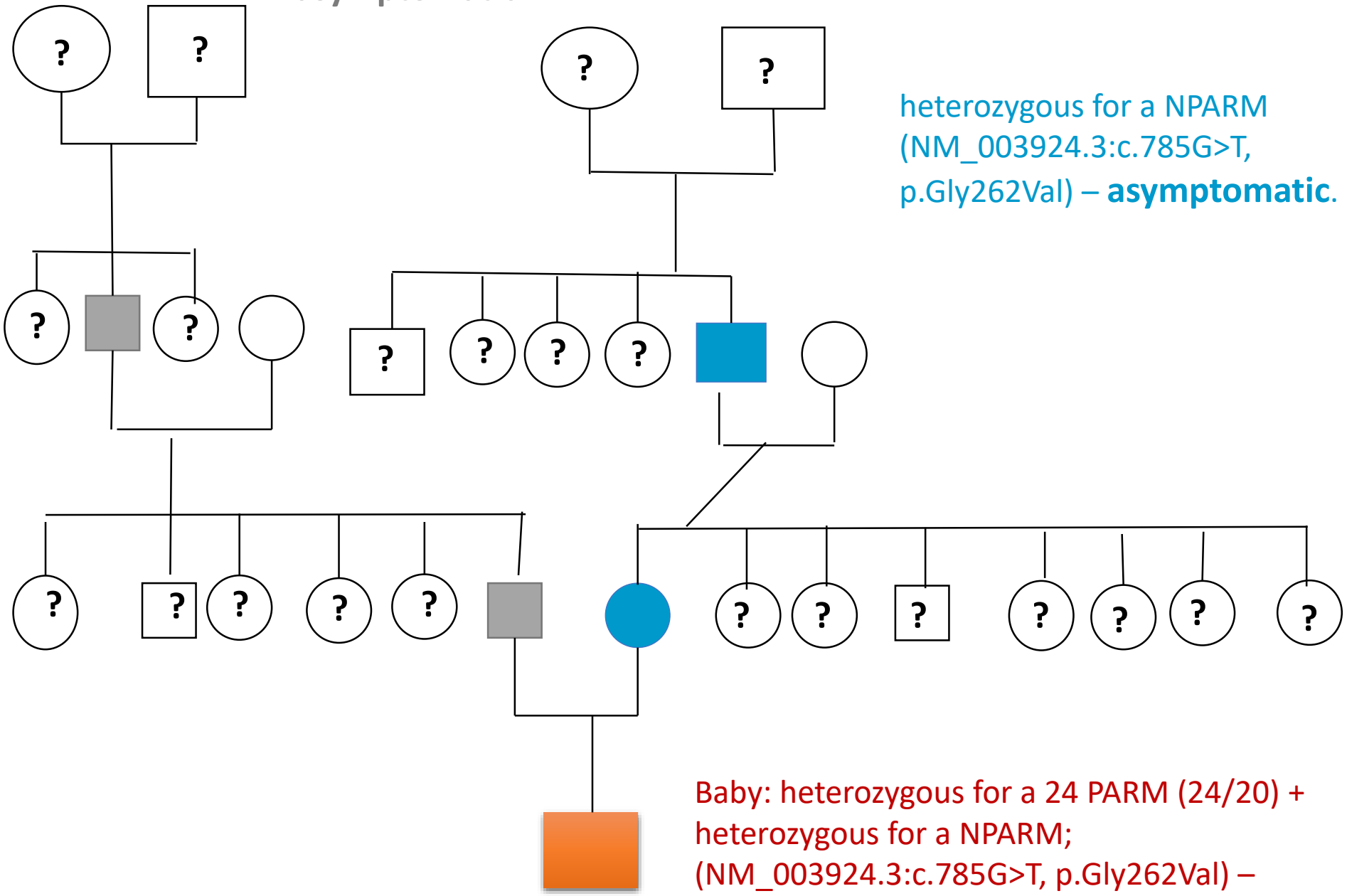
heterozygous for a PARM (24/20) – **asymptomatic**.



heterozygous for a NPARM (NM_003924.3:c.785G>T, p.Gly262Val) – **asymptomatic**.

Baby: heterozygous for a 24 PARM (24/20) + heterozygous for a NPARM; (NM_003924.3:c.785G>T, p.Gly262Val) –

Current Research and Directions

Basic considerations

- Is it a loss-of-function or a toxic gain-of-function mechanism?
- Is it a gene that needs to be replaced or one that needs to be knocked-down?
- Is it a developmental disorder that can only be treated before birth or early infancy?
- Is there ongoing toxic damage?
- Is there a deficiency effect of a non functional protein that can be treated throughout life?
- Candidate drugs that modulate expression of *PHOX2B* gene (animal studies ongoing)

Current Research and Directions

- Candidate drugs that enhance a non-*PHOX2B* respiratory stimulation (studies ongoing)
- Promoting clearance of mutant proteins (animal studies ongoing)
- Ventilatory assist modes
- Enhancement of respiratory response to chemosignals
- Lessons learned from other amino acids expansion diseases (polyQ)
- “on” / “off” studies

בית החולים
אדמונד ולילי ספרא
לילדים

המרכז הרפואי ע"ש ח. שיבא, תל השומר
בית החולים של המדינה

CCHS Center

- שרון רגב ירושלמי
- דר' אבישי להד
- דר' שי טאיימן-ירדן, דר' אריאל כץ, ד"ר רועי ביינרט
- צוות המכון להתפתחות הילד - דר' לידיה גביס
- צוות נוירולוגיה ילדים – פרופ' ברוריה בן זאב
- מכות הדימות - דר' מיכל סודק בן-נון
- דר' תמרה ויגננסקי
- דר' אמיר שינברג
- פרופ' אורי אפרתי
- עמותת יד לנשימה

יד לנשימה
החלום שלי זה לנשום - CCHS ישראל


Ondine

Ondine, a water nymph, fell in love with a knight and married him

"every waking breath would be a testimony of my love"

She bore his child. In doing so, she lost her eternal youth and immortality. Catching her husband in bed with another women, she placed a curse on him:

"You swore faithfulness to me with every waking breath, and I accepted your oath. So be it. As long as you are awake, you shall have your breath, but should you ever fall asleep, then that breath will be taken from you and you will die!"

CCHS - mutations in the *PHOX2B* gene.

PHOX2B gene regulates a protein synthesis that acts early in development:

1. help promote the **formation** of neurons.
2. regulate maturation and **differentiation** of neurons.
3. **function** of neurons.

The protein is active in the neural crest cells that migrate to form parts of the ANS to many tissues.

Mutations interfere with **neuron formation** and **differentiation**, especially in the ANS resulting in problems regulating breathing and other autonomic body functions.

PHOX2B required and expressed in breathing circuits

Nodose ganglion

↓ **responsible for**

pulmonary stretch
Herring-Breuer reflex

Petrosal ganglion

↓ **innervates**

Carotid body

↓ **responsible for**

CO_2 sensors
O_2 sensors

Solitary tract nucleus

↓ **Inegration of**

Mechano-receptors
Chemo-receptors
Baro-receptors

Noradrenergic centers

Locus ceroleus
A5

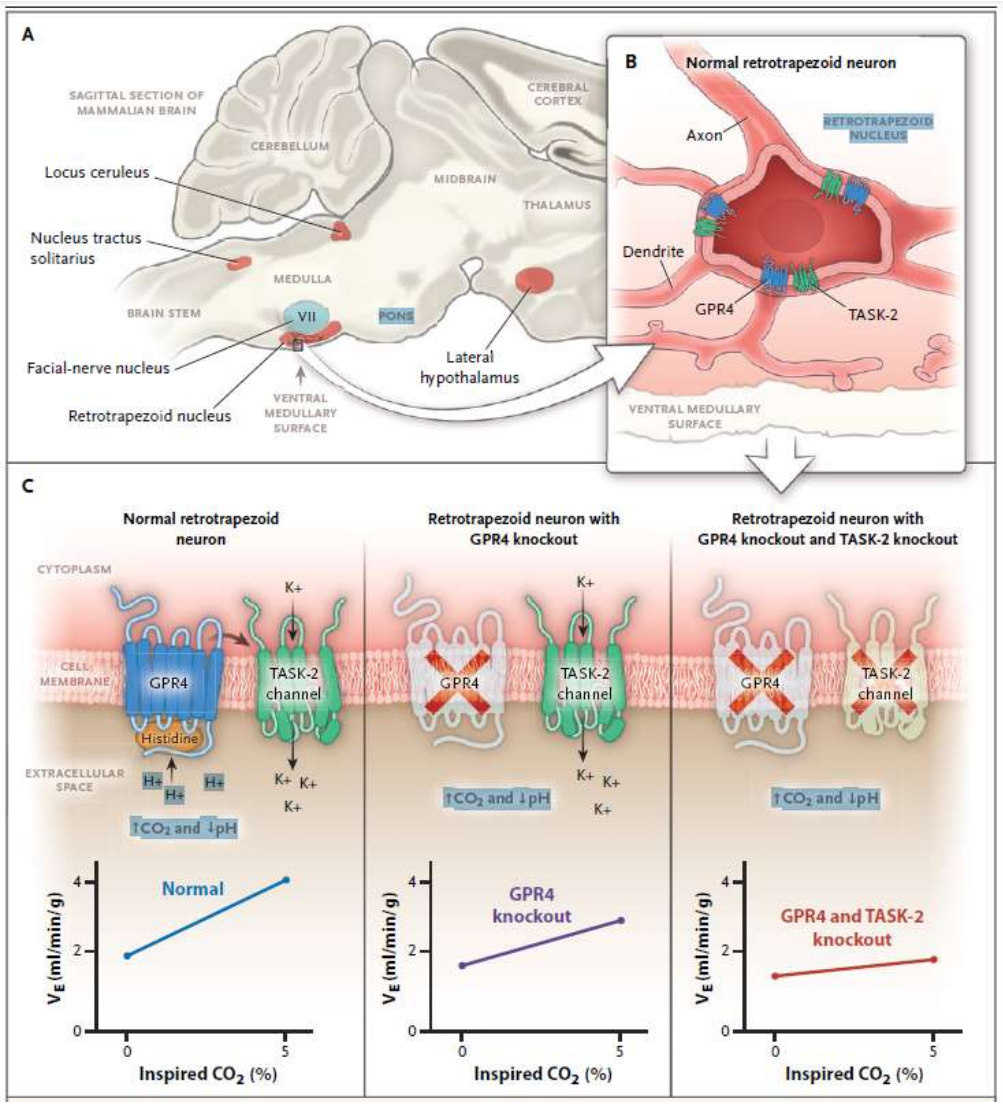
↓ **Modulation of**

Respiratory rhythm

GPR4 occurs in neurons expressing *PHOX2B* plays an essential role in detecting blood CO_2 and pH levels

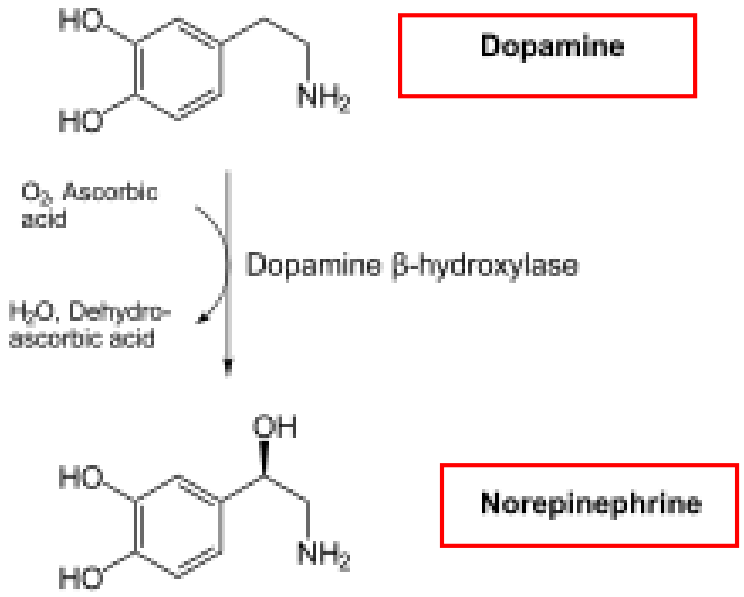
G-protein coupled receptor 4 is a protein that in humans is encoded by the *GPR4* gene

G protein coupled receptors and TASK-2 channels are activated when extracellular **pH** falls into the range of 6.4-6.8 and when **CO_2** rises.



Function reversed by reintroduction of GPR4 (via a lentivirus vector). A path to potential therapies?

PHOX2B protein activates Dopamine beta-Hydroxylase



Norepinephrine is a modulator of RTN chemoreceptors and therefore important in the control of respiration and chemoreception.

fMRI data

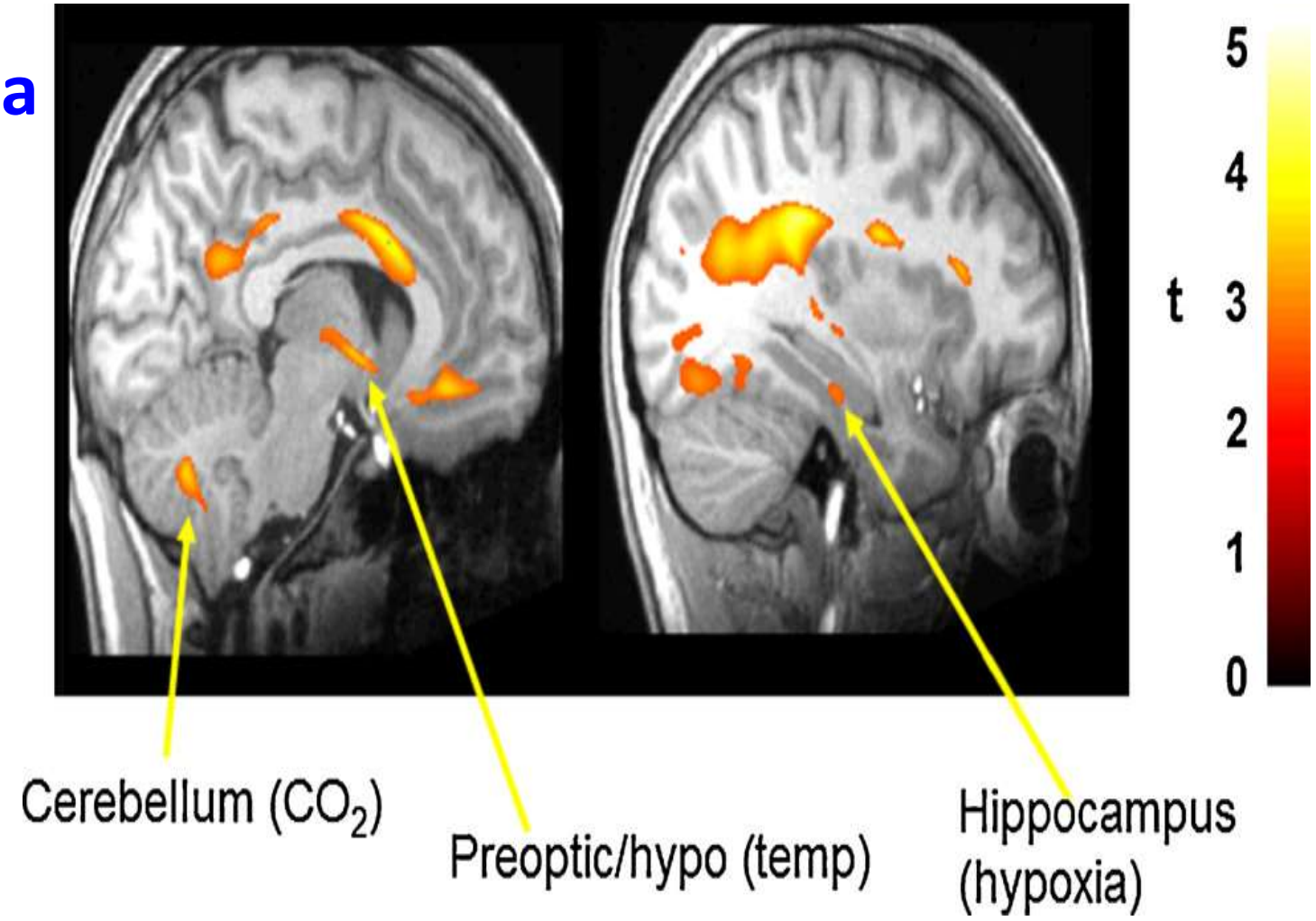
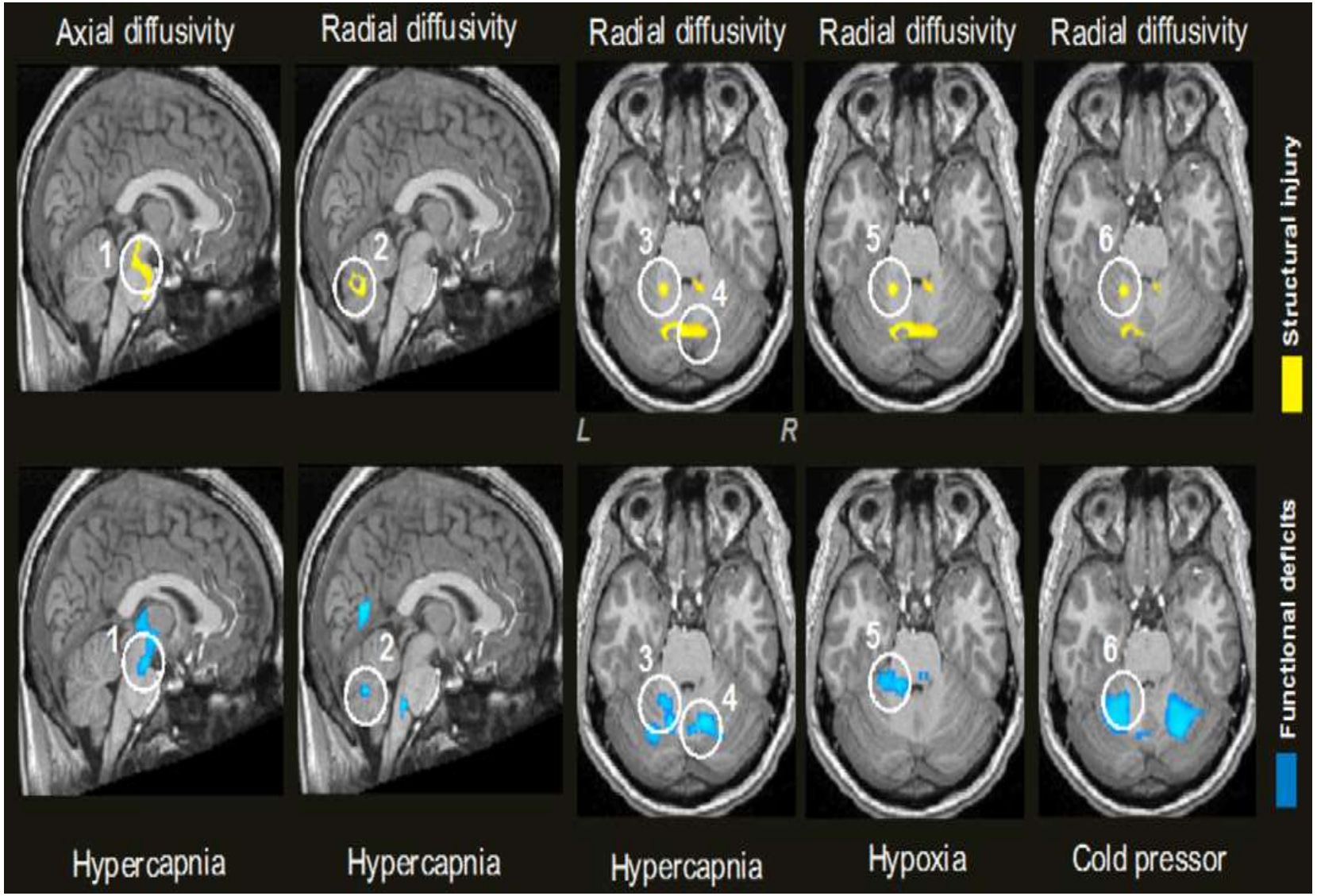


Fig. 2. T2-relaxometry procedures, which quantify free water content, indicate **neural injury or failed development of neurons in CCHS children**



Structural injury and functional deficits appear in cerebellum, lateral medulla, and a region of tissue extending from the posterior thalamus through the midbrain [Harper et al. (2005), Kumar et al. (2008), and Macey et al. (2005)].

In most case the mutation arise **de novo (AD)**. However, 15 to 20% of unaffected parents show **somatic mosaicism** for the mutation identified in the child. (chance for inheritance depends on degree of mosicism in germ cells).

As a germ line mosaicism cannot be ruled out, parents with no somatic mosaicism detected are counseled at 1% recurrence risk in siblings.

Alanine **contractions** (−5, −7 and −13 alanines) in the 20-alanine stretch can be observed with no phenotypic consequences reported to date.

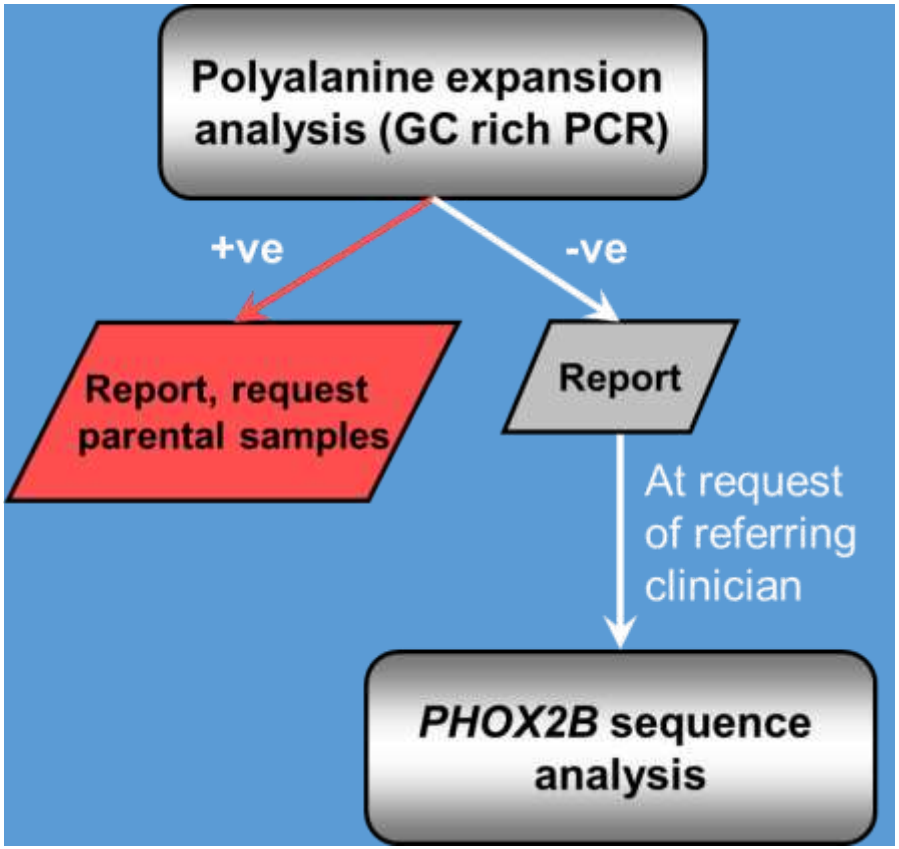
Knockout mice *PHOX2B*$^{+/-}$ do not reproduce CCHS phenotype

Knockin mice *PHOX2B*$^{27ala/+}$ *hypopneic/*apneic after birth, no response to hypercapnia, die in few hours – RTN failed to develop in utero.

Initial investigations

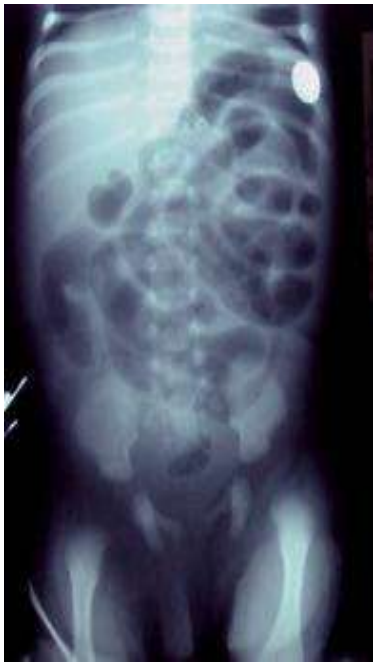
PHOX2B testing confirmation is now **required** for a diagnosis of CCHS (ATS statement on CCHS 2010).

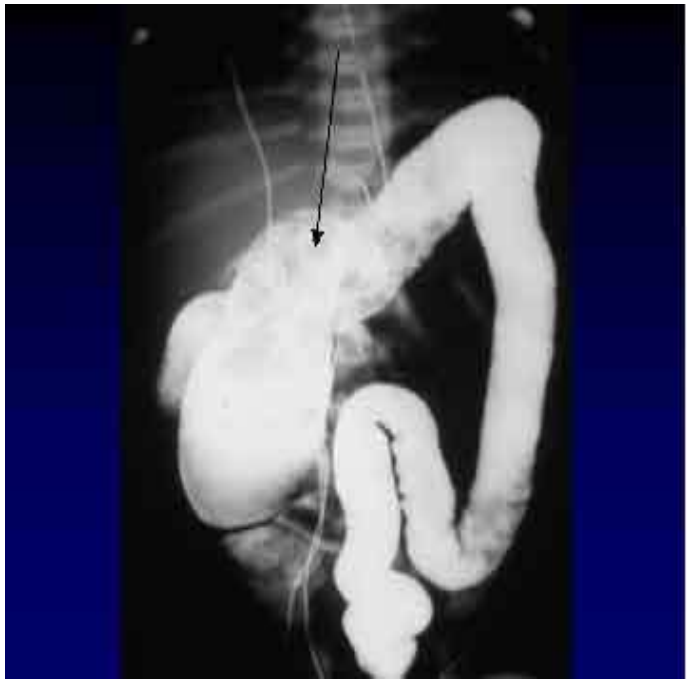
PHOX2B Testing Strategy



Other investigation – for consideration

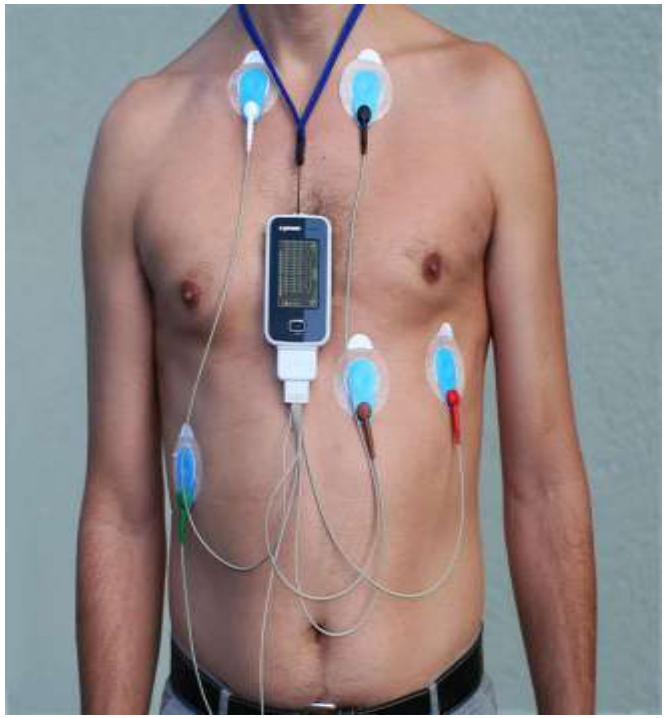
- R/O **Hirschsprung's disease**, Barium enema or rectal biopsy should be performed for patients with constipation or abdominal distension.

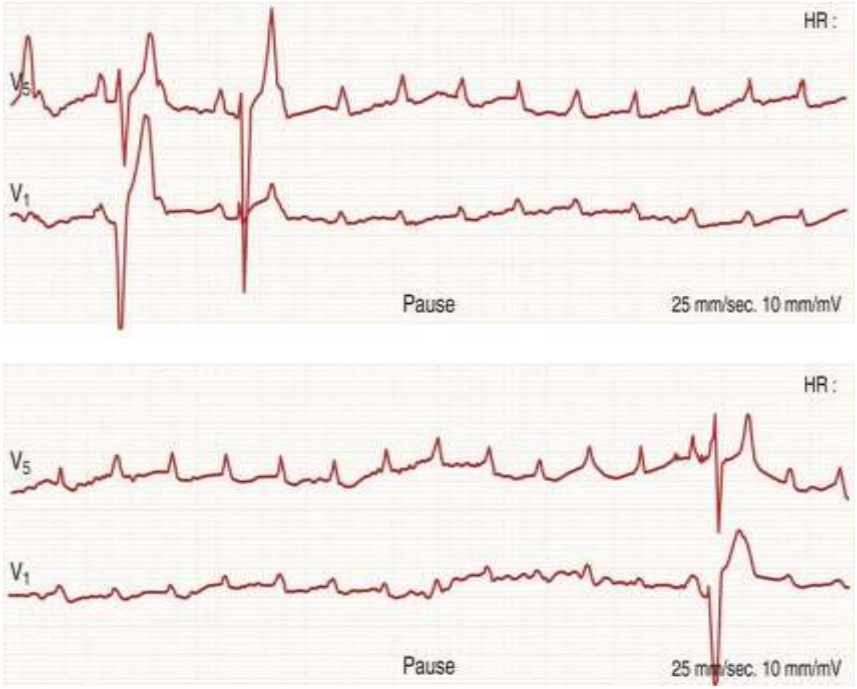




Other investigation – for consideration

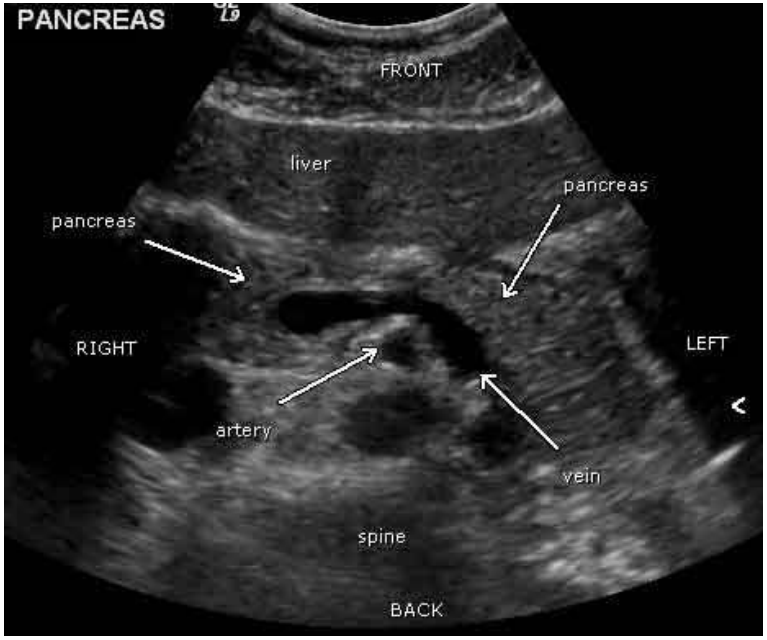
- 24-72-hour Holter ECG monitoring once a year to exclude bradyarrhythmias and asystoles.
- Criteria for pacing (compared to general population have not been established)

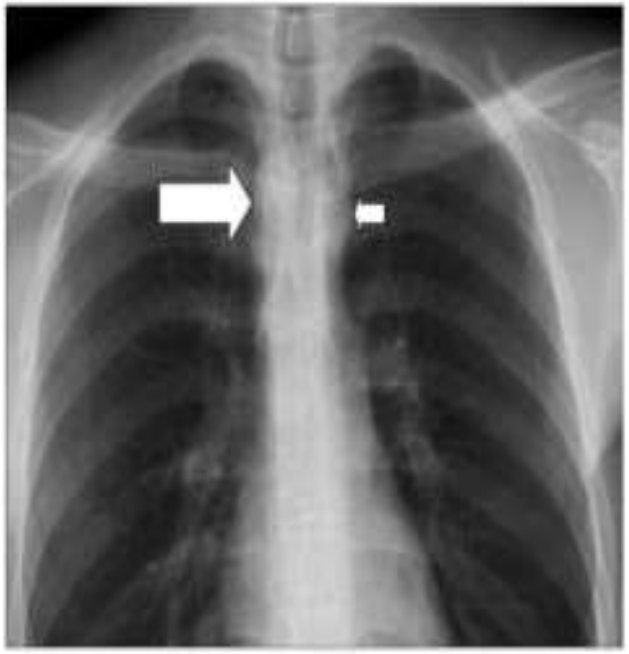




Other investigation – for consideration

- Chest and abdominal imaging every 6 months up to 3 y. then once a year up to 7 y, particularly in patients with the corresponding mutations.





Other investigation – for consideration

- A comprehensive ophthalmologic examination to identify eye involvement for early intervention to avoid interference with learning.

Other investigation – for consideration

- Neurocognitive testing every year or if there is developmental delay or learning disability.

Other investigation – family genetic screening

Prenatal diagnosis

אבחון גנטי טרום השרשה

Preimplantation Genetic Diagnosis (PGD)

children with CCHS already demonstrate reduced neurocognitive performance.
Do deviations in neurocognitive performance are intrinsic to the CCHS genotype or due to diffuse central nervous system insult (e.g, hypoxia).

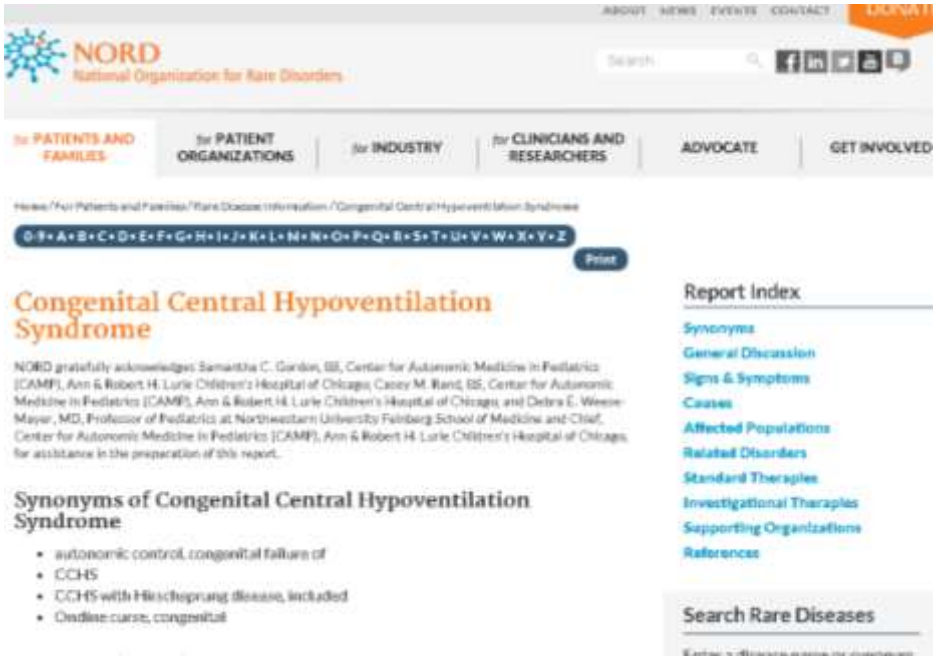
8 of the 19 cases (42%) with 25 PARM were complicated by mental retardation

Shimokaze et al. Journal of Human Genetics 6.2015

Are LO-CCHS children at greater risk for ND deficiencies due to unrecognized sleep hypoxemia?

Table 4. Mental and Motor Development Scores for CCHS-related *PHOX2B* Genotype Groups

	Mental Score*	Motor Score**
Genotype (frequency)		
Polyalanine Repeat Expansion Mutations (PARMs)(25)		
20/25 (7)	103.29 ± 13.76	93.33 ± 2.33[a]
20/26 (9)	76.89 ± 22.17	70.22 ± 19.24
20/27 (8)	66.00 ± 13.58	65.13 ± 15.08
20/33 (1)	83.00 ± N/A	54.00 ± N/A
Non-PARMs (NPARMs) (5)	84.00 ± 29.40	62.60 ± 18.99
Whole Gene Deletion (1)	138.00 ± N/A	120.00 ± N/A
Values provided as mean ± SD		
*P < 0.001; **P < 0.006 (for comparison of 20/25, 20/26, and 20/27 PARM genotypes) [a]N = 6 for Motor Score in 20/25 group		



יד לנשימה

החלום שלי זה לנשום - CCHS ישראל

Case 3

A full term baby. Soon after birth – desaturations, required assisted ventilatory support.

Consultation – suggested PHOX2B testing. Result: 22/20 (normal), sequencing – no NPARM mutations.

Investigation – unrevealing.

Transferred to our PICU for further assessment and preparation for home ventilation

Clinical presentation – apneas and desats mainly during nursing – highly suspicious for CCHS. Hypoventilation during sleep.

Tracheostomy, discharged home.

Blood samples were sent to DWM lab in Chicago.

Baby: heterozygous for a 24 alanine repeat expansion mutation (PARM) (24/20) + heterozygous for a non-polyalanine repeat expansion mutation (NPARM; NM_003924.3:c.785G>T, p.Gly262Val)

Father: heterozygous for a 24 alanine repeat expansion mutation (PARM) (24/20) – **asymptomatic**.

autosomal dominant disease

Mother: heterozygous for a non-polyalanine repeat expansion mutation (NPARM; NM_003924.3:c.785G>T, p.Gly262Val) – **asymptomatic**.

Grandfather (father's father) – PARM 24/20 - asymptomatic
Grandfather (mother's father) – NPARM c.785G>T, p.Gly262Val – asymptomatic
Both grandmothers – normal genetics

Parents were evaluated:

PSG – normal
ECG holter – normal
CXR - normal

Potential research projects

- Neuroimaging (fMRI +), cognitive performance in CCHS: disease or gas exchange deficiency
- Understanding of the PHOX2B genotype/CCHS phenotype relationship
- Pharmacologic agents that might improve the CCHS phenotype
- Basic molecular research – understanding the mechanism and potential therapies

Neuroimaging (fMRI +), neurocognitive performance in CCHS: disease or gas exchange deficiency

- UCLA cohort are mid teenagers and not all are PHOX2B mutation-confirmed.
- Need more diverse ages
- What results from CCHS/PHOX2B mutations and what is due to sequelae of postnatal exposure?
- Hypothesis:
- Neuroanatomical "pathology" in CCHS is due in small part (15%) to the PHOX2B effects but the remaining 85% is due to postnatal hypoxemia/hypercarbia.
- A cohort with fMRI (and potentially with PET) beginning in early infancy and followed longitudinally – patients diagnosed and treated soon after birth, later (> 1m.), late-onset and optimal vs. suboptimal treatment (compliance, technique, monitoring, medical coverage).
- Neurocognitive assessment by the NIH Toolbox + wearable technology, so we would be able to obtain longitudinal physiologic measures in activities of daily living in the home (and in the lab).

Understanding of the PHOX2B genotype/CCHS phenotype relationship

Research questions

- What increases vulnerability to development of Hirschsprung disease, neural crest tumors, cardiac sinus pauses etc.?
- What determines the clinical expression in the different PARM (why are some 24/20 completely disease free compared to others? Why some are late-onset and once diagnosed become symptomatic? Why some 25/20 need almost 24h assisted ventilation vs. 25/20 who may not need support?)

Chicago group developed a bank of skin biopsies-->fibroblast cultures from children with CCHS and developed them into stem cells. To date we have not identified the ideal partner to help us differentiate these cells to determine what about the specific PHOX2B mutations heightens a child's vulnerability to the varied aspects of the CCHS phenotype.

Investigation of the PHOX2B mutations in the zebrafish model has potential not only to understand CCHS but also to better understand autonomic nervous system development.

A star in stem cell differentiation into neural crest derivatives would be essential to the success of this project.

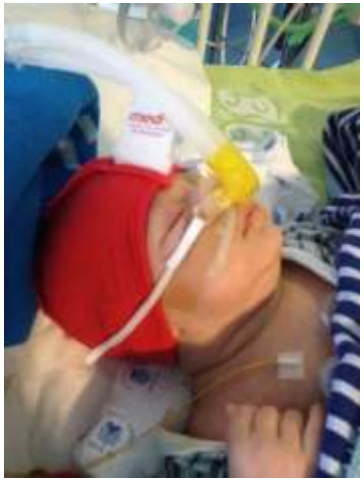
Pharmacologic agents that might improve the CCHS phenotype

Carbon dioxide responsiveness is more robust in infancy and early childhood than it is in school age and older children.

Look for pharmacologic agents that might preserve that early ventilatory responsiveness to carbon dioxide (pharma collaboration).

Case presentation

Dan & Yonathan – monozygotic bi-chorionic twins were born after 34 weeks gestation.

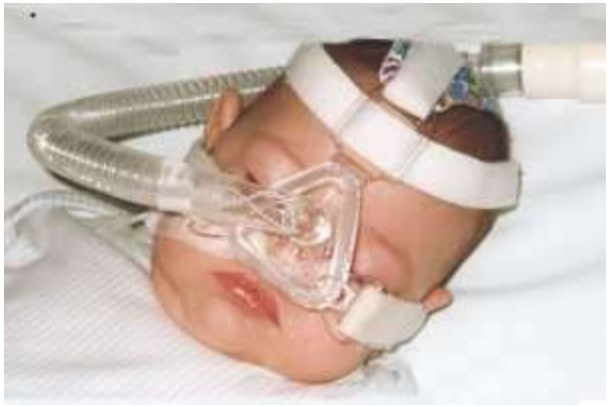


Post natal course: had apneas & bradycardias – considered as A&B of prematurity. Y required non-invasive ventilation started via nasal prongs most of the time.

At age 1.5 months - PHOX2B – positive for CCHS.

Transferred to our PICU.
Requirement of assisted ventilation during sleep was documented.

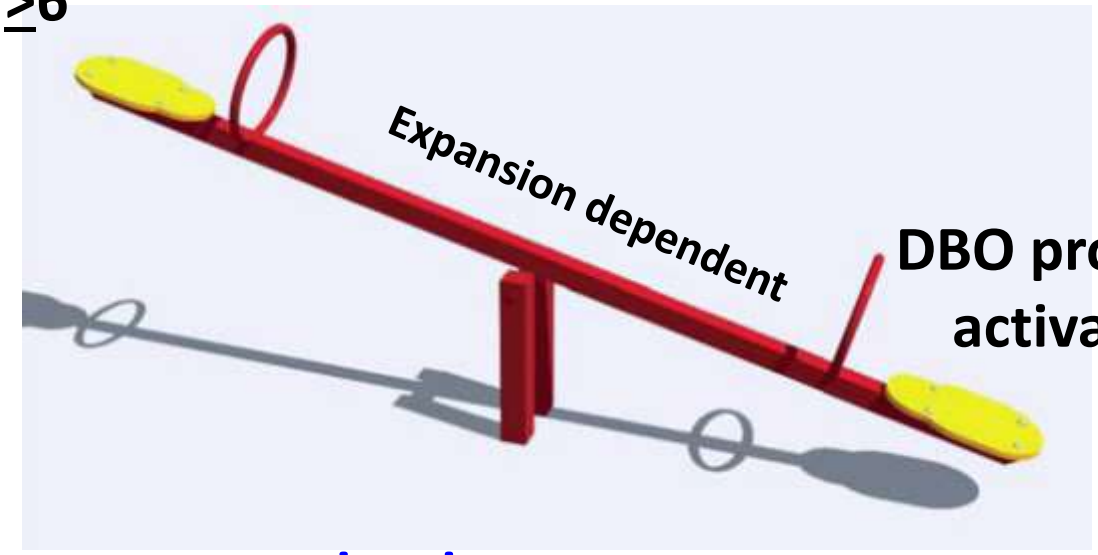
Tracheostomy was addressed. Parents were reluctant.



Nasal pronges – failed - ineffective ventilation – leaks, changes with position and wake state.

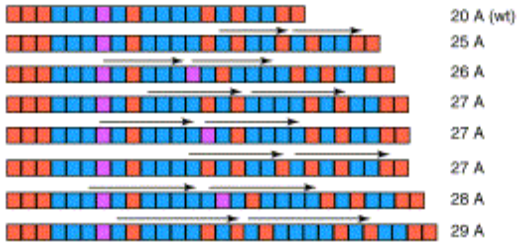
Nasal mask – failed – effective ventilation but significant inconvenience and pain.

ala ≥6

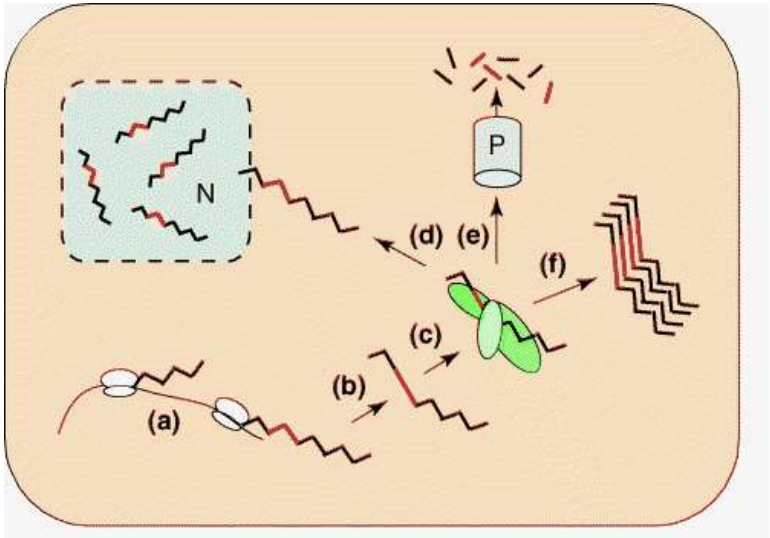


DBO promotor activation

Polyalanine repeat expansions



protein misfolding (oligomers instead of dimers), aggregation, reduced mobility within cell



Diagnosis

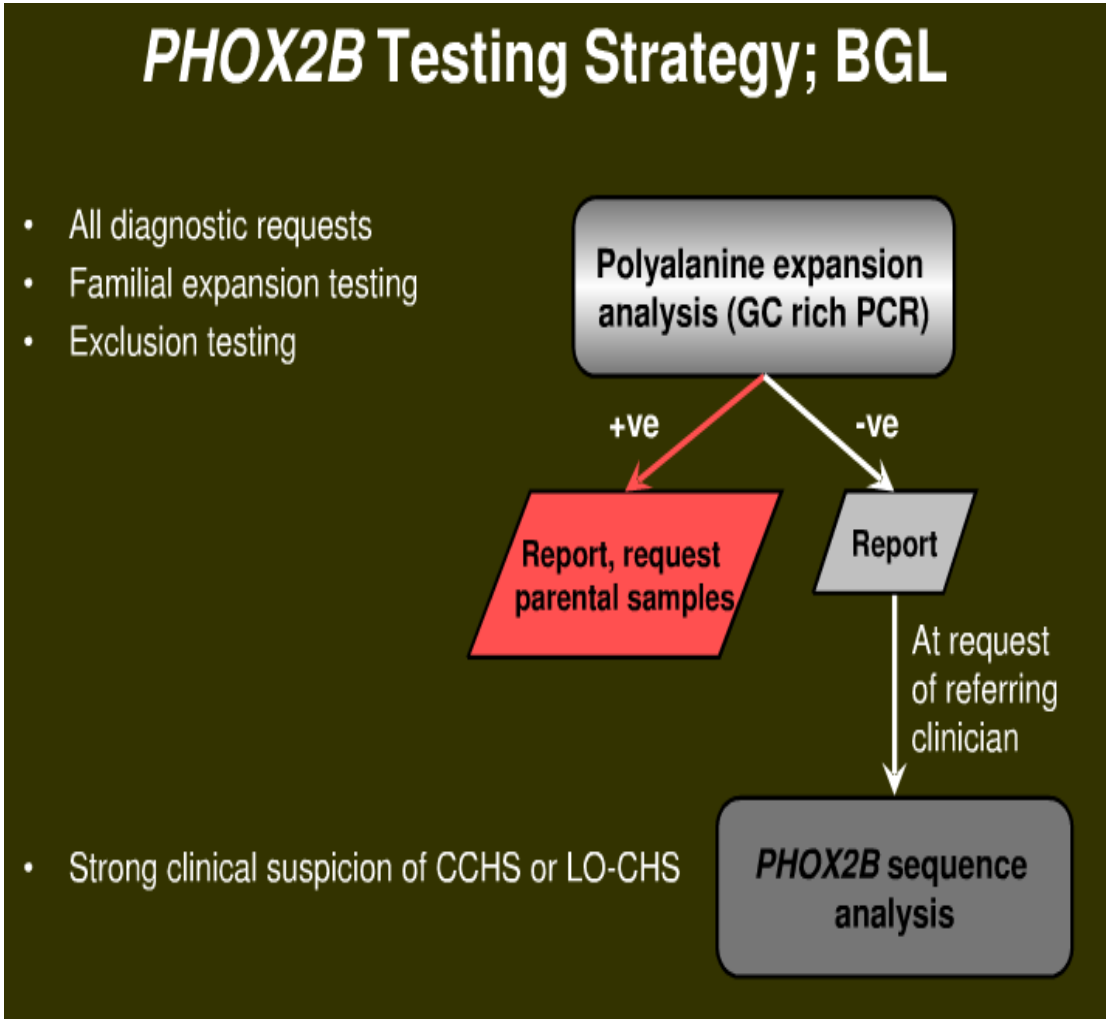
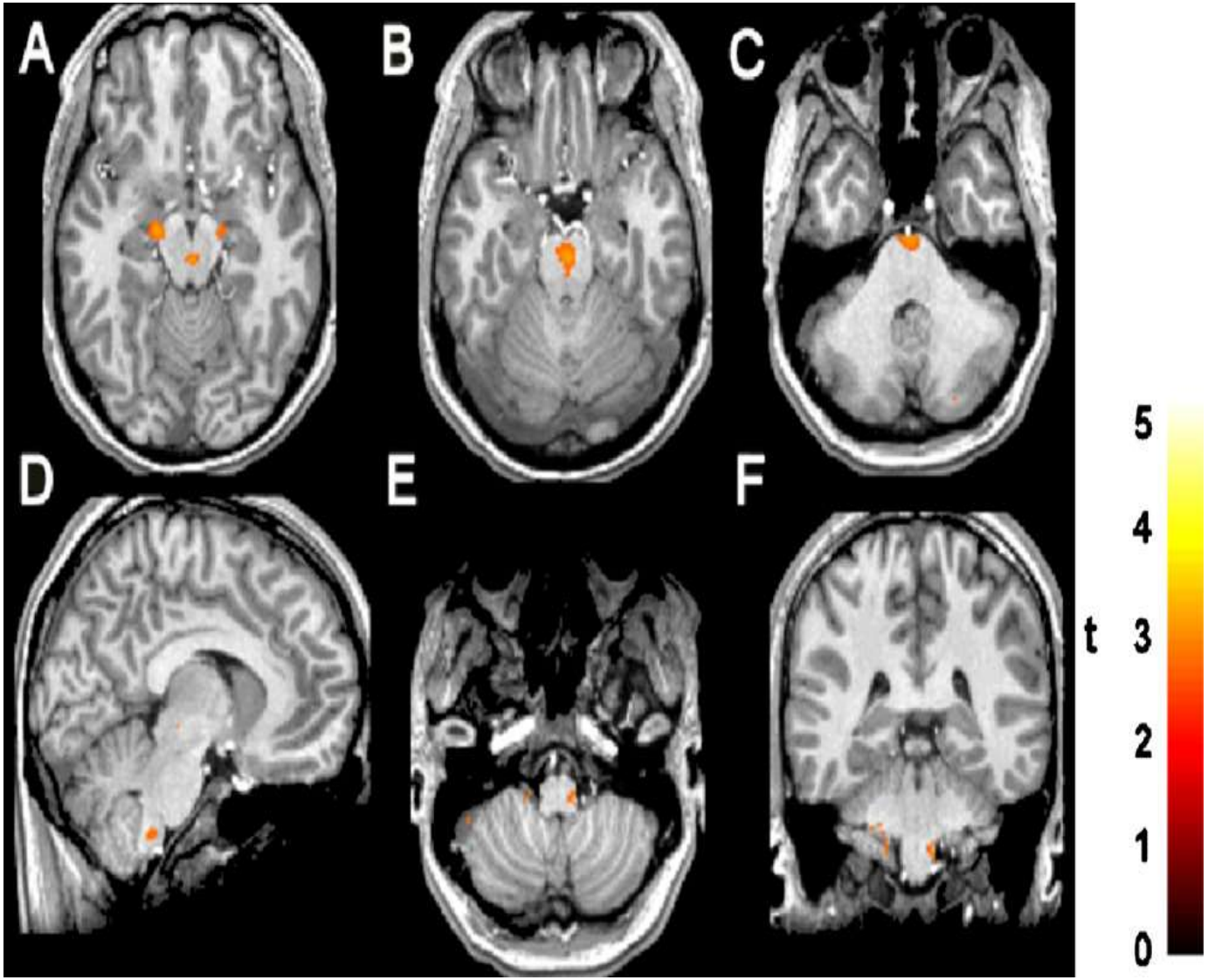

PHOX2B Testing Strategy; BGL
All diagnostic requests
Familial expansion testing
Exclusion testing
Polyalanine expansion analysis (GC rich PCR)
+ve
-ve
Report, request parental samples
Report
At request of referring clinician
Strong clinical suspicion of CCHS or LO-CHS
PHOX2B sequence analysis

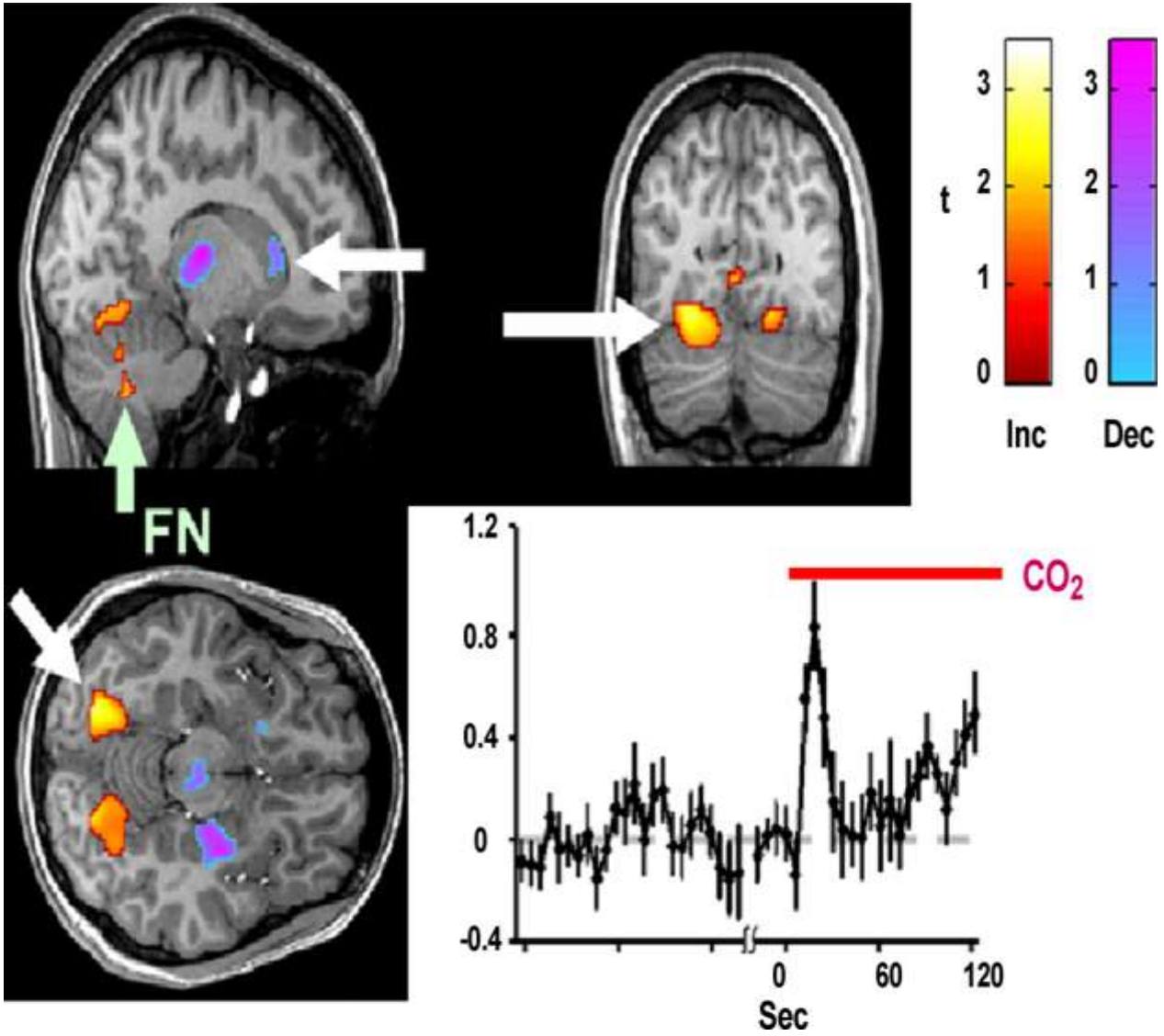
TABLE 1. CLINICAL PRESENTATION AND *PHOX2B* CODING SEQUENCE STATUS FOR PATIENTS WITH LATE-ONSET CENTRAL HYPOVENTILATION SYNDROME

Cases	Sex	Age at Dg of CHS	Triggering Factor for Decompensation	Chronic Hypoventilation before Decompensation	Severe RI	Assisted Ventilation Required	Other Clinical Manifestations	PHOX2B Status	Mosaic	Heredity
O26	M	2.3 yr	URI	PAH	+	During sleep	Strabismus	+5 ala	No	ND
O34	M	4 yr	U	PAH	+	During sleep	Ptosis, ataxia, seizures, strabismus	CDS normal	—	—
O51	F	1.5 yr	RI	PAH, RVH	U	During sleep	No	+5 ala	No	*de novo*
O55	F	2.5 yr	U	U	U	During sleep	Congenital epilepsy, cardiac defect	CDS normal	—	—
O86	F	6 mo	U	U	U	During sleep	No	+5 ala	No	*de novo*
O103	M	7 mo	U	U	+	During sleep	temperature instability	+5 ala	No	*de novo*
O104	M	2 yr	U	U	U	U	temperature instability	+5 ala	No	*de novo*
O106	F	9 mo	U	U	U	U	temperature instability	+5 ala	No	Paternal
O115	F	12 yr	Anesthesia	RVH	+	During sleep	No	+5 ala	No	*de novo*
O160	M	1.5 yr	U	U	U	During sleep	No	CDS normal	—	—
O188	M	1.5 yr	U	Apnea, hypercapnia	U	U	No	c.692delG	U	*
O200	F	6 mo	RI	RHH, apnea	+	During sleep	PDA	CDS normal	—	—
O201	F	8 mo	RI	PAH, RVH	+	During sleep	Hypotonia, hypoglycemia	c.419C>A, p.A140E	No	*de novo*
O205	M	2.8 yr	RI	U	U	During sleep	Hypotonia, epilepsy, strabismus, ptosis	CDS normal	—	—
O211	F	6 yr	U	No	+	During sleep	Developmental delay	CDS normal	—	—
O234	M	7 mo	Bronchiolitis (RSV)	VA at birth	−	During sleep	GER	+5 ala	No	*de novo*
O235	F	13 mo	No	VA at birth	−	During sleep	No	+5 ala	No	ND
O237	M	17 yr	U		+	During sleep	No	+5 ala	No	ND
O266	M	8 mo	No	PAH, RVH	No	During sleep	Abnormal pupillary	+5 ala	No	ND
O281	M	29 yr	URI	No	U	During sleep	Obstructive apneas	CDS normal	—	—
O274	F	12 yr	RI		No	During sleep	No	+5 ala	No	ND
O297	F	50 yr	No	VA, fatigue	No	During sleep	Unilateral vocal cord paralysis	CDS normal	—	—
O299	F	3 mo	U	VA at birth	No	During sleep	—	+5 ala	No	Paternal
O299f	M	25 yr	No	PAH, RVH	No	During sleep	Epilepsy	+5 ala	No	ND
O44GM	F	55 yr	Anesthesia	U	U	U	Familial case	+5 ala	No	ND

the present study shows that all cases with LO-CHS reported herein who had a 15 alanine expansion harbored a germinal mutation, and that this was also the case in two asymptomatic parents. Interestingly, one of these parents (patient 0106f) has a child with LO-CHS. These observations support the hypothesis that a 15 alanine expansion can remain incompletely penetrant for the ventilator phenotype.



Increased axial diffusivity from diffusion tensor imaging (DTI) in children with CCHS. Abnormalities appear in the midbrain (A), raph) ˈB), midline of the caudal basal pons (C), and the right lateral medulla (D, E, and F). Adapted from Kumar et al. (2008b)



fMRI responses to 5% CO2/95% O2 (red bar) in control adolescents. Signal increases in yellow-red scale appear in the cerebellar cortex as indicated with arrows. “FN” is cerebellar fastigial nucleus. Signal decreases in blue-green scale appear in the caudate nucleus, posterior thalamus, hippocampus and medial midbrain. Adapted from Harper et al. (2005)

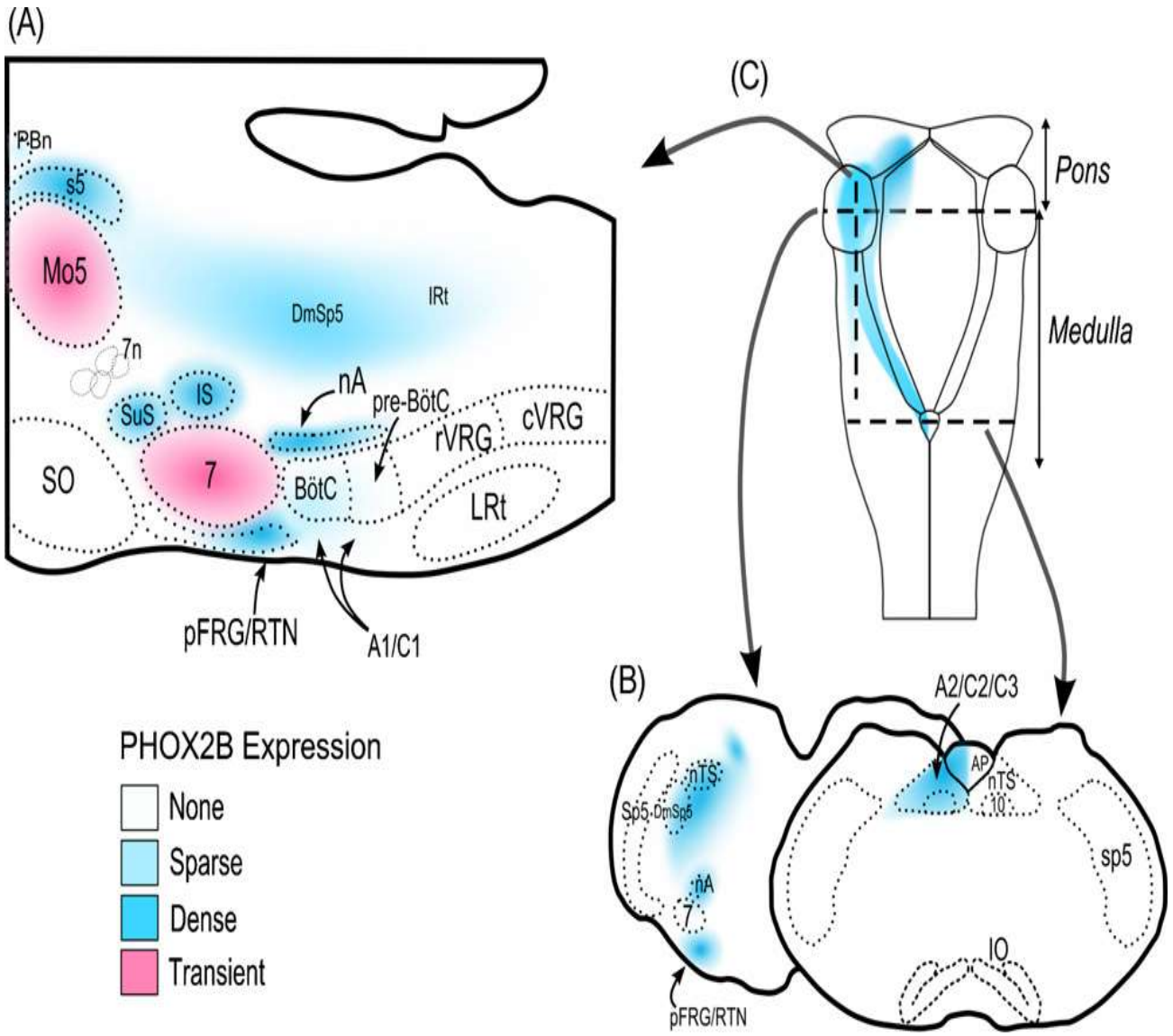
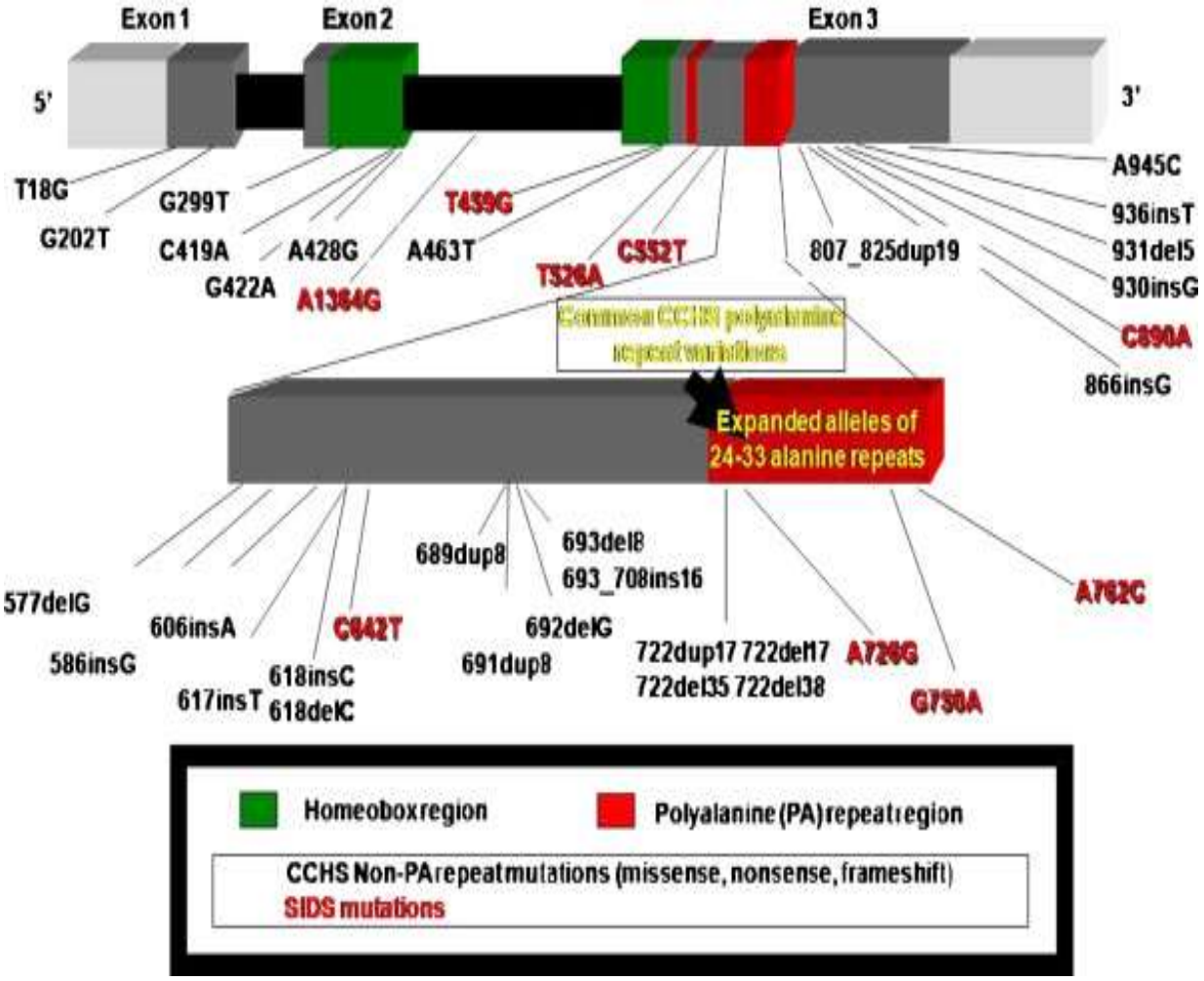


Fig. 1. Schematic representation of expression profile and developmental dependence on phox2b in rodent pontomedullary structures associated with autonomic regulation and respiratory control.


Exon 1
Exon 2
Exon 3
5'
3'
T18G
G202T
G299T
C419A
A428G
G422A
A1364G
T459G
A463T
T526A
C552T
807_825dup19
A945C
936insT
931del5
930insG
C890A
866insG
Expanded alleles of
24-33 alanine repeats
577delG
606insA
586insG
C642T
617insT
618insC
618delC
689dup8
693del8
693_708ins16
692delG
691dup8
722dup17 722del17
722del35 722del38
A726G
G750A
A762C
Homeoboxregion
Polyalanine (PA)repeatregion
CCHS Non-PArepeatmutations (missense, nonsense, frameshift)
SIDS mutations

Using techniques such as **Valsalva maneuver** which provides an **autonomic challenge** eliciting a sequence of sympathetic and parasympathetic actions several brain sites showed functional deficits:

- delayed responses in **medullary sensory** regions as well as decreased activity in **cerebellar and pontine sensorimotor coordination areas**, suggest origin of cardiorespiratory integration deficits.
- Abnormalities noted in the **cingulate, parietal cortex, the amygdala, insula** – areas that regulate respiratory timing, cardiac rate, cardiorespiratory integration.

Congenital Central Hypoventilation Syndrome; a polyalanine repeat disorder-

Amiel J et al. Nat Genet 2003

Weese-Mayer DE et al. Am J Med Genet 2003

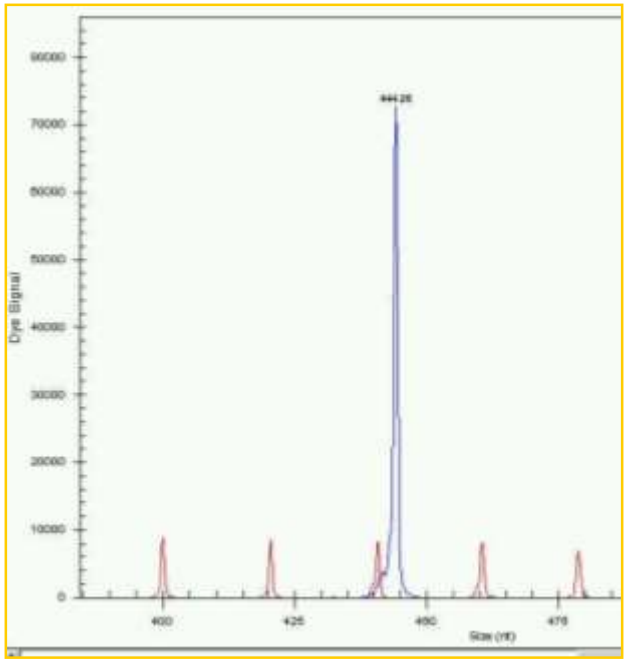
- found heterozygous de novo mutations in PHOX2B in 18 of 29 individuals with CCHS.
- Most mutations consisted of 5-9 alanine expansions within a 20-residue polyalanine tract probably resulting from non-homologous recombination.

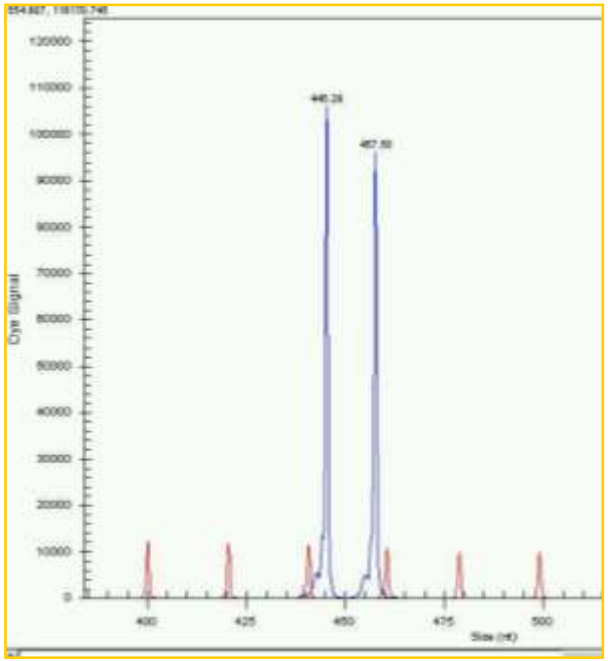
Polyalanine repeat expansions

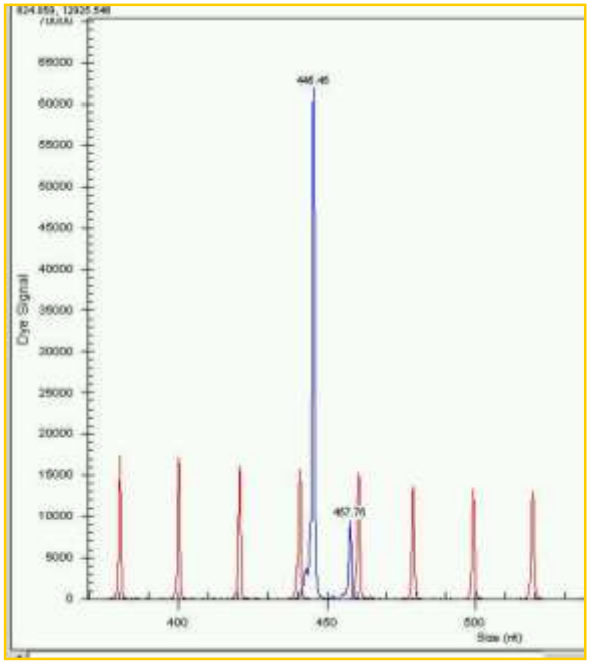
Polyalanine expansion disorders.

Gene	Condition	Inheritance	Phenotype	Mutation	Function	References
FOXL2	Blepharophimosis-ptosis-epicanthus inversus syndactyly	AD	Blepharophimosis, ptosis, epicanthus inversus and ovarian failure	14→22,24	Helix/forkhead TF expressed in developing eye and ovaries	[5,6]
ZIC2	Holoprosencephaly (HPE5)	AD	Malformation of midline structures of the forebrain and facial cranium	15A→25A	odd-paired TF, development of brain and limbs	[7,8]
PHOX2B	Cong. central hypoventilation, Haddad syndrome	AD	Loss of ventilary response to high CO_2 and low O_2, also in combination with Hirschsprung disease (Haddad)	20A→25–33A	Homeodomain-containing TF, development of brain	[9••,10•]
ARX	Mental retardation, epilepsy, West syndrome, Partington syndrome	XR	A spectrum of conditions including to variable extents of mental retardation, various forms of epilepsy, and dystonia	1) 16A→18,23A 2) 12A→20A	TF with role in development of cerebral cortex and axonal guidance	[13,14,16]
SOX3	Mental retardation with growth hormone deficiency	XR	Combination of X-linked mental retardation and short stature caused by growth hormone deficiency	15A→26A	SRY-related TF, neuronal differentiation in brain and spinal chord	[17]
RUNX2 (CBFA1)	Cleidocranial dysplasia	AD	Skeletal dysplasia with hypoplastic clavicles, open fontanelles, tooth abnormalities, short stature	17A→27A	Runt domain TF, central role in morphogenesis of skeleton, osteoblast differentiation	[19]
HOXA13	Hand–foot–genital syndrome	AD	Hand/foot malformation with short thumbs/great toes, abnormal genitalia	1) 14A→24,24A 2) 12A→18A 3) 18A→24–30A	Homeobox TF of A-cluster, patterning of dorsal axis, limbs, genitals	[22,23••,27]
HOXD13	Synpolydactyly	AD	Hand/foot malformation with syndactyly and polydactyly, brachydactyly, hypodactyly in homozygous individuals	15A→22–25A,29A	Homeobox TF of D-cluster, patterning of dorsal axis, limbs, genitals	[25–27]
PABPN1	Oculopharyngeal muscular dystrophy	AD, AR	Progressive, late onset muscular weakness of oculopharyngeal muscles, nuclear inclusion bodies in affected tissues	10A→12–17A (AD) 10A→11A(AR)	Poly(A) binding, regulates length of poly(A) mRNA tails	[30,33•]

Phenotype and references refer only to those conditions associated with polyalanine repeat expansions. Abbreviations: AD, autosomal dominant; AR, autosomal recessive; TF, transcription factor; XR, X-linked recessive. Numbering of mutations refers to polyalanine tracts counted from the N terminus of the protein.

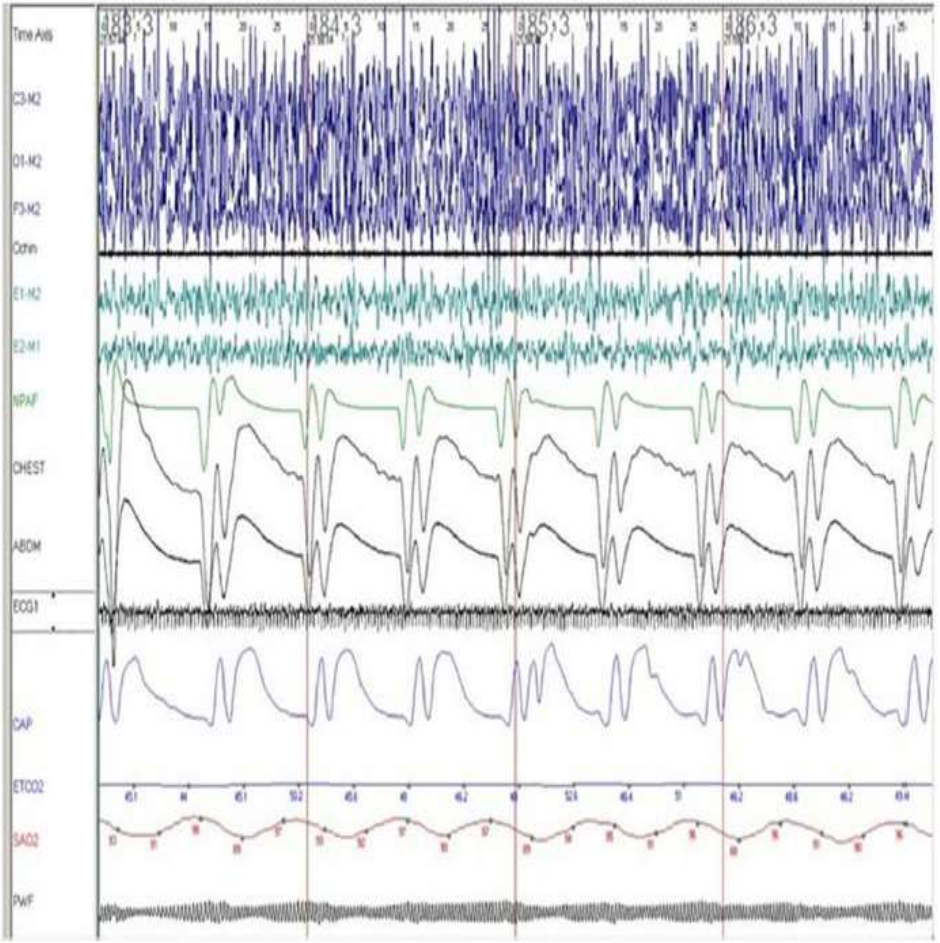

Dye Signal
444.26
Size (nt)


Dye Signal
446.28
457.50
Size (nt)

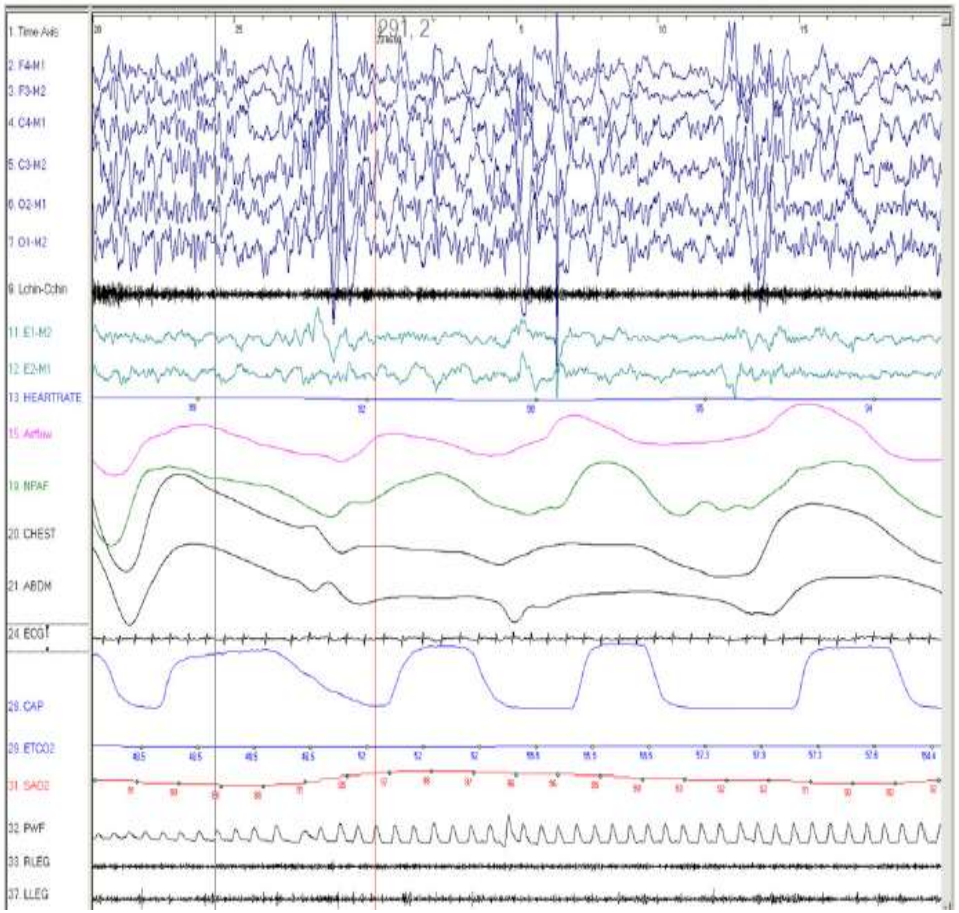

Dye Signal
446.46
457.76
Size (nt)

Genetic Counseling

- Germline Mosaicism of PHOX2B Mutation Accounts for Familial Recurrence of Congenital Central Hypoventilation Syndrome (CCHS) Casey M. Rand,1Min Yu,2 Lawrence J. Jennings,2Kelvin Panesar,1 Elizabeth M. Berry-Kravis,3 Lili Zhou,3 and Debra E. Weese-Mayer1 * Am J Med Genet A. 2012
- Recurrence of CCHS associated PHOX2B poly-alanine expansion mutation due to maternal mosaicism. Bachetti T, Di Duca M, Della Monica M, Grappone L, Scarano G, Ceccherini I.. Pediatr Pulmonol. 2014
- These cases suggest that up to 25% are inherited from asymptomatic parents with somatic mosaicism for these mutations



120 sec. epoch



30 sec. epoch