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Expanding the phenotype of children presenting with hypoventilation with biallelic *TBCK* pathogenic variants and literature review

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Abstract:

Individuals with biallelic TBCK pathogenic variants present in infancy with distinctive facial features, profound hypotonia, severe intellectual impairment and epilepsy. Although rare, it may mimic other neurogenetic disorders leading to extensive investigations. Improved understanding of the clinical phenotype can support early monitoring of complications due to respiratory insufficiency. We present six individuals who were found to have pathogenic biallelic TBCK variants. The clinico-radiological and diagnostic records were reviewed. Five individuals were diagnosed with hypoventilation, requiring respiratory support, highlighting the need for early respiratory surveillance. Characteristic brain imaging in our cohort included periventricular leukomalacia-like changes. We recommend screening for *TBCK* in hypotonic children with periventricular leukomalacia-like changes, particularly in the absence of prematurity.

Introduction :

TBCK (TBC1 domain-containing kinase) encoding a putative Rab-specific GTPase-activating protein (GAP), is associated with downregulated mTOR (mechanistic target of rapamycin) signalling, although the mechanism is yet to be elucidated. The mTOR pathway regulates fundamental cellular processes such as growth, autophagy, energy production and consumption.(1, 2) Downstream TBCK mediated inhibition of mTOR can alter autophagy of oligosaccharides, demonstrated by significantly reduced lysosomal proteolytic function in TBCK-deficient fibroblasts.(3)

Homozygous or compound heterozygous variants in *TBCK* lead to an intellectual impairment phenotype with hypotonia and characteristic facies type 3 (IHPRF3; OMIM: 616900). It is frequently associated with white matter changes, and rarely with cerebellar hypoplasia.(4, 5) The largest world-wide case series to-date identified the main clinical and radiological features in TBCK-related disorders as: i) profound global developmental delay (100%, n=32), ii) severe hypotonia (88%, n=29/33), iii) seizures (69%, n=22/32) iv) bitemporal narrowing (38%, n=12/32) and v) abnormal white matter changes on brain MRI (41%, n=12/29).(5) Neuro-imaging features of TBCK-related disorders include normal imaging (10%, n=3/29), thinning of corpus callosum (45% n=13/29), diffuse brain atrophy (45% n=13/29) and cerebellar hypoplasia (24%, n=7/29).(4, 5) More than 80% of published cases presented with severe hypotonia, which was frequently neonatal in onset (64%, n=9/14).(5) Respiratory failure was reported in 46% (n=12/26).(5) In this series, we report respiratory involvement in five of six children (aged 18 months to 5 years) with biallelic pathogenic *TBCK* variants. We describe their clinico-radiological and diagnostic details. This study supports the clinician faced with an hypotonic infant, to develop a phenotypic approach to TBCK-related disorders.

This approach complements agnostic diagnostic tools such as whole exome and whole genome sequencing.

Methods: Clinical records were reviewed for all individuals with genetically confirmed *TBCK* variants, seen at a tertiary paediatric hospital. For each individual, all the clinical records, investigations; sleep studies, genetic testing, muscle pathology and brain imaging were reviewed.

Individuals 1, 2, 4 and 6 were identified through trio exome sequence analysis of the coding region and conserved splice sites of 23,244 genes by next generation sequencing (Twist Core Human Exome/Illumina NextSeq) at Exeter. Individual 3 was identified by gene sequencing of the familial variant and individual 5 identified through whole genome sequencing by reanalysis of data from the 100,000 Genomes Project.

Ethical approval

As the data analysis was retrospective and no additional data were collected beyond that required for standard medical care of the patient, a full ethics review under the terms of the Governance Arrangements of Research Ethics Committees in the UK was deemed not necessary by the study team. Any data not published within the article will be shared upon request from any senior investigator. Full informed consent was obtained from the family of individual 5 to participate in the 100 000 Genomes Project. Written informed consent for publication was provided by the five unrelated families.

Clinical Report:

All children presented by 4 months of age with hypotonia and developmental delay. Five (83.3% n=5/6) required non-invasive ventilation, the youngest at 14 months (median age 34 months, IQR 24-60 months).

Individual 1 had a baseline routine sleep study at 1.5 years prior to diagnosis. This identified mild sleep disordered breathing. Isolated central hypopneas and apnoea were seen in active sleep resulting in mild oxygen desaturations, with normal gas exchange. (Apnoea Hypopnea Index (AHI) 3.3/hour of which 2.6/hour were central). Aged 1 year, she was admitted to PICU with a respiratory infection (rhinovirus positive). Aged 2 years and 3 months, she was admitted for an MRI brain under general anaesthetic but had desaturations during the anaesthetic. A repeat sleep study the same month showed mild sleep disordered breathing (central and obstructive events, mean TcCO2 47mmHg, SpO2 nadir 83%) and BiPAP was commenced. She had one further PICU admission with respiratory infection but recovered quicker than her first PICU admission.

Individual 2 was admitted aged 2 years to paediatric intensive care unit (PICU) following a seizure. He had 2 further admissions to PICU aged 8 years due to respiratory depression post-seizure. A month later, he had a cluster seizure which self-terminated but subsequently had respiratory depression requiring intubation and ventilation. A sleep study identified centrally driven hypoventilation and hypercapnoea. (Unclassified AHI of 7.5ev/hr which reduced to normal of a UnAHI of 0.5ev/hr on supplemental O2 of 0.6L/min. TcCO2 values were very elevated throughout; mean 82.9mmHg, SpO2 nadir 78%).

Individual 3 presented with hypotonia and developmental delay. His sibling was diagnosed with biallelic *TBCK* pathogenic variants. *Individual 3* underwent targeted genetic testing and was identified to have the same biallelic *TBCK* pathogenic variants. He does not have seizures or respiratory involvement at last review aged 18 months.

Individual 4 was admitted aged 2 weeks old with an aspiration pneumonia which led to his first PICU admission. He had 4 apnoea between 2.5 and 3 years of age which led to further PICU admissions. These events were thought to be due to respiratory infections secondary to aspiration. He was commenced on non-invasive ventilation (NIV) following a sleep study carried out during the 3rd PICU admission. The study identified his oxygen desaturation index was 4/h and his AHI was 2/h. Analysis of his breathing pattern revealed central hypopneas associated with an increase in his carbon dioxide level and, at times, a drop in his oxygen saturations. In summary, there was severe hypoventilation. Two of the apnoeic episodes (including one while on NIV) were associated with limb jerking raising the possibility of seizure-related apnoea, EEG during an event did not show any ictal change. Due to ongoing recurrent severe apnoea requiring ICU admission he had a tracheostomy with dramatic reduction in apnoea thereafter.

Individual 5 had 9 hospital admissions for respiratory illnesses prior to his first admission to PICU aged 1 year 2 months for a lower respiratory chest infection. He had 3 further PICU admissions with respiratory causes in the next 2 months. He subsequently started nocturnal NIV aged 1 year 4 months but continued to have 9 further respiratory led PICU admissions in the following 2.5 years. Salbutamol (1mg four times a day) was started at 3 years of age, prior to diagnosis, for possible congenital fibre type disproportion. It appeared to help reduce respiratory admissions so he has been continued on it by respiratory team.

Individual 5 was recruited to the 100,000 Genomes project. He had metopic ridging and turricephaly on clinical examination although no other overt evidence of craniosynostosis. He underwent trio whole genome sequencing with the following panels applied: genetic epilepsy syndromes (1.34), craniosynostosis (1.48), intellectual disability (2.7), mitochondrial disorders (1.12) and undiagnosed metabolic disorders (1.95). Initial analyses did not reveal any tier 1 or 2 significant variants. However, further clinical correlation led to the suggestion of a TBCK which led to focused re-analyses of the data This showed biallelic variants in *TBCK*: a maternally inherited exon 23 deletion and a paternal variant c.276C>T,p.Arg126Ter which had previously been reported in the literature. It is likely that the bioinformatic pipeline excluded the paternal variant during filtering in the absence of a second variant. It was only through targeted analyses that the maternal exon 23 deletion was identified and further search on the paternal allele revealed the second single nucleotide variant, confirming the diagnosis of a TBCK-related disorder.

Individual 6 presented in neonatal period with hypotonia and went on to develop epilepsy, cortical visual impairment and required gastrostomy feeding by age 4 years. CSF neurotransmitters identified low CSF 5-methyltetrahydrofolate (5MTHF). She had an initial sleep study age 3 years which did not identify any disordered breathing. Snoring was reported age 5 years so had a sleep study repeated. This study identified a mixed picture of central and obstructive apnoea and subsequently commenced BIPAP. (Unclassified AHI of 5.6ev/hr). Despite initial whole exome sequencing being reported as negative, re-analysis of TBCK was performed when she commenced non-invasive ventilation which identified exon 23 homozygous deletion.

Typical neuro-imaging findings of white matter abnormalities were seen in our cohort.(5) In individual 4, we identified periventricular scarring on neuroimaging leading to irregular scalloping of the lateral ventricles, mimicking periventricular leukomalacia (PVL). We identified additional novel findings of subtle cortical dysgyria and a small pons. (Figure 1).

Discussion :

Hypoventilation leading to chronic respiratory failure was reported in six of eight Puerto-Rican boys with homozygous *TBCK* p.Arg126Ter pathogenic variants, five of whom were tracheostomy-ventilator dependent.(2) All eight cases had hypotonia from infancy and areflexia. The youngest two children, aged 3 and 5 years old, had no evidence of respiratory failure. The authors suggested the respiratory failure results from progressive neuromuscular weakness with age.(2) In another cohort of patients with homozygous *TBCK* variants one child was reported as commencing ventilation at 4 months of age.(4) Their cohort of five children (aged 24 months to 14 years), all had evidence of hypoventilation and four were on respiratory support.(4) The authors suggested decreased respiratory drive in *TBCK* resulting in central respiratory failure. Respiratory failure due to fatigue of respiratory muscles or failure in the respiratory control centre can be difficult to differentiate. A test of central respiratory drive reportedly unaffected by respiratory muscle weakness is the measurement of mouth pressure in the first 0.1 second of inspiration after an occlusion (P0.1).(6) However, this is difficult to do in practice, particularly in young children. Either way, the surveillance and anticipation of respiratory difficulties is important in TBCK-related disorders.

Neuromuscular involvement in TBCK-related disorders is variable. Electrophysiology in 5 children was more consistent with anterior horn cell disease. There was fasciculation of intramuscular components on real-time ultrasound.(2) This denervation over time may be due to an abnormal accumulation of storage material in spinal cord motor neurons.(7) However, non-specific myopathic changes in muscle biopsies have also been reported in TBCK-related disorders, highlighting its ubiquitous involvement in multiple systems.(8) Zapata-Aldana et al. note in one 4 year old child, that initial contralateral brisk reflexes disappeared over a year time frame. Over this year, there was resolution of previous myokymia in electromyography (EMG) with persistence of an essentially normal nerve conduction study (NCS).(5) All our individuals had EMG and NCS in infancy. Repeated testing over time may have identified further abnormalities. Individual 2 developed tongue fasciculations after the age of 7 years which suggests a progressive neuronopathy. He has not had repeat EMG or NCS. Tongue fasciculations were described in a 14-year-old with normal NCS, despite muscle and nerve biopsy demonstrating axonal damage.(4)

Homozygous p.R126X mutations (Boricua) were reported with a more severe TBCK phenotype in 8 males aged 3-18 years.(2) EMG and NCS were done after the age of 10 years in 5 cases and suggested denervation. Our cohort had a similar severe phenotype which was not restricted to the Boricua mutation and were diagnosed in infancy with normal EMG and NCS investigations. However, if EMG and NCS were repeated over time, we postulate that denervation changes may become apparent.

A number of other conditions can present with distinctive facial features and central hypoventilation. These include mitochondrial disorders, *P4HTM, TECPR2* and *PHOX2B.*(9-12) Hypotonia is not a common feature of *TECPR2* and *PHOX2B*, but is seen in mitochondrial

disorders and *P4HTM*. However patients with *P4HTM*-related disorder usually have normal brain imaging and this can be a very useful clue to distinguish the two.(9) (Table 2)

Mitochondrial disorders are usually suspected when there is multi-system involvement and was investigated for individuals 1, 4, 5 and 6 of this cohort. This and previous cohorts have commented on a range of additional clinical features in TBCK-related disorders, such as endocrine involvement (thyroid dysfunction and precocious puberty), fragile bones, and abnormal eye movements.(2, 13) Magnetic resonance spectroscopy (MRS) in mitochondrial disorders may have an abnormal lactate peak and deep brain structures such as the basal ganglia are particularly vulnerable.(14)

Due to the downstream effects of alteration in the mTOR pathway, metabolic and mitochondrial investigations may be abnormal in TBCK- related disorders.(3, 15) This cohort had one individual with a low urate, three individuals with abnormal neurotransmitter profile and three individuals with abnormal lipid profile. MR Spectroscopy in individual 1 showed a slightly elevated lactate peak but was normal in individual 5. Three children in our cohort had a muscle biopsy which showed myopathic findings with one borderline abnormality in respiratory chain enzymes.

In two of the six children, hypoventilation was a useful diagnostic clue which lead to a review of the genetic data, specifically looking for *TBCK* variants. The review revealed compound heterozygosity for a single exon deletion and a pathogenic variant in *TBCK*. (Table 1a-c; individuals 2 and 5). To determine central hypoventilation versus peripheral muscle weakness, requires use of diaphragmatic EMG or oesophageal pressure monitoring which are

challenging in children.(16) However there can be a paradoxical effect of prolonged muscle weakness on central sleep disordered breathing. Secondary reduction in chemosensitivity following long standing hypercapnia may develop.(17) In summary it is likely a mixed hypoventilation mechanism in TBCK related disorders.

The pertinent clinical features of hypotonia, intellectual disability and hypoventilation, along with neuro-radiological white matter abnormalities can focus diagnostic testing towards TBCK-related disorders.

Conclusion: Respiratory involvement and hypoventilation is an important clinical feature within the TBCK-related disorders and can present in infancy. Clinical suspicion should be raised in hypotonic infants with PVL-like changes on brain MRI with no history of prematurity. Assessment for sleep disordered breathing should be sought at diagnosis with surveillance sleep studies at least yearly due to the high risk of hypoventilation, and the significant co-morbidity this complication brings.

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