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USE OF ULTRA-RAPID WHOLE EXOME SEQUENCING TO DIAGNOSE CONGENITAL CENTRAL HYPOVENTILATION SYNDROME

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**USE OF ULTRA-RAPID WHOLE EXOME SEQUENCING TO DIAGNOSE CONGENITAL
CENTRAL HYPOVENTILATION SYNDROME**

Dear Editor,

Infants presenting with hypoventilation are clinically challenging, with a wide array of diagnoses possible. Whilst patients can be managed without an underlying diagnosis through the provision of respiratory support, there are benefits to determining the underlying cause such as screening for associated complications, facilitating timely clinical decision-making (especially regarding provision of long-term respiratory support) and rarely initiating disease-specific treatment. Given the broad range of potential causes, there are numerous investigations, which may be helpful (see table 1). It can be challenging deciding which investigations to perform, in which order, and with what degree of urgency, given that there are not always clinical clues to aid clinicians in choosing which test will likely have the highest yield. As a result, one guideline suggests that all investigations be performed concurrently ¹ however given that some of the investigations are invasive and not widely available this is not appropriate in all cases. When all tests are performed concurrently there is a large financial burden ², and this is exacerbated by children frequently remaining inpatients of the hospital, often in intensive care units (ICU), whilst awaiting results.

Congenital central hypoventilation syndrome (CCHS) is one cause of infantile hypoventilation where confirming the diagnosis, and characterizing the underlying

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genetic mutation in the *PHOX2B* gene, is important due to the genotype phenotype correlation. Knowledge of a patient's exact mutation allows accurate prognostication and targeted screening for complications of CCHS¹. Current guidelines¹ recommend genetic testing for CCHS consisting of *PHOX2B* screening with sequencing of the *PHOX2B* gene if the initial screen is negative and there is a high clinical suspicion. This process of testing is not readily available at all centres meaning that often samples will have to be sent internationally for testing, with associated cost and delay.

Genomic testing, such as whole exome sequencing (WES) is transforming rare disease diagnosis in pediatrics³, and rapid genomic diagnosis programs are becoming increasingly available at major pediatric hospitals, particularly for ICU patients^{2,4,5}.

Recently at our centre, WES was used to diagnose CCHS in a 6-week old term infant. The infant presented with apnoea and hypoxemia during sleep, necessitating treatment with supplemental oxygen. On investigation, serial blood gases showed a respiratory acidosis, worse after sleep, in keeping with hypoventilation. Initial investigations for infection, structural brain abnormality, and seizure disorder were normal. Rather than pursue a complete metabolic workup, nerve conduction studies, electromyography and *PHOX2B* testing (which in our setting is performed overseas with a result available after 6 weeks), the patient had an in-house ultra-rapid trio WES, the results of which were available in 69 hours. WES showed a *de novo* heterozygous polyalanine repeat expansion in *PHOX2B* with a 20/25 genotype, in keeping with CCHS. The size of the expansion was confirmed on Sanger sequencing within 2 weeks. The rapid result allowed the family to make informed decisions about the patient's

care, appropriate ventilator support to be provided, and a targeted screening program for other complications to be formulated.

This case demonstrates that WES can be used to diagnose CCHS, and that a result can be available within 3 days. It is important that pediatric pulmonologists are aware of developments in genetic testing so that they can provide optimal care for their patients. In this case, had international guidelines been followed there would have been a 6 week delay until results were available, however due to the availability of rapid trio WES a result was available within 3 days. This highlights that appropriate ordering of genetic testing will likely vary between countries and even individual centres. The parameters which determine the most suitable genetic test for any patient include the suspected conditions, time until results are available, and expense.

Infantile hypoventilation can be a relatively undifferentiated condition or there may be an indicator to the cause, (i.e. a history of Hirschprung's disease being suggestive of CCHS). In patients with an undifferentiated phenotype, the use of a broader genetic screen (such as WES) is more attractive than a single gene test (as suggested by current guidelines) as it can simultaneously test for a range of pathologies. In another case with an undifferentiated presentation of infantile hypoventilation, WES successfully diagnosed a rare treatable condition, that would not have been tested for had single gene tests been ordered.² In presentations that are more suggestive of an individual condition, a targeted gene test is more appropriate. Another consideration for testing when a specific condition is suspected is the nature of the mutation being assessed. The utility of WES in assessing for certain mutation types, including repeat expansions

is still being determined but in this case it accurately confirmed the size of the polyalanine tract expansion.

The time delay until results are available will be highly variable between centres. For many centres it will be possible to get results for a single gene test in a more timely fashion than WES testing. However, for other centres, including in this case, the results of WES may be available much faster. There is also between centre variation regarding time to results of ultra-rapid next generation genetic testing which clinicians must be aware of. Other centres⁶ report a longer time to results for ultra-rapid testing of 5 days, when compared to the 3 days in this case.

The cost of genetic testing is another important consideration when determining the most appropriate test to order. Currently single gene tests are usually cheaper than WES. However, also factored into any cost analysis needs to be the expense of any delay to getting a result, especially if the patient remains in an ICU setting during this time. The savings from avoiding other tests should also be factored in to decision making.

Advances in genetic testing hold the promise of improved patient care, however decisions about which is the best test to order for individual patients are complex and will vary from centre to centre. For centres looking after the case described in this letter, where *PHOX2B* testing yield fast results and WES is relatively slow and expensive, single gene testing would have been a better choice than WES. However, for many other centres, for the reasons described, WES may be superior. The decision regarding which test is the best to order will not always be simple, and is likely to change with time, so it will be prudent for pediatric pulmonologists to be aware of the

different options available and to utilise the expertise of geneticists. If used in a considered manner, genetic testing, whether it be single gene testing or broader testing such as WES, has the potential to drastically improve the care of patients.

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Statement of Ethics

The parents of the subject gave informed consent for the publication of this case report.

Disclosure Statement

The authors have no conflicts of interest to declare

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Author Contributions

All authors were involved in the clinical care of the patient, the formulation of the idea for the report, and writing and editing of the manuscript.

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Table 1. Potential Investigations in an Infant with Hypoventilation

Site	Investigation
Central Nervous System	Computed Tomography or Magnetic Resonance Imaging Brain (may need general anaesthesia), Lumbar Puncture, Electroencephalography, Metabolic screen, Genetic Testing for <i>Phox2B</i> mutation

Peripheral Nervous System	Nerve Conduction Studies, Electromyography, Muscle Biopsy, Genetic tests for conditions associated with neuromuscular weakness, Metabolic screen
Diaphragm	Chest X-RAY, Ultrasound or Fluoroscopy, May need peripheral nervous system tests
Thoracic Cage	Chest X-RAY
Lung	Chest X-RAY, Computed Tomography of Lungs (may need general anaesthesia), Lung Biopsy