FIRST APPLICATION OF THE EXOME SEQUENCING IN MOROCCOIN A CASE OF CONGENITAL CENTRAL HYPOVENTILATION SYNDROME WITH A MUTATION OF THE PHOX2B GENE

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Abstract

Congenital central hypoventilation syndrome (CCHS) is a rare genetic disorder with apnea and cyanosis during sleep requiring immediate endotracheal intubation during the first day of life. The *PHOX2B* gene has been identified as the major gene involved in CCHS. This is the first report of a Moroccan neonate with CCHS confirmed to have a heterozygous mutation in exon 2 of the *PHOX2B*. This mutation was detected with the complete exome sequencing based on Ion Proton system and shows that this technique is highly efficient, fast and cheap as a mutation detection method.

Key words: Congenital central hypoventilation syndrome (CCHS);PHOX2B; exome sequencing.

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Résumé

Le syndrome d'Ondine ou syndrome d'hypoventilation centrale congénitale est une maladie génétique rare qui se caractérise par une ventilation anormale chez une personne éveillée et une hypoventilation durant le sommeil nécessitant une intubation endotrachéale immédiate au cours de la première journée de la vie. Le gène PHOX2B a été identifié comme le gène majeur impliqué dans Le syndrome d'Ondine. Cet article reporte le premier cas découvert au Maroc d'un nouveau-né ayant une mutation hétérozygote dans l'exon 2 de la PHOX2B. Cette mutation a été détectée avec le séquençage complet de l'exome basé sur le système Ion Proton et montre que cette technique est très efficace et rapide.

Mots clés : Le syndrome d'Ondine ; *PHOX2B*; Séquençage Haut Débit de l'exome.

Introduction

Congenital central hypoventilation syndrome (CCHS) is a rareneuro-respiratory disorder associated with several possible mutationsof the *PHOX2B*gene. Animal models have shown thatthese mutations result in major alterations in the development ofthe parafacial region of the brainstem that is primordial torespiratory chemosensitivity.[1] Patients with these mutationsexperience hypoventilation when sleeping, resulting in sleeprelatedventilator-dependency. They lack ventilatory andperceptual responses to hypercapnia or hypoxia .[2] However,most CCHS patients have normal or subnormal resting ventilationwhen awake. The dramatic discrepancy between sleeping and wakeningsuggests the contribution of cortical mechanisms,but theprecise nature of these mechanisms is unknown.[3]The paired-like homeobox 2b gene (*PHOX2B*) encodes a transcription factor involved in the development of several noradrenergic neuron populations in mice. In the murine model, the *PHOX2B*expression starts as soon as enteroblasts invades the foregut mesenchyme and it is maintained throughout the development into enteric neurons, so that homozygous disruption of *PHOX2B* in mice leads to an absence of enteric ganglia.[4]*PHOX2B* is the major disease causing gene in Congenital Central Hypoventilation Syndrome (CCHS, OMIM 209880), that is associated with sympathetic tumor and Hirschsprung disease (HSCR, OMIM 142623) in 5 and 20% of cases respectively. *PHOX2B*

mutations are mainly alanine expansions within the 20-residue ofpolyalaninetract and less frequently frameshift, missense and nonsense mutations, and by partial or whole gene deletions.[5,6]

Case-report

The pediatric department identifieda girl who had required hospitalization since birth; she was referred toa neonatal intensive care unit for failure to wean from the ventilator. She was born of a cesarean with a BW of 3.4kg. She remained hospitalized because of recurrent apnea requiring mechanical ventilation (MV).

Her physical examination (PE) while awakeand the metabolic screening produced normal results. The doctors suspected Ondine's curse, and given the urgency of the case and in order to target several genes at once that may are responsible for the signs of the disease; a completeexome sequencing was performed to search for the gene responsible for the disease in the molecular biology department of Anoual Laboratory.

Methods

Genomic DNA were isolated from 4 ml of peripheral blood using Wizard® Genomic DNA Purification Kit (Promega, Madison, WI, USA) according to the manufacturer's protocols.100 ng of DNA were used to amplify coding region using Ion AmpliSeqExome Ready Panel (Ion Torrent, Thermo Fisher Scientific) according to the manufacturer's protocols.The library was normalized to a final concentration at ~100 pM. Emulsion PCR was performed using the Ion PI™ Template OT2 200 Kit (Ion Torrent™, Thermo Fisher Scientific) according to the manufacturer's protocols. Following Amplification and recovery an ion sphere quality control Kit (Ion Torrent™, Thermo Fisher) wasused to evaluate the library's quality according to manufacturer's protocols.Next,the enrichmentwascompleted by selectivelybinding the ISPscontainingamplifiedlibrary fragments to streptavidincoatedmagneticbeads, removingemptyISPsthroughwashingsteps, and denaturing the librarystrands to allow the collection of the positive template ISPs. Sequencing primer and polymerase were added to the final enriched spheres ISPs prior to loading onto an Ion PI chip according to the Ion PGM™ 200 sequencing kit v3 protocols. Sequencing was carried out on the Ion Proton System (Life

technologies). For a sequence variant to be considered authentic, sequencing coverage of 250 wasused as a minimum requirement in this study.

Data analysis

Sequence data were processed using the Torrent Suite software v 4.4.3 (Ion Torrent™, Thermo Fisher Scientific) to align reads to the genome reference (HG19) and to generate run metrics, including chip loading efficiency and total read counts and quality. Following data analysis, annotation of a single-nucleotide variants, insertions, deletions, and splice site alterations was performed by the Ion Reporter Server System (Life Technologies).

Sanger sequencing

The variant of interest was confirmed by Sanger sequencing of amplified PCR products.

Discussion

The pediatric department identified a girl who had required hospitalization since birth; she was referred to a neonatal intensive care unit for failure to wean from the ventilator.She was presenting a severe sleep apnea caused by a mutation in *PHOX2B* gene which is responsible of Congenital Central Hypoventilation Syndrome.The baby carried a heterozygous mutation in the *PHOX2B* gene which is the first mutation reported in Morocco to the best of our knowledge.The mutation was detected with the complete exome sequencing using the Ion Proton System and the Ion Reporter Server System; this is the first application in Morocco. The mutation detected by the Ion Proton System was confirmed by direct target Sanger sequencing.Previous studies have reported various types of *PHOX2B* gene mutations in patients with CCHS. Approximately 90% of individuals with the CCHS phenotype are heterozygous for a polyalanine repeat expansion mutation(PARM), and remaining 10% approximately of individualswith CCHS are heterozygous for a non-PARM (NPARM)(including missense, nonsense, and frameshift mutation) inthe *PHOX2B* gene.[9]In contrast to the PARMs, the majority of NPARMs are associated with very severe phenotypes, including Hirschsprung disease with extensive gut involvement, needfor continuous ventilatory support, and increased tumor risk.We detected in this case a heterozygous

mutation in exon 2 of the *PHOX2B* gene c.428A>G (figure 1), it has been previously described as a disease causing central hypoventilation syndrome by Trochet et al., 2009.[7]The c.428A>G mutation is expected to produce p.Q143R, which may have a pathogenic effect than other NPARMs.[10]

It is reported that central sleep apnea is a relatively common phenomenon in normal infants.[8] However, the frequency of central apnea events in this case was extremely high, suggestive an unusual etiology, which was the reason for our considering genetic testing for CCHS. PHOX2B is the disease-defining gene for CCHS.CCHS patients usually present hypoventilation andhypoxemia. They lack both of the hypercapnicventilator and hypoxic ventilatory responses.[11, 12]

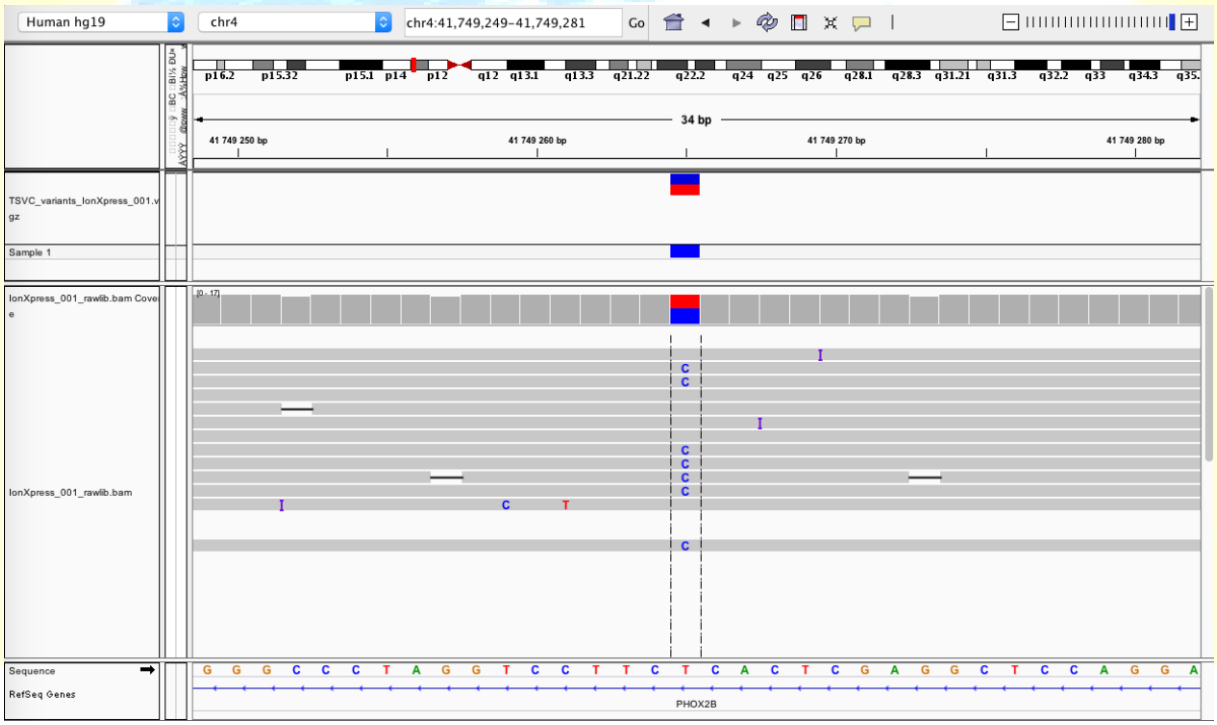

Figure 1: profile of the gene mutation *PHOX2B*

Conclusion

We report in this article the first heterozygous mutation in exon 2 of the *PHOX2B* gene c.428A>G in Morocco to the best ofour knowledge.This mutation was detected with the complete exome sequencing which is the first application in Morocco. These results support that NGS approach based on ampliseqlibraries, Ion Proton system and the Ion Reporter Server System, is highly efficient, fast and cheap high throughout mutation detection method to integrate in clinical diagnostics for the detection of genetic abnormalities. It will lead to a better management of patients and genetic counseling of relatives at risk.

ACKNOWLEDGMENT

We thank Thermo Fisher Scientific and their representative in Morocco Genome Biotechnologies.

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