Short Report

Exploring the utility of whole-exome sequencing as a diagnostic tool in a child with atypical episodic muscle weakness


The advent of whole-exome next-generation sequencing (WES) has been pivotal for the molecular characterization of Mendelian disease; however, the clinical applicability of WES has remained relatively unexplored. We describe our exploration of WES as a diagnostic tool in a 3 1/2-year old female patient with a 2-year history of episodic muscle weakness and paroxysmal dystonia who presented following a previous extensive but unrevealing diagnostic work-up. WES was performed on the proband and her two parents. Parental exome data was used to filter potential de novo genomic events in the proband and suspected variants were confirmed using di-deoxy sequencing. WES revealed a de novo non-synonymous mutation in exon 21 of the calcium channel gene CACNA1S that has been previously reported in a single patient as a rare cause of atypical hypokalemic periodic paralysis. This was unexpected, as the proband’s original differential diagnosis had included hypokalemic periodic paralysis, but clinical and laboratory features were equivocal, and standard clinical molecular testing for hypokalemic periodic paralysis and related disorders was negative. This report highlights the potential diagnostic utility of WES in clinical practice, with implications for the approach to similar diagnostic dilemmas in the future.

Conflict of interest

J. R. L. is a consultant for Athena Diagnostics, has stock ownership in 23andMe and Ion Torrent Systems, and is a co-inventor on multiple United States and European patents for DNA diagnostics. R. A. G. was a founding shareholder in Seq-Wright, Inc. Some of the authors are based in the Department of Molecular and Human Genetics at Baylor College of Medicine, which derives revenue from genetic laboratory testing, including whole-exome sequencing. The remaining authors have no conflicts of interest to declare.

Key words: CACNA1S – hypokalemic periodic paralysis – hypotonia – next-generation sequencing

Corresponding author: Chester W. Brown, Department of Molecular and Human Genetics, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030, USA. Tel.: 713-798-3994; fax: 713-798-7418; e-mail: cbrown@bcm.edu

Received 11 July 2012, revised and accepted for publication 3 August 2012

Studies of Mendelian disease have been transformed by the advent of whole-exome next-generation sequencing (WES) (1), which has enabled the identification and molecular characterization of a wide spectrum of Mendelian traits (2, 3). More recently, however, the role of whole-exome sequencing in the armory of clinical genetics has come to the fore, with implications for the diagnostic and therapeutic aspects of disease (4–8).

Primary hypokalemic periodic paralysis (HypoKPP) is an autosomal dominant disorder typically characterized by acute, episodic, usually flaccid loss of skeletal muscle tone in the context of low serum potassium (between 1 and 3 mmol/l). The typical age of onset is...
between 5 and 20 years (9), with episodes that last hours to days and are often precipitated by carbohydrate-rich meals and rest after prolonged exercise. Myopathy may develop in some patients, but myotonia is not a prominent feature. HypoKPP is caused by mutations in the gene encoding a voltage gated calcium channel – CACNA1S in 50–75% of cases, or sodium channel – SCN4A in 8–10% of cases (9). Both genes are fairly large, and all reported disease-causing mutations involve substitution of highly conserved arginine residues found in voltage sensing segments. In CACNA1S, four mutations in exons 11 and 30 account for >90% of known cases; with a similar percentage of known SCN4A cases accounted for by three mutations in exon 12 (10). This distribution of mutations has made targeted testing the mainstay of the current clinical testing paradigm (11). It is clear, however, that the full genetic spectrum of the disease is still unknown, as these two genes only account for about 80% of all cases (10, 12). A molecular diagnosis of HypoKPP is important for the future reproductive choices of the family, the testing of other family members at risk, and can influence appropriate pharmacologic therapy; for instance, although the diuretic acetazolamide is reported to be efficacious for the majority of patients with HypoKPP, it has been associated with less benefit or even worsening of symptoms in some patients with specific mutations in SCN4A (12). Furthermore, individuals with HypoKPP are at an increased risk for post-operative complications related to anesthesia, including malignant hyperthermia. Therefore, a precise and secure molecular genetic diagnosis is important for clinical management, prognosis, and genetic counseling.

We report a 3 1/2-year-old female with early-onset episodic muscle weakness associated with other neuromuscular abnormalities. After an extensive diagnostic evaluation, including equivocal therapeutic trials and negative clinical sequencing, WES was undertaken to evaluate the hypothesis that she had a rare, de novo mutation in a novel gene causing her symptoms. We advocated for this approach as a potential means of reducing the time, effort, and cost associated with arriving at a genetic diagnosis.

Materials and methods

Participants

Adult members of the study family, which consisted of the proband, mother, and father, provided written informed consent for themselves and their child for enrollment in the study. The study protocol was approved by the Baylor College of Medicine (BCM) Institutional Review Board in accordance with the Declaration of Helsinki.

Whole-exome next-generation sequencing

Paired-end whole-exome-enriched libraries were prepared from genomic DNA isolated from the peripheral blood of the proband and parents using an in-house-developed capture reagent from Roche NimbleGen (Madison, WI). Libraries were sequenced on the HiSeq 2000 platform (Illumina, San Diego, CA), and sequencing reads (with 20× or more coverage in 95% of targeted regions) were aligned to the March 2006 human reference assembly (NCBI36/hg18). Annotated high-quality variants were subsequently filtered to exclude common variants (>1% minor allele frequency). Variants of interest were subsequently confirmed by di-deoxy sequencing. Details of the sequencing and filtering protocols are given in the Appendix S1, supporting information.

Results

Clinical report

The patient was a 3 1/2-year-old female with a 2-year history of episodic lower limb weakness, who had previously received an extensive but non-diagnostic evaluation. Her episodes manifested as difficulty with weight bearing, stumbling and clumsiness, and subjective descriptions of pain. They usually occurred in the morning and were often associated with prolonged sitting or compromised sleep. There was no association with meals or diet. Episodes occurred two to three times per week, with occasional daily attacks, lasting from 30 min to 4 h. The frequency and intensity of attacks had been relatively stable over the previous 2 years.

The medical history included early-onset scoliosis, high arched feet, lower limb hypertonia, clumsy gait and frequent toe-walking. Her cognitive development was normal, but she had received developmental services between 18 months and 2 years of age for mild speech delay. She had a healthy 2-year-old sister and there was no family history of similar symptoms, musculoskeletal or neurologic disease. On physical examination, she was non-dysmorphic with tight heel cords, pes cavus, increased plantar reflexes, lordosis, a positive Gower sign, and showed stiff toe-walking and in-toeing of the right foot.

Biochemical evaluations (including measurements of potassium and creatine kinase) were reported as normal at baseline (see Appendix S2). An electromyogram was normal and nerve conduction studies showed decreased amplitudes with normal latency and conduction velocities. A 48-h video electroencephalogram during an episode confirmed the episodes to be non-epileptogenic.

Previously considered diagnoses included paroxysmal dyskinesia (carbamazepine subjectively worsened the episodes); paroxysmal dystonia (a trial of L-dopa was unsuccessful) and HypoKPP (an empiric trial of acetazolamide resulted in more frequent and intense episodes). Serum potassium during an episode was 3.0 mmol/l (lower limit of normal 3.5 mmol/l). Subsequent potassium levels during episodes ranged from 2.2 to 3.3 mmol/l.
Clinical molecular testing

Clinical testing (see Appendix S2) for HypoKPP, including evaluation of CACNA1S exons 11 and 30 and exons 12, 13, 23, and 24 of SCN4A, was negative. Testing for Myotonia Congenita (MIMs 160800, 255700) was similarly unrevealing.

Whole-exome next-generation sequencing

At the completion of the available clinical testing (accounting for >90% of CACNA1S/SCN4A cases), we clinically considered that the proband’s atypical presentation was more likely the consequence of a rare, de novo mutation in a novel gene constituting some fraction of the 15–40% of HypoKPP cases that are not attributable to CACNA1S or SCN4A. Therefore, since any additional work-up would necessarily be done on a research basis, rather than attempting full sequencing of either gene or ad hoc sequencing of other potential candidate genes, we carried out WES on the patient and her parents, with the goal of determining the utility of WES in a clinical setting. In our analysis, we first utilized a trio-based model of a fully penetrant dominant disorder, from which we would anticipate one or two true de novo mutations (13, 14). Inherited variants were excluded from the list of rare deleterious variants identified in the patient (Table 1). This subtractive process revealed a total of five apparently de novo variants (before validation) in the patient. We were surprised to find among these a single variant in the CACNA1S gene (Fig. 1a) that resulted in an arginine to serine amino-acid change in exon 21 (c.2691G>T; p.R897S). Sanger di-deoxysequencing confirmed this variant as truly de novo in the proband (Fig. 1b). This variant was molecularly consistent with typical HypoKPP-causing CACNA1S mutations (arginine residue substitution in voltage-sensing domain), was predicted to be deleterious by PolyPhen2 (15), and has been reported as a pathogenic mutation in another patient (of different ethnicity) who had severe, early-onset, HypoKPP (16). None of the other identified gene variants (see Appendixes S1 and S2) were known to be disease-causing or relevant to the patient’s phenotype.

The previous report of CACNA1S R897S (16) involved a 2-year old Turkish male who also had an atypical presentation of HypoKPP with early onset and predominant lower extremity involvement. Our patient had several noteworthy differences (Table 2), which made it difficult to associate her presentation with the previous case report. The reason for these differences remains unclear, but may reflect modifier effects of other gene variants on the HypoKPP phenotype. The clinical similarities between the two patients, however, raise the possibility that a genotype–phenotype correlation exists with early age of onset, predominant lower limb involvement, and a spectrum of atypical symptoms. The clinical and molecular attributes of the R897S variant strongly suggest that it is pathogenic; however, additional cases and functional data in model organisms such as zebrafish will be important to ultimately establish variant pathogenicity and genotype–phenotype correlations.

Discussion

This report illustrates the clinical power of the WES approach – identifying a molecular cause in a patient with an atypical presentation and equivocal diagnosis, while potentially obviating a number of invasive, expensive, and time-consuming diagnostic evaluations. In this case, the molecular confirmation of the diagnosis could provide additional evidence for the necessity of long-term potassium supplementation and allow the physician to counsel the family appropriately, including addressing concerns that the family’s youngest child might also be affected and providing reassurance to the parents that their recurrence risk in future pregnancies would be low.
Table 2. Comparison of clinical features reported with the CACNA1S R897S mutation

<table>
<thead>
<tr>
<th></th>
<th>This patient</th>
<th>Chabrier et al. (16)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at onset</td>
<td>1 year</td>
<td>1 year</td>
</tr>
<tr>
<td>Age at diagnosis</td>
<td>4 years</td>
<td>2 years</td>
</tr>
<tr>
<td>Ethnic background</td>
<td>African American</td>
<td>Turkish</td>
</tr>
<tr>
<td>Perinatal period</td>
<td>IUGRa</td>
<td>Neonatal hypotonia</td>
</tr>
<tr>
<td>Muscle presentation</td>
<td>Persistent hypertonia; Predominantly lower limb</td>
<td>Global hypotonia; Lower limbs more affected than upper</td>
</tr>
<tr>
<td>Maximum frequency of attacks</td>
<td>Daily</td>
<td>Daily</td>
</tr>
<tr>
<td>Temporal relation of attacks</td>
<td>Morning</td>
<td>Evening</td>
</tr>
<tr>
<td>Initial hypokalemia (with attack)</td>
<td>3.0 mmol/l</td>
<td>3.1 mmol/l</td>
</tr>
<tr>
<td>Additional clinical features</td>
<td>Scoliosis</td>
<td>Pes Cavus</td>
</tr>
<tr>
<td></td>
<td>Toe-walking</td>
<td></td>
</tr>
</tbody>
</table>

*Intrauterine Growth Restriction.

A very important consideration is whether the ability to make an actionable diagnosis justifies the cost of WES. In our case, we found that WES might actually result in relative cost savings – conservatively, the cost for the trio-based exome analysis on a research basis, including reagents, equipment and expertise to carry out, interpret and confirm the sequencing results was two to three thousand (US) dollars less than the cost of the previous diagnostic work-up (Table S1); this would have been even more cost effective had we included the costs for hospitalizations, emergency room visits, medications, and physician services. Moreover, the cost of the WES technology is steadily decreasing, and for those patients whose clinical evaluation is likely to involve extensive, expensive, or invasive diagnostic testing to evaluate a broad differential diagnosis, it has been argued that WES should be considered early in the diagnostic approach (17); although a formal cost comparison between the current sequential clinical diagnostic approach and now clinically available WES needs to be undertaken to determine this conclusively.

We identified a rare, de novo cause for a genetic disease that was not detected by standard clinical molecular testing strategies. For CACNA1S-associated HypoKPP, a tiered approach of targeted mutation testing followed by sequencing of exons 11 and 30 is the accepted clinical diagnostic laboratory approach and is sufficient in the vast majority of positive cases. Full sequencing of CACNA1S or SCN4A for HypoKPP is not routinely performed on a clinical laboratory basis, and the R897S CACNA1S mutation, having only been described once before in the medical literature, is not routinely tested. As a result, our patient’s mutation was not identified by standard clinical diagnostic laboratory testing. Were suspicions for classical HypoKPP higher, we might have considered the extensive task of sequencing the 44 and 26 exons of the CACNA1S and SCN4A genes (respectively) in their entirety; however, we viewed this as unlikely given the clinical presentation, and it would have been challenging to accomplish. Further, full sequencing of individual genes in diseases with a restricted and established disease-allele spectrum can be cost prohibitive for clinical diagnostic laboratories given the lower likelihood of finding rare pathogenic mutations, and accordingly, this is usually reserved for clinically unambiguous cases. Thus, for unusual clinical presentations, WES is likely to be a viable alternative to costly stepwise clinical sequencing of suspected disease genes because WES allows one to simultaneously query all known genes and pathogenic variants, as well as to identify mutations within novel candidate genes.

In conclusion, by identifying a de novo mutation as the cause of HypoKPP in this atypical case, we have illustrated the utility of WES in the armory of clinical diagnostics. Viewed in the context of previous reports (4–6), there is now a growing body of evidence to suggest that WES should be considered as a diagnostic option in disorders for which clinical and molecular diagnoses are challenging, especially those for which clinical management may be affected (17).

Supporting Information

The following Supporting information is available for this article:

Appendix S1. Supplementary methods.

Appendix S2. Supplementary results.

Table S1. Comparison of estimated costs for trio-based whole-exome sequencing (WES) versus clinical diagnostic costs (base cost before institutional mark-up) in the affected family.

Additional Supporting information may be found in the online version of this article.

Acknowledgements

The authors would like to thank the family for their participation in the study. This study was funded by US National Human Genome Research Institute (NHGRI) grant 2-U54HG003273-09 to R.A. G.

References


Exploring whole-exome sequencing as a diagnostic tool


