Whole-Exome Sequencing as a Diagnostic Tool in a Family With Episodic Ataxia Type 1

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Abstract

Complex neurologic phenotypes are inherently difficult to diagnose. Whole-exome sequencing (WES) is a new tool in the neurologist’s diagnostic armamentarium. Whole-exome sequencing can be applied to investigate the “diagnostic odyssey” cases. These cases involve patients with rare diseases that likely have a genetic etiology but have failed to be diagnosed by clinical evaluation and targeted gene testing. We describe such a case, a 22-year-old man who had mild intellectual developmental disability and episodes of jerking ataxic movements that affected his whole body. He underwent numerous multidisciplinary and multicentric evaluations throughout his life that failed to establish a clear diagnosis. Following his visit to Mayo Clinic in Jacksonville, Florida, WES was applied for genetic determination of the unknown disorder in the proband and his biological parents and sister. Additional clinical evaluation, magnetic resonance neuroimaging, electromyography, and electroencephalography of the proband were performed to verify the phenotype after the WES results were available. To our knowledge, this is the first report of the application of WES to facilitate the diagnosis of episodic ataxia type 1. This case illustrates that WES supported by clinical data is a useful and time-saving tool in the evaluation of patients with rare and complex hereditary disorders.
Recently, the Mayo Clinic Center for Individualized Medicine has initiated a program that uses WES to support personalized care through genetic diagnosis. It targets patients with rare complex diseases that are likely of genetic origin, e.g., those with a family history of disease or early age at onset. These patients are sometimes referred to as “diagnostic odyssey” cases because of the numerous prior unsuccessful attempts to establish a definite diagnosis.

As a diagnostic odyssey case, the patient was offered WES. He agreed to this procedure and signed the authorization form for genetic counseling and collection of his medical records. After the first genetic counseling appointment, contact with his biological family was established, and further counseling was provided to all participating biological family members. The assessment was accomplished from August 13, 2013, through July 15, 2014. Whole-exome sequencing and cosegregation analysis were performed by a Clinical Laboratory Improvement Amendments–certified laboratory utilizing samples collected from the proband, his biological parents, and his sister (Ambry Genetics Corp). Samples were prepared using the SeqCap EZ VCRome 2.0 system (Roche NimbleGen, Inc). Detailed methodology for WES and analysis have been described previously. After the WES results were available, additional neurologic evaluation, brain magnetic resonance imaging (MRI), electromyography (EMG), and electroencephalography (EEG) were performed on the proband to verify the phenotypic characteristics of the disorder. All examinations were performed for diagnostic purposes only; no approval of the institutional review board was sought or required.

Genealogical Investigations

According to the proband’s adoptive mother, the proband’s sister, father, and 4 paternal relatives have or had a chronic neurologic disorder with symptoms similar to those of the proband, including his deceased paternal grandfather. No autopsy was performed. The sister’s motor complaints worsened after a recent pregnancy, which was her second pregnancy. The proband’s mother has mild intellectual disability and diabetes. Her daughter, who was the proband’s half sister, was reported to have an intellectual disability and possibly seizures. The pedigree structure is presented in Figure 1.

Molecular Genetic Studies

A review of the proband’s medical records revealed that prior karyotyping, chromosomal microarray, and screening for mutations in the myotonic dystrophy protein kinase (DMPK) and huntingtin (HTT) genes did not bring clarification. Exome sequencing of the family trio (proband, mother, and father) resulted in an average of approximately 11 giga base pairs of sequence per sample, with approximately 95% of bases within the captured region covered at least 10-fold. The removal of common single nucleotide polymorphisms, intergenic and 3′/ 5′-untranslated region variants, non–splice-related intronic variants, and synonymous variants through stepwise filtering resulted in approximately 12,000 variants associated with each trio member. Subsequent filtering based on family history and inheritance modeling (autosomal dominant/recessive, X-linked dominant/recessive, and Y-linked) within the trio narrowed the list of candidates to 165 genes and 171 unique alterations. After manual review of

![Pedigree structure of the proband’s family. Standard symbols were used. Round symbols indicate females, squares indicate males, and diagonal lines indicate that the individual is deceased. Diamonds were used to disguise sex, and numbers inside symbols indicate number of children. The arrow indicates the proband. Orange symbols indicate individuals with clinical features of episodic ataxia type 1. Asterisks indicate family members who underwent whole-exome sequencing (proband and parents) or cosegregation analysis (sister). Blue symbols represent individuals who did not present with ataxia.](image-url)
each alteration to rule out sequencing artifacts and polymorphisms, the list of candidates was narrowed to 110 genes and 111 unique alterations. Each gene/variant was then assessed for potential clinical importance and subsequently divided into “characterized” or “novel” classifications depending on previous literature documenting a role in disease. Of the 110 genes, only 3 genes were labeled as “characterized,” and 1 of these was the potassium voltage-gated channel, shaker-related subfamily, member 1 gene (KCNA1) on chromosome 12p13.32, known to harbor mutations causing episodic ataxia type 1 (EA1). In this trio, WES identified the KCNA1 c.1210G>A (p.V404I) heterozygous missense mutation in the proband, inherited from his affected father. Follow-up cosegregation studies showed that the affected sister also carries this mutation.

Clinical and Laboratory Investigations

Only the proband was clinically investigated. Neurologic evaluations of other family members for clinical assessment are planned in the near future. The proband was a white man who had development of episodes of “shaking spells” at the age of 18 months. The spells consisted of uncontrollable “jerking” movements of his trunk and limbs that would keep him awake at night. Sometimes the movements were seen all over his body. During these episodes, the patient had difficulty with swallowing, speaking, and ambulation. The episodes could last for a few minutes and would appear in rapid succession more than 15 times a day, leading to severe exhaustion. They could occur spontaneously or be induced by fatigue, emotional stress, or sudden postural changes. The spells became more severe and frequent. He started to walk at around 14 months of age, but further motor development was delayed. At 12 years of age, he was noted to have a short stature, mildly dolichocephalic head shape, and facial asymmetry with right eye downslanting.

Prior evaluations included an ophthalmology examination that did not reveal Kayser-Fleischer rings. He had normal 24-hour urinary copper excretion. Blood tests were negative for antistreptolysin O and anti-DNAse, and his erythrocyte sedimentation rate was normal. Results from long-term closed-circuit television EEG, brain MRI, and multiple routine EEGs, including studies performed during his spells, were all reported to be negative or normal. He was diagnosed as having attention-deficit/hyperactivity disorder, anxiety, depression, hereditary spastic paraparesis, chorea, myotonic dystrophy, and “generalized jerking and shaking.” He was treated with unknown doses of multiple medications alone or in combination. These included lisdexamfetamine, risperidone, valproic acid, alprazolam, and clonazepam; he was also given haloperidol for alleged auditory hallucinations. The medications produced no benefit.

At his first visit to Mayo Clinic, multiple spells were observed. During his spells, neurologic examination revealed high-amplitude and low-velocity, involuntary, uncoordinated movements involving the trunk and upper limbs that increased when he tried to stand up. His gait and the results of his finger-pointing test were ataxic, and he needed temporary assistance for mobility. Results of a Romberg test and eye movements were unremarkable (Supplemental Video, available online at http://www.mayoclinicproceedings.org). Increased muscle tone was noted, predominantly in his lower extremities, due to mild spasticity with brisk deep tendon reflexes and an increase in tone on passive movements of his legs. Babinski sign, ankle clonus, muscle weakness, or visible myokymias were not observed. The neuropsychological and psychiatric evaluations requested at the time of initial evaluation led to the diagnosis of mild intellectual developmental disorder with a full-scale IQ of 59, auditory hallucinations, and lower frustration tolerance.

Brain MRI detected a mild vermian hypoplasia and prominent cisterna magna (Figure 2, A and B), and EEG revealed a generalized background slowing of 7 to 8 Hz without epileptiform activity. Nerve conduction studies of the sural, peroneal, and tibial nerves yielded normal results. Concentric needle EMG of the left biceps brachii, triceps brachii, first dorsal intersosseus, vastus lateralis, tibialis anterior, and gastrocnemius muscles documented myokymic discharges during and between the ataxic spells (Figure 3). However, they were absent in the left oribcularis oculi muscle.

Acetazolamide therapy at an initial dosage of 125 mg once daily produced a severe rash...
that appeared after 2 doses. After a successful desensitization procedure, the dosage was increased to 250 mg twice daily and led to an approximately 50% reduction in the frequency and duration of his spells.

DISCUSSION

Based on successful implementation of WES studies, the diagnosis of EA1 was accurately established 11 months after the patient’s initial visit to Mayo Clinic. The cost of WES was approximately $7000, whereas the previous 4 genetic tests were estimated to be approximately the same amount. This success with such a low cost underlines the importance of WES in the clinical setting. With this tool, we are able to correctly diagnose complicated neurologic disorders that have a potential genetic nature even in difficult circumstances, such as in this case.

Episodic ataxia is a group of 7 rare familial disorders characterized by brief attacks of generalized ataxia with normal or almost normal neurologic function between attacks. Episodic ataxia type 1 is an autosomal dominant disorder, has a childhood or early adolescence onset, and is a potassium channelopathy. Its cardinal symptoms include ataxia with loss of both motor coordination and balance accompanied by constant myokymia of the skeletal muscles of the head and limbs, detectable clinically or on EMG. Other symptoms may include vertigo, blurred vision, diplopia, nausea, headache, diaphoresis, clumsiness, dystarthric speech, difficulty breathing, epilepsy, delayed motor development, cognitive disability, choreoathetosis, and carpal spasm. Episodic ataxia type 1 was first described in 1975 by van Dyke et al, and since then, the EA1 phenotype has been reported in little more than 100 individuals.

Although most individuals with EA1 present with episodes of ataxia, myokymia on EMG, and normal findings on brain MRI, establishing a diagnosis may be challenging because of phenotypic variations. The diagnosis will depend on the underlying mutation and consideration of different presenting phenotypes, which can include only myokymia or distal weakness or a lack of episodic ataxia or myokymia. There is also intrafamilial and interfamilial phenotypic variability.

In the described family, analysis of the pedigree suggested an autosomal dominant disorder. However, the family history suggesting mental retardation on both the mother’s and father’s side and the vermian hypoplasia with prominent cisterna magna on MRI of the proband were difficult to interpret. Additionally, there is only one report of cerebral atrophy in EA1. Thus, the proband underwent extensive diagnostic testing for Wilson disease, Huntington disease, Sydenham chorea, epilepsy, and Curschmann-Steinert myotonic dystrophy, but these investigations did not establish a diagnosis.

The application of WES in a clinical diagnostic setting has accelerated and simplified the diagnosis and management of genetic diseases. In addition, WES is becoming more cost-effective and as a result more extensively applied. Current diagnostic panel testing for complete ataxia evaluation offered by commercial laboratories costs almost twice as much as WES and does not include a specific test for EA1. Thus, in the case of clinical atypical ataxia, it may be beneficial to consider WES first, especially if clinical and genealogical data are sparse. However, it should be noted that WES would not detect triplet repeat mutations, which represent an estimated 40% to 50% of genetic ataxias worldwide.

For the first time in molecular diagnosis, WES can simultaneously interrogate virtually all genes, including those most recently discovered as well as genes that are both related to and outside of the clinician’s differential diagnoses. Therefore, it is particularly useful for patients who have genetic disorders with profound and heterogeneous phenotypes and atypical and incomplete presentations. It is also useful for patients who are suspected to have a

**FIGURE 2.** Magnetic resonance images of proband. A, Sagittal T1-weighted, and B, axial T2-weighted with fat saturation images revealed mild vermian atrophy (white arrow) and prominent cisterna magna (red arrow). L = left-sided.
genetic diagnosis but have incomplete histories, and it can help those who have rare or newly discovered diseases or disorders. However, the limitations of this approach need to be cautioned. Whole-exome sequencing is not emphasized or optimal for analyzing the following types of mutations: gross deletions/duplications, gross rearrangements, deep intronic variations, long repeat sequences, trinucleotide repeat sequences, mutations involved in triallelic inheritance, mitochondrial genome mutations, epigenetic effects, and oligogenic inheritance. Although some of these mutations may be detected by whole-genome sequencing, large expanded-repeat sequences present a considerable diagnostic challenge.

The limitations of WES are not just restricted to technical methodology. In the present case, we had a familial trio available for filtering variants and reached the short list of candidate genes. In most cases, only the proband may be available, and each individual carries a substantial number of rare/unique variants that could make genetic diagnosis difficult. In our recent study of 500 unselected families for WES, the diagnostic rate was significantly higher among families undergoing a trio whole-exome testing strategy (37%) as compared with a singleton whole-exome testing strategy (21%). In the present case, the likely pathogenic mutation was also identified in a gene known to cause EA1, which fits with the clinical phenotype. However, if the causative mutation is in a novel gene that has not been connected with the phenotype, it will fall into a long list of candidates. Finally, the KCNA1 c.1210G>A (p.V404I) mutation has previously been reported in a family with EA1, but a scenario could arise in which a novel mutation in a characterized gene could be identified but with an unclear pathogenicity; functional studies with a pathogenic readout may be helpful in these cases.

To our knowledge, this is the first report of the application of WES to facilitate the diagnosis of EA1. Currently, more than 20 reported KCNA1 mutations have been identified by standard gene analysis in familial cases, and only 1 de novo mutation has been reported. All carriers are heterozygous for KCNA1 mutations that are mostly missense mutations, with a very few nonsense and in-frame deletion mutations.

The KCNA1 c.1210G>A (p.V404I) mutation results in a valine to isoleucine substitution at a highly conserved position in the sixth transmembrane segment of the potassium voltage-gated channel subunit hKv1.1. This leads to a rather subtle disturbance of channel function and a milder phenotype than other mutations, such as the p.R417* mutation.

Although antiepileptic drugs may considerably reduce the frequency of the attacks in EA1, the response is heterogeneous. In a second family with the KCNA1 c.1210G>A (p.V404I) mutation, carbamazepine was reported to have good results. In our proband, a trial of 2 benzodiazepines proved ineffective; acetazolamide therapy first became beneficial at the daily dosage of 500 mg. Of note, the first reported family with the KCNA1 c.1210G>A (p.V404I) mutation did not respond well to acetazolamide.10

CONCLUSION
This case illustrates that WES supported by evaluation of clinical data is a useful, time-saving,
and cost-effective diagnostic tool for patients with a complex phenotypic presentation of EA1. This diagnostic approach may help determine early therapeutic intervention strategies and directly affect patient care.

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**SUPPLEMENTAL ONLINE MATERIAL**

Supplemental material can be found online at http://www.mayoclinicproceedings.org.

**Abbreviations and Acronyms:** DMPK = myotonic dystrophy protein kinase gene; EA1 = episodic ataxia type 1; EEG = electroencephalography; EMG = electromyography; HTT = huntingtin gene; MRI = magnetic resonance imaging; WES = whole-exome sequencing

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**Potential Competing Interests:** Dr Tang and Ms El-Khechen are employed by and receive a salary from Ambry Genetics Corp. Exome sequencing is among its commercially available tests.

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**REFERENCES**