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Potential Role of Genomic Sequencing in the Early Diagnosis of Treatable Genetic Conditions

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Abstract

We present cases of 3 children diagnosed with the same genetic condition, Gitelman syndrome, at different stages using various genetic methods: panel testing, targeted single gene sequencing, and exome sequencing. We discuss the advantages and disadvantages of each method and review the potential of genomic sequencing for early disease detection.

At least 7000 rare diseases have been identified, most of which are genetic disorders. Rare diseases collectively affect approximately 30 million people in the US and have a significant impact on patients and families.[1,2] Many go undiagnosed for years, even after numerous medical evaluations.[3] Rapid advances in genetic testing and sequencing technology, such as gene panel and whole exome sequencing (WES), offer the potential to significantly augment rare disease diagnostics; however, the use of tests in diagnostic workups is often variable. Appropriate and early implementation of genomic sequencing technology could lead to earlier detection of these disorders and possibly identify patients at early symptomatic stages. Especially when treatment is available, early identification of genetic disorders could have profound implications for patients with respect to disease management and the prevention of complications. However, one challenge to the use of genomic sequencing is insufficient familiarity with the yield and limitations of the tests, results interpretation, and optimizing application to different patients.[4-7]

Herein, we examine different approaches to diagnosis of a rare treatable genetic disorder by presenting 3 children with varying presentations that led to the diagnosis of Gitelman syndrome. WES led to a diagnosis in a patient who had only mild nonspecific symptoms

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that had gone unrecognized. In these examples, we discuss classical genetic testing and the potential of genomic sequencing for early detection of treatable genetic conditions.

Case Presentations

Case 1: Classic Symptomatic Presentation

A 16-year-old boy presented to the hospital with paresthesias, muscle cramps, and perioral numbness and was admitted to the intensive care unit with severe hypokalemia. Initial laboratory work revealed significantly low serum potassium (2.0 mEq/L; normal range 3.5-5.0) and magnesium (0.7 mg/dL; normal range 1.5-2.5), and elevated bicarbonate. An electrocardiogram showed U waves, but no prolonged QT interval or arrhythmia. Prenatal and birth history were uncomplicated. He had normal growth measures. Motor and speech development were delayed (walked at 2.5 years of age, first words at 3 years of age). At 9 years of age, he had also presented to the emergency room with hand cramps in the setting of vomiting and dehydration, but received limited workup. His parents and his 2-year-old brother were reportedly healthy.

Given the history of a prior similar presentation, he underwent further workup to identify the etiology of his electrolyte abnormalities. His urine studies revealed an elevated spot urine potassium:creatinine ratio (65 mEq/g; normal range <13), an elevated fractional excretion of magnesium in the setting of hypomagnesemia (2.7%; where >2% indicates inappropriate renal wasting), and a low 24-hour urinary calcium (<75.4 mg/day; normal range 100-250), which together were suggestive of a renal potassium and magnesium wasting disorder such as Bartter or Gitelman syndrome. During the 4-day hospitalization in the intensive care unit, he received intravenous potassium, his electrocardiogram normalized, and symptoms improved. He was then started on spironolactone and a regimen of potassium, magnesium, and calcium supplements. Genetic testing was performed for definitive diagnosis and to optimize pharmacological therapy. A gene panel for Gitelman and Bartter syndromes (types I-IV) was sent, including testing of *SLC12A1, KCNJ1, CLCNKB, BSND,* and *SLC12A3* genes, which identified a pathogenic variant.

Case 2: Familial Asymptomatic Testing

The 2-year-old brother of patient 1 was subsequently seen for a genetic evaluation to determine his risk of hereditary renal tubulopathy, given patient 1’s presentation. His birth, development, and past medical history were unremarkable and physical examination was normal. Electrolytes were checked and targeted DNA sequencing for the familial gene variant was performed.

Case 3: Early Diagnosis

A 7-year-old boy was evaluated for developmental delay, hypotonia, and possible autism spectrum disorder after being referred to genetics clinic. He was an only child of nonconsanguineous parents. There were no known maternal prenatal exposures. His motor and language development were delayed. He spoke in short sentences with a paucity of spontaneous speech, although he was able to understand well and convey his needs. He had difficulty with reading and spelling, but was comfortable with numbers, counting, and

addition. Socially, he seemed interested in interacting with other children and exhibited play behaviors, but seemed more engaged with objects. Parents also reported unique stereotypical behaviors. Past medical history included constipation, poor sleep, and unexplained knee arthralgias with normal radiographs. There was no known family history of autism. On examination, he had normal growth measures and no physical anomalies. He made limited eye contact and had low tone. Audiology and ophthalmology assessments were normal. He had a normal brain magnetic resonance imaging study with spectroscopy and prior electroencephalographs were negative for seizure activity. A biochemical workup for inborn errors of metabolism was unrevealing. He had a normal karyotype, fragile X testing, and microarray. Diagnostic WES, trio, was performed in clinical laboratory testing as part of his developmental delay and autism workup. Written informed consent was obtained and the family chose to receive results of secondary findings. Exon capture was performed using Agilent's SureSelect XT2 All Exon V4 kit (Agilent, Santa Clara, California). DNA was sequenced using the Illumina HiSeq 2000 sequencing system (Illumina, San Francisco, California) with 100 bp paired-end reads. Resulting reads were aligned to the human reference genome (hg19). Variants in genes associated with autism, intellectual disability, metabolic, and other neurologic disorders, hypotonia, and speech delay were assessed in accordance with the phenotype information provided.

Diagnosis and Outcome

Genetic testing identified mutations in the solute carrier family12, member 3 (*SLC12A3)* gene encoding the thiazide-sensitive sodium-chloride co-transporter in all 3 patients. *SLC12A3* mutations are associated with Gitelman syndrome, an autosomal-recessive, salt-losing tubulopathy that results in hypokalemia, hypomagnesemia, metabolic alkalosis, and hypocalciuria. Patients often present in adolescence or early adulthood with muscle cramping, paresthesias, low blood pressure, and cardiac arrhythmia. It is a treatable condition with management of electrolytes by administration of potassium and/or potassium-sparing diuretics. The prognosis is generally good if patients are recognized, treated, and monitored to prevent episodes of electrolyte imbalance that can lead to complications such as fatal cardiac arrhythmias, especially in the setting of intercurrent illness.[8]

In patient 1, gene panel testing revealed a homozygous missense variant in the *SLC12A3* gene (c. 533C>T; p. Ser178Leu). Two previous reports have demonstrated this specific gene alteration (p. Ser178Leu) in patients with Gitelman syndrome.[9,10] At follow-up, he was without signs of electrolyte imbalance. He continued on magnesium oxide, potassium chloride, calcium carbonate, and spironolactone with serial monitoring of electrolyte levels. Genetic testing was offered to other family members including his 2-year-old sibling (patient 2), who was asymptomatic at testing. By familial targeted DNA sequencing, patient 2 was also found to be homozygous for the missense variant in the *SLC12A3* gene (c. 533C>T;p. Ser178Leu). Laboratory evaluation for him revealed intermittently low serum potassium (range 3.1-5.6 mmol/L) and elevated urine potassium (82.3 mmol/L). Serum magnesium, calcium, and bicarbonate were consistently normal. He was followed for long-term monitoring. On 2 separate occasions, he presented with severe hypokalemia in the setting of viral illnesses with poor intake and diarrhea, but quickly responded to potassium

supplementation. Eventually, spironolactone was initiated with stabilization of serum potassium levels.

Exome sequencing for patient 3 surprisingly found compound heterozygous disease-associated variants in *SLC12A3* (p.(P643L) and p.(G741R)), but did not find any definitive pathogenic variants in well-known disease genes associated with developmental delay or the autism spectrum. Both of the *SLC12A3* mutations identified in him have been previously reported in Gitelman syndrome.[9,11] On follow-up, his electrolytes demonstrated borderline low potassium, mildly low magnesium, and low urine calcium consistent with Gitelman syndrome on multiple tests. He was also noted to have polydipsia, polyuria, and enuresis, which had not been brought to clinical attention. Potassium and magnesium supplementation was started to prevent complications. Notably, his unexplained arthralgias resolved after the initiation of treatment, suggesting that these symptoms were an early manifestation of his condition. Developmental delay and autism were not the expected presentations for this renal condition, but interestingly growth retardation and developmental delays have been reported in a few patients with Gitelman syndrome.[12]

Discussion

The cases presented herein demonstrate different paths to diagnosis for a treatable genetic condition and highlight the usefulness of each genetic diagnostic approach. The first 2 patients were diagnosed by more traditional testing methods after a severe presentation (patient 1, gene panel testing) or in an asymptomatic sibling (patient 2, familial targeted genetic testing). The third patient, who had mild symptoms from the disease that had gone unrecognized, was diagnosed via exome sequencing.

Genetic testing is increasingly performed by high-throughput sequencing for a gene panel or an exome.[13] Exome sequencing interrogates the protein-coding regions of the genome, which is the approximately 2% of the genome where 85% of disease-causing mutations have been found.[14] Compared with more traditional targeted sequencing, it has the advantage of simultaneously analyzing many genes and identifying mutations in any disease-associated gene. As such, it can be particularly helpful in disorders with high clinical heterogeneity or atypical manifestations,[3,15] or potentially in presymptomatic or early symptomatic individuals, such as in patient 3.

Whether diagnostic genomic sequencing could improve diagnosis rates, patient outcomes, or cost effectiveness has not been evaluated rigorously in clinical trials yet. However, data from initial studies on usefulness seem to be encouraging so far. Studies in large cohorts of patients across many clinical indications have reported a diagnostic success rate of around 25%,[16-20] which is higher than that of other genetic tests such as chromosome analysis (3%-10%)[21,22] or microarray (15%-20%).[23] The yield may vary by indication for testing (some indications as high as 40%-50% with detailed phenotyping) and is higher in pediatric populations,[24] with trio sequencing,[18,19] and in patients with a family history of consanguinity.[25] When WES can lead to a diagnosis, studies suggest that it can have important implications regarding patient management, such as initiation of targeted treatments or disease surveillance. Furthermore, exome sequencing can uncover a different

genetic disease from the initial clinical diagnosis, which better accounts for the proband's presenting symptoms and in some cases affects patient management (up to 8% of the time in 1 study[26]). Other studies have suggested that, when used early in the diagnostic workup, WES may even be cost effective compared with the relatively high costs of the entire traditional diagnostic trajectory that many patients with rare genetic disorders undergo.[16,19,27,28] These studies suggest that there could be a significant benefit in using genetic sequencing early as a diagnostic tool in oligosymptomatic pediatric patients who come to clinical attention with nonspecific symptoms, such as our third patient. The early diagnosis of Gitelman syndrome in our third patient, for example, provided an opportunity to treat early and implement monitoring before he presented with an acute severe manifestation, such as in patient 1.

Despite the power of diagnostic genomic sequencing, there are important limitations (Table I) and challenges to consider.[29] As a result, genetic tests such as microarrays, metabolic screening, or traditional gene testing may still be more suitable for consideration given a clear diagnostic indication,[30] such as in cases 1 and 2 here that underwent panel or single gene testing. Interpretation of variants of unknown significance remains a significant challenge in diagnostic WES, and the issue of secondary (incidental) findings continues to evolve. Although there is an emerging effort to report certain pathogenic secondary findings, there is less agreement on what should be included in secondary findings reports. We and others[31-33] agree that a useful framework for identifying the most clinically useful findings, especially in a pediatric population, should include whether the finding is known to be associated with a serious condition, whether it is actionable with significant clinical consequences if diagnosis is delayed, and whether it will ultimately serve the best interest of the child. A consensus comprehensive list of actionable genes has not fully emerged yet, but various recommendations have been made. The most widely recognized recommendation is by the American College of Medical Genetics and Genomics, which currently recommends reporting secondary findings in a minimum set of nearly 60 genes, mostly associated with conditions such as cancer predisposition and cardiomyopathy (Table II; available at www.jpeds.com).[34,35] Some testing laboratories offer expanded analyses of secondary findings beyond this minimum list, and others have proposed recommendations for disclosure of findings in a larger set of 114 genes.[36] Further efforts by the Actionability Working Group at ClinGen are aimed at developing rigorous and standardized procedures for categorically defining "clinical actionability" and have produced lists of gene–condition pairs that meet their clinical actionability threshold with an associated metric for the strength of the evidence. It is important to consider that some currently used actionable disease lists are likely incomplete.[34] Because they had generally prioritized disorders in which individuals with pathogenic mutations might be asymptomatic for long periods of time, actionable disorders with onset in childhood were not initially well-represented. As such, solely relying on static recommendations may lead to missed opportunities for reporting important actionable secondary findings when genomic sequencing is performed in the pediatric population. Furthermore, disease genes continue to be discovered at an astonishing pace and the mechanisms for updating lists should try to keep pace. For example, several novel and potentially treatable and actionable rare genetic conditions have been identified via exome sequencing in recent years (Table III; available at www.jpeds.com) that have not

yet been incorporated into testing workflows and recommendations. Recently, a more comprehensive curated gene list has been developed using the ClinGen clinical validity classification framework criteria, age of onset, penetrance, and mode of inheritance and includes 954 genes that met criteria for return.[48] This list was developed for reporting results in a study on the feasibility of newborn genomic sequencing, but the gene list could also serve as a useful resource when applied to diagnostic genomic sequencing in the pediatric population. Our third patient's secondary finding was not among the initial genes recommended by the American College of Medical Genetics and Genomics; however, we reported this gene to the family as a secondary finding because of its significant clinical implications, treatability, and the potential for disclosure to prevent severe medical consequences. Notably, the gene for Gitelman syndrome, *SLC12A3*, is now included in the list of 954 reportable genes mentioned, in addition to *SC12A1* for Bartter syndrome and several other *SLC* family members.

The benefits rendered by the reporting of secondary findings raise the question of who should have genomic sequencing more broadly. Indeed, there has been a lot of interest in whether it can also be used in population screening applications, given the breadth of information that can be obtained. At the present time, interpretive challenges of sequence data as well as the limited data on clinical usefulness and cost effectiveness make genome-wide population screening applications, especially in newborns, difficult, leading some organizations to recommend against use for now.[49,50] To address some of the challenges, however, 4 investigator groups in the Newborn Sequencing In Genomic medicine and public HealTh (NSIGHT) program are currently exploring the implications, challenges, and opportunities associated with the possible use of genomic sequence information in the newborn period.[51] Groups are also attempting to answer questions about broader sequencing in an adult population and in preconception carrier screening.[52,53] The pioneering work of our collective community may well lead the path to the use of genomic sequencing for the identification of patients with treatable genetic disorders at presymptomatic or early stages; however, further work on the best use of testing, optimal genomic interpretation, and linking to outcomes is recommended.

Glossary

WES	Whole exome sequencing

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Table I.
Comparison of DNA-based genetic testing modalities

Testing methods	Yield or advantages	Variants missed and disadvantages
Genome sequencing	"All" variants detectable in the genome, including exons, introns, intergenic sequences and regulatory regions CNV detection May be better for repetitive regions of the genome	Currently expensive, therefore, typically lower coverage per nucleotide More computational time for analysis Increased ambiguity in interpretation owing to a higher number of variants and poor understanding of noncoding sequences Does not detect methylation or epigenetic abnormalities Interpretation of many VUS can be challenging
WES	Variants in ~2% of the genome that is in the coding portion (exons) Also includes sequence surrounding exons for variants that change splicing	Does not detect CNVs routinely, methylation or epigenetic abnormalities, trinucleotide repeat expansions, or variants in genes with highly homologous pseudogenes Does not identify variants in introns or regulatory regions Up to 5% of targeted exons may not be well-covered with the current technology, leading to false-negative results Interpretation of many VUS can be challenging
Gene panel testing	All variants in the coding region and splice site junctions of the genes on the testing panel Sometimes offered with gene exon deletion or duplication testing	Is limited to identifying variants in the selected known disease associated genes Exon deletions and duplications are not typically detected, unless specifically included in the sequencing test
Gene Sanger sequencing	Can be targeted to nearly any gene in the genome Time efficient for single-gene diagnosis or familial variants Has been the traditional gold standard	Requires an index of suspicion for the involvement of a single gene Sequencing multiple genes by Sanger can be expensive
Gene deletion and duplication testing	Detects smaller deletions or duplications that may not be detected in a broad microarray (within gene exonic or multi-exonic deletions and duplications)	Does not detect single nucleotide variants Can be tested by MLPA; 1 or multiple genes can be tested by specific arrays. (deletion duplication testing)
Microarray *	Detects CNVs (larger deletions or duplications or the presence or absence of specific segments of DNA) (eg, DiGeorge or velocardiofacial deletion) May show regions of homozygosity, as with exome	Does not provide the gene sequence, so any small variant will not be detected Some chromosomal rearrangements or balanced translocations may be missed Rare or unique family mutations may be missed

CNV, Copy number variant; *MLPA*, multiplex ligation-dependent probe amplification; *VUS*, variants of unknown significance.

* First-tier test for intellectual disability, developmental delay, autism, or multiple congenital anomalies single nucleotide polymorphism microarray is preferred compared with CGH microarray for broad deletion and duplication testing. (Moeschler JB, Shevell M. Comprehensive evaluation of the child with intellectual disability or global developmental delays. Pediatrics. 2014;134(3):e903-e918. doi:10.1542/peds.2014-1839).

Table II.

Select genetic conditions with actionable management recommendations*

Categories	% of genes	Example disorders (associated genes)
Cardiovascular conditions	44.8	Brugada syndrome (*SCN5A*) Long QT/arrhythmia (*KCNQ1, KCNH2, SCN5A, RYR2*) Familial aortic aneurysms (*MYH11, ACTA2, MYLK*) Cardiomyopathies (*TMEM43, DSP, PKP2, DSG2, DSC2, LMNA, MYBPC3, MYH7, TPM1, PRKAG2, TNNI3, TNNT2, MYL3, MYL2, ACTC1*) Hypercholesterolemias (*APOB, LDLR, PCSK9*)
Cancer predisposition conditions	41.1	Adenomatous polyposis (*APC, MUTYH*) Familial breast-ovarian cancer (*BRCA1, BRCA2*) Li-Fraumeni syndrome (*TP53*) Lynch syndrome (*MLH1, MSH2, MSH6, PMS2*) Multiple endocrine neoplasias (*RET, MEN1*) Paragangliomas (*SDHD, SDHAF2, SDHC, SDHB*) Neurocutaneous conditions (*NF2, TSC1, TSC2, VHL*) Other (*STK11, PTEN, RB1, WT1*)
Connective tissue conditions with cardiovascular manifestations	10.5	Ehlers-Danlos type 4 (*COL3A1*) Marfan syndrome (*FBN1, TGFBR1*) Loeys-Dietz syndrome (*TGFBR1, TGFBR2, SMAD3*)
Lysosomal storage condition	1.8	Fabry disease (*GLA*)
Anesthesia Susceptibility condition	1.8	Malignant hyperthermia (*RYR1, CACNA1S*)

*American College of Medical Genetics and Genomics Recommendations - ClinVar - NCBI. http://www.ncbi.nlm.nih.gov/clinvar/docs/acmg/.

Table III.

Novel potentially treatable and actionable rare genetic conditions identified via exome sequencing

Authors	Year	Gene	Condition
Liu et al[37]	2014	*ADCK3*	Autosomal-recessive cerebellar ataxia associated with CoQ10 deficiency
Tarailo-Graovac et al[38]	2016	*CA5A*	Mitochondrial carbonic anhydrase VA deficiency
Fan et al[39]	2014	*GCH1*	Dopa-responsive dystonia
Tarailo-Graovac et al[38]	2016	*GOT2*	Mitochondrial glutamate oxaloacetate transaminase deficiency
Romberg et al[40]	2014	*NLRC4*	Enterocolitis and auto-inflammatory syndrome
Stray-Pedersen et al[41]	2014	*PGM3*	Congenital disorder of glycosylation with severe immunodeficiency and skeletal dysplasia
Imperatore et al[42]	2016	*RAPSN*	Congenital form of myasthenic syndrome
Flønes et al[43]	2016	*SLC19A3*	Biotin-responsive basal ganglia disease
Haack et al[44]	2014		
Kohrogi et al[45]	2015		
Foley et al[46]	2014	*SLC52A2*	Riboflavin-responsive childhood neuropathy
Wortheyetal[47]	2011	*XIAP*	X-linked inhibitor of apoptosis deficiency