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Exome report

Clinical utility of whole-exome sequencing in rare diseases: Galactosialidosis

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ABSTRACT

Rare genetic disorders can go undiagnosed for years as the entire spectrum of phenotypic variation is not well characterized given the reduced number of patients reported in the literature and the low frequency at which these occur. Moreover, the current paradigm for clinical diagnostics defines disease diagnosis by a specified spectrum of phenotypic findings; when such parameters are either missing, or other findings not usually observed are seen, the phenotype driven approach to diagnosis may result in a specific etiological diagnosis not even being considered within the differential diagnosis. The novel implementation of genomic sequencing approaches to investigate rare genetic disorders is allowing not only the discovery of new genes, but also the phenotypic expansion of known Mendelian genetic disorders. Here we report the detailed clinical assessment of a patient with a rare genetic disorder with undefined molecular diagnosis. We applied whole-exome sequencing to this patient and unaffected parents in order to identify the molecular cause of her disorder. We identified compound heterozygous mutations in the *CTSA* gene, responsible for causing galactosialidosis; the molecular diagnosis was further confirmed by biochemical studies. This report expands on the clinical spectrum of this rare lysosomal disorder and exemplifies how genomic approaches are further elucidating the characterization and understanding of genetic diseases.

1. Introduction

Thousands of single-gene, mitochondrial, and chromosomal disorders have been described. Often, patients who are followed in genetics clinics remain without a diagnosis for years. Application of whole-exome sequencing (WES) has enabled identification and characterization of new diseases and has expanded the phenotypes of known syndromes [Lupski et al., 2010; Ng et al., 2010a, 2010b]. However, WES technologies are under scrutiny due to implications of identifying unrelated conditions or findings of unknown clinical significance [Levenson 2012].

We describe a patient who was diagnosed with a Williams-like syndrome as a child based upon the aortic stenosis and facial features. Clinically-available testing was unrevealing. WES was consequently pursued through the research initiative of the Centers for Mendelian Genomics (http://www.mendelian.org). This approach identified two mutations in the cathepsin A gene, *CTSA*. Mutations in this gene are causative for late infantile galactosialidosis.

Galactosialidosis (MIM #256540) is a lysosomal storage disease caused by a deficiency of the enzyme protective protein cathepsin A (PPCA). This protein is required for the integrity of the lysosomal enzymes β–D-galactosidase and neuraminidase. A deficiency of cathepsin A, therefore, results in a combined phenotype of GM-1 gangliosidosis and sialidosis. Galactosialidosis has been divided into three phenotypic presentations based upon age of onset. Early infantile onset is characterized by hydrops fetalis, hepatosplenomegaly, coarse facial features, cardiomyopathy, a macular "cherry red spot", and dysostosis multiplex. Symptoms are evident

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early including prenatally or within the first 3 months of life with death occurring in the first few years of life. The late infantile form usually presents after 6 months of age, but within the first few years of life. The symptoms are similar to the early infantile form with hepatosplenomegaly, coarse facial features, cardiomyopathy, cherry red spot and dysostosis multiplex. Patient survival is variable from childhood to adulthood. The juvenile/adult form, in contrast, has a very different presentation with symptoms including ataxia, myoclonus, seizures, progressive intellectual disability, angiokeratomas, and dysostosis multiplex. The average age of onset is 16 years of age [d'Azzo et al., 2011; Lehman et al., 2012].

All three forms of galactosialidosis are considered extremely rare and there is no known prevalence. Out of all diagnosed cases, over 60% are considered to be the juvenile-adult type and the majority of affected individuals are of Japanese descent [d'Azzo et al., 2011; Lehman et al., 2012]. The pathophysiologic consequences of total deficiency of cathepsin A are still unclear, given that most phenotypic abnormalities characteristic of the disease have been attributed to the secondary, profound loss of neuraminidase activity [Lehman et al., 2012].

Application of WES approaches has been shown to be able to identify a molecular diagnosis in approximately 25%–30% of clinically unsolved cases [Dixon-Salazar et al., 2012]. Fewer than 15 patients have been reported in the literature with late infatile galactosialidosis [Zhou et al., 1996]. This case expands the phenotype of this type of galactosialidosis and illustrates the ability of whole exome sequencing to expand the phenotypic spectrum of known rare Mendelian disorders.

2. Methods

2.1. Participants

Proband, mother, and father provided written informed consent for themselves for enrollment into the Baylor-Hopkins Center for Mendelian Genomics genomic sequencing protocol. The study protocol was approved by the IRB of Baylor College of Medicine (BCM). Phenotype and other clinical data were annotated and stored using the recently-developed PhenoDB, a web-based tool for the collection and analysis of phenotypic features [Hamosh et al., 2013].

2.2. Exome sequencing

We performed trio exome sequencing of the proband and unaffected parents. We used the BCM Human Genome Sequencing Center (HGSC) Core exome for sequencing on the Illumina HiSeq platform. Variant calling from the aligned BAM files was performed using the ATLAS and SAMtools suites. Annotation and variant filtering was performed using the in-house developed SACBE annotation pipeline. Details of the approach have been described previously [Bainbridge et al., 2013; Lupski et al., 2013].

2.3. Mutation confirmation and segregation

Specific primers for the mutations in the *CTSA* gene were designed in order to PCR amplify the target regions containing the mutations and verify them by Sanger sequencing.

3. Results

3.1. Clinical description

We describe a 24-year-old female with coarse facial features, short stature, mild cognitive disability, mild conductive hearing loss, and aortic stenosis. Her mother's pregnancy history was uncomplicated with no exposures to alcohol, tobacco, or drugs. Her parents were healthy and unrelated. She was born at 40 weeks gestational age with a birth weight of 3 kg and length of 50 cm. Family history was negative for learning disabilities and congenital heart disease. Her previous genetic workup included a normal karyotype (46,XX), normal FISH for Williams syndrome, and a small (423.4 kb) paternally inherited microduplication of 15q13.3 detected by SNP microarray of unknown clinical significance.

On examination, her height was 149 cm (−2.2SD), weight was 45.7 kg (3rd centile), and head circumference was 53 cm (10th centile). The physical exam was remarkable for mildly coarse facial features, hypertelorism, short palpebral fissures, arched eyebrows, broad nasal tip, flat nasal bridge, macrostomia, prominent lips, widely spaced teeth, normally shaped and set ears, short and broad neck, mild pectus excavatum, short trunk, and kyphosis (Fig. 1). Eye examination was negative for corneal opacities or retinal abnormalities, including absence of a cherry red spot of the macula. She was tachycardic and had a grade I/VI systolic ejection murmur. There was no hepatosplenomegaly. Her gait was stable with no ataxia. Joints had a normal range of motion. She had generalized low muscle tone and no extrapyramidal signs or tremors. Skin was thick without additional abnormalities, including no angiokeratomas.

Her medical history was complicated by a recent onset of dyspnea after an episode of adenovirus pneumonia. Her pulmonary

Fig. 1. Facial characteristics include hypertelorism with a depressed nasal bridge, malar hypoplasia and a prominent pre-maxilla.