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CLINICAL REPORT

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Massive parallel sequencing of dried umbilical cord remnants

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Abstract

Genetic diagnosis depends on having available tissue to test. This can be important for many reasons, such as related to familial diagnosis in the case of another pregnancy. When blood or DNA samples from affected family members are not available, accurate prenatal diagnosis may be much more difficult and hence additional effort may be needed to obtain a genetic diagnosis in such families. We report two families with suspected monogenic disorders where attempts were made to establish the genetic etiology in deceased offspring using dried umbilical cord remnants which had been preserved by the family.

KEYWORDS

massive parallel sequencing, monogenic disorders, postmortem genetic testing

1 | INTRODUCTION

Monogenic disorders have an estimated prevalence of approximately 0.4% of live births (Chong et al., 2015). The development of massive parallel sequencing techniques has enabled more accurate and available genetic diagnosis of monogenic disorders. When the causative variant in a family is known, accurate prenatal diagnosis can be performed by Sanger sequencing of fetal DNA isolated from amniotic fluid or chorionic villus. Prenatal diagnosis is more challenging when the familial variant is not known. Additional effort may be necessary to obtain a genetic diagnosis of an index patient in monogenic disorders to enable prenatal diagnosis in subsequent pregnancies. We report two families with monogenic disorders in whom genetic testing was performed on dried umbilical cord remnants of affected members more than a year after their demise.

2 | CASE 1

A consanguineous couple (first cousins) were referred to our fetal clinic for preconception genetic counseling. Their first child had died at 11 months of age. The pregnancy had been uneventful. The male child had been evaluated at 8 months of age for developmental delay, hypotonia, and frequent respiratory infections. A clinical diagnosis of Pompe disease was made based on the presence of concentric left ventricular hypertrophy on echocardiogram, typical electrocardiographic changes of a short P-R interval and increased QRS voltage, as well as low alpha acid glucosidase activity on purified lymphocytes. However, molecular genetic testing had not been offered and the infant died at 11 months of age. There was no family history of cardiomyopathy or unexplained death in childhood. The family were referred to us 15 months after the death of the infant. In India, it is traditional in most families to store the dried umbilical cord remnants of newborn babies and the parents had stored the cord of the infant. The remnants were sent for exome sequencing.

3 | CASE 2

A nonconsanguineous couple were referred to our fetal clinic for preconception genetic counseling after two first trimester spontaneous abortions and the demise of their first-born child in early infancy. The infant was born premature at 31 weeks' gestation after a pregnancy complicated by pregnancy induced hypertension. The early neonatal period had been complicated by seizures that were difficult to control, abnormal liver enzymes, cholestasis, and coagulopathy. A liver biopsy had suggested a metabolic liver disease. However, a detailed report was not available with the parents. The baby had died at 2 months of age while being evaluated for an orthotopic liver transplantation. A genetic etiology for seizures and cholestasis had been suggested but

genetic testing was not performed for the baby and DNA isolation or storage had not been advised. The parents did not report any chronic medical illness or liver disorders in their family. The family were referred 13 months after the death of the infant. The parents again had the dried umbilical cord remnant of the baby which was sent for exome sequencing.

In both the cases, genomic DNA isolated from the dried umbilical cord was enzymatically fragmented and regions of interest were selectively enriched using capture probes targeted against coding regions of 4,100 genes of known clinical significance. Libraries were sequenced using an Illumina platform (Illumina Inc., San Diego, CA) at a mean depth of 80-100X. The Genome Analysis Toolkit (GATK) best practice framework was followed for variant identification. The Burrow Wheels Alignment (BWA) algorithm BWA-mem aligner was used to align the obtained sequences to the human reference genome (GRCh37/hg19). The identification and removal of duplicate as well as recalibration and re-alignment of reads based on indels were performed using inbuilt modules. The Haplotype caller module (Sentieon Inc., San Jose, CA) was used to identify the variants relevant to the patient phenotypes. The Deep Variant analysis pipeline on Google cloud platform was used as a secondary pipeline to call genetic variants. Varseq variant analysis software (GoldenHelix Inc., Bozeman, MT) was used for variant annotations as well as copy number variant analysis. This software provided access to published genomic databases including Online Mendelian Inheritance in Man (OMIM), the Genome Wide Association Studies (GWAS) catalog, 1000Genomes and ClinVar. The Mastermind database (Genomenon Inc., Ann Arbor, MI) was used to ascertain the relevant literatures related to the prioritized genetic variants of interest.

Exome sequencing in the Case 1 revealed a homozygous missense variant (c.1939 G>A, p. Asp645Asn) (Figure 1) in the *GAA* gene (NM_000152.4) responsible for Pompe disease (OMIM phenotype number #232300). This variant met criteria for pathogenic according to the American College of Medical Genetics and Genomics (ACMG) guidelines on variant interpretation(Richards et al., 2015). In Case 2 exome sequencing revealed a homozygous variant (c.1209 G>C, p. Arg403Ser) in the *RFT1* gene (NM_052859.3). Pathogenic variants in the *RFT1* gene cause a variant of Congenital Disorders of Glycosylation—CDG Type 1n (OMIM phenotype number # 612015), which leads to early onset epilepsy and hepatomegaly. Although cholestasis of infancy is a feature of a number of CDG types, the phenotype has not previously been reported in *RFT1* gene (Quelhas et al., 2019). The variant detected was found to be absent in the 1000 Genomes database (https://www.internationalgenome.org) and also in general South Asian population (based on the Lifecell internal database of 1,300 clinical exome samples). In-silico predictions from Sorting Intolerant from Tolerant (SIFT) and Polyphen suggested a deleterious effect. However, due to lack of scientific evidence in terms of functional studies or other data, this variant was classified as a variant uncertain significance according to the ACMG guidelines(Richards et al., 2015). Further segregation analysis revealed that the parents were each carriers for the same variant.

Advances in diagnosis and therapy have improved outcomes for a number of monogenic disorders. Enzyme replacement therapy has been shown to improve survival and cardiovascular morbidity in Pompe disease (Chen, Zhang, & Quan, 2017). However, such treatment is expensive and is often not realistic in developing countries. Prenatal diagnosis hence provides an opportunity for the parents to make informed decisions about the course of pregnancy. When the variant in the family is not known, the diagnostic options are limited. Sequencing of the DNA of both parents can help identify carrier status. Stals and colleagues performed exome sequencing of 50 couples

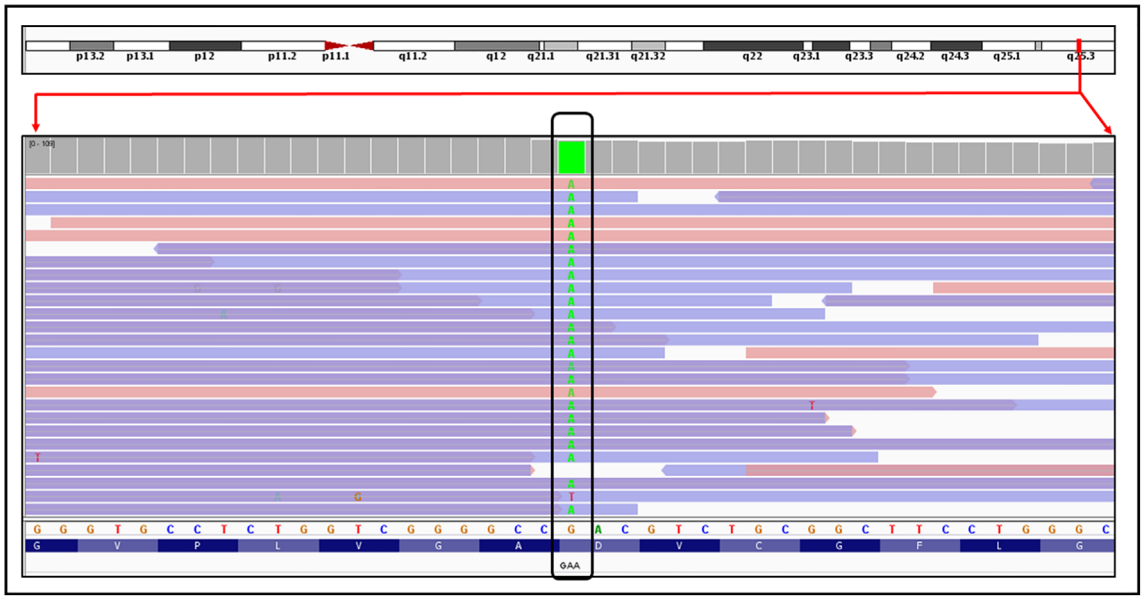

FIGURE 1 Integrative Genome Viewer (IGV) snapshot depicting the homozygous variant in the *GAA* gene. *Gene & Transcript*: GAA, NM_000152.4, *Variant*: c.1933G>A (p.Asp645Asn), Location: Exon 14, Zygosity: Homozygous [Color figure can be viewed at wileyonlinelibrary.com]

who had one or more children with a suspected disorder of autosomal recessive inheritance and identified a causative variant in more than 50% of cases (Stals et al., 2018). However, this approach increases the cost of testing. There are ethical concerns regarding identification of other potential disease-causing variants that are unrelated to the phenotype in question. Genetic analysis by fetal exome or genome sequencing, though available in some countries, is currently not recommended routinely outside the research setting (International Society for Prenatal, Society for, Fetal,, & Perinatal Quality, 2018) and is typically only applicable in disorders which result in structural abnormalities or other findings diagnosed by ultrasound imaging or other prenatal testing approaches. This approach is not usually possible in metabolic disorders. The technique of DNA extraction from remains after many years has been an integral part of forensic pathology (Evison, Smillie, & Chamberlain, 1997). The practice of preserving the umbilical cord of the newborn baby exists in a few Asian communities but this is rarely utilized for disease diagnosis. The quality of genomic DNA isolated from fresh umbilical cord has been found to be equivalent to that of peripheral venous blood in research settings (Rajatileka et al., 2013). Using a PCR based method, Kabra and colleagues isolated DNA from dried umbilical cord remnants to detect *SMN* gene deletions in a child suspected to have died of Spinal Muscular Atrophy (Kabra, Arora, Maria, & Aggarwal, 2003). Koyano and colleagues had shown evidence of congenital cytomegalovirus (CMV) infection in a 1-year old boy by isolating CMV DNA from his umbilical cord remnant (Koyano et al., 2004). However genetic testing of umbilical cord remnants by massive parallel sequencing in a clinical context has not been reported to the best of our knowledge. Our report confirms the feasibility of DNA extraction and massive parallel sequencing from dried umbilical cord remnants to evaluate for possible monogenic disorders in families with rare inherited disorders.

CONFLICT OF INTEREST

The authors report no conflict of interest with regard to this work.

AUTHOR CONTRIBUTIONS

Usha Nandhini Sennaiyan and Mani Ram Krishna were involved in the clinical management of both the patients, reviewed the literature and prepared the manuscript. Nagaraj M. Phani, Vuppu Deepak, and Giridharan Appaswamy were involved in the genetic testing and reporting process. All the authors have approved the final version of the manuscript. Mani Ram Krishna will act as the guarantor for the manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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