Clinica Chimica Acta 444 (2015) 50–53

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/clinchim

Case report

Application of whole exome sequencing to a rare inherited metabolic disease with neurological and gastrointestinal manifestations: A congenital disorder of glycosylation mimicking glycogen storage disease

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ARTICLE INFO

Article history:
Received 11 December 2014
Accepted 3 February 2015
Available online 11 February 2015

Keywords:
Congenital disorders of glycosylation
PMM2
Inherited metabolic diseases
Whole-exome sequencing
Genetic diagnosis

ABSTRACT

Background: Rare inherited metabolic diseases with neurological and gastrointestinal manifestations can be misdiagnosed as other diseases or remain as disorders with indeterminate etiologies. This study aims to provide evidence to recommend the utility of whole exome sequencing in clinical diagnosis of a rare inherited metabolic disease.

Methods and results: A 4-month-old female baby visited an outpatient clinic due to poor weight gain, repeated seizure-like episodes, developmental delay, and unexplained hepatomegaly with abnormal liver function test results. Although liver biopsy revealed moderate fibrosis with a suggested diagnosis of glycogen storage disease (GSD), no mutations were identified either by single gene approach for GSD (*G6PC* and *GAA*) or by next generation sequencing panels for GSD (including 21 genes). Whole exome sequencing of the patient revealed compound heterozygous mutations of *PMM2*: c.580C>T (p.Arg194*) and c.713G>C (p.Arg238Pro) which mutations were associated with congenital disorder of glycosylation Ia (CDG-Ia: PMM2-CDG).

Conclusions: We successfully applied exome sequencing to diagnose the first reported Korean patient with CDG-Ia, which was misdiagnosed as GSD. Whole exome sequencing may prove to be the preferred strategy for analysis of clinical features that do not readily suggest a specific diagnosis, such as those observed in inherited metabolic diseases, including CDG.

1. Introduction

Abnormal neurological manifestations accompanied by gastrointestinal manifestations in children are common features in a variety of inherited metabolic diseases. Some metabolic diseases may be misdiagnosed as another disease, or remain diagnosed as disorders with indeterminate etiologies. Confirmatory diagnoses can only be made by molecular genetic testing in many cases. Molecular testing of single genes would not be the best option for these individuals if there is no indication of which gene may be defective, and such an approach could lengthen the time to diagnosis, and is not cost effective [1]. One recent advancement in the clinical diagnostic laboratory is the use of next generation sequencing (NGS) technology with gene panel testing of multiple genes involved in particular disorders, or whole exome sequencing (WES) [1]. There have been reports on the clinical utility of WES for the detection of rare variants in patients with a phenotype suspected to be due to a Mendelian genetic disorder [2].

Here, we report a successful application of WES in a patient with a neurologic manifestation accompanied by gastrointestinal manifestations. The results of the patient's liver biopsy mimicked glycogen storage disease (GSD), but she was identified to have congenital disorder of glycosylation type Ia (PMM2-CDG; CDG-Ia). CDG-Ia is here reported for the first time in Korea, and this study provides further evidence to recommend the clinical utility of WES in diagnosing patients with rare inherited metabolic diseases.

Abbreviations: ALT, alanine transaminase; AST, aspartate transaminase; CDG, congenital disorder of glycosylation; GSD, glycogen storage disease; IEF, isoelectric focusing; LFT, liver function tests; MS, mass spectrometry; NGS, next generation sequencing; PAS, Periodic acid–Schiff; PMM, phosphomannomutase; WES, whole exome sequencing.

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http://dx.doi.org/10.1016/j.cca.2015.02.008
0009-8981/

2. Case

A four-month-old female baby visited an outpatient clinic due to poor weight gain, repeated seizure-like episodes, and developmental delay. She was the third baby born to non-consanguineous Korean parents, at term. She had two older brothers; the oldest brother had cerebral palsy and died of pneumonia in early childhood. The patient had no facial dysmorphism or other phenotypic abnormalities at birth. At two months of age, she received supportive care for failure to thrive (weight plot below the 3rd percentile for age), until her body weight reached within the 3rd to 10th percentile group. At four months of age, a diagnostic work-up for repeated seizure-like episodes was performed. On physical examination, she had hepatomegaly, with a 4-5 cm palpable liver with sharp edges of soft consistency. Laboratory studies at that time showed a Complete Blood Count (hemoglobin–white blood cells–platelets) of 10.7 g/dL–10,670/mm^3–532,000/mm^3. Abnormal liver function tests (LFT) were observed, with aspartate transaminase (AST) and alanine transaminase (ALT) levels of 202 IU/L (reference range, 0–37 IU/L) and 246 IU/L (reference range, 0–41 IU/L), respectively. Her blood glucose level was 110 mg/dL (reference range, 70–110 mg/dL). Other biochemical tests including blood urea nitrogen, serum creatinine, total protein, and serum albumin were not remarkable. Prothrombin time was not markedly elevated (1.10 INR, reference range 0.90–1.10 INR). Viral studies including hepatitis A, B, and C virus and TORCH infections [Toxoplasmosis, others (syphilis, varicella-zoster, parvovirus B19), Rubella, Cytomegalovirus, and Herpes infections] were normal. Ceruloplasmin level was 7.9 mg/dL, with a follow-up result of 11.5 mg/dL (reference range: 14.0–41.0 mg/dL). Her brain magnetic resonance imaging revealed a small cerebellum with cistern magna. Video electroencephalography examination showed no epileptic-form discharges. During a 5-month follow-up period, her AST/ALT levels fluctuated between 253/331 IU/L and 644/669 IU/L. *ATP7B* mutation analysis for Wilson disease using the Sanger sequencing method was performed at the age of 8 months, but no pathogenic mutations were identified. Liver biopsy was performed at 9 months of age due to unexplained LFT abnormalities. The biopsy results seemed to be compatible with GSD with crovascular fatty change (18%) and stage 3 moderate fibrosis (portal-to-portal bridging fibrosis) as per the grading system proposed by the International Association for the Study of the Liver [3]. Periodic acid–Schiff (PAS) stain was positive with ballooning hepatocytes (because of glycogen), while PAS stain used in combination with diastase, an enzyme that breaks down glycogen, was negative, indicating the presence of glycogen in the specimen. Based on the histologic suspicion of GSD, molecular investigation by direct sequencing was performed for *G6PC* (von Gierke disease, GSD type 1a) and *GAA* (Pompe disease, GSD type 2). However, no pathogenic mutations were identified in those two genes. The patient was placed on an uncooked corn starch diet when she was 18 months old, and her AST/ALT levels normalized to 47/44 IU/L after a 6-month treatment period. At age 4, her height plot was at the 25th percentile, but weight plot was still at the 3rd percentile for age.

NGS with exome sequencing with GSD panels was performed using a Wizard Genomic DNA purification kit according to the manufacturer's instructions (Promega, Madison, WI, USA). GSD panels included 21 causative genes for different types of GSDs as per the OMIM database (*GYS1* and *GYS2* for GSD 0, *G6PC* for GSD Ia, *SLC37A4* for GSD Ib, *GAA* for GSD II, *AGL* for GSD III, *GBE1* for GSD IV, *PYGM* for GSD V, *PYGL* for GSD VI, *PFKM* for GSD VII, *PHKA2* for GSD IXa, *PHKB* for GSD IXb, *PHKG2* for GSD IXc, *PHKA1* for GSD IXd, *PHKG1* for GSD IXe, *PGAM2* for GSD X, *LDHA* for GSD XI, *ALDOA* for GSD XII, *ENO3* for GSD XIII, *PGM1* for GSD XIV, and *GYG1* for GSD XV). Exonic sequences were enriched in the DNA sample using SureSelect Target Enrichment kit (Agilent Technologies, Santa Clara, CA, USA). Sequences were determined by HiSeq2000 (Illumina, San Diego, CA, USA) and 150–200 bp were read paired-end. The patient's variants passing quality filters were filtered against public databases [National Heart, Lung, and Blood Institute Exome Sequencing Project; 1000 Genomes Project; dbSNP] for global minor allele frequency < 1.0%. Protein-altering variants were then selected. Because the patient had no family history of disease, the possibility of compound heterozygous alleles of variants related to a disease listed in the OMIM database was posited. The resulting sequence was analyzed for single nucleotide variants and small insertions and deletions (indels) differing from the reference genome (Human genome 19, hg 19). The variants derived from the variant filtering strategy were then prioritized based on their likelihood to affect protein function using public algorithms such as SIFT, and/or to totally or partially match the patient's phenotype.

Because no pathogenic mutations were identified in NGS for GSD, whole exome sequencing was performed for accurate identification of the genetic cause of the patient's disease. WES from the proband revealed only 2 heterozygous variants of *PMM2* genes (NM_000303.2): c.580C>T (p.Arg194*) and c.713G>C (p.Arg238Pro) which were confirmed by Sanger sequencing. A familial study was performed with targeted mutation analysis for two *PMM2* variants. The patient's mother was a heterozygous carrier for c.713G>C while the patient's father and the surviving older brother were heterozygous carriers for c.580C>T (Fig. 1). *PMM2* is a causative gene for CDG-Ia, and *PMM2* mutation leads to an abnormal glycoform of transferrin. Phosphomannomutase (PMM) 2 enzyme deficiency induces an early disruption of the N-glycan assembly and transfer of glycoproteins. We performed a carbohydrate-deficient transferrin assay using capillary electrophoresis (Sebia, Every, France) according to the manufacturer's instructions and it revealed an increase in asialotransferrin and disialotransferrin (30.8%, reference range: <1.3%), which is an abnormal pattern observed in patients with CDG-I, due to defects in the assembly of dolichol-lipid linked oligosaccharides.

3. Discussion

CDG is a rapidly growing family of genetic diseases of over 60 different disorders with the majority of defects residing within the N-linked glycosylation biosynthesis pathway [4]. This post-translational modification is essential for the proper functioning of many systems within the body and is characterized by multi-organ dysfunction with significant morbidity and mortality [4]. Clinical features are shared across many CDGs, and include developmental delay, seizures, intellectual disability, hypotonia, and microcephaly [5]. The overlapping symptoms make it difficult to determine the specific genetic defect based on phenotype alone [1]. Liver is a commonly affected organ in various CDG types (CDG-Ia, -Ib, -Ih, -Ii, -Ik, -IL, -IIe, -IIg, and -IIh) [4].

The most commonly defective gene by far in CDGs is *PMM2*, with which more than 700 individuals have been identified [5]. Defects in the enzyme PMM2, stemming from *PMM2* mutation, lead to an early disruption of the N-glycan assembly of glycoproteins, resulting in CDG-Ia [4]. The phenotypic spectrum of CDG-Ia is broad. The nervous system is affected in all patients, and most other organs are involved in a variable way [5].

Diagnosis of CDG in Western countries usually begins with screening tests such as transferrin isoelectric focusing (IEF) or capillary electrophoresis. The predominant glycoform of transferrin contains four oligosaccharide branches (tetrasialotransferrin) on two N-linked glycans, while hypoglycosylation of transferrin results in partial deficiency of terminal glycosylation, and an increase in a- and disialotransferrin glycoforms with decreased tetrasialotransferrin is observed in patients with abnormal glycosylation [4]. However, a secondary CDG (mainly galactosemia or hereditary fructose intolerance), or genetic abnormalities that lead to amino acid changes in transferrin other than *PMM2*, may alter the charge of the protein, leading to cathodal shifts which cause false-positive results in transferrin tests [5]. PMM enzyme activity in fibroblasts and leukocytes also can be used to identify a PMM deficiency responsible for CDG-Ia. However, overlap with values in healthy controls or carriers has been reported [6]. Moreover, those biochemical