European Journal of Medical Genetics 65 (2022) 104518

Contents lists available at ScienceDirect

European Journal of Medical Genetics

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

Glycogen Storage Disease type IA refractory to cornstarch: Can next generation sequencing offer a solution?

Or Steg Saban [a,k], Ben Pode-Shakked [b,c,h,k], Bassam Abu-Libdeh [d], Maya Granot [g,k], Galia Barkai [a,f,k], Yael Haberman [c,g,h,k], Inon Roterman [g,k], Avishay Lahad [g,k], Dror S. Shouval [g,k], Batia Weiss [g,k], Dina Marek-Yagel [b,k], Ortal Barel [l,m], Nurit Loberman-Nachum [g,k], Smadar Abraham [b], Raz Somech [e,i,k], David A. Weinstein [j], Yair Anikster [b,k,m,*]

[a] Pediatrics Department B, Edmond and Lily Safra Children's Hospital, Sheba Medical Center, Tel-Hashomer, Israel
[b] Metabolic Disease Unit, Edmond and Lily Safra Children's Hospital, Sheba Medical Center, Tel-Hashomer, Israel
[c] The Talpiot Medical Leadership Program, Sheba Medical Center, Tel-Hashomer, Israel
[d] Departments of Pediatrics and Genetics, Makassed Hospital and Al-Quds Medical School, East Jerusalem, Palestine
[e] Pediatrics Department A, Edmond and Lily Safra Children's Hospital, Sheba Medical Center, Tel-Hashomer, Israel
[f] Pediatric Infectious Disease Unit, Edmond and Lily Safra Children's Hospital, Sheba Medical Center, Tel-Hashomer, Israel
[g] Division of Pediatric Gastroenterology, Hepatology and Nutrition, Edmond and Lily Safra Children's Hospital, Sheba Medical Center, Tel-Hashomer, Israel
[h] Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA
[i] Pediatric Immunology Service, Jeffrey Modell Foundation Center, Edmond and Lily Safra Children's Hospital, Sheba Medical Center, Tel-Hashomer, Israel
[j] Glycogen Storage Disease Program, University of Connecticut School of Medicine (Farmington, CT), Connecticut Children's Medical Center, Hartford, CT, USA
[k] Sackler Faculty of Medicine, Tel-Aviv University, Tel-Aviv, Israel
[l] The Genomic Unit, Sheba Cancer Research Center, Sheba Medical Center, Tel-Hashomer, Israel
[m] The Wohl Institute for Translational Medicine, Sheba Medical Center, Tel-Hashomer, Israel

ARTICLE INFO

Keywords:
Glycogen storage disease
Exome sequencing
Sucrase isomaltase deficiency
Uncooked cornstarch

ABSTRACT

Avoidance of fasting and regular ingestion of uncooked-cornstarch have long been the mainstay dietary treatment of Glycogen Storage Disease type Ia (GSD-Ia). However, GSD-Ia patients who despite optimal dietary treatment show poor glycemic control and are intolerant to cornstarch, present a complex clinical challenge. We pursued Whole Exome Sequencing (WES) in three such unrelated patients, to both confirm a molecular diagnosis of GSD-Ia, and seek additional variants in other genes (e.g. genes associated with amylase production) which may explain their persistent symptoms. WES confirmed the GSD-Ia diagnosis, with all three probands harboring the homozygous p.R83C variant in *G6PC*. While no other significant variants were identified for patients A and B, a homozygous p.G276V variant in the *SI* gene was detected in patient C, establishing the dual-diagnosis of GSD-Ia and Sucrase-Isomaltase Deficiency. To conclude, we suggest that WES should be considered in GSD-Ia patients who show persistent symptoms despite optimal dietary management.

1. Introduction

Glycogen Storage Disease (GSD) Type I, also known as Von Gierke disease, is a group of autosomal recessive disorders with an overall incidence of approximately 1:100,000. The disease is caused by a defect in the Glucose-6-Phosphatase Catalytic unit (G6PC). G6PC is a key enzyme for the maintenance of glucose homeostasis between meals, catalyzing the hydrolysis of glucose-6-phosphate (G6P) to glucose and phosphate in the terminal step of gluconeogenesis and glycogenolysis. GSD-Ia patients are unable to maintain glucose homeostasis. As in other hepatic glycogenoses, the disease hallmark manifestation is hypoglycemia following a short fast.

The prognosis of GSD Type Ia has dramatically improved since the introduction of nocturnal nasogastric infusion of a high glucose formula

* Corresponding author. Metabolic Disease Unit, Edmond and Lily Safra Children's Hospital Sheba Medical Center, Tel-Hashomer, Israel Sackler Faculty of Medicine, Tel-Aviv University, Tel-Aviv, Israel.
E-mail address: Yair.Anikster@sheba.health.gov.il (Y. Anikster).

https://doi.org/10.1016/j.ejmg.2022.104518
Received 7 July 2021; Received in revised form 18 March 2022; Accepted 1 May 2022
Available online 9 May 2022

in 1976 in addition to usual frequent meals during daytime (Greene et al., 1976). By constantly maintaining a nearly normal level of blood glucose, this treatment causes a remarkable decrease, although not normalization, of blood lactate, urate and triglyceride levels, as well as bleeding time values. A second major improvement in the treatment, introduced in 1984 (Chen et al., 1984), was the use of uncooked starch in the regimen. This allows maintenance of a normal blood glucose level for as long as four to 6 h. In 2007 (Bhattacharya et al., 2007; Correia et al., 2008), an extended release cornstarch preparation was presented, for maintaining glucose concentrations during the overnight period. The preparation, Glycosade, was approved in the United Kingdom and in the United States in 2009 and 2012, respectively (Ross et al., 2016), and allows 7–10 h of coverage without sacrificing metabolic control (Dambska et al., 2017). Under this combined treatment, glycemic control is achieved, and to a great extent the multisystem complications of the disease are expected to be avoided (Dambska et al., 2017). Indeed, for most GSD-Ia patients, adherence to this dietary regimen achieves good glycemic control. However, occasional patients for whom uncooked cornstarch is not tolerated or otherwise does not achieve glycemic control, present a unique and complex challenge in the management of GSD.

2. Patients and methods

2.1. Patient recruitment

At the Metabolic Disease Unit of the Edmond and Lily Safra Children's Hospital, Sheba Medical Center, we have encountered three unrelated patients with a clinical diagnosis of GSD-Ia who are refractory to uncooked cornstarch. The patients were treated with the same strict diet that is customary and described above, but persistently showed poor glycemic control, as well as resistance to different therapeutic interventions, including dietary alternatives to cornstarch. The dietary treatment was tailored and planned by metabolic disease specialists and dedicated certified dieticians highly experienced with the management of pediatric and adult patients with GSDs and other inborn errors of metabolism. As each of these patients experienced persistent hypoglycemic episodes with or without diarrhea, despite optimal treatment as described above, we sought to pursue Whole Exome Sequencing (WES) for the patients and their parents (trio exome). This, in order to both confirm the clinical suspicion of GSD-Ia, and to try and unveil any other potential genetic contributors to the complex phenotype.

Written, informed consent for genetic analysis was obtained from the patients or their legal guardians. Approval for human subject research was obtained from the Institutional Review Board of the Sheba Medical Center.

2.2. Case presentations

Patient A is a 31 years-old male, born to non-consanguineous parents of Ashkenazi-Jewish descent. He showed difficulties to maintain good metabolic control since infancy. In addition, he has chronic diarrhea. He was treated with uncooked cornstarch as usual, however hypoglycemic events occurred regularly. He was also treated with Glycosade, the aforementioned extended release cornstarch preparation, with no improvement. The patient experiences multi-systemic complications due to his unbalanced disease, including hyperlipidemia, hypertriglyceridemia, proteinuria, osteoporosis, chronic anemia, hyperuricemia and hepatic adenomas. One adenoma was resected from the left lobe of his liver. As a part of his evaluation at the Gastrointestinal Unit, he had starch tolerance test that showed intolerance to cornstarch. Duodenal biopsies were considered normal. After cornstarch treatment was halted, diarrhea stopped as anticipated. He was treated with other dietary alternatives to cornstarch, including mazza-flour and Tapioca, with no change in his metabolic control. To date, the only way to maintain his glucose levels within the desired range during night time, is a continuous drip of Dextrose10% PG.

Patient B is an 11 years-old girl, born to non-consanguineous parents of Ashkenazi-Jewish descent. She was followed by the Glycogen Storage Disease Program team at the University of Florida (UF Health), under a working diagnosis of GSD-I. However, similar to Patient A, despite optimal adherence to dietary treatment, she shows intolerance to uncooked cornstarch, with poor glycemic control and subsequent multisystemic complications of GSD-I, proving a challenge in her routine care.

Patient C is an 18 months-old boy, born to highly consanguineous parents of Arab-Muslim descent. He was admitted from another hospital for evaluation due to poor glycemic control and chronic diarrhea. He was managed as is usually done in GSD-Ia patients, gastrostomy feeding tube was inserted and cornstarch was added to his meals. During his hospitalization, despite full adherence with the strict dietary regimen, he experienced recurrent events of hypoglycemia 30–40 min after every meal. In order to avoid the hypoglycemia, he was connected to a maintenance amount of continuous intravenous fluids with 10% Dextrose. All trials to wean him off IV fluids failed, and he showed lactic acidosis, hyperlipidemia and hypertriglyceridemia. In addition, the patient exhibited severe failure to thrive (FTT) and chronic diarrhea, which was reported to have started at the age of 6 months. Before this age, he was fed with breast milk only, and his stools were reportedly normal. At 6 months, regular infant formula was added to breast milk, and at 9 months breastfeeding was stopped. A large variety of formulas were tried, but no improvement was noticed-these included an extensively hydrolyzed formula, hypoallergenic formula with 55% MCT, amino acid-based formula, and 84% MCT. None of the above helped. Diarrhea continued, and the patient spent his life up to 18 months in the hospital as weaning him off IV fluids was not achievable. He underwent a thorough clinical assessment, including exhaustive laboratory tests (CBC, ESR, CRP, Celiac serology, thyroid function tests, metabolic screening tests: urine organic acids profile, plasma free carnitine, as well as infectious evaluation in stool: *Salmonella*, *Shigella*, *Campylobacter Pylori*, *Yersinia enterocolitica*, and *E. Coli*)- which were all normal. The diarrhea was characterized as watery (Thiagarajah et al., 2018), and was noted to be diet-induced. Endoscopies were done and biopsies were considered normal with no evidence of inflammation. The patient was then fed with Galactomin-19 (formula containing minimal levels of lactose, galactose and glucose), with cessation of his diarrhea. Based on his response to Galactomin-19, two possible disorders of malabsorption were considered in the differential diagnosis, as co-morbid conditions to his suspected GSD-Ia: Glucose Galactose Malabsorption and Sucrase Isomaltase Deficiency (Haberman et al., 2017). To rule out the former, glucose 10% solution was infused to his gastrostomy. With this infusion, the patient showed normal glucose levels, and there were no events of hypoglycemia. However, none of the formulas that were infused PG (including uncooked cornstarch) managed to maintain glycemic control. Thus, the child was suspected to have Sucrase-Isomaltase Deficiency.

2.3. Whole exome sequencing

For Patient A and B, Whole exome sequencing (WES), variant detection, and filtering were performed at the Genomic Unit, Sheba Cancer Research Center, Sheba Medical Center, as previously described (Tirosh et al., 2019). Mutation calling was performed by a team of clinician scientists, who had knowledge of the clinical phenotypes and pedigree structure, as well as genetic expertise in exome evaluation. Exome data were interpreted according to the American College of Genetic and Genomic Medicine (ACMG) guidelines (Richards et al., 2015), and pathogenicity of the variants was also evaluated using VarSome (Kopanos et al., 2019).

3. Results

For all three unrelated probands, trio-WES yielded a shared homozygous p.R83C variant in the *G6PC* gene, previously reported and

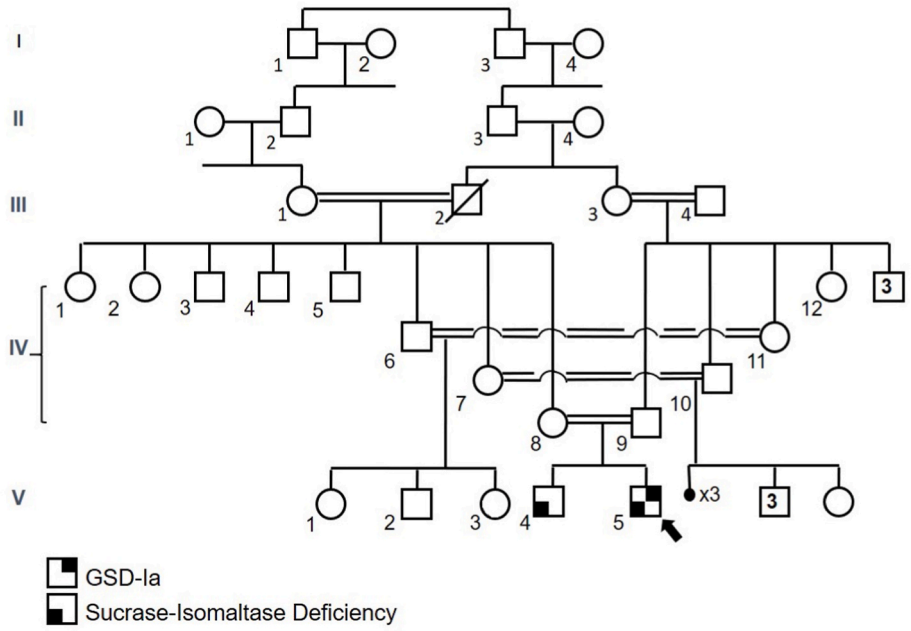

Fig. 1. Pedigree of family C. The index patient (arrow) was revealed by WES to harbor homozygous pathogenic variants in both the *G6PC* gene and *SI* gene. His brother (V:4) was found to be homozygous for the *SI* variant, while their parents were found to be heterozygous carriers of both variants.

considered a known and common pathogenic variant in the Ashkenazi-Jewish population, as well as in GSD-Ia patients of other ethnic origins. This served to confirm a molecular diagnosis of GSD-Ia in all three patients. However, for patients A and B, no other pathogenic variants were identified in other genes, and the explanation of their intolerance to cornstarch is unfortunately still elusive.

In contrast, for Patient C, who had previously undergone Trio WES, a revision of the WES data interpretation was pursued, with a specific question of pathogenic variants in genes associated with malabsorption. Upon re-analysis, the patient was found to be homozygous also for a c.827G > T, p.(G276V) mutation in the Sucrase Isomaltase (*SI*) gene (NM_001041.4), thus establishing a second diagnosis of Sucrase-Isomaltase deficiency in the proband (Fig. 1). Subsequently, targeted treatment with enzyme replacement therapy (ERT) with sacrosidase was initiated, and after spending most of his life in hospitals, the patient was able to be discharged home, and began gaining weight. Interestingly, his older brother, who was at first reported as healthy, was later reported by the parents to have intermittent diarrhea from infancy, without further manifestations of GSD. Following the revision of the proband WES data, the older brother was also tested for both pathogenic variants and was found to harbor the *SI* variant, thus confirming him to also have SI deficiency.

4. Discussion

The past few decades have seen significant advancements made in the efficiency and clinical utility of Next Generation Sequencing (NGS) technology, including targeted gene panel sequencing, whole exome- and whole genome sequencing (WES, WGS). Along with the continued decrease in their associated costs, these NGS methods brought a revolution to clinical pediatrics at large, and specifically to pediatric gastroenterology (Ki, 2021). This has enabled elucidation of the molecular basis of a myriad of genetic disorders with gastrointestinal involvement affecting infants and children, and allows to increase the diagnostic yield and shorten the diagnostic odyssey for many families.

In our study, we pursued research-based whole exome sequencing (WES) in three unrelated families, in which the proband presented with a clinical diagnosis of GSD, most consistent with GSD-Ia, for whom the well-established dietary management based primarily on uncooked cornstarch, was not tolerated and thus failed to achieve glycemic control. For specialists well-experienced with the management of patients with GSD and inborn errors of metabolism, these patients constituted the most complex and difficult-to-treat cases, challenging the treatment guidelines. Indeed, the inability to achieve glycemic control in these three patients brought severe multi-systemic complications, as well as significant impairment of their quality of life.

The role of NGS in the diagnostic evaluation of patients with glycogen storage disorders is growingly recognized (Beyzaei et al., 2020), first and foremost due to the potentially overlapping phenotypic features of GSD-Ia, other hepatic glycogenoses, related IEMs and other conditions mimicking GSD, which often hamper the ability to reach a definitive diagnosis based on clinical, laboratory and imaging findings alone.

The striking finding of an additional pathogenic variant in the *SI* gene in patient C in our study, establishing a second diagnosis of Sucrase-Isomaltase Deficiency, not only brought to a targeted therapeutic intervention which benefited the patient, but enabled genetic counselling and identification of additional affected and at-risk individuals in his family. Furthermore, our experience with this clinical scenario underscores the importance of considering the possibility of more than a single genetic disorder in a patient or family. This is especially true for patients born to consanguineous parents. As discussed by Kurolap et al. (2016), clinicians are often guided by a single disorder paradigm, but sometimes the patient's diagnosis does not explain all of the clinical findings. With the increased use of NGS in the clinical setting, such cases of dual diagnosis are not an unheard-of phenomenon (Kurolap et al., 2016). We suggest that WES or WGS should be considered in such cases, even more so than gene panel sequencing, and especially in consanguineous kindreds.

In conclusion, the families reported herein underscore some of the benefits of next generation sequencing (NGS) in the evaluation of complex patients with GSD. We suggest that in selected patients, if the initial diagnosis does not explain the whole phenotype, NGS might reveal an alternative or even a second diagnosis, and especially in consanguineous families.

Source of funding

No funding to acknowledge.

Authors' contributions

Conceptualization- O.S.S., B.P.-S., Y.A.; Data curation- O.S.S., B.P.-S., B.A.-L., M.G., G.B., Y.H., I.R., A.L., D.S.S., B.W., D.M.-Y., O.B., N.L.-N., S. A., R.S., D.A.W., Y.A.; Formal analysis- D.M.-Y., O.B.; Supervision- Y.A.; Roles/Writing - original draft- O.S.S., B.P.-S., Y.A.; Writing - review & editing- O.S.S., B.P.-S., Y.A.. Finally, all authors critically read and approved the final version of the manuscript.

Declaration of competing interest

The authors declare no conflicts of interest.

Acknowledgements

The authors wish to thank the patients and their families for their kind cooperation, as well as Prof. Orly Elpeleg, Department of Genetics, Hadassah Medical Center, for her kind assistance with Whole Exome Sequencing of family C.

References

Beyzaei, Z., Geramizadeh, B., Karimzadeh, S., 2020. Diagnosis of hepatic glycogen storage disease patients with overlapping clinical symptoms by massively parallel sequencing: a systematic review of literature. Orphanet J. Rare Dis. 15 (1), 286.

Bhattacharya, K., Orton, R.C., Qi, H., et al., 2007. A novel starch for the treatment of glycogen storage diseases. J. Inherit. Metab. Dis. 30, 350–357.

Chen, Y.T., Cornblath, M., Sidbury, J.B., 1984. Cornstarch therapy in type I glycogen-storage disease. N. Engl. J. Med. 310, 171–175.

Correia, C.E., Bhattacharya, K., Lee, P.J., et al., 2008. Use of modified cornstarch therapy to extend fasting in glycogen storage disease types Ia and Ib. Am. J. Clin. Nutr. 88, 1272–1276.

Dambska, M., Labrador, E., Kuo, C.L., Weinstein, D.A., 2017. Prevention of complications in glycogen storage disease type Ia with optimization of metabolic control. Pediatr. Diabetes 18, 327–331.

Greene, H.L., Slonim, A.E., O'Neill Jr., J.A., Burr, I.M., 1976. Continuous nocturnal intragastric feeding for management of type 1 glycogen-storage disease. N. Engl. J. Med. 294, 423–425.

Haberman, Y., Di Segni, A., Loberman-Nachum, N., et al., 2017. Congenital Sucrase-Isomaltase Deficiency: a novel compound heterozygous mutation causing aberrant protein localization. J. Pediatr. Gastroenterol. Nutr. 64 (5), 770–776.

Ki, C.S., 2021. Recent advances in the clinical application of next-generation sequencing. Pediatr. Gastroenterol. Hepatol. Nurt. 24 (1), 1–6.

Kopanos, C., Tsiolkas, V., Kouris, A., et al., 2019. VarSome: the human genomic variant search engine. Bioinformatics 35 (11), 1978–1980.

Kurolap, A., Orenstein, N., Kedar, I., et al., 2016. Is one diagnosis the whole story? Patients with double diagnoses. Am. J. Med. Genet. 170, 2338–2348.

Richards, S., Aziz, N., Bale, S., et al., 2015. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of medical genetics and genomics and the association for molecular pathology. Genet. Med. 17 (5), 405–424.

Ross, K.M., Brown, L.M., Corrado, M.M., et al., 2016. Safety and efficacy of chronic extended release cornstarch therapy for glycogen storage disease type I. JIMD Rep. 26, 85–90.

Thiagarajah, J.R., Kamin, D.S., Acra, S., et al., 2018. Advances in evaluation of chronic diarrhea in infants. Gastroenterology 154 (8), 2045–2059.

Tirosh, I., Spielman, S., Barel, O., et al., 2019. Whole exome sequencing in childhood-onset lupus frequently detects single gene etiologies. Pediatr. Rheumatol. Online J. 17 (1), 52.