The Application of Clinical Genetics

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ORIGINAL RESEARCH

Effect of Genetic Testing on Diagnosing Gastrointestinal Pediatric Patients with Previously Undiagnosed Diseases

This article was published in the following Dove Press journal:
The Application of Clinical Genetics

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Purpose: Four consanguineous Jordanian families with affected members of unknown gastrointestinal related diseases were recruited to assess the utility and efficiency of whole exome sequencing (WES) in reaching the definitive diagnosis.

Patients and Methods: Members from four consanguineous Jordanian families were recruited in this study. Laboratory and imaging tests were used for initial diagnosis, followed by performing WES to test all affected members for the detection of causative variants. Sanger sequencing was used for validation.

Results: We had a 100% success rate identifying each case presented in this study.

Conclusion: This is the first study applying a WES testing approach in the diagnosis of pediatric diseases in Jordan. Our results strongly suggest the need to implement WES as an evident diagnostic tool in the clinical setting, as it will subsequently allow for proper disease management and genetic counseling.

Keywords: pediatric diseases, whole exome sequencing, gastrointestinal diseases, metabolic diseases

Introduction

Pediatric diseases of genetic origin are a group of broad, overlapping conditions that often involve multiple organs. Due to the overlapping symptoms, many children remain undiagnosed and face repeated admissions and prolonged hospital stay.[1,2] Although many of those diseases are associated with poor prognosis and even premature deaths, some are compatible with a good quality of life if they are diagnosed early and offered optimal management.[3] Diagnostic approaches have often involved standard laboratory, imaging studies, and even more sophisticated interventions that can be invasive, time consuming, and costly. Hence, the need to diagnose such disorders at the molecular level seems most promising. The emergence of whole exome sequencing (WES) gave a momentum chance to look at difficult to diagnose diseases from a different perspective through an individual's genetic makeup. The first successful use of WES in medical diagnosis was through the identification of the causative variant responsible for a rare form of inflammatory bowel disease in an infant.[4] This allowed the extensive use of WES in diagnostic settings where it has proved its success.[5–7]

In this study, members from four consanguineous Jordanian families were recruited to test the utility of WES in detecting the causative genetic variant responsible for each case.

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DovePress
http://doi.org/10.2147/TACG.S275992

Patients and Methods

Subject Recruitment and Clinical Evaluation Phase

This study was approved by the institutional review board (IRB) of the Faculty of Medicine and the Research Committee at Jordan University of Science and Technology (JUST), in accordance with the recommendations of the declaration of Helsinki. Written informed consent was obtained from each participant (the legal guardian in the case of the children). Four consanguineous families with children having rare undiagnosed gastrointestinal related diseases (Figure 1) were recruited into this study after rigorous evaluation and consultation between a specialized pediatric gastroenterologist and geneticist. Sample collection took place at the pediatric clinics in King Abdullah University Hospital, Irbid, Jordan. Clinical data and history details were collected, followed by a thorough clinical examination. Standard laboratory testing in addition to any required imaging studies took place for each participant family.

Sample Collection and DNA Testing Phase

Blood samples were collected from affected individuals and their unaffected relatives in the family in EDTA tubes. The DNA was isolated using the QIAprep Spin Mini-prep Kit according to the manufacturer's instructions. Genomic purity and quality were assessed using nanodrop and gel electrophoresis prior to performing molecular testing.

Whole Exome Sequencing and Analysis

DNA preparation for WES, variant detection, filtration, validation, and segregation analysis were performed as described by Azab et al.[8]

Results

Available clinical findings of the proband patients recruited in this study are available in Table 1. Identified pathogenic variants for each family can be found in Table 2.

Clinical Assessment and Variant Detection in Family F1

Family F1 had a reported family history of neurodevelopmental delay, kidney problems, and early neonatal deaths. The 13-year-old girl (proband IV-3), labeled as a case of renal tubular acidosis and thalassemia intermedia, was referred due to repetitive bouts of vomiting, hematemesis,

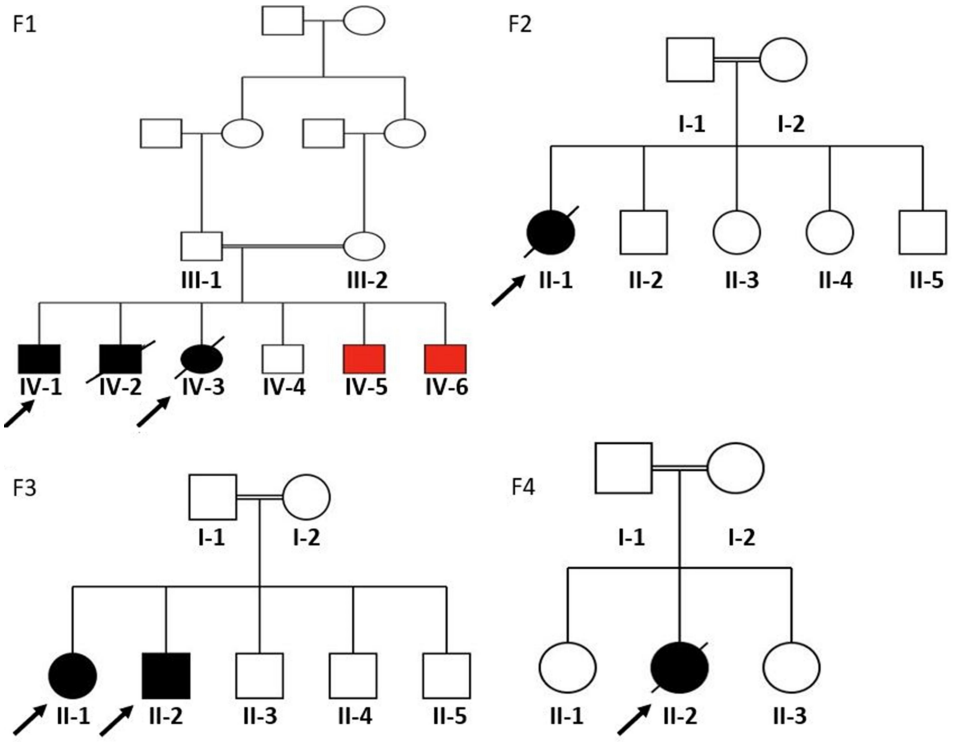

Figure 1 Pedigrees of the four participating families. F1 refers to family #1; F2, family #2; F3, family #3, and F4 refers to family #4. Squares and circles indicate male and female members, respectively. Black filled symbols represent affected individuals of relevance in this study, red filled symbols represent affected siblings with different disease, empty symbols for unaffected individuals, crossed symbols for deceased individuals, double lines represent consanguinity, and finally, arrows refer to probands in each case.

Table 1 Clinical Data for Participated Probands in Each Family

Family	Member	Gender	Age Onset	Initial Diagnosis	Symptoms	Outcome
F1	IV-3	Female	AB	Renal tubular acidosis, thalassemic, growth deficiency	Growth delay, splenomegaly, vomiting, diarrhea, painful distended abdomen, anemia, liver steatohepatitis and cirrhosis	Dead (15 Y)
	IV-1	Male	1.5 M	Suspected mitochondrial/ metabolic disorder	Vomiting, hypotonia, failure to thrive, anemia, hypothyroidism, distended abdomen, gastroesophageal reflux, skin peeling, chronic diarrhea	Stabilized through respiratory support (3 Y)
F2	II-1	Female	AB	Hirschsprung's disease	Distended abdomen, fecaloma, food intolerance, enterocolitis, vomiting, failure to thrive	Dead (1.5 Y)
F3	II-1	Female	2 M	Severe cholestatic hepatitis; metabolic disease	Prolonged neonatal jaundice	Clinically stable (11 M)
F4	II-2	Female	AB	Hypertrichosis related syndrome	Developmental delay, breathing difficulties including apnea, hypertrichosis, elevated liver enzyme, feeding difficulties with dysmorphic features, hypotonic and severe anasarca	Dead (2.5 M)

Abbreviations: AB, at birth; M, month; Y, year.

and chronic non-bloody diarrhea. Upon examination, the petite girl, with growth parameters far below the third centile for age, had hepatosplenomegaly, with significant abdominal distention. Her work-up revealed abnormal non-specific amino acid levels, anemia, elevated ferritin levels, elevated liver enzymes, prolongation of prothrombin time (PT), and international normalized ratio (INR). Her abdominal ultrasound confirmed hepatosplenomegaly with no features of portal hypertension and her upper endoscopy was normal. Her liver biopsy unexpectedly showed steatohepatitis with evolving liver cirrhosis (Figure 2). The patient underwent splenectomy due to hypersplenism, which partially corrected her hematological abnormalities. Her serum ammonia level was elevated, but amino acid chromatography and organic acid abnormalities were thought to reflect renal dysfunction and chronic liver disease. The patient stayed in hospital for almost a year due to recurrent symptoms of diarrhea, vomiting, abdominal pain, respiratory infections, etc., with no settled diagnosis. During her hospitalization, her younger brother (proband IV-1) was admitted to the hospital at the age of 1.5 months with persistent vomiting, hypotonia, respiratory distress, and poor weight gain. Persistent diarrhea developed with no improvement even on amino acid formula. He had anemia, mildly elevated liver enzymes, and thrombocytopenia. His metabolic work-up showed hyperammonemia, significant hyperferritinemia, and non-diagnostic amino and organic profile. WES seemed to be the proper course of action for both siblings. Both probands' exome sequencing identified a homozygous variant in the *SLC7A7* gene (Figure 3, Table 2), which is responsible for lysinuric protein intolerance (LPI). Retrospective analysis of metabolic work-up identified supporting evidence of the LPI diagnosis (Table 3).

Clinical Assessment and Variant Detection in Family F2

A one-year-old female (proband II-1), born to consanguineous parents, started showing symptoms soon after birth, such as difficulty in passing stool associated with increasing abdominal distention. At five-months-old, she was presented with a picture of intestinal obstruction. Her thyroid function and electrolytes were normal. The patient was labeled as Hirschsprung's disease and underwent colostomy creation and appendectomy. Soon after surgery her symptoms recurred with more distention and infrequent stooling. The patient underwent diagnostic laparotomy which showed stool impaction in the proximal and

Table 2 Identified Variants in Each Participating Family

F#	Gene Transcript_ ID	MI	Variant coordinate (hg19)[a]	Exon	Genotype	HGVS cDNA	HGVS aa	Consequence	GNOMAD Max MAF	dbSNP ClinVar HGMD IDs	Classification
1	*SLC7A7* ENST00000555702.1	AR	chr14: 23243620–23243623	9	Homozygous	c.1185_1188del	p.Ser396fs	Frameshift deletion	0.0084%	rs386833800 56352 CD000276	Pathogenic
2	*ACTG2* ENST00000345517.3	AD	chr2: 74141962	7	Heterozygous	c.769C>T	p.Arg257 Cys	Missense	N/A	rs587777387 132803 CM143757	pathogenic
3	*HSD3B7* ENST00000297679.5	AR	chr16: 30997024–30997025	2	Homozygous	c.45_46del	p.Gly17fs	Frameshift deletion	0.0128%	rs786200876 2885 CD031506	Pathogenic
4	*DPAGT1* ENST00000409993.2	AR	chr11: 118968581	8	Homozygous	c.901C>T	p.Arg301 Cys	Missense	0.0028%	rs776632995— CM126444	VUS

Abbreviations: F, family; MI, mode inheritance; AR, autosomal recessive; AD, autosomal dominant; HGVS, Human genome variation society; cDNA, coding DNA sequence; aa, amino acid level; MAF, minor allele frequency; N/A, not available; VUS, virulence of uncertain significance; [a]Variant coordinate according to the Genome Reference Consortium Human Build 37 (hg19).

distal loop of colostomy and megacolon. The bowel was evacuated, multiple biopsies were taken, and an ileostomy was created following closing colostomy. Her intestinal pathology confirmed the presence of ganglions at the rectum, which excluded Hirschsprung's disease (Figure 4). However, even with the ileostomy, the patient continued to have feeding intolerance and severe distention. This raised the possibility of dealing with chronic intestinal pseudo-obstruction (CIPO). WES analysis was later performed, and the results showed a heterozygous variant in the *ACTG2* gene (Figure 3, Table 2).

Clinical Assessment and Variant Detection in Family F3

A two-month-old baby (proband II-1), born to consanguineous parents, suffered from prolonged neonatal jaundice. Her family history showed early neonatal deaths, and her older brother (proband II-2) is diagnosed with cholestatic liver disease, which started in early childhood (now aged 26 years old). Her work-up was normal except for elevated liver enzymes (AST, ALT, ALP, but normal levels of GGT), and prolonged PTT and PT (Table 4). The patient's liver ultrasound was normal, and her hepatobiliary iminodiacetic acid (HIDA) scan showed no evidence of biliary atresia (Supplementary Material Text S1, Video S1 and Video S2). Thus, a liver biopsy was required for further testing, and the H&E staining revealed severe cholestatic hepatitis (Figure 5). Clinicians then decided to test both probands for urine bile salts through fast atom bombardment mass spectrometry (FAB-MS) (Table 5). Later, the two were recruited for WES analysis. The results showed a homozygous variant in the *HSD3B7* gene (Figure 3, Table 2). Subjects with this type of variant fail to synthesize bile acids and end up developing a form of progressive liver disease (cholestatic).

Clinical Assessment and Variant Detection in Family F4

A one-month-old female (proband II-2), born to consanguineous parents, was admitted to the NICU for two weeks on respiratory and nutritional support. A week following her discharge, she was presented to the emergency room due to difficulty in breathing and poor feeding, and she had required readmission to the pediatric ICU. Oral feeding could not be established due to poor sucking; therefore, she was fed through a nasogastric tube. The patient had several symptoms that are shown in Table 1. Her work-up included a normal metabolic profile, karyotyping, echocardiogram, and abdominal

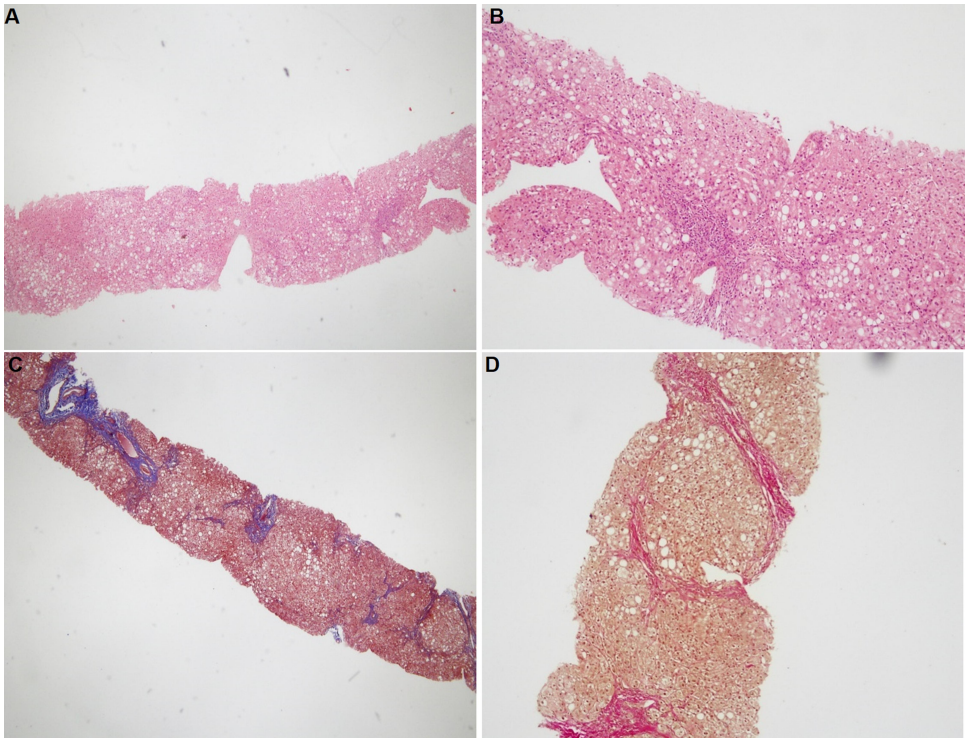

Figure 2 Histopathological findings for patient IV-3 in family F1. (**A** and **B**) Hematoxylin and eosin staining shows steatosis, hepatocytes ballooning, and portal lymphocytic infiltration. (**C** and **D**) Masson's trichrome and Van Gieson's stain, respectfully, showing bridging fibrosis partially forming half nodules. Magnification: 40× (**A** and **C**) and 100× (**B** and **D**).

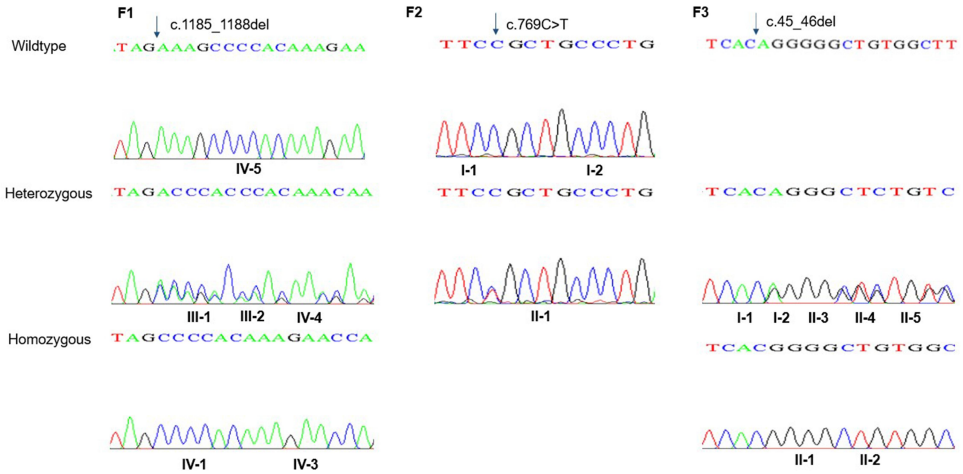

Figure 3 Sanger sequence chromatography for family F1 (left panel), family F2 (middle panel), and family F3 (right panel). In F1: the two probands (IV-1 and IV-3) have a pathogenic homozygous variant in *SLC7A7* gene. Heterozygous carriers in the family are shown in middle part, while homozygous for the wild type allele is shown at the top. In F2 (middle panel): proband II-1 carries a de novo heterozygous variant in *ACTG2* gene; whilst her parents carry the wild type homozygous allele. In the right panel, family F3, the two probands II-1 and II-2 have a homozygous deletion in *HSD3B7* gene. While the parents along their children have the heterozygous allele.

ultrasound. Her head ultrasound showed a thin corpus callosum and a brain MRI then followed to confirm the finding. The MRI result was normal; hence, congenital brain abnormalities were excluded in the diagnosis. This raised suspicion of dealing with an unknown genetic disease, and thus the patient was a candidate for WES. The analysis showed a missense variant in the *DPAGT1* gene (Table 2). The proband's MRI findings and characteristic features can be seen in Figure 6.

Discussion

The completion of the human reference genome in 2003, along with the availability of large databases of known single

Table 3 Laboratory Tests for Both Patients in Family F1

Proband	Plasma Amino Acid Levels (μmol/L)			Urine Amino Acid Levels (nmol/mg)			Ammonia (μmol/L)	Ferritin (ng/mL)	LDH[a] (U/L)
	Lysine (120–290)	Arginine (44–120)	Ornithine (44–90)	Lysine (19–1988)[b] (10–240)[c]	Arginine (10–560)[b] <153 [c]	Ornithine <265[b] <18[c]	(11–32)	(12–64)[b](15–77)[c]	(180–435)[b](110–283)[c]
IV-1	72	0	0	5957	473	0	111.2	1814	2698
IV-3	216	46	83	2614	257	0	128.5	1917	4231

Notes: [a]LDH: lactate dehydrogenase. Reference ranges designated with [b] refer to group < 24 months old. Reference ranges designated with [c] refer to group between the ages 9 and 17 years.

nucleotide polymorphisms (SNPs) and known pathogenic variants, changed our understanding of genetic variations and their relevance to health and disease.[9] As a result, the use of next generation sequencing (NGS) platforms, and WES in particular, has been extensively incorporated in the diagnosis of rare, novel, and hard to diagnose diseases.[10] Over the past decade, multiple platforms have been introduced to the diagnostic field of genetics, providing increased efficiency and reduced cost. These advances enabled clinicians to widely use WES in order to reach causative variants in multiple diseases, and, consequently, provide proper treatment.[9]

In 2018, Wojcik et al highlighted the need to improve the genetic diagnostic evaluation for infants suspected of genetic diseases.[1] As their study proved that WES or whole genome sequencing (WGS) have only been performed after traditional testing consisting of cytogenetic and molecular tests, biochemical testing, enzyme analysis, and tissue biopsies, all reported as negative. The extremely low usage rate of WES and WGS is an indication of missed opportunities children suffer in their odyssey of getting a proper diagnosis.

Herein, we evaluated the utility of WES in diagnosing challenging cases of five members in four consanguineous Jordanian families, where traditional laboratory and clinical testing failed to accurately reach a definitive diagnosis. We successfully reached a 100% diagnostic rate for each family and were able to detect the causative variant responsible for each case (Table 2).

In family F1, two siblings of consanguineous parents displaying complex, multisystem manifestations raised the suspicion of inborn error of metabolism (IEM).[11] Diagnosis of IEM represents a real challenge considering the overlapping clinical presentations of multisystem disorders and limitations of specific biomarkers. In some cases, the metabolic changes may be transient and affected by substrate intake.[12] In family F1, this was further complicated by other comorbidities; such as being a thalassemia carrier, where organomegaly and hematological abnormalities were thought to be secondary, and renal tubular defect, which was blamed for the aminoaciduria proband IV-3 manifested. Both siblings' exome sequencing identified a homozygous variant in the *SLC7A7* gene, and this variant is one of the 60 already reported variants in this gene causing LPI, an inherited genetic metabolic disorder caused by defective cationic amino acid (CAA) transport at the basolateral membrane of epithelial cells in the kidney and intestines.[13,14] Functional studies on this variant confirmed failing to

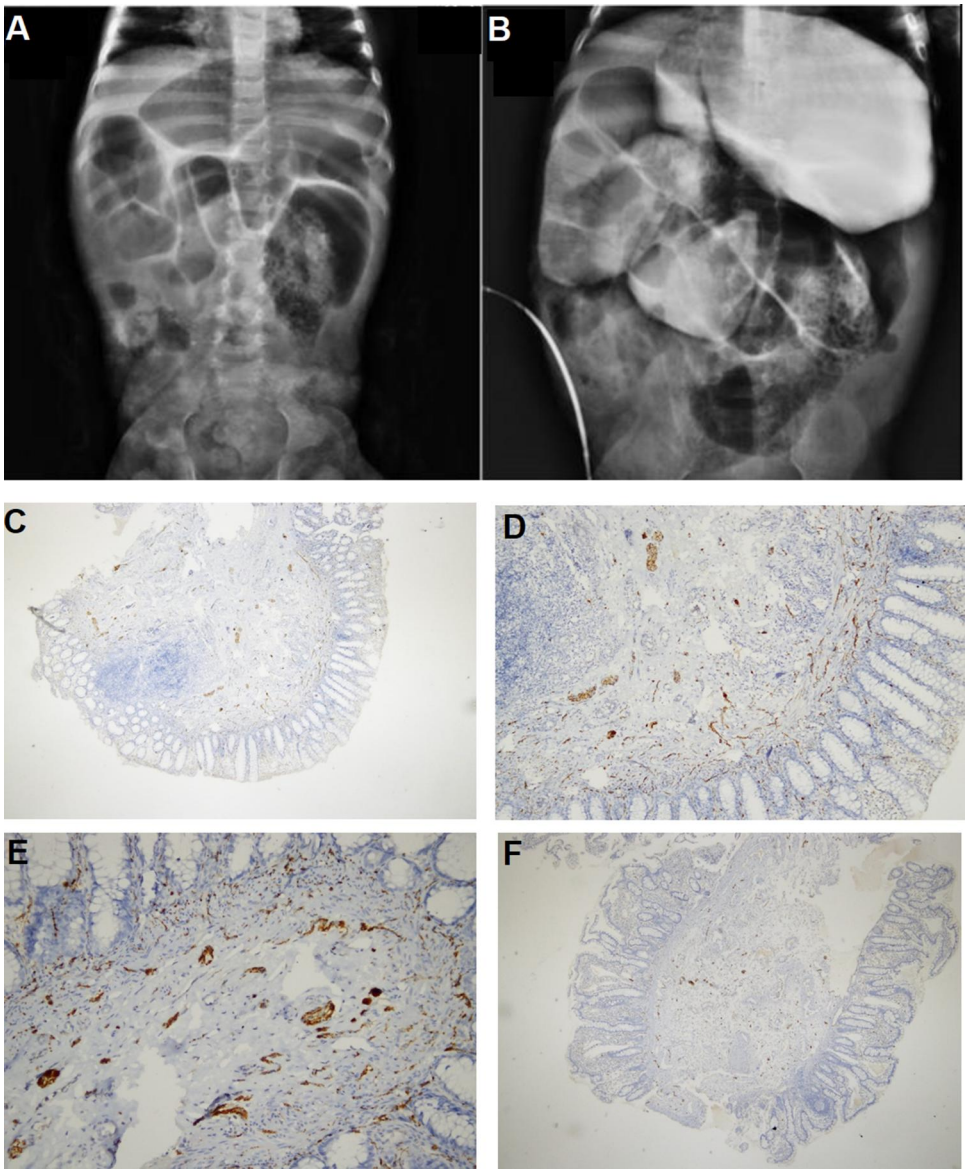

Figure 4 Imaging tests for proband II-1 in family F2. (**A** and **B**) Abdominal X-ray and barium study showing severe dilated bowel loops. (**C** and **D**) Calretinin staining 2 cm away from the anal verge, 4 cm from the anal verge (**E**), and from the ileostomy site (**F**). Ganglion cells are present (magnification: 40×, 100×, 200×, and 40× for **C, D, E**, and **F**, respectively).

induce the CAA transport.[15] Considering the non-diagnostic biochemical findings in our patients, WES settled the diagnosis for this family.

The delayed diagnosis and utility of WES in diagnosing LPI was previously reported; Posey et al reported a five-year-old boy with osteoporosis and recurrent fracture that followed for three years before the diagnosis of LPI was confirmed by WES.[16] Another report by Cimbalistienè et al described a 17-year delay in diagnosing LPI in a child with hepatosplenomegaly, recurrent episodes of drowsiness, and osteoporosis. WES confirmed the diagnosis.[17]

We believe that without WES the two patients would have continued to be misdiagnosed. Following WES results, better management was provided to the two patients, and they were put on L-carnitine and special formula. Unfortunately, at the age of 15 years, proband IV-3 developed a picture of intestinal obstruction and passed away as a complication of her illness. Meanwhile, her brother is stabilized on oxygen support and regular citrulline supplement.

In family F2 (proband II-1), the infantile presentation of progressive intestinal obstruction suggested congenital ganglionic colon. Presence of colonic ganglions and the small intestinal nature of involvement suggested CIPO.

Table 4 Liver Function Tests and Blood Coagulation Tests Conforming Liver Damage in Proband II-1 from Family F3

Liver and Coagulation Tests	Proband II-1	Reference Range
Aspartate aminotransferase (AST)	68.5 U/L	8–50 U/L
Alanine aminotransferase (ALT)	89 U/L	7–45 U/L
Alkaline phosphatase (ALP)	691 U/L	122–469 μmol/L
Total bilirubin	50.9 μmol/L	2.0 to 21 μmol/L
Direct bilirubin	45.9 μmol/L	<8 μmol/L
Partial thromboplastin time (PPT)	41.8 seconds	30–45 seconds
Prothrombin time (PT)	17 seconds	12–13 seconds

Table 5 FAB-MS Test Results for Both Probands in Family F3 Indicate the Presence of Peaks Characteristic of 3-β-HSD Deficiency

Test	Proband II-1	Proband II-2	Reference Range
Creatinine	1.5 mmol/L	21.9 mmol/L	0.5–2.2 mmol/24 h
Cholic acid	1.08 μmol/L	0.47 μmol/L	Negative
Chenodeoxycholic acid	0.12 μmol/L	0.57 μmol/L	Negative
Deoxycholic acid	0.00 μmol/L	0.25 μmol/L	Negative
Lithocholic acid	0.00 μmol/L	0.0 0μmol/L	Negative
Ursodeoxycholic acid	0.00 μmol/L	136 μmol/L	Negative
Hyocholic acid	0.00 μmol/L	0.7 μmol/L	Negative
Hyodeoxycholic acid	0.00 μmol/L	0.00 μmol/L	Negative
3β,7α-Dihydroxy-5-cholestenoate	0.00 μmol/L	51.54 μmol/L	Negative
DiOH oxycholeoic acid	0.00 μmol/L	1.00 μmol/L	Negative

Although isolated hypoganglionosis has been reported to cause CIPO, however, the diagnosis of hypoganglionosis can be misleading. Moreover, no unbiased genetic study was used in these diseases to determine if there is an underlying genetic cause. Many cases of CIPO are caused by well characterized variants, so we decided to perform WES. The analysis matched what Ravenscroft et al have previously reported in their study; a de novo heterozygous variant in the *ACTG2* gene that is associated with CIPO.[18] *ACTG2* dysfunction is associated with visceral myopathy with bladder and intestinal involvement.[19] Our patient manifested only intestinal involvement by recurrent intestinal obstruction in the absence of an organic blockage. Unfortunately, the proband's status worsened, as she suffered multiple septic episodes related to her central line and died aged 16.5 months.

In the case of family F3, cholestasis is marked as defective bile secretion with build-up levels of bile salts in the body. A significant portion of infantile cholestasis disorders are secondary to genetic defects,[20] with limited biomarkers and non-diagnostic histological features. The availability of WES can test rapidly, accurately, and cost-effectively for a large number of genetic causes.[21]

Furthermore, WES is now proposed in diagnostic algorithms of neonatal cholestasis. Nicastro et al reported a genetic testing detection rate of 60% in their infantile cholestatic cohort.[22,23]

Considering that infantile cholestasis represents a heterogeneous hereditary group and owing to the history of hepatic diseases and early neonatal deaths in family F3, the use of WES analysis was prompted to identify the genetic cause. WES results showed a homozygous variant in the *HSD3B7* gene in both siblings. Subjects with this type of variant produce abnormal bile acids that fail to leave the liver and end up developing progressive liver disease and even liver cirrhosis and failure.[24,25] Untreated patients eventually require liver transplant.[25] Proband II-1 is now 11 months old, maintained on the second-choice medication (ursodeoxycholic acid), waiting for the first-choice drug (cholic acid) to be available. It is worthy to mention that her successful diagnosis could have been a blessing had her older brother, proband II-2, been properly diagnosed at the beginning of his onset. Even though he is alive now, he suffers from liver cirrhosis which could have been preventable with the use of proper medication earlier.

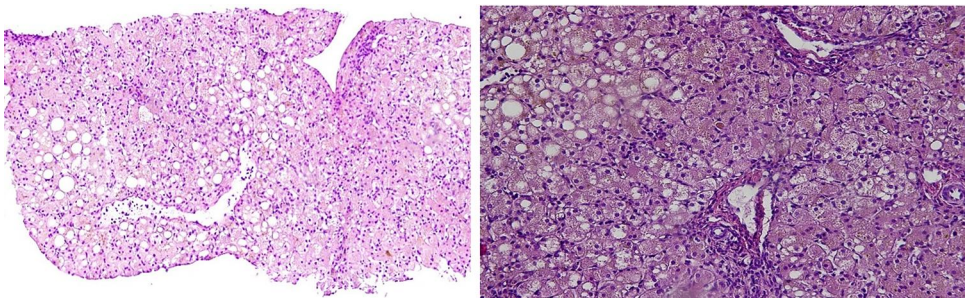

Figure 5 Histopathological findings for patient II-1 in family F3. Hematoxylin and eosin staining showing hepatocytes ballooning and feathery changes with marked intrahepatic cholestasis. Mild periportal inflammation and foci of spotty necrosis are also present (magnification: 100× and 200× left to right, respectively).

In family F4, whole exome analysis of proband II-2 showed a missense variant in the *DPAGT1* gene. Pathogenic variants in *DPAGT1* are known to manifest two distinct phenotypes, either limb-girdle congenital myasthenic syndrome (CMS) with tubular aggregates, or congenital disorder of glycosylation (DPAGT1-CDG).[26] Interestingly, in this study, our proband shared the same homozygous missense variant and diagnosis that has been reported only once before; where a Spanish non-consanguineous family had a baby boy with similar clinical features and prognosis as proband II-2.[27] Although this variant has only been reported once before, we believe it harbors a deleterious outcome. Imtiaz et al previously reported a novel missense variant in the same amino acid position (Arg 301), this residue is highly conserved among 42 different species, and no change over this residue has ever been found in healthy controls.[28] Moreover, prediction tools such as SIFT,

Figure 6 MRI and clinical features of proband II-2 in family F4. (**A**) Brain MRI; myelination is appropriate for proband's age. Both thalami, both basal ganglia, corpus callosum, brainstem, cerebellum, and ventricular system appear normal. Central midline structures with no mass effect, no hemorrhage or collection seen. (**B**) Patient's characteristic features; soft long ears, U-shaped vermilion of the upper lip, thick skin, hypertrichosis, moderate multiple contractures, hypotonia, and severe hypokinesia.

Polyphen 2, and MutationTaster gave predicted outcome of deleterious, probably damaging, and disease causing, respectively, as this variant seems to likely affect the protein misfolding. We believe that our patient presents further clinical evidence that this homozygous variant is the candidate disease causing variant. Both infants in the Spanish case report and in our study passed away aged 1.5 months and 2.5 months, respectfully, due to multiorgan failure.

In this study, all patients were successfully diagnosed following failed attempts due to the nonspecific and overlapping symptoms in each case. Of worthy notice, those patients were brought to our attention due to their gastrointestinal related problems, which led to primary assumption of dealing with GI diseases. However, through WES we managed to identify two families suffering from metabolic disorders with multisystem involvement. This is an achievement that would not have been possible without the use of WES. Furthermore, we managed to provide better management of disease through the right administration of proper medication in proband II-1 of family F3. And although three of the patients passed away while waiting for the WES results, better awareness and genetic counseling have been provided to their families.[29]

Conclusion

Children with suspected genetic conditions have a great chance of getting properly diagnosed and managed through WES, as it has proved its efficiency and great diagnostic yield. Moreover, WES cost-effectiveness is maximized by early application in the diagnostic process. Therefore, pediatricians are highly encouraged to consider early referral of children with undiagnosed syndromes to clinical geneticists.[30]

Abbreviations

CAA, cationic amino acid; CDG, congenital disorders of glycosylation; CIPO, chronic intestinal pseudo-obstruction; IEM, inborn error of metabolism; IRB, Institutional Review Boards; LPI, lysinuric protein intolerance; NGS, next generation sequencing; SNP, single nucleotide polymorphisms; WES, whole exome sequencing; WGS, whole genome sequencing.

Data Sharing Statement

The authors confirm that the data supporting the findings of this study are available within the article and its supplementary material. For additional inquiries, please refer to the corresponding author.

Ethics Approval and Informed Consent

The study was approved by the institutional review board of the Faculty of Medicine and the Research Committee at Jordan University of Science and Technology (JUST). Written informed consent to participate in this study and to publish each case's details was provided by the participants (the legal guardian in case of the children). Additionally, the parents of the proband in family F4 have provided their written informed consent to publish the patient's images and videos.

Consent for Publication

Consent for publishing data in this study was obtained from each participating family.

Acknowledgments

The authors thank the patients' families for participating in this project.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Funding

This work was supported by a grant from the Deanship of Scientific Research, Jordan University of Science and Technology (Number 20180083).

Disclosure

The authors report no conflicts of interest for this work and declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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