

ORIGINAL ARTICLE

Gastroenterology

A novel nutritional approach to infants and children with congenital diarrhea due to homozygous DGAT1 mutations

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Abstract

Objectives: Diacylglycerol acyltransferase (DGAT) catalyzes the final step in triglyceride synthesis. DGAT1 is expressed in human enterocytes and is essential for fat absorption. Homozygous DGAT1 deficiency often presents with severe diarrhea and protein-losing enteropathy (PLE) in the 1st weeks of life. Because severe restriction of fat intake controls diarrhea and decreases PLE, total parenteral nutrition (TPN) was the initial standard therapy in infants and children. We present tertiary center experience managing infants and children with DGAT1 deficiency resulting in the development of a nutritional approach that minimizes the use of TPN.

Methods: From 2014 to 2020, 12 infants with DGAT1 deficiency were treated. Stool output, growth, and development, as well as essential fatty acid status, were monitored. This retrospective experience formed the basis for treatment recommendations, which include an ultralow fat formula with intermittent peripheral intravenous lipid infusions during the 1st year of life.

Results: All patients with prolonged intestinal fat exposure had PLE, which resolved when treated with the nutrition protocol. Essential fatty acid status as measured by triene:tetraene ratios normalized in all treated patients. Over time, early genetic diagnosis and prompt initiation of an ultralow fat diet with peripheral intravenous lipid infusions replaced the need for TPN.

Conclusions: Children with DGAT1 deficiency respond to dietary restriction of lipids. Management with a novel nutritional approach provides effective treatment for infants with DGAT1 deficiency, treats diarrhea and PLE, promotes growth and development, avoids TPN dependency, and decreases the potential for essential fatty acid deficiency.

KEYWORDS

congenital diarrhea, DGAT1, essential fatty acid deficiency

Abbreviations: DGAT, diacylglycerol acyltransferase; EFA, essential fatty acid; PIV, peripheral IV; PLE, protein losing enteropathy; SMOF, SMOf lipid (Fresenius Kabi, Bad Homburg, Germany), given with fat-soluble vitamin supplementation dosed by age; TG, Triglyceride; T:T, triene:tetraene ratio; TPN, total parenteral nutrition; ULFF, ultra low-fat formula 0.2%; VLFD, very low-fat diet 0%–2% advancing with age.

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1 | INTRODUCTION

Acyl-CoA:diacylglycerol acyltransferase (DGAT) catalyzes the final step in triglyceride (TG) biosynthesis. Because in humans, only isotype DGAT1 is expressed in the intestine, adequate DGAT1 function is essential for intestinal dietary fat absorption.^{1,2} Patients without DGAT1 activity develop symptoms in the 1st weeks of life, but children with partial deficiency may present later. Infants with homozygous DGAT1 deficiency present with congenital diarrhea and protein-losing enteropathy (PLE), and are typically total parenteral nutrition (TPN)-dependent in early life.

The nutritional approach reported in this manuscript developed as experience with treatment with DGAT1 deficiency increased, resulting in control of diarrhea, preservation of growth and development, prevention of essential fatty acid (EFA) deficiency, and avoidance of the need for prolonged TPN.

2 | METHODS

From 2014 to 2020, 12 patients with DGAT1 deficiency were treated at a single tertiary center. Treatment goals were to prevent intestinal failure, PLE, EFA deficiency, as well as minimize TPN dependency, while maintaining growth and development. Avoiding intestinal fat exposure prevents enterocyte damage and intestinal dysfunction, thereby allowing for the resolution of PLE and normal nutrient absorption. Early diagnosis of DGAT1 deficiency allowed initiation of ultralow fat feeding and prevented the onset of severe PLE, avoiding prolonged TPN. Ultra-low-fat formula 0.2% (ULFF), very low-fat diet (VLFD), and intermittent peripheral IV lipids SMOFlipid (SMOF) (0%–2% depending upon age) replaced the use of prolonged TPN. Details of nutritional management are provided in Table S1.

2.1 | Formula

The ULFF used in this cohort is the amino acid-based formula Tolerex^R. Tolerex^R at full strength is 1.7% fat, half of which is essential fatty acids, from a safflower oil source. Tolerex^R full strength is 30 calories per 30cc. Tolerex^R powder was diluted with water to adapt it for infant use. In the 1st week or two of life or after diagnosis, half strength Tolerex^R (15 calories per 30cc) was given. 2/3 strength Tolerex^R is 20 calories per 30cc. Tolerex^R powder is diluted with water. Formula guidelines are provided in Table 1.

Basic F^R is an alternative ULFF used by some groups. However, in this patient cohort, a high incidence of cow's milk protein intolerance prohibited the use of Basic F^R. A high medium chain triglyceride-containing formula, Monogen^R has been used in some patients.

What is Known

- Homozygous DGAT1 deficiency causes congenital diarrhea/intestinal failure associated with protein-losing enteropathy.
- Affected infants are often dependent on total parenteral nutrition (TPN) in early life.

What is New

- DGAT1 deficient patients treated with ultra-low-fat formula and periodic peripheral intravenous lipid supplementation maintained normal growth and development.
- Triene:tetraene ratios were consistent with essential fatty acid status.
- In infants with DGAT1 deficiency, ultra-low-fat formula coupled with periodic peripheral intravenous lipid supplementation prevents the need for prolonged TPN, while maintaining adequate growth and essential fatty acid status as well as achieving enteral autonomy.

However, Monogen^R was not tolerated in this patient cohort, presumably either because of the cow's milk protein content, or because medium chain triglycerides could not be absorbed across the damaged enterocyte border, or because the long chain triglyceride content, although relatively low compared to normal formulas, is still 4.5% and may be too high for some cases of DGAT1 deficiency.

2.2 | Peripheral IV lipid infusion

To prevent EFA deficiency in the newborn period, peripheral IV SMOF with Vitalipid was given at a dose of 2 mg/kg per week. Based on tolerance of enteral fat as well as EFA assessments, dosing of peripheral IV SMOF was compressed, intervals spaced, and eventually weaned by age 9 months to 2 years, according to the guidelines in Table S1.

2.3 | Diet

The VLFD carefully introduces and advances fat content according to age. The purpose of introducing dietary fat is not for caloric content, which can be supplied by protein and carbohydrate, but rather to stimulate presumed alternate mechanisms of fat absorption compensating for DGAT1 deficiency, as well as to supplement essential fatty acids allowing weaning off intermittent PIV SMOF infusions. Therefore, the focus of dietary fat is on food high in EFA content. Tastes of food

TABLE 1 DGAT1 deficiency sample menu and nutrition recommendations (for well child with DGAT1 deficiency).

Age	Formula	Solids/general	Breakfast	Lunch	Dinner	Peripheral intravenous lipid infusion (SMOF) ^c
0–2 months	Tolerex 50% strength—2/3 strength 60–120cc 6–8x per day	None				2–3 g/kg/day once per week in first 6 weeks, then 3 g/kg/day once in 2 weeks
2 to 4–6 months	Tolerex 2/3 strength 90–120cc 6–8x per day	None				4 g/kg/day once in 2 weeks, beginning age 4 months once in 3–4 weeks
4–6 to 7 months	Tolerex 2/3—3/4 strength 120–180cc 6x per day	Tastes of fat-free solids, increasing volume as tolerated ^b	Fruit such as banana, potato, apple, pear		Fruit or vegetable such as carrot, green beans, broccoli, cauliflower	4 g/kg/day once per month
7–9 months	Tolerex 2/3 strength 180–240 4–6x per day	Tastes of low-fat solids ^b —As fat is included, should ideally be concentrated on fat relatively rich in EFA ^a	Fruit—add citrus fruit as tolerated	Peas (0.2% fat) graduating to lentils (% fat), graduating to small volume chicken breast (3%–4% fat)	Vegetable— Such as Kohlrabi, sweet potato, zucchini, squash; May slowly add as tolerated fingertips of avocado, humus, tahina, olive oil	4 g/kg/day once per month
9–12 months	Tolerex 2/3 strength 180–240cc 3–4x per day	Low-fat solids 1% total dietary fat ^b —As fat is included, should ideally be concentrated on fat relatively rich in EFA ^a	Fruit cereal	Lentils/peas with 1–3 teaspoons chicken breast; Advance to bread/rice as tolerated	Vegetable with 1 teaspoon of avocado, hummus, or tahina or fingertip of olive oil; Advance to bread/rice as tolerated	4 g/kg/day once per month
Toddler	May continue Tolerex as supplement	1%–2% dietary fat ^b	Fruit cereal	Lentils/peas with 1–3 teaspoons chicken breast; Bread/rice; trial fingertips advancing to 1–2 teaspoons of low-fat fish such as sol or canned tuna in water; trial fingertips advancing to 1–2 teaspoon lean meat or turkey as tolerated	Vegetable with 1 teaspoon of avocado, hummus, or tahina or fingertip of olive oil; advance to bread/rice as tolerated; advance to pasta/couscous as tolerated	4 g/kg/day once in 2 months; may stop infusions once reaches age 2 years assuming absorption of low-fat diet
School age	May continue Tolerex as supplement	2% dietary fat ^b	As above; add low-fat 1%–2% yogurt, cheese, or puddings as tolerated, advance volumes of lean meat, chicken breast, and low-fat fish as tolerated			None unless apparent EFA deficiency (not expected at this age provided good dietary management)
Adolescence	May continue Tolerex as supplement	2% dietary fat ^b				

^aOils/foods rich in EFA include: primrose, safflower, sunflower, sesame, almond, canola, peanut, olive, fish, red-brown algae, chestnut, walnut, avocado.

^bThese fat intake recommendations presume that the child's nutritional status and absorptive capacity for protein and carbohydrates are normal.

^cSMOF recommendations are maximal rate of 0.17 g/kg/h; to complete infusion within 8 h the rate can be 0.5 g/kg/h—We have not encountered lipid overload syndrome or other side effects at this rate.

containing fat are begun once the infant reaches 7 months of age. Dietary fat is advanced to 1% from age 9 to 12 months, and to 2% between age 1–2 years. See Table 1 for sample menu guidelines by age.

2.4 | EFA supplementation

Oils rich in EFA was dosed as tolerated to help wean PIV lipid infusions. See Table S2 for dosing recommendations.

Patients were started on the nutritional protocol at diagnosis or at the time of presentation to the tertiary care center. Patients 1a and 2a were diagnosed in the second decade of life, and were started on this nutritional protocol at that time. Patients 3b and 7 were diagnosed in the first 2 weeks of life and were started on the nutritional protocol at that time. The rate of enteral fat exposure and weaning of IV or tube feeding depended on intestinal function and absorption.

3 | RESULTS

3.1 | Genetic mutations

Ten patients are of Ashkenazi Jewish descent and homozygous for mutation in intron 8c.751+2T>C that results in a protein without DGAT activity. One patient is of Ashkenazi and Tunisian/Moroccan descent and has a compound heterozygote mutation for c.751+2T> and a new mutation, p.Gln305*. One patient is of consanguineous Arab descent and has a homozygous mutation for p.R395X, and an unreported nonsense mutation.

3.2 | Clinical presentation

All 12 patients presented with severe congenital diarrhea within the 1st month of life (Table S3). One affected sibling was diagnosed in the 1st week of life with diarrhea, promptly treated with the nutritional protocol, and never developed PLE. The other 11 patients presented with diarrhea, PLE, and some developed malnutrition, hypoalbuminemia, hypogammaglobulinemia, and fat-soluble vitamin deficiency. One infant had transient exocrine pancreatic insufficiency demonstrated by low fecal elastase levels before the diagnosis of DGAT1 deficiency. Diarrhea and metabolic abnormalities resolved within 3 months of instituting the nutrition protocol. Response to nutritional therapy took longer in the older patients, who were diagnosed later in life and therefore exposed to excess intestinal fat for a prolonged period, presumably leading to more intestinal compromise.

3.3 | Treatment

The two oldest patients were treated with TPN for years, younger patients were treated with TPN for months, whereas, the most recently born patients were never treated with TPN or tube feeding.

Patients born between 2002 and 2007 were managed with TPN before diagnosis. TPN was weaned in patient 1a by age 5 years to a VLFD containing 1%–2% fat (VLFD). Before age 14 formal genetic diagnosis, his working diagnosis was congenital disaccharidase deficiency, his VLFD was self-imposed, and interpreted as selective disordered eating, rather than prescribed by the medical team. Patient 2a was managed on a very low-fat formula containing 0.2% fat (VLFF) via gastrostomy. This management was initiated in response to enterocyte fat droplets found on intestinal biopsy. Gastrostomy feeds were stopped at 5 years of age as she transitioned to a VLFD.

Patients born between 2014 and 2019 (except 3b and 7) were diagnosed between the ages of 3 months and 2 years. They were weaned off TPN and tube feeding to VLFF/VLFD within the 1st or 2nd year of life.

In contrast to the older patients, the youngest patients 3b and 7 were diagnosed by genetic testing before 2 months of age and were managed with only oral VLFF and peripheral intravenous SMOF infusions, and then transitioned gradually to VLFD. Neither required TPN.

All patients with prolonged intestinal fat exposure had clinical PLE, which resolved with initiation of the nutritional protocol. No patients currently require TPN or tube feeding. All patients currently have formed stools without evidence of steatorrhea, but accidental dietary fat exposure greater than 2% causes diarrhea that may last 12–24 h.

3.4 | Growth

With nutritional management, low weight and BMI resolved in all patients. Patient 1a required growth hormone in adolescence for familial short stature. Patients 5 and 9 both have short stature, but their height percentiles were maintained (9) or improved (5) after weaning TPN. For detailed growth parameters, see Table S4.

3.5 | Small bowel biopsy

Small bowel biopsies of 1 of 12 patients (2a and 3a) performed at the time of clinical intestinal failure and dietary fat exposure demonstrated lipid droplets within enterocytes. Small bowel biopsies of 5 of 12 patients performed during treatment with bowel rest or VLFF/VLFD did not demonstrate lipid droplets (Figure 1).

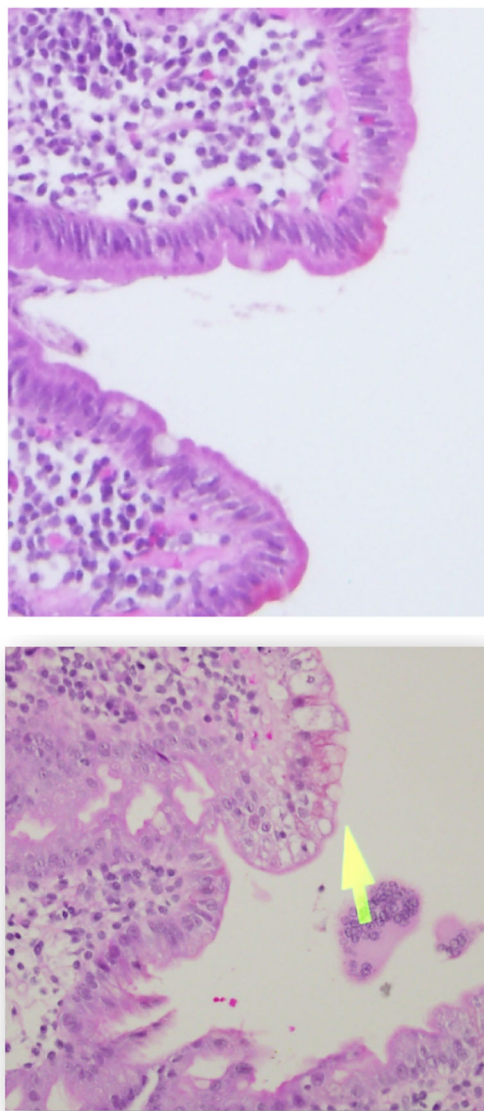


FIGURE 1 (A) (18yM) Hematoxylin and eosin (H&E) staining of small bowel biopsy taken during ultra-low fat diet with good intestinal function—enterocyte lipid droplets are absent. (B) (7yM) Enterocyte lipid droplets on H&E staining of small bowel biopsy taken during period of dietary fat intake and intestinal failure.

3.6 | Assessment of essential fatty acid status

Fatty acid profiles were batched and performed on plasma of 10 patients (Figure 2). In 7 of 10 patients the triene: tetraene (T:T) ratios were normal, indicating EFA sufficiency. In 3 of 10 patients, initial T:T ratios were >0.2 consistent with EFA deficiency, including patient 9 who was not yet treated with the protocol and presented to our center at the age of 4 years with a severe diffuse dry scaly rash. Two asymptomatic infants (3b and 7) received suboptimal SMOF infusions because of nonadherence. Except for patient 1a, who

was empirically given monthly PIV SMOF from age 14 to 16 years during treatment with growth hormone, all patients discontinued IV SMOF between ages 9 months and 2 years.

After 3 months, follow-up fatty acid profiles (final values) were repeated in all patients (Figure 2). Those patients with previously normal T:T ratios still had normal results. The 4-year-old patient with EFA deficiency remained EFA deficient; however, his rash and diarrhea resolved with implementation of the oral nutritional protocol, and his T:T ratio improved. With improved adherence to the nutritional protocol, the two asymptomatic infants with EFA deficiency had normalization of T:T ratios by indicating EFA sufficiency.

3.7 | Hypertriglyceridemia

Although most lipid measurements were normal, peak hypertriglyceridemia occurred between 6 and 12 months of age and normalized over time (Figure 3). In two patients (3b and 7), hypertriglyceridemia correlated with EFA deficiency. In one patient (patient 8), TG and EFA were normal, but TG increased as peripheral SMOF was weaned, suggesting that EFA deficiency may have occurred transiently. The two oldest patients (1a and 2a) had normal carotid artery ultrasounds in adolescence.

3.8 | Discussion

DGAT1 and DGAT2 catalyze the final step in TG biosynthesis and are central to intestinal fat absorption.^{1,2} Unlike humans whose intestinal mucosa only expresses DGAT1, in the mouse intestine, both DGAT1 and DGAT2 are expressed.^{1,2} DGAT1-knockout mice have decreased adiposity, increased insulin sensitivity, and resistance to diet-induced obesity and fatty liver.³ In humans, however, the absence of intestinal DGAT2 expression renders DGAT1 activity essential for fat absorption.

Von Rijn et al. developed patient-derived organoids to study the molecular mechanism of DGAT1 deficiency.⁴ They demonstrated aberrant lipid metabolism with reduced lipid droplet and TG formation after incubation with fatty acid in both patient-derived organoids and fibroblasts. DGAT1 deficient cells appear to be more susceptible to lipid-induced toxicity and these data support the hypothesis that lipid toxicity is likely the cause of PLE. In a later study, von Rijn et al. demonstrated that DGAT enzyme function is critically important in protecting enterocytes from toxic concentrations of fatty acids.⁵ Epithelial stem cells express both functional DGAT1 and DGAT2, but DGAT2 expression is lost upon differentiation toward an enterocyte phenotype. DGAT1-dependent lipid droplet formation is required for tolerance of fatty acids in

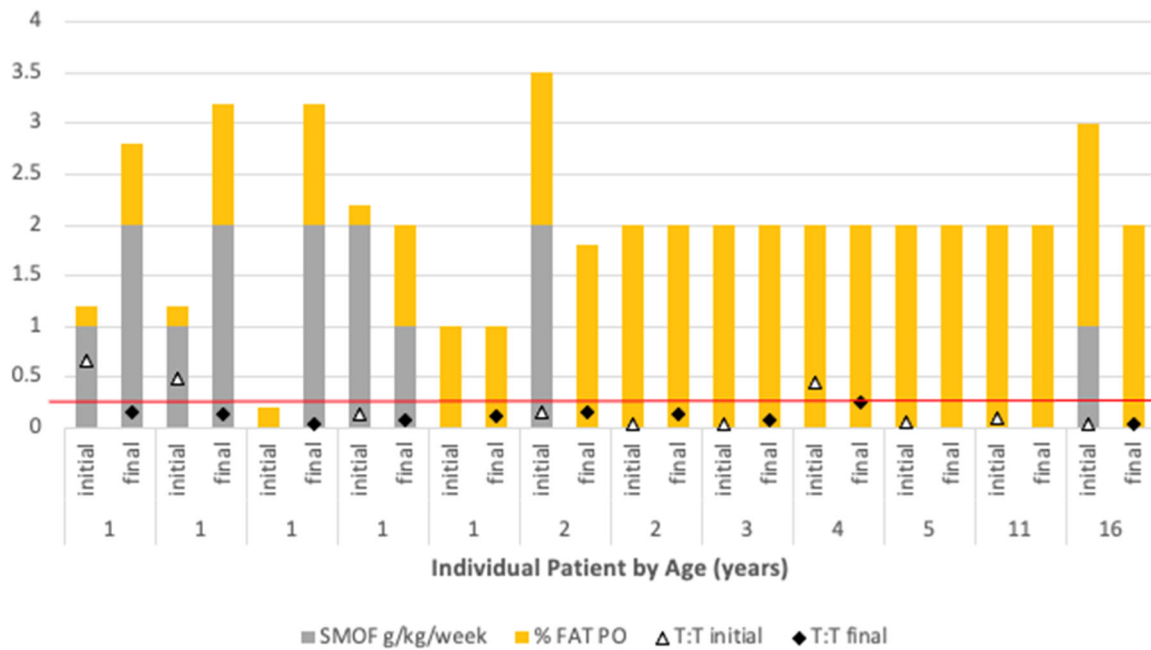


FIGURE 2 Essential fatty acid (EFA) status as measured by T:T (triene:tetraene ratio) ratio compared to age when EFA levels were measured. Yellow bars represent the percent of fat consumed orally; gray bars represent intravenous fat intake. Individual patients are plotted along the x-axis by increasing age. T:T ratio (initial white triangle, final black diamond) is normal if below the horizontal red line, which represents the threshold normal value. At initial EFA assessment, three patients were EFA deficient (white triangle above the red line): (i) a symptomatic 4-year-old patient who had not yet started treatment with the nutritional protocol; (ii) two asymptomatic infants whose caregivers did not adhere to the schedule of SMOFlipid (SMOF) infusions, and received only half of the prescribed monthly dose. At follow-up EFA assessment (3 months later for all patients), patients with previously normal T:T ratios retained EFA sufficiency. The symptomatic 4-year-old was not able to obtain SMOF, and remained EFA deficient, although his T:T ratio trended toward normal and his symptoms resolved with implementation of the remainder of the nutritional protocol. The two asymptomatic EFA deficient infants had normalization of T:T ratios, consistent with adherence to prescribed SMOF infusions. This figure estimates dietary fat intake and peripheral intravenous SMOF doses sufficient to maintain EFA sufficiency at a given age in DGAT1 deficient patients.

Year of birth	1-3 months	4-6 months	6-12 months	2-4 years	5-8 years	9-12 years	13-16 years	17-18 years
2002				122			203	141
2007	195	273	339			186	166	
2014	111		354	168	186			
2014				213*				
2015	168	53	89	96	165			
2017		80	212	186				
2017			691	106				
2017	154	197	33	106				
2018	27	212	389	127				
2019	115	168	230*	186				
2019	262	98	319**					
2019	80	151	221					

FIGURE 3 Lipid measurements in patients with DGAT1 deficiency. Highest triglyceride (TG) measurements (mg/dL) per patient at age intervals (elevated TG highlighted—normal range 0–99). Peak TG generally occurred at 6–12 months. *Elevated TG correlated with essential fatty acid (EFA) deficiency. **EFA deficiency was not measured, but TG peaked as peripheral infusions of SMOF were weaned.

human intestinal stem cells. The protective effect of lipid droplet formation was connected to diminished lipid-induced endoplasmic reticulum stress. When DGAT1 function was inhibited in intestinal stem cells, DGAT2 compensated lipid droplet formation conferred resistance to lipotoxicity.⁵

DGAT1 deficiency is a rare autosomal recessive condition caused by loss of function mutations in the DGAT1 gene. The first report in humans of DGAT1 deficiency was by Hass and Winter et al. in an Ashkenazi family in whom two children presented with diarrhea, protein-losing enteropathy (PLE), and poor weight gain in the 1st week of life.^{6,7} Identification of a deletion in chromosome 8 145541756A → G, results in the formation of an unstable protein product and complete loss of DGAT1 activity. These children were managed initially with TPN and only one of the siblings survived. Subsequently, other mutations have been identified that result in complete or partial absence of DGAT1 activity. Although the clinical phenotype is similar, the severity of the disease may vary depending on the level of enzyme activity. Deaths from complications of malnutrition and central-line sepsis have been reported.⁶

The variability of clinical presentation and outcome may be related to genotype, or to other genetic modifiers. Thus far, 23 individual mutations in the DGAT1 gene are described in the literature.^{8,9} First described in 2012,⁶ the most common mutation is the c.751+2T>C variant, in which there is a rare splice site mutation in intron 8 resulting in a 25 amino acid deletion in the highly conserved mBOAT domain of the DGAT1 protein causing a highly unstable protein.¹⁰ Fibroblasts expressing this genetic variant produce mRNA, but the DGAT1 protein does not have functional activity. The c.751+2T>C variant is found with high frequency in the Ashkenazi Jewish population, likely due to founder effect.

NM_012079.4: c.884T>C, a novel homozygous missense variant in exon 10 predicted to lead to a p.(Leu295Pro) in the active site of the DGAT1 protein, was described in 2016.¹¹ This causes a loss of a hydrogen bond in the helix that affects protein structure, leading to impaired activity. The mutated gene caused 70% reduced expression in fibroblasts. Children with this mutation demonstrated later onset disease and absent or only mild hypertriglyceridemia.¹¹ In 2017, the c.314T>C, p.L105 missense mutation was described. In patient-derived fibroblasts, the mutant allele was less abundant, resulting in only partial loss of triglyceride synthesis, and a less severe phenotype.¹²

The first case of a compound heterozygote patient with DGAT1 deficiency was reported in 2018, with two new mutations described: c.1013_1015delTCT; p.Phe338del and c.1260C>G; P.Ser240Arg. This patient presented at age 1 month with PLE, and responded well to dietary intervention, with normal triglyceride measurements throughout the reported course.⁴

Five new mutations in DGAT1 were described in 2018 by von Rijn et al.⁴ The c.1202G>A, p.W401X mutation leads to an early stop codon, resulting in the absence of intestinal DGAT1 protein expression. C.573_574delAGinsCCCATCCCACCCTGCCCATCT is an insertion–deletion at exon 6. This mutation caused aberrant splicing, leading to an undetectable protein product. C.937-1G>A is a splice site acceptor mutation preceding exon 12, which results in highly reduced expression of DGAT1. The c.953insG, p.1319Hfs*31 mutation is a single base pair insertion that leads to frameshift and an early stop codon and the C.629_631delCCT, p.S210_Y211delinsY mutation is a 3 base pair deletion in exon 7, which yielded normal mRNA levels, but absent protein expression.

Xu et al.¹³ described in 2020 a new DGAT1 splice site mutation 145541376c.895-1G>A in a Chinese patient who was a compound heterozygote with another known mutation. Interestingly, this patient's presentation was atypical with neonatal onset of hypertriglyceridemia, but later onset of diarrhea at 8 months of age. All these mutations result in varying levels of DGAT1 activity and are likely to account for the variation in clinical presentation and tolerance of oral fat.

Li et al. describe three new DGAT1 mutations in two Chinese patients. Two patients had a frameshift mutation leading to an early stop codon at amino acid 74 (c.1215_c1216delAG). Both were compound heterozygotes for other mutations—One nonsense mutation leading to an early stop codon preventing translation of half the protein (c.838C>T) and the second a missense mutation in exon 13 leading to alanine/valine substitution at position 350 (C/1049c>t). Both presented with failure to thrive, diarrhea and hypoproteinemia, and hypogammaglobulinemia suggestive of PLE. Both responded remarkably well to dietary fat restriction, highlighting the importance of early diagnosis, leading to early dietary treatment and improved prognosis.⁹

Two new genetic variants are found in our cohort: The p.Gln305* variant creates a stop codon at exon 11 out of 17 exons and is predicted to result in a truncated protein or nonsense mediated mRNA decay. The nonsense mutation Chr8:145,54,750G>8, p.R395X was also identified.

In our cohort, clinical course, outcome, requirement for and duration of parenteral nutrition, and management of hypertriglyceridemia are more related to age at diagnosis and the timing of initiation of appropriate dietary intervention, rather than with genotype, which may be more relevant to subsequent tolerance of fat.

Homozygous DGAT1 deficiency causes severe congenital diarrhea along with vomiting, PLE, and intestinal failure.⁶ Most patients have a grossly normal endoscopy, with pathology varying from normal to villous flattening with enterocyte lipid vacuolization. PLE resolves with severe restriction of dietary fat. Tolerance of enteral fat and medium-chain triglyceride

supplementation vary with the specific activity of the mutation and in some children may increase with age. Nutritional management of infants and toddlers may include intravenous essential fatty acids, TPN with subsequent weaning to nasogastric tube or enteral feeding. Outcomes are mostly positive, but deaths have been reported due to metabolic or infectious complications. Small bowel transplantation failed in one case.¹³ Although not universal, abnormal lipid profiles are common, including low HDL and hypertriglyceridemia.¹³

Clinical experience with DGAT1 deficiency led to the development of a TPN-free protocol for infants and children (Table S1). When the diagnosis of DGAT1 deficiency can be determined in the first 2 weeks of life, such as in a sibling of a patient known to have the disease, as soon as the diagnosis is established breast feeding and fat-containing formulas should be avoided. Small frequent feeds every 2 h with a fat-free formula such as Tolerex^R (Nestle) (1/2 strength for the first 2 weeks and then increasing the concentration to 2/3 strength around 2–4 weeks of life as tolerated) or Basic F^R (Nutricia) should be started. Monogen^R (Nutricia) has also been used. Monitoring stool and urine output, weight, electrolytes, Mg, PO₄, Ca, pH, and bicarbonate is needed in the 1st weeks of life. Fat with peripheral IV SMOF with Vitalipid 2 g/kg/week is given for the 1st month. Between 1 and 3 months of age, the dose can be modified to 4 g/kg every other week and then changed to monthly from 3 to 12 months of age depending on the level of EFA. At 1 year of age the dose of SMOF can be decreased to every other month and only continued after 2 years of age if the child has EFA deficiency.

Since fat is not needed for calories, dietary fat should be limited to fats with high concentrations of EFA. Oils such as safflower oil¹⁴ (linoleic acid, 76%) or primrose oil¹⁵ (linoleic acid, 60%–80% and γ -linoleic acid, 8%–14%) can be provided frequently in small doses (Table S2). A more commercially available oil such as canola oil, which contains approximately 35% EFA, can be administered by doubling the dose recommended for safflower oil. In early infancy, oil can be applied to infant's skin as part of an effort to prevent EFA deficiency, but the amount needed to prevent EFA deficiency by cutaneous application in the setting of DGAT1 deficiency is not known.

Fat-free solids may be introduced around 4 months of age if developmentally appropriate and the child is not having diarrhea. Slow introduction of fat-containing foods (1%–2% fat) such as avocado which has a high content of EFA may be beneficial if tolerated. By 2–4 years of age children can generally tolerate a low-fat diet. Children may self-restrict fat from their diets if they associate eating fat-containing foods with discomfort, abdominal pain, or diarrhea. Intravenous SMOF can be weaned as the tolerance of dietary fat increases. Fat soluble vitamins should be supplemented, and levels

monitored every 3 months. Measuring an EFA profile every 3–6 months to maintain the triene:tetraene ratio ≤ 0.2 is recommended. Lipid profile should be monitored every 3 months. In older children with dyslipidemia, assessment of carotid artery and liver ultrasound could be considered, but there are no data to suggest that dyslipidemia in these patients increases their risk for atherosclerosis. For children in whom malabsorption and PLE continue to be an issue, an endoscopy with duodenal biopsy to assess villous atrophy and/or enterocyte lipid storage (Figure 1) will identify individuals who may need additional lipid restriction.

Infants with prolonged exposure to fat before initiation of fat restriction may have delayed tolerance of oral fat and require continuous nasogastric feeding of Tolerex^R, Basic F^R, or Monogen^R. Persistent intolerance should raise suspicion of pancreatic insufficiency and consideration of pancreatic enzyme supplementation. Monitoring stool alpha-1 antitrypsin is a good indicator of the severity of mucosal injury and is a biomarker for fat intolerance when introducing low-fat foods.

Vitamin D deficiency and rickets are reported in children with DGAT1 mutations.¹⁶ The formation of triacylglycerol from diacylglycerol is catalyzed by DGAT1 and is essential for incorporation of vitamin D into chylomicrons. Absorption of chylomicrons results in the acquisition of both triacylglycerol and vitamin D. When vitamin D is activated into calcitriol it binds to the vitamin D receptor to upregulate calcium-binding protein expression that enhances calcium absorption.¹⁶ Without a functioning DGAT1 protein, vitamin D levels in chylomicrons will be diminished resulting in lower vitamin D and impaired calcium absorption. Patients with DGAT1 deficiency should be monitored for hypovitaminosis D, calcium, phosphorus, and potential rickets.

The relationship between DGAT1 deficiency and hypogammaglobulinemia is not established, but the association has been reported.¹⁷ The loss of protein in the stool might explain the low IgG in some patients, but very few children require intravenous gammaglobulin.

4 | CONCLUSION

We describe a novel nutritional approach for the management of DGAT1 deficiency that evolved over 6 years while treating 12 patients with this rare disease. The protocol effectively treats diarrhea and PLE while preventing EFA deficiency and intestinal failure. The nutritional protocol avoids TPN dependency and maintains growth and development. This report is limited not only by the sample size, but also because only three specific DGAT1 mutations are represented in the patient cohort; however, patients with other mutations should benefit from this therapeutic approach. Further study is needed to prove that this protocol is generalizable to other patients with DGAT1 deficiency.

Comparison of disease severity, manifestations, and treatment requirements in our older versus younger patients supports the concept that enteral fat exposure modulates PLE in DGAT1 deficiency. These observations in a limited cohort of 12 patients suggest that early diagnosis and appropriate dietary intervention will mitigate the onset of PLE, intestinal failure, and TPN dependence.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

ETHICS STATEMENT

Ethical approval was obtained by Peri Millman, MD by the Ethical Review Board at Hadassah Hospital.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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