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CASE REPORT

NOVEL DGAT1 MUTATIONS IDENTIFIED IN CONGENITAL DIARRHEAL DISORDER 7: A CASE REPORT WITH THERAPEUTIC EXPERIENCE

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ABSTRACT

Congenital diarrheal disorders (CDD) are a group of rare inherited intestinal disorders, among which CDD7 was recently identified to be associated with only 24 mutations in gene coding for diacylglycerol-acyltransferase 1 (*DGAT1*).

We report on a female patient who presented with diarrhea, vomiting, hypoalbuminemia, and failure to thrive after birth. Two novel variants of c.1215_1216delAG and c.838C>T were found in the *DGAT1* gene by whole exome sequencing, which was confirmed to be compound heterozygous by Sanger sequencing. Her symptoms and nutritional status improved significantly after 1 year of a fat-restricted enteral diet. Weight for age and weight for length increased from -5.0 SDS and -4.0 SDS at 3 months to +0.08 SDS and +1.75 SDS at 15 months, respectively.

This report expanded the mutation spectrum of *DGAT1*-related CDD7 and enriched our knowledge of the clinical features. Moreover, early fat-restricted enteral diet intervention was suggested for the treatment of such patients.

Keywords: congenital diarrheal disorders, diacylglycerol-acyltransferase 1, nutrition

INTRODUCTION

Congenital diarrheal disorders (CDD) are rare inherited intestinal disorders characterized by diarrhea, nutrient malabsorption, and sometimes life-threatening [1]. Genetic background of CDD is heterogeneous. Recent studies have shown that mutations in diacylglycerol-acyltransferase 1 (*DGAT1*, OMIM* 604900), a gene encoding a protein involved in lipid metabolism, were associated with CDD7 (OMIM# 615863). DGAT1 is a microsomal enzyme that is highly expressed in several organs, such as the small intestine, adrenal medulla, adrenal cortex, and testes [2]. DGAT1 and its isozyme diacylglycerol-acyltransferase 2 (DGAT2) are responsible for the conversion of diacylglycerol and fatty acyl-CoA to triacylglycerol in humans [1]. The human intestine might be particularly vulnerable to DGAT1 deficiency, as the human intestine expresses *DGAT1* exclusively and *DGAT2* is mainly expressed in the liver [1], whereas mice and other mammalian intestines express both DGAT1 and DGAT2 [3-4]. CDD7, caused by bi-allelic variants of the *DGAT1* gene, mostly develops in the neonatal period with severe diarrhea, vomiting, hypoalbuminemia, and failure to thrive. To date, few mutations of the *DGAT1* gene have been reported on.

Herein, we present an infant with CDD7 caused by two novel *DGAT1* mutations. The clinical features and physical growth parameters were analyzed and followed up at 12 months. The efficacy of nutritional therapy is instructive for pediatricians to consider as early treatment for such patients.

CASE REPORT

A girl aged 3 months was admitted with the main complaint of slow weight gain. She was born by vaginal delivery without complications at 40 weeks' gestation.

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The birth weight was 2.75 kg (weight for age: -1.13 SDS), and the birth length was 49.0 cm (length for age: -0.05 SDS). Her parents were not consanguineous and denied any familial history of genetic diseases. She was the first child of her parents. She had mixed feeding (breastfed and regular formal) and she presented watery diarrhea (twice a day) 2 days after birth, accompanied by occasional vomiting and slow weight gain. At 3 months, her height was 53.0 cm (length for age: -3.24 SDS). She was underweight and emaciated with the weight of 2.8 kg (weight for age: -5.0 SDS; weight for length: -4.0 SDS). The stool routine indicated increased fat globules without red or white blood cells. The serum 25-hydroxyvitamin D was as low as 3.29 ng/mL (normal range: 20-40 ng/mL). Both albumin (25.0 g/L, normal range: 35-53 g/L) and prealbumin (0.107 g/L, normal range: 0.18-0.45 g/L) were significantly reduced. The levels of serum potassium (3.48 mmol/L, normal range: 3.5-5.5 mmol/L) and sodium (130 mmol/L, normal range: 136-145 mmol/L) were slightly low. Aspartate aminotransferase was elevated (60 U/L, normal range: 5-34 U/L). Blood sugar, hemoglobin, and plasma amino acids were within the normal ranges. The urine routine and urinary organic acids tests were also normal. She was diagnosed with severe malnutrition and hospitalization was recommended, which was not accepted by the parents. Take-home high-energy formula was thus provided to improve nutrition. However, the diarrhea worsened rapidly after 2 days of Nutricia formula feeding (5-6 times per day), and she was admitted to the Intensive Care Unit (ICU) for dehydration and low serum bicarbonate (13.6 mmol/L, normal range: 22-28 mmol/L) ten days later. The stool routine was normal, and the virus and bacterial pathogens were negative. Immunoglobulin G was significantly decreased (IgG 0.99 g/L, normal range: 5.19-10.79 g/L), and lymphocyte subset analysis was normal. Ultrasonography showed gallbladder stones. During that hospitalization, she received albumin, intravenous immunoglobulin infusions, red blood cell transfusion, and parenteral nutrition. Treatment with extensively hydrolyzed formula was effective with no more diarrhea. She was discharged from the hospital after 20 days.

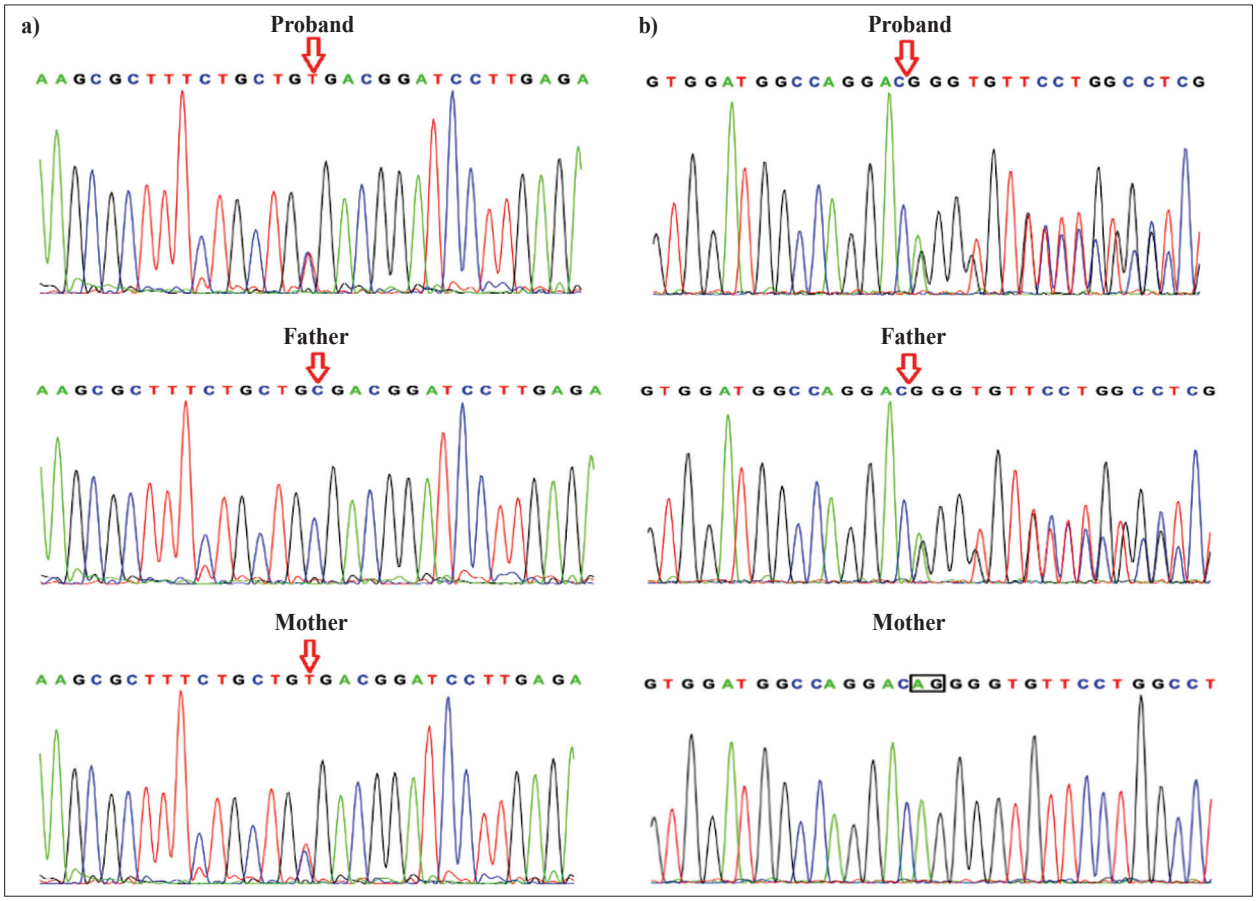

Figure 1. Sanger sequencing results show mutations in *DGAT1* detected in the proband`s family. (a) The proband was maternally inherited the variant of c.838 C>T in the *DGAT1* gene. (b) The proband was paternally inherited the variant of c.1215 _ 1216 del AG in the *DGAT1* gene.

Due to the unknown etiology of diarrhea and slow weight gain, a whole exome sequencing (WES) was performed using peripheral blood samples from the proband after obtaining written informed consent from the parents. Compound heterozygous mutations in *DGAT1* were identified by WES and confirmed by Sanger sequencing (Figure 1a, b): a paternally inherited variant of c.1215_1216delAG (p.Phe408fsTer74) and a maternally inherited variant of c.838C>T (p.Arg280*). Neither of the mutations has been reported in the human gene mutation database (HGMD), in the literature, nor found in the public or in-house databases. The variant of c.838C>T (p.Arg280*) was evaluated as "likely pathogenic" according to the American College of Medical Genetics and Genomics (ACMG) guidelines with evidence of PVS1+PM2. The variant of c.1215_1216 del AG (p.Phe408fsTer74) was evaluated as "pathogenic" according to the ACMG guideline with evidence of PVS1+PM2+PM3. No other variants in the WES data were found to be related to digestion and absorption. By reviewing the literature and disease database, a total of 26 *DGAT1* variants (including ours) have been reported on in patients with CDD7. The schematic presentation of the *DGAT1* mutation spectrum is depicted in Figure 2a. By constructing a three-dimensional molecular model, using Pymol software, these variants caused an abnormal DGAT1 protein structure, which might be destructive for the normal function of protein (Figure 2b). The major clinical features of these patients are summarized in Table 1.

The patient was referred to the developmental pediatrics for feeding guidance and physical monitoring since discharge. At first, an extensive hydrolyzed formula (a limited fat to 45.2% of total calories) was provided. There were no more complaints about diarrhea, yet there was also no weight gain observed for 2 months (Figure 3). She was recommended to consume a fat-restricted diet based on the definitive genetic diagnosis, with several explorations and modifications according to previous reports [1,3,5]. Treatment with adult low-fat milk powder (a limited fat of 3.6% of total calories) was subsequently chosen as an alternative. The consumption and ratio of milk powder and water was intensively calculated based on the weight and energy requirements. The patient's growth parameters rapidly improved during regular follow-up (Figure 3). At of 15 months of age, her malnutrition was corrected with a catch-up with a weight of 9.7 kg (weight for age: +0.08 SDS, weight for length: +1.75 SDS). The short stature was slightly ameliorated (recumbent length 70.6 cm, length for age: -2.56 SDS). The absorptive parameters were satisfactory, with normal albumin and fat-soluble vitamin levels (Vitamin A, D, E, K). Triglycerides was also slightly elevated (1.7 mmol/L,

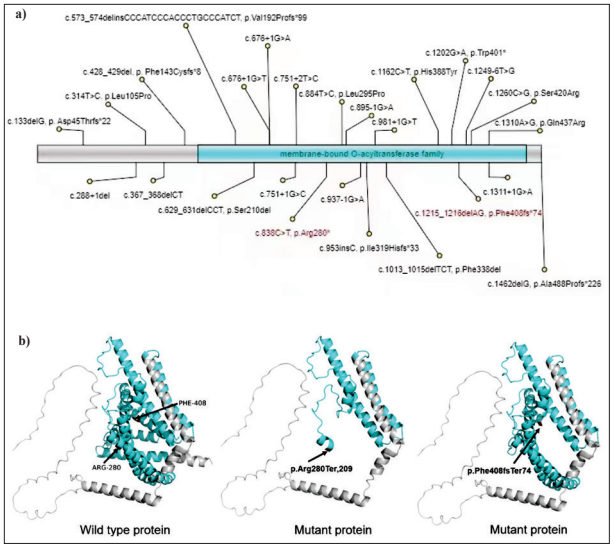

Figure 2. (a) The schematic presentation of the *DGAT1* mutation spectrum in previous studies, and the mutations identified in this study are shown in red. (b) DGAT1 structure model with *DGAT1* mutations identified in this study. Note: these pictures are drawn by using DOG2.0 (http://dog.biocuckoo.org/) and PyMOL (www.pymol.org). Reference database for protein domain (https://www.ebi.ac.uk/interpro/protein/UniProt/O75907/entry/pfam/#table).

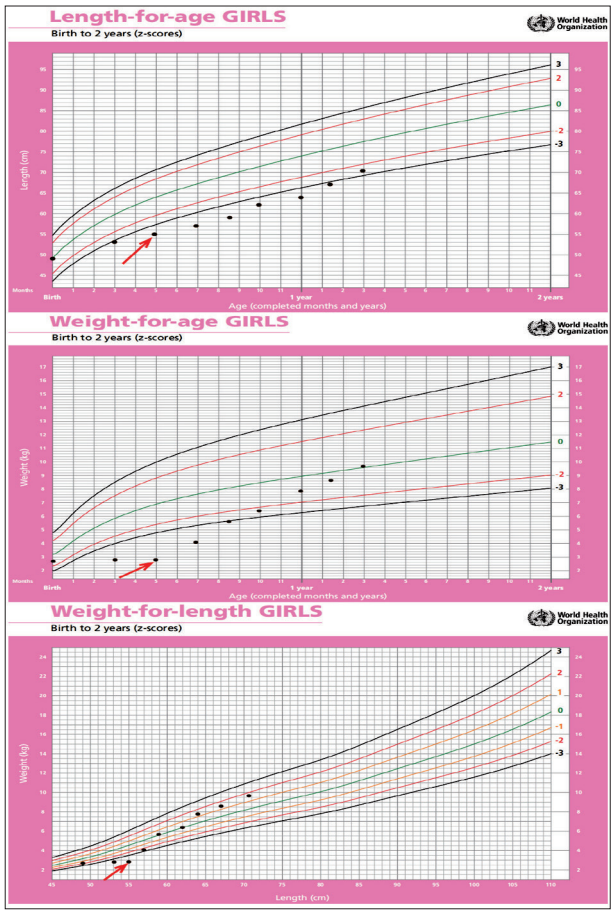

Figure 3. The growth curve of the proband. Red arrows indicate the time when the proband started a fat-restricted diet.

Table 1. Characteristics of published DGAT1 deficiency patients

Descent	DGAT1 mutation site	Protein position	Onset age	Phenotype
Turkish [1]	c.1202G>A	p. Trp401Ter	Birth (diarrhea)	FTT, vomiting, hypoalbuminemia, hypogammaglobulinemia, edema
Turkish [1]	c.573_574delAGinsCCCAT CCCACCCTGCCCATCT	-	3 weeks (diarrhea)	Vomiting, hypoalbuminemia, edema, FTT, hypogammaglobulinemia, hypertriglyceridemia
Turkish [1]	c.937-1G>A	-	2 months (diarrhea)	FTT, diarrhea, vomiting, hypoalbuminemia, hypogammaglobulinemia
Turkish [1]	c.953insC	p. Ile319Hisfs*31	Before the age of 2.5 months (diarrhea)	FTT, vomiting, diarrhea, hypoalbuminemia, hypogammaglobulinemia
Caucasian [1] and NM [6]	c.629_631delCCT	p. Ser210del	Before the age of 2 weeks (diarrhea, vomiting)	Vomiting, diarrhea, FTT, malnutrition, hypoalbuminemia.
NM [5]	c.288+1del c.629_631del	p.? p. Ser210del	Before the age of 17 day (vomiting, diarrhea, weight loss)	FTT, vomiting, diarrhea, hypoalbuminemia
NM [5]	c.428_429del c.629_631del (paternal)	p. Phe143Cysfs*8 p. Ser210del	The early postnatal period (feed intolerance)	FTT, vomiting, diarrhea
Mexican [6]	c.676+1G>A	-	11 days (vomiting)	Vomiting, Diarrhea, feeding difficulties, FTT, mild developmental delay
NM [6]	c.1311+1G>A c.1462delG	- p. Ala488Profs*226	1 week (vomiting, tired, and sleepy during a feed)	FTT, diarrhea, vomiting, nutritional microcephaly, anemia, arachnodactyly and mild dysmorphic facial features, hypogammaglobulinemia
NM [6]	c.1310A>G (de novo) c.981+1G>T(maternal)	p. Gln437Arg -	3 weeks (poor feeding and vomiting)	FTT, poor feeding, vomiting, short stature, rickets, abnormal brain MRI, anemia, hypoglycemia.
Ashkenazi Jewish [3] and Hispanic [7]	c.751+2T>C	-	Before the age of 7 weeks (vomiting)	Diarrhea, vomiting, malnutrition, hypertriglyceridemia, or triglyceride levels were normal, and hypoalbuminemia
South Asian [8]	c.314T>C	p. Leu105Pro	Shortly after birth (diarrhea)	Diarrhea, FTT, hypertriglyceridemia, hypoalbuminemia
Chinese [11]	c.895-1G>A (paternal) c.751+1G>C (maternal)	- -	Soon after birth (vomiting)	Vomiting, diarrhea, hypoalbuminemia, hypertriglyceridemia
Chinese [12]	c.895-1G>A	-	Birth (diarrhea, vomiting)	Diarrhea, vomiting, Malnutrition, hypoalbuminemia, intestinal lymphangiectasia,
Chinese [12]	c.1249-6T>G	-	30 months (edema)	Malnutrition, hypoalbuminemia, lymphopenia, edema
Arab-Muslim [4]	c.884T>C	p. Leu295Pro	2 months (diarrhea)	Diarrhea, hypoalbuminemia, hypogammaglobulinemia, FTT, edema, anemia
Chinese [13]	c.676+1G>T(maternal) c.367_368delCT(paternal)	- -	Birth (diarrhea, vomiting)	FTT, diarrhea, vomiting, hypoalbuminemia, and triglyceride levels were normal
Latin America [14]	c.1162C>T c.838C>T	p. His388Tyr p. Arg280*	2 months (diarrhea)	Diarrhea, growth retardation, anemia, hypoalbuminemia, thrombocytosis, hypogammaglobulinemia.
Caucasian [15]	c.1013_1015delTCT(maternal) c.1260C>G (paternal)	p. Phe338del p. Ser420Arg	1 months (vomiting)	FTT, vomiting, diarrhea, malnutrition, hypoalbuminemia, rickets
Chinese [16]	c.133delG	p. Asp45Thrfs*22	20 days (vomiting)	FTT, feeding difficulties, vomiting, diarrhea, hypoalbuminemia, hypertriglyceridemia
Chinese (This study)	c.1215_1216 del AG (paternal) c.838C>T (maternal)	p. Phe408fsTer74 p. Arg280*	Birth (diarrhea)	FTT, feeding difficulties, vomiting, hypoalbuminemia, hypertriglyceridemia

FTT: Failure to thrive; NM: not mentioned

normal range: 0.4-1.69 mmol/L). Our therapeutic experience was supportive for early fat-restricted enteral diet in *DGAT1*-related CDD7.

DISCUSSION

CDDs are a group of uncommon, clinically varying enteropathies that are often missed or misdiagnosed, and usually present with persistent diarrhea in the first few months of life [6]. If unrecognized, patients can suffer from malnutrition, failure to thrive, and even death [7]. CDD7 is a rare autosomal recessive condition caused by loss of function mutations in the *DGAT1* gene. Since the first case with *DGAT1* mutation was described in 2012 [3], only 32 patients with 24 *DGAT1* mutations have been identified, with varied severity in the disease phenotype [7]. More data about the clinical features are needed to enhance our awareness of the disease. All of the patients with *DGAT1* mutations suffer from diarrhea or vomiting within the first week of life, as well as hypoalbuminemia, and failure to thrive. The severity of the disease is correlated with the amount of residual DGAT1 activity [8]. Our patient had an

early disease onset at 2 days and aroused the attention of the parents as late as 3 months. Moreover, a wrong treatment of high energy formula was provided to deteriorate the diarrhea. The necessity of early genetic diagnosis is critical for the correct breeding strategy of these patients.

To date, 26 variants (including ours) in the *DGAT1* gene have been identified (Table 1), including various types of nonsense, missense, splicing, frameshift, and insertion-deletion. We added two novel mutations to the *DGAT1* mutation spectrum, both of which were supposed to be loss-of-function by creating frame-shift and premature termination codon. The symptoms of our patient were also more severe than in the previously reported cases. It is interesting to see that the construction of DGAT1 deficient mice were lean and resistant to obesity but did not recapitulate the diarrhea observed in human patients [9-10]. The etiology of diarrhea due to DGAT1 deficiency is still unknown [3]. One of the hypotheses is that increased levels of DGAT1 lipid substrates from the diet in the intestine mucosa or lumen could result in cellular dysfunction due to lipotoxic stress in enterocytes [3]. In addition, toxicity to enterocytes could also lead to protein-losing enteropathy which occurs in all patients. Furthermore, a deficiency of DGAT1 could affect bile acid metabolism, and bile acid malabsorption can cause diarrhea [3]. Mild hypertriglyceridemia occurred in some affected patients. Some reasons may be from overcompensation of hepatic DGAT2 or the interruption of bile acid absorption in the distal small intestine [3]. However, not all patients with *DGAT1* mutations present with hypertriglyceridemia. In addition, hypertriglyceridemia also did not appear to be associated with homozygous or heterozygous mutations in *DGAT1*. Therefore, more clinical cases and experiments are needed to clarify this question.

Our patient was promptly switched to a fat-restricted diet as soon as the genetic diagnosis was obtained. We carefully explored the amount of dietary fat, which was on one hand tolerable to the patient, and on the other hand satisfactory for growth. A limited fat to 2%-10% of total calories intake was found effective. This constitution was similar to previous experience suggested by Eldredge et al. [5]. Moreover, the patient was suggested to be fed with small amounts of fat multiple times, which was supposed to increase the tolerance of dietary fat in these patients [8]. Patients with a fat-restricted diet must be monitored for the levels of essential fatty acids, fat-soluble vitamins, serum lipid, and total protein levels. As described in previous literature, most patients develop catch-up growth and normal development after diet modification [11]. Our experience provides an alternative method using calculated adult low-fat milk powder for children who are unable to obtain low-fat infant formula. Our follow-up data showed a satisfactory rate of weight gain and normal metabolic parameters. However, the increase in length was less satisfactory. We speculated that it may be related to the short treatment and follow-up period or the fact that the two novel mutations in the *DGAT1* gene in the child might cause short stature. Therefore, more cases and longer follow-up times will need to be studied in the future.

CONCLUSIONS

We report the clinical presentation, diagnosis, and treatment of an infant with CDD7 caused by two novel variants in the *DGAT1* gene. Our data expanded the mutation spectrum, emphasized the importance of early genetic diagnosis, and shared our successful experience in diet therapy, which might be instructive for pediatricians to better understand the rare *DGAT1*-related CDD7.

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DECLARATIONS

Declaration of Interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

Declaration of Participate Consent

All procedures performed in this study involving human participants were following with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Written consent was obtained from the parents of the proband for the collection of samples and for DNA sequencing.

Declaration of Patient Consent

The authors certify that informed consent for publication of identifying images or the clinical details was obtained from the parents or legal guardians of any participant under the age 18.

Authors' contributions

Zhao Y. designed the research. Li X., Liu X. and Zhao Y. conducted the research. Shi C., Liu X. and Zhao Y. collected and analyzed the data. Shi C. and Liu X. were major contributors to writing the manuscript. Zhao Y. had the primary responsibility of the final content. The authors offered critical comments, read, and approved the final manuscript.

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Availability of data and materials

All datasets generated or analyzed during the current study are included in this published article and available from the corresponding author on reasonable request.

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