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Case Report

A *de novo* homozygous missense mutation of the GUSB gene leads to mucopolysaccharidosis type VII identification in a family with twice adverse pregnancy outcomes due to non-immune hydrops fetalis

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ABSTRACT

Non-immune hydrops fetalis (NIHF) is a common and severe manifestation of many genetic disorders. The ultrasound is an ideal method for diagnosing hydrops fetalis during pregnancy. Since most NIHFs do not have an identifiable cause, determining the underlying etiology remains a challenge for prenatal counseling. Due to advancements in exome sequencing, the diagnostic rates of NIHF have recently increased. As reported here, DNA was extracted from the amniotic fluid of a pregnant woman who was prenatally diagnosed with a NIHF type of unclear origin. Amniocentesis sampling demonstrated a normal female karyotype and copy number variation (CNVs) without alterations. Tri-whole exome sequencing (WES) was conducted to identify possible causative variants. In the fetus, a *de novo* genetic mutation was identified as a homozygous form. The mutation was located on the glucuronidase beta (GUSB) gene: NM_000181.3: c.1324G > A; p. Ala442Thr; Chr7:65439349, which leads to mucopolysaccharidosis type VII. This mutation was inherited from the parents and was first reported to be related to NIHF. We conclude that the use of WES is beneficial for NIHF cases whose prognosis has not been explained by standard genetic testing.

1. Introduction

Non-immune hydrops fetalis (NIHF) has several known genetic etiologies. There are at least 131 genes with strong evidence of causing NIHF and 46 additional genes that are potentially involved in this disease. Variants of these genes can impact the individuals at several levels including metabolic, lymphatic, neuromuscular, cardiovascular, and hematologic [1]. In a large case series, lysosomal storage disorders were responsible for 5.2% of otherwise unexplained NIHF cases [2]. According to a retrospective case control study, the most common lysosomal storage diseases in NIHF were galactosialidosis, sialic acid storage disease, mucopolysaccharidosis VII, and Gaucher disease [3]. An infant with fetal nonimmune edema will likely have a poor prognosis, depending on the etiology, diagnosis, gestational age, Apgar score, degree of resuscitation, and whether the newborn needs transportation [4].

There is a highly rare lysosomal disease known as mucopolysaccharidosis VII (MPS VII, or Sly syndrome) caused by an inability to generate glucuronidase (GUS) enzyme [5]. MPS VII was first described in 1973, thereafter, the glucuronidase beta (GUSB) gene that codifies beta-glucuronidase was cloned and mapped. Patients with MPS VII often present with severe prenatal diseases, such as NIHF. About half of patients with MPS VII die from complications related to NIHF [6].

We describe a new variant of the GUSB gene associated with severe recurrent fetal hydrops in this study. In this study, the parents had a previous fetal loss caused by NIHF. During the second gestation, and similarly to the previous gestation of the couple, a generalized edema was detected. Whole exome sequencing (WES) was performed on a fetal sample. We identified the NM_000181.3: c.1324G > A; p. Ala442Thr; Chr7:65439349 homozygous mutation in the GUSB gene. The pregnant patient terminated the pregnancy at 27 weeks and 4 days. In this study, we describe a rare variant of the GUSB gene that was associated with a severe fetal hydrops case related to MPS VII. MPS VII can be diagnosed early in pregnancy with these data, as well as diagnosed through genetic counseling and preimplantation genetic testing. Additionally, inborn metabolic errors should be considered, in the event of more common etiologies are excluded. MPS VII is the most common lysosomal storage diseases diagnosed in NIHF.

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1.1. Case description

A 23-year-old pregnant woman was referred to our fetal medicine center due to fetal hydrops. A thorough systematic ultrasound examination of the fetus was done at 22 weeks of gestation. According to the ultrasound examination, there was generalized skin edema, bilateral pleural effusions, and peritoneal effusions in the fetus. A normal Doppler, normal placenta, and amniotic fluid were found, without any structural anomalies or signs of fetal anemia (Fig. 1). TORCH screening, blood routine examinations, the hepatorenal function, and the maternal serum indirect Coombs test showed no abnormal changes. Thus, we performed an amniocentesis, which indicated no abnormalities in chromosome karyotype analysis or in copy number variations (CNVs). The pregnant patient had previously been evaluated in our fetal medicine center for a fetus with nonimmune fetal hydrops at 20 weeks. (Fig. 2). Amniocentesis sampling of the first fetus demonstrated a normal male karyotype and no alterations in the CNVs test. WES was not performed during the first pregnancy. Considering the previous history of fetal edema of the pregnant patient, her WES test was targeted at fetal hydrops phenotype disorders. A trio-based WES targeted to the phenotype was requested after informed consent was obtained. The patient was explained the meaning, limitations, and time necessary for results of WES.

2. Methods and materials

2.1. DNA extraction

DNA samples were collected from the amniotic fluid and the peripheral blood of the pregnant patient, using EDTA K2 tubes. 10 mL amniotic fluid was collected in a centrifuge tube. Using the Whole Blood DNA Extraction Kit, genomic DNA was extracted from whole blood (Qiagen Company, Hilden, Germany).

2.2. Whole exome sequencing and data analysis

Using GenCap liquid phase capture target gene technology, a DNA library was built. In this library, the target region was captured and sequenced using a MGISEQ-2000 high throughput sequencer (Shenzhen Hua Da Gene Technology Co., Ltd., Shenzhen, China).

2.3. Bioinformatics analysis of variants

Using the BWA v0.7.17 software, we compared the filtered gene fragment sequence with the human genome reference sequence (hg19), after removing low-quality variation reads. An analysis of single nucleotide polymorphisms (SNP) and insertion deletions (InDel) was performed, using the GATK v4.0.8.1 software. Variations were annotated using the ANNOVAR software. The Search Exome Aggregation

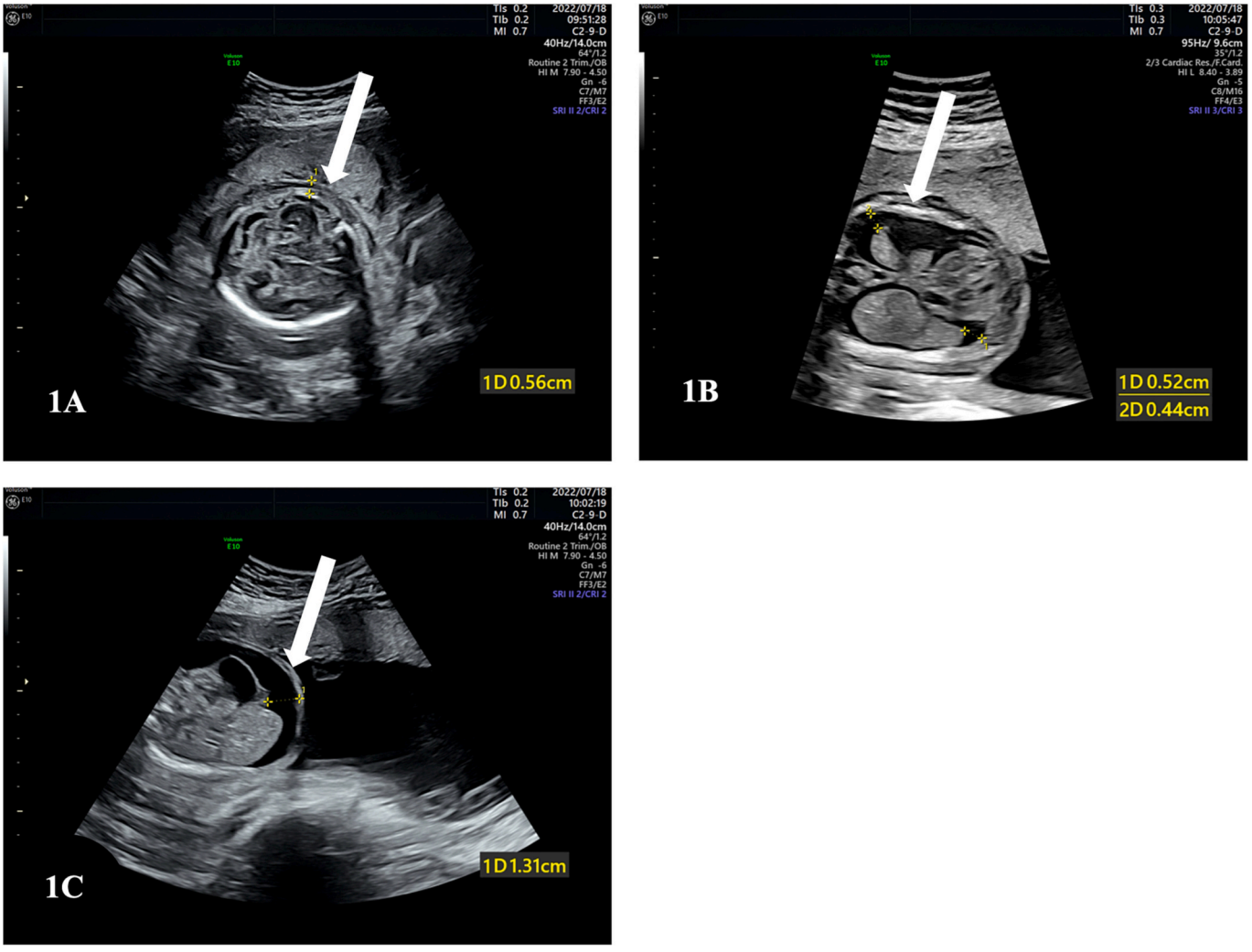

Fig. 1. Fetal system ultrasonography findings at 22 weeks of gestation in the NIHF case with GUSB mutations: (A)subcutaneous edema: 5.6 mm (B) bilateral hydrothorax: 4.4 mm, 5.2 mm (C) peritoneal effusion:13.1 mm.

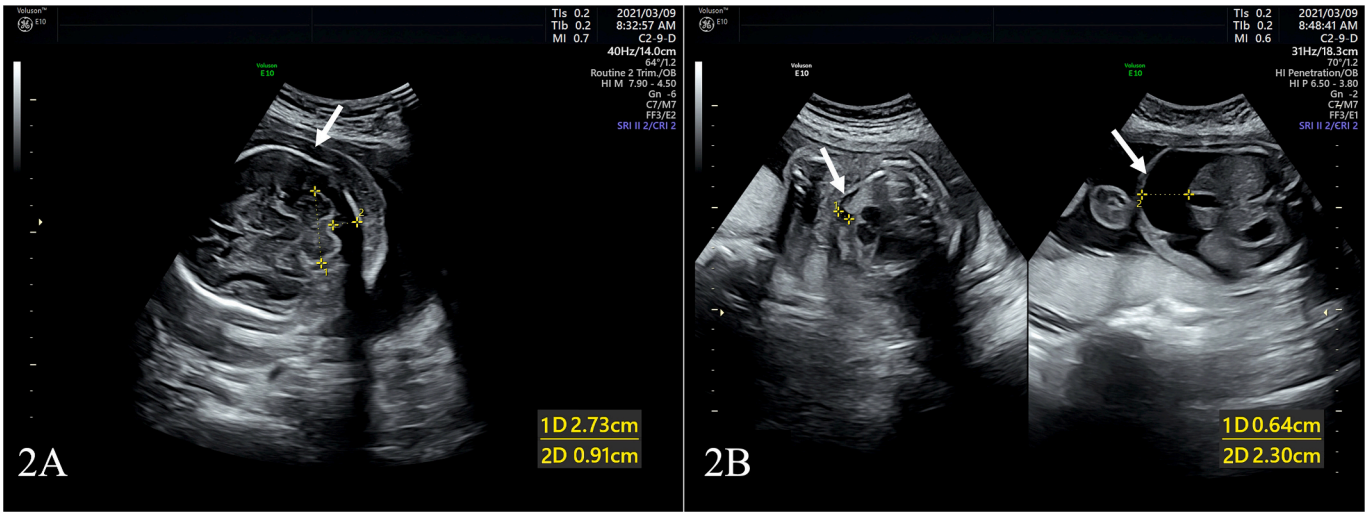

Fig. 2. Fetal system ultrasonography findings at 20 weeks of gestation in the last NIHF case with GUSB mutations: (A)subcutaneous edema (B) pleural effusion and peritoneal effusion.

Consortium, the Genome Aggregation Database, the 1000 Genomes database, the Genome Asia 1000 k database, and other databases were used for population frequency analysis of the mutations. The pathogenicity of the mutation was evaluated by querying the HGMD and the ClinVar databases. The functional effect of the mutation at the protein level was predicted using the SIFT, PolyPhen-2, Mutation Taster, and GERP++ software. To compare GUSB protein sequences among different species, including *Homo sapiens*, *Pan troglodytes*, *Macaca mulatta*, *Canis lupus*, *Bos taurus*, *Mus musculus*, and *Rattus norvegicus*, using NCBI HomoloGene and DNAMAN, multiple sequence alignments were performed. As of the time of documentation, as we currently understand the disease, the ACMG classification and variant interpretation are based on the information we have.

2.4. Conservative analysis

To find homologous genes in other species, the NCBI HomeloGene database and the transcript number of the target protein (NM_000181.3) were used. The UGENE v45.0 software was used to select mammalian sequences for multiple sequence alignment and to find target sites.

3. Results

There were two homozygous variants in GUSB and one in CHD7,

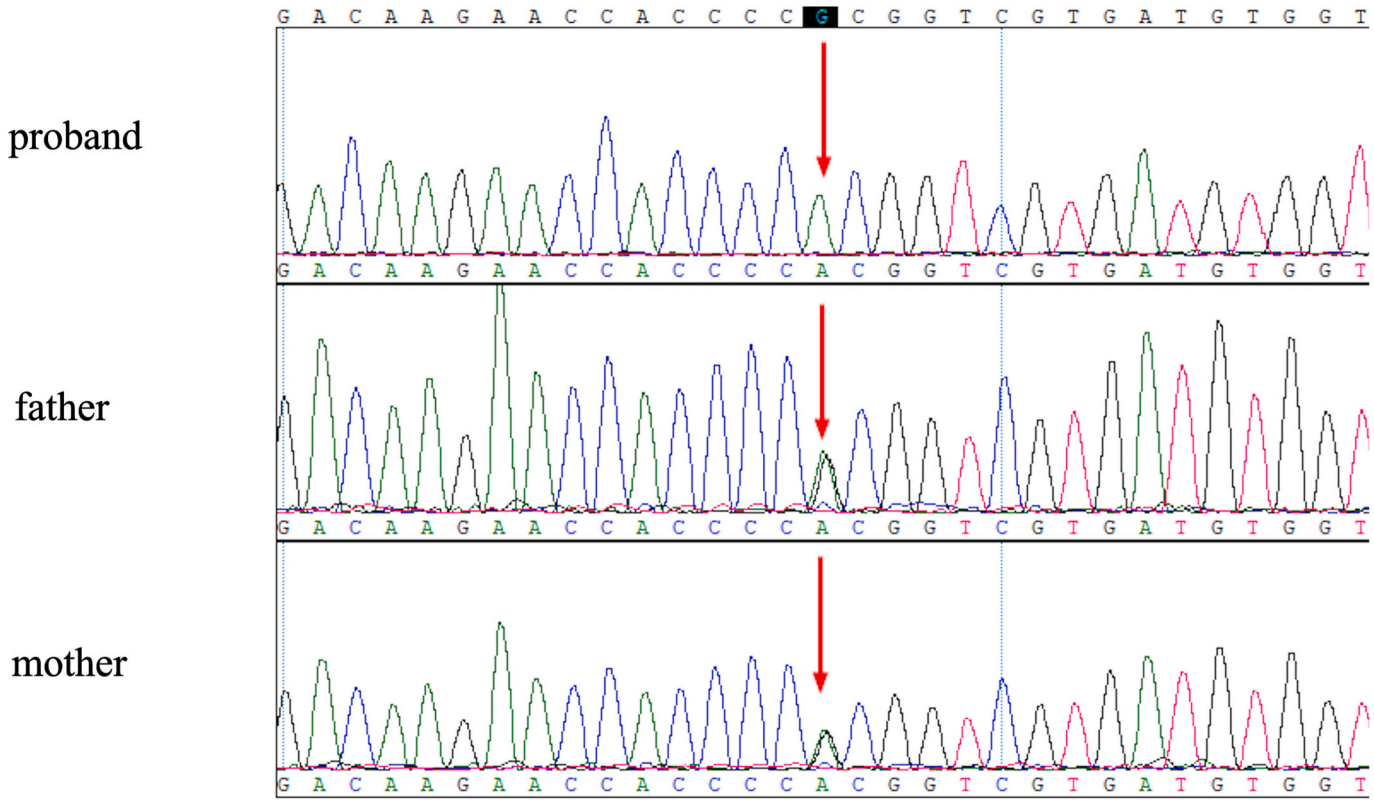

Fig. 3. WES illustrating a *de novo* homozygous GUSB missense variant in the present case.

Genetic analysis was conducted on the results. We excluded the variant of the CHD7 gene as the cause of our observations, because its clinical description in the OMIM database was not related to hydrops. A homozygous missense variant in the GUSB gene was identified, whose associated phenotypes were compatible with the patient's clinical features.

In exon 8 of the GUSB gene on chromosome 7, c.1324G > A causes a single amino acid substitution (from Ala to Thr) at position 442 (p. Ala442Thr). (Fig. 3). To the present date, the c.1324G > A variant has not been included in the HGMD, Clinvar, ExAC, gnomAD, or the 1000 Genomes database (PM2). Bioinformatics analysis using SIFT and Condel predicted the novel variant (NM_000181.3: c.1324G > A, p. Ala442Thr) to be deleterious. According to Mutation Taster, this mutation occurred at a non-conservative site, causing the amino acid sequence to change. Protein features might also be affected (as documented in PM3_Supporting). The mutation associated with the amino acid positions was located in non-conserved functional domains.

WES results revealed that both parents were heterozygous carriers of the GUSB gene variant previously identified in the fetal sample. To further clarify the pathogenicity of the above genes, we sequenced the DNA of the previous edema fetus, by Sanger sequencing. We found that the mutation was also present in the previous fetus (Fig. 4). When the related genetic cause was identified, the parents opted to terminate the pregnancy at 27 weeks and 4 days.

4. Discussion

The autosomal recessive mucopolysaccharide metabolic disorder MPS VII is caused by a deficiency of the GUS enzyme. There are very few accurate epidemiologic data available for MPS VII. The phenotypes range from a severe type with fetal hydrops to a milder type with a late onset and a normal intellectual capacity, MPS VII patients display a wide range of clinical variability [6]. GUS is a glycosyl hydrolase with a relative molecular mass of 332,000 kDa, which hydrolyzes β-glucuronic acid at the end of myxopolysaccharides, in lysosomes. Its four subunits form an enzymatically active tetramer [7]. In lysosomes, GUS is a housekeeping enzyme that degrades proteoglycans. It plays a vital role in the degradation of dermatan sulfate, heparan sulfate and chondroitin sulfate. Therefore, GUS deficiency leads to impaired degradation of these three substances, which are deposited and accumulated in lysosomes of various tissues, leading to the dysfunction of cells and organs.

In the long arm of chromosome 7 (7q11.21–7q11.22), the GUSB gene encodes the GUS enzyme. A 651-amino acid precursor and a mature 629-residue protein are encoded by its 12 exons. It displays significant genetic heterogeneity [8]. In total, 80 variants of the GUSB gene have been described. 74% of these are missense mutations, 11% are nonsense mutations, 5% are splicing mutations, and 7% are small deletions or indel mutations [9].

Recently, WES has been used to genotype NIHF patients with normal karyotypes and CNVs. WES has been successfully used in 29–50% of samples from studies exploring the genetic etiology of prenatally diagnosed NIHF since the development of molecular genetics [10–12]. It also enables identification of carriers, genetic counseling for families, and prenatal genetic testing for future pregnancies.

In our study, the fetus was homozygous for a missense variant of the GUSB gene, which has not been previously reported. The homozygous mutation c.1324G > A, which was inherited from the parents, was found in exon 8. c.1324G > A was a missense mutation that caused the alanine at position 442 of the β-glucuronidase protein to be replaced by a threonine, which could cause the loss of function of the protein. The pathogenicity of the c.1304 G > C mutation has been reported in other countries [13].

According to Sanger sequencing results, the last edema fetus of this couple carried homozygous missense variants of the GUSB gene. Thus, the phenotypes of the patients and of their previous fetus could be considered as supporting evidence (PP4). Due to the lack of *in vivo* or *in vitro* functional studies of the deleterious effects of the mutations and relevant reports on pathogenicity, the mutation was classified as a variant of unknown clinical significance (VUS), based on ACMG guidelines (c.1324G > A: PM2 + PM3 + PP4).

There are several genetic variants associated with MPS VII. The most common of these variants is p. Leu176Phe, which is found in different cohorts worldwide. The clinical manifestations of p. Leu176Phe homozygotes have been reported in more recent reports as variable, including the severe spectrum, suggesting that the genotype–phenotype correlation is not as straightforward as previously thought. As such, it could be affected by other factors [14]. In the lysosome, the lack of GUS activity causes the sialic acid residue at the end of the sugar chain to be unable of degradation, which affects the subsequent hydrolysis of dermatan sulfate. As a result of the large amounts of dermatan sulfate deposited in lysosomes, cells become vacuolated, which further damages their function [13]. Based on the phenotype of homozygotes and the predicted change in tertiary structure of the protein, individual mutations and disease severity are correlated, and the observed *in vitro* levels of enzyme activity. Hence, additional functional experiments are needed to confirm the mutation's biological effects.

Managing patients affected with MPS is a joint effort that involves the patient's family members and a dedicated team of specialists including pediatricians, endocrinologists, cardiologists and ophthalmologists. Hematopoietic stem cell transplantation and enzyme replacement therapy have been performed in some institutions for patients with MPS VII. In 1998, Yamada [15] conducted allogeneic hematopoietic stem cell transplantation on a 12-year-old Japanese girl, resulting in improved daily living abilities; however, there was no significant enhancement observed in cognitive function. Fox [16] conducted a clinical trial of recombinant human β-glucuronidase on a 12-year-old girl with late-stage MPS VII, resulting in improved liver, kidney, and lung function as well as enhanced physical activity. Gene therapy is currently only in the stage of animal model research [17,18], and further human trials are necessary to gain a better understanding of its effects and potential complications.

Inconclusion, this study highlights the benefits of WES in prenatal diagnosis, which suggests that the novel variant identified in the GUSB gene could be associated with NIHF. When chromosome and CNV testing were not able to identify the cause of NIHF, exome sequencing proved valuable. Genetic analysis is very helpful for genetic counseling and prenatal diagnosis. The application of genetic analysis enables us to achieve early detection, accurate diagnosis, and effective treatment of this disease, potentially leading to less intricate disease progression [19].

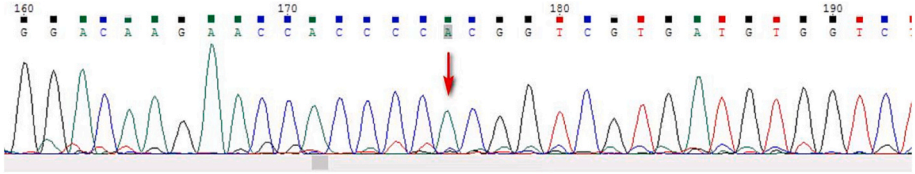

Fig. 4. Sanger sequencing illustrating the *de novo* homozygous GUSB missense variant in the last case.

CRediT authorship contribution statement

Runxuan Du: Writing – original draft. **Haishen Tian:** Writing – review & editing. **Bingyi Zhao:** Software. **Xuedong Shi:** Formal analysis. **Yanmei Sun:** Investigation. **Bo Qiu:** Software. **Yali Li:** Funding acquisition, Writing – review & editing.

Declaration of Competing Interest

None.

Data availability

No data was used for the research described in the article.

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