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A Boy with Sandestig-Stefanova Syndrome and Genital Abnormalities

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Established Facts

- Sandestig-Stefanova syndrome is an autosomal recessive developmental syndrome characterized by microcephaly, trigonocephaly, congenital cataracts, microphthalmia, facial findings, camptodactyly, periventricular white matter loss, thin corpus callosum, delayed myelination, and poor prognosis.
- Sandestig-Stefanova syndrome, caused by biallelic loss-of-function mutations in the *NUP188* gene, has been described in 2 studies in the literature to date.

Novel Insights

- Our patient is the first case of Sandestig-Stefanova syndrome reported from Turkey.
- Moreover, immunodeficiency, congenital hypothyroidism, biotinidase deficiency, undescended testis, hypospadias, and ambiguous genitalia are defined for the first time in this syndrome.

Keywords
Hypotonia · Microcephaly · Mutation · *NUP188* gene · Sandestig-Stefanova syndrome

Abstract
Introduction: Sandestig-Stefanova syndrome is an autosomal recessive developmental syndrome characterized by microcephaly, trigonocephaly, congenital cataracts, microphthalmia, facial findings, camptodactyly, periventricular white matter loss, thin corpus callosum, delayed myelination, and poor prognosis. This syndrome is caused by biallelic loss-of-function mutations in the *NUP188* gene. ***Case Presentation:*** In the physical examination of our patient, whose mother and father were third-degree relatives, hypotonia, bilateral congenital cataracts, ambiguous genitalia, hypospadias, undescended testis, and facial dysmorphic findings (hypertelorism, high palate, micrognathia, microphthalmia, low-set ears) were detected. ***Discussion:*** In our patient, a homozygous c.1087C>T (p.Gln363Ter) variant was detected in exon 11 of the *NUP188* (NM_015354.3) gene. The mother and father were found to be heterozygous carriers of this variant. All patients with the diagnosis of Sandestig-Stevanova syndrome reported in the literature are female. Our patient is the first male patient reported with this syndrome. In addition, immunodeficiency, congenital hypothyroidism, biotinidase deficiency, undescended testis, hypospadias, and ambiguous genitalia are defined for the first time in this

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syndrome. Our patient is the first case of Sandestig-Stefanova syndrome reported from Turkey. In this study, Sandestig-Stefanova syndrome with a novel pathogenic *NUP188* gene variant is presented.

Introduction

Sandestig et al. [2020] reported for the first time that biallelic loss-of-function mutations in the *NUP188* gene caused a new autosomal recessive developmental syndrome named Sandestig-Stefanova syndrome (SANDSTEF; OMIM #618804). This syndrome is characterized by microcephaly before and after birth, trigonocephaly, congenital cataracts, microphthalmia, facial findings, camptodactyly, periventricular white matter loss, thin corpus callosum, delayed myelination, and poor prognosis [Sandestig et al., 2020]. Although pathogenic nucleoporin (NUP) 188 variants cause multisystem disorders, affected individuals usually present with central nervous system (CNS) findings.

Sandestig-Stefanova syndrome is a newly defined syndrome and has been described in only 2 studies in the literature to date [Muir et al., 2020; Sandestig et al., 2020]. We describe a novel variant in the *NUP188* gene in a male patient, which has not been reported in genetic databases and in the literature before.

Case Report

A male baby with a length of 48 cm, head circumference of 34 cm, and weight of 2,480 g, was delivered by cesarean section at 37 weeks of gestation as the first living child from the 4th pregnancy of a 20-year-old mother. Our patient was born small for gestational age. The first baby from the other 3 pregnancies of the mother died when it was aged 37 days of congenital heart disease. One died intrauterine, and the other pregnancy was detected as an empty sac. Our patient, whose mother and father are third-degree rela-

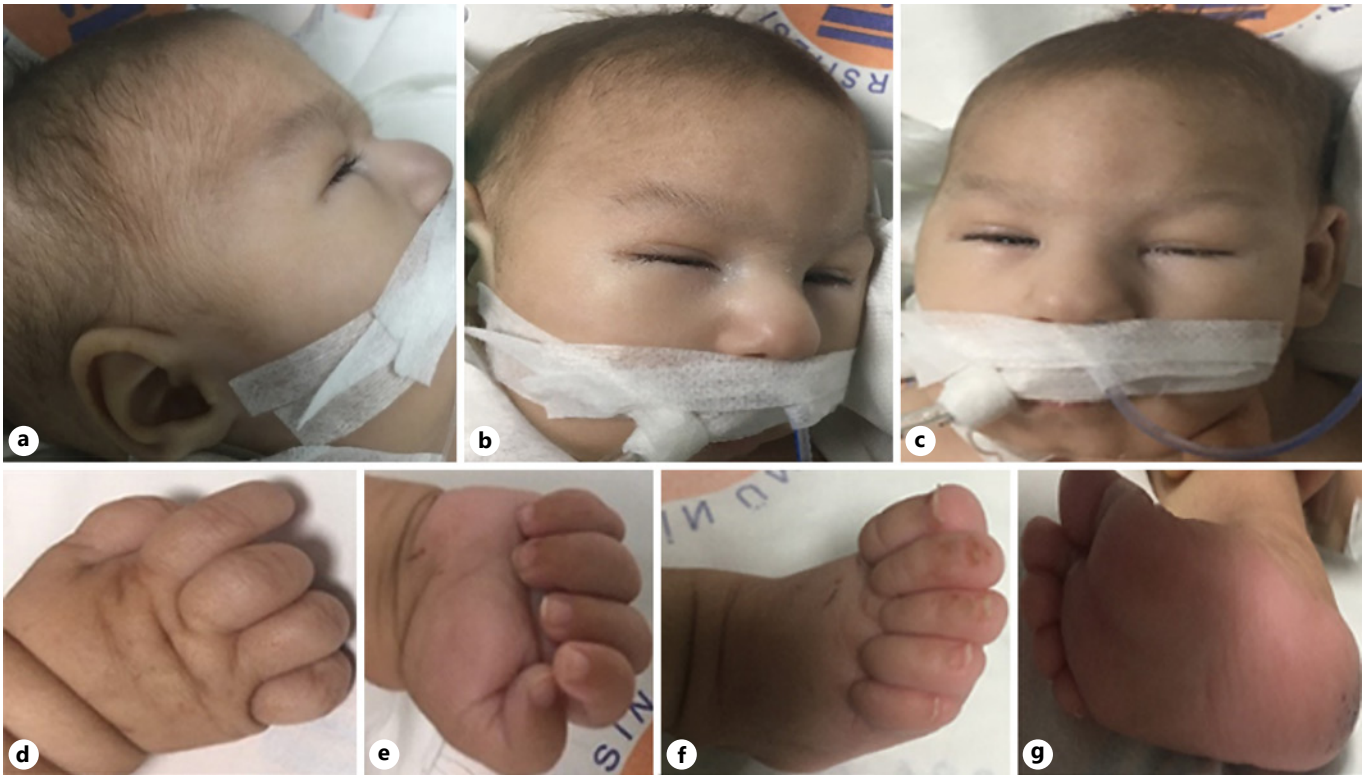

Fig. 1. Facial features and digit anomalies of the patient. **a** Low-set ears, hypoplastic tragus, prominent angulated antihelix. **b** Sparse medial eyebrows, laterally extended arched eyebrows, metopic ridge, wide prominent nasal bridge and wide convex nasal ridge. **c** High anterior hair line, narrow lateral forehead, broad forehead, microcephaly, trigonocephaly, microphthalmia, epicanthus, retrognathia, short neck, small and downslanting palpebral fissures. **d**, **e** Clinodactyly and camptodactyly. **f**, **g** Overlapping toes, hammer toe, and camptodactyly.

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tives, was hospitalized in the neonatal intensive care unit due to postpartum respiratory distress and dysmorphic findings. In his physical examination, hypotonia, bilateral congenital cataracts, ambiguous genitalia, hypospadias, undescended testis, bifid scrotum, and facial dysmorphic findings (hypertelorism, high palate, micrognathia, microphthalmia, low ear) were recorded (Fig. 1; Table 1). Metabolic screening tests and chromosome and microarray analyses of the patient were found to be normal. Thereupon whole-exome sequencing (WES) analysis was performed. Biotinidase deficiency was detected in newborn screening tests.

When he had a seizure in the form of a spasm in the upper extremities, levetiracetam was started at the age of 2.5 months. The patient's electroencephalogram (EEG) was reported as cerebral dysfunction and dysmaturity. In addition, active epileptiform encephalopathy was reported. He underwent surgery on his left eye due to a congenital cataract. With the diagnosis of congenital hypothyroidism, levothyroxine treatment was started. The patient, who developed respiratory failure during his follow-up, was admitted to the pediatric intensive care unit (PICU) as intubated when he was aged 4 months. Due to septic shock, inotropic drugs and appropriate antibiotic therapy were started. Although 2 doses of the hepatitis B vaccine were administered, anti-Hbs and HbsAg were negative. In the cluster of differentiation (CD) panel, CD8 was low (9.9%), and the patient had lymphopenia. The patient was followed up with the diagnosis of combined immunodeficiency. Intravenous immunoglobulin was given twice due to hypogammaglobulinemia. In cerebral magnetic resonance imaging, corpus callosum and cerebral atrophy were detected, the third and lateral ventricles were dilated, and the extra-axial cerebrospinal fluid distance was enlarged. No congenital anomaly was detected in the thorax and abdomen computed tomography. Using scrotal ultrasonography, it was determined that neither testicle could be ob-

Table 1. Clinical findings of the 8 published female cases and our patient

Features	Our patient	Patients in the literature (*n* = 8)
NUP188 variant	Homozygous	Homozygous/compound heterozygous
Gender	Male	Female
Age of death, months	6	1–31
Hypotonia	+	8/8
Preterm/SGA	+	8/8
Bilateral congenital cataract	+	6/8
Microphthalmia	+	8/8
Microcephaly	+	8/8
Metopic ridge	+	8/8
Trigonocephaly	+	8/8
Respiratory failure	+	8/8
Heart anomalies	+ (LV hypertrophy, LV aberrant band, PDA [small])	7/8 (1 patient not tested)
Brain MRI		
Wide ventricles	+	7/8
Loss of white matter	+	4/8
Thin corpus callosum	+	7/8
Delayed myelination	+	5/8
Epilepsy	+	3/8
Narrow lateral forehead	+	8/8
Broad forehead	+	8/8
Wide prominent nasal bridge	+	8/8
Wide convex nasal ridge	+	8/8
Micrognathia	+	8/8
Short neck	+	8/8
Camptodactyly	+	8/8
Clinodactyly	+	8/8
Overlapping toes	+	8/8
Rocker-bottom feet	+	8/8
Low-set ears	+	8/8
Immunodeficiency	+	0/8
Congenital hypothyroidism	+	0/8
Biotinidase defect	+	0/8
Undescended testicle	+	0/8
Ambiguous genitalia	+	0/8
Hypospadias	+	0/8

SGA, small for gestational age; LV, left ventricle; PDA, patent ductus arteriosus; +, present.

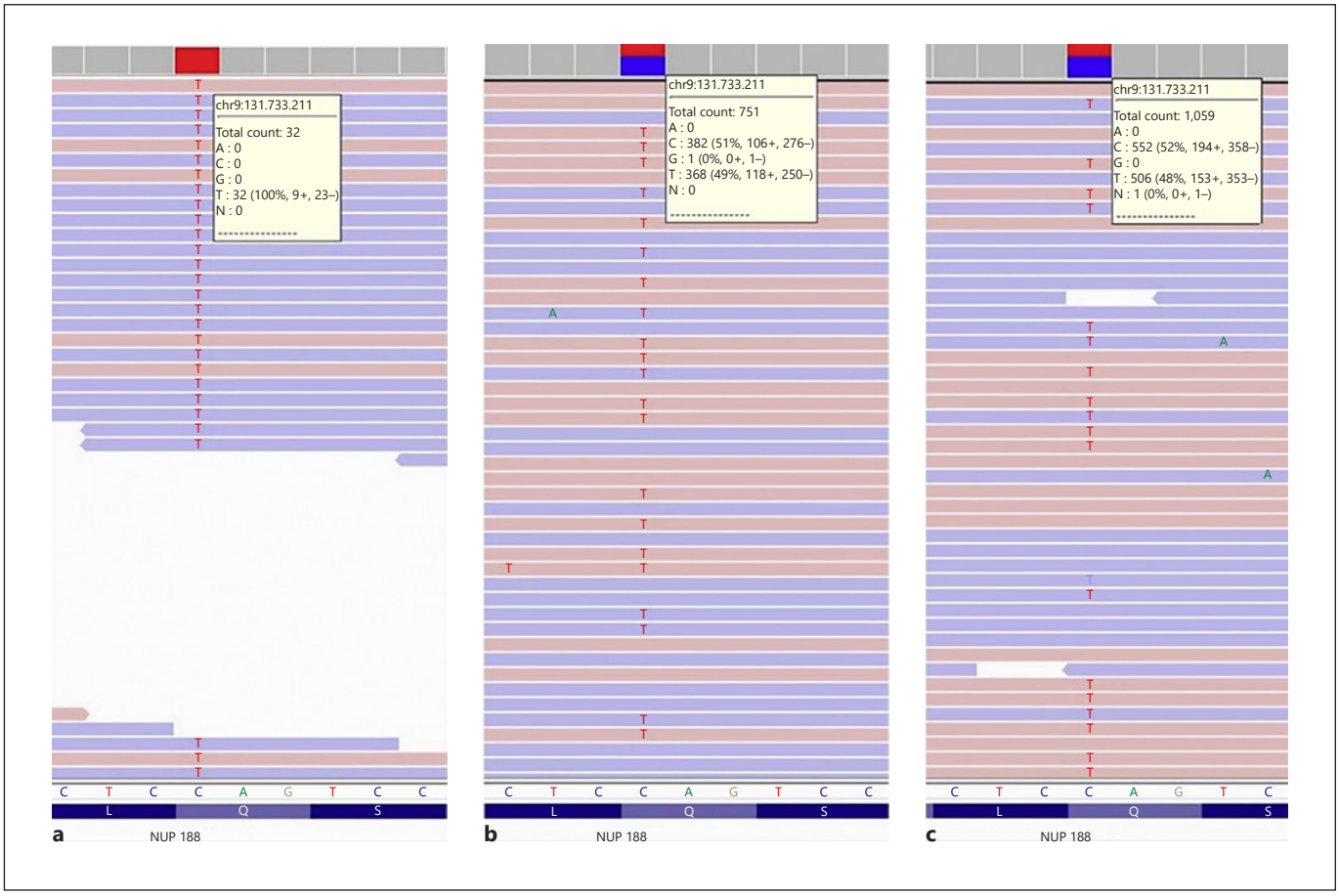

Fig. 2. Detected novel *NUP188* gene pathogenic variant. **a** Proband, homozygous (affected). **b**, **c** Father (**b**) and mother (**c**) are heterozygous carriers.

served in the scrotum. Peripheral blood chromosome analysis of the patient revealed a 46,XY karyotype. In the microarray (Affymetrix CytoScan® Optima Suite (315K)) study for the detection of copy number variations (CNVs), no CNV was describing the patient's clinic. Then, WES (TWIST Human Core Exome®) analysis was performed using next-generation sequencing. A homozygous c.1087C>T (p.Gln363Ter) variant was detected in exon 11 of the *NUP188* (NM_015354.3) gene in the patient. This variant has not been previously reported in the gnomAD exomes, gnomAD genomes, ClinVar, and dbSNP databases or in the literature, so it was considered a novel variant. This novel variant was considered pathogenic because it met the criteria for PVS1, PM2, PP3, and PP4 according to the American College of Medical Genetics (ACMG) classification. The mother and father were found to be heterozygous carriers of the *NUP188* c.1087C>T change. According to the results of the WES analysis shown in Figure 2, our patient was diagnosed as having Sandestig-Stefanova syndrome. He was followed up in the PICU for a total of 2 months, 3 weeks with a high-flow nasal cannula and 5 weeks as intubated. The patient died at the age of 6 months of central respiratory failure. However, the patient's parents did not permit an autopsy.

Discussion

All patients with the diagnosis of Sandestig-Stevanova syndrome reported in the literature are female. Our patient is the first male patient reported with this syndrome. In addition, genitourinary disorders such as ambiguous genitalia, undescended testis and hypospadias, immunodeficiency, congenital hypothyroidism and biotinidase deficiency, which we found in our patient, are described for the first time in Sandestig-Stevanova syndrome.

The nuclear pore acts as a highly specific controller, regulating the exchange of macromolecules between the nucleus and the cytoplasm. NUP188 is predicted to interact with other proteins to form the basic skeleton of the nuclear pore [Miller et al., 2000]. The *NUP188* gene is located at 9q34.11 and encodes the nucleoporin NUP188 homolog (hNup188), which is part of the large nuclear pore complex (NPC). NPCs are water channels built by a

complex network of proteins known as nucleoporins that control all entry and exit between the nucleus and cytoplasm [Jühlen and Fahrenkrog, 2018].

Sandestig et al. [2020] performed trio-exome sequencing on 2 unrelated girls with similar congenital anomalies and their parents. Both girls died a few months after birth due to central respiratory failure. The authors detected homozygosity for 2 different truncating mutations in the *NUP188* gene for the first time. They defined Sandestig-Stefanova syndrome according to the genetic characteristics, dysmorphic appearance, and laboratory and imaging results of these 2 patients. Microcephaly, trigonocephaly, congenital bilateral cataract, cleft lip/palate or high-arched palate, camptodactyly, rocker-bottom feet, congenital heart disease, cerebral changes (such as periventricular white matter loss), thin corpus callosum, and delayed myelination were detected in both patients. In addition to these, the facial shapes were also found to be similar. The homozygous truncating *NUP188* mutations observed in both children underlay all these abnormalities suggesting the presence of "nucleoporin 188 deficiency syndrome" [Sandestig et al., 2020].

A literature review showed that Muir et al. [2020] also defined the same syndrome in 6 children from 4 families in 2020, with phenotype and genetic results. They described homozygosity or compound heterozygosity for mutations in the *NUP188* gene in the identified patients. Functional analysis showed that disruption of *NUP188* was associated with low active protein transport to the nucleus. All 6 affected individuals had similar clinical features. All individuals were small at birth, and growth parameters were <10th percentile for gestational age. Dysmorphic features such as a tubular nose and a wide nasal bridge, small palpebral fissures, epicanthal folds, micrognathia, camptodactyly, and digital anomalies including a wide big toe were detected. Congenital cataracts in 4 patients and congenital heart anomalies including bicuspid aortic valve, partial abnormal pulmonary venous return, and patent ductus arteriosus were seen in 5 patients. All 6 patients presented with congenital hypotonia, delayed myelination, and white matter abnormalities. Most had a thin corpus callosum, and at least 3 patients had microcephaly with progressive neurodegeneration.

In our patient, phenotypic features such as microcephaly, trigonocephaly, microphthalmia, high-arched palate, retrognathia, clinodactyly, camptodactyly, rocker-bottom feet; CNS pathologies such as hypotonia, epilepsy, thin corpus callosum and ventriculomegaly; organ anomalies such as cardiac anomalies, central respiratory failure, and congenital cataracts were found to be similar to the patients described by both Sandestig et al. [2020] and Muir et al. [2020]. However, immunodeficiency, congenital hypothyroidism, biotinidase deficiency, undescended testis, hypospadias, and ambiguous genitalia, detected in our patient, have not been previously defined in the literature in patients with Sandestig-Stefanova syndrome.

Although pathogenic *NUP188* variants cause multisystem disorders, affected individuals usually present with CNS findings. Generalized hypotonia, progressive microcephaly, seizures, cerebral atrophy, thin corpus callosum, and central hypoventilation are the most important CNS findings. Neurologic functions are progressive neurodegenerative and patients cannot gain functions such as sitting and walking. As in our patient, patients who are fed orally after birth lose their swallowing function over time and become dependent on nasogastric/orogastric tubes. Similarly, epileptic seizures begin over time, and cerebral dysfunction and dysmaturity are usually detected in EEG. Patients die in the early period due to respiratory failure secondary to CNS anomalies. No signs of congenital pulmonary or tracheal disease were detected in the identified patients.

The nucleus is a specific and distinctive organelle in all eukaryotic cells surrounded by a double membrane called the nuclear envelope. The nuclear envelope mainly consists of 3 parts: the double membrane, the nuclear lamina, and the NPC. The outer leaf of the bilayer membrane surrounding the perinuclear cavity is continuous with the lumen of the granular endoplasmic reticulum, and the inner leaf contains some specialized nuclear envelope transmembrane proteins [Swift and Discher, 2014]. The protein composition of NPCs varies in different organisms and between different cells of the same organism. Therefore, in addition to their role in nucleocytoplasmic transport, they also play a role in other cellular processes such as gene expression, chromatin organization, and regulation of the cell cycle [Sakuma and D'Angelo, 2017].

The *NUP188* gene encodes a protein involved in the NPC and controlling the passage of membrane or transmembrane proteins as part of the hNup93 subcomplex [Beck and Hurt, 2017]. Apart from its role in nuclear transport, NUP188 also plays a role in various cellular functions, including ciliogenesis [Del Viso et al., 2016], chromatin organization [Labade et al., 2016], transcriptional regulation [Labade et al., 2016], and chromosome segregation [Itoh et al., 2013]. In the study by Muir et al. [2020], clinical features were reported that can be seen in heterotaxy or ciliopathies, especially in some patients, such as polysplenia, preaxial polydactyly, possible rib

anomalies, and cerebellar malformations. The authors speculate that although their study assumes that the reduction in nuclear transport is at least partially responsible for the phenotype of the affected individuals, the possibility that disruption of one or more of the other functions of NUP188 may also contribute to disease progression [Muir et al., 2020]. Loss of NUP188 results in a reduction of nuclear import and mRNA export in yeast [de Bruyn Kops and Guthrie, 2018].

WES analysis was performed using next-generation sequencing for genetic diagnosis, and a homozygous c.1087C>T (p.Gln363Ter) variant was detected in the 11th exon of the *NUP188* (NM_015354. 3) gene in our patient. In the literature reports, Sandestig et al. [2020] determined homozygosity for mutations in the *NUP188* gene of 2 girls from different families. Muir et al. [2020], investigating 6 girls from 4 different families, defined homozygosity for mutations in the *NUP188* gene in 2 of them and compound heterozygosity in the other 4 children. Nine patients, including our patient, were of European descent, 3 of whom were non-Finnish, 1 Syrian, 1 Indian, 1 Turkish, and 3 Ashkenazi Jews. It may therefore be too early to suggest an ethnic or geographic prevalence in this syndrome.

The patients described by Sandestig et al. [2020] were born small for gestational age, developed respiratory failure, and died of central respiratory failure at the age of 67 and 140 days, respectively. All patients described by Muir et al. [2020] died of respiratory failure or respiratory-related disease before the age of 3 years. Five of the 6 patients died in the first 7 months of their lives, the 6th patient lived for 2 years and 7 months, but the patient was severely mentally disabled and unable to walk. Our patient, like the patients reported in the literature, was born small for gestational age. He was followed up for a long time after his birth due to respiratory distress and died of central respiratory failure at the age of 180 days.

Evaluation of *NUP188* should be considered in infants with neurologic deficits, congenital cataracts, congenital heart defects, and unexplained respiratory failure. In our patient, a novel pathogenic variant was detected in the *NUP188* gene. Our patient had Sandestig-Stefanova syndrome, which is newly defined and rarely encountered in the literature. Our case will contribute to the literature because it is the first case reported from Turkey. The present case represents the first male patient with Sandestig-Stefanova syndrome and significantly broadens the phenotypic spectrum of this novel disorder.

Acknowledgement

We sincerely thank the patient's family for cooperation.

Statement of Ethics

Written informed consent was obtained from the patient's family for publication of this case report and accompanying images. According to the clinical research ethics committee of Mersin University, where the study was conducted, ethics committee approval is not required for case reports.

Conflict of Interest Statement

The authors declare no conflicts of interest.

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Author Contributions

Project design: A.K., B.B., and A.E.A. Data collection and clinical evaluation: A.K. and M.A. Sequence analysis: A.K. and B.B. Preparation of the manuscript: A.K., B.B., A.E.A., and M.A.

Data Availability Statement

All data generated or analyzed during this study are included in this article. Further inquiries can be directed to the corresponding author.

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