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case report

A Rare Etiology of 46, XY Disorder of Sex Development and Adrenal Insufficiency: A case of MIRAGE syndrome caused by mutations in *SAMD9* gene

Running Head:MIRAGE Syndrome

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What is already known on this topic?
The recently described MIRAGE syndrome, which has autosomal dominant inheritance, is a very rare form of syndromic adrenal hypoplasia.

What this study adds?
Here, we presented the first syndromic adrenal hypoplasia case which was diagnosed with MIRAGE syndrome in Turkey.

Abstract
Adrenal hypoplasia is a rare congenital disorder. In spite of biochemical and molecular genetics evaluation, etiology in many patients with adrenal hypoplasia is not clear. MIRAGE syndrome is a recently recognized congenital disorder characterized by myelodysplasia, infection, growth restriction, adrenal hypoplasia, genital phenotypes, and enteropathy. We present here a case of MIRAGE syndrome due to a heterozygous missense variant c.2920G>A; p.E974K mutation in sterile alpha motif domain-containing protein-9 (*SAMD9*) gene. This report describes the first MIRAGE syndrome patient in Turkey.
Key words: Adrenal hypoplasia, 46, XY Disorder of Sex Development, MIRAGE Syndrome

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Introduction

Normal gonadal differentiation and sex development depend on the meticulous choreography and synchrony of a network of endocrine, paracrine, and autocrine signaling pathways reflecting the actions and interactions of specific genes, transcription factors, and hormones. Perturbations of this intricate network of gene regulation and gene expression governing fetal gonadal development result in disorders of sex development (DSD). These disorders are a congenital condition involving a spectrum of abnormalities in which the chromosomal, genetic, gonadal, hormonal or anatomical aspects of the sex are atypical (1). DSD patients were grouped according to karyotypes: 46, XY DSD, 46, XX DSD and sex chromosome DSD (1). Because of the complexities of chromosomal and gonadal development, some diagnoses can be included in more than one of the three major categories. The number of genes identified to be involved in sex development continues to increase. Nevertheless, despite many genetic recent advances the specific molecular etiology of the genital ambiguity in an individual cannot always be identified.

The recently described MIRAGE syndrome (OMIM# 617053), which has autosomal dominant inheritance, is a very rare form of syndromic adrenal hypoplasia. Its prevalence is <1/1000000 and six core characteristic features are myelodysplasia, recurrent invasive infections, growth restriction, adrenal hypoplasia, genitalia anomalies, and enteropathy. Additional associated features are variable and include prematurity, chronic lung disease, developmental delay, dysmorphism, and central nervous system anomalies (2, 3).

The cause of the syndrome is germ line heterozygous *SAMD9* variants that usually occur *de novo*. The syndrome is caused by gain of function mutations in the *SAMD9*. Cytogenetic location; 7q21.2, which is the long (q) arm of chromosome 7 at position 21.2. This gene encodes a sterile alpha motif domain-containing protein and widely expressed (expressed in 208 organs). The encoded protein localizes to the cytoplasm and may play a role in regulating cell proliferation and apoptosis (2, 3). *SAMD9* is likely to act as a growth restriction and a lesser degree of fibroblasts expressed in endothelial cells. Pathogenic variants in the *SAMD9* gene consequence in excessive growth-restricting activity intrinsic to the protein (4).

We present here a case of MIRAGE syndrome due to a heterozygous missense variant c.2920G>A; p.E974K mutation in the *SAMD9* gene.

Case presentation

The patient who was five months old presented to our hospital with fever, lack of oral intake, vomiting, and diarrhea. Due to a diagnosis of adrenal insufficiency, the patient was consulted to us after being cared in the in patient clinics of infectious diseases. The medical history revealed that the patient, the third live-born among five pregnancies of a healthy 32-year-old mother, was prematurely born by cesarean section in the 31st gestational week with a birth weight of 930 gr (<3rd percentile) and severe intrauterine growth retardation (IUGR). The patient in this case was nonconsanguineous parents with no family history of adrenal insufficiency. It was reported that the patient who required resuscitation followed by end tracheal intubation due to the postnatal respiratory difficulty was treated with surfactant with regard to respiratory distress syndrome (RDS). It was reported that mechanical ventilation was required for six weeks, and intravenous immunoglobulin (IVIG) treatment was started for thrombocytopenia that was detected during the follow-up. Steroid therapy and oral salt supplementation were started after the patient was diagnosed with adrenal insufficiency after skin hyperpigmentation was observed on the postnatal 15th day. Table I. shows the results of hormone profile evaluation. The patient was discharged from an external medical center after four months of treatment in the intensive care unit. Currently, at age five months old. The initial physical examination we performed resulted in body measurements such as 3850 gr (<3rd percentile) of weight, 57 cm (<3rd percentile) of height, and 36 cm (3rd percentile) of head circumference. The patient lacked head support, and frontal bossing was present. His pubic hair is stage 1 in the external genital examination. His testes are 1 ml bilaterally in the middle and proximal portions of the inguinal canal. His stretched penile length is 2.0 cm. The patient was reported to receive 30mg/m^2/day hydrocortisone therapy. During the follow-up, diarrhea progressed to be resistant, and there was enteropathy that indicated colitis. Our patient also had thrombocytopenia and/or anemia that required recurrent transfusions. IVIG therapy for thrombocytopenia was administered, too. As the patient could not tolerate oral intake, nutritional support was provided by nasogastric tube feeding. For respiratory distress, intermittent oxygen was supplied by nasal cannula. Combined antibiotic therapy was started for the sepsis. A stress dose of intravenous hydrocortisone (100 mg/m^2/day) was administered. The patient's serum electrolytes were normal. Therefore, fludrocortisone acetate and sodium chloride were not administered. Table 1 presents the requested laboratory tests for the adrenal insufficiency and disorder of sex development in the patient. **Scrotal ultrasonography imaging**; the right testis, which was measured as 9x8x4.5 mm (vol: 0.2 cc), was localized in the middle portion of the right inguinal canal, while the left testis, which was measured as 6x8x8 mm (vol: 0.2 cc), was observed in the proximal portion of the left inguinal canal. **Surrenal ultrasonography imaging:** there was no image that indicated the presence of adrenal glands, so bilateral adrenal hypoplasia was concluded. The peripheral chromosomal analysis resulted in 46, XY karyotype. The presence of adrenal insufficiency, adrenal hypoplasia, the anomaly of the genitalia, resistant diarrhea, invasive infections and recurrent thrombocytopenia with bouts of anemia were compatible with MIRAGE syndrome. The results of bone marrow aspiration biopsy for delineating the etiology of thrombocytopenia, anemia, and neutropenia were not compatible with myelodysplastic syndrome (MDS). The cytogenetic investigation of bone marrow revealed 45, XY, -7 [4]/46, XY [3] karyotype. This result was described as mosaic monosomy 7. Therefore, SAMD9 sequencing was performed and identified a heterozygous missense variant c.2920G>A; p.E974K mutation in exon 3 in the *SAMD9* gene. As the patient developed intolerance to oral intake due to vomiting and diarrhea, the decision to cease enteral feeding and to start total parenteral nutrition was made accordingly. The patient was admitted to the intensive care unit upon worsening of the general condition, tacypnea, tachycardia, and fever. Hypotension, decrease in respiratory sounds, and coagulopathy developed during the follow-up and the patient was lost due to multisystem organ failure.

Result

With these clinical findings, the case was diagnosed as MIRAGE syndrome. Full gene sequencing of the patient was done. In the patient, a heterozygous 1-base change (c.2920G>A) leading to a missense mutation p.E974K (p.Glu974Lys) was indentified in the *SAMD9* gene. The variant was previously reported to be pathogenic with gain-of-function effect in a patient with MIRAGE syndrome (2, 5). This variant was not found in public SNP databases (dbSNP136, 1000 genomes, the NHLBI Exome Sequencing Project Exome Variant Server, or The Exome Aggregation Consortium). In slica prediction methods, SIFT, and Clinvar, indicated that mutation would be harmful.

Genetic analysis of parents could not be performed to confirm the de novo nature of the variant because the parents' blood samples were not available. The family was called for blood sample collection, but they did not come.

Ethics

Ethics Committee Approval:

Informed Consent: A written informed consent was obtained from the patient's family

Discussion

Adrenal hypoplasia is a rare, congenital and life-threatening disease. The patients with adrenal hypoplasia are clinically classified into two categories: the first one is without any extra-adrenal features (non-syndromic adrenal hypoplasia), and the other is with such features (syndromic adrenal hypoplasia).

The responsible genes for the former category include the genes that code for corticotropin receptor (*MCR2*) or its accessory protein (*MRAP*), *DAX1* transcription factor (*NR0B1*), nicotinamide nucleotide transhydrogenase (*NNT*) and mitochondrial thioredoxin reductase (*TXNRD2*). The syndromic category includes four different forms which are AAA syndrome (*AAAS* mutations), IMAGE syndrome (*CDKN1C* mutations), MIRAGE syndrome (*SAMD9* mutations), and a syndrome with *MCM4* mutations (2).

New advances in molecular genetics technologies have led to the identification of various rare gene genes in patients with primary adrenal insufficiency. However, 20%–60% of patients with primary adrenal insufficiency remain genetically undiagnosed (6). Previous molecular genetic studies in MIRAGE syndrome have specifically targeted patients with adrenal insufficiency. The first two studies of MIRAGE syndrome were reported in studies by Japan and the United Kingdom and 94% of patients with *SAMD9* variant had adrenal insufficiency in these studies (2, 3).

MIRAGE syndrome was first described by Narumi et al. (2016) in 11 patients that showed strikingly similar phenotypes including prenatal and postnatal moderate to severe growth restriction. The presence of skin hyperpigmentation even before the onset of salt-losing symptoms in these patients led to a suspicion of adrenal insufficiency, and adrenal hypoplasia was detected via ultrasonography in 7 patients. The extent of neurodevelopmental effects varied among patients as four patients

out of 8, who survived the first year of life, did not have head support and any speech. Out of the seven patients with the 46, XY karyotype, underdevelopment of the genitalia with microphallus, cryptorchidism, and hypospadias was observed in six; and one of the patients had complete female external genitalia at birth. During early toddler years, all of the patients had thrombocytopenia and/or anemia that required transfusions; however, they were spontaneously resolved. Serious invasive infections, such as sepsis, meningitis and fungal infections were observed at all times; 6 patients were lost before the age of 2 years mainly due to invasive infections. Two patients, who were diagnosed with mosaic 7 monosomy and developed myelodysplastic syndrome were lost due to complications. The myelodysplastic syndrome in these patients developed at 2 and 3 years of age. Heterozygote *SAMD9* gene mutations were detected in all of the patients (2).

The clinical features of our case were similar to the patients' findings described in the previous paragraph. Although mosaic monosomy 7 was detected in our patient, myelodysplastic syndrome did not develop. However, our patient was deceased in a short time due to invasive infections; therefore, we could not establish any future MDS development. The relationship between MIRAGE syndrome and MDS is complex. MDS is a heterogeneous disease characterized by clonal hematopoiesis, the proliferation of ineffective blood cells and the risk for acute leukemia. In half of the patients with MDS, there are chromosomal abnormalities that most commonly include interstitial or complete deletion of chromosome 7 (7, 8).

SAMD9 is known to be a potent and widely expressed growth repressor (9, 10). The first described *SAMD9* mutation responsible for the human disease was the homozygote p.K1495E variant that caused familial normophosphatemic tumoral calcinosis (9).

The cells with *SAMD9* mutations are characterized by structural and functional variations in the endosomal system (2).The instinct of cells for overcoming the growth restriction of mutant *SAMD9* protein, somatic monosomy 7, 7q deletion or even somatic deletion-nondisjunction mutations are observed in MIRAGE patients with or without any evidence of MDS (2, 3). In spite of losing the whole chromosome 7, the cells with loss-of-function mutations would likely gain a survival advantage over mutated cells with growth restriction. A similar "aneuploidy adaptation" mechanism in disordered cells has been reported in a mouse model with fumarylacetoacetate hydrolase deficiency (11). The first evidence of the adaptation-by-aneuploidy mechanism in humans by deletion of chromosome 7 in *SAMD9* mutation carriers was reported by Narumi et al. (2016) (2). In our case, a heterozygous 1-base change (c.2920G>A) leading to a missense mutation p.E974K (p.Glu974Lys) was indentified in the *SAMD9* gene. The variant was previously reported to be pathogenic with gain-of-function effect in a patient with MIRAGE syndrome (2, 5).

Gonadal differentiation has a significant effect on gender development in human embryos. Understanding the developmental biology and embryology of the urogenital system is crucial to categorize and define the molecular basis of the disease and, if possible, the treatment of an individual patient. Sexual differentiation refers to the process through which male or female phenotype develops. Throughout first two months of human gestation, both sexes develop in the same way. The gonads, internal genital ducts, and external genital structures all develop from bipotential embryologic tissues. Each cell in the developing gonad has the potential to differentiate into either a testicular or ovarian cell. The gonads are derived from intermediate mesoderm. In humans, 4-6. at gestational weeks, the urogenital ridges develop as paired protrusions of the coelomic epithelium (mesothelium). The gonads, adrenal cortex, kidney, and reproductive tract originate from the urogenital ridge. Several genes are necessary for the development of the bipotential gonad. Due to their origin as part of the developing urogenital system, ovaries and testes are initially located high in the abdomen near the kidneys. One of the earliest morphologic changes is increased proliferation and size of developing 46, XY gonads. 46, XY DSDs include disorders of testicular development, disorders of androgen synthesis and action (replacing and expanding the former category of male pseudohermaphroditism), and XY sex reversal (12).

The *SAMD9* gene is expressed in many tissues including the adrenal gland and gonad. Mutations of this gene occur with many disorders including these tissues. Histological analyzes of placenta tissues obtained from patients with MIRAGE syndrome (to reveal DSD mechanisms) have been performed and characteristic placental villous deterioration has been demonstrated. Mutated SAMD9 proteins have potent growth-restricting capacity, and thus they can directly cause systemic growth restriction and testicular hypoplasia. Additionally, SAMD9 variants also affect placenta, resulting in weak blood providing and suboptimal HCG stimulation. Testicular hypoplasia and insufficient HCG stimulation result in lack of testosterone synthesis. The assumption that the coexistence of the two mechanisms (direct effect by a pathogenic variant and indirect effect by placental insufficiency) would be responsible for serious clinical phenotypes seems reasonable (13).

Although the inheritance mode is autosomal dominant, the fact that less than 25% of all reported patients are female suggests that MIRAGE syndrome might be overlooked in girls. Female patients with 46, XX karyotype do not show any external genitalia abnormalities although ovarian dysgenesis is histologically revealed (2). In all of the 15 patients with 46, XY karyotype, there were external genitalia anomalies that ranged from hypospadias to full female phenotype (13). Also, the early diagnosis in the current case is mainly due to the 46, XY DSD of our patient. We may conclude that female patients without external genitalia anomalies are lost before any diagnosis is reached.

There is a broad spectrum of phenotype variation related to *SAMD9* (14). These variations can pose difficulties in the clinical diagnosis of MIRAGE syndrome; however, some features should be taken into account. Adrenal insufficiency seems to be a more consistent feature and reflects the reason for the identification of the largest cohort described up to date. Previous reports underlie the contents of systemic disorder and the high rates of death in MIRAGE syndrome. In order to advance the outcome, an early diagnosis that might lead to appropriate medical intervention is required. We reviewed a case of MIRAGE syndrome with a previously identified p.E974K mutation by paying particular attention to the dysmorphology and other findings that might assist in the detection of this disorder.

Conclusion

It is difficult to diagnose correctly in patients with syndromic adrenal hypoplasia due to various genetic etiologies and overlapping clinical and biochemical features. Here, we presented the first syndromic adrenal hypoplasia case which was diagnosed with MIRAGE syndrome in Turkey.

Ethics

Informed Consent: A written informed consent was obtained from the patient's family

Authorship Contributions
Surgical and Medical Practices: Eda Mengen, Aynur Kucukcongar Yavas
Concept: Eda Mengen
Design: Eda Mengen
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Analysis or Interpretation: Eda Mengen
Literature Search: Eda Mengen
Writing: Eda Mengen
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Table 1. Hormonal results of the patient

	15 days	Normal Range	5 months	Normal Range
Glucose (mg/dL)	71	70-100	86	70-100
Sodium (mmol/L)	133	136-145	138	136-145
Potassium (mmol/L)	5.33	3.5-5.1	4.14	3.5-5.2
ACTH (pg/mL)	>1250	6-48	92.2	6-48

Cortisol (mcg/dL)	2.7	2.8–23	2.93	2.8-23
Aldosterone (ng/dL)	3.63	19-141	2.21	5-90
Plasma Renin Activity (ng/mL/h)	15.14	11-167	3,93	2.35 – 37
TSH (mIU/ml)			7.9	0.9-7.7
Free T4 (ng/dL)			1.02	0.75-1.49
Testosterone (ng/dl)			23	75 -400
FSH (mIU/ml)			0,69	0.16 - 4.1
LH (mIU/ml)			0,36	0.02-7
DHT (ng/dL)			24.35	*
Androstenedione (ng/dL)			18	6 -68
17-OHP (ng/dL)			38	**
AMH (ng/mL)			141	39.1 – 91.1

ACTH, adrenocorticotropic hormone; FSH, follicle-stimulating hormone; LH, luteinizing hormone; T4, thyroxine; TSH, thyroid-stimulating hormone; AMH, Anti-Mullerian Hormone; DHT, Dihydrotestosterone and 17-OHP, 17a Hydroxyprogesterone.

*DHT decreases rapidly the first week, then increases to 12–85 ng/dL between 30–60 days. Levels then decrease gradually to prepubertal values by seven months.(Prepubertal children < 3 ng/dl)

**Levels increase after the first week to peak values ranging from 40 – 200 ng/dl between 30 and 60 days. Values then decline to prepubertal range before one year.(1 – 10 Years: 3 – 90 ng/dl)