







GENETIC AND CLINICAL PROFILING OF MENDELIAN SUSCEPTIBILITY TO MYCOBACTERIAL DISEASE PATIENTS; SINGLE-CENTER EXPERIENCE

MİKOBAKTERİYEL HASTALIKLARA MENDEL DUYARLILIĞI HASTALARININ GENETİK VE KLİNİK PROFİLİ; TEK MERKEZ DENEYİMİ

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Citation/Atf: Nepesov S, Firtina S, Aygun FD, Isikgil B, Camcioglu Y, Kiykim A, et al. Genetic and clinical profiling of mendelian susceptibility to mycobacterial disease patients; single-center experience. Journal of Journal of Advanced Research in Health Sciences 2022;5(3):140-146. <https://doi.org/10.26650/JARHS2022-1119899>

ABSTRACT

Objective: Mendelian susceptibility to mycobacterial disease (MSMD) is a subgroup of primary immunodeficiencies which develops with the Bacille Calmette–Guérin (BCG) vaccine or non-tuberculous mycobacterial infections. The clinical symptoms have a broad spectrum, from localized to disseminated infections.

Materials and Methods: Herein, we performed whole-exome sequencing (WES) on 13 patients with MSMD phenotype. All variants were confirmed by Sanger sequencing. The mean age was 8.41 years (min 3 – max 14 years), and the mean age of symptom onset was 4.6 years in our cohort.

Results: We found previously identified *IFNGR1* (*n*=1), *IFNGR2* (*n*=1), *TYK2* (*n*=1), *IL12RB1* (*n*=1), and *CYBB* (*n*=1) gene variants in nine patients. Our patients mostly suffered from lymphadenitis (61.5%), osteomyelitis (38%), and miliary tuberculosis (31%). All patients except one had had the BCG vaccination. Two patients developed BCGitis after vaccination. Three patients suffered from disseminated BCG infection (BCGosis).

Conclusion: Our findings show the importance of molecular diagnosis in patients with severe infections as an approach for understanding the genetic basis of infectious diseases and deciding on treatment options. The deficiency of IFN-mediated immunity genes plays a crucial role in the pathogenesis of MSMD and must be considered in pediatric patients with BCGitis.

Keywords: MSMD, BCGitis, whole exome sequencing

ÖZ

Amaç: Mikobakteriyel hastalığa (MSMD) Mendel duyarlılığı, Bacille Calmette–Guérin (BCG) aşısı veya tüberküloz dışı mikobakteriyel enfeksiyonlarla gelişen primer immün yetmezliklerin bir alt grubudur. Klinik semptomlar, lokalize enfeksiyondan yayılmış enfeksiyona kadar geniş bir spektruma sahiptir.

Gereç ve Yöntem: Bu çalışmada; MSMD fenotipli 13 hastada tüm ekzom dizileme (WES) yaptık. Tüm varyantlar Sanger dizileme ile doğrulandı. Bizim kohortumuzda ortalama yaş 8.41 yıl (en az 3 – en fazla 14 yıl) ve ortalama semptom başlangıç yaşı 4.6 idi.

Bulgular: Dokuz hastada; *IFNGR1* (*n*=2), *IFNGR2* (*n*=1), *TYK2* (*n*=1), *IL12RB1* (*n*=1) ve *CYBB* (*n*=1) gen varyantları bulduk. Hastalarımızda en çok lenfadenit (%61,5), osteomyelit (%38) ve miliyer tüberküloz (%31) mevcuttu. Biri hariç tüm hastalara BCG aşısı yapıldı. İki hastada aşılamadan sonra BCGitis gelişti. Üç hasta, yayılmış BCG enfeksiyonundan (BCGosis) muzdaripti.

Sonuç: Bulgularımız, enfeksiyon hastalıklarının genetik temelini anlamasında ve tedavi seçeneklerine karar verilmesinde bir yaklaşım olarak ağır enfeksiyonlu hastalarda moleküler tanının önemini göstermektedir. IFN aracılı bağışıklık genlerinin eksikliği, MSMD'nin patogeneğinde çok önemli bir rol oynar ve BCGitis'li pediatrik hastalarda düşünülmelidir.

Anahtar Kelimeler: MSMD, BCGitis, tüm ekzom dizileme

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Submitted/Başvuru: 03.06.2022 • **Revision Requested/Revizyon Talebi:** 01.08.2022 • **Last Revision Received/Son Revizyon:** 05.08.2022

• **Accepted/Kabul:** 10.08.2022 • **Published Online/Online Yayın:** 25.08.2022



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INTRODUCTION

Mendelian susceptibility to mycobacterial disease (MSMD) is a type of primary immunodeficiency characterized by susceptibility to mycobacterial infections or other intracellular pathogens. Infectious agents in patients with MSMD tend to be weakly virulent environmental organisms including mycobacteria (the nontuberculous mycobacteria (NTM), BCG, occasionally *Mycobacterium tuberculosis*); bacteria (salmonellae, some *Burkholderia*, *Listeria*); fungi, especially the dimorphic molds (*Histoplasma*, *Blastomyces*, *Coccidioides*, cryptococci); and the intracellular parasite *Leishmania*. In addition, herpes virus family infections are common and can be severe, including herpes simplex virus (HSV) type 1 and 2, cytomegalovirus (CMV), and Epstein–Barr virus (EBV). Bacillus Calmette–Guerin (BCG) infection and disseminated BCG infection are seen quite frequently in MSMD disorders because of reduced IL-12 and IFN- γ cytokines (1). *IFNGR1*, *IFNGR2*, *STAT1*, *IL12B*, *IL12RB1*, *IRF8*, *CYBB*, and *NEMO* genes are the best-known disease-causing genes underlying MSMD disorders, all of which are related to IFN- γ mediated immunity (2,3). To date, more than 200 pathogenic variants have been identified in MSMD-related genes (4).

IL-12RB1 deficiency (autosomal recessive inheritance) is the most common gene defect to cause MSMD (5). *IL-12RB1* is responsible for the formation of IL-12 and IL-23 receptors. It has been reported that IFN- γ production is low and there is no IL-12 response (T and NK cells) in patients with *IL-12RB1* deficiency (6,7).

IFNGR1 and *IFNGR2* deficiencies are a rare type of MSMD that includes recessive or dominant inheritance leading to complete and partial IFN receptor defects (8). *IFNGR1* and *IFNGR2* encode the alpha and beta chain of the IFN receptor and play a key role in mediating the IFN- γ cytokine production and response (9,10).

The *TYK2* (Tyrosine Kinase 2) gene is an autosomal recessive inherited deficiency that encodes Tyk2 protein. Tyk2 protein regulates signal transduction of IL-6, IL-10, IL-12, and IL-23 receptors and IFN α / β cytokines. Patients with *TYK2* deficiency have a better clinical course than those with other MSMD-related genes. However, *TYK2* deficiency poses problems in the regulation of IL-12 and IL-23 receptors and is associated with susceptibility to MSMD and tuberculosis (11,12).

Most of the genes involved in the formation of MSMD are inherited autosomal. However, the *CYBB* gene shows X-linked recessive inheritance. The *CYBB* gene is expressed in large amounts on phagocytic cells and in small amounts on B cells. Most of the germline mutations occurring in *CYBB* have been associated with chronic granulomatous disease (CGD) and disruption of the respiratory burst of phagocytic cells. Some mutations in the *CYBB* gene have been reported to cause MSMD (13,14).

Clinical genetic testing has an important role in resolving the uncertainty of clinical diagnosis in heterogeneous disorders,

managing treatment, and genetic counseling for the family. In addition, delayed genetic testing can contribute to morbidity and mortality (15). Whole exome sequencing approaches have enabled accurate and rapid genetic diagnoses and improved diagnostic success rates (16). Understanding the genetic background in MSMD disorders plays a key role in targeting treatment and determining management options (17).

Herein, we report five variants in the *IFNGR1&2*, *TYK2*, *IL12RB1*, and *CYBB* genes in 13 MSMD patients. In this study, we were able to identify pathogenic variants in MSMD patients with high accuracy, confirm the familial segregations, and evaluate the genotype–phenotype correlations. The importance of identifying gene defects in the clinical diagnosis of MSMD is discussed.

MATERIAL and METHODS

Approval for this study was obtained from the “Non-Invasive Clinical Research Ethics Committee” of Istanbul Medipol University (January 2022, Decision no: 56). Written informed consent was obtained from the patients and the parents included in the study.

Clinical characteristics of the patients

Thirteen patients (8 male with mean age 5.5 and 5 female with mean age 5.4) with the diagnosis of BCG or non-tuberculous mycobacterial infections were enrolled in this study. Gastric juice after prolonged fasting, sputum, cerebrospinal fluid, thoracentesis fluids, and lymph node biopsy materials in cases with lymphadenopathy were used for mycobacterial culture. Löwenstein–Jensen and Bactec media were used in culture examinations. Patient samples were evaluated for acid-resistant bacilli (ARB) positivity in Ehrlich Ziehl Neelsen (EZN) staining. X-ray and computed tomography (CT) were used for radiographic examinations. Data on demographic characteristics, parental consanguinity status, family history, clinical manifestations (age at onset of the disease, age at diagnosis, and infection history), treatment modality, and follow-up were retrieved from patient files and electronic records. Immunologic investigations included complete blood count analyses (white blood cell, lymphocyte, and neutrophil counts), CD3+, CD4+, and CD8+ T cell, CD19+ B cell, and CD16/56+ natural killer (NK) cell, serum IgG, -A, -M, -E levels, nitro blue tetrazolium (NBT) tests, and genetic analyses.

Whole Exome Sequencing and Sanger Sequencing

The genomic DNA of the patients and their family members were isolated with a genomic DNA isolation kit. A WES was applied by using SureSelect Human All Exon kit v.5 (Agilent Technologies, Santa Clara, California) by NextSeq 550 system (Illumina Inc., San Diego, California, U.S). Sanger sequencing was applied with region-specific primers. Fifty nanograms of genomic DNA were used for PCR reaction. The following conditions were used for PCR amplification: The initial denaturation step at 94°C for 5 min, 30 cycles of 94°C for 30 s, 60°C for 40 s, 72°C for 1 min, and a final step at 72°C for 5 min. Two percent agarose gel was used for the PCR amplifications check. DNA sequence variations were determined by bidirectional sequencing.

DATA ANALYSIS

FASTQ files were mapped to a reference genome (hg19) with the Burrows–Wheeler Aligner (BWA), and sequence alignment/map (SAM) tools were used to generate a BAM file and then a vcf file. Variants with min 20X depth and 20% frequency were filtered for further analysis. To investigate the disease-causing variant, variants were filtered according to their MAF (<0.01). For the clinical interpretation, open-source programs such as Sorting Intolerant from Tolerant (SIFT) (<http://sift.jcvi.org/>), Polymorphism Phenotyping v2 (Polyphen2) (<http://genetics.bwh.harvard.edu/pph2/>), variant effect predictor (VEP) (<https://www.ensembl.org/Tools/VEP>), Varsome (<https://varsome.com/>), and Mutation Taster (<https://www.mutationtaster.org/>) were used. The available public databases were used for the allele frequency of the variants.

RESULTS

We evaluated 13 MSMD patients. The mean age was 8.41 years (min 3 – max 14 years), and the mean age of symptom onset was 4.6 years. The nationality of patients was as follows: Turkish (n=10, 77%), Syrian (n=2, 15%, patients: P13 and P14), and Albanian (n=1, 8%, patient: P11). Two patients died during follow-up. Clinical characteristics (Table 1) and laboratory and immunological findings of the patients (Table 2) are shown.

The most frequent clinical findings were lymphadenitis (61.5%), osteomyelitis (38%), and miliary tuberculosis (31%). All patients except one had had the BCG vaccination. Two patients developed BCGitis after vaccination. Three patients suffered from disseminated BCG infection (BCGosis). We performed whole exome sequencing on 13 patients with the MSMD phenotype and found *IFNGR1* (n=1), *IFNGR2* (n=1), *TYK2* (n=1), *IL12RB1* (n=1), and *CYBB* (n=1) gene variants in nine patients (Table 3). All variants were confirmed by Sanger sequencing.

IFNGR1 Deficiency: a five-year-old boy (P01) carried a monoallelic *IFNGR1* missense variant (NM_000416.3:c.589G>A; p.E197K, rs55666220), and a three-year-old boy (P02) carried a biallelic *IFNGR1* frameshift variant (NM_000416.3:c.523delT, p.R175Efs*33, rs749956849) individually. P01 had a history of sepsis and fever at one month old without any additional features. His laboratory findings, cell count, lymphocyte subsets, serum IgG, IgA, IgM, and IgE levels, and the NBT were normal. P02 had left axillary lymphadenitis at six months old and osteomyelitis of the ulna bilaterally. This patient underwent hematopoietic stem cell transplantation (HSCT) but died due to organ failure six months after the HSCT.

IFNGR2 Deficiency: Two siblings (P3 and P4) were born to a consanguineous family at full term and without any complications. We identified a homozygous missense variant in the *IFNGR2* gene in both siblings. This variant (NM_005534.3:c.421G>A, p.G141R, rs1196094724) was located in exon 4 of the gene. The index patient (P3) is a two-year-old boy who was admitted to the hospital with axillary erythematous axillary inflation and abscess at the vaccination site of a BCG vaccine when he was

six months old. A clinical sample of the gastric aspirate did not yield bacilli. The excised lymph node biopsy confirmed the presence of AFB, and the histopathological examination showed cellular infiltration and granulomatous infiltration. The culture yielded the *Mycobacterium Tuberculosis* complex with the *Mycobacterium Bovis* species. The patient was compatible with tuberculosis lymphadenitis. Complete blood count, laboratory findings, and detailed T, B, and NK immunophenotyping and NBT test results were all normal. According to current findings, the patient was diagnosed with BCGitis. He was treated successfully with isoniazid, rifampin, and ethambutol. His sister (P4) is an 11-month-old girl with a history of axillary lymphadenitis and hepatosplenomegaly. She was given antituberculosis treatment when she was eight months of age. We do not have any information about her culture and histopathologic results. Neither sibling had pulmonary involvement, and they showed a dramatic clinical response to anti-tuberculosis treatment.

TYK2 deficiency: We detected a homozygous frameshift variant in the *TYK2* gene (NM_003331.5:c.647delG, p.P216Rfs*14, rs1555719963) in two siblings. They were treated with antibiotics and IFN- γ . Both siblings (P8 and P9) were born to a consanguineous family. No complications developed after the BCG vaccination. They were treated in the intensive care unit at the age of one month and four months due to pneumonia, and no infectious agents were detected. A diffuse varicella zoster infection developed after varicella vaccination at the age of one year.

IL12RB1 deficiency: We identified a homozygous splice site mutation (NM_001290024.1:c.184+2T>G, rs765825621) in the *IL12RB1* gene in two siblings (P6 and P7) born to consanguineous-marriage parents. They were diagnosed with recurrent fever and enteritis symptoms. *Salmonella spp.* was positive in their blood and stool culture. P6 died due to salmonella sepsis. P7 was treated with antibiotics and IFN- γ .

CYBB deficiency: We showed a hemizygous frameshift (NM_000397.4:c.1308delC, p.K438Rfs*64) variant in the *CYBB* gene in one patient (P10). This patient was hospitalized four times because of fever and cervical lymphadenitis. His NBT test was positive, and he was treated with antibiotics and IFN- γ .

DISCUSSION

Mendelian susceptibility to MSMD is a rare disorder that mostly starts during childhood and has a broad spectrum, from localized to disseminated BCG infections. Patients are characterized by a defective IFN- γ immune response leading to a predisposition to selective infections (5). Today, X-linked (*NEMO* and *CYBB*) and autosomal dominant and/or recessive-inherited *IFNGR1*, *IFNGR2*, *IL12B*, *IL12RB1*, *IL12RB2*, *TYK2*, and *IL23R* genes are the most known genes related with MSMD etiology. More than 80% of MSMD patients suffer from *IL12RB1/2* and *IFNGR1/2* gene deficiencies (11).

Herein, we analyzed 13 MSMD patients and found *IFNGR1*, *IFNGR2*, *TYK2*, *IL12RB1*, and *CYBB* gene defects in nine of them. These variants are known as damaging in the literature.

Table 1: Clinical Features of the MSMD patients

Patients	P01	P02	P03	P04	P05	P06	P07	P08	P09	P10	P11	P12	P13
Gene	IFNGR1	IFNGR1	IFNGR2	IFNGR2	NEG	IL12RB1	IL12RB1	TYK2	TYK2	CYBB	NEG	NEG	NEG
Gender	M	M	M	F	M	F	F	F	M	M	M	F	M
Current Age	5	3	2	11 mo	4	8	13	4	6	8	10	14	14
Diagnosis Age	1	1	1	8 mo	10 mo	6	11	1	5	6	NA	NA	NA
Symptoms	Recurrent Fever	Axillary lymphadenitis	Axillary lymphadenitis	Axillary lymphadenitis	Osteomyelitis	Enteritis, Fever, Lymphadenitis	Enteritis, Fever, Lymphadenitis	Pneumonia	Pneumonia	Fever, Lymphadenitis	Pain in left ankle	Pain in right knee and hip	Fever, Back pain
Lymphadenitis	No	Yes	Yes	Yes	No	Yes	Yes	No	No	Yes	No	Yes	Yes
Osteomyelitis	Right Wrist	Bilateral Ulna	No	No	Sternum	No	No	No	No	No	Left Tibia	Right knee and hip	Vertebra, left scapula
Miliary Tuberculosis	No	No	Yes	Yes	No	No	No	No	No	No	No	Yes	Yes
Enteritis	No	No	No	No	No	Yes	Yes	No	No	No	No	No	No
Hepatosplenomegaly	No	No	No	Yes	No	No	No	No	No	Yes	No	No	No
Infections	<i>M.Bovis</i> /joint aspiration	<i>M. Tuberculosis</i> (lymph tissue)	<i>M. Tuberculosis</i> (lymph tissue)	<i>M. Tuberculosis</i> (lymph tissue)	<i>M. Bovis</i>	<i>Salmonella</i> spp/in blood and stool	<i>Salmonella</i> spp/in blood and stool	Varicella-zoster virus	Varicella-zoster virus	<i>S.Aureus</i> (from the lymph node)	<i>M.Bovis</i> /joint aspiration	<i>M.Bovis</i> /joint aspiration	NA
BCG Vaccination	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
BCGIt	No	No	Yes	No	No	No	Yes	No	No	No	No	No	No
BCGosis	No	Yes	Yes	Yes	No	No	No	No	No	No	No	No	No
Last Status	Alive	ex	Alive	Alive	Alive	ex	Alive	Alive	Alive	Alive	Alive	Alive	Alive

M: Male, F: Female, Mo: Months, Neg: Negative, NA: Not available, *M.Bovis*: *Mycobacterium bovis*, BCG: Bacillus Calmette–Guerin, Ex: Exitus

Table 2: Laboratory and Immunological Features of the MSMD patients

Patients	P01	P02	P03	P04	P05	P06	P07	P08	P09	P10	P11	P12	P13
WBC x10 ⁹ /L	9700	8500	20000	17000	9970	11400	6600	11700	6640	9225	4110	7490	6300
ANS	3000	2800	5030	6600	4820	8830	4400	3270	2160	4540	1550	4920	4870
ALS	4300	4800	13100	9000	3960	2600	2200	6240	3830	2960	2160	1880	860
Eosinophil x10 ⁹ /L	1100	300	1580	600	50	30	330	1000	240	120	60	190	6
Monocyte x10 ⁹ /L	500	1100	990	1100	1090	730	990	690	380	1600	330	480	800
IgG mg/dl	1070	800	620	976	1151	1001	1100	721	880	890	1097	1327	1680
IgM mg/dl	78	94	121	130	88	137	144	105	110	88	70	105	210
IgA mg/dl	178	68	59	77	35	117	110	33	88	77	172	176	110
IgE IU/L	140	77	34	66	19	33	90	54	44	17	196	48	20
CD3%	69	42 ↓	66	68	71	57	71	82	77	66	74	80	73
CD4%	41	30 ↓	36	36	50	24 ↓	39	34	32	36	35	50	39
CD8%	36	13 ↓	28	24	19	21	27	31	26	22	26	40	24
CD19%	14	22	11 ↓	17	23	28	15	13 ↓	12 ↓	14	9	15	15
CD16%	8	8	6	14	5	12	8	11	8	5	12	4	6
NBT test	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Positive	Normal	Normal
IFN-γ	No	No	No	No	No	No	Yes	No	No	Yes	No	No	No

WBC: White Blood Cells; ANS: absolute neutrophil count; ALS: Absolute Lymphocyte Count; NBT: Nitroblue tetrazolium Test, IFN-γ: interferon gamma

Table 3: Genetic variants in our patients

Patients	Gene	cDNA position	Protein	dbSNP	Allelic Inheritance	MAF	ACMG/AMP	ClinVar
P01	<i>IFNGR1</i>	NM_000416.3:c.589G>A	p.E197K	rs55666220	Het	0.000272 (ExAC)	Likely benign	VUS
P02	<i>IFNGR1</i>	NM_000416.3:c.523delT	p.R175Efs*33	rs749956849	Hom	0.000014/2 (GnomAD)	Pathogenic	Pathogenic
P03 and P04	<i>IFNGR2</i>	NM_005534.3:c.421G>A	p.G141R	rs1196094724	Hom	0.000007 (GnomAD)	VUS	NA
P06 and P07	<i>IL12RB1</i>	NM_001290024.1:c.184+2T>G	NA	rs765825621	Hom	0.00001 (ExAC)	Likely pathogenic	NA
P08 and P09	<i>TYK2</i>	NM_003331.5:c.647delG	p.P216Rfs*14	rs1555719963	Hom	NA	Pathogenic	Likely pathogenic
P10	<i>CYBB</i>	NM_000397.4:c.1308delC	p.K438Rfs*64	NA	Hemi	NA	Likely pathogenic	NA

NA: Not Available, MAF: Minor Allele Frequency, Het: Heterozygous, Hom: Homozygous, Hemi: Hemizygoty, ACMG/AMP: American College of Medical Genetics / Association for Molecular Pathology, VUS: Variant of Unknown Significance

IFNGR1/2 was one of the most common variants seen in our study. We identified a monoallelic missense E197K (P1) and a biallelic frameshift R175Efs*33 (P2) variant in two different individuals. E197K variant was in the IFN-binding domain of the protein, which is essential for the protein function (18). The other frameshift variant, R175Efs*33, might lead to loss of protein (19).

A functional IFN-γ receptor signaling must consist of two functional *IFNGR1* and two additional *IFNGR2* chains (20). Monoallelic or biallelic mutations of this gene cause the complete or partial loss of IFN-γ signaling (21). Disease-causing variants in the *IFNGR1* gene can be monoallelic or biallelic. Although both monoallelic and biallelic variants cause impaired IFN-γ signaling, biallelic variants lead to a complete lack of signaling whe-

reas monoallelic variants have been associated with blocking the IFN- γ /IFN- γ R1 complex or JAK1 binding site of the receptor.

In addition, we identified a missense variant (G141R) in the *IFNGR2* gene in two siblings. This variant leads to misfolded proteins with abnormal *N*-glycosylation resulting in partial *IFNGR2* deficiency. Both siblings were admitted with skin complications and axillary lymphadenitis that developed after they received a BCG vaccine at approximately eight months of age. They were treated with anti-tuberculosis treatment.

We detected a splice site variant (c.184+2T>G) in the *IL21RB1* gene in two siblings. *IL21RB1/2* genes encode the p40 subunit of IL-12 and IL-23; deficiency of this gene is responsible for the lack of IL12 and IL23 production. Although this splice site variant has not been shown in an MSMD patient before, pathogenic variants in this gene have been associated with MSMD (22).

Apart from the common mechanisms of MSMD disorders, *TYK2* deficiency is a rare causative genetic disorder for this disease. The tyrosine kinase 2 (*TYK2*) gene is a Janus kinase (*JAK*) family member and plays a pivotal role in the signaling of some important cytokines, such as IL-6, IL-10, IL-12, IL-23, and IFN α / β (23). Besides *TYK2* deficiency, which is a rare condition, patients have clinical features such as intracellular bacterial infections, high IgE levels, and B/T cell lymphoma (12,24).

Here, a biallelic frameshift deletion (c.647delG) in the *TYK2* gene was found in two siblings. Biallelic variants in this gene have been associated with an impaired immune response to microbial and viral infections (23).

A hemizygous c.1308delC in the *CYBB* gene was detected in one MSMD patient. The *CYBB* variant in this case has not been previously published in the literature. MSMD patients with *CYBB* are diagnosed with X-linked CGD and this gene mutation cause a respiratory burst of phagocytes. Some CGD patients develop disseminated tuberculosis mycobacterial disease, which is named X-linked recessive (XR) MSMD due to *CYBB* deficiency (13).

Next-Generation Sequencing panels give a powerful advantage to understanding the genetic background of the patients and improving the success rate by detecting both mutations and CNVs. Screening MSMD patients with NGS panels leads to discovering new genetic etiologies of the MSMD pathogenesis and enlightens the genotype-phenotype correlation.

Our report points to the importance of genetic diagnosis in patients with severe infections as an approach for understanding the genetic basis of infectious diseases and deciding treatment options. In MSMD patients, the diagnosis is made late because the results of first-line immunological tests are mostly found to be normal. Patients with severe BCG and disseminated BCG infections should receive molecular diagnosis due to the increased risk of the diagnosis of inborn errors of human disorders.

Ethics Committee Approval: This study was approved by Istanbul Medipol University Non-Interventional Clinical Research Ethics Committee (Date: 06.01.2022, No: 56).

Peer Review: Externally peer-reviewed.

Author Contributions: Conception/Design of Study- S.N., A.A., S.F.; Data Acquisition- S.N., A.A., S.F.; Data Analysis/Interpretation- B.I., S.N., Y.Ç., S.F., Y.K.D., A.A.; Drafting Manuscript- S.F., F.D.A., S.N.; Critical Revision of Manuscript- S.N., S.F.; Final Approval and Accountability- S.N., S.F.

Conflict of Interest: The authors have no conflict of interest to declare.

Financial Disclosure: This project was supported by Istanbul Medipol University.

Etik Komite Onayı: Bu çalışma için etik komite onayı İstanbul Medipol Üniversitesi Girişimsel Olmayan Klinik Araştırmalar Etik Kurulu'ndan alınmıştır (Tarih: 06.01.2022, No: 56).

Hakem Değerlendirmesi: Dış bağımsız.

Yazar Katkıları: Çalışma Konsepti/Tasarım- S.N., A.A., S.F.; Veri Toplama- S.N., A.A., S.F.; Veri Analizi/Yorumlama- B.I., S.N., Y.Ç., S.F., Y.K.D., A.A.; Yazı Taslağı- S.F., F.D.A., S.N.; İçeriğin Eleştirel İncelemesi- S.N., S.F.; Son Onay ve Sorumluluk- S.N., S.F.

Çıkar Çatışması: Yazarlar çıkar çatışması beyan etmemişlerdir.

Finansal Destek: Bu proje İstanbul Medipol Üniversitesi tarafından desteklenmiştir.

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