



골수형성이상증후군/급성골수성백혈병 환자에서 발견된 *DDX41*의 새로운 생식세포 돌연변이

A Novel Germline Mutation in *DDX41* Predisposed to Myelodysplasia/Acute Myeloid Leukemia

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Germline *DDX41* lesions indicate a hereditary myelodysplastic syndrome and acute myeloid leukemia (MDS/AML). Canonical somatic mutations in this gene often coincide as a second hit with germline *DDX41* mutations. We report a patient with inherited MDS/AML containing novel germline *DDX41* mutations that harbor somatic mutations in the other *DDX41* allele. The 72-year-old woman was diagnosed with myelodysplastic syndrome with excess blasts-2 (MDS-EB-2) and had gone through 16 rounds of chemotherapy. However, an increase in leukemic myeloblasts was observed in the bone marrow aspiration, resulting in transition to AML. Targeted gene panel sequencing revealed *DDX41* c.308_309del (p.Glu103Valfs*31) with a variant allele frequency (VAF) of 49%, and *DDX41* c.1589G > A (p.Gly530Asp) with a VAF of 6%. The c.308_309del variant was confirmed as a germline variant after analyzing buccal DNA. An identical germline *DDX41* mutation was detected in her unaffected daughter and son. The patient was repeatedly hospitalized for neutropenic fever and eventually expired on account of sepsis. Genetic investigation is crucial for providing appropriate medical management to patients and determining the prognosis of a disease. In addition, it helps to provide appropriate counseling and raise awareness of inherited hematologic malignancies for family members.

Key Words: DEAD box helicase 41, Genetic variations, Myelodysplastic syndromes, Acute myeloid leukemia, Next-generation sequencing

INTRODUCTION

Myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML) typically present as sporadic diseases. However, some cases of MDS or AML are associated with germline mutations harboring specific genetic and clinical characteristics [1, 2]. To date, 10 genes

have been identified in familial MDS or AML: *RUNX1*, *CEBPA*, *TERC*, *TERT*, *GATA2*, *SRP72*, *ANKRD26*, *ACD*, *ETV6*, and *DDX41* [3].

MDS or AML with the germline *DDX41* variant is characterized by a long latency, an advanced disease (high-risk MDS/AML), and a normal karyotype. [4]. The *DDX41* gene, located on chromosome 5q35, is composed of 17 exons and encodes a DEAD (Aspartic acid-Glutamic acid-Alanine-Aspartic acid)-box helicase. As a tumor suppressor gene, it participates in pre-mRNA splicing and processing [4, 5]. The reported frequency of germline *DDX41* variants is approximately 1–5% of all myeloid neoplasms [6–10]. In addition to germline *DDX41* variants, somatic variants of this gene are often concomitant. Approximately 70% of MDS/AML cases that develop in germline carriers harbor a mutation on the other allele of *DDX41* [9, 11]. To date, 177 different *DDX41* gene mutations have been listed in the Bibliome Variant Database (<https://bibliome.ai>). However, a frameshift mutation of *DDX41* c.308_309del, p.(Glu-

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103Valfs*31) introduced in this case has never been reported earlier. In this report, we present a patient with inherited MDS/AML possessing a novel germline *DDX41* variant that harbors a somatic mutation in the other allele.

CASE

A 72-year-old woman presented to the emergency department with fever and general weakness. Complete blood count (CBC) results revealed pancytopenia with a hemoglobin (Hb) value of 9.2 g/dL, white blood cell (WBC) count of $1.50 \times 10^9/L$, and platelet count of $31 \times 10^9/L$. Peripheral blood smear (PBS) results revealed a few blasts without any dysplastic cells. A bone marrow smear revealed slightly hypocellular marrow with 16.2% leukemic blasts of all nucleated cells. The patient was diagnosed with myelodysplastic syndrome with excess blasts-2 (MDS-EB-2) displaying a normal karyotype. She started chemotherapy with decitabine because of a high-risk score evaluated (risk score 6.5) according to the revised International Prognostic Scoring System (IPSS-R) category [12]. Following the 4th chemotherapy treatment, the PBS result showed no leukemic blasts with increased CBC parameters (Hb of 11.8 g/dL, WBC count of $2.70 \times 10^9/L$ and platelet count of $143 \times 10^9/L$).

A follow-up bone marrow smear revealed normocellular marrow with 2.0% leukemic blasts of all nucleated cells, indicating complete remission. However, despite the 16th chemotherapy, pancytopenia persisted for 8 weeks. Moreover, leukemic myeloblasts (23.4% of all nucleated cells) were observed in the bone marrow sample, implying a transformation from MDS-EB-2 to AML. Chromosome analysis showed a normal karyotype in the

follow-up studies. A multiplex nested reverse-transcription polymerase chain reaction analysis with HemaVision (DNA Diagnostic A/S, Risskov, Denmark), which enables the detection of 28 chromosomal rearrangements associated with acute leukemia, did not detect any rearrangement abnormalities. Targeted gene panel sequencing included AML-related 49 genes that were sequenced with MiSeq (Illumina, San Diego, CA, USA) according to manufacturer's instructions. The sequencing results revealed the following: NM_000546.5:c.646G>A, p.(Val216Met), with an 8% variant allele frequency (VAF) of *TP53*, and NM_016222.2:c.1589G>A, p.(Gly530Asp), with 6% VAF of *DDX41*. These variants were confirmed as somatic variants because they were not detected in the buccal swab of the patient.

The panel also detected one suspected germline variant, NM_016222.2:c.308_309del, p.(Glu103Valfs*31) of *DDX41*, with a 49% VAF. This variant was confirmed to be germline in origin as it was detected in the buccal swab of the patient. The variant was also predicted to undergo non-mediated decay in exon 4 and form a frameshift change. In addition, the variant was not found in the gnomAD exome database or KRGDB (Korean Registry Gene Database). Consequently, it was interpreted as a likely pathogenic variant according to the 2015 American College of Medical Genetics and Genomics and the Association for Molecular Pathology Guidelines [13]. According to OMIM, the *DDX41* gene is associated with familial myeloproliferative/lymphoproliferative neoplasms (OMIM 608170) in an autosomal dominant inheritance pattern. Upon clinical and genetic investigations of the patient's family, an identical germline *DDX41* gene variant was found in her 54-year-old unaffected daughter and 42-year-old unaffected son (Fig. 1). After being diagnosed with AML, the patient was re-

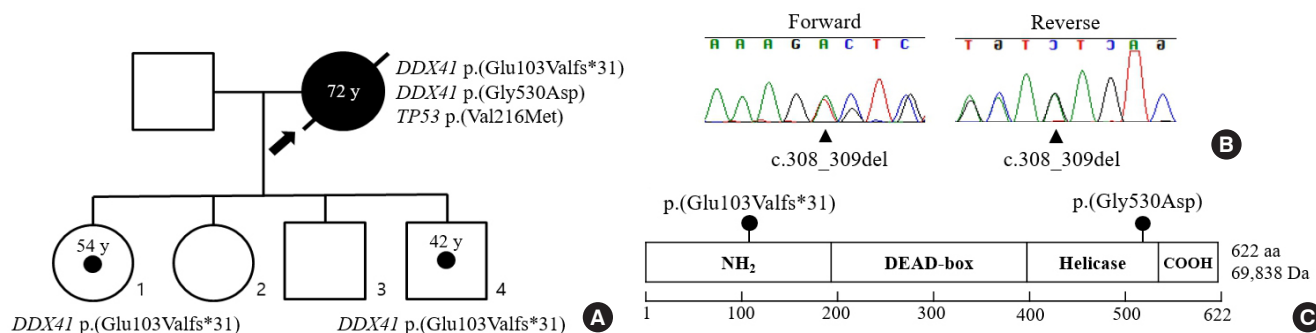


Fig. 1. (A) Family pedigree of the patient. The arrow indicates the proband. Circled dots indicate heterozygous carriers of the germline *DDX41* p.(Glu103Valfs*31) variant. Child 2 and 3 refused genetic testing. (B) Electropherogram showing the germline heterozygous c.308_309del variant detected in the peripheral blood sample of the patient. (C) *DDX41* protein structure with germline *DDX41* variants reported in this case.

peatedly hospitalized owing to neutropenic fever, but eventually expired on account of sepsis.

DISCUSSION

DDX41 encodes an RNA helicase, and also acts as a tumor suppressor gene [6]. It contributes to specific processes within the cell, including pre-mRNA splicing, innate immunity, and ribosome biogenesis [14]. Specifically, mRNA splicing and ribosome biogenesis may be closely related to *DDX41*-driven leukemogenesis. *DDX41* can be divided into four main domains: N-terminal, DEAD-box, helicase, and C-terminal [14]. The germline p.(Glu103Valfs*31) and somatic p.(Gly530Asp) variants of the *DDX41* gene are located in the N-terminal and helicase domains, respectively. Defects in the helicase domain can lead to altered RNA splicing, such as exon skipping or exon retention. A specific mis-splicing pattern influences the altered expression of specific downstream genes, leading to leukemogenesis [4]. *DDX41* also contributes to the processing of precursor ribosomal RNA to mature rRNAs in ribosome biogenesis [15, 16]. Kadono et al. [16] reported that an overexpression of the *DDX41* R525H mutant located in the helicase domain leads to decreased hematopoietic stem cell growth. However, the effect of the *DDX41* mutation on cell growth via ribosome biogenesis has yet to be elucidated [14]. Compared to the helicase domain, little information about the N-terminal domain has been revealed.

A *DDX41* germline variant was first introduced by Polprasert et al. in 2015 [4]. Since then, novel variants of *DDX41* have been continuously discovered. The germline p.(Glu103Valfs*31) frameshift variant found in this case is a novel variant, and the p.(Gly530Asp) missense somatic variant has been reported in four patients with AML, according to the literature [6, 14].

Some genetic predisposing factors for myeloid malignancies (e.g., *RUNX1*, *CEBPA*, and *GATA2*) [17-19] are often associated with a younger age of malignancy onset. Compared with these predisposing genes, familial myeloid malignancies associated with *DDX41* are late-onset at an age similar to those observed in *de novo* MDS or AML [6]. In the reported germline *DDX41* mutation cases, the median age at which myeloid hematologic malignancies were diagnosed was 65 years, according to Cheah et al. [14], and 62 years, according to Lewinsohn et al. [6], outlining the long latency of this case. This feature of a long latency period makes it

difficult for physicians to investigate the histories of other family members.

Germline *DDX41* variants induce MDS/AML with substantial penetrance [4, 6]. In this case, both the unaffected 42-year-old son and the 54-year-old daughter were carriers of this variant. Currently, there are no consensus guidelines for family genetic counseling. However, Lewinsohn et al. reported germline variant carriers who develop MDS or AML that usually present with leukopenia (83.3%) with or without macrocytosis or other cytopenias [6]. In this case, the son and daughter had no abnormal findings on the CBC and PBS examination. Thus, CBC and PBS monitoring may be needed for clinical follow-up of asymptomatic carriers.

The prognostic significance of myeloid neoplasms with germline *DDX41* variants remains unclear. Polprasert et al. reported a shortened overall survival in patients with *DDX41*-related MDS/AML than the wild-type cases [4]. In contrast, Sébert et al. reported that patients with *DDX41*-related inherited hematological malignancies had a longer median overall survival (5.2 years) than the wild-type matched controls (2.7 years) [9]. Li et al. also reported that all affected patients with germline *DDX41* variants had complete remission following bone marrow transplant [11]. In this case, despite chemotherapy, the disease progressed rapidly, and the patient died two years after the diagnosis of MDS-EB-2, implying poor prognosis.

Germline *DDX41* variants are also associated with hypocellular bone marrow, a higher risk of MDS/AML, and a normal karyotype [14]. In our case, the patient showed similar characteristics at the time of diagnosis. When these features are found in patients with late-onset MDS/AML, the possibility of harboring a *DDX41* variant should be considered.

Germline alterations may cause predisposal to somatic mutations in the same gene [4]. Similar to *CEBPA* and *JAK2* [17, 20], germline variants of *DDX41* serve as a first hit that significantly enhances the likelihood of subsequent development of leukemia. Canonical somatic variants in the other allele of this gene coincide as a second hit with the germline *DDX41* mutation, often at a low VAF during the initiation of leukemogenesis [4, 14]. Consequently, we hypothesize that our patient with the p.(Glu103Valfs*31) germline variant of the *DDX41* had a long latency in an asymptomatic carrier state. However, owing to an unexplained cause, a somatic mutation p.(Gly530Asp) occurred in the same gene, as a second hit and caused a loss of function in the tumor suppressor gene, re-

sulting in MDS/AML.

In conclusion, we have identified a novel germline *DDX41* mutation in a 72-year-old female patient diagnosed with MDS/AML. Her clinical features were consistent with those of other patients with previously reported *DDX41* variants. Genetic investigation is crucial for providing appropriate medical management to patients and determining the prognosis of the disease. In addition, it is helpful to provide appropriate genetic counseling and raise awareness of familial hematologic malignancies to family members. Guidelines for family genetic counseling and management of carriers in the family should be implemented.

요약

DDX41 유전자의 생식세포 변이는 유전성 골수형성이상증후군/급성골수성백혈 병(MDS/AML)에서 나타난다. *DDX41* 유전자는 생식세포 변이와 더불어 해당 유전자에서의 체세포 변이도 빈번하게 동반된다. 저자들은 이전에 보고되지 않았던 생식세포 *DDX41* 돌연변이를 가지면서 동일한 유전자의 체세포 돌연변이도 함께 가지고 있는 유전성 MDS/AML 환자 1예를 경험하였기에 이를 보고하고자 한다. 72세 여자환자는 MDS with excess blasts-2를 진단받고 항암치료를 진행하였으나, 골수검사상 진단 전보다 증가된 백혈병성 골수모구가 관찰되면서 AML로 재진단 받게 되었다. 추가로 시행한 표적 유전자 패널 염기서열분석법 결과 대립유전자빈도 (VAF) 49%의 *DDX41* c.308_309del (p.Glu103Valfs*31) 변이와 VAF 6%의 *DDX41* c.1589G>A (p.Gly530Asp) 변이가 발견되었다. c.308_309del 변이는 구강 DNA 분석을 통해 생식세포 변이임이 확인되었으며, 환자의 딸과 아들에서도 동일한 생식세포 *DDX41* 돌연변이가 관찰되었다. 환자는 호중구감소성 발열로 인해 반복적인 입원치료를 하였으나 결국 패혈증으로 사망하였다. 유전자 검사는 질병의 예후를 결정하고 환자에게 적절한 치료를 제공하는 데 있어서 매우 중요하다. 또한, 환자의 가족들에게 적절한 유전 상담을 시행하여 유전성 혈액암에 대한 인식을 높이는 것이 도움이 될 것이다.

Conflicts of Interest

None declared.

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