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Letter to the Editor

Novel multiple heterozygous *NUDT15* variants cause an azathioprine-induced severe leukopenia in a patient with systemic lupus erythematosus

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To the Editor:

The efficacy of azathioprine (AZA) has been demonstrated for the maintenance treatment of autoimmune diseases including systemic lupus erythematosus (SLE) [1]. However, some patients are unable to continue AZA treatment because of serious adverse events including leukopenia and alopecia. Therefore, there is a need to establish a strategy for predicting the drug susceptibility of AZA. The US Food and Drug Administration recommends TPMT status such as the TPMT enzyme activity and *TPMT* gene testing to prevent AZA-induced leukopenia before AZA treatment. However, it has been shown that the frequency of *TPMT* mutation is considerably lower in the Asian populations than in Caucasians [2].

In addition to the identification of single nucleotide polymorphisms (SNPs) in the *thiopurine S-methyltransferase* (*TPMT)* gene that define the cytotoxicity of thiopurine [3], recent studies on patients with inflammatory bowel diseases have shown that *nucleoside diphosphate-linked moiety X-type motif 15* (*NUDT15)* gene was strongly associated with thiopurine-induced leukopenia in the Asian populations [4–6].

A 57-year-old Japanese man, who had been treated with 5 mg/day of prednisolone for SLE, presented with a tongue ulcer and slight fever 4 weeks following the introduction of AZA (50 mg/day) as maintenance therapy for SLE. He was admitted to our department because laboratory tests revealed severe neutropenia and high C-reactive protein levels. After admission, his body temperature was 37.3 °C, blood pressure was 111/79 mmHg, and heart rate was 89 bpm. Laboratory investigations revealed the following: hemoglobin level 11.4 g/dL, white blood cell count 0.9×10^3/μL (neutrophils 5.7%, lymphocytes 76.4%, monocytes 2.2%, eosinophils 15.7%), platelet count 48×10^3/μL, albumin 4.0 g/dL, total bilirubin 1.0 mg/dL, lactate dehydrogenase 228 IU/L, alkaline phosphatase 239 U/L, aspirate aminotransferase 23 IU/L, alanine aminotransferase 18 IU/L, γGTP 19 IU/L, blood urea nitrogen 16 mg/dL, creatinine 1.14 mg/dL, and ferritin 472 ng/mL. Peripheral blood smear showed burr and helmet cells. Immunological studies showed the following: anti-dsDNA antibody 2.5 IU/mL, Complement C3 72.0 mg/dL (normal 60–115 mg/dL), C4 26.8 mg/dL (normal 15–50 mg/dL), and CH50 31.4 U/mL (normal 25–50 U/mL). Cytomegalovirus antigenemia (C7-HRP) and Epstein-Barr virus (EBV) DNA were negative. Computed tomography scan revealed no evidence suggestive of lymphadenopathy or infections. A bone marrow aspiration smear showed no abnormality in the cell density of the three systems of blood cells, red blood cells, and platelets; moreover, there was no increase in dysplasia or blast cells.

Based on these findings, we diagnosed thiopurine-induced early severe hair loss and leukopenia. Thus, we discontinued AZA and initiated a daily injection of G-CSF; however, his tongue ulcer did not subside, and severe neutropenia had been sustained for 3 weeks. Furthermore, although hair loss was not clear at admission, it progressed to total alopecia 3 weeks following admission. His SLE activity was stable during admission; therefore, we thought that immunological mechanisms were not responsible for these clinical manifestations. Four weeks after AZA discontinuation, the pancytopenia improved to normal level, and hair loss reduced 2 months after the discontinuation.

Genetic analysis revealed multiple heterozygous adjacent mutations (c.415C > T; c.416G > A) within the 139th amino acid of the *NUDT15* gene (Fig. 1). A homozygous variant of p.Arg139Cys in the *NUDT15* gene results in severe neutropenia and total hair loss similar to that in this case [5,7]. However, with heterozygous mutation of p.Arg139Cys, the clinical course is relatively mild [7,8]. Consistent with our report, a previous study has indicated that patients with compound-heterozygous *NUDT15* genotypes (p.Val18Ile/p.Arg139Cys and p.Val18_Val19insGlyVal/p.Arg139Cys) are as sensitive to mercaptopurine, as patients with homozygous variant of p.Arg139Cys [4].

Both mutations (p.Arg139Cys and p.Arg139His) in the *NUDT15* gene affect the residue Arg 139 located in the α 2 α helix at the base of the NUDT 15 substrate binding pocket [9]. It is hypothesized that a disulfide bond that interferes with TGTP binding is introduced at this position resulting from the replacement of arginine with cysteine (p.Arg139Cys) [9]. In contrast, arginine-histidine changes caused by the mutation of p.Arg139His may result in a reduced eletrophilicity and compromise substrate interaction [9]. Since we were not able to examine *NUDT15* sequences in his family members, it is not certain whether these mutations were on the same allele (p.Arg139Arg/p.Arg139Tyr) or the different allele (p.Arg139Cys/p.Arg139His). In our case, although we did not perform detailed analysis for the structural and functional changes caused by the substitution of arginine with tyrosine (p.Arg139Tyr), we speculate that it may significantly affect thiopurine metabolism compared to other amino acid substitutions.

To conclude, this case suggests that genetic testing of *NUDT15* is useful for predicting the severity of the adverse effects of AZA in

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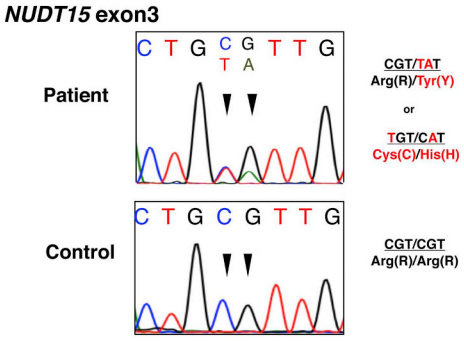

Fig. 1. The gene analysis of *NUDT15*. (A) The mutations found in the present patient are multiple heterozygous adjacent mutations (c.415C > T; c.416G > A, shown in the chromatogram), leading to p.Arg139Tyr or p.Arg139Cys/p.Arg139His amino acids substitution.

patients with SLE and that novel *NUDT15* multiple heterozygous adjacent mutations associated with higher AZA-induced toxicities than a p.Arg139Cys heterozygous mutation. Further accumulation of cases and functional studies are required to clarify the utility of measuring *NUDT15* gene in SLE patients and how multiple heterozygous adjacent mutations (c.415C > T; c.416G > A) within the 139th amino acid of the *NUDT15* gene is involved in the metabolism of AZA.

Informed consent

Written informed consent was obtained from the patient for publication of this case report and any accompanying images.

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