Clonal Cytopenia of Undetermined Significance in a Patient with Congenital Wilms’ Tumor 1 and Acquired DNMT3A Gene Mutations

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Abstract:
Congenital mutations of the Wilms’ tumor 1 (WT1) gene can lead to various abnormalities, including renal/gonadal developmental disorders and cardiac malformations. Although there have been many reports of somatic WT1 mutations in patients with acute myeloid leukemia and myelodysplastic syndrome, congenital WT1 mutations have not been reported in hematological disorders. We herein report a patient with early-onset clonal cytopenia of undetermined significance that was associated with a congenital mutation of WT1 and an acquired mutation of DNMT3A (encoding DNA [cytosine-5]-methyltransferase 3A).

Key words: Germline WT1 mutation, clonal cytopenias of undetermined significance, CCUS, Meacham syndrome


Introduction

The Wilms’ tumor 1 gene (WT1) is involved in cell growth and differentiation; congenital mutations of this gene reportedly cause various abnormalities, including renal/gonadal developmental disorders and cardiac malformations, as observed in Denys-Drash, Frasier, and Meacham syndromes (1-3). To our knowledge, congenital mutations of WT1 have not been identified in patients with hematological disorders; however, somatic mutations of WT1 have been detected in patients with hematopoietic stem cell disorders, such as acute myeloid leukemia and myelodysplastic syndrome (4-6). DNA (cytosine-5)-methyltransferase 3A (DNMT3A) is one of the most frequently mutated genes in adult and elderly patients with myeloid and lymphoid malignancies, as well as in patients with clonal hematopoiesis (7).

We herein report a case of early-onset clonal cytopenia of undetermined significance (CCUS) with an acquired mutation of DNMT3A in a patient who had Meacham syndrome associated with a congenital mutation of WT1.

Case Report

The patient was a 41-year-old woman. Because of tricuspid regurgitation, she had undergone the Fontan operation at four years old. At 22 years old, she had been admitted to her local hospital for the treatment of a fever. Laboratory studies upon admission showed leukocytopenia (white blood cell count, 1,320/μL; hemoglobin, 12.1 g/dL; platelet count, 106×10³/μL). A bone marrow examination showed normocellular marrow for her age without abnormal findings. In addition, various examinations indicated no obvious infectious disease, autoimmune disease, or tumor. However, the patient’s hyperthermia persisted for several months but resolved spontaneously without treatment; because of persistent pancytopenia, she transitioned to outpatient follow-up. At 40 years old, the patient was transferred to our hospital because of a change in her medical care team. At that time, she reported no history of hematological or congenital diseases among family members; she also was not a heavy drinker.

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The patient’s laboratory findings upon admission to our hospital are shown in Table. Pancytopenia was observed, but no blast or immature cells were found in the peripheral blood; the reticulocyte count was normal. There were no metabolic abnormalities that could cause pancytopenia; no abnormalities were evident in the secretion of thyroid hormones or erythropoietin. She was receiving monthly IV iron supplementation, and her ferritin levels were within normal limits. Her vitamin B12 levels were at the lower limit of normal, but a bone marrow examination showed no findings that would raise suspicion of megaloblastic anemia. There were no findings suggestive of intravascular hemolysis. Computed tomography showed splenomegaly but no obvious mass lesion. Abdominal echography showed fibrosis of the liver. Bone marrow aspiration showed no marked increase in the number of blast cells; it also did not indicate myelodysplasia, lymphoma, or hemophagocytic syndrome. A bone marrow biopsy showed normocellular marrow for her age, with no evidence of fibrosis or lipidosis (Figure). Finally, the karyotype was normal.

We performed targeted panel sequencing that included 377 genes implicated in hematologic disorders. The patient consented to collection and analyses of bone marrow aspiration and buccal swab samples. WT1 (p.H271N; chr11: 25463593 C>A; AF: 0.469) and DNMT3A (p.E697*; chr2: 32449563G>T; AF: 0.137) mutations were observed in bone marrow cells; WT1 mutations were also observed in the buccal mucosa, suggesting a germline mutation (Figure 2a and b).

Our patient exhibited a congenital mutation of WT1 and congenital heart disease but showed no renal dysfunction and no gonadal developmental disorder. Accordingly, we diagnosed the patient with Meacham syndrome. In addition, the patient showed no obvious hematologic malignancy but had a mutant clone of DNMT3A and concurrent pancytopenia, which supported a diagnosis of CCUS.

We encountered a patient with CCUS who had a congenital mutation in WT1 and an acquired mutation in DNMT3A. Syndromes associated with congenital mutations in WT1 include Denys-Drash, Frasier, and Meacham syndromes. Denys-Drash and Frasier syndromes are known to cause glomerulonephritis, gonadal developmental disorders, and solid tumors (3). Meacham syndrome is characterized by cardiac malformations without renal impairment or impaired gonadal development, as in our patient; some patients have...
Dysplastic syndrome (4-6), we found no reports of congenital mutations in patients with acute myeloid leukemia or myelodysplasia; furthermore, WT1 loss leads to impaired hematopoietic differentiation, similar to the impairment observed after TET2 loss. It was also reported in 2018 (15) that the amount of intracellular 5-hmC was reduced in mice with WT1 heterozygous loss and that this was ameliorated by vitamin C administration, as in TET2 mutant mice. Furthermore, in 2015, Wang et al. (16) reported that TET2 and WT1 mutations were exclusively found in human AML cases, suggesting the involvement of a WT1 point mutation in the pathogenesis of hematological malignancies by causing TET2 dysfunction in human cases. In 2020, Bick et al. (17) reported that some patients with CHIP have a germline mutation in the TET2 enhancer sequence; this mutation is present in all patients with CHIP, regardless of the specific mutated gene. This mutation in the TET2 enhancer sequence reduces the binding abilities of GATA-binding factors 1 and 2, thereby decreasing the expression of TET2. The congenital loss of TET2 function presumably promotes the appearance of CHIP. Although the relationship between WT1 and TET2 has only recently been reported, and the findings in human cases are still unclear, in our patient, it is quite possible that the congenital mutation of WT1 caused the dysfunction of TET2, which in turn promoted the appearance of mutant clones of DNMT3A.

It is also possible that WT1 directly affects DNMT3A, thus promoting pancytopenia. In 2012, Szemes et al. (18) reported that WT1 directly transactivates DNMT3A expression; furthermore, the loss of the WT1 function causes reduced DNMT3A expression. Therefore, DNMT3A haploinsufficiency and a WT1 germline mutation may have worked together to reduce the expression of DNMT3A in our patient.

Although pancytopenia reportedly develops after Fontan surgery because of liver fibrosis and splenomegaly caused by Fontan circulation (19, 20), it is unlikely that the pancytopenia in our patient was caused by Fontan surgery. A previous study (19) showed that the mean neutrophil count after Fontan surgery is approximately 3,000-4,000/μL; no patients in that study exhibited severe neutropenia similar to the extent observed in our patient (693/μL).

An important limitation of this report is that we were unable to ascertain whether or not the WT1 function had been lost due to the germline mutation of WT1. To resolve this issue, mouse model analyses should be conducted in future studies.

**The authors state that they have no Conflict of Interest (COI).**

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**Figure 2.** (a) Structure of the WT1 gene. Our patient exhibited a germline mutation (p.H271N) in exon 3. In patients with acute myeloid leukemia, WT1 mutation sites are commonly found in exons 7 and 9 (6). (b) The structure of the DNMT3A gene. The DNMT3A mutation in our patient is commonly seen in patients with clonal hematopoiesis of indeterminate potential (7). Act: activation domain, Rep: repression domain, ZF: zinc-finger domain, PWWP: PWWP domain, ADD: ADD domain, MTase: methyltransferase domain
References


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