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Case report

Case report of pediatric TTMV-related acute promyelocytic leukemia with central nervous system infiltration and rapid accumulation of RARA-LBD mutations

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ABSTRACT

TTMV::RARA is a recently reported fusion gene associated with acute promyelocytic leukemia (APL), caused by the integration of torque teno mini virus (TTMV) genomic fragments into the second intron of the *RARA* gene. Currently, there have been only six documented cases, with clinical presentations showing significant variability. Although initial responses to all-trans retinoic acid (ATRA) treatment may be observed in patients with *TTMV::RARA*-APL, the overall prognosis remains unfavorable among infrequent reported cases. This article presents a pediatric case that manifested as *PML::RARA*-negative APL with central nervous system involvement at onset. The patient experienced both intramedullary and extramedullary relapse one year after undergoing allogeneic hematopoietic stem cell transplantation. Upon identification as *TTMV::RARA*-APL and subsequent administration of two rounds of ATRA-based treatment, the patient

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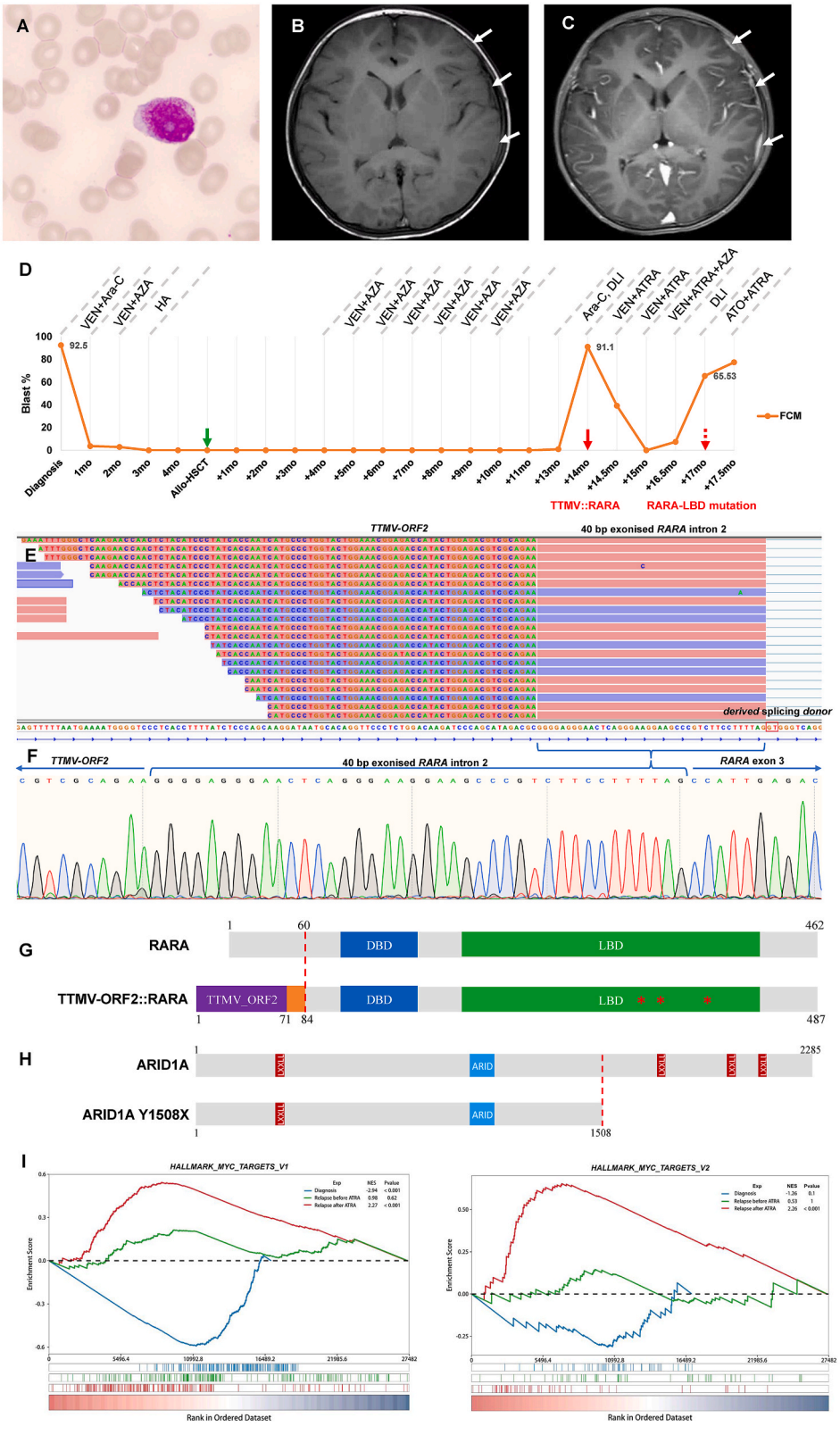

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Fig. 1. Clinical features and genetic analysis of the *TTMV::RARA*-positive acute promyelocytic leukemia (APL) case.
(A) Wright's staining of the bone marrow aspiration specimen at diagnosis showed aberrant promyelocytes with abundant granules.
(B and C) Cranial contrast-enhance magnetic resonance imaging indicated leukemic infiltration of the meninges. B is MRI T1WI and C is MRI T1WI C+; the MRI findings reveal bilateral, asymmetrical strip-like and spindle-shaped enhancements located inferior to the frontal and parietal cranial plates. Notably, the left side exhibits pronounced enhancements, accompanied by thickening and enhancement of the dura mater within the left temporal region. White arrows indicate the specific location of leukemic infiltration of the meninges.
(D) Clinical timeline of the patient from diagnosis to the end of follow-up. Treatment at different time points is indicated along the top of the timeline; the orange line shows the percentage of blast cells in bone marrow detected by flow cytometry (FCM). The green arrow represents the time point of allo-HSCT. The red arrows represent the time point of whole transcriptome sequencing (WTS) at relapse after allo-HSCT; The red dashed arrow represents the time point of the third WTS after ATRA treatment.
(E) Alignment of the whole transcriptome sequencing reads in the integrative genomics viewer revealed abundant RARA fusion transcripts in tandem spliced by 5' non-human sequence, 40 bp exonized *RARA* intron 2, and *RARA* exon 3. The 5' fusion sequence is not homologous to any human sequence, and further analysis confirmed it belongs to the open reading frame 2 (ORF2) sequence of the torque teno mini virus (TTMV).
(F) Sanger sequencing confirmation of the *TTMV::RARA* fusion transcript.
(G) Schematic diagram of the normal RARA protein (upper), and the predicted TTMV::RARA chimeric protein (lower). Splicing and fusion sites are shown in red dashed lines. The TTMV_ORF2 part encodes the first 71 amino acids at the N-terminus, followed by 13 derived amino acids encoded by the 40bp exonized *RARA* intron 2 sequence. The *RARA* part of the fusion protein retains the full length of its DNA-binding domain (DBD) and ligand-binding domain (LBD). The red asterisks represent the three *RARA*-LBD mutations.
(H) Schematic diagram of the normal ARID1A protein (upper), and the predicted disruptive ARID1A protein with stop-gain mutation (lower).
(I) Comparative to the initial diagnosis, gene set enrichment analysis of two whole transcriptome sequencing data demonstrated a progressed activation of the MYC-target gene sets following relapse. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

rapidly developed multiple *RARA* ligand-binding domain mutations and demonstrated extensive resistance to ATRA and various other therapeutic interventions. Additionally, the patient experienced *ARID1A* mutant clone expansion and progressed MYC-targeted gene activation. This case represents the first documentation of extramedullary involvement at both the initial diagnosis and relapse stages, emphasizing the intricate clinical features and challenges associated with the rapid accumulation of multiple ATRA-resistant mutations in *TTMV*::*RARA*-APL, characterizing it as a distinct and complex sub-entity of atypical APL.

1. Introduction

The torque teno mini virus (TTMV) is a ubiquitous single-stranded DNA virus characterized by a minimalist genome and high sequence variability [1]. It is also considered a symbiotic virus with humans. While research on TTMV has seen rapid growth in recent years, its only established correlation with human disease is known to cause atypical acute promyelocytic leukemia (APL) [2–6]. This occurs through the insertion of its genome fragment, comprising part of the open reading frame 2 (*ORF2*) and its 5' untranslated region, into the human genome's second intron of the *RARA* gene, resulting in the formation of the *TTMV*::*RARA* fusion gene [2–6]. The fundamental etiology by which this viral variant induces APL has been elucidated, but much remains to be investigated regarding the characteristics and effective treatment of this distinctive disease subgroup. Here we report a new clinical case with a unique disease course to help provide a deeper understanding of the characteristics of this group of diseases.

2. Case presentation

A 9-year-old boy presented with recurrent abdominal pain for one month and fever for five days. Blood cell counts revealed leukocytosis (white blood cells 41.9 $\times$ 10^9/L), anemia (hemoglobin 86g/L), and thrombocytopenia (platelets 28.0 $\times$ 10^9/L). Morphological examination showed 70% blast cells in the peripheral blood and 95.5% aberrant promyelocytes with abundant granules in the bone marrow (BM) aspiration sample (Fig. 1A). Immunophenotype investigation indicated that 92.5% of blast cells were positive for CD13, CD33, CD38, CD123, MPO, and CD371, negative for HLA-DR, and weakly expressed CD34. Chromosomal analysis revealed a karyotype of 46, XY, i(17) (q10) [3]/47, XY, idem, +8 [9]/46, XY, idem, add(16) (q24) [8]. Whole transcriptome sequencing (WTS) with a conventional bioinformatics pipeline for calling pathological fusion transcripts, reported a negative result. Cranial contrast-enhance magnetic resonance imaging indicated leukemic infiltration of the meninges (Fig. 1B and C).

He was then diagnosed with *PML*::*RARA*-negative APL with central nervous system (CNS) involvement, and immediate treatment with venetoclax (200 mg/m^2 days1-28) and low-dose cytarabine (100 mg/m^2 days 1-7) was initiated (Fig. 1D). However, BM morphology assessments on days 21 and 28 still showed 11.5% and 16.0% abnormal promyelocytes, respectively. Another induction regimen of venetoclax (200 mg/m^2 days 1-27) and azacitidine (75 mg/m^2 days 1-7) was then administered, and morphological remission of and minimal residual disease (MRD) by flow cytometry was acquired. This was followed by consolidation with homoharringtonine (3 mg/m^2 days 1-7) and cytarabine (1g/m^2 days 1-3). A total of five intrathecal injections MTX (10mg), Ara-C (50 mg), and dexamethasone (5 mg) were performed to treat CNS infiltration.

Following remission as indicated by both positron emission tomography-computed tomography (PET-CT) and BM examination, the patient underwent allogeneic hematopoietic stem cell transplantation (allo-HSCT) with his father as the donor in the fifth month of

diagnosis. The patient received total body irradiation/cyclophosphamide/fludarabine (TBI/Cy/Flu) based conditioning regimens, whole brain and spinal cord irradiation (1.6 Gy × 5 d, days -13 to -9); total body irradiation (2 Gy bid × 3 d, days -11 to -9); fludarabine (30 mg/m^2/d, days -8 to -4); cyclophosphamide (1.0g/m^2/d, days -5 to -4); Me–CCNU (250mg/m^2/d, day -3); ATG (2.5 mg/kg/d, Genzyme, days -5 to -4); ALG (35mg/kg/d, Sinopharm, days -3 to -2, [Medication was changed due to an adverse infusion reaction of ATG]). Regular BM examinations in the first year after allo-HSCT consistently indicated remission. During this period, the patient underwent three cycles of venetoclax (100 mg/m^2 days 1-23) and azacytidine (35 mg/m^2 days 1-5) and three cycles of venetoclax (200 mg/m^2 days 1-23) and azacytidine (75 mg/m^2 days 1-5) consolidation therapy.

At 13 months after allo-HSCT, the patient developed thrombocytopenia. Chimerism analysis showed that the percentage of donor-derived cells was 94.0% in BM. Flow cytometry investigation reported 1.0% blast cells positive for CD13, CD33, MPO, CD64, and CD9, negative for CD2, CD7, CD56, CD3, CD34, HLA-DR, and weakly expressed CD38. The WT1/ABL1 ratio by quantitative reverse transcription PCR was 61.3%. Therefore, laboratory tests indicate relapse. After treatment with cytarabine (1g/m^2 bid days 1-3) and donor lymphocyte infusion (5 × 10^7/kg, day 6), blast cells increased to 92.0% in BM on day 23, with complex chromosome karyotype of 47, XY, t(1;11) (p13;p11.2), del(15) (q11.2q21), add(16) (q24), i(17) (q10),+21[20]. PET-CT showed high-density shadows in the T3 vertebral body and intervertebral foramen, suggesting extramedullary infiltration.

Given our center's accumulated experience in analyzing variant APL fusion genes, we conducted WTS on the relapsed BM samples and analyzed them alongside the WTS data from the initial onset. Although a validated bioinformatics pipeline for calling pathological fusion transcripts reported a negative result [7], abundant chimeric *TTMV*::*RARA* sequences were identified upon manual investigation through the Integrative Genomics Viewer in both initial-onset and relapse specimens (Fig. 1E). This *TTMV*::*RARA* fusion transcript was confirmed by PCR and Sanger sequencing (Fig. 1F and G). Furthermore, gene mutation analysis revealed that the patient's initial diagnosis sample carried the *ARID1A* c.4524T > A/p.Y1508X mutation with a variant allele frequency (VAF) of 4%, and its VAF increased to 57.4% in the relapsed sample (Fig. 1H, Supplementary Fig. 1A).

Therefore, the diagnosis was amended to *TTMV*::*RARA*-APL. One cycle of ATRA (20 mg, days 1-28) combined with venetoclax (200 mg/m^2, days 1-28) therapy was administered, resulting in remission confirmed by both BM morphology and flow cytometry investigation. Ten days later, the second cycle of ATRA (25 mg, days 1-14) combined with venetoclax (200 mg/m^2, days 1-28) and azacitidine (75 mg/m^2, days 1-5) therapy, and donor lymphocyte infusion (1 × 10^7/kg, day 34) was performed. Unfortunately, BM investigation revealed 65.5% blast cells at the time of this post-treatment evaluation by flow cytometry, indicating a recurrence. A third WTS was performed.

The treatment approach was then shifted to ATRA (20 mg, days 1-14) combined with arsenic trioxide (0.15mg/kg, days 1-14). However, on the 14th day of this treatment, the blast cells in BM increased to 77.5% by flow cytometry (Fig. 1D). Mutation analysis utilizing the third WTS data identified three *RARA* ligand-binding domain (LBD) mutations with VAF exceeding 2%: c.826C > G/p.R276G (VAF 3.7%), c.860C > G/p.S287W (VAF 27.6%), and c.1061C > G/p.P354R (VAF 37.2%) (Fig. 1G, Supplementary Fig. 1B). Comparative to the initial diagnosis and the first relapse, gene set enrichment analysis of the three WTS data demonstrated a gradually increased activation of the MYC targeted gene sets following relapse (Fig. 1I). The patient underwent continuous chemotherapy and targeted therapy, resulting in persistent agranulocytosis and severe pulmonary infection. Currently, the primary treatment approach involves anti-infective therapy. Simultaneously, we are actively searching for suitable donors for a potential second allo-HSCT.

3. Discussion

The pediatric case of *TTMV*::*RARA*-APL described in this article presented with CNS infiltration at onset and experienced both intramedullary and extramedullary relapse 13 months after allo-HSCT. A prior report from our team documented a *TTMV*::*RARA*-APL case that initially presented as myeloid sarcoma [6]. These instances suggest a propensity for CNS or other extramedullary lesions within this group of diseases. High incidences of *ARID1A/B* mutations in relapsed *PML*::*RARA*-positive APL [8,9] and atypical APL [10,11] have been reported. In a previous pediatric *TTMV*::*RARA*-APL case, we observed an *ARID1B* mutation at relapse [5]. In this case, a notable increase in *ARID1A* mutation VAF was noted at relapse following allo-HSCT, indicating that *ARID1A/B* mutations might be resistance mutations that frequently emerge under non-ATRA regimens in *TTMV*::*RARA*-APL.

Three *RARA*-LBD resistance mutations were identified in this patient after only two cycles of ATRA treatment, reflecting the characteristic rapid accumulation of resistance mutations. This further supports the therapeutic pressure of ATRA on *TTMV*::*RARA* leukemia cells. However, the swift accumulation of *RARA*-LBD mutant clones implies a predisposition to rapid ATRA resistance within *TTMV*::*RARA*-APL, limiting the efficacy of ATRA. TTMV is a single-stranded DNA virus with highly variable sequences [1], which might contribute to the rapid mutation mechanism, warranting further investigation. Gene set enrichment analysis revealed this patient acquired sustained enhanced activation of the MYC target gene sets at relapse following allo-HSCT and subsequent resistance to ATRA. Aberrant activation of the MYC target gene sets is a common molecular etiology of refractory hematological malignancies [12] and may be an important etiology conferring extensive drug resistance in this patient.

Within the two years since the *TTMV*::*RARA*-APL was reported, a total of seven cases, including this one, have been documented by three centers, six of which were children [2–6]. This suggests that this group of diseases, which was previously under-recognized and under-identified, may not occur infrequently. Although initial responses to ATRA treatment may be observed, the overall prognosis remains unfavorable. Based on this case and documented cases, *TTMV*::*RARA*-APL may exhibit the following disease characteristics: primarily affecting children, which may be related to widespread TTMV-acquired infection in early human life; prone to extramedullary lesions; prone to accumulate *ARID1A/B* mutations under non-ATRA based regimen; ATRA treatment is initially effective, but prone to the rapid accumulation of *RARA*-LBD mutant resistant clones. Rapid and accurate identification of *TTMV*::*RARA* is important for identifying such patients but presents challenges in bioinformatics analysis due to the high variability of TTMV genome

sequences. Initial treatment with ATRA and venetoclax-containing regimens may provide short-term benefits, but there is a high risk of progression to refractory APL. Therefore, effective treatment strategies necessitate further research.

Ethical approval statement

This article was reviewed and approved by Medical Ethics Committee of Beijing Children's Hospital, Capital Medical University. Written informed consent was obtained from the patient's legal guardian for publication of this article.

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Data availability statement

The datasets presented in this article are not readily available because of ethical/privacy restrictions. Requests to access the datasets should be directed to the corresponding author.

CRediT authorship contribution statement

Linya Wang: Resources, Project administration, Investigation. **Jiaqi Chen:** Writing – original draft, Resources, Methodology. **Bei Hou:** Resources, Project administration. **Ying Wu:** Resources, Project administration. **Jun Yang:** Resources, Project administration. **Xiaosu Zhou:** Formal analysis. **Qihui Chen:** Visualization, Software. **Xue Chen:** Formal analysis. **Yang Zhang:** Visualization, Data curation. **Fang Wang:** Visualization, Data curation. **Jiancheng Fang:** Visualization, Software. **Panxiang Cao:** Visualization, Software. **Mingyue Liu:** Visualization, Data curation. **Yanan Li:** Resources, Project administration. **Pan Zhang:** Resources, Project administration. **Yan Liu:** Resources, Project administration. **Ruidong Zhang:** Resources, Project administration. **Hongxing Liu:** Writing – review & editing, Methodology, Conceptualization. **Huyong Zheng:** Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e27107.

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