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CORRESPONDENCE

# A novel loss of function mutation in the *HAVCR2* gene in a patient diagnosed with Hodgkin's lymphoma

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To the Editor,

TIM3, encoded by the *HAVCR2* gene, belongs to the T cell immunoglobulin and mucin domain (TIM) protein family. This protein is expressed by both innate and adaptive immune system cell types. Activation of TIM3 occurs through its interaction with ligands such as galectin-9, high-mobility group protein B1, carcinoembryonic antigen cell adhesion molecule 1, and phosphatidylserine. Studies have shown that TIM3 regulates immune responses in cancer, autoimmunity, and infection [1].

This study examined the T cell immune response in a patient with a *HAVCR2* c.799A > G (p.I267V) homozygous nucleotide variant using whole exome sequencing (WES) analysis.

A 6-year-old male patient with neck swelling and fever was diagnosed with stage IV Hodgkin's lymphoma (mixed cell type) by lymph node excisional biopsy. The COPP/ABVD treatment protocol was administered, and the patient achieved remission as confirmed by PET CT imaging. The patient was referred to us at the age of 12 because of the presence of cervical and supraclavicular lymphadenopathy, as well as pancytopenia. Lymph node biopsies performed at both initial diagnosis and relapse confirmed Hodgkin lymphoma, while bone marrow biopsies and aspirations at both time points showed no involvement. The patient received ICE (ifosfamide, carboplatin, etoposide) and brentuximab treatments, and the *HAVCR2* gene mutation was identified through whole exome sequencing (WES) analysis. During the physical examination of the patient, hyperpigmented follicular papules and macules were observed on both the anterior and posterior aspects of the trunk. Evaluations of other systems revealed no significant abnormalities. The patient was scheduled to undergo allogeneic stem cell transplantation. Due to the absence of an HLA-matched related donor, the transplantation was carried out using a matched unrelated donor (MUD) with a 10/10 HLA match. The patient

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had c.799A > G homozygous nucleotide change in *HAVCR2* which were passed on by the carrier parents (Fig. 1A). The variant was identified as rare in population databases with reported allele frequencies of $C = 0.000008$ (2/264690, TOPMED), $C = 0.000024$ (6/251158, GnomAD_exome), $C = 0.000008$ (1/121360, ExAC), and $C = 0.00003$ (1/35432, ALFA). Polyphen predicts the mutation as probably damaging (0.981), while SIFT classifies it as deleterious. Additionally, a CADD score of 24.2 supports its likely pathogenicity, whereas Alphamissense (0.12) predicts it as benign. Familial segregation analysis via Sanger sequencing revealed that both of the patient's siblings were heterozygous for the variant. The sequencing results are shown in Figure S1. A carton drawing of the TIM3 protein structure highlighting the location of the patient's I267V mutation is shown (Fig. 1B). I267 (mutation site) was conserved across multiple species, suggesting the importance of this conserved amino acid (Fig. 1C). Structural ribbon model showing the position of the TIM3 protein in the cytoplasmic tail with the mutated region (I267V) is highlighted in Fig. 1D.

TIM3 was initially identified as a Th1-specific cell surface protein responsible for producing IFN-γ in 2002 [2]. Subsequently, it was found that the dysregulation of TIM3 expression was associated with certain autoimmune diseases. For example, TIM3 regulates an autoimmune disease such as experimental autoimmune encephalomyelitis (EAE) through macrophage activation [2]. The interaction of TIM3 with its ligand leads to suppression of Th1 response; therefore, it has an inhibitory function. As a result of in vivo application of anti-TIM3 fusion protein, TIM3/ligand interaction is blocked, Th1 activation is increased and cytokine production is strengthened, so the disease is exacerbated. Similarly, decreased TIM3 expression on T cells was correlated with increased IFN-γ production in human experiments [3]. In another study focused on patients with multiple sclerosis, blocking TIM3 during T cell stimulation showed no impact in the untreated group but led to a significant increase in IFN-γ secretion in both the controls group and the treated patients. This revealed a defect in TIM3 immunoregulation [4]. In addition, dysregulation of TIM3 signaling has been linked to various autoimmune conditions, including rheumatoid arthritis, SLE, Crohn's, type 1 diabetes, thrombocytopenia, and aplastic anemia [5]. All these studies concluded that T cell tolerance would be correlated with TIM3 expression [6]. TIM3 MFI values of CD4 + (top left) and CD8 + (bottom left) cells in PBMCs stimulated with different mitogens for 1 night were similar in patients and healthy controls (Fig. 1E). We performed ex vivo Th1 differentiation from memory CD4 + T cells selected from patients and healthy donors. TIM3 MFIs of IFNy + (middle top) and IFNy − (middle bottom) cells of the patient were significantly decreased compared to the healthy donor (Fig. 1E). The PBMCs of the patient and healthy donors were stimulated with PHA + IL2 for 12 days and activated with CD3 + CD28 for 4 days. TIM3 MFIs of CD8 − (top right) and CD8 + (bottom right) cells of the patient were significantly decreased compared to healthy controls (Fig. 1E). Ex vivo Th1 cells of the patient with TIM3 defect were significantly increased compared to the healthy controls (Fig. 1F). All these results are consistent with the literature. TIM3-deficient mouse spleen cells were found to have increased proliferation and increased IL-2 production [7]. Furthermore, in the peripheral blood of breast cancer patients, T follicular helper cells showed increased expression of PD1 and TIM3. These cells displayed signs of exhaustion, including decreased proliferation and cytokine production [8]. The proposed pathogenic mechanism in this case report may entail dysregulated B cell receptor signaling facilitated by galectin-9, which is a TIM3 ligand, necessitating additional research to determine causality and generalization. This study identifies a novel correlation between a homozygous *HAVCR2* variant and B-cell lymphoma, requiring additional research for validation.

We re-examined the TIM3 expression level in the blood obtained from the patient after allogeneic HSCT. We used both a healthy controls and a donor who had allogeneic HSCT for another reason (other than TIM3 mutation as controls). TIM MFI was increased in the patient after transplantation and was as high as the healthy controls (Fig. 1G). We revisited the ability of ex vivo Th1 differentiation after allogeneic HSCT. Our results showed that the frequency of Th1 cells were comparable between transplanted TIM3 patient and the healthy controls (Fig. 1H), suggesting a restoration of the TIM3 variant-mediated Th1 dysregulation.

Altered proliferation profiles of cells with TIM3-deficiency have been demonstrated in studies with cancers [9]. We showed that proliferation of blood lymphocytes of the patient with TIM3 defect is increased (Figure S2). T cell activation markers of the patient were examined. CD69 and HLA-DR expressions were found to be higher, and T cells had memory characteristics (Figure S3, S4), suggesting aberrant T cell activation due to this variant.

Although initially discovered as a Th1-specific molecule, TIM3's regulatory role has since been shown to extend to other cell types, including Th17 cells, FOXP3 + T cells (Tregs), CD8 + T cells, and some innate immune cells. Blocking TIM3 with an anti-TIM3 antibody reduced the number of both CD4 + CD25 + Foxp3 + Treg and TIM3 + Treg cells, stimulated an antitumor immune response, and inhibited tumor growth in a mouse xenograft model of head and neck cancer [10]. In our patient, the percentage of Tregs in PBMCs was as high as the control (Figure S5).

Missense mutations in the *HAVCR2* gene have been associated with sporadic subcutaneous panniculitis-like T-cell lymphoma, which was not observed in our patient [11, 12]. However, galectin-9, which appears to be TIM3-ligand

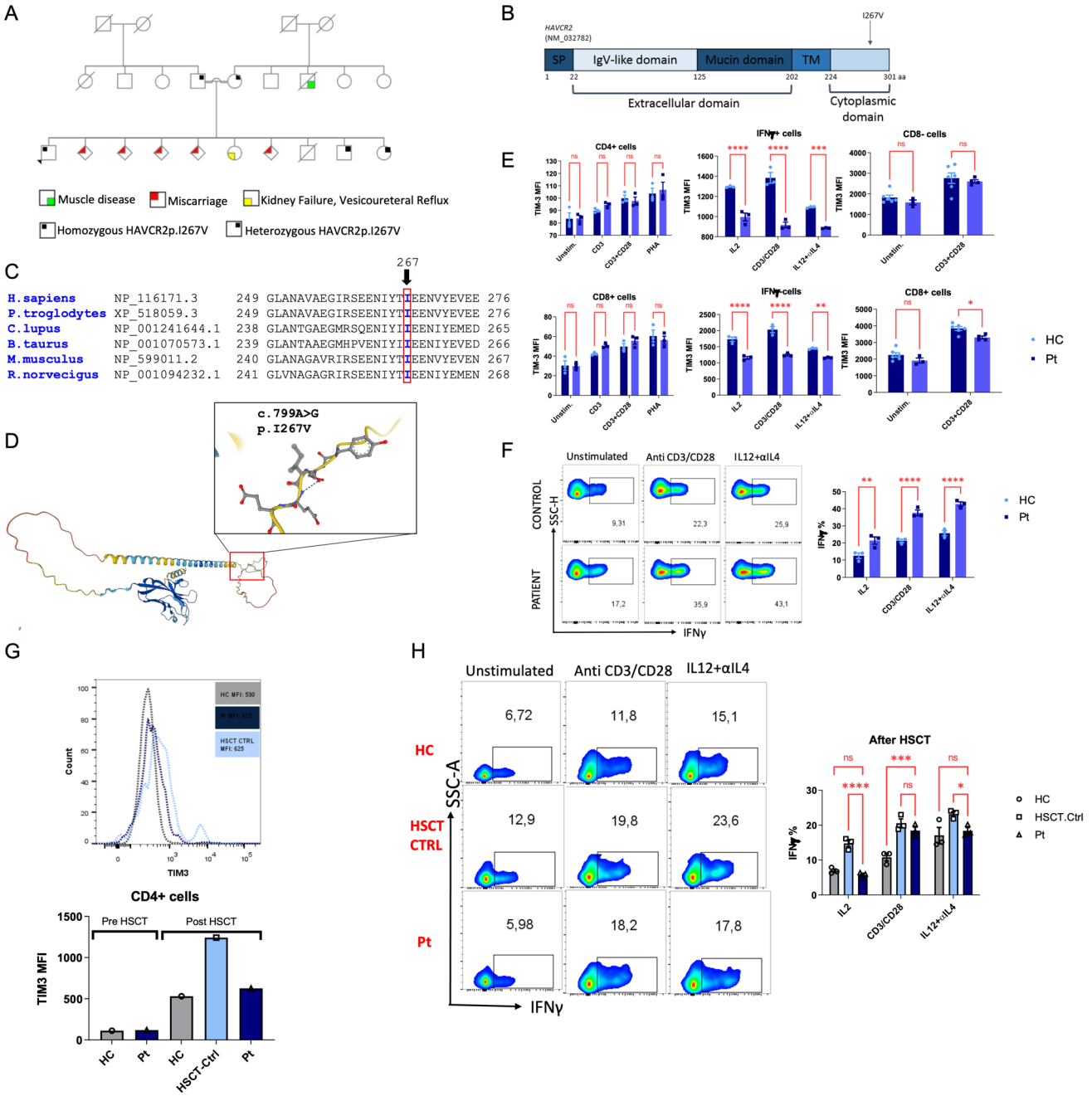


**Fig. 1** I267V substitution alters TIM3 protein expression on prolonged activation, TIM3 expression, and ex vivo Th1 cell frequency after allogeneic HSCT are at the same levels as controls. **A** Pedigree analysis showing inheritance of the homozygous HAVCR2 p.I267V mutation (denoted by squares with a black dot) within the family. Colored symbols indicate clinical manifestations: muscle disease (green), abortion (red), and kidney failure (yellow). **B** Schematic of the TIM3 protein structure, highlighting the location of the I267V mutation within the cytoplasmic domain. **C** Evolutionary conservation of the I267V mutation site in HAVCR2 across multiple species, showing conservation of the affected amino acid (boxed). **D** Structural ribbon model of the TIM3 protein with the mutated site (I267V) highlighted, demonstrating the position within the cytoplasmic tail. **E** TIM3 MFI plot of CD4 + (top left) and CD8 + (bottom left) cells from PBMCs 1 day after activation. TIM3 MFI plot of IFN-γ + (middle top) and IFN-γ (middle bottom) cells after ex vivo Th1 differentiation from memory CD4. TIM3 MFI plot of CD8-(top right) CD8 + (bottom right) cells proliferated with PHA + IL2 for 12 days and then activated with CD3 + CD28 for 4 days. **F** SSC-H versus IFN-γ flow cytometry plots (left) and quantification plots (right) after 5 days of ex vivo Th1 differentiation of selected memory CD4 + T cells. Samples from patients and healthy controls were run in triplicate. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$, $^{****}P<0.0001$. Error bars indicate ± SEM. HC, healthy controls; Pt, patient; MFI, mean fluorescence intensity. **G** Patient, HSCT controls, healthy controls CD4 + T cell TIM3 MFI histogram (top) and bar graphs (bottom) after allogeneic HSCT. **H** Patient, HSCT controls, healthy controls ex vivo Th1 (5 days) percentage after allogeneic HSCT. Samples from patients and healthy controls were run in triplicate. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$, $^{****}P<0.0001$. Error bars indicate ± SEM. HC, healthy controls; Pt, patient; HSCT, hematopoietic stem-cell transplantation. MFI, mean fluorescence intensity

[13], was shown to restrain B cell receptor signaling [14, 15]. Our data and the case presented suggest that human patients with TIM3 variants may also present with B cell malignancies. Cutaneous T-cell lymphoma has been treated with HSCT [16, 17]. A patient with TIM-3 compound mutations (p.Ile97Met and p.Thr101Ile, also in the Ig domain) developed panniculitis-like T-cell lymphoma and hemophagocytic lymphohistiocytosis and was treated with HSCT [18]. Our report also supports that HSCT may be curative in malignancies associated with this rare IEI. The majority of documented TIM3 variants are missense mutations that impact the immunoglobulin or mucin domains, resulting in hemophagocytic lymphohistiocytosis (HLH) or autoimmune disorders (Supplemental Table 1). Our patient underwent assessments for clinical and laboratory indicators of HLH, including ferritin levels, triglycerides, and soluble CD25 levels, all of which were within normal limits. The variant in the current report lies in the cytoplasmic domain of TIM3 and underscores a distinctive phenotype associated with Hodgkin's lymphoma, indicating a potential novel genotype–phenotype link.

**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1007/s12026-025-09618-5.

**Author contribution** SE and ANC conducted the experiments and contributed to the manuscript preparation. EY, AO, MK, and EU were involved in patient care, data analysis, and manuscript revision. AB performed whole exome data analysis. MED performed Sanger sequencing. AE designed the study, interpreted the results, and critically revised the manuscript.

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**Data availability** No datasets were generated or analysed during the current study.

## Declarations

**Ethics approval** All experimental procedures were reviewed and approved by the Erciyes University Clinical Research Ethics Committee (approval no. 2021/17). The study was conducted in full compliance with institutional guidelines, the ethical principles outlined in the Declaration of Helsinki, and applicable regulations for clinical research.

**Conflict of interest** The authors declare no competing interests.

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