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ORIGINAL ARTICLE

Treatment of STING-associated vasculopathy with onset in infancy in patients carrying a novel mutation in the TMEM173 gene with the JAK3-inhibitor tofacitinib

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ABSTRACT

Objectives: This study aimed to reveal the genetic background of patients in the two-generation family suffering from rheumatoid arthritis, psoriatic arthropathy pain, scratches, and bruises.

Patients and methods: A clinical exome sequencing analysis was performed in 10 individuals in the same family using the Sophia Genetics clinical exome solution kit.

Results: A novel V194L mutation in the TMEM173 gene was identified in three members of the family. Two of the family members were treated with the JAK3 inhibitor tofacitinib and recovered completely one month after the treatment.

Conclusion: The V194L mutation was reported for the first time in this study, and a positive response was achieved with tofacitinib.

Keywords: STING–associated vasculopathy with onset in infancy, tofacitinib, V194L mutation.

The stimulator of interferon genes (STING) is an endoplasmic reticulum (ER)-resident transmembrane protein and was first recognized as part of the ER translocon system.[1,2] STING, cyclic guanosine monophosphate-adenosine monophosphate (cGAMP), and cGAS (cGAMP synthase) form a DNA-sensing mechanism in the cytoplasm of mammalian cells. During the infection of pathogens and DNA viruses, this pathway leads to induce the expression of proinflammatory cytokines regulated by the type I interferon and NF-κB (nuclear factor kappa B).[3] STING can also be known as transmembrane protein 173 (TMEM173), ERIS, MITA, and MPYS and plays important roles in controlling the transcription of proinflammatory cytokines. STING can be expressed in hematopoietic cells, such as T cells, macrophages, dendritic cells, endothelial cells, and epithelial cells.[4] STING protein has four transmembrane domains, a CDN binding domain, and interaction sites for TBK1 and IRF3 (interferon regulatory factor 3) at the tail.[5]

STING-associated vasculopathy with onset in infancy (SAVI) is a rare autoinflammatory disease with autosomal dominant inheritance, caused by gain-of-function mutations in the

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human TMEM173 gene, which is located at the chromosome 5q31.2 encoding a STING protein,[6,7] and is identified in infants who suffer from severe and chronic vasculopathy, cutaneous vasculitis, systemic inflammation, and interstitial lung disease.[8,9] Lung involvement is a cause of death in SAVI.[8] Eight activating mutations in the TMEM173 gene are known to lead to SAVI. These mutations are represented in Figure 1.[10] This study aimed to reveal the genetic background of patients in the two-generation family suffering from rheumatoid arthritis, psoriatic arthropathy pain, scratches, and bruises.

PATIENTS AND METHODS

A 45-year-old female (II:5) with rheumatoid arthritis, psoriatic arthropathy, and swelling of the ankles, hands, face, eyes, and abdomen described that she never had 5 min of comfortable time in her life without pain, scratches, and bruises. The patient had difficulty stepping on the ground in the morning for approximately 30 min and mentioned that bruises appear without a reason on her body. Three of her children applied to our department. Two of them (III: 8,9,10) had similar symptoms with their mother. The 20-year-old elder daughter was also suffering from severe symptoms similar to her mother (Figure 2). A 14-year-old boy had a general skin eruption that does not compatible with urticaria. Proband's elder sister (II:4) lost her life at the age of 59 as a result of acute lung complications. Posteroanterior chest radiographs were taken twice a year for follow-up purposes. Her elder sister (II:2) and brother (II:1) also had some complications. The pedigree of the entire family is shown in Figure 2.

(a)

***TMEM173*-activating mutations in SAVI patients**

Inherited *TMEM173*-activating mutations	Affected individuals	De novo *TMEM173*-activating mutations	Affected individuals
G166E	5	N154S	4
V155M	6	V155M	5
		V147M	2
		V147L	1
		C206Y	1
		R284G	1
		R281Q	1
		S102P-F279L	1

(b)

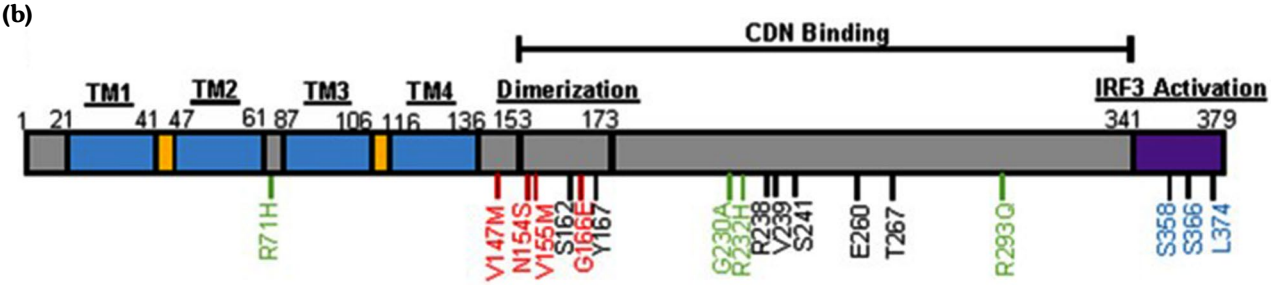

Figure 1. **(a)** Activating mutations in SAVI patients. **(b)** Representative presentation of functional domains in the human STING protein.[10]

SAVI: STING-associated vasculopathy with onset in infancy; STING: Stimulator of interferon genes.

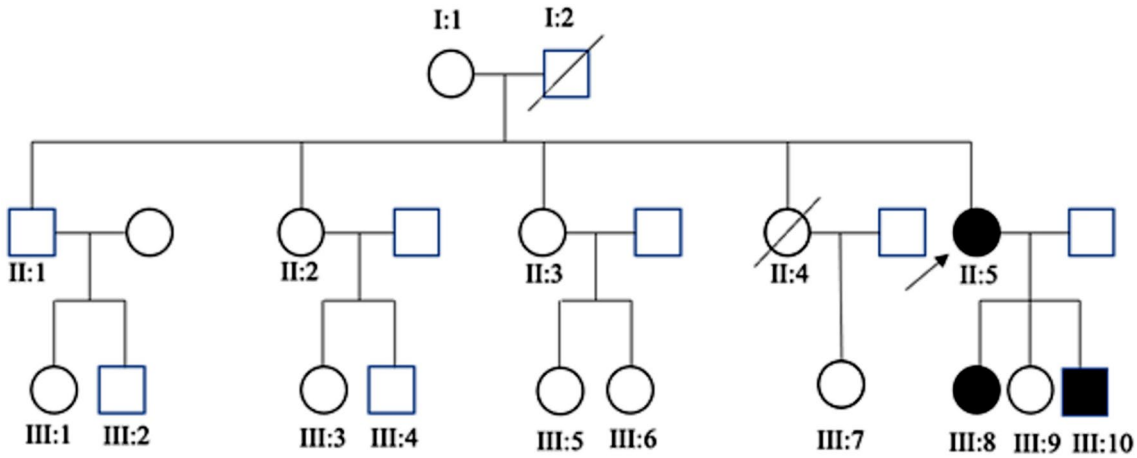

Figure 2. Pedigree of the family

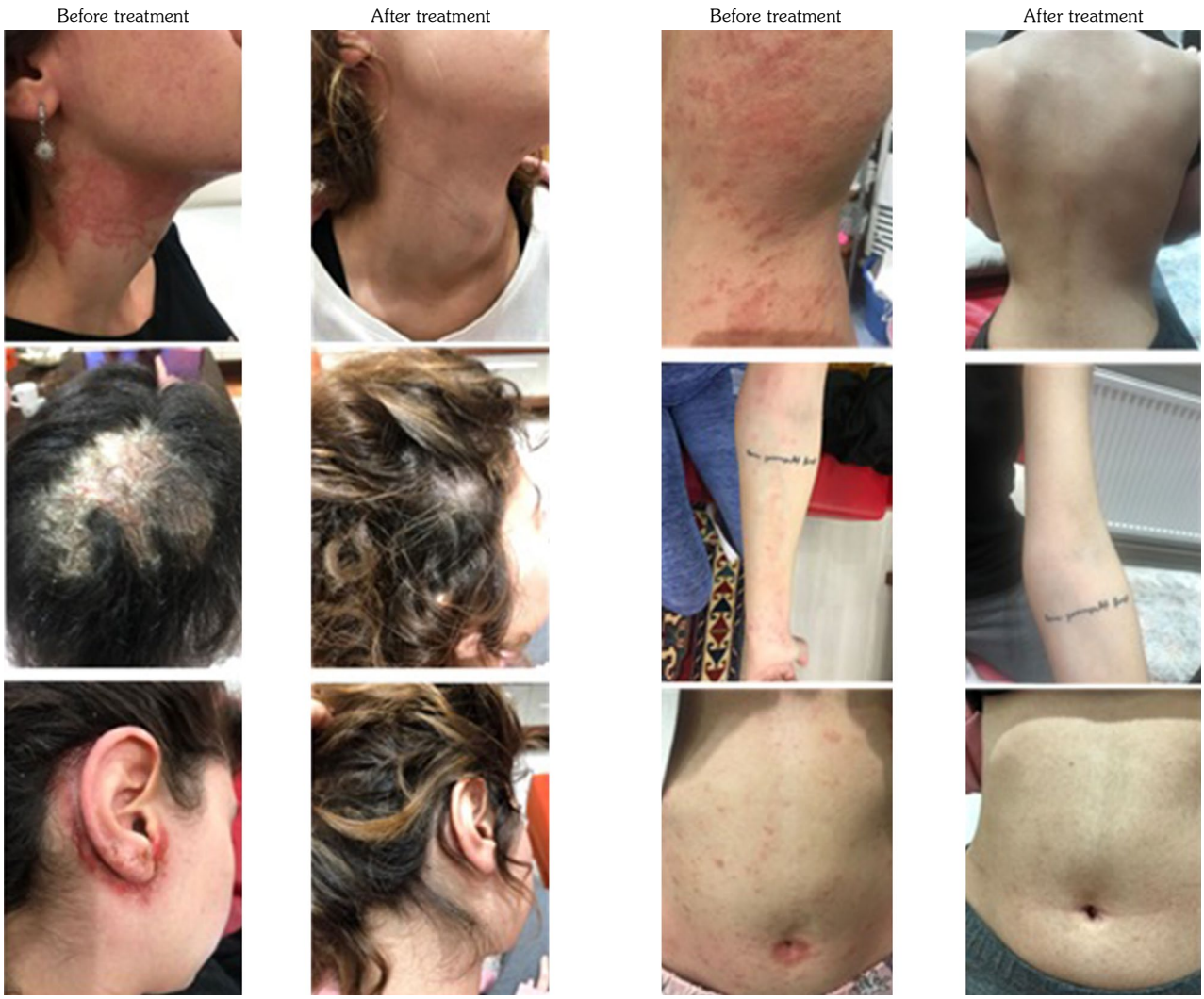

Figure 3. Proband's daughter after tofacitinib treatment.

Specimen collection

Whole blood was collected in tubes with EDTA. DNA was extracted from 200 µL of whole blood on a QIAcube instrument with QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany) according to the instructions of the manufacturer.

Clinical exome sequencing and data analysis

Samples underwent clinical exome sequencing using Sophia Genetics clinical exome solution kit (Sophia Genetics, Lausanne, Switzerland). Libraries were pooled together, quantified, and fragment size was assessed by Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). Reads were obtained with the NextSeq-500 sequencer (Illumina Inc., San Diego, CA, USA) with a coverage of at least 100× for 80-90% of all sequences. Fastq files were uploaded to the SOPHiA DDM platform (Sophia Genetics, Lausanne, Switzerland) to analyze single nucleotide variants, indels, and copy number variations. The pathogenicity of the mutations was evaluated based on in silico prediction tools (Varsome, SIFT, PolyPhen-2, MutationTaster), the inheritance mode (OMIM), database entries (HGMD, ClinVar), and the American College of Medical Genetics and Genomics recommendations.

RESULTS

We evaluated 10 individuals in the family (II:1,2,3,5; III:3,5,7,8,9,10). Clinical exome sequencing was performed for the proband and her three children at the first step. Coding variants were filtered against allele frequencies from public and local databases, and virtual gene panels composed of genes causing autoinflammatory diseases (n=95) and psoriasis (n=5) were created using the SOPHiA DDM platform. No pathogenic mutations have been observed in psoriasis-related genes (CTLA4, IL36RN, GATA3, CARD14, and ZNF750). When filtering the variants for genes related to autoinflammatory diseases, we identified a novel heterozygous mutation in exon 6 of the TMEM173 gene (c.580G>T, p.Val194Leu) in the proband and her two children (III:8, III:10) who developed similar symptoms. After finding the novel mutation in these patients, we checked whole TMEM173 mutations in the literature and found four activating mutations in the protein region close to our finding site that also cause pathologic symptoms. Those who are neighboring V194L mutations are V147L, V147M, V155M, and C206Y mutations that are shown to be related to severe skin lesions, systemic inflammation, and interstitial lung disease.[11-13] Further, the proband's two siblings and their children, who do not have

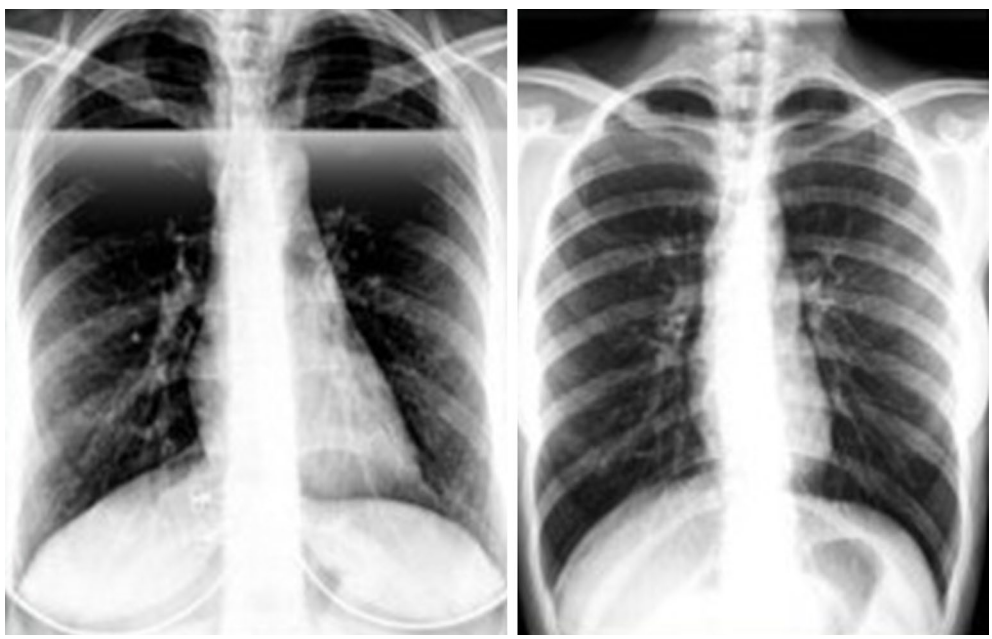

Figure 4. Posteroanterior chest radiographs of patients.

symptoms, were also evaluated for the novel TMEM173 mutation, and none of them were found to carry the TMEM173 V194L mutation.

Proband, who had been followed up in the rheumatology department, did not respond to any treatment despite using colchicine, methylprednisolone (4 mg), leflunomide (10 mg), adalimumab, and indomethacin for her conditions. Her daughter used adalimumab, secukinumab, colchicine, and leflunomide, which were also not effective. Posteroanterior chest radiographs of both the proband and her daughter did not show any signs of lung complications (Figure 4). As a result of the V194L mutation detected in the TMEM173 gene, she and her daughter were diagnosed as SAVI, and the treatment strategy was exchanged with 5 mg oral dosage of JAK3 (Janus kinase 3)-inhibitor tofacitinib two times a day. The patients positively responded to the treatment from the initial use. Complaints decreased by about 70% in two weeks and completely disappeared one month after the treatment (Figure 3). Tofacitinib treatment has been continued for more than one year.

Initial blood results demonstrated elevated levels of alanine aminotransferase (mother: 52 IU/L, daughter: 40 IU/L; reference: <32 IU/L) and aspartate transaminase (mother: 42 IU/L, daughter: 37 IU/L; reference: <33 IU/L). The rest of the biochemistry, hormone, and hematology markers were between the reference ranges. In addition, the daughter had an increased ferritin saturation (53%; reference: 15-45%). Their latest blood test results revealed that the levels of alanine aminotransferase (mother: 26 IU/L, daughter: 21 IU/L) and aspartate transaminase (mother: 22 IU/L, daughter: 18 IU/L) were decreased, and the ferritin saturation of the daughter was 13%.

DISCUSSION

We report three patients in the same family with a novel mutation in the TMEM173 gene encoding the STING adaptor protein. STING is known to regulate several pathways in two manners: cGAMP dependent or independent. In SAVI, STING activation is cGAMP independent. In the case of a gain of mutation, STING is activated in Golgi via a cGAMP-independent manner and augments type I interferon production via activating the NF-κB pathway through inflammatory cytokine production and IRF3 phosphorylation. This activation induces ER stress and leads to cell death.[14]

SAVI with the de novo TMEM173 mutations tend to have an early onset (<8 weeks) and severe phenotype,[3,15] whereas familial TMEM173 mutations have late-onset (teenager or adulthood) and milder clinical manifestations.[9,16] Two genetic variants have been described, of which p.Val155Met is the most common.[10] Eight novel mutations were also identified (N154S, V155M, V147M, V147L, C206Y, R284G, R281Q, and S102P-F279L). STING has four amino-terminal transmembrane domains, a dimerization domain, known as helix α1, and a CDN binding domain that contains a dimerization domain carboxy-terminal tail. These identified mutations are located in the CDN binding domain, and the V194L mutation are also found in the same domain.

The activating mutations that are neighboring the V194L mutation are found to be related to the SAVI phenotype. It has been shown that inherited V155M mutations in SAVI patients have less severe penetration compared to the ones with de novo V155M mutation. A de novo V147L mutation in a patient with Chilean ancestry was identified in a SAVI patient.[3] Patients carrying a N154S mutation were found to have an earlier disease onset and more severe skin lesions compared to patients with a p.V155M mutation.[11] Munoz et al.[12] reported a de novo V147M mutation in a patient with SAVI. Melki et al.[13] reported three individuals with de novo TMEM173 mutations (C206Y, P281Q, R284G) who were harboring the features of the SAVI phenotype including systemic inflammation, interstitial lung disease, and skin lesions.

Anti-inflammatory treatment strategies in SAVI patients including corticosteroid, anti-TNF (tumor necrosis factor), intravenous immunoglobulin, steroids, and anti-CD20 were found to be ineffective.[8,17] Lung damage is irreversible in SAVI patients, resulting in death.[11] JAK inhibitors are used for the treatment of SAVI. It has been suggested that ruxolitinib (JAK1 inhibitor) and baricitinib (JAK2 inhibitor) are more suitable than tofacitinib (JAK3 inhibitor) for SAVI treatment,[10] but ruxolitinib appears to be a therapeutic option

for SAVI.[17] TMEM173 mutations (V147M [n=1], V155M [n=3], R281Q [n=1], N154S [n=1], and R284G [n=1]) in SAVI patients and their response to JAK inhibitor treatment are also described. All patients carrying those mutations undergone for ruxolitinib treatment were found to have clinical responses in the first weeks of treatment.[17-19] A novel homozygous R281W mutation was also identified in two siblings who had undergone ruxolitinib treatment with a positive response. Two patients with N154S mutations were treated with baricitinib and reported to have no lesions since the treatment.[20,21] A 9-year-old Korean male carrying two de novo mutations, S102P, and F279L, responded positively to the tofacitinib treatment.[22]

Although the prevalence of SAVI is less than 1/1,000,000, there is a limitation for enlightening the effects of JAK inhibitors on SAVI treatment. Nevertheless, the small number of cases worldwide have shown that JAK inhibitors relieve the symptoms of SAVI. Our study has importance in showing a novel TMEM173 variant and the effect of tofacitinib on relieving the symptoms in SAVI patients.

In conclusion, we described a novel V194L mutation in the TMEM173 gene for the first time by three members out of 10 in the same family. Treatment with the JAK3 inhibitor tofacitinib has been found to be effective in fully recovering symptoms in two family members suffering from the disease.

Ethics Committee Approval: The study protocol was approved by the Pamukkale University Faculty of Medicine Ethics Committee (date: 03/02/2021, no: 26289). The study was conducted in accordance with the principles of the Declaration of Helsinki.

Patient Consent for Publication: A written informed consent was obtained from each patient.

Data Sharing Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Author Contributions: Concept: P.E.T., N.K.; Design: P.E.T., N.K., O.T., S.T., U.K., A.D., H.A., S.Y.; Supervision: N.K., S.Y., H.A.; Data collection and/or processing: P.E.T., N.K., O.T., S.T., U.K., A.D., H.A., S.Y.; Analysis and/or interpretation: P.E.T., N.K., O.T., S.T.; Literature search: P.E.T., N.K., O.T., S.T., U.K., A.D., H.A., S.Y.; Writing: P.E.T., N.K.; Critical review: P.E.T., N.K., O.T., S.T., U.K., A.D., H.A., S.Y.

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