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LETTER TO EDITOR

A Novel *FOXN1* Variant Is Identified in Two Siblings with Nude Severe Combined Immunodeficiency

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To the Editor,

Severe combined immunodeficiency (SCID) is the most severe form of primary immunodeficiencies (PIDs) caused by gene variants that lead to a failure of functional T cell development, with or without accompanying defects in the production of B and/or NK cells [1]. Deleterious variants in more than 20 genes have been implicated in SCID [2]. The *FOXN1* (Forkhead Box N1) deficiency, also known as the nude SCID, is a very rare autosomal recessive form of SCIDs. It has a unique phenotype with severe T cell immunodeficiency with normal B and NK cells, thymus dysgenesis, congenital alopecia, and nail dystrophy [3]. Due to the pleiotropic effects of *FOXN1*, the patients present with non-immunological features in addition to the classical $T^-B^+NK^+$ SCID, with mainly the skin and hair being affected including abnormal hair keratinization and absence of hair [4].

FOXN1 gene (located in 17q11.2) encodes a transcription factor that regulates the development, differentiation, and function of thymic epithelial cells (TECs) both in the prenatal and postnatal thymus [5, 6]. *FOXN1* mutations disrupt T cell lineage commitment, development, and selection [7]. Currently three different variants (p.R255*, p.R320W, and p.S188fs) have been reported in the different domains of *FOXN1* [8–10]. In this report, we describe the clinical features of two siblings with nude SCID phenotype who are homozygous for a novel variant in *FOXN1*.

A two-month-old male (P1), born to consanguineous parents (second-degree cousins) presented with pneumonia, otitis, diarrhea, and absence of a thymus (Fig. S1) and was diagnosed with $T^-B^+NK^+$ SCID. Although the patient was referred to a bone marrow transplantation center, the family refused any further diagnostic tests or treatment; he died at home 1 month after diagnosis.

Fourteen months after the passing of their first child, the mother gave birth to a daughter (P2), who suffered from oral candidiasis, recurrent lung infections, and sepsis. Immunophenotyping results showed T cell deficiency and although she had very low amount of B and NK cells, due to a very low absolute lymphocyte count, she was diagnosed as $T^-B^+NK^+$ SCID (Table S1).

P2 was referred for hematopoietic stem cell transplantation (HSCT) from a HLA-match unrelated donor.

An amplicon-based targeted next generation sequencing (NGS) panel that contains 18 of the most common SCID-related genes (*IL2RG*, *JAK3*, *L7RA*, *PTPRC*, *CD3D*, *CD3E*, *CD3Z*, *CORO1A*, *DCLRE1C*, *PRKCD*, *AK2*, *ADA*, *RAG1*,

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RAG2, *NHEJ1*, *LIG4*, *PNP*, and *ZBTB24*) was designed by our group and used to screen the patients but found no disease-causing variant in P1 [11]. The second child of the family (P2) had additional features besides T cell immunodeficiency, including nail dystrophy (Fig. 1a) and total alopecia (Fig. 1b). The x-ray examinations revealed that she had no thymus (Fig. 1c). All coding regions of the *FOXN1* gene (NM_003593.2) were examined in the two siblings by bidirectional sequencing. Amplicon sequences were evaluated with CLC workbench 3.6.1 (Denmark). To predict the functional impact of the variant, SIFT [12], Polyphen [13], and mutation taster [14] prediction tools were used. The available public databases including dbSNP, The Human Gene Mutation Database (HGMD), GenomAD, and the Exome Aggregation Consortium (ExAC) were used for frequency data. TREC and KREC copy numbers of the patients, parents, and 15 healthy subjects were detected by quantitative real-time PCR (qRT-PCR) as previously described [11]. The Istanbul Medical Faculty ethics board approved this study and informed consents were obtained from the parents.

Due to the additional clinical features, P2 was diagnosed as nude-SCID and mutation screening of *FOXN1* gene identified a novel homozygous single-nucleotide substitution (NM_003593.2:c.880G > A, p. (V294I)) located in exon 5 in the siblings (II-1 and II-2). Parents (I-1 and I-2) were also identified as carrying the heterozygous form of this variant (Fig. 1d). The novel missense variant is located on the forkhead domain that is a highly conserved region. This variant predicted as "disease-causing" in prediction tools and was absent in the public databases. The novel *FOXN1* mutation that we describe here has been submitted to the LOVD database (www.lovd.nl/FOXN1 (patient ID 00148287).

Clinical presentation of the affected sibling from a consanguineous family with recurrent infections, alopecia, nail dystrophy, and $T^{-}B^{+}NK^{+}$ SCID fits with the characteristics of *FOXN1* deficiency.

In addition, TREC/KREC copies were evaluated in all members of the family and absent T cell maturation was found with normal B cells in the patients. TRECs and KRECs originates from a stable circular DNA product during V(D)J recombination

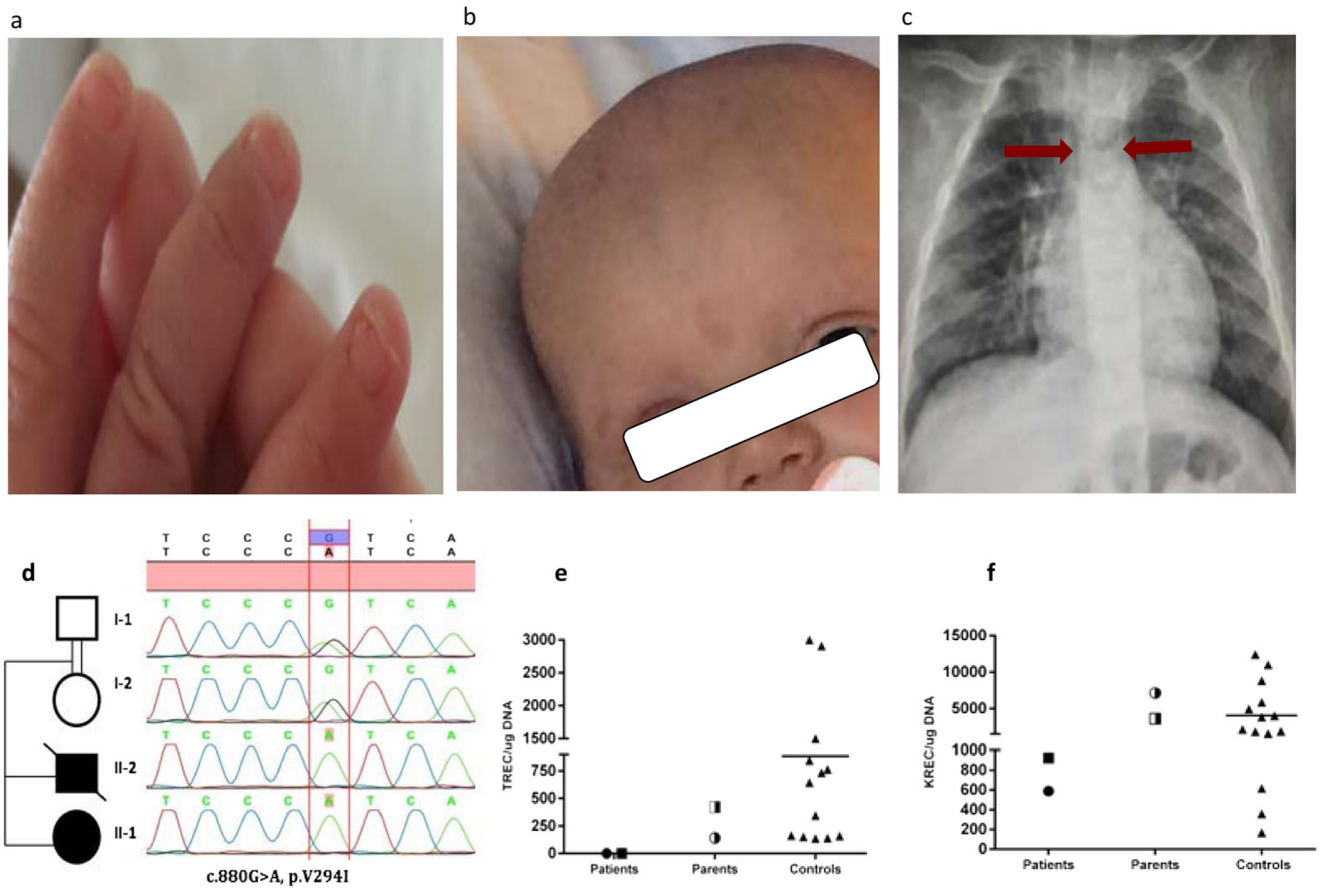

Fig. 1 Clinical and genetic features of the nude SCID patient (P2) with c.880G > A (p.V294I) variant in *FOXN1* gene **a** Nail dystrophy. **b** Nude phenotype of the patient, no hairline, and no eyebrows. **c** X-ray of proband showing thymus atrophy. **d** Sanger sequencing results of the proband and the family members. **e**, **f** T cell receptor excision circles (TREC) and Kappa-deleting recombination excision circle (KREC) analysis was performed to verify the T and B cell maturation levels in variant-detected patients (square P1, circle P2), heterozygous parents and healthy subjects by qRT-PCR. *TRAC* gene was used for normalization. Horizontal lines represent the mean values of TREC/KREC copy numbers in adult healthy subjects in the graphics (GraphPad Prism 7)

when the lymphocytes proliferate, are used to quantify T cell and B cell replication history [15, 16] and TRECs are reduced due to T cell immunodeficiency in nude SCID patients [3]. Heterozygotes parents showed reduced TREC levels as compared with their age-matched controls with normal KREC levels. Although the heterozygous carriers did not have any immunodeficiency, they had decreased T cell counts (Fig. 1e, f).

FOXN1 deficiency is an extremely rare form of SCID associated with a specific phenotype. So far, only three variants (p.R255*, p.R320W and p.S188 fs) have been described in nine patients [10, 17–19]. The first variant c.763C > T, p.(R255*), resulting in a truncated protein, was identified in an isolated area in Italy. The second variant c.987C > T, p.(R320W) is a homozygous missense variant that affects the DNA binding domain of the FOXN1 protein. The last variant of *FOXN1* is a frameshift variant c.562_562delA, p.(S188fs) which causes a premature truncation of the protein. All SCID patients with *FOXN1* deficiency reported so far presented with absent T cell counts. The p.(V294I) missense variant was identified in the nude SCID patient (this study) located in the forkhead domain part of the protein which is a highly conserved, winged-helix DNA binding part of the protein that regulates target genes of *FOXN1* [20].

The two cases described herein presented with the typical $T^{-}B^{+}NK^{+}$ SCID features, namely very early onset of severe infections, susceptibility to infections with opportunistic microorganisms and lack of T cells in the peripheral blood examination. It is known that thymus defects can only be cured by a thymus transplantation but during the first admission to the hospital, due to the patients' severe condition, she was administered HSCT immediately without any genetic diagnosis. The key to the diagnosis were additional features of P2 associated with the *FOXN1* deficiency, namely, the nail dystrophy and absent hair, which led us to sequence the *FOXN1* gene. *FOXN1* gene mutation screening was performed later and a pathogenic variant was identified in both siblings. There was no thymus reconstitution in the x-ray examinations (Fig. S2) and immunophenotyping showed low lymphocyte counts (Table S2). However, T cell proliferation after HSCT was confirmed by in vitro stimulation with CD3/CD28 antibodies (Fig. S3). Thirteen months after transplantation, hyperpigmentation of the skin was observed and a skin biopsy was performed for GVHD evaluation but pathologic examination did not show any evidence of GVHD. In thymus deficient SCIDs only geno-identical HSCT without the use of serotherapy is suggested and with the absence of a geno-identical sibling, thymus transplantation is still the only curative option.

In conclusion, a novel *FOXN1* variant (c:880G > A:p.(V294I)) described in a Turkish family, is consistent with nude SCID phenotype. High consanguineous marriage frequencies in certain regions of the country are associated with rare clinical and genetic events. Early genetic testing guides clinical diagnosis, provides accurate genetic counseling for the family and definitive management improves outcomes. Our study shows the importance of the correct diagnosis for the appropriate treatment choice and screening for rare but important genes like *FOXN1* in NGS panels for SCID for early evaluation of atypical/incomplete phenotypes. Thymus transplantation should be the only unique cure in the absence of a geno-identical donor in these thymus-deficient SCIDs.

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Compliance with Ethical Standards

Conflict of Interest The authors declare no conflict of interest.

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